REVIEW ARTICLE

# *Cryptosporidium* species in Australian wildlife and domestic animals

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SUMMARY

*Cryptosporidium* is an important enteric parasite that is transmitted via the fecal-oral route, water and food. Humans, wildlife and domestic livestock all potentially contribute *Cryptosporidium* to surface waters. Most species of *Cryptosporidium* are morphologically indistinguishable and can only be identified using molecular tools. Over 24 species have been identified and of these, 7 *Cryptosporidium* species/genotypes are responsible for most human cryptosporidiosis cases. In Australia, relatively few genotyping studies have been conducted. Six *Cryptosporidium* species (*C. hominis*, *C. parvum*, *C. meleagridis*, *C. fayeri*, *C. andersoni* and *C. bovis)* have been identified in humans in Australia. However, little is known about the contribution of animal hosts to human pathogenic strains of *Cryptosporidium* in drinking water catchments. In this review, we focus on the available genotyping data for native, feral and domestic animals inhabiting drinking water catchments in Australia to provide an improved understanding of the public health implications and to identify key research gaps.

Key words: *Cryptosporidium*, zoonotic, genotype, marsupials, sheep, cattle.

## INTRODUCTION

*Cryptosporidium* spp. are parasitic protists that infect a wide range of vertebrates including humans (Xiao and Ryan, 2004; Xiao, 2010). The parasite causes self-limiting diarrhoea in immunocompetent individuals but may be chronic and life threatening to those that are immunocompromised (Hunter *et al*. 2007). Humans can acquire *Cryptosporidium* infections through various transmission routes, such as direct contact with infected persons (person-to-person transmission) or animals (zoonotic transmission) and ingestion of contaminated food (foodborne transmission) or water (waterborne transmission) (Karanis *et al*. 2007; Xiao, 2010). Molecular data indicate that 7 *Cryptosporidium* species/genotypes are responsible for most human cryptosporidiosis cases, including *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. ubiquitum*, *C. cuniculus* (Xiao and Feng, 2008; Xiao, 2010; Chalmers *et al*. 2011) with *C. parvum* and *C. hominis* by far the most common species in humans worldwide (Xiao, 2010).

In Australia, 3 *Cryptosporidium* species (*C. hominis*, *C. parvum* and *C. meleagridis*) have been identified in humans in Western Australia (WA), New South Wales (NSW), Victoria (VIC) and South Australia (SA), with *C. hominis* being the most frequently identified species of the 3 (Robertson *et al*. 2002; Chalmers *et al*. 2005; Jex *et al*. 2007; Ng *et al*. 2008; O'Brien *et al*. 2008, Jex *et al*. 2008; Alagappan *et al*. 2008; Waldron *et al*. 2009*a*, *b*; Ng *et al*. 2010*a*,*b*; 2012; Waldron and Power, 2011; Waldron *et al*. 2011*a*,*b*). Recently, *C. fayeri*, *C. andersoni* and *C. bovis* have been identified in individual human cases in NSW (Waldron *et al*. 2010, 2011*a*; Ng *et al*. 2012).

A major mode of transmission of *Cryptosporidium* is via contaminated drinking and recreational waters. The oocyst is the environmentally stable stage and is able to survive and penetrate routine wastewater treatment and is resistant to inactivation by commonly used drinking water disinfectants (Fayer *et al*. 2000). Of the waterborne protozoan parasitic outbreaks that have been reported worldwide between 2004 and 2010, *Cryptosporidium* was the aetiological agent in 60·3% (120) of the outbreaks (Baldursson and Karanis, 2011). Oocyst transport to surface water can occur by deposition of manure directly in the water or by wash off in surface runoff. Humans, wildlife and domestic livestock all potentially contribute *Cryptosporidium* to surface waters. A significant knowledge gap in assessing microbial risks to surface waters is the lack of genotyping data on the

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Table 1. Estimates of wildlife animal density for native vegetation land use areas

| Animal | Country | Density (per km$^2$) | Reference |
|---|---|---|---|
| Kangaroos | Australia | 400–500 | Fletcher (2007) |
| Kangaroos | Australia | ~ 500 | West (2008) |
| Kangaroos | Vic | 178 | Ramp (2002) |
| Pigs (feral) | WA | 2 | Dr Peter Adams, *personal communication* |
| Pigs (feral) | Qld | 1–>20 | Dexter (1990) |
| Pigs (feral) | NSW | 2 | Saunders and Kay (1991) |
| Rodents | UK | Similar to human population | O'Keefe *et al*. (2003) |
| Rabbits | Australia | 50–400 | Williams *et al*. (1995) |
| Foxes | Australia | 1–7·2 | Saunders *et al*. (1995) |
| Dogs (feral) | Australia | 0·25 | Ferguson (2010) |
| Cats (feral) | Australia | 1–57 | Dickman (1996) |

Table 2. Manure production rates for wildlife animals

| Animal | Country | Kg manure/ animal$^{-1}$/d$^{-1}$ | Reference |
|---|---|---|---|
| Kangaroos | Australia, Canberra | 0·3 | Dr Peter Adams, *personal communication* |
| Pigs (feral) | Western Australia | 4–5 | Dr Peter Adams, *personal communication* |
| Deer | USA | 1–2 | Anonymus (2003) |
| Rabbits | Netherlands | 0·019 | Medema (1999) |
| Dogs (feral) | Australia | 0·003–0·014 | Anonymus (2002) |

contribution of animal hosts to human pathogenic strains of *Cryptosporidium* in drinking water catchments in Australia. Identification of the sources/carriers of human pathogenic strains is essential for accurate risk assessment and catchment management.

This review examines the current information from Australia on the prevalence and genotypes of *Cryptosporidium* identified in wildlife and domestic livestock to assist our understanding of the key host species contributing human pathogenic *Cryptosporidium* species to Australian water catchments.

## SPECIES AND STRAINS OF *CRYPTOSPORIDIUM* INFECTING WILDLIFE

### *Wildlife population density*

Animal density by area is an important determinant of pathogen loadings as higher animal density results in a larger volume of manure excreted per unit area. Thus there is an increase in pathogen source material that may be transported in runoff to surface waters and/or deposited directly to streams (Ferguson, 2010). It is difficult to quantify animal densities for wildlife, because animal movement is uncontrolled and because animal populations vary with season and environmental conditions, with many species being migratory but published values and estimates are summarized in Table 1.

### *Volume of wildlife manure*

Manure excretion rates and volumes for wildlife are less well documented than for domestic animals. An estimate of the volume of manure produced by wildlife, however, is important to assess the impact of wildlife manure on catchments. Estimates of manure production rates for wildlife are shown in Table 2.

### *Marsupials*

In Australia, marsupials are the dominant animals inhabiting many water catchment areas (Power, 2010) with densities >500 per square km sometimes recorded (West, 2008). Therefore, it is important to determine the potential role that marsupial species play in the dissemination of *Cryptosporidium* to drinking water sources and the associated human health risks associated with this. To date, *Cryptosporidium* has been identified in 15 Australian marsupial species and 7 of these host species belong to the family Macropodidae (kangaroos and wallabies) (Power, 2010; Yang *et al*. 2011; Dowle *et al*., *unpublished observations*).

The prevalence of *Cryptosporidium* in marsupials varies, as does the oocyst shedding rate. In New South Wales (NSW), the prevalence of *Cryptosporidium* in fecal samples from eastern grey kangaroos (*Macropus giganteus*) was 6·3% (239/3,557) (Power

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Table 3. *Cryptosporidium* species identified in marsupial hosts in Australia

| Host species | *Cryptosporidium* species identified | Prevalence | Location | Reference |
|---|---|---|---|---|
| Red kangaroo (*Macropus rufus*) | *C. fayeri* | N/A* | NSW | Morgan *et al.* (1997) |
| Koala (*Phascolarctos cinereus*) | *C. fayeri* | N/A | NSW | Morgan *et al.* (1997) |
| Eastern grey kangaroos (*Macropus giganteus*) | *C. fayeri* and *C. macropodum* | 6·7% | NSW | Power *et al.* (2003, 2004, 2005) |
| Eastern grey kangaroos (*Macropus giganteus*) | *C. macropodum*, *C. parvum*, *C. hominis* | 16·9% | NSW | Ng *et al.* (2011*a*) |
| Yellow-footed rock wallaby (*Petrogale xanthopus*), | *C. fayeri* | N/A | NSW | Power *et al.* (2009) |
| Wallaby (no species identification) | *C. parvum* | 5·9% | NSW | Ng *et al.* (2011*a*) |
| Swamp wallaby (*Wallabia bicolour*) | *C. macropodum* | N.A | NSW | Ryan *et al.*, *unpublished observations* |
| Western-barred bandioot (*Peremeles bougainville*) | *C. fayeri* | N/A | WA | Weilinga *et al.*, *unpublished observations* |
| Western grey kangaroos (*Macropus fuliginosus*) | *C. fayeri*, *C. macropodum*, kangaroo genotype I, *C. xiaoi* | 9·3% | WA | McCarthy *et al.* (2008); Yang *et al.* (2011) |
| Bilby (*Macrotis lagotis*) | *C. muris* | N/A | WA | Warren *et al.* (2003) |
| Long-nosed bandicoot (*Perameles nasuta*) | *C. parvum/hominis* like | 9·3% | NSW | Dowle *et al.*, *unpublished observations* |
| Southern Brown Bandicoot (*Isoodon obesulus*) | *C. parvum/hominis* like | 16·7% | NSW | Dowle *et al.*, *unpublished obsevations* |
| Brush tail possum (*Trichosuris vulpecula*) | Brushtail possum genotype I, *C. parvum/hominis*-like. | 11·3% (urban) 5·6% (wild) | NSW | Hill *et al.* (2008) |

* N/A, not attempted (in many cases only 1 sample was available).

*et al.* 2005). Oocyst shedding ranged from 20/g feces to $2{\cdot}0\times10^6$/g feces (Power *et al.* 2005). Another study in NSW on common brushtail possums (*Trichosurus vulpecula*) reported that *Cryptosporidium* occurred with a higher prevalence in possums from urban habitats (11·3%) than in possums from woodland habitats (5·6%) (Hill *et al.* 2008). In Western Australia (WA), the prevalence of *Cryptosporidium* in wild western grey kangaroos (*Macropus fuliginosus*), was 9·3% (Yang *et al.* 2011). Prevalences can be seasonal with one study in NSW on eastern grey kangaroos reporting the highest rate was found in autumn (Power *et al.* 2004), while a study in WA on western grey kangaroos reported that the highest prevalence was detected in summer (Yang *et al.* 2011).

The main species identified in marsupials are *C. fayeri* and *C. macropodum* (previously marsupial genotype I and II) (Morgan *et al.* 1997; Power *et al.* 2004, 2005, 2009; McCarthy *et al.* 2008; Power and Ryan, 2008; Ryan *et al.* 2008; Power, 2010; Ng *et al.* 2011*a*). *Cryptosporidium fayeri* and *C. macropodum* have been reported in a red kangaroo (*Macropus rufus*), a koala (*Phascolarctos cinereus*), eastern grey kangaroos, (*M. giganteus*), western grey kangaroos (*M. fuliginosus*), a yellow-footed rock wallaby (*Petrogale xanthopus*), a swamp wallaby (*Wallabia bicolour*), a wallaby (no species identification) and a western-barred bandicoot (*Peremeles bougainville*) (Morgan *et al.* 1997; Power *et al.* 2004; 2009; McCarthy *et al.* 2008; Ryan *et al.*, *unpublished observations*; Weilinga *et al.*, *unpublished observations*; Yang *et al.* 2011; Ng *et al.* 2011*a*) (Table 3). Neither of these species is associated with diarrhoea in their marsupial hosts (Power and Ryan, 2008; Ryan *et al.* 2008); however, *C. fayeri* has recently been identified in a 29-year-old woman in Sydney in 2009 (Waldron *et al.* 2010). The woman was immuno-competent but suffered prolonged gastrointestinal illness. The patient resided in a national forest on the east coast of New South Wales, Australia, an area where marsupials are abundant. She had frequent contact with partially domesticated marsupials (Waldron *et al.* 2010). Identification of *C. fayeri* in a human patient is a concern for water catchment authorities in the Sydney region. The main water supply for Sydney, Warragamba Dam, covers 9050 $km^2$ and is surrounded by national forest inhabited by diverse and abundant marsupials. The same *C. fayeri* subtype (IVaA9G4T1R1) was also identified in eastern grey kangaroos in Warragamba Dam (Power *et al.* 2009).

In addition to *C. fayeri* and *C. macropodum*, there have been several other host-adapted strains identified in Australian marsupials. Possum genotype I has been described in brush tail possums, a host species found in a range of habitats throughout Australia (Hill *et al.* 2008) and the novel kangaroo genotype I in western grey kangaroos (Yang *et al.* 2011). These *Cryptosporidium* genotypes were genetically distinct and the range of genetic similarities to all other

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*Cryptosporidium* species at the 18S rRNA locus was 87·4–97·4% and at the actin locus it was 77·9–86·5%. This is within the range of the percentage similarities between currently accepted *Cryptosporidium* species at the 18S rRNA locus (89–99·8%) and the actin locus (76–98·7%) and is one of the criteria used to delimit species within the genus *Cryptosporidium* (Xiao and Ryan, 2004). Possum genotype 1 and kangaroo genotype I have not been reported in humans or other animals, and the zoonotic potential is unknown.

*Cryptosporidium* species typically found in non-marsupial mammalian hosts have also been reported in marsupial species. *Cryptosporidium muri*s, commonly found in rodents, has been identified in bilbies (*Macrotis lagotis)* (Warren *et al*. 2003). In that report, oocyst morphology combined with molecular analyses confirmed the presence of *C. muris* in 11/28 (39·2%) bilbies housed at a captive breeding colony in WA (Warren *et al*. 2003). One mouse was trapped in the breeding enclosures and found to be positive for *C. muris*. It is likely that the bilbies acquired the infection from mice by fecal contamination of food and water. *Cryptosporidium xiaoi*, commonly found in sheep, has been identified in 6 wild western grey kangaroos in WA (Yang *et al*. 2011). The identification of *C. xiaoi* in the kangaroos suggests that they may have picked up the infection from grazing on sheep pastures. In a previous study on sheep, *C. fayeri* was identified in 4 sheep fecal samples (Ryan *et al*. 2005), indicating that grazing on contaminated pastures can result in transmission. Whether the kangaroos were actually infected or simply mechanically transmitting the organism remains to be determined.

There have also been reports of *C. parvum* and *C. hominis* in kangaroos (*M. giganteus*, a wallaby (no species identification), possums (*Trichosuris vulpecula)* and bandicoots (*Isoodon obesulus)* (Ng *et al*. 2011*a*; Hill *et al*. 2008; Dowle *et al., unpublished observations*). These identifications were based on direct DNA extraction from feces and subsequent PCR screening. In all cases *Cryptosporidium* could only be amplified at the multi-copy 18S rDNA locus, other loci tested were single copy targets (Ng *et al*. 2011*a*; Hill *et al*. 2008; Dowle *et al., unpublished observations*). The inability to amplify other loci may be due to low levels of *Cryptosporidium* present in the samples. Immunomagnetic separation coupled to cell sorting was used to determine oocyst numbers in *C. parvum* and *C. hominis* positive fecal samples in bandicoots and possums (Hill *et al*. 2008; Dowle *et al., unpublished observations*). The authors reported that most samples contained less than 10 oocysts per gramme of feces. In those studies, the marsupial hosts were inhabiting areas associated with humans. It remains to be determined whether these marsupials were actually infected with *C. parvum* or *C. hominis* or whether they were simply passively transmitting the oocysts. Further studies are required to clarify the potential role that marsupials play in contamination of the catchment with human-infectious oocysts.

### *Feral and domestic pigs*

Pigs were introduced to Australia with the First Fleet in 1788 and today domestic pigs are agriculturally important, in addition to being pest species. Feral pig populations are now found within 40% of Australia's ecosystem (West, 2008) and represent a potential risk to drinking water supplies. It has been reported that Australia has the largest number of wild pigs in the world with an estimated 23 million feral pigs (Hampton *et al*. 2006). The high density of feral pigs, their foraging and wallowing behaviour of pigs, which can markedly increase the turbidity of water supplies and their ability to transmit and excrete a number of infectious waterborne organisms pathogenic to humans have made them a target for intense management for the protection of source (drinking) water supplies (Atwill *et al*. 1997; Hampton *et al*. 2006).

Little is known about the prevalence of *Cryptosporidium* in feral pigs. A study in California reported that 12 (5·4%) of 221 feral pigs were shedding *Cryptosporidium* oocysts (Atwill *et al*. 1997). The authors also reported that younger pigs (< or = 8 months) and pigs from high-density populations (> 2·0 feral pigs/km$^2$) were significantly more likely to shed oocysts compared to older pigs (> 8 months) and pigs from low-density populations (< or = 1·9 feral pigs/km$^2$) (Atwill *et al*. 1997). This trend makes reduction of feral pig abundance in high-density catchment areas even more important to reduce the risk of waterborne feral pig pathogens being introduced to reservoirs. In Spain, a prevalence of 7·6% was reported for wild boar and infections were significantly higher in juvenile male wild boars (22%) than in adult males (6%) (Castro-Hermida *et al*. 2011). The mean intensity of infection by *Cryptosporidium* was 5 to 200 oocysts per gramme of faeces (Castro-Hermida *et al*. 2011). A study in WA reported a prevalence of 0·3% (1/292) (Hampton *et al*. 2006). Genotyping attempts were unsuccessful. A more recent study of 237 wild pigs in WA, did not identify *Cryptosporidium* by PCR (Pallant *et al., unpublished observations*).

Studies in Australia on domestic pigs have identified prevalence rates of 6–22·1% (Ryan *et al*. 2003, 2004; Johnson *et al*. 2008). The main *Cryptosporidium* species identified in pigs in Australia and worldwide are *C. suis* and pig genotype II, although *C. muris, C. tyzzeri* and *C. parvum* have also been reported (Ryan *et al*. 2003; Xiao *et al*. 2006; Zintel *et al*. 2007; Johnson *et al*. 2008; Kváč *et al*. 2009*a*; Jeníková *et al*. 2010; Jenkins *et al*. 2010; Sevá Ada *et al*. 2010; Xiao, 2010; Wang *et al*. 2010*a*;

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Budu-Amoako *et al*. 2012; Chen *et al*. 2011; Farzan *et al*. 2011; Fiuza *et al*. 2011*a*; Yin *et al*. 2011). *Cryptosporidium suis* has been reported in humans (Xiao *et al*. 2002*a*; Xiao, 2010) and has frequently been recovered from water samples (Feng *et al*. 2011*a*). Pig genotype II has also been reported in an immunocompetent human (Kvác *et al*. 2009*b*).

*Cryptosporidium parvum* has been reported once in pigs from an indoor farm in Western Australia, in four 19-day-old pre-weaned piglets with diarrhoea (Morgan *et al*. 1999*a*). There have been 4 additional reports of *C. parvum* in pigs internationally; in asymptomatic sows from intensive commercial pig production units in Ireland (Zintel *et al*. 2007); in 2 piglets from Prince Edward Island, Canada (Budu-Amoako *et al*. 2012), in piglets in Ontario where it was the post prevalent species detected (55·4%) (Farzan *et al*. 2011) and in pig slurry lagoons in the US (Jenkins *et al*. 2010). This suggests that pigs may play an important role in the transmission of zoonotic *Cryptosporidium*. However, further research is required to understand the prevalence of *Cryptosporidium* species in feral pigs.

### *Deer*

*Cryptosporidium* species have been found in various species of deer and the prevalence rates differed by study locations and animal species ranging from 0–100% (cf. Feng, 2010). *Cryptosporidium ubiquitum*, the deer genotype, *C. parvum* and a *C. hominis-like* genotype have been reported in wild deer (cf. Amer *et al*. 2009; Jellison *et al*. 2009; Feng, 2010).

Few studies have been conducted in wild deer in Australia (Cinque *et al*. 2008; Ng *et al*. 2011*a*). In a recent study in NSW, only 1 deer was positive out of 137 isolates screened (0·7%) (Ng *et al*. 2011*a*) and the target 18S rRNA sequence was identical to *Cryptosporidium* environmental sequence isolate 8059 (GenBank Accession no. AY737603) from water previously identified in New York storm water in the US (Jiang *et al*. 2005). The other study was conducted on Sambar deer (*Cervus unicolor*) from Melbourne catchments (Cinque *et al*. 2008). In that study, 16/32 pooled fecal samples were positive for *Cryptosporidium* and 7 of these were identified as *C. parvum* by sequence analysis of the 18S ribosomal RNA gene (Cinque *et al*. 2008). Analysis of a further 600 samples using PCR-based (single strand conformation polymorphism (SSCP) analysis and selective sequencing of the second internal transcribed spacer (ITS-2) as well as 18S rRNA and the glycoprotein 60 (*gp60*) gene did not identify *C. parvum* (Cinque *et al*. 2008). As both *C. parvum* and *C. ubiquitum* are infectious to humans, further research is required to understand the contribution of deer to catchment contamination with human-infectious species of *Cryptosporidium*.

### *Rodents*

Mice are closely associated with human activity and are now distributed throughout the Australian continent, especially in agricultural and urban areas. The black rat (*Rattus rattus*), which originated in tropical mainland Asia and, later spreading to Europe and the rest of the world (Musser and Carleton, 1993), is now found throughout much of coastal Australia including urban and peri–urban habitats (West, 2008). Rodents, which are abundant and widespread, have been considered reservoirs of cryptosporidiosis in humans and farm animals (Lv *et al*. 2009). Nearly 40 rodent species belonging to 11 families (Sciuridae, Muridae, Cricetidae, Castoridae, Geomyidae, Hystricidae, Erethizontidae, Myocastoridae, Caviidae, Hydrochoeridae, and Chinchillidae) have been reported as hosts of *Cryptosporidium* spp. (cf. Lv *et al*. 2009; Feng, 2010). These include mice (*Mus musculus*, *M. spretus Apodemus flavicollis*, *A. sylvaticus*, *A. speciosus*, *Peromyscus* sp.), rats (*Rattus norvegicus*, *R. rattus*), voles (*Clethrionomys glareolus*, *Clethrionomys gapperi*, *Microtus arvalis*, *M. agrestis*, *M. pennsylvanicus*, *Myodes gapperi*), muskrat (*Ondatra zibethicus*) and squirrels (*Spermophilus beecheyi*, *Sciurus carolinensis*, *Tamiasciurus hudsonicus*, *Sciurus vulgaris*) (Lv *et al*. 2009; Feng, 2010).

Prior to genotyping studies, it was thought that rodents were infected with *C. parvum* and *C. muris* (Feng, 2010). However, it is now believed that most infections in house mice are *C. tyzzeri* (formerly mouse genotype I), which differs significantly from *C. parvum* (Xiao *et al*. 2004; Ren *et al*. 2011). Thus, house mice are commonly infected with *C. muris* and *C. tyzzeri*, and occasionally with the mouse genotype II (Morgan *et al*. 1999*c*, 2000; Foo *et al*. 2007). Confirmed *C. parvum* infections have been reported in only a few rodents (Morgan *et al*. 1999*c*; Lv *et al*. 2009; Feng, 2010).

Several species/genotypes have been identified in rats including *C. tyzzeri* and 4 rat genotypes (1-IV) (Lv *et al*. 2009). Rat genotype 1 has previously been identified in a boa constrictor in the US (Xiao *et al*. 2004) and in wastewater in Shanghai (Feng *et al*. 2009) and the UK (Chalmers *et al*. 2010). Rat genotypes II and III have previously been described from brown rats (*Rattus norvegicus*) and Asian house rats (*Rattus tanezumi*) from China (Lv *et al*. 2009). Rat genotype IV (previously W19) has been identified in storm-water (Jiang *et al*. 2005; Lv *et al*. 2009). Despite the identification of *Cryptosporidium* rodent genotypes from stormwater and wastewater (Jiang *et al*. 2005; Feng *et al*. 2009; Lv *et al*. 2009; Chalmers *et al*. 2010), the contribution of rodents to contamination of drinking water supplies with *Cryptosporidium* is not well understood.

In Australia, *C. tyzzeri*, mouse genotype II and rat-like genotypes have been identified in mice

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(Morgan *et al*. 1999*b*, *c*; Foo *et al*. 2007) and rat-like genotypes have been identified in black rats (Paparini *et al*. 2012). Recent evidence suggests that *C. tyzzeri*, however, could be a human pathogen, as subtype analysis at the hypervariable *gp60* locus identified a *C. tyzzeri* subtype in a symptomatic Kuwaiti child (Sulaiman *et al*. 2005; Feng *et al*. 2011*b*). Further studies are needed to determine the zoonotic potential of *C. tyzzeri*. This species was previously assumed to be *C. parvum* but subsequent re-analysis identified it as *C. tyzzeri* (Feng *et al*. 2011*b*). *Cryptosporidium parvum* has not been identified in Australian rodents but has been identified in mice in the UK (Morgan *et al*. 1999*c*). Limited studies of *Cryptosporidium* in rodents have been undertaken in Australia. Given the occurrence of zoonotic species in this host in other countries, it may represent an important reservoir for human infective species and requires further study.

### *Rabbits*

Rabbits (*Oryctolagus cuniculus*) were introduced in the mid- to late 1800s, are presently found in all states and territories throughout Australia and are one of the most widely distributed and abundant mammals in Australia. Rabbits presently inhabit an estimated 70% (i.e. 5·33 million square kilometres) of Australia and populations can reach 300–400 per square kilometre (Williams *et al*. 1995).

Our current knowledge and understanding of *Cryptosporidium* in rabbits is limited. In Australia, only 2 studies have been conducted. One study screened 176 fecal samples from rabbits from 4 locations northeast of Melbourne (< 90 km apart) in Victoria. The prevalence rate was 6·8% and all positives were identified as *C. cuniculus* (Nolan *et al*. 2010). Another screened 3 rabbits near the Denmark River in WA and found 1 positive, which was identified as *C. cuniculus* (Ferguson, 2010). The prevalence of *Cryptosporidium* in rabbits in other countries ranges from 0·9 to 42·9% (Robinson and Chalmers, 2010; Shi *et al*. 2010) and genotyping studies have all have identified that rabbits habour *C. cuniculus* (previously the 'rabbit' genotype (cf. Robinson and Chalmers, 2010; Nolan *et al*. 2010; Ferguson, 2010; Shi *et al*. 2010).

*Cryptosporidium cuniculus* was initially thought to be host-specific until the recent discovery that *C. cuniculus* was linked to a human cryptosporidiosis outbreak in the UK (Chalmers *et al*. 2009), which has raised considerable awareness about the importance of investigating rabbits as a source of *Cryptosporidium* transmissible to humans. A recent study in the UK reported that *C. cuniculus* was the third most commonly identified *Cryptosporidium* species in patients with diarrhoea (Chalmers *et al*. 2011). *Cryptosporidium cuniculus* has a close genetic relationship with *C. hominis* with limited differences at the 18S rRNA, *hsp70* and actin genes (0·51%, 0·25% and 0·12%, respectively) and only 0·27% of base pairs when combined multiple loci (4469 bp) were investigated (Robinson *et al*. 2010). *C. cuniculus* has also recently been identified in children in Nigeria (Molloy *et al*. 2010).

Rabbits are susceptible to experimental infection with *C. cuniculus*, *C. parvum* and *C. meleagridis* (Robinson and Chalmers, 2010). All these species are human pathogens and the role of rabbits as a potential source of zoonotic *Cryptosporidium* must be considered, although direct contact with rabbits or their feces has not been identified as a risk factor for human cryptosporidiosis (Robinson and Chalmers, 2010). However, our understanding of the potential risks from rabbits for human infection with *Cryptosporidium* is still at an early stage and its genetic similarity to *C. hominis* and the recent finding of the parasite in humans in the UK and children in Nigeria, indicate that rabbits can be a potential reservoir of zoonotic cryptosporidiosis. More systematic characterization of the parasite is needed to understand the taxonomic status of *C. cuniculus* and its public health significance.

### *Foxes, wild dogs and feral cats*

The wild dog population of Australia comprises all wild-living dogs and includes dingoes (*Canis lupus dingo*), feral dogs (*Canis lupus familiaris*) and their hybrids. They are distributed widely throughout the country and are pests in many agricultural areas. Wild dogs presently inhabit an estimated 82·8% (i.e. 6·3 million square kilometres) of Australia (West, 2008). Dingoes are thought to be descendants of East Asian dogs that were first introduced to Australia about 3500–4000 years ago (Corbett, 1995). Feral dogs are descendants of European domestic dogs that were introduced over the past 200 years.

The European red fox is one of the most widely spread feral animals in Australia and Australia's number one predator threatening the long-term survival of a many native wildlife species. The fox is found ranging from Australia's arid centre to the alps and coastal areas, and is also abundant in urban areas (West, 2008).

Domestic cats (*Felis catus*) were introduced to Australia either before or during European settlement and have been released deliberately in many areas to control rabbits, mice and rats (McLeod, 2004). Feral cat populations have now established in almost every significant habitat type throughout Australia, they also inhabit many of Australia's small islands. It is estimated that there are about 18 million feral cats (McLeod, 2004) and populations can reach as high as 57 cats per square kilometre (Dickman, 1996).

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Table 4. Livestock numbers in Australia by State

(NSW, New South Wales; Vic, Victoria; Qld, Queensland; SA, South Australia; WA, Western Australia; Tas, Tasmania; NT, Northern Territory; ACT, Australian Capital Territory.)

| Animal | Total no. | NSW | Vic | Qld | SA | WA | Tas | NT | ACT |
|---|---|---|---|---|---|---|---|---|---|
| Sheep and lambs | 68 085 497 | 24 366 338 | 14 377 696 | 3 622 141 | 8 989 472 | 14 691 553 | 1 991 282 | 6 | 47010 |
| Dairy cattle | 2 542 363 | 348 318 | 1 588 693 | 162 200 | 138 501 | 113 023 | 1 916 221 | 1 | 5 |
| Meat cattle | 24 007 730 | 5 107 062 | 2 079 529 | 11 193 348 | 903 861 | 2 206 183 | 445 751 | 2 065 746 | 6250 |
| Pigs | 2 289 292 | 584 614 | 509 884 | 583 144 | 381 131 | 219 393 | 11 092 | 35 | 0 |

Recently published studies of *Cryptosporidium* infection in cats and dogs, worldwide, have reported prevalence rates in dogs ranging from 0·5% to 44·1% and in cats from 0% to 29·4% (cf. Lucio-Forster *et al*. 2010). In foxes, prevalence rates of 7·9–8·5% have been reported (Feng, 2010). Genotyping studies of *Cryptosporidium* oocysts in feces of dogs and cats, have demonstrated that most infections in these animals are caused by *C. canis* and *C. felis*, respectively. *Cryptosporidium felis* has a restricted host range and has been identified in cats, immunocompetent and immunocompromised humans and a cow (Bornay-Llinares *et al*. 1999; Lucio-Forster *et al*. 2010). Similarly, *C. canis* has been identified in dogs, foxes, wolves and immunocompetent and immunocompromised humans (Lucio-Forster *et al*. 2010). In children in developing countries, *C. felis* and *C. canis* are responsible for as much as 3·3% and 4·4% respectively of overall cryptosporidiosis cases (Lucio-Forster *et al*. 2010). *Cryptosporidium muris* and *C. parvum* have also occasionally been reported in dogs and cats (cf. Lucio-Forster *et al*. 2010). *Cryptosporidium muris* has a wide host range and has also been identified in a few humans in developing countries (Palmer *et al*. 2003; Gatei *et al*. 2006; Muthusamy *et al*. 2006). However, most human cases of cryptosporidiosis, worldwide, are associated with *C. hominis* and *C. parvum* (Xiao, 2010) and therefore *C. muris, C. canis* and *C. felis* are likely to be of low zoonotic risk to humans.

In Australia, a prevalence of 22·7% ($n=44$) was reported for dingoes and wild dogs and genotyping identified *C. canis* and a *C. hominis*-like genotype (Ng *et al*. 2011*a*). In domestic dogs in Australia, only *C. canis* has been identified and *C. felis* and *C. muris* have been identified in domestic cats in Australia (Sargent *et al*. 1998; Morgan *et al*. 1998, 2000; Palmer *et al*. 2008; FitzGerald *et al*. 2011).

Of the few genotyping studies have been conducted in foxes, 3 species have been identified; the *Cryptosporidium* fox genotype, *C. canis* fox subtype (a variant of *C. canis*), and *C. canis* (Xiao *et al*. 2002*b*). In Australia, a prevalence of 10·5% ($n=19$) was reported in foxes and *C. canis* and a *C. macropodum*-like genotype were identified (Ng *et al*. 2011*a*). Foxes, wild dogs and feral cats are unlikely to be a major source of zoonotic *Cryptosporidium* in catchments but further research is required.

## SPECIES AND STRAINS OF *CRYPTOSPORIDIUM* INFECTING DOMESTIC LIVESTOCK

Over the past 20 years, sheep and and particularly pre-weaned cattle have been identified as being one of the main reservoir hosts for the zoonotic *C. parvum* (Davies *et al*. 2003, 2004, Ferguson *et al*. 2003; Fayer *et al*. 2006, 2007, 2008; Santin *et al*. 2004, 2008; Xiao and Feng, 2008; Xiao, 2010). However, studies worldwide suggest that cattle are infected with at least 5 *Cryptosporidium* parasites. In sheep a total of 8 species/genotypes of *Cryptosporidium* have been reported (Fayer *et al*. 2005, 2008; Xiao and Feng, 2008; Xiao, 2010).

Conclusive molecular evidence linking contamination of water supplies by sheep or cattle with outbreaks of cryptosporidiosis in human populations is scant; however, there have been several studies in which outbreaks of cryptosporidiosis have been strongly linked with sheep and cattle grazing near the implicated reservoir, catchment or river (Anonymus, 1999; Qamruddin *et al*. 1999; Yang *et al*. 2008; Ruecker *et al*. 2007). In addition, many studies have reported that *C. andersoni*, which is predominantly a parasite of adult cattle, *C. ubiquitum*, which is a common *Cryptosporidium* species in sheep and *C. parvum* are the dominant *Cryptosporidium* species detected in watersheds and raw and drinking water (Yang *et al*. 2008; Ruecker *et al*. 2007; Nichols *et al*. 2010; Smith and Nichols, 2010). In addition, the high density of cattle and sheep across Australia, which contribute a large volume of manure to catchments indicate that they are the main species that present a risk to public health in Australian catchments.

### *Population density of domestic livestock*

An assessment of stock numbers within Australia was obtained from the Australian Bureau of Statistics Agricultural Commodities, Australia, 2009/2010 (Table 4). In 2009/2010, NSW had the highest

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number of sheep (24·3 million), followed by WA (14·6 million) and Victoria (Vic) (14·3 million). Dairy cattle were reported at 2·5 million for 2009/2010, with Victoria continuing to dominate the dairy industry with 62% of Australia's total dairy herd at 1·5 million. Meat cattle were reported as 24 million in 2009/2010 with the highest number in Queensland (Qld) (11·1 million), followed by NSW (5·1 million), and WA (2·2 million). Pigs were reported as 2·2 million in 2009/2010, with the highest density in NSW at 0·58 million.

### *Volume of domestic livestock manure*

Livestock excretion rates and volumes are reasonably well documented compared to those for wildlife. Estimates for manure production rates for domestic livestock were obtained from a 2003 revision of the Manure Production and Characteristics produced by the American Society of Agricultural Engineers (Anonymus, 2003). The data was combined from a wide base of published and unpublished information on livestock manure production and characterization (Anonymus, 2003). It has been estimated that a 400 kg adult beef cow will produce on average 23 kg of feces per day and a 400 kg dairy cow 34·4 kg of feces per day. A 45 kg adult sheep will produce on average 1·8 kg of feces per day and a 40 kg pig will produce on average 3·4 kg of feces per day (Anonymus, 2003).

### *Cattle*

In cattle, cryptosporidiosis causes significant neonatal morbidity, resulting in weight loss and delayed growth, which leads to large economic losses (McDonald, 2000). Contamination of food or water by cattle manure has been identified as a cause of several foodborne and waterborne outbreaks of cryptosporidiosis (Glaberman *et al*. 2002; Blackburn *et al*. 2006). In case–control studies, contact with cattle was implicated as a risk factor for human cryptosporidiosis in the United States, United Kingdom, Ireland and Australia (Robertson *et al*. 2002; Goh *et al*. 2004; Hunter *et al*. 2004; Roy *et al*. 2004).

The environmental loading rate of *Cryptosporidium* in cattle has been estimated at between 3900 and $1{\cdot}7 \times 10^5$ oocysts $cow^{-1}$ $day^{-1}$ (Hoar *et al*. 2000; Atwill *et al*. 2003). In eastern Australian cattle feedlot manures, the occurrence of *Cryptosporidium* and other pathogens was quantified using quantitative PCR. High counts of *Cryptosporidium* ($>10^5$ $g^{-1}$) were sporadically identified in all manures (Klein *et al*. 2010). Cattle can therefore potentially contribute significantly to contamination of drinking water catchments with *Cryptosporidium*. It is essential, however, to determine the proportion of oocysts shed that are infectious to humans. Studies worldwide suggest that cattle are infected with at least 5 *Cryptosporidium* parasites: *C. parvum*, *C. bovis*, *C. andersoni*, *C. ryanae* (previously called deer-like genotype) and *C. suis* (Santin *et al*. 2004; Fayer *et al*. 2006, 2007; Starkey *et al*. 2006; Coklin *et al*. 2007; Feng *et al*. 2007; Geurden *et al*. 2007; Langkjaer *et al*. 2007; Mendonça *et al*. 2007; Plutzer and Karanis, 2007; Halim *et al*. 2008; Nuchjangreed *et al*. 2008; Wielinga *et al*. 2008; Liu *et al*. 2009; Keshavarz *et al*. 2009; Santin *et al*. 2008, 2009; Xiao and Feng, 2008; Ondrácková *et al*. 2009; Paul *et al*. 2008, 2009; Amer *et al*. 2009, 2010; Ayinmode *et al*. 2010; Diaz *et al*. 2010; Silverlås *et al*. 2010; Fayer *et al*. 2010; Karanis *et al*. 2010; Khan *et al*. 2010; Xiao, 2010; Kváč *et al*. 2011; Maikai *et al*. 2011; Meireles *et al*. 2011; Muhid *et al*. 2011; Nazemalhosseini-Mojarad *et al*. 2011). Of these only *C. parvum* is a major human pathogen (Xiao, 2010).

There also appear to be geographical differences in the age-related prevalence of different *Cryptosporidium* species in cattle (Table 5). Few longitudinal studies have been conducted but a study in the US reported that the highest prevalence of infection occurs in calves <8 weeks of age (45·8%), followed by post-weaned calves (3–12 months of age) (18·5%) and heifers (12–24 months of age) (2·2%) (Santin *et al*. 2008). Other studies have reported prevalences as high as (75·9%) in 11 to 22-day-old calves, which subsequently decreased (Coklin *et al*. 2010). In parts of the US, Belgium, Ireland, Germany, Malaysia, the UK and Sweden, it has been reported that the zoonotic *C. parvum* is responsible for the majority of *Cryptosporidium* infections in pre-weaned calves and only a small percentage of *Cryptosporidium* infections in post-weaned calves and heifers (Santin *et al*. 2004, 2008; Brook *et al*. 2009; Coklin *et al*. 2007; Geurden *et al*. 2007; Thompson *et al*. 2007; Xiao *et al*. 2007; Broglia *et al*. 2008; Halim *et al*. 2008; Paul *et al*. 2008; Fayer *et al*. 2010; Silverlås *et al*. 2010). Post-weaned calves were mostly infected with *C. bovis*, *C. andersoni* and *C. ryanae* (Fayer *et al*. 2010). Other studies in China, India, Georgia, Nigeria and western North Dakota however, have reported that *C. bovis* was the most common species found in pre-weaned calves (Feng *et al*. 2007; Feltus *et al*. 2008; Maikai *et al*. 2011). A recent study in Nigeria reported that there were no significant differences ($P>0{\cdot}05$) in *Cryptosporidium* infection rates by sex, herd location, management system, breed of calves, or fecal consistency but that calves 180 days or younger had a higher infection rate of *Cryptosporidium* than older calves ($P=0{\cdot}034$) and that younger calves also had higher occurrence of *C. bovis* and *C. ryanae* ($P=0{\cdot}022$) (Maikai *et al*. 2011).

In Australia, the prevalence of *Cryptosporidium* in cattle ranges from 2 to 58·8% (Becher *et al*. 2004; Nolan *et al*. 2009; Ng *et al*. 2011*b*; Izzo *et al*. 2011). The most recent study reported that the total prevalence of *Cryptosporidium* in calves from

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Table 5. Prevalence and species of *Cryptosporidium* identified in pre- and post-weaned cattle in Australia

| Country | Age Preweaned/ postweaned | Prevalence | No genotyped | *Cryptosporidium species* | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *C. parvum* | *C. bovis* | *C. ryanae* | *C. andersoni* | Others | |
| Australia – WA | 0–12 weeks | 48·3% (36/54) | 6 | 100% (6) | 0 | 0 | 0 | 0 | Becher *et al.* (2004) |
| Australia – Vic | Preweaned | 46·3% (124/268) | 124 | 100% | 0 | 0 | 0 | 0 | Nolan *et al.* (2009) |
| Australia – WA | Postweaned | 15% (17/111) | 4 | 0 | 100% (4/4) | 0 | 0 | *C. ubiquitum* 25% (1/4) | Ferguson (2010) |
| Australia – WA | Preweaned | 47% (9/19) | 4 | 0 | 75% (3/4) | 0 | 0 | | Ferguson (2010) |
| Australia – WA and NSW | Preweaned | 22·5% (82/364) | 71 | 38% (27/71) | 39·5% (28/71) | 21% (15/71) | 0 | 1·5% (1/71) | Ng *et al.* (2011*b*) |
| Australia – NSW | Preweaned | 73·5% (144/196) | 142 | 60% (85/142) | 20·4% (29/142) | 9·8% (14/142) | 0 | 9·8%* (14/142) | Ng *et al.* (2012) |

* Mixed *C. parvum/bovis/ryanae* infections.

84 dairy and dairy beef properties across Australia was 58·5% (Izzo *et al.* 2011) (Table 6). In Victoria, the prevalence of *Cryptosporidium* in fecal samples from 268 individual calves on pasture-based dairy farms in three regions (Northern Victoria, South Gippsland and Western District) was 46·3% (124/268) (Nolan *et al.* 2009). The detection tool employed, however, (PCR analysis of the *gp60* locus) was specific to *C. parvum*/*C. hominis* and therefore only *C. parvum* was detected in all samples typed (Nolan *et al.* 2009). *Cryptosporidium andersoni* is usually only found in older cattle and is morphologically distinct (7·4 × 5·5 $\mu$m) from the intestinal species, which includes *C. parvum* (5·0 × 4·5 $\mu$m) (Ralston *et al.* 2010). The prevalence of *C. andersoni* in fecal samples from 10 groups of feedlot beef cattle in Western Australia ranging in age from 11 to 36 months, ranged from 0% to 26% (Ralston *et al.* 2010). *Cryptosporidium andersoni* is commonly detected in water samples in the US and UK but is not considered a human pathogen (Xiao and Feng, 2008; Xiao, 2010; Wang *et al.* 2011).

A recent study screened a total of 364 fecal specimens from randomly selected pre-weaned calves, aged up to 4 months, from 5 different farms in the south of Western Australia and 1 farm from New South Wales (Ng *et al.* 2011*b*). There were substantial differences in prevalence between the farms with the highest prevalence in a WA farm (37·5%). The overall prevalence was 22·3% (81/364) (Ng *et al.* 2011*b*). *Cryptosporidium bovis* was the most common species detected (39·5%) followed by *C. parvum* (38%) and *C. ryanae* (21%) (Ng *et al.* 2011*b*).

In NSW, a preliminary study in 2006 that examined the species/genotypes and subgenotypes of *Cryptosporidium* in 7 human and 15 cattle cases of sporadic cryptosporidiosis in rural western NSW, reported that 4 of the 6 *C. parvum* subtypes found in humans were also found in the cattle, indicating that zoonotic transmission may be an important contributor to sporadic human cases of cryptosporidiosis in rural NSW (Ng *et al.* 2008). A more extensive study conducted in 2009, screened 196 fecal samples from diarrhoeic (scouring) calves on 20 farms and 63 fecal samples from humans on 14 of these farms (Ng *et al.* 2012). The overall prevalence of *Cryptosporidium* in cattle and humans by PCR and sequence analysis of the 18S rRNA was 73·5% (144/196) and 23·8% (15/63) respectively. Three species were identified in cattle; *C. parvum*, *C. bovis* and *C. ryanae*, and from humans, *C. parvum* and *C. bovis* (Ng *et al.* 2012). This is only the second report of *C. bovis* in humans. The previous report *C. bovis* was in a dairy farm worker in India, where the infection was asymptomatic (Khan *et al.* 2010). Subtype analysis at the *gp60* locus identified the same *C. parvum* subtype in the calves in some of the humans, suggesting that zoonotic transmission may have occurred but more

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Table 6. Prevalence and species of Cryptosporidium identified in pre- and post-weaned sheep in Australia

| Country | Age Pre-weaned/ post-weaned | Prevalence | No genotyped | *Cryptosporidium species* | | | Others | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | *C. parvum* | *C. ubiquitum* | *C. xiaoi* | | |
| Australia-WA | Post-weaned | 26·2% (131/500) | 60 | 0 | 55% (33/60) | 23·3% (14/60) | Pig genotype II (6·6%), *C. fayeri* (6·6%), *C. suis* (3·3%), *C. andersoni* (1·6%), *C. hominis* (1·6%), unknown genotype (1·6%) | Ryan *et al.* (2005) |
| Australia-WA | Pre-weaned | 24·5% (117/477) | 66 | 4·5% (3/66) | 15·1% (10/66) | 79% (52/66) | 90·1% (60/66) (Mixed *C. parvum*/ *C. xiaoi*). | Yang *et al.* (2009) |
| Australia-WA | Post-weaned | 17% (12/70) | 7 | 14·3% (1/7) | 28·5% (2/7) | 57% (4/7) | 0 | Ferguson (2010) |
| Australia-WA | Pre-weaned | ~ 29% | 134 | 3% (4/134) | 16·4% (22/134) | 61% (82/134) | 1·5% (2/134) Sheep 0 *C. andersoni* 10·4% (14/134) mixed | Sweeny *et al.* (2011*a*) |
| Australia-WA | Post-weaned | ~ 35% | 251 | 7·5% (19/253) | 3·2% (8/253) | 80·3% (203/253) | 1·6% (4/253) Sheep I 1·6% (4/253) *C. andersoni* 6% (15/253) mixed | Sweeny *et al.* (2011*b*) |

* Combined data from several samplings and farms.

studies involving extensive sampling of both calves and farm workers are needed for a better understanding of the sources of *Cryptosporidium* infections in humans from rural areas of Australia.

### *Sheep*

In sheep, cryptosporidiosis presents as a mild to severe yellowish liquid diarrhoea with a strong odour, loss of weight, depression, abdominal pain, and death usually involving animals up to 1 month of age and is associated with reduced lamb carcase productivity (cf. Fiuza *et al.* 2011*b*; Sweeny *et al.* 2011*a*). *Cryptosporidium* has been reported in sheep worldwide, with prevalences ranging from 2·6 to 82% for *Cryptosporidium* (cf. Ryan *et al.* 2005; Yang *et al.* 2009, Wang *et al.* 2010*b*; Sweeny *et al.* 2011*b*). In Australia, reported prevalences for ewes in Western Australia ranged from 6·3–8·3% (Sweeny *et al.* 2011*b*) and for lambs from 9·3–56·3% on different properties (Ryan *et al.* 2005; Yang *et al.* 2009; Sweeny *et al.* 2011*a*, *b*).

A total of 9 species/genotypes of *Cryptosporidium* have been reported in sheep and lambs in Australia; *C. parvum*, *C. hominis*, *C. xiaoi*, *C. bovis*, *C. ubiquitum*, sheep genotype I, *C. andersoni*, pig genotype II, *C. fayeri* and *C. suis* and sheep genotype I (Ryan *et al.* 2005; Giles *et al.* 2009; Yang *et al.* 2009; Robertson, 2009; Sweeny *et al.* 2011*a*, *b*), with *C. xiaoi* and *C. ubiquitum* most common although Yang *et al.* (2009) found high proportions of *C. parvum* isolates in pre-weaned sheep in Western Australia when a *C. parvum*-specific PCR was used. In that study, using both 18S and a *C. parvum*-specific PCR for *Cryptosporidium* produced very different results. At the 18S locus, *C. bovis* was the most common species identified (95% of positives) in the pre-weaned lambs and *C. parvum* was only identified in two samples (0·4%) (Yang *et al.* 2009). However, using a *C. parvum*-specific PCR and additional 53 *C. parvum*-positives were identified (mostly mixed *C. bovis*/ *C.parvum* infections). Quantitative PCR revealed that *C. parvum* was present in low numbers compared to *C. bovis* and it is likely that the 18S PCR preferentially amplified the more abundant template. It may be that the use of *C. parvum*-specific primers is necessary to determine the true prevalence of *C. parvum*. In a previous study on post-weaned sheep (Ryan *et al.* 2005), *C. parvum* was not detected; however, *C. parvum*-specific primers were not used and it is possible that *C. parvum* was present in those animals.

*Cryptosporidium ubiquitum* (previously known as the cervine genotype) has been identified in humans worldwide (Ong *et al.* 2002; cf. Xiao, 2010) but has not been detected in any human cryptosporidiosis cases in Australia to date. Quantification analysis using quantitative PCR (qPCR) and microscopy

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indicated that oocyst output in sheep feces varies widely and ranges from ~1 to $10^6$ oocysts per gramme (Yang *et al*. 2009; Ryan *et al*., *unpublished observations*). Because sheep can harbour C. *parvum*, they should be considered a potential source of infection of *Cryptosporidium* either by direct transmission or by contamination of the environment.

As with cattle, there appears to be both geographical and age-related differences in the prevalence of zoonotic and non-zoonotic genotypes in sheep based on recent molecular characterization studies worldwide (Santin *et al*. 2007; Geurden *et al*. 2008; Quilez *et al*. 2008; Mueller-Doblies *et al*. 2008; Paoletti *et al*. 2009; Diaz *et al*. 2010; Robertson *et al*. 2010; Wang *et al*. 2010; Fiuza *et al*. 2011*b*; Shen *et al*. 2011). A recent longitudinal study of *Cryptosporidium* in meat lamb farms in southern Western Australia reported that *Cryptosporidium* prevalences at individual samplings ranged between 18·5 and 42·6% in lambs and were <10% in the ewes. *Cryptosporidium xiaoi* was the most prevalent species detected at all 5 samplings and was also isolated from lamb dam water on 1 farm. *Cryptosporidium ubiquitum* was most commonly detected in younger lambs and *Cryptosporidium parvum* was detected in lambs at all 5 samplings, typically in older lambs and as part of a mixed species infection with *C. xiaoi*. The novel sheep genotype I, was identified in 6 *Cryptosporidium* isolates from 1 farm. The longitudinal study revealed that sampling a random selection of animals from a flock/herd on 1 occasion (point prevalence), underestimates the overall prevalence of these parasites in the flock/herd across an extended time-period (Sweeny *et al*. 2011*b*).

## CONCLUSIONS AND PERSPECTIVES

Wildlife host-adapted species of *Cryptosporidium* are likely to have evolved in close association with marsupials. Confirmation of human-infectious species in these animals is rare, with detection of such isolates based on sequence data from direct fecal DNA extraction. There is a need to confirm whether molecular detection of zoonotic *Cryptosporidium* species is associated with actual infections. Additionally the presence of an atypical species in one or two individual marsupial hosts does not indicate that the parasite will be successful in all marsupials. The low abundance detected in individuals and low prevalence of human-infectious species in marsupial host groups, suggests that even if marsupial hosts are infected, it may not be a very successful host parasite interaction. However, marsupial hosts should still be monitored given the risk of emergence and the public health implications.

Non-marsupial hosts in Australia fall into 2 categories. (1) Those that have been introduced and become feral (cats, deer, dingos, foxes, pigs, rabbits, rodents, etc.) and (2) those that have become domesticated (sheep, cattle and pigs). Many of these hosts represent important agricultural resources. Although there have been limited studies in Australia, deer, rabbits and rodents appear to be potential reservoirs of human infectious species and more systematic studies of the prevalence, oocyst numbers and species infecting these hosts are essential to understand the public health significance.

Cattle and sheep are present in high numbers across Australia, contribute a large volume of manure to catchments and can shed oocysts in high concentrations. It has been reported that manure from cattle and sheep are the second-most significant source of pathogens that cause waterborne disease (Hrudey and Hrudey, 2004). Preliminary evidence suggests that pre-weaned lambs and cattle in Australia may be an important source of *C. parvum,* which is of concern for public health. Larger numbers of both pre-weaned and post-weaned sheep and cattle from different geographical areas within Australia need to be screened at different times of the year at multiple loci and the numbers of oocysts quantified to confirm this.

The 2011 Australian Drinking Water Guidelines recommend managing water quality risks at source, within the catchment. Therefore management practices, particularly of cattle and sheep (including vegetation management of riparian zones) play a major role in reducing the risk of water contamination with *Cryptosporidium*. This can be achieved by compulsory land acquisition by government and drinking water companies, but this is an expensive option that is not widely practiced. It is more usually achieved by restricting stock access to riparian areas. In areas where permanent fencing is not an option, minimizing infection (and *Cryptosporidium* shedding) rates in stock – especially calves and lambs, by implementing good animal health practices such as excluding calves from pastures grazed by infected cows, is important.

Australia has a unique ecosystem with wildlife species specific to the continent. The introduction of non-marsupial animals and their parasites has resulted in complex and often devastating effects on the endemic fauna. It is therefore important to understand these intricate associations between wildlife, domestic animals and humans in the Australian context to enable management of the zoonotic risk of *Cryptosporidium*.

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