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CHAPTER SIX

A Perspective on *Cryptosporidium* and *Giardia*, with an Emphasis on Bovines and Recent Epidemiological Findings

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Abstract

Cryptosporidium and *Giardia* are two common aetiological agents of infectious enteritis in humans and animals worldwide. These parasitic protists are usually transmitted by the faecal–oral route, following the ingestion of infective stages (oocysts or cysts). An essential component of the control of these parasitic infections, from a public health perspective, is an understanding of the sources and routes of transmission in different geographical regions. Bovines are considered potential sources of infection for humans, because species and genotypes of *Cryptosporidium* and *Giardia* infecting humans have also been isolated from cattle in molecular parasitological studies. However, species and genotypes of *Cryptosporidium* and *Giardia* of bovids, and the extent of zoonotic transmission in different geographical regions in the world, are still relatively poorly understood. The purpose of this article is to (1) provide a brief background on *Cryptosporidium* and *Giardia*, (2) review some key aspects of the molecular epidemiology of cryptosporidiosis and giardiasis in animals, with an emphasis on bovines, (3) summarize research of *Cryptosporidium* and *Giardia* from cattle and water buffaloes in parts of Australasia and Sri Lanka, considering public health aspects and (4) provide a perspective on future avenues of study. Recent studies reinforce that bovines harbour *Cryptosporidium* and *Giardia* that likely pose a human health risk and highlight the need for future investigations of the biology, population genetics and transmission dynamics of *Cryptosporidium* and *Giardia* in cattle, water buffaloes and other ruminants in different geographical regions, the fate and transport of infective stages following their release into the environment, as well as for improved strategies for the control and prevention of cryptosporidiosis and giardiasis, guided by molecular epidemiological studies.

1. INTRODUCTION

Many pathogens, including bacteria, viruses and parasites, are known to cause gastrointestinal infections in humans and animals. Diarrhoea caused by these pathogens is one of the principal reasons for high morbidity in humans in developed and developing countries, often resulting in mortality in underprivileged communities (Fletcher et al., 2013). Among parasitic pathogens, *Cryptosporidium* and *Giardia* are frequently associated with diarrhoea and pose a considerable threat to human and animal health globally (Jex et al., 2011a; Thompson et al., 2008).

Cryptosporidium and *Giardia* can be transmitted via the faecal–oral route, following direct or indirect contact with infected hosts (Cacciò et al., 2005). Members of these genera have been responsible for community-wide outbreaks associated with drinking or recreational water (Robertson, 2009). Although infections are often self-limiting in immunocompetent individuals (Chalmers and Davies, 2010; Feng and Xiao, 2011), they can become severe

and chronic in infants, elderly people and immunocompromised or -suppressed individuals (Hunter and Nichols, 2002; Petri et al., 2008). Investigations of outbreaks and case–control studies have shown clearly that cryptosporidiosis and giardiasis can be transmitted from humans to humans (anthroponotic) or from animals to humans (zoonotic) (Xiao and Fayer, 2008). Therefore, identifying pathogen sources and understanding transmission routes are essential components of the prevention and control of infections and disease.

Of the zoonotic sources of infection, cattle are recognised as a major contributor, because species and genotypes of *Cryptosporidium* and *Giardia* infecting humans have also been isolated from cattle (reviewed by Xiao and Fayer, 2008). Although there is a considerable amount of information on *Cryptosporidium* and *Giardia* infections in cattle, little is known about them in other livestock animals. For instance, there have been only few molecular investigations of water buffalo (*Bubalus bubalis*) (Amer et al., 2013; Cacciò et al., 2007; Feng et al., 2012; Helmy et al., 2013; Maurya et al., 2013), in spite of their importance as agricultural animals and their close association with humans in some countries.

Apart from their veterinary relevance relating to morbidity, mortality and production losses, *Cryptosporidium* and *Giardia* of domestic bovids receive major attention as zoonotic pathogens (Robertson et al., 2014). However, the prevalence of infections in human and animal populations and the extent of zoonotic transmission vary across different geographical regions of the world (Feng et al., 2007b). Most molecular studies of *Cryptosporidium* and *Giardia* have been conducted mainly in a limited number of countries in the developed world (reviewed by Feng and Xiao, 2011; Jex and Gasser, 2010), and there is a paucity of information from other regions of the world, particularly in underprivileged countries.

The purpose of this article is to (1) provide a brief background on *Cryptosporidium* and *Giardia*, (2) concisely review aspects of the molecular epidemiology of cryptosporidiosis and giardiasis in animals, with an emphasis on bovines, (3) summarize some recent progress in research of *Cryptosporidium* and *Giardia* from bovines in parts of Australasia and Sri Lanka, considering public health aspects and (4) provide a perspective on future avenues of study.

2. HISTORICAL PERSPECTIVE AND TAXONOMY

2.1 *Cryptosporidium*

Cryptosporidium was first discovered by Ernest Edward Tyzzer in 1907. He identified life cycle stages of a parasitic protist in the gastric glands of

laboratory mice and proposed the name *Cryptosporidium muris* for this new species (Tyzzer, 1910). Subsequently, another new species, called *Cryptosporidium parvum*, was described by Tyzzer in 1912. *C. parvum* infected the small intestine, and the oocysts were smaller than those of *C. muris*. After its first discovery by Tyzzer, *Cryptosporidium* was not recognized as an economically or medically important parasite for the next 50 years. In 1955, it was first identified as a potentially disease-causing agent, when it was isolated from diarrhoeic turkeys (Slavin, 1955). In 1971, *Cryptosporidium* was reported to be associated with diarrhoea in young calves, for the first time (Panciera et al., 1971). Five years later, the first two human cases of cryptosporidiosis were reported (Meisel et al., 1976; Nime et al., 1976). Then, in 1982, the Centers for Disease Control (CDC) reported cryptosporidiosis in 21 HIV/AIDS patients in the USA (MMWR, 1982), after which *Cryptosporidium* received major attention around the world.

Traditionally, *Cryptosporidium* taxa have been identified based on oocyst morphology, host specificity and/or predilection site within the host (Ryan and Xiao, 2014). Subsequently, using immunological or molecular methods, many distinct species and genotypes have been identified within *Cryptosporidium*. Over the years, the taxonomy of *Cryptosporidium* has undergone substantial change, leading to some controversies. The development of new molecular tools has allowed the identification and characterisation of species and/or genetic variants (genotypes) (reviewed by Jex et al., 2011a; Ryan and Xiao, 2014).

The genus *Cryptosporidium* belongs to the Phylum Apicomplexa, Order Eucoccidiorida and Family Cryptosporidiidae (Fayer and Xiao, 2008). Presently, more than 26 species of *Cryptosporidium* have been recognised (Chalmers and Katzer, 2013; Ryan and Xiao, 2014). In addition, there are more than 40 genotypes that have not yet been formally recognised as species, because of a lack of sufficient morphological, biological and molecular data to comply with the International Code for Zoological Nomenclature (ICZN) rules of describing new species (Table 1) (cf. Jex et al., 2011a; Ryan and Xiao, 2014).

2.2 *Giardia*

Although *Giardia* was first observed in 1681 by Antonie van Leeuwenhoek, the first detailed description of this protist was not published until 1859. Subsequently, species of *Giardia* were described based on the host occurrence, because of a lack of characteristic or differentiating morphological features. Later, in 1952, an increasing number of *Giardia* species and uncertainty

Table 1 Recognised species of *Cryptosporidium*

Species	Host group	Site of infection	References
Cryptosporidium andersoni	Cattle	Abomasum	Lindsay et al. (2000)
Cryptosporidium baileyi	Birds	Bursa, cloaca, intestine	Current et al. (1986)
Cryptosporidium bovis	Cattle	Intestine	Fayer et al. (2005)
Cryptosporidium canis	Canids	Intestine	Fayer et al. (2001)
Cryptosporidium cuniculus	Rabbit, human	Intestine	Inman and Takeuchi (1979)[a]
Cryptosporidium fayeri	Marsupials	Intestine	Ryan et al. (2008)
Cryptosporidium felis	Cats	Intestine	Iseki (1979)
Cryptosporidium fragile	Amphibians	Intestine	Jirku et al. (2008)
Cryptosporidium galli	Birds	Proventriculus	Pavlasek (1999)[b]
Cryptosporidium hominis	Humans	Intestine	Morgan-Ryan et al. (2002)
Cryptosporidium macropodum	Marsupials	Intestine	Power and Ryan (2008)
Cryptosporidium meleagridis	Birds (humans)	Intestine	Slavin (1955)
Cryptosporidium molnari	Fishes	Stomach	Alvarez-Pellitero and Sitja-Bobadilla (2002)
Cryptosporidium muris	Rodents	Stomach	Tyzzer (1910)
Cryptosporidium parvum	Mammals (humans)	Intestine	Tyzzer (1912)
Cryptosporidium ryanae	Cattle	Intestine	Fayer et al. (2008)
Cryptosporidium scophthalmi[c]	Fishes	Intestine	Alvarez-Pellitero et al. (2004)
Cryptosporidium scrofarum	Pigs	Intestine	Kváč et al. (2013)
Cryptosporidium serpentis	Snakes	Stomach	Levine (1980)
Cryptosporidium suis	Pigs	Intestine	Ryan et al. (2004)
Cryptosporidium varanii	Reptiles	Intestine	Pavlásek et al. (1995)
Cryptosporidium viatorum	Humans	Intestine	Elwin et al. (2012)
Cryptosporidium wrairi	Rodents	Intestine	Vetterling et al. (1971)
Cryptosporidium xiaoi	Sheep	Intestine	Fayer and Santín (2009)

[a]See Robinson et al. (2010).
[b]See Ryan et al. (2003).
[c]No molecular data available for this 'species'.

regarding host specificity led to a taxonomic rationalisation. Based on this classification, most members infecting vertebrates were named as one group, *Giardia duodenalis* (Filice, 1952). Although this 'species' was isolated from humans and many animal hosts, the zoonotic significance of *Giardia* was controversial until the World Health Organization (WHO) recognised it as a zoonotic agent in 1979 (WHO, 1979).

Giardia is a binucleate, flagellate protist belonging to Phylum Metamonada, Order Giardiida and Family Giardiidae (Plutzer et al., 2010). Other recognised species of *Giardia* include *Giardia agilis*, *Giardia ardae*, *Giardia microti*, *Giardia muris* and *Giardia psittaci* (Table 2) (Thompson, 2011). At the trophozoite stage, they can be distinguished based on morphological characteristics using light and electron microscopy. *Giardia duodenalis* (syn. *Giardia intestinalis*, *Giardia lamblia*) is known to infect at least 40 host species, including humans (Thompson, 2011). The other species are known to be host specific or have a limited host range: *G. agilis* in amphibians, *G. ardae* and *G. psittaci* in birds and *G. microti* and *G. muris* in rodents (Adam, 2001). Currently, *G. duodenalis* is recognised as a complex of at least eight different assemblages (A–H) (Koehler et al., 2014a; Monis et al., 2003). Although there are little or no morphological differences, there is

Table 2 Currently recognised species of *Giardia* and genetic groupings (assemblages) within *Giardia duodenalis*

Species/assemblages	Hosts
G. duodenalis	
Assemblage A	Humans, primates, dogs, cats, livestock, rodents, wild mammals
Assemblage B	Humans, primates, dogs, cattle, some species of wild mammals
Assemblage C	Dogs, other canids
Assemblage D	Dogs, other canids
Assemblage E	Cattle and other hoofed livestock
Assemblage F	Cats
Assemblage G	Rodents
Assemblage H	Marine vertebrates
Giardia agilis	Amphibians
Giardia ardeae	Birds
Giardia microti	Rodents
Giardia muris	Rodents
Giardia psittaci	Birds

substantial genetic diversity among these assemblages (Nash et al., 1985; Nash, 1992). Assemblages A and B are described to have a relatively broad host range, including humans and various other mammals. The other assemblages are either host specific or have narrow host ranges. Assemblages C and D are commonly found in dogs, whereas assemblage F mainly infects cats. Assemblage E is found in cloven-hoofed animals, and G is found in rodents (Feng and Xiao, 2011; Xiao and Fayer, 2008). Recently, assemblage H was identified in marine vertebrates (Lasek-Nesselquist et al., 2010).

3. LIFE CYCLES

3.1 *Cryptosporidium*

Cryptosporidium has a direct life cycle consisting of asexual and sexual reproductive phases (Ramirez et al., 2004). The infective oocyst stage (4–6 μm) is excreted in the faeces from the infected hosts (Kosek et al., 2001). Susceptible hosts usually acquire infection by ingesting oocysts from contaminated water or food. Oocysts will then excyst at the predilection site in the host (gastrointestinal tract of mammals, birds and reptiles or respiratory tract of birds), and oocyst-derived sporozoites invade the epithelial cells and develop into trophozoites. Once they are intracellular, they start further development. Developing stages can be found in the extra-cytoplasmic, parasitophorus vacuoles in epithelial cells on the luminal surface (O'Donoghue, 1995). Then, trophozoites undergo asexual reproduction (merogony or schizogony) to produce type I meronts (schizonts). Each of the type I meronts contains 16 merozoites, which are capable of invading new host cells to replicate and develop into a new type I or type II meronts. Type I meronts continue schizogony, and repeat the cycle, whereas type II meronts initiate sexual reproduction. Each of type II meronts contains four merozoites, each of which develops either into a microgamont (containing 12–16 microgametes; male) or a macrogamont (female). Mature microgametes are released and fertilize the unicellular macrogametes. Fertilized macrogametes develop into zygotes, which subsequently develop into oocysts, and four sporozoites develop within each oocyst (Fayer and Xiao, 2008; O'Donoghue, 1995). There are two types of oocysts; thick-walled oocysts (80%) leave the host body with the faeces, whereas thin-walled oocysts (20%) can induce autoinfection directly the gut (de Graaf et al., 1999; Kosek et al., 2001).

3.2 *Giardia*

Giardia has a direct life cycle and reproduces by asexual replication (longitudinal binary fission) (Adam, 1991). Infective stages (cysts) are excreted in the faeces from infected hosts into the environment. Following ingestion by a susceptible host, cysts enter the small intestine, where they excyst by the action of gastric acids and pancreatic enzymes (Thompson et al., 2008); the emerging trophozoites consume bile salts, causing deconjugation (Sinha et al., 2012). Each cyst produces two motile trophozoites, which are 12–15 μm long and 5–9 μm wide (Adam, 1991). The trophozoites attach to the intestinal mucosa by their ventral sucking disc, a unique organelle composed of microtubules and tightly associated micro-ribbons (Schwartz et al., 2012). The trophozoites usually colonize the duodenum and jejunum of the host and multiply by binary fission. Although it is widely accepted that *Giardia* reproduces by longitudinal binary fission, some studies have suggested that *Giardia* can reproduce sexually (Birky, 2005; Thompson, 2011). Some of the trophozoites encyst in the posterior intestine and are excreted as cysts in the faeces.

4. TRANSMISSION

Generally, infective stages of *Cryptosporidium* and *Giardia* are excreted in the faeces from infected hosts, and are capable of infecting susceptible hosts following ingestion. Humans can acquire infection directly from contact with infected humans (anthroponotic transmission) or animals (zoonotic transmission), or indirectly from contaminated food or water sources (Smith et al., 2007). *Cryptosporidium* oocysts and *Giardia* cysts are capable of remaining infective for long periods, particularly in cold and moist environments (Smith et al., 2006). The microscopic size and low specific gravity of infective stages facilitate their dissemination in water (Gajadhar and Allen, 2004). These stages are resistant to most of the routine disinfection methods used for drinking water, swimming pools and irrigation systems (Gajadhar and Allen, 2004). Both *Cryptosporidium* and *Giardia* can infect their hosts at very low doses (<10 oocysts/cysts) (Okhuysen et al., 1999; Rendtorff, 1954). Among different *Cryptosporidium* species infecting humans, *C. parvum* has a broad host range, including livestock, companion animals and wild animals; therefore, it can be transmitted via both anthroponotic and zoonotic routes (Cacciò et al., 2005). In contrast, *Cryptosporidium hominis* primarily infects humans, although experimental (Giles et al., 2001) and

natural infections in livestock (Smith et al., 2005) and other animals (Morgan et al., 2000b) have been reported in some instances. Therefore, with few exceptions, *C. hominis* is usually transmitted by anthroponotic pathways (Jex et al., 2011a). Epidemiological and genetic studies of *Cryptosporidium* and *Giardia* isolates from different sources have enhanced knowledge of transmission routes and animal reservoirs.

Different species of *Cryptosporidium*, including *C. parvum*, *C. hominis* and *Cryptosporidium meleagridis*, have been identified from human cases associated with waterborne cryptosporidiosis outbreaks. According to a review (Baldursson and Karanis, 2011), *Cryptosporidium* and *Giardia* have been responsible for 120 (60.3%) and 70 (35.2%) of 199 waterborne outbreaks from 2004 to 2010. Many of these waterborne outbreaks have been reported from New Zealand (40.2%), followed by North America (30.6%) and Europe (16.5%). In contrast, 3.5% of outbreaks have been reported from developing countries in Asia (Baldursson and Karanis, 2011). However, the greater number of waterborne outbreaks in developed countries is likely due to advanced reporting and surveillance systems, rather than to a higher prevalence of infections. Furthermore, a review of environmental transmission of *Cryptosporidium* and *Giardia* infections in humans has reported that *C. hominis* was responsible for more cryptosporidiosis outbreaks than *C. parvum* (see Smith et al., 2006). However, this interpretation might be biased in that the majority of data were from developed countries, where many infections are likely acquired anthroponotically via swimming pools and/or daycare centres (Xiao, 2010).

Foodborne transmission of cryptosporidiosis and giardiasis has also been reported on a number of occasions, due to the consumption of contaminated food. Use of fertilizer produced from livestock waste, surface run-off from feedlots and untreated water used for irrigation can lead to the contamination of food with infective stages. Food may also be contaminated during harvesting, packing, transportation, selling and/or preparation under unhygienic conditions (Escobedo et al., 2010; Robertson and Chalmers, 2013). Various studies have isolated oocysts and cysts from different fresh produce, including water spinach, lettuce, Chinese cabbage, potatoes and carrots (Amahmid et al., 1999; Amoros et al., 2010; Monge and Chinchilla, 1996; Takayanagui et al., 2000; Vuong et al., 2007). Other studies have examined the presence of *Cryptosporidium* and *Giardia* in water used for irrigation and food production or packing, and have demonstrated that contaminated water is a major source of oocysts or cysts in fresh produce (Chaidez et al., 2005; Thurston-Enriquez et al., 2002). In addition, it has been shown that such infective

stages are capable of remaining viable in stored fresh food for long periods (Macarisin et al., 2010; Thurston-Enriquez et al., 2002).

An investigation of the efficacy of different treatments used in food processing (i.e. chlorine, blanching, blast freezing and microwave heating) on the viability of *C. parvum* has showed that oocysts can be destroyed by heat and, to some extent, by freezing, but not by safe concentrations of chlorine (Duhain et al., 2012). Usually, *Giardia* cysts are more susceptible to chlorine disinfection than *Cryptosporidium* oocysts (Sterling, 1990). *Giardia* cysts tend to have shorter longevity in the environment. In one study, *Giardia* cysts were shown to survive at -4 °C in water and soil for <1 week, whereas *Cryptosporidium* oocysts survived for >12 weeks (Olson et al., 1999). Nonetheless, the persistence of these oocysts/cysts in water, soil or in/on food suggests that the consumption of fresh produce, which receives minimal washing and heat treatment, can pose an infection risk. With the increasing number of outbreaks and sporadic cases associated with *Cryptosporidium* and *Giardia*, new techniques have been developed for the concentration and detection of *Cryptosporidium* and *Giardia* oocysts/cysts in food produce (Cook et al., 2007). Although these techniques have been able to recover *Cryptosporidium* and *Giardia* from contaminated foods, the occurrence of these protists might be underestimated, because of the suboptimal efficacy or sensitivity of the methods used (Cook et al., 2006).

In animals, *Cryptosporidium* and *Giardia* infections also can occur via multiple pathways. For instance, infections can spread among calves by direct contact or indirectly via utensils or udders contaminated with calf faeces containing oocysts/cysts (Geurden et al., 2010). Because young animals are usually more susceptible to *Cryptosporidium* and *Giardia*, they can act as a major source of infections to other animals (Mark-Carew et al., 2010). Inadequate hygiene and poor husbandry practices are significant contributors to the high prevalence of *Cryptosporidium* and *Giardia* infections in cattle herds. Generally, intensive management systems on dairy farms can increase the transmission of infections among calves, when young animals are kept at high density (Geurden et al., 2010). The introduction of infective stages from wild animals is another potential route of infection for domestic animals (Jex et al., 2011a).

5. EPIDEMIOLOGY

Cryptosporidium and *Giardia* are major causes of parasitic diarrhoea in humans and animals globally (McCormick, 2013; Shirley et al., 2012).

Disease surveillance projects have provided epidemiological information on the prevalence of cryptosporidiosis and giardiasis in relation to demographic features, such as age, sex and geographical location. However, information on the epidemiology of these diseases is limited in developing countries compared with developed countries, mainly because of poor health care and reporting systems, and a lack of diagnostic facilities and tools.

Sources and transmission of *Cryptosporidium* and *Giardia* can be difficult to establish because of the ubiquitous nature and the multiple routes of transmission of these protozoan parasites. Based on the analyses of outbreaks, the incubation period of cryptosporidiosis varies, but has been reported to be within 5–7 days in many cases, depending on the immune status of the host and the dose of infective stages (Berge et al., 2009; Causer et al., 2006; Insulander et al., 2005; Millard et al., 1994; Naumova et al., 2003; Yamamoto et al., 2000). The duration of symptoms in cryptosporidiosis outbreaks has been reported to commence at 5–9 days (range: 2–13), following the ingestion of infective stages (Nichols, 2008). In *Giardia*, the incubation period can range from 3 days to 6 weeks (Thompson, 2011). Prospective and retrospective serological surveys (in spite of diagnostic test performance issues; Ndao, 2009) have been used to estimate seroprevalence and the risk of infections in human populations. However, the reported seroprevalence is highly variable, and depends on many factors including social and hygienic conditions and geographical location (Uehlinger et al., 2011). Low seroprevalence in communities can be predictive of a high infection rate in outbreak situations (Nichols, 2008). In contrast, a high seroprevalence might indicate a high rate of immunity in a population (Uehlinger et al., 2011).

According to epidemiological studies (Black, 1990; Flanagan, 1992; Gerba et al., 1996; Hunter and Nichols, 2002; Rodriguez-Hernandez et al., 1996), high risk groups for cryptosporidiosis and giardiasis usually include infants, young children and staff in daycare centres, immunocompromised people (e.g. HIV/AIDS, solid organ and bone marrow transplant patients), farmers, animal handlers and international travellers (Robertson and Chalmers, 2013). HIV-infected people are particularly susceptible to a number of *Cryptosporidium* species and genotypes, and can develop chronic and severe infections (Hunter and Nichols, 2002). High rates of infections in young children can be related to exposure as well as reduced immune status (Nichols, 2008). Therefore, protection of these vulnerable populations is important for the prevention of disease transmission. Asymptomatic infections of *Cryptosporidium* and *Giardia* are also common in humans, particularly in endemic areas in developing countries (Chalmers and Davies,

2010; Dawson, 2005; Esteban et al., 1998; Ortega and Adam, 1997). Therefore, some individuals can act as asymptomatic carriers for other family members and people in the same community (Ortega and Adam, 1997).

Based on the results of epidemiological studies, a number of risk factors have been associated with cryptosporidiosis and giardiasis in humans. For instance, case–control studies conducted in Australia (Robertson et al., 2002a), the UK (Hunter et al., 2004) and the USA (Roy et al., 2004) have described that contact with infected persons, particularly young children with diarrhoea, is a major risk factor for sporadic cryptosporidiosis. Other risk factors associated with sporadic cryptosporidiosis include travelling abroad, swimming in fresh water and contact with cattle (Hunter et al., 2004; Robertson et al., 2002a; Roy et al., 2004). Studies conducted in developing countries (e.g. Abdel-Messih et al., 2005; Katsumata et al., 1998; Molloy et al., 2011) have identified multiple risk factors for cryptosporidiosis, including young age (<2 years), rainy season, absence of breast feeding, contact with pets, overcrowded living conditions, low birth weight, malnutrition and/or coinfections with other pathogens (e.g. malaria).

Cryptosporidiosis and giardiasis have also been reported to display temporal variation in human and animal populations. Generally, a high prevalence of parasitic infections is reported in warmer and wetter months of the year (Pawlowski et al., 2009). A recent review of the seasonality of enteric zoonotic diseases in Australasia, Europe and North America (Lal et al., 2012) has reported that there is a bimodal pattern of human cryptosporidiosis cases in spring and late summer–early autumn. Similarly, high incidences of giardiasis have been observed in summer in Canada, the UK and the USA. In tropical climates, the number of cryptosporidiosis cases has been reported to be higher in hot and humid months in the rainy season (Nath et al., 1999; Reinthaler, 1989).

6. PATHOGENESIS OF DISEASE AND CLINICAL MANIFESTATION

Cryptosporidium and *Giardia* primarily infect the gastrointestinal tracts of a wide variety of vertebrates including mammals, birds and reptiles. In addition, *Cryptosporidium* is known to cause respiratory or renal infections in birds (O'Donoghue, 1995; Ryan, 2010). Usually, *Cryptosporidium* invades the epithelial cells of the terminal jejunum and ileum in immunocompetent people (Kosek et al., 2001), but can affect the entire gastrointestinal tract, including bile and pancreatic ducts, in immunocompromised individuals

(Hunter and Nichols, 2002). Epithelial destruction due to parasite invasion and inflammatory reactions in the intestine leads to villous atrophy and fusion, which causes malabsorption and diarrhoea in cryptosporidiosis (Kosek et al., 2001; Thompson et al., 2003). *Giardia* usually colonises the duodenum and jejunum of the small intestine. It has been shown that *Giardia* causes strain-dependent induction of enterocyte apoptosis, which may lead to the loss of function of epithelial barrier and increased permeability (Chin et al., 2002). In addition, it has been shown that hypersecretion of chloride is responsible for the accumulation of fluid in the intestinal lumen in chronic giardiasis (Troeger et al., 2007).

Clinical manifestations and patterns of oocyst excretion in patients with cryptosporidiosis depend on host factors (including immune status) as well as the pathogen species, variant, virulence, origin and/or infective dose (Goodgame et al., 1993; Okhuysen et al., 1999). Cryptosporidiosis in immunocompetent individuals is usually self-limiting and lasts for 5–10 days (Pantenburg et al., 2008). In addition, fever, nausea, anorexia, fatigue, abdominal cramps and vomiting are also common (Kosek et al., 2001; MacKenzie et al., 1994). Infection in immunocompromised individuals (e.g. HIV-infected patients, malnutrition and/or defects in the CD40-CD154 system) may lead to chronic enteritis/diarrhoea and sometimes death (Hunter and Nichols, 2002). Based on the results of in vivo and in vitro studies, both innate and adaptive immunities are involved in host defences against cryptosporidiosis (On et al., 2011; Pantenburg et al., 2008). Mucosal epithelial cells provide initial defence against *Cryptosporidium* infection by producing different inflammatory mediators, such as antimicrobial peptides (e.g. β-defensins), inflammatory chemokines and cytokines (On et al., 2011). These chemokines/cytokines then activate and mobilise the immune effector cells (e.g. neutrophils, lymphocytes and macrophages). The adaptive immunity is activated by recognition of specific antigens by T-cells and B-cells. Cell-mediated immunity involving $CD4^+$ T-cells and IFN-γ plays a major role in adaptive immunity (Borad and Ward, 2010). Mucosal antibodies are also involved in the control of infection. Therefore, both humoral and cellular components of immunity play a role in the control of cryptosporidiosis.

Giardia infections can be asymptomatic or can cause a severe malabsorption syndrome, depending on the immune status of the host (Faubert, 2000). Clinical signs of giardiasis include acute or chronic diarrhoea, abdominal pain, nausea, vomiting and weight loss (Eckmann, 2003). Pathophysiological consequences of giardiasis are multifactorial and depend on

both host and parasite factors (Cotton et al., 2011). The attachment of *Giardia* trophozoites to intestinal epithelial cells activates a series of events that leads to diarrhoea by increasing rates of enterocyte apoptosis, small intestine barrier dysfunction, anion hypersecretion, lymphocyte activation, shortening of brush border microvilli and/or malabsorption (Cotton et al., 2011). Host defence against *Giardia* involves a number of immunological and non-immunological events in the gut mucosae (Roxstrom-Lindquist et al., 2006). The intestinal epithelium plays a major role in innate and adaptive immunity in giardiasis (Roxstrom-Lindquist et al., 2006). Studies conducted using laboratory mice have demonstrated that IL-6 is important for clearance and control of acute infections (Zhou et al., 2003).

Cryptosporidiosis in cattle is typically symptomatic in neonates and pre- and postweaned calves, but adults are usually asymptomatic (Ramirez et al., 2004). *Cryptosporidium parvum* causes high morbidity linked to profuse diarrhoea in preweaned calves. Sometimes infection can lead to death in severely affected animals. Older calves and adults are usually infected with host-adapted species of *Cryptosporidium* including *Cryptosporidium bovis*, *Cryptosporidium andersoni* and *Cryptosporidium ryanae* (see Robertson et al., 2014). Although giardiasis in ruminants is often asymptomatic, diarrhoea, lethargy and poor weight gain in calves can lead to production losses and/or deaths (O'Handley and Olson, 2006). The duration of infection can range from a few days to several months, and intermittent excretion of *Giardia* cysts has been observed in both humans and animals (Thompson, 2011). Also dogs and cats may develop acute or chronic self-limiting diarrhoea. Asymptomatic infections are also common in various companion animals (Thompson et al., 2008). Clearly, understanding host responses against *Cryptosporidium* and *Giardia* infections could provide a foundation for the development of novel intervention strategies (On et al., 2011).

7. ASPECTS OF TREATMENT, PREVENTION AND CONTROL

Although *Cryptosporidium* and *Giardia* infections are often self-limiting in humans and animals, treatments can help control disease and its complications. Although more than 100 compounds have been tested for treating cryptosporidiosis in humans, only a few of them can reduce the severity of symptoms, including paromomycin, azithromycin and nitazoxanide (Leder, 2009). However, there was no registered therapeutic agent for treating cryptosporidiosis in humans until nitazoxanide was licensed in the USA

(Rossignol, 2010). Halofuginone, a synthetic product of quinazolinone group, is known to have therapeutic effect against clinical signs of cryptosporidiosis, particularly in ruminants (Jarvie et al., 2005). For example, a study of the efficacy of halofuginone lactate against cryptosporidiosis in calves reported a reduction in oocyst excretion and diarrhoea, and delayed the onset of infection in calves exposed to challenge infection with *C. parvum* (see Lefay et al., 2001). On the other hand, a number of therapeutic agents, including nitroimidazole derivatives (e.g. metronidazole, tinidazole and ornidazole), nitrofuran and benzimidazole drugs have been shown to be effective against *Giardia* in both humans and animals (Farthing, 2006). However, metronidazole has been the most commonly used drug for treating giardiasis for the past 40 years. Metronidazole treatment failures against giardiasis have been reported; although metronidazole resistance can be readily induced in *G. duodenalis* in vitro, little is known regarding the extent and spread of resistance in natural populations of this protist (Müller et al., 2011). Interestingly, nitazoxanide is the first new compound developed for treating giardiasis in more than 20 years (Rossignol, 2010), but its efficiency and safety are controversial (Hemphill et al., 2006).

Although there are different treatment options available, prevention is the most effective means of protecting humans and animals from *Cryptosporidium* and *Giardia* infections. Identifying sources, routes of transmission and risk factors are key requirements for the prevention and/or control of these parasites. Because of the robust nature and low infective doses of *Cryptosporidium* and *Giardia*, prevention and control are challenging, and management components are critical (Cacciò et al., 2005). In humans, control measures should essentially include good hygiene and avoiding the ingestion of contaminated water and/or food. Because of the frequent detection of zoonotic *Cryptosporidium* and *Giardia* in water, it is important to maintain water quality by protecting water catchments and interventions via water treatment processes, in order to prevent waterborne outbreaks. Many major cities in developed countries have ongoing monitoring programs for the detection of *Cryptosporidium* and *Giardia* in catchments and drinking water (Feng et al., 2007a; Nolan et al., 2013). In contrast, people in most of the countries in the developing world do not have access to clean and safe drinking water (Sobsey et al., 2008). People in such countries use water from irrigation canals, unprotected wells, boreholes and public standpipes for drinking and other household requirements (Clasen and Bastable, 2003; Shortt et al., 2006). Therefore, in such regions, various household water treatment methods, such as boiling and filtration, can be used to remove

or inactivate the infective stages of *Cryptosporidium* and *Giardia* from/in water (Sobsey et al., 2008).

Cryptosporidiosis and giardiasis of livestock are also controlled through proper management and husbandry practices. Because of the presence of infective stages in faeces, manure and sewage management is crucial for disease prevention and control. The measures used to prevent or reduce transmission from animal-to-animal and farm-to-farm include good hygiene (regular cleaning of pens, proper disposal of faecal waste and disinfection of utensils), proper management (avoiding overcrowding, keeping young and adult animals in separate areas and ensuring that newborn animals receive immediately an adequate amount of colostrum), prevention of concurrent infections and early diagnosis and treatment of infected animals (e.g. Jex et al., 2011a). Because of the high prevalence of *C. parvum* reported for preweaned calves, additional measures need to be taken into account to control diarrhoea and prevent transmission to humans. According to a study conducted in dairy cattle herds in Canada (Trotz-Williams et al., 2007), removing neonatal calves from the dam within 1 h of birth, while providing adequate initial colostrum, and calving in winter months may decrease the risk of neonatal diarrhoea. Keeping calves in individual pens and using an 'all-in-all-out' management strategy (Silverlås et al., 2009) also decrease the risk of transmission and the rapid spread of disease (Castro-Hermida et al., 2002a; Maddox-Hyttel et al., 2006).

8. BRIEF ACCOUNT OF *CRYPTOSPORIDIUM* AND *GIARDIA* OF HUMANS

Cryptosporidium and *Giardia* are frequent causes of gastrointestinal infections in humans worldwide. Both parasites are considered as significant waterborne pathogens due to their ubiquitous nature, frequent association with waterborne outbreaks and their resistance to most of the disinfectants used in water treatment (Savioli et al., 2006). *Giardia* is the most commonly diagnosed intestinal parasite of humans, with more than 2.5×10^8 cases estimated annually (e.g. Lane and Lloyd, 2002). According to an estimation by the Centers for Disease Control and Prevention (CDC), the number of giardiasis cases in the USA is ~2 million per year (Yoder and Beach, 2007). It has been estimated that ~200 million people have symptomatic giardiasis in Asia, Africa and Latin America, with some 500,000 new cases reported each year (Cacciò and Sprong, 2011). Cryptosporidiosis is also global in distribution. It has been suggested that cryptosporidiosis is responsible for up to 20%

of all cases of childhood diarrhoea in developing countries (McCormick, 2013; Mosier and Oberst, 2000; Robertson, 2014).

Most human cryptosporidiosis cases are due to *C. hominis* and/or *C. parvum* (See Cacciò, 2005), although some are attributed to *Cryptosporidium canis*, *Cryptosporidium felis* and *C. meleagridis*, *Cryptosporidium suis* and 'cervine and monkey' genotypes (which usually infect animals) (Xiao and Feng, 2008), and have been reported mainly from immunocompromised (e.g. HIV-infected) or -suppressed patients (Cama et al., 2006; Morgan et al., 2000a). Interestingly, *Cryptosporidium cuniculus*, usually a parasite of rabbits, is genetically closely related to *C. hominis* and *C. parvum*, and has been responsible for outbreaks of cryptosporidiosis in humans in the UK (Chalmers et al., 2009; Puleston et al., 2014; Robinson et al., 2010). The only species of *Giardia* infecting humans is *G. duodenalis* (syn. *G. lamblia* and *G. intestinalis*). Human infections due to *G. duodenalis* assemblages A and B have been reported, with variable prevalences in different countries (reviewed in Cacciò and Ryan, 2008). Based on molecular epidemiological data, it has been suggested that *G. duodenalis* assemblage B, which is believed to be anthroponotic, constitutes a greater public health risk than assemblage A, which is zoonotic and particularly prevalent in cattle (Cacciò et al., 2005).

Although cryptosporidiosis and giardiasis have been reported globally, the prevalences of infections appear vary considerably, depending on geographical region. For example, the prevalence of cryptosporidiosis in asymptomatic people (immunocompetent and healthy individuals) has been estimated to range from 0% to 6.4% in developed countries, and from 2.3% to 7.5% in developing countries (reviewed in Jex et al., 2011a). A higher prevalence of infections in developing countries might be due to the poor sanitation, poverty and lack of diagnostic and treatment facilities (Putignani and Menichella, 2010). Studies conducted in developing countries have reported that cryptosporidiosis is common in children younger than 5 years, with the highest prevalence in children of less than 2 years of age (Bern et al., 2000, 2002; Bhattacharya et al., 1997; Perch et al., 2001; Sulaiman et al., 2005). However, detailed information allowing meaningful interpretation of the epidemiology of cryptosporidiosis and giardiasis from developing countries is very limited. The Malnutrition and Enteric Disease (MAL-ED) project was developed to significantly enhance the knowledge of these diseases in developing countries and has highlighted their major contribution to the global burden of diarrhoeal diseases (McCormick, 2013). However, no detailed information is available from developing countries for a meaningful interpretation of the epidemiology of cryptosporidiosis and giardiasis.

In contrast, better statistics are available for many developed countries (Graczyk and Fried, 2007). Marked seasonal patterns of *Cryptosporidium* and *Giardia* infections have been observed in a number of developed countries, including Australia, Canada, New Zealand (Snel et al., 2009), the UK (McLauchlin et al., 2000) and the USA (Yoder et al., 2012). A recent review, which analysed information on patterns of important human enteric zoonotic diseases in temperate climatic zones in developed countries (Lal et al., 2012), reported that there is a clear (bimodal) peak of cryptosporidiosis cases in spring and summer seasons. In contrast, giardiasis showed a relatively small peak in summer (Lal et al., 2012). Critically, from a disease burden perspective, the majority of studies in developed and developing countries are cross-sectional surveys, and a greater focus on longitudinal cohort studies is needed (McCormick, 2013), particularly considering that many of the health impacts of these pathogens are believed to be chronic and extend beyond the patency of infection (Guerrant et al., 2013; Haillez and Buret, 2013).

Interestingly, most of the outbreaks associated with drinking and recreational waters have been reported from developed countries. According to the literature, from 1984 to 1996, there were 19 documented cryptosporidiosis outbreaks affecting more than 427,000 individuals (Smith and Rose, 1998), the largest being the outbreak in Milwaukee (USA) in 1993. An appraisal of waterborne outbreaks of parasitic protozoan diseases (Karanis et al., 2007) indicates that North American and European countries accounted for 93% of the 325 reported water-associated outbreaks; *Cryptosporidium* and *Giardia* were responsible for 165 and 132 outbreaks, respectively (Karanis et al., 2007). These numbers reflect an ascertainment bias for developed countries, with the greater resources to track outbreaks of cryptospordiosis and giardiasis.

In addition, foodborne outbreaks of cryptosporidiosis and giardiasis have also been reported from developed and developing countries, due to the consumption of fresh vegetables, salads and fruits contaminated with infective stages of *Cryptosporidium* and *Giardia* (Dawson, 2005). Therefore, understanding the molecular epidemiology of human cryptosporidiosis and giardiasis is important for the prevention and control of diseases caused by these two parasitic diseases in both developed and developing countries. The identification of potential infection sources, awareness of important preventive measures (hygiene and behavioural practices) and improved water treatment and disinfection strategies can aid in preventing disease transmission to humans (Jex et al., 2011a).

9. *CRYPTOSPORIDIUM* AND *GIARDIA* OF ANIMALS, WITH AN EMPHASIS ON BOVINES

Cryptosporidium and *Giardia* have been recorded in a wide variety of animals including mammals, birds, reptiles, amphibians and fishes (Adam, 2001; O'Donoghue, 1995; Ryan and Xiao, 2014; Yang et al., 2010). Currently, more than 150 mammalian species have been identified as hosts for various *Cryptosporidium* species (Ryan and Xiao, 2014). Generally, young animals are more susceptible to cryptosporidiosis and giardiasis than adults. Most *Cryptosporidium* species are infective to a range of host species, while some are host specific. *Cryptosporidium* and *Giardia* infections have increasingly been recorded in livestock animals, particularly cattle. In young calves, high morbidity caused by *Cryptosporidium* and *Giardia* can lead to substantial production losses to the livestock industry (de Graaf et al., 1999). According to studies conducted in dairy and beef cattle, cryptosporidiosis is mostly detected in dairy calves from 8 to 15 days of age, and in beef calves from 1 to 2 months of age (Ramirez et al., 2004). *Cryptosporidium parvum* and *G. duodenalis* (assemblages A and B) have been identified as potentially zoonotic, because these species/genotypes/assemblages are also capable of infecting humans. Among other livestock animals, *C. parvum* has been reported from lambs and goat kids of less than 3 months of age (Castro-Hermida et al., 2007; Geurden et al., 2008b; Ortega-Mora and Wright, 1994; Santín et al., 2007). However, the extent of zoonotic transmission of cryptosporidiosis or giardiasis from lambs and goat kids is unclear, given limited studies of these animals (Robertson, 2009; Ryan et al., 2005). In sheep, *Cryptosporidium ubiquitum*, *Cryptosporidium xiaoi* and *C. parvum* have been reported relatively frequently, whereas for goats, species and genotypes of *Cryptosporidium* are not comprehensively documented (Santín, 2013). Cryptosporidiosis and giardiasis in pigs and horses are often asymptomatic, even though there were some reports of disease in very young animals (Hamnes et al., 2007; Rotkiewicz et al., 2001; Tacal et al., 1987; Xiao and Herd, 1994).

Evidence for zoonotic transmission of *Cryptosporidium* and *Giardia* infections from companion animals is limited (Palmer et al., 2008). Dogs and cats are primarily infected with host-adapted species and genotypes; therefore, the actual risk of zoonotic transmission from companion animals appears to be minimal (Lucio-Forster et al., 2010). *Cryptosporidium* infection in dogs is usually due to *C. canis* (see Abe et al., 2002b; Palmer et al., 2008), although *C. parvum* has been detected in some studies (Abe et al., 2002a). Cats are

usually infected with *C. felis*, which is also host specific (Fayer et al., 2006a; Morgan et al., 1998). Giardiasis of dogs is commonly caused by *G. duodenalis* assemblages C and D (Abe et al., 2003; Souza et al., 2007). However, *G. duodenalis* assemblage A has been reported in dogs from Germany (Leonhard et al., 2007), Mexico (Lalle et al., 2005a) and Brazil (Volotao et al., 2007). Giardiasis of cats is often due to *G. duodenalis* assemblage F, but *G. duodenalis* assemblage A has also been reported infrequently (Souza et al., 2007). Case–control studies conducted in Australia, the UK and the USA (Hunter et al., 2004; Robertson et al., 2002a) have found that there is a negative association between sporadic cryptosporidiosis and contact with domestic pets. There are some studies of *Cryptosporidium* and *Giardia* in wild animals (Bednarska et al., 2003, 2007; Perz and Le Blancq, 2001; Rickard et al., 1999). The potentially zoonotic species of *Cryptosporidium* and *G. duodenalis* (assemblages A and B) have been reported from wild animals including artiodactyles, rodents, primates and carnivores (reviewed by Appelbee et al., 2005). However, the role of wild animals in the zoonotic transmission of cryptosporidiosis and giardiasis to humans is still unclear, because limited information is available for different host species, and most studies to date have not used molecular techniques for the identification and genetic characterisation of isolates (e.g. Appelbee et al., 2005; Ramirez et al., 2004; Xiao and Fayer, 2008).

9.1 Cattle

Cryptosporidium and *Giardia* are two of the commonest parasitic protists infecting cattle of all age groups (Olson et al., 2004). Clinical signs in infected animals can vary from acute to chronic diarrhoea, which often leads to anorexia, weight loss, ill thrift and sometimes death (Olson et al., 2004; Thompson et al., 2005). Cattle can harbour a number of *Cryptosporidium* species/genotypes and *G. duodenalis* assemblages. Currently, *C. andersoni*, *C. bovis*, *C. ryanae* and *C. suis* have been commonly reported from cattle (Santín, 2013). In addition *C. canis*, *C. felis*, *C. hominis*, *C. suis*-like genotype and pig genotype II have been reported sporadically (Trout and Santın, 2008). For *G. duodenalis*, assemblages A, B and E are known to infect cattle (Geurden et al., 2010; Thompson, 2004). *G. duodenalis* assemblage E has been reported at a higher prevalence than assemblage A (Xiao and Fayer, 2008), whereas assemblage B has been less commonly reported (Budu-Amoako et al., 2012a; Coklin et al., 2007; Lalle et al., 2005b; Liu et al., 2012; Winkworth et al., 2008).

Most dairy calves seem to be infected with *Cryptosporidium* and/or *Giardia* during the first few months of life, but only some animals develop diarrhoea

(O'Handley et al., 1999; O'Handley, 2002; Ralston et al., 2003). Although *Cryptosporidium* and *Giardia* infect cattle of all ages, various studies have reported an age-related variation of *Cryptosporidium* species in cattle (Fayer et al., 2006b, 2007; Kváč et al., 2006; Santín et al., 2004). *Cryptosporidium parvum* is usually predominant in preweaned calves (<3 months); *C. bovis* and *C. ryanae* are common in postweaned calves, whereas *C. andersoni* is commonly isolated from older calves and adult cattle (Brook et al., 2009; Dixon et al., 2008; Santín et al., 2004). Based on longitudinal and cross-sectional surveys of bovine cryptosporidiosis, the highest rates of *Cryptosporidium* infection have been reported in calves of 1–3 weeks of age (Becher et al., 2004; Castro-Hermida et al., 2002b; Huetink et al., 2001; Santin et al., 2008). Furthermore, it has been reported that, in calves, the shedding of *Cryptosporidium* oocysts in faeces starts as early as 2 days of age and can peak around 14 days of age (Olson et al., 2004). Therefore, it is believed that calves might acquire infection during or shortly after birth (Xiao and Herd, 1994). Usually, oocyst shedding occurs within a relatively short period, lasting for 4–16 days (Fayer et al., 1998). Although there is an apparent age-related distribution (Xiao and Fayer, 2008), *Giardia* has been mostly recorded in calves of 1–6 months of age, rather than animals of >6 months of age (reviewed in Geurden et al., 2010). The prevalence of *Giardia* in calves of <6 months has been reported to be 20–73% globally (Geurden et al., 2008a). However, *Giardia* has been detected in dairy calves as early as 3 days of age in some studies (Xiao, 1994). Based on longitudinal and cross-sectional surveys of bovine cryptosporidiosis, the highest rates of *Cryptosporidium* infection have been found in calves of 1–3 weeks of age (Becher et al., 2004; Castro-Hermida et al., 2002b; Huetink et al., 2001; Santin et al., 2008).

Infections of cattle with *Cryptosporidium* species and *G. duodenalis* assemblages of zoonotic potential (i.e. *C. parvum* and *G. duodenalis* assemblages A and B) have been frequently reported, indicating that cattle are a reservoir for human cryptosporidiosis and giardiasis (e.g. Hunter and Thompson, 2005; Ryan and Cacciò, 2013). Contact with infected calves has been identified as a main reason for a number of small cryptosporidiosis outbreaks in veterinary students (Gait et al., 2008; Pohjola et al., 1986; Preiser et al., 2003), research technicians and children attending agricultural camps (Kiang et al., 2006; Smith et al., 2004), providing evidence for zoonotic transmission of cryptosporidiosis from cattle. In addition, case–control studies have reported that contact with cattle is a significant risk factor for sporadic cryptosporidiosis in humans (Hunter et al., 2004; Robertson et al., 2002a; Roy et al., 2004).

Giardia duodenalis assemblage A is increasingly being detected in cattle (reviewed in Ryan and Cacciò, 2013). For example, this assemblage has been reported at prevalences of 43−70% of cattle in the USA (Santín et al., 2009) and Canada (Uehlinger et al., 2006). A recent review (Ryan and Cacciò, 2013) on zoonotic transmission of *Giardia* has reported that the prevalence of assemblage A in France, Germany, Italy and the UK ranged from 28% to 61%. However, by contrast, most of the studies of bovine giardiasis in Australia, Europe and North America have reported that the livestock-specific assemblage E is predominant in cattle (Becher et al., 2004; Langkjær et al., 2007; Santín et al., 2009; Trout et al., 2004, 2005), except in New Zealand, where assemblages A and B appear to be common, and assemblage E was reported to be largely absent from cattle (Feng and Xiao, 2011). It has been estimated that ~80% of *Giardia* infections in dairy cattle and 98% in beef cattle are due to assemblage E (reviewed in O'Handley and Olson, 2006). Thus, it has been suggested that the public health risk of bovine giardiasis is minimal, at least in the countries where assemblage E is the predominant genotype (Cacciò et al., 2005). Mixed infections of assemblages A and E have also been reported frequently in cattle (Geurden et al., 2008a; Khan et al., 2011; Santín et al., 2009). Furthermore, longitudinal studies of cattle have indicated that infections of zoonotic genotypes of *Giardia* in cattle might be transient and, when the frequency of transmission with assemblage E is high, a competition of genotypes might occur (Becher et al., 2004; Feng and Xiao, 2011).

Recently, molecular tools using markers in the 60 kDa glycoprotein (*gp60*) gene have improved the ability of identifying and differentiating zoonotic *Cryptosporidium* at the genotypic and subgenotypic levels. Such tools can be applied to investigate the distribution of *C. parvum gp60* variants in cattle and human populations in different geographical regions (Robertson et al., 2014). Based on *gp60* gene sequence data, *C. parvum* genotype IIa was the commonest genotypes found in cattle globally (reviewed in Jex and Gasser, 2010). Many of the IIa subgenotypes detected in cattle have also been found in humans (Xiao and Fayer, 2008). For example, several studies conducted in Australia, Canada and Portugal have isolated the same *C. parvum* subgenotypes from cattle and humans in the same regions (Alves et al., 2003; Budu-Amoako et al., 2012b; Ng et al., 2008), which indicates *C. parvum* transmission between human and cattle populations in those regions.

Usually, infected animals excrete as many as 10^6−10^7 oocysts/cysts per gram of faeces, which can massively contaminate the environment, even

in the situation of a low prevalence of infection (Robertson et al., 2014; Thompson, 2011). Surface water and groundwater sources can be contaminated by these infective stages from run-off from farm paddocks. Therefore, these stages can ultimately contaminate food and water sources used for human consumption (Ramirez et al., 2004). *Cryptosporidium* and *Giardia* oocysts/cysts have been isolated from fresh fruits and vegetables in studies conducted, for example, in Costa Rica (Monge and Arias, 1996; Monge and Chinchilla, 1996; Calvo et al., 2004), Peru (Ortega et al., 1997) and Norway (Robertson and Gjerde, 2001; Robertson et al., 2002b). Although the sources of infective stages were not identified in these studies, cattle were suspected as a source in some instances, because molecular detection has revealed that the subgenotypes found in the infected humans and food are common also in cattle (Blackburn et al., 2006; Smith et al., 2007). In addition, a small outbreak of cryptosporidiosis occurred following the consumption of unpasteurized cow's milk (due to poor udder hygiene), which also provides evidence for zoonotic transmission (Harper et al., 2002).

Although many studies of cryptosporidiosis and giardiasis in cattle have been conducted, most of them have been on dairy farms, whereas only a small number of studies have focused on beef cattle (Table 3). Few studies have used molecular diagnostic tools to identify and differentiate the species/genotypes infecting beef cattle. However, *C. parvum* and *G. duodenalis* assemblages A and B have been reported from beef animals (Atwill et al., 2003; Budu-Amoako et al., 2012a; Geurden et al., 2007, 2008a; Ng et al., 2008). Studies conducted in Canada and the USA have reported prevalences of *Cryptosporidium* infection in beef cattle of 7.1−43% (Atwill et al., 2003; Budu-Amoako et al., 2012a; Fayer et al., 2010; Gow and Waldner, 2006; McAllister et al., 2005). On the other hand, the prevalence of *Giardia* infection ranged between 6.5% and 34% (Budu-Amoako et al., 2012a; Hoar et al., 2001; McAllister et al., 2005). Very little information is available on cryptosporidiosis in beef cattle for other countries. Studies have suggested that beef cattle are of limited significance in the zoonotic transmission of *Giardia* relative to dairy cattle (Hoar et al., 2001). The prevalence of *G. duodenalis* assemblage E, which is not typically zoonotic, is high ($\leq$100%) in both beef and dairy cattle (Appelbee et al., 2003; Ng et al., 2011; Trout et al., 2004, 2006). In contrast, however, *G. duodenalis* A, which is zoonotic, is reportedly much less prevalent in beef relative to dairy cattle (Appelbee et al., 2003; Dixon et al., 2011; Geurden et al., 2008a). Caution in the interpretation of this information is warranted, as there are limited molecular investigations of beef cattle, particularly for *Cryptosporidium*

Table 3 Studies of *Cryptosporidium* and *Giardia* in beef cattle

Country	Host age	Total number	Molecular marker(s), *Cryptosporidium* species or genotype (% of positive)	Molecular marker(s), *Giardia duodenalis* assemblage (% of positive)	References
Australia	≤3 or 3–9 months	201	SSU-rRNA and *gp60* *Cryptosporidium parvum* 3% *Cryptosporidium bovis* 10% *Cryptosporidium ryanae* 3%	*tpi* Assemblage A 1% Assemblage E 24%	Abeywardena et al. (2013a)
Belgium	<10 weeks	333	–	β-giardin Assemblage A 16% Assemblage E 84% *tpi* Assemblage A 6% Assemblage E 71% Mixed A and E 22%	Geurden et al. (2008a)
Canada	<6 months	739	SSU-rRNA and *hsp70*	SSU-rRNA and β-giardin	Budu-Amoako et al. (2012a)
	>6 months		*C. parvum* 24% *C. bovis* 20% *C. ryanae* 7% *Cryptosporidium andersoni* 49%	Assemblage A 4% Assemblage B 7% Assemblage E 89%	
Canada	Pooled manure samples from different ages	112	SSU-rRNA and *hsp70* *C. andersoni* 100%	SSU-rRNA and β-giardin Assemblage E 100%	Dixon et al. (2011)

Japan	2 months to 15 years	113	SSU-rRNA *C. bovis* 13% *C. ryanae* 5% Mixed *C. bovis* and *C. ryanae* 2%		Murakoshi et al. (2012)
USA	Calves (6–8 months) and cows (>2 years)	212	SSU-rRNA *C. bovis* 9.4% *C. andersoni* 1.4% *Cryptosporidium* deer-like genotype 6.6%	–	Feltus et al. (2008)
USA	6–18 months	819	SSU-rRNA *C. andersoni* 68% *C. bovis* 23% *C. ryanae* 9%	–	Fayer et al. (2010)
USA	6–18 months	819	–	SSU-rRNA Assemblage E 31.5% Assemblage A 1.2%	Santin et al. (2012)
Vietnam	2–6 months	232	SSU-rRNA *C. bovis* (0.8%) *C. ryanae* (3.9%)	–	Nguyen et al. (2012)
Zambia	2–70 days	238	SSU-rRNA *C. parvum* 2.9% *Cryptosporidium suis* 0.4% *hsp70* *C. parvum* 1.3% *C. suis* 0.4%	–	Geurden et al. (2006)

(Budu-Amoako et al., 2012a; Fayer et al., 2010; Feltus et al., 2008; Geurden et al., 2006), and the extent to which these studies are representative of beef cattle generally is not yet established.

A review of *gp60* sequence data for *Cryptosporidium* (Jex and Gasser, 2010) reported that the information on cattle originated mainly from 14 countries, most of them representing only six countries. Therefore, a major gap in current knowledge is the lack of data from other regions in the world, particularly from developing countries. According to studies conducted in developing countries, *C. hominis* is responsible for 70–90% of human infections (reviewed in Xiao and Fayer, 2008). Therefore, some authors suggest that the zoonotic cryptosporidiosis is less common in developing countries. However, this might not be entirely true, because there is insufficient information on *Cryptosporidium* and *Giardia* in bovids or other animal hosts in those countries. In some instances, *C. hominis* has been inferred to infect cattle in countries including India, Korea and Scotland (Smith et al., 2005), and it is possible that *C. hominis* might infect animals other than humans in geographical regions in which no molecular investigations have been conducted. Furthermore, the molecular epidemiology of cryptosporidiosis and giardiasis in cattle in developed countries might be different from developing countries, because cattle farms in developed countries are managed intensively, whereas most of the farms in developing countries are extensive or semi-extensive. Household, small cattle farms (<10 animals) are common in developing countries, particularly in Asia. Cattle in developing countries might have a closer contact with humans than in developed countries. Therefore, the transmission dynamics of cryptosporidiosis and giardiasis might not be the same in developed as in developing countries.

9.2 Water buffalo

Water buffalo (*B. bubalis*) is one of the important livestock animals in many regions of the developing world. The world's water buffalo population was estimated as 195 million in 2011, being mostly distributed in Asia, some Mediterranean regions and Latin American countries (Robertson et al., 2014). They contribute significantly to the agricultural economy in many parts of Asia through milk, meat, leather and draught power (Vijh et al., 2008). There are two broad lineages of water buffaloes, namely the river and swamp types. The river type is found mainly in Italy and the Indian subcontinent, whereas the swamp type is distributed from Assam in India through Southeast Asia to China (Kumar et al., 2006). Based on an early

report (Iannuzzi, 1998), the karyotypes (2n) of riverine (50) and swamp (48) buffaloes differ, suggesting that they are distinct species.

Although *Cryptosporidium* has been detected in the faeces of water buffaloes in a number of studies (Table 4), reports of *Giardia* in this host species are very limited. Specifically, *Cryptosporidium* and *Giardia* have been detected in the faeces from water buffaloes from Egypt (El-Khodery and Osman, 2008; Shoukry et al., 2009), India (Bhat et al., 2012; Maurya et al., 2013), Italy (Cacciò et al., 2007; Rinaldi et al., 2007a,b), Nepal (Feng et al., 2012), Pakistan (Nasir et al., 2009), the Philippines (Villanueva et al., 2010) and Spain (Gómez-Couso et al., 2005), but most of these studies have been based on the use of microscopy and coproantigen detection techniques. Although some studies have reported '*C. parvum*' in water buffalo (Nasir et al., 2009; Rinaldi et al., 2007a,b), these reports should be interpreted with some caution, as immunological methods do not allow specific diagnosis. In some studies, molecular techniques were used for the specific or genotypic identification of *Cryptosporidium* or *Giardia* (e.g. Amer et al., 2013; Cacciò et al., 2007; Feng et al., 2012; Gómez-Couso et al., 2005; Venu et al., 2012). For the first time, Gómez-Couso et al. (2005) conducted a molecular analysis of *Cryptosporidium* in water buffaloes in Spain. Subsequently, Cacciò et al. (2007) detected and genetically characterised *C. parvum* and *G. duodenalis* assemblages A and E from water buffaloes from Central Italy. Here, the small subunit of ribosomal RNA gene (*SSU*) and the β-giardin (*bg*) gene were used for the amplification of *Cryptosporidium* and *Giardia* DNA, respectively. Although the significance of water buffaloes as reservoirs of cryptosporidiosis and giardiasis is limited, findings from various studies (Amer et al., 2013; Cacciò et al., 2007; Maurya et al., 2013) do suggest that water buffaloes might contribute to the zoonotic transmission of species or genotypes of *Cryptosporidium* and *Giardia*. Oocysts and cysts in the faeces from buffaloes can enter into food and water sources through a number of pathways. Buffaloes usually wallow in rivers, streams and other water sources. Buffalo dung is used as a fertilizer for crops. In some countries, dung is also used to line the floors and walls of houses, which represents a potential risk of transmission to humans. By these and other means, zoonotic genotypes of *Cryptosporidium* and *Giardia* in buffaloes might directly or indirectly infect humans in these developing countries. The extent to which water buffaloes contribute to the zoonotic transmission of these protists is largely unknown (Robertson et al., 2014), emphasizing the need for further investigations of riverine and swamp buffaloes in developing and developed countries.

Table 4 Summary of information (country, clinical symptoms, age, diagnostic techniques, % of positive samples, species/genotypes and references) relating to *Cryptosporidium* infections/cryptosporidiosis in water buffaloes

Country	Symptoms	Host age	Diagnostic techniques used	% of test positive (no. test positive/ total no.)	Species/genotype detected	References
Australia	Not assessed	≤6, 6−24, or >24 months	Molecular analysis (SSU-rRNA)	13% (62/476)	*Cryptosporidium parvum*, *Cryptosporidium bovis*, Genotypes 1−3	Abeywardena et al. (2013b)
Egypt	Diarrhoeic and non-diarrhoeic	<3 months	Modified Ziehl −Neelsen (MZN)	14% (65/458)	Not detected	El-Khodery and Osman (2008)
Egypt	Diarrhoeic and non-diarrhoeic	Not stated	MZN	22% (16/71)	Not detected	Shoukry et al. (2009)
Egypt	Not stated	1 week to 4 months and adults	Molecular analysis (SSU-rRNA, *gp60*)	Calves 10% (17/179), adults: 0	*C. parvum* (IIdA20G1 and IIaA15G1R1), *Cryptosporidium ryanae*	Amer et al. (2013)
Egypt	Diarrhoeic adults, not stated for calves	Calves and adults	Antibody-based coproantigen test and molecular analysis (SSU-rRNA and *gp60*)	1 day to 3 months: 40% (34/85), 3 months to 1 year: 11% (6/56%), >1 year: 4% (3/70)	*C. parvum*, *C. bovis*, *C. ryanae*	Helmy et al. (2013)
India	Diarrhoeic and non-diarrhoeic	<3 months	MZN and molecular analysis (SSU-rRNA)	24% (64/264)	*C. parvum*	Maurya et al. (2013)

India	Diarrhoeic and non-diarrhoeic	<5 months	MZN	38% (62/162)	Not detected	Bhat et al. (2012)
India	Diarrhoeic and non-diarrhoeic	Not stated	Modified Ziehl—Neelsen (mZN)	25% (76/305)	Not detected	Mohanty and Panda (2012)
Italy	Asymptomatic	Calves	ELISA (Enzyme-linked immunosorbent assay), IFA (Immunofluorescence assay) and molecular analysis (SSU-rRNA)	14% (8/57)	*C. parvum*	Cacciò et al. (2007)
Italy	Not stated	2—60 days	ELISA	20% (35/177)	Not detected	Rinaldi et al. (2007a)
Italy	Asymptomatic	1—9 weeks	ELISA	15% (51/347)	Not detected	Rinaldi et al. (2007b)
Nepal	Not stated	2—7 months	molecular analysis (SSU-rRNA)	37% (30/81)	*C. ryanae*	Feng et al. (2012)
Pakistan	Diarrhoeic and non-diarrhoeic	1 day to 1 year, >1 year	MZN	24% (60/250)	Not detected	Nasir et al. (2009)
Philippines	Diarrhoeic and non-diarrhoeic	1—12 days calves and their dams	Kinyoun acid fast stain	Calves 3% (1/38), adults 26% (10/38)	Not detected	Villanueva et al. (2010)
Spain	Not stated	Calves, heifers and adults	IFA (Immunofluorescence antibody assay), molecular analysis (*COWP*)	8% (1/12)	An isolate closely relate to '*Cryptosporidium* pig-genotype'	Gomez-Couso et al. (2005)
Sri Lanka	Not assessed	Calves (<6 or ≥6 months)	Molecular analysis (SSU-rRNA)	10% (29/297)	Genotypes 9—11	Abeywardena et al. (2014)

9.3 Bovids as possible reservoirs of human cryptosporidiosis and giardiasis

There has been an increasing concern about cryptosporidiosis and giardiasis in bovids as gastrointestinal illnesses as well as infections of zoonotic significance. However, direct evidence for zoonotic transmission of cryptosporidiosis and giardiasis from animal reservoirs is limited, because of the challenges in conducting cross-transmission studies (Cacciò and Ryan, 2008; Xiao and Fayer, 2008). Nonetheless, the application of molecular techniques has provided some insights into zoonotic species and genotypes present in animals. Molecular genetic studies have shown that domestic bovids, including cattle and water buffaloes, can harbour the same *C. parvum* and *G. duodenalis* genotypes/assemblages, and thus likely constitute a public health risk as reservoirs (Hunter and Thompson, 2005).

Although there are not many experimental studies, several small outbreaks have been reported in cattle farm workers, veterinarians and veterinary students, who had been exposed to cattle faeces (Gait et al., 2008; Muyzer and Smalla, 1998; Pohjola et al., 1986; Preiser et al., 2003; Robertson et al., 2006; Siwila et al., 2007). A report also indicated that the risk of being seropositive for *Cryptosporidium* infection is higher in dairy farmers than in other people not involved in dairy farming (Lengerich et al., 1993). According to the case–control studies conducted in Australia and the USA (Robertson et al., 2002a; Roy et al., 2004), contact with calves has been identified as a risk factor for sporadic cryptosporidiosis in humans.

Comparatively limited information is available on zoonotic transmission of giardiasis from cattle to humans. The commonest reported *G. duodenalis* assemblage in cattle is E, which is usually not infective to humans. However, zoonotic assemblage A is increasingly being detected in cattle, indicating that this assemblage might be more widespread in cattle than previously assumed (reviewed in Ryan and Cacciò, 2013). In contrast, assemblage B has been reported from cattle only on some occasions (Coklin et al., 2007; Lalle et al., 2005b; Mendonca et al., 2007; Ng et al., 2011; Winkworth et al., 2008).

A long-term survey in the UK (Bodley-Tickell et al., 2002) found *Cryptosporidium* in surface water draining from a livestock farm and reported the presence of oocysts throughout the year; the highest levels coincided with the calving season. In some studies, *C. parvum* and other *Cryptosporidium* species infecting cattle have been detected in different water sources in Europe (Alves et al., 2006; Ward et al., 2002) and North America (Jiang et al., 2005; Ruecker et al., 2007; Xiao et al., 2001), suggesting that cattle manure is a

significant source of water contamination. A recent field study conducted in Spain (Amoros et al., 2010) reported *Cryptosporidium* oocysts in fresh vegetables irrigated with contaminated water, and another investigation conducted in Poland isolated *Cryptosporidium* oocysts from vegetables and fruits grown in an area of high livestock production (Rzezutka et al., 2010). Therefore, these studies suggest that farm animals, particularly cattle, are an important potential source of human cryptosporidiosis (Hunter et al., 2004; Robertson et al., 2002a; Roy et al., 2004). Although the extent of zoonotic transmission of giardiasis from bovids is not entirely clear, infected animals can pose a public health risk, because they can excrete 10^5 to 10^6 *Giardia* cysts per gram of faeces (Thompson, 2011). Therefore, future research should focus on exploring in detail the zoonotic potential and the extent of transmission of *Cryptosporidium* and *Giardia* from bovids to humans in different geographical regions of the world. This can be achieved using advanced molecular tools, which allow the characterisation of *Cryptosporidium* and *Giardia* using particular genetic markers (see Table 5).

9.4 The need to use effective molecular tools

The accurate identification and characterisation of *Cryptosporidium* and *Giardia* are central to exploring and understanding the epidemiology of cryptosporidiosis and giardiasis, respectively. However, there are significant limitations in the detection or diagnosis of these protists using conventional microscopic, biochemical and immunological techniques, such that reliable and practical molecular methods need to be used. Using molecular methods, recognised species and genotypes of these protists can be identified and classified (Chalmers and Katzer, 2013; Jex et al., 2008b; Koehler et al., 2014a; Ryan and Caccio, 2013; Ryan and Xiao, 2014; Thompson, 2011; Thompson and Monis, 2012), although the specific status of some taxa and new records need confirmation. Species and genotypes of *Cryptosporidium* and *Giardia* cannot be distinguished solely based on host origin or parasite morphology. Key microscopic and immunological methods used for the detection of *Cryptosporidium* and *Giardia*, as well as nucleic acid-based approaches for the diagnosis of cryptosporidiosis or giardiasis and the analysis of genetic variation within and among species of these protists have been reviewed elsewhere (e.g. Jex and Gasser, 2009; Koehler et al., 2014a; Ryan and Cacciò, 2013; Xiao et al., 2004) and are thus not covered in detail here. In previous reviews (Jex et al., 2008b; Koehler et al., 2014a), we discussed the advantages of particular genetic markers and PCR-coupled methods, and emphasised the benefits of using molecular tools to enhance the understanding of the

Table 5 List of key genetic loci used for the genetic characterisation of *Cryptosporidium* and *Giardia*, their features and main applications[a]

Genetic marker or coding gene	Description	Main applications
Cryptosporidium		
Small subunit (*SSU*) of nuclear ribosomal RNA	Small subunit of the ribosome	Specific and genotypic identification
70 kDa heat shock protein (*hsp70*) gene	Molecular chaperons	Specific and genotypic identification
Actin gene	Structural protein	Specific and genotypic identification
β-tubulin	Structural protein	Specific and genotypic identification
Cryptosporidium oocyst wall protein (cowp) gene	Structural protein	Specific and genotypic identification
Second internal transcribed spacer of nuclear ribosomal DNA (ITS-2)	Ribosomal	Specific identification and some subspecific characterisation
Thrombospondin-related attachment protein (TRAP) genes	Sporozoite motility	Specific and genotypic identification
Microsatellite locus 1 (ML1)	Untranslated regions	Genotypic and subgenotypic identification
Microsatellite locus 2 (ML2)	Untranslated regions	Genotypic and subgenotypic identification
60 kDa glycoprotein (*gp60*) gene	Encodes surface glycoproteins GP45 and GP15	Genotypic and subgenotypic identification
Giardia		
Glutamate dehydrogenase	Housekeeping enzyme	*G. duodenalis* assemblage and subassemblage identification
Triose phosphate isomerase	Housekeeping enzyme	*G. duodenalis* assemblage and subassemblage identification
Beta-giardin	Structural protein	*G. duodenalis* assemblage and subassemblage identification

Elongation factor 1-α	Involved in translation	Species and *G. duodenalis* assemblage identification
Ferredoxin	Mediates electron transfer	Species and *G. duodenalis* assemblage identification
Histone H2B	Nucleosomal protein	Species and *G. duodenalis* assemblage identification
Histone H4	Nucleosomal protein	Species and *G. duodenalis* assemblage identification
Actin	Structural protein	Species and *G. duodenalis* assemblage identification
α-tubulin	Structural protein	Species and *G. duodenalis* assemblage identification
Chaperonin 60	Heat shock protein	Species and *G. duodenalis* assemblage identification
Open reading frame C4	Hypothetical heat shock protein	Species and *G. duodenalis* assemblage identification
18S rDNA	Small subunit of the ribosome	Species and *G. duodenalis* assemblage identification
Intergenic ribosomal spacer	Noncoding ribosomal	Species and *G. duodenalis* assemblage identification
ITS-1, ITS-2 and 5.8S rDNA	Ribosomal	Species and *G. duodenalis* assemblage identification
Ribosomal protein 17a	Ribosomal	Species and *G. duodenalis* assemblage identification
Mlh1	Function in DNA repair	Species and *G. duodenalis* assemblage identification

[a]Information taken from key review articles (including Xiao et al., 2004; Jex et al., 2008b; Nolan et al., 2010b; Ryan and Cacciò, 2013; Koehler et al., 2014a).

epidemiology and population genetics of these protists. Of the many nucleic acid-based methods available, we have shown consistently that PCR-based single-strand conformation polymorphism (SSCP) is a practical and efficient mutation scanning technique for both diagnostic and analytical applications (cf. Gasser et al., 2006); this technique has served us well for genetic investigations of *Cryptosporidium* and *Giardia* using well-accepted genetic markers (e.g. Jex et al., 2007a, 2008a; Jex and Gasser, 2009; Koehler et al., 2014b; Nolan et al., 2009, 2010a,b, 2013). In the following section, this mutation scanning method, together with targeted DNA sequencing, was used to gain new insights into these protists in bovines.

10. RECENT INSIGHTS INTO *CRYPTOSPORIDIUM* AND *GIARDIA* OF BOVINES IN PARTS OF AUSTRALASIA AND SRI LANKA

Recently, we undertook some investigations of *Cryptosporidium* and *Giardia* in domestic bovines (*Bos taurus* and *B. bubalis*) in different geographical regions in New Zealand, Australia and Sri Lanka (Abeywardena et al., 2012, 2013a,b, 2014). We genetically characterised these protists and explored their zoonotic potential. Overall, *Cryptosporidium* and *Giardia* were detected in a considerable percentage (8.3−62% for *Cryptosporidium* and 0.7−41.5% for *Giardia*, respectively) of cattle and water buffaloes in all three geographical regions studied (Abeywardena et al., 2012, 2013a,b, 2014), providing evidence for a relatively widespread occurrence of these protists. This section discusses salient findings and proposes avenues for future research.

In New Zealand, using a PCR-based approach, we explored whether dairy calves in the Canterbury region of the South Island of New Zealand harboured genotypes of *Cryptosporidium* and *Giardia* known to infect humans (Abeywardena et al., 2012). The results revealed the presence of potentially zoonotic species/genotypes of these parasites. An important finding was evidence of *C. hominis* in faecal samples collected from pre- and post-weaned calves (n = 12 of 180). *Cryptosporidium hominis* found represented subgenotype IbA10G2R2, the most widely distributed subgenotype of *Cryptosporidium* infecting humans globally (Jex and Gasser, 2010). Although evidence of patent infection was not available, this result raises questions as to possible routes of transmission of *C. hominis* between human and cattle populations. It is not clear how these calves might have come into contact with *C. hominis*, although the dispersal of wastewater effluent on farms, which is

common practice in New Zealand, is one possible source. Naturally acquired *C. hominis* infection has been reported in animals including cattle, goats, marsupials and dugongs (Feng et al., 2007b; Morgan et al., 2000b; Park et al., 2006; Ryan and Power, 2012; Smith et al., 2005). The findings of our study (Abeywardena et al., 2012) might suggest a need for future investigations of the host specificity of *C. hominis*, and to understand its transmission between human and cattle populations. Noting this, *C. parvum* (subgenotypes IIaA15G3R1, IIaA19G3R1 and IIaA23G4) was the commonest species detected, reinforcing the potential of cattle as zoonotic reservoirs for this species. According to a report on foodborne diseases in New Zealand (On et al., 2011), the highest rates of cryptosporidiosis notifications in humans have been recorded in the Canterbury region. Furthermore, contact with farm animals has been reported as the main risk factor for human cryptosporidiosis in New Zealand (On et al., 2011). It should be remarked, however, that the molecular methods chosen for this study (based on the detection and characterisation of the *gp60* gene) did not use primers able to detect other common species and genotypes of *Cryptosporidium* known to commonly infect cattle (e.g. *C. andersoni*, *C. bovis* and *C. ryanae*; see Feng et al., 2007b; Quilez et al., 2008; Xiao, 2010). We also examined dairy cattle in Canterbury for the presence of *Giardia* (Abeywardena et al., 2012). Both *G. duodenalis* assemblage A and E were detected in faecal samples from these animals. Surprisingly, this is the first report of assemblage E in New Zealand, although it is the most commonly reported assemblage of *G. duodenalis* in cattle globally. This may be explained by the fact that only a small number of molecular studies have been conducted in this country (Hunt et al., 2000; Learmonth et al., 2003; Winkworth et al., 2008), rather than a limited presence of this assemblage. Together, these findings suggest considerable potential for zoonotic transmission in the Canterbury region, and indicate that additional studies of the zoonotic potential of cattle in other regions of New Zealand are warranted.

In Australia, a molecular epidemiological survey of *Cryptosporidium* and *Giardia* was conducted for dairy and beef calves from five farms in an open drinking water catchment area in Victoria (Abeywardena et al., 2013a). The results of this study showed that a considerable proportion of cattle, particularly dairy calves, harboured *C. parvum* and *G. duodenalis* assemblage A. These results are in agreement with previous investigations from other parts of Australasia (Becher et al., 2004; Ng et al., 2008, 2011; Nolan et al., 2009; Winkworth et al., 2008). In addition to than *C. parvum*, *C. bovis*, *C. ryanae* and a new genotype of *Cryptosporidium* were detected.

Based on the analysis of *gp60* gene sequences, all of the *C. parvum* isolates from Victorian cattle (Abeywardena et al., 2013a) were identified as genotype IIa and subgenotype A18G3R1. Previous studies conducted in Australia have reported that *C. parvum* IIaA18G3R1 was a common subgenotype in humans (Jex et al., 2007b, 2008a; Waldron et al., 2009) and cattle (Ng et al., 2011). Therefore, published reports (Abeywardena et al., 2013a; Ng et al., 2008, 2011; Nolan et al., 2009) indicate potential transmission of *C. parvum* between cattle and human populations in Australia. Intriguingly, although *C. parvum* IIaA18G3R1 has been reported with high prevalence in Australia, it is rarely reported elsewhere in the world (Jex and Gasser, 2010). Why this genotype should be so common in Australia and not elsewhere is not clear, but may indicate a predilection for Australian cattle.

Another interesting finding was that most of the *C. parvum* cases found (82.9% of 35 infections) were linked to a particular dairy farm (two sampling time-points) (Abeywardena et al., 2013a). This result could be associated with differences in management practices, because calves on this farm were reared at a relatively high stocking density in pens with sawdust bedding, whereas calves on all other farms were kept on paddocks with less crowding. Previous epidemiological studies have reported that there is a link between management practices and the occurrence of *C. parvum* in calves (Atwill et al., 1999; Garber et al., 1994; Maddox-Hyttel et al., 2006; Trotz-Williams et al., 2007). A number of potential risk factors were identified in these studies, including large herd size, use of multi-cow maternity facilities, cleanliness of calf pens and a long calving season. The stocking density of calves, particularly prior to weaning, and their housing in pens rather than on paddocks are known risk factors for *Cryptosporidium* transmission (Castro-Hermida et al., 2002a; Mohammed et al., 1999). Noting this, adequate consumption of colostrum by neonatal calves is a protective factor against *Cryptosporidium* and other pathogens causing diarrhoea (e.g. Coronavirus, Rotavirus and *Escherichia coli*) (Berge et al., 2009; Duranti et al., 2009) and may also have contributed to these differences.

Giardia duodenalis assemblage E was the most frequently identified assemblage in both dairy and beef cattle in Victoria (Abeywardena et al., 2013a), which is consistent with the earlier studies conducted in other states of Australia (Becher et al., 2004; Ng et al., 2011; O'Handley et al., 2000) and in other countries (Appelbee et al., 2003; Langkjær et al., 2007; Trout et al., 2004). In contrast, *G. duodenalis* assemblage A was the most frequently isolated from Victorian water buffaloes (Abeywardena et al., 2013b). This finding is consistent with reports from water buffaloes from Italy (Cacciò

et al., 2007). Although these data suggest potential for these animals to act as zoonotic sources of giardiasis, the limited molecular data for water buffalo elsewhere strongly suggest that these findings are preliminary, and more investigation is needed. Similarly, studies of *Cryptosporidium* infection in water buffaloes are also limited, with only a small number utilizing molecular tools (Amer et al., 2013; Feng et al., 2012; Gómez-Couso et al., 2005; Maurya et al., 2013; Venu et al., 2012). These studies have reported a unique *Cryptosporidium* genotype, which is closely related to *C. ryanae*, in most of the samples from buffaloes. Recent findings show that the genotypes of *Cryptosporidium* found in water buffaloes are clearly different from those found in cattle (Abeywardena et al., 2013b). Of the two genotypes (1 and 2) identified in Victorian water buffaloes (Abeywardena et al., 2013b), genotype 1 was identical to those previously reported for isolates from water buffaloes (Amer et al., 2013; Feng et al., 2012; Venu et al., 2012), whereas genotype 2 had not been reported previously. Therefore, further investigations are required to understand taxonomic status, host specificity and virulence of these genotypes found in water buffaloes.

Although zoonotic species/genotypes of *Cryptosporidium* and *Giardia* were detected in a considerable proportion of samples collected from Australia and New Zealand (Abeywardena et al., 2012, 2013a,b), this was not the case in bovines in Sri Lanka, where the zoonotic risk of these protists appeared to be minimal in the regions studied (Abeywardena et al., 2014). Whilst previous studies in Sri Lanka have established the occurrence of cryptosporidiosis and giardiasis in humans (Perera, 1988; Perera et al., 1999; Perera and Lucas, 1990) and animals (Bahirathan et al., 1987; Ekanayake et al., 2006; Noordeen et al., 2000, 2001), ours is the first study documenting species and genotypes of *Cryptosporidium* and *Giardia* in domestic bovids in this country using molecular tools (cf. Jex et al., 2008b; Koehler et al., 2014a). Interestingly, eight new genotypes of *Cryptosporidium* were identified. The absence of *C. parvum* from any of the samples collected from 1- to 3-month-old calves was unexpected, because it is the most commonly reported species of *Cryptosporidium* in preweaned calves throughout the world (Jex et al., 2011a; Xiao, 2010; Xiao and Feng, 2008). The identification of many new genotypes and the unexpected absence of *C. parvum* suggest that the molecular epidemiology of cryptosporidiosis in Sri Lanka might be distinct from Australasia and other regions in the world. This particular hypothesis needs further testing. As in other countries, *G. duodenalis* assemblage E was more common than assemblage A in dairy calves, whereas only livestock-specific assemblage E was detected in

water buffaloes. These findings all suggest that zoonotic transmission of giardiasis from cattle and water buffaloes in our study regions in Sri Lanka was limited at the time of investigation. However, this proposal needs testing in the future. In addition, it would be useful to conduct studies of *Cryptosporidium* and *Giardia* in other neighbouring countries in South Asia.

The identification of zoonotic species and genotypes of *Cryptosporidium* and *Giardia* in some water buffalo populations (Abeywardena et al., 2013b; Amer et al., 2013; Cacciò et al., 2007; Helmy et al., 2013; Maurya et al., 2013) indicates their public health significance. Like cattle, water buffaloes pass substantial amounts of faeces, which may contain large numbers of oocysts/cysts when they are infected with *Cryptosporidium* and/or *Giardia*. Although these parasites have been reported in water buffaloes in a number of studies (Bhat et al., 2012; El-Khodery and Osman, 2008; Nasir et al., 2009; Rinaldi et al., 2007a,b), most investigations did not employ molecular tools. Advanced PCR-based techniques have been developed and applied extensively to the genetic characterisation of *Cryptosporidium* and *Giardia* of cattle, but not water buffaloes. Furthermore, there are very few publications reporting *Cryptosporidium* and *Giardia* in water buffaloes from Asian countries which have the largest proportion of the world's water buffalo population (>90%). Buffaloes usually wallow in water sources, which likely make them a major source of water contamination through faecal contamination during wallowing. Given these knowledge gaps, there is major scope for future studies of the molecular epidemiology of cryptosporidiosis and giardiasis in water buffaloes and other bovids.

Currently, it is widely accepted that there is an age-related distribution of *Cryptosporidium* species and genotypes in cattle. Several studies conducted in Denmark, Germany, Ireland, the UK and the USA have shown that *C. parvum* is more common in neonates and very young calves. In contrast, *C. bovis* and *C. ryanae* predominate in older calves, and *C. andersoni* is more prevalent in adults (Broglia et al., 2008; Fayer et al., 2007; Feltus et al., 2008; Feng et al., 2007b; Maddox-Hyttel et al., 2006; Santín et al., 2004; Sturdee et al., 2003; Thompson et al., 2007). One of our recent studies (Abeywardena et al., 2013a) showed a similar pattern in Victorian dairy and beef cattle, in which *C. parvum* was detected exclusively in <3-month-old and *C. bovis* was detected mostly in >3-month-old calves. However, such a pattern was not evident in New Zealand (Abeywardena et al., 2012) likely due to the relatively small sample size in the study. In contrast, *C. parvum* was not detected in faecal samples collected from dairy calves in Sri Lanka (Abeywardena et al., 2014), rather a new genotype with 99% homology to *C. bovis* was the

commonest. Interestingly, other studies conducted in China, India, Georgia, Nigeria and Sweden have also reported a similar situation, where *C. bovis* has been the predominant species in 1−60-day-old calves, instead of *C. parvum* (see Feng et al., 2007b; Maikai et al., 2011; Silverlås et al., 2010; Wang et al., 2011; Zhang et al., 2013). Therefore, evidence provided in this review suggests that the age-related distribution of *Cryptosporidium* species in cattle is not the same for all countries or geographical regions.

For *Giardia*, assemblages A and E were isolated from both cattle and water buffaloes, although there were differences in proportions of test-positive samples in different geographical regions studied (Abeywardena et al., 2012, 2013a,b, 2014). Overall, *G. duodenalis* assemblage E was most frequent in cattle sampled from all geographical regions studied. This finding was consistent with previous studies (Appelbee et al., 2003; Berrilli et al., 2004; Langkjær et al., 2007; Ng et al., 2011; Trout et al., 2004). *G. duodenalis* assemblage A was also detected in cattle in the geographical regions studied (Abeywardena et al., 2012, 2013a, 2014), but with fewer occurrences than assemblage E. These two assemblages were also identified in water buffaloes (Abeywardena et al., 2013b, 2014). However, *G. duodenalis* was detected only on some farms in Australia and Sri Lanka (Abeywardena et al., 2013b, 2014). Interestingly, one of the two water buffalo farms in Victoria, Australia, had a higher occurrence of *G. duodenalis* assemblage A than assemblage E at two samplings (6 months apart) (Abeywardena et al., 2013b). In contrast, all the samples collected from the other farm in Victoria were test-negative for *Giardia* (Abeywardena et al., 2013b) on both occasions. In Sri Lanka, only two of 297 water buffaloes had *G. duodenalis* assemblage E (Abeywardena et al., 2014). The limited presence of assemblage A compared with assemblage E suggested that the public health risk of giardiasis in bovines does not appear to be as great as previously thought.

Another interesting finding was that samples collected from selected dairy farms had a higher occurrence of *Cryptosporidium* and *Giardia* than those from beef farms in the same geographical region in Victoria (Abeywardena et al., 2013a). Prior to this report, only few studies have compared occurrence and zoonotic potential of *Cryptosporidium* and *Giardia* in dairy and beef cattle in the same region (Dixon et al., 2011; Geurden et al., 2006; Uehlinger et al., 2011). Consistent with the previous findings (Dixon et al., 2011; Kváč et al., 2006; O'Handley and Olson, 2006), both *C. parvum* and *G. duodenalis* assemblage A were detected in higher proportions of dairy than beef herds. Factors that might contribute to differences between dairy and beef calves could relate to variations in environmental and/or host factors. A high

prevalence of *C. parvum* in neonates is likely linked to the immature immune status of young animals (Fayer et al., 2007). Therefore, the transfer of passive immunity is likely a major host factor preventing infection at a young age (Duranti et al., 2009; Godden, 2008; O'Handley, 2007). Beef calves usually have immediate and ready access to colostrum, because they are usually kept with their dam for a longer time than dairy calves are. In addition, it has been shown that beef cows produce a significantly higher concentration of immunoglobulin in their colostrum than dairy cows (Guy et al., 1994). In addition, overcrowding (high population density) in dairy calf production systems is an important environmental factor, which can increase infection rates of *Cryptosporidium* and *Giardia* in dairy calves (McAllister et al., 2005). Overcrowding causes stress that reduces immunity in animals (Proudfoot et al., 2012; Thompson et al., 2008). Therefore, proper animal management is a key requirement for the control of cryptosporidiosis, giardiasis and other infectious diseases in cattle herds.

11. CONCLUDING REMARKS

Recent studies (Abeywardena et al., 2012, 2013a,b, 2014) have contributed to knowledge of the genetic diversity and public health significance of *Cryptosporidium* and *Giardia* from domestic bovids (*B. taurus* and *B. bubalis*) in selected geographical areas of Australasia and Sri Lanka. The findings from these studies highlight the need for expanded research into the epidemiology of *Cryptosporidium* and *Giardia*.

The detection of zoonotic species/genotypes of *Cryptosporidium* and/or *Giardia* from domestic bovids (*B. taurus* and *B. bubalis*) reinforces the importance of studying their prevalence and transmission dynamics in different regions of the world. Although *Cryptosporidium* and *Giardia* have been detected in bovids in many studies, their fate and transport in the environment and water are not well documented or understood. Therefore, future research might focus on investigating the fate of oocysts or cysts of *Cryptosporidium* and *Giardia* released by bovids and other animals into the environment. In addition, methods that can determine oocyst and cyst viability and infectivity should be developed. Advanced molecular tools (cf. Jex and Gasser, 2010; Koehler et al., 2014a; Ryan and Xiao, 2014; Xiao and Fayer, 2008) could be used for the detailed genetic characterisation of *Cryptosporidium* and *Giardia* in clinical and environmental samples. Studies might also focus on cryptosporidiosis and giardiasis in various livestock and wild

animals, to assess their significance as reservoirs for transmission to humans. There is also a need to expand future research to less privileged countries, where cryptosporidiosis and giardiasis are particularly prevalent in human populations. Many people in rural communities in Asian countries keep cattle, water buffaloes and/or other livestock animals as a source of income. However, very little is known about the zoonotic transmission in such countries. Therefore, molecular studies might focus on understanding the epidemiology of zoonotic infections in regions where animals live in close proximity to humans. In the future, we expect to see a substantial expansion in the use of genome-wide sequencing (e.g. Jex et al., 2011b, 2013; Jex and Gasser, 2014) for the characterisation of *Cryptosporidium* and *Giardia* isolates as well as the definition of a broad range of genetic markers for use in diagnostic and analytical tools. Such tools could offer unique opportunities to address questions regarding the complex network of epidemiological and biological factors involved in the interactions among these protists, their hosts and the environment. The integrated use of advanced genomic and bioinformatic tools will also be crucial to underpin studies of the systems biology of these protists on an unprecedented scale, and might also provide prospects for the development of new interventions.

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