Parasitol Res (2007) 101:1169–1182
DOI 10.1007/s00436-007-0598-4

REVIEW

Giardia and *Cryptosporidium* and public health: the epidemiological scenario from the Italian perspective

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Received: 28 April 2007 / Accepted: 23 May 2007 / Published online: 26 June 2007

Abstract *Giardia* and *Cryptosporidium* spp. are protozoa that cause human and animal disease worldwide and often exhibit zoonotic transmission. This review gives ample information concerning the epidemiology of these parasites in Italy, i.e. prevalence data in humans, farm and pet animals, shellfish and aquatic environment. Moreover, it reports genotyping results obtained from different isolates, with particular emphasis on the spread of host-specific and zoonotic species/genotypes of various origin, and on molecular data that make the Italian situation different from that of other countries. Finally, possible explanations are given for the infrequent reports of *Giardia* and *Cryptosporidium* spp. outbreaks, despite widespread faecal contamination by these parasites.

This work was supported by a research grant for Research of Relevant National Interest (PRIN 2005–2007) from the Ministry of the University and Research, Italy.

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Introduction

Giardia and *Cryptosporidium* are well-known food- and waterborne protozoa affecting humans and a wide range of domestic and wild animals. They are significant etiological agents of intestinal disorders, mainly in immunocompromised subjects. Over the last few decades, the scientific community has focussed much attention on *Giardia* and *Cryptosporidium* to understand how they spread in humans, animals and the environment and to figure out whether infected animals can serve as reservoirs of infection for humans. This is especially important because of the latest molecular studies which state that mainly *Giardia duodenalis* Assemblage A and *Cryptosporidium parvum* have potential zoonotic relevance due to their wider host range (Fayer 2004; Thompson and Monis 2004; Xiao and Ryan 2004). Hence, mapping and understanding the worldwide epidemiology of these protozoa—from prevalence in humans, animals and the environment to molecular genotyping of isolates—is an urgent task for the scientific community.

Italy (long. 6–18°E; lat. 47–35°N) is a peninsula in Southern Europe covering 301,302 km^2 and surrounded on three sides by the Mediterranean Sea. The country has 58,751,711 inhabitants and the fifth highest population density in Europe. The largest farm animal population is represented by poultry (171 million), followed by pigs (9.3 million), sheep (6.8 million), cattle (6.3 million), goats (1 million), buffaloes (250,000) and horses (200,000). Pet animals include 6.6 million cats and 5.8 million privately owned dogs (ISTAT 2005).

In recent years, Italian researchers have contributed greatly to knowledge about giardiosis and cryptosporidiosis in the country, and most debated topics have been dealt with. Therefore the present review reports the prevalence

studies carried out in humans, animals and groundwater, including the molecular background when available, to give a homogeneous picture of the epidemiology of *Giardia* and *Cryptosporidium* in a national framework. It also presents the diagnostic tools and clinical aspects. Finally, there is a discussion of the potential impact on public health in comparison with other countries, and the main objectives to be pursued in the future are set out.

Human infection

Human giardiosis and cryptosporidiosis are not notifiable diseases in Italy, and prevalence data are based on specialised studies, which suggest that *G. duodenalis* and *Cryptosporidium* spp. are common protozoa in humans (Table 1).

The first indexed reports of human giardiosis in Italy date back to the 1960s (Pucci et al. 1967; De Riu et al. 1967), but only later were larger-scale studies carried out, showing that *Giardia* clinically affects 3.2% of adult patients (Libanore et al. 1991) and 0.9% (Caprioli et al. 1996) to 4.7% of children (Cevenini et al. 1985; Scotti et al. 1996).

Later on, a significant decline in prevalence from 4.66 to 0.94% and from 10.99 to 2.41%, independent of age, was registered in a 4-year survey (1994–1998) of hospitalised and non-hospitalised subjects (Ballone et al. 2001), and the latter prevalence was recently confirmed by Capelli et al. (2003) and Crotti et al. (2005).

Subjects at risk include residents in psychiatric institutions and also Human Immunodeficient Virus (HIV) patients. In mental patients, *G. lamblia* (syn. *G. duodenalis*) was detected in about 2.5% of the population in two different studies made in Central (Giacometti et al. 1997) and Northern Italy (Gatti et al. 2000). Although *Giardia* is not considered a common opportunistic pathogen in immunocompromised patients, the prevalence of giardiosis in HIV-infected people, before the introduction of the Highly Active Anti-Retroviral Therapy (HAART), ranged from 3.47 to 6.15% (Angarano et al. 1997; Brandonisio et al. 1999; Giacometti et al. 2000).

Imported giardiosis is also documented in Italy, and an outbreak of *G. lamblia* infection associated with *Entamoeba histolytica* was identified in travellers returning from the tropics (Phuket, Thailand; de Lalla et al. 1992).

From a clinical point of view it has been shown that besides diarrhoea, other unexpected symptoms can be detected in patients affected by *Giardia*. In adult subjects, giardiosis can be associated with chronic atrophic gastritis, intestinal metaplasia of the gastric mucosa and *Helicobacter pylori* infection (Doglioni et al. 1992), and structural alterations of the retinal pigment epithelium were found to be quite common in paediatric patients with current or past giardiosis (Corsi et al. 1998). Finally, the emerging role of intestinal protozoa in the pathogenesis of Irritable Bowel Syndrome (IBS; Stark et al. 2007) was also confirmed in Italian patients (D'Anchino et al. 2002). In particular, 6.5% of patients with IBS and dyspepsia presented *Giardia* infection (Grazioli et al. 2006).

The prevalence of cryptosporidiosis in immunocompetent patients, independent of age, was usually lower than giardiosis and ranged from 1.27% (Moretti et al. 1988) to 1.7% (Brandonisio et al. 1996). A low percentage of cryptosporidiosis (0.84%) was also found in subjects living in a mental institution (Giacometti et al. 1997). The high prevalence (5.4%) recently registered in immunocompetent diarrheic patients in the Tuscany Region (Central Italy) needs to be confirmed because of the conflicting results obtained from microscopy and molecular data (Magi et al. 2006).

A high prevalence of cryptosporidiosis was found in HIV+ patients, before the introduction of the HAART. In fact, a prevalence of 8.33–11% was registered throughout the Italian Regions (reviewed by Brandonisio et al. 1999); it is interesting that in Southern Italy 21% of HIV+ subjects with diarrhoea were infected, with a higher incidence in the rainy seasons (Brandonisio et al. 1999).

Evidence of high *Cryptosporidium* infection rates in Italy derives indirectly from serological investigations. In Central Italy, 5.3% of healthy people presented antibodies against an oocyst soluble antigen (Gomez Morales et al. 1992); however, these data do not correspond with those obtained in Northern Italy, where an IgG serological response was registered in 62–83% of blood donors, depending on the antigen used (Frost et al. 2000).

Table 1 Range of prevalence (in percentages) of *G. duodenalis* and *Cryptosporidium* in different host species in Italy

Host	*Giardia*	*Cryptosporidium*
Humans	0.9–6.15	0.84–11
Dogs	3.6–80	3.3
Cats	5.6–15.8	–
Cattle	66.6[a]	58.3[a]
Sheep	1.5	–
Water	30[a]	24.4[a]
buffaloes	18.1	14.7
Horse	13	8
Pigs	–	10.3–19.8
Poultry	–	26
Fallow deer	11.5	–

[a] Prevalence on farms

Animal infection

Giardiosis and cryptosporidiosis in animals have not received as much attention in Italy as other endoparasitoses.

Although data on these protozoa, as for human infections, date back to the 1960s, it is only recently that parasitologists and veterinary clinicians have used more reliable epidemiological and diagnostic methodology to reach a better understanding of the spread of these parasites. Table 1 summarises the prevalence in different animal host species.

Companion animals After the first report on the presence of *Giardia* by De Carneri and Castellino (1964) in 3 out of 31 dogs subjected to postmortem examination from a public animal shelter, surveys carried out subsequently showed that giardiosis prevalence ranges from 3.6 to 9.56% (Agresti et al. 1977; Piergili-Fioretti and Moretti 1989; Rosso et al. 1989) and can reach 80% when puppies and/or imported dogs are studied (Traldi and Castiglioni 1993). Since 2000, more statistically significant epidemiological surveys involving privately owned dogs and animal shelter dogs have been carried out and risk factors have been analyzed. Shelter animals constantly presented higher levels of *Giardia* infection (14–74%) compared to pet dogs (4.3–19%), and young animals and subjects with diarrhoea were significantly more affected (Capelli et al. 2003; Bianciardi et al. 2004; Berrilli et al. 2006a; Papini et al. 2005; Paoletti et al. 2006).

As to *Cryptosporidium*, a clinical case was reported in 1990 in a 6-year-old Pyrennean Mountain dog, whose infection was confirmed by experimental challenge in newborn mice (Traldi 1990). However, no surveys were carried out until very recently when a molecular epidemiological study revealed the presence of *Cryptosporidium* spp. in 3.3% of animal shelter and privately owned dogs; here again, shelter dogs and dogs with gastrointestinal symptoms were significantly more affected (Giangaspero et al. 2006).

Regarding *Giardia* infection in cats, De Carneri and Castellino (1963) found the parasite for the first time in fresh intestinal smears from 5.6% of 90 autopsied stray cats. The prevalence of 4% reported by Bianciardi et al. (2004) appears to be underestimated; when larger cat populations are investigated, giardiosis prevalence reaches 15% (Canestri-Trotti et al. 1990; Papini et al. 2007b) and no risk factor seems to influence such infection (Papini et al. 2007b).

No correlation between infection and immunological status in feline immunodeficiency virus (FIV)-positive cats—except for the amount of cysts excreted—was evidenced by Diaferia et al. (2006); however, such unexpected data require more extensive published reports to draw conclusions. Finally, no data are available for cryptosporidiosis in the feline population.

Farm animals Cryptosporidiosis (Canestri-Trotti et al. 1982; Genchi et al. 1984) and giardiosis (Lalle et al. 2005) affect Italian cattle, although wide prevalence studies are only related to cattle farms; 66.6% of the Italian farms examined harbour *Giardia* and 58.3% *Cryptosporidium*, and in most cases both protozoans were present (Grana et al. 2006).

Giardiosis does not seem to be a serious problem in a flock of sheep, where a prevalence of only 1.5% was found in adults (Giangaspero et al. 2005b), but it has been the cause of a malabsorption syndrome, decreased weight gain and impaired feed efficiency in lambs from the same area (Central Italy; Aloiso et al. 2006).

Out of 150 surveyed horses, *Giardia* has recently been detected in 13% and *Cryptosporidium* in 8% (Veronesi et al. 2006).

It has been known since the 1980s that water buffaloes harbour *Cryptosporidium* (Canestri-Trotti and Quesada 1983; Canestri-Trotti et al. 1984b), and this was confirmed later (Galiero et al. 1994; Saralli et al. 2001). However, an interesting survey has been published very recently in which *Giardia* infection has also been reported for the first time. Copro-antigens of *G. duodenalis* and *C. parvum* occurred in 30 and 24.4% of farms and in 18.1 and 14.7% of animals, respectively. Co-infection was present but animals rarely presented diarrhoea (Rinaldi et al. 2006). Finally, in the past, *Cryptosporidium* has been registered in pigs (Canestri-Trotti et al. 1984a) and also recently (Manfredi et al. 2006) with prevalence levels of up to 19.8%.

Poultry Only one survey is available for poultry in which cryptosporidiosis was detected, with a prevalence of 26% among roosters and broilers bred in Central Italy and affected by gastro-intestinal symptoms (Piergili-Fioretti et al. 1991). This research came 20 years after a first contribution on the presence of *Cryptosporidium* in the same species (Mandelli and Valeri 1972).

Wild animals In a survey involving 139 hunted fallow deer (*Dama dama*), 16 (11.5%) subjects where found to excrete *Giardia* cysts (Lalle et al. 2007).

Water contamination

Drinking, raw and reclaimed water are not routinely monitored for *Cryptosporidium* and *Giardia* in Italy; however, studies on the presence of oo/cysts in different kinds of water and wastewater have been recently carried out throughout Italy, and the results are shown in Tables 2, 3 and 4.

Surface water All river water samples appeared to be heavily contaminated because 100% of examined samples tested positive for *Giardia* cysts and *Cryptosporidium* oocysts, with the *Giardia* load higher than that of *Cryptosporidium* (Carraro et al. 2000; Briancesco and Bonadonna 2005; Di Benedetto et al. 2005).

Table 2 *Giardia* and *Cryptosporidium* in surface water in Italy

Region	Years	Type of water	Number of samples	*Giardia*		*Cryptosporidium*		References
				Positive samples (%)	Mean cyst N/L±SD	Positive samples (%)	Mean oocyst N/L±SD	
Piedmont	1997	River	22	22 (100)	1.16±0.78	22 (100)	0.19±0.08	Carraro et al. 2000
Lazio	2001–2002	River	10	10 (100)	80±90	10 (100)	5±4	Briancesco and Bonadonna 2005
Sicily	2003–2004	River	7	7 (100)	465±413	7 (100)	9.86±6.79	Di Benedetto et al. 2005
Sicily	2003–2004	Watersheds	10	1 (10)	0.12±0.36	1 (10)	0.23±0.73	Di Benedetto et al. 2005
Lazio	2001–2002	Watersheds	4	2 (50)	0.006±0.009	0	0	Briancesco and Bonadonna 2005
Apulia	2000–2001	Watersheds	27	0	0	4 (14.8)	0.13±0.07	Brandonisio et al. 2004
Apulia	2001–2002	Watercourse	7	3 (42.9)	0.01±0.02	0	0	Briancesco and Bonadonna 2005
Tuscany	2003–2004	Watercourse	16	14 (87.5)	2.05	10 (62.5)	0.19	Sacco et al. 2006
Lazio	2001–2002	Lakes	3	1 (33.3)	0.28	N.I.	N.I.	Di Cave et al. 2005

N/L number of oo/cysts per liter; *N.I.* Not investigated

In watersheds and watercourses used as source water by the local Municipal Water Companies, *Giardia* cysts were found in 31% of samples while *Cryptosporidium* oocysts were present in 23% (Brandonisio et al. 2004; Briancesco and Bonadonna 2005; Di Benedetto et al. 2005; Sacco et al. 2006).

Ground water Oo/cysts were occasionally found in well water used for agricultural purposes (Briancesco and Bonadonna 2005; Di Benedetto et al. 2005; Lonigro et al. 2006) and especially in wells with a depth of less than 31 m, where faecal bacteria were also found (Di Benedetto et al. 2005).

Drinking and recreational water Neither of the parasites was detected in any of 19 drinking water samples examined (Briancesco and Bonadonna 2005); however, *Cryptosporidium* and/or *Giardia* were occasionally detected in swimming pool water (Bonadonna et al. 2004; Briancesco and Bonadonna 2005; Oliveri et al. 2006).

Wastewater In raw sewage and primary effluent, more *Giardia* cysts were found (up to 108,000/l) than *Cryptosporidium* oocysts (Carraro et al. 2000; Cacciò et al. 2003; Briancesco and Bonadonna 2005; Di Benedetto et al. 2005).

High concentrations of *Giardia* cysts, and to a lesser extent of *Cryptosporidium* oocysts, were also present after secondary treatment of wastewater by activated sludge and sedimentation (Bonadonna et al. 2002; Di Benedetto et al. 2005; Lonigro et al. 2006), while it has been shown that tertiary treatments significantly reduce cyst and oocyst density in wastewater (Brandonisio et al. 2000; Carraro et al. 2000). Final disinfection with chlorine has little effect

Table 3 *Giardia* and *Cryptosporidium* in groundwater in Italy

Region	Years	Number of samples	*Giardia*		*Cryptosporidium*		References
			Positive samples (%)	Mean cyst N/L±SD	Positive samples (%)	Mean oocyst N/L	
Lazio	2001–2002	14	0	0	0	0	Briancesco and Bonadonna 2005
Apulia	2004–2005	18	2 (11.1)	0.28±0.31	0	0	Lonigro et al. 2006
Sicily	2003–2004	14	2 (14.3)	0.33±0.25	1 (7.1)	1.5	Di Benedetto et al. 2005

Table 4 *Giardia* cysts and *Cryptosporidium* oocysts in wastewater in Italy

Region	Type of wastewater	Number of samples	*Giardia*		*Cryptosporidium*		References
			Positive samples (%)	Mean/Range cyst N/L	Positive samples (%)	Mean/Range oocyst N/L	
Piedmont	Raw sewage	3	3 (100)	54	3 (100)	4.5	Carraro et al. 2000
Lombardy Campania Sardinia Sicily	Raw sewage	16	16 (100)	2,100–42,000	3 (18.75)	107	Cacciò et al. 2003
Lazio	Raw sewage	10	10 (100)	800–7,000	10 (100)	0.4–30	Briancesco and Bonadonna 2005
Lazio	Raw sewage	5	5 (100)	3,500–34,000	N.I.	N.I.	Di Cave et al. 2005
Sicily	Primary effluent	8	8 (100)	108,000	8 (100)	702	Di Benedetto et al. 2005
Sicily	Secondary effluent[a]	8	8 (100)	6,160	7 (87.5)	56.9	Di Benedetto et al. 2005
Apulia	Secondary effluent[a]	4	4 (100)	1,800	2 (50)	56	Lonigro et al. 2006
Apulia	Tertiary effluent[b]	4	4 (100)	253	4 (100)	0.26	Brandonisio et al. 2000
Apulia	Tertiary effluent[c]	4	4 (100)	6.65	4 (100)	0.23	Brandonisio et al. 2000
Piedmont	Tertiary effluent[d]	11	11 (100)	1.4	11 (100)	0.21	Carraro et al. 2000
Apulia	Chlorinated effluent	14	11 (78.6)	37.5	3 (21.4)	0.29	Brandonisio et al. 2004
Lazio	Chlorinated effluent	6	6 (100)	60	6 (100)	30	Briancesco and Bonadonna 2005
Lazio	Chlorinated effluent	2	2 (100)	31–670	N.I	N.I.	Di Cave et al. 2005
Apulia	Chlorinated effluent	11	9 (81.82)	98.85	4 (36.4)	6.99	Brandonisio et al. 2007
Lazio	UV-treated effluents	3	3 (100)	72.33	N.I	N.I.	Di Cave et al. 2005
Apulia	UV-treated effluent	10	7 (70)	37.8	1 (10)	2.67	Brandonisio et al. 2007

N.I. Not investigated
[a] Activated sludge
[b] Chemical flocculation
[c] Chemical flocculation followed by slow sand filtration
[d] Chemical dephosphorization and multilayer filtration

on oo/cyst density (Brandonisio et al. 2004; Briancesco and Bonadonna 2005), and chlorine and UV treatments may not influence *Giardia* cyst viability (Brandonisio et al. 2007).

Food contamination

In a historical work performed in 1966, samples of *Lactuca sativa* sold in local markets yielded *Giardia* cysts (Mastandrea and Micarelli 1968). Almost 50 years later, studies on food have switched to edible shellfish harvested in the sea, lagoons and lakes; *Giardia* was detected only in *Chamelea gallina* (Molini et al. 2004), whereas *Cryptosporidium* oocysts were detected in *C. gallina* and *Ruditapes philippinarum* (Giangaspero et al. 2005a; Molini et al. 2007). Contamination by both parasites was also found in *Mytilus galloprovincialis* (Sorgi et al. 2007).

Molecular findings

The occurrence and identification of *Giardia* and *Cryptosporidium* isolates in Italy from different sample types are reported in Tables 5, 6, 7 and 8.

Table 5 Occurrence and identification of *Giardia* isolates in Italy from different origins

Origin	Number of isolates	PCR target	Genotypes (N)	References
Human	30	β-giardin	Ass A (24), Ass B (6)	Cacciò et al. 2002b
Human	37	β-giardin	Ass A (17), Ass B (15), Ass (A+B) 5	Lalle et al. 2005
Human	11	β-giardin	Ass A (5), Ass B (5), Ass (A+B) 1	Crotti et al. 2005
Human	42	18S	Ass A (19), Ass B (13), Ass (A+B) 10	Berrilli et al. 2006c
Companion animals				
Dog	17	18S	Ass A (2), Ass C (11), Ass D (1), Ass (A+C) 2 Ass (C+D) 1	Berrilli et al. 2004
Dog	21	β-giardin 18S GDH	Ass A (6), Ass C (1), Ass D (13), Ass (A+D) 1	Lalle et al. 2005
Dog	30	β-giardin 18S	Ass A (2), Ass C (3), Ass D (25)	Paoletti et al. 2006
Dog	26	18S	Ass A (8), Ass C (14), Ass D (4)	Berrilli et al. 2006a
Cat	1	18S	Ass A (1)	Berrilli et al. 2004
Cat	1	β-giardin GDH	Ass F (1)	Lalle et al. 2005
Cat	10	18S	Ass A (10)	Papini et al. 2007a
Cat	8	Tpi	Ass A (8)	Diaferia et al. 2006
Rabbit	2	18S	Ass A (1), Ass (A+B) 1	Berrilli et al. 2006a
Farm Animals				
Calf	3	18S	Ass E (3)	Berrilli et al. 2004
Calf	24	β-giardin	Ass A (12), Ass B (5), Ass E (3), Ass (A+B) 2 Ass (A+E) 2	Lalle et al. 2005
Calf	18	Tpi β-giardin	Ass A (7), Ass E (11)	Grana et al. 2006
Sheep	5	β-giardin GDH	Ass A (5)	Giangaspero et al. 2005b
Sheep	2	Tpi	Ass B (2)	Aloisio et al. 2006
Wild Animals				
Fallow deer	8	Tpi β-giardin	Ass A (8)	Lalle et al. 2007
Water				
Wastewater	16	β-giardin	Ass A (8), Ass (A+B) 8	Cacciò et al. 2003
Wastewater	10	18S	Ass A (10)	Di Cave et al. 2005
Wastewater	6	18S	Ass A (5), Ass B (1)	Lonigro et al. 2006
Wastewater	14	18S Tpi	Ass A (10), Ass B (1) Ass A or B (3)[a]	Brandonisio et al. 2007
Surface water	3	18S	Ass A (3)	Di Cave et al. 2005
Watercourses	7	18S Tpi	Ass A (6), Ass B (1)	Brandonisio et al. 2007

[a] Incoherent results given by using two different loci

Humans One hundred and twenty different *Giardia* DNA samples from human stools have been genotyped, 54.16% displayed *Giardia* Assemblage A, 32.5% Assemblage B and 13% harbour mixed Assemblage A/B. *Giardia* intra-Assemblage variations have also been detected in Italy for both Assemblages, within Assemblage A, seven subgenotypes (referred to as 'A1–A7') were identified and three subgenotypes (B1, B3 and B4) within Assemblage B (Cacciò et al. 2002b; Lalle et al. 2005; Crotti et al. 2005). Out of ten genotyped *Cryptosporidium* isolates, nine isolates were identified as *C. parvum*, and in one case, *C. felis* was detected (Cacciò et al. 2000, 2001, 2002a).

Companion animals Ninety-four *Giardia* isolates from dogs have been molecularly identified, 76.5% belonged to species-specific Assemblages (C and D) while 19% belonged to Assemblage A and 4% to mixed Assemblages. Twenty isolates have been characterised from cats in Northern and Central Italy and all belonged to Assemblage A (Berrilli et al. 2004; Papini et al. 2007a; Diaferia et al. 2006) except for one belonging to Assemblage F (Lalle et al. 2005). Two isolates from rabbits harboured Assemblage A and mixed Assemblages (A + B; Berrilli et al. 2006a).

As to *Cryptosporidium*, nine isolates have been genotyped from dogs and one from hamsters, *C. canis* was detected in dogs while, more notably, both animal species harboured *C. parvum* (Giangaspero et al. 2006; Cencioni 2005; Berrilli et al. 2006a).

Farm animals *Giardia* and *Cryptosporidium* genotypes/species have been detected in cattle, sheep, goats and pigs for a total of 52 and 68 isolates typed, respectively.

Table 6 *Giardia* genotypes from different sample types in Italy

Origin	Total samples	Ass A (%)	Ass B (%)	Ass A or B[a] (%)	Ass C (%)	Ass D (%)	Ass E (%)	Ass F (%)	Mixed assemblage (%)
Human	120	65 (54.2)	39 (32.5)		–	–	–	–	(A + B) 16 (13,3)
Dog	94	18 (19.1)	–		29 (31.0)	43 (45.7)	–	–	(A + C) 2 (C + D) 1 (A + D) 1 (4.2)
Cat	20	19 (95.5)	–		–	–	–	1 (0.5)	–
Rabbit	2	1 (50.0)	–		–	–	–	–	(A + B) 1 (50.0)
Calf	45	19 (42.2)	5 (11.1)		–	–	17 (37.8)	–	(A + B) 2 (A + E) 2 (8.9)
Sheep	7	5 (71.4)	2 (28.6)		–	–	–	–	–
Fallow deer	8	8 (100)	–		–	–	–	–	–
Water	56	42 (75.0)	3 (5.35)	3 (5.35)	–	–	–	–	(A + B) 8 (14.2)
TOT	352	177 (50.3)	49 (13.9)	3 (0.8)	29 (8.2)	43 (12.2)	17 (4.8)	1 (0.3)	33 (9.3)

[a] Incoherent results given by using two different loci

Forty-five *Giardia* isolates from cattle were mainly identified as Assemblage A (42%) followed by the species-specific Assemblage E (38%), Assemblage B (11%) and mixed Assemblages (9%; Berrilli et al. 2004; Lalle et al. 2005; Grana et al. 2006); in these isolates, a number of subgenotype (A1, A2, A3, A4, B3 and the new subgenotypes B5 and B6) were identified (Lalle et al. 2005).

Seven isolates have been typed in sheep, and Assemblages A1 and B have been detected (Giangaspero et al. 2005b; Aloisio et al. 2006).

As regards *Cryptosporidium*, only *C. parvum* isolates were identified in all farm animals (Cacciò et al. 2000, 2001; Cencioni 2005; Grana et al. 2006).

Finally, *C. suis* and the pig genotype II were identified in 12 faecal pools from pigs (Manfredi et al. 2006).

Wild animals Eight faecal samples collected from fallow deer were all identified as a new unique subtype within Assemblage A (Lalle et al. 2007).

Water and wastewater Molecular typing of *Giardia* and *Cryptosporidium* in Italy was performed in wastewater (Cacciò et al. 2003; Di Cave et al. 2005; Lonigro et al. 2006) and in surface water (Di Cave et al. 2005). Although this referred to a limited number of *Giardia* isolates (57), most (up to 74%) belonged to Assemblage A, while

Table 7 Occurrence and identification of *Cryptosporidium* isolates from different origins in Italy

Origin	Number of isolates	PCR target	Species/Genotypes (N)	References
Human	9	Microsatellite	*C. parvum* (9)	Cacciò et al. 2000, 2001
Human	1	SSUrRNA COWP	*C. felis* (1)	Cacciò et al. 2002a
Dog	1	COWP	*C.canis* (1)	Cencioni 2005
Dog	8	COWP	*C.canis* (1) *C.parvum* (7)	Giangaspero et al. 2006
Hamster	1	COWP	*C.parvum* (1)	Berrilli et al. 2006a
Calf	29	Microsatellite	*C.parvum* (29)	Cacciò et al. 2000, 2001
Calf	3	COWP	*C.parvum* (3)	Cencioni 2005
Calf	15	COWP 18S rDNA	*C.parvum* (15)	Grana et al. 2006
Kid	11	Microsatellite	*C.parvum* (11)	Cacciò et al. 2000, 2001
Lamb	10	Microsatellite	*C.parvum* (10)	Cacciò et al. 2000, 2001
Pig	12	18S rDNA	*C. suis* (8) pig genotype II (4)	Manfredi et al. 2006
Shellfish (*C. gallina*)	2[a]	COWP	*C.parvum* (2)	Traversa et al. 2004; Giangaspero et al. 2005a
Shellfish (*R.philippinarum*)	7[a]	COWP	*C.parvum* (6) *C.hominis* (1)	Molini et al. 2007
Wastewater	2	COWP	*C.parvum* (2)	Lonigro et al. 2006
Watercourses	1	COWP	*C.parvum* (1)	Brandonisio et al. 2007

[a] The number refers to pooled samples.

Table 8 *Cryptosporidium* species/genotypes of different origins in Italy

Origin	Total samples	*C. parvum* (%)	*C. hominis* (%)	*C. felis* (%)	*C.canis* (%)	*C. suis* (%)	Pig genotype II (%)
Human	10	9 (90.0)	–	1 (10.0)		–	–
Dog	9	7 (77.8)	–	–	2 (22.2)	–	–
Hamster	1	1 (100)	–	–	–	–	–
Calf	47	47 (100)	–	–	–	–	–
Kid	11	11 (100)	–	–	–	–	–
Lamb	10	10 (100)	–	–	–	–	–
Pig	12	–	–	–	–	8 (66.7)	4 (33.3)
Shellfish	9[a]	8(88.9)	1 (11.1)	–	–	–	–
Water	3	3 (100)	–	–	–	–	–
TOT	112	96 (85.7)	1 (0.8)	1 (0.8)	2 (1.7)	8 (7.1)	4 (3.5)

[a] The number refers to pooled samples.

Assemblage B (7%) was poorly represented and mixed Assemblages (A + B) were detected in 16%. Interestingly, incoherent results were obtained in three cases when two different loci targets were used (Brandonisio et al. 2007). Two isolates of *Cryptosporidium* from secondary-treated wastewater (Lonigro et al. 2006) and one from a watercourse were identified as *C. parvum* (Brandonisio et al. 2007).

Food Sequencing analysis was carried out on samples from *C. gallina* and *R. philippinarum* clams harvested along the Adriatic coast; *C. parvum* was found in both clam species, and *C. hominis* was also detected in *R. philippinarum* (Giangaspero et al. 2005a; Molini et al. 2007).

Discussion

Data on the prevalence of giardiosis and cryptosporidiosis in humans, animals and the environment vary greatly in Italy according to geographical location, the population studied and diagnostic methods used.

Well-standardised procedures are available for carrying out laboratory diagnosis of human giardiosis/cryptosporidiosis (http://www.dpd.cdc.gov/dpdx/HTML/Giardiasis.htm; http://www.dpd.cdc.gov/dpdx/HTML/Cryptosporidiosis.htm; Cacciò and Pozio 2006; Savioli et al. 2006), but special acid-fast staining or immunodiagnostic techniques are needed for *Cryptosporidium* in particular. Because of its greater sensitivity and specificity, immunofluorescence microscopy is the method of choice in the detection of this coccidian parasite, followed closely by enzyme immunoassays (Savioli et al. 2006). Unfortunately, these methods are not routinely performed in parasitological stool examination, so that *Cryptosporidium* and other intestinal coccidians may often be missed in humans.

In animals, most prevalence investigations in the Italian arena have principally been based on the detection of oo/cysts by light microscopy followed by immunofluorescence or enzyme-linked immunosorbent assay (ELISA) tests; however, the application of immunoassays in animals for epidemiological survey is still a matter for debate. This due to the fact that the test has not yet been approved for use in veterinary medicine, and, more importantly, the need to confirm positive ELISA results by (repeated) faecal examination using other detection methods has been demonstrated (Cirak and Bauer 2004).

Techniques based on DNA detection such as polymerase chain reaction (PCR) assays are too expensive for prevalence studies in humans and animals and thus are not widely applied in Italy. Different methods have also been used to identify protozoa in water—especially in wastewater—and consequently, the results are often difficult to compare. In Italy, methods suggested by the National Institute of Health (Annali ISTISAN 2003) are mainly used for detecting *Giardia* and *Cryptosporidium* in raw and drinking water (concentration by filtration capsules or a compressed foam filter system, followed by purification by Percoll-sucrose or immunomagnetic separation and identification by immunofluorescent antibodies). Different methods were used for wastewater because there are no well-standardised protocols available.

While the prevalence of human giardiosis and cryptosporidiosis evaluated by stool examination overlaps that of other industrialised countries (de Wit et al. 2001; Ali and Hill 2003; Horman et al. 2004; Fayer 2004; Joachim 2004), the seroprevalence of cryptosporidiosis seems to be unexpectely high in asymptomatic subjects, at least in a geographically limited area of Northern Italy (Frost et al. 2000) when compared to other countries (Frost et al. 2004; Fayer et al. 2004). If confirmed on larger subject numbers, this serological condition may suggest a lower risk of symptomatic cryptosporidiosis in that area, because of recent seroepidemiological studies showing that people with high anti-*Cryptosporidium* antibody levels have less risk of diarrhoeal or gastrointestinal illness (Frost et al. 2005a; Frost et al. 2005b).

In recent years, HAART has drastically reduced the prevalence of cryptosporidiosis in HIV+ patients in Italy (Conti et al. 2000; Manfredi 2000; Maggi et al. 2001), also due to direct effects on *Cryptosporidium* of aspartyl protease inhibitors of the HIV, which interfere with the life cycle of the parasite (Mele et al. 2003). However, the presence of such infections must be monitored in HIV+ patients because relapses have been recorded in patients who interrupted the combined anti-retroviral therapy (Maggi et al. 2000).

No epidemiological surveys are available for immigrant populations; however, this may allow for some considerations. Immigration is on the increase in Italy, an impressive new phenomenon, and most immigrants arrive from Eastern European Countries and Albania *in primis* (ISTAT 2005). From an epidemiological point of view, this sociological event may facilitate the spread of giardiosis, considering that *G. duodenalis* prevalence has recently been found to be as high as 12.27% in healthy (Spinelli et al. 2006) and symptomatic (Berrilli et al. 2006b) Albanians. In addition, foreign travel is confirmed as a risk factor for giardiosis (Faustini et al. 2006).

Among animals, dogs are the most studied species for giardiosis in Italy and prevalence appears to be of concern mainly in dogs that are well cared for (up to 19%) compared to other European countries (Barr and Bowman 1994; Borkovcova 2003).

Interesting data are emerging from cats because the high prevalence level (15.8%) registered in Italy confirms that cats are not poor or inadequate hosts nor limited reservoirs as previously reported (Hill et al. 2000; McGlade et al. 2003; Vasilopulos et al. 2006).

Besides the confirmed risk factors of young age and confinement, diarrhoea and *Giardia* seems to be closely associated in dogs. In addition, the possibility that dogs fed on commercial wet food are more susceptible to *Giardia* infection (Papini et al. 2005) is an intriguing point for future investigation.

Thanks to more incisive information, it seems that in the last few years greater attention has been paid by both Italian veterinary practitioners and owners to screening and treating puppies for giardiosis; this is also due to the availability of safe and effective treatment (Giangaspero et al. 2002). The Italian epidemiological data regarding *Cryptosporidium* prevalence are too scarce to allow a conclusion about pets and farm animals, and clinicians in the country display no such awareness. Further limits are created by the difficulty of detecting *Cryptosporidium* in clinical laboratory conditions and the lack of new and effective molecules for control (Benbow et al. 1998; Yu and Choi 2000).

As regards environmental dispersion of these parasites, although wastewater, surface, ground and recreational water were contaminated by oo/cysts throughout Italy, drinking water was not. However, *Giardia* and *Cryptosporidium* testing is not routinely performed in drinking water, raw water and reclaimed water, in accordance with the European Directive 91/271/CEE on the treatment of municipal wastewater, Italian Law (Decree 152/1999) and the European Directive 98/83/CE on the quality of water intended for human consumption. Although a high concentration of *Giardia* has been detected in water, only one outbreak of waterborne cryptosporidiosis has been reported in Italy. It occurred in January and February 1995, and involved 294 out of 1731 members of a community for the rehabilitation of drug users. The rate of clinical cryptosporidiosis was 13.6% among HIV negative subjects and 30.7% among HIV positive subjects. *Cryptosporidium* oocysts were found in the sandy sediment collected from the bottom of two water storage tanks serving the community (Pozio et al. 1997).

It has also been shown in Italy that disinfection of wastewater is not always sufficient to ensure good oo/cysts removal and that the removal capacity varied greatly depending on the technology used in the treatment plants. These findings suggest the need for monitoring the presence of oo/cysts, especially in wastewater for reuse, and the necessity of insisting on advanced tertiary treatment for producing reclaimed water with no negative impact on public health. In this respect, recent work carried out in Southern Italy has demonstrated that a new wastewater tertiary treatment system based on membrane ultrafiltration is useful in removing pathogenic protozoa from secondary-treated municipal wastewater without chlorine disinfection (Lonigro et al. 2006).

An important point is that contaminated faeces and rainfall-initiated runoff and wastewater carrying protozoa oo/cysts run into rivers, estuaries and coastal waters, thus contaminating sea and shellfish. The identification of *Giardia* and *Cryptosporidium* in two clam species (*R. philippinarum* and *C. gallina*; Giangaspero et al. 2005a; Molini et al. 2007) provided evidence of the high level of faecal pollution along the Italian Adriatic coast and above all in the Venice Lagoon, where contaminated shellfish were found in different seasons (Molini et al. 2007). The overall prevalence of contamination by *Giardia* and *Cryptosporidium* in harvested Italian shellfish is still unknown, and considering that (a) each shellfish may transport more than 10^3 oocysts (Gomez-Couso et al. 2003), (b) shellfish depuration processes do not totally remove *Cryptosporidium* and moreover, that (c) usual shellfish cooking methods (steaming) do not eliminate the presence and the infectivity of *C. parvum* oocysts, as demonstrated in *M. galloprovincialis* by Gomez-Couso et al. (2006), such findings are a matter for concern in Italy. Shellfish represent more than 10% of Italian fishing products (Mattei and Pellizzato 1997) and are a major food

source in the country, where large quantities are typically eaten raw or uncooked and sometimes even sold illegally.

Molecular characterization of *Giardia* and *Cryptosporidium* isolates appears important for providing accurate information about host specificity, zoonotic potential and possible routes of transmission.

Different DNA targets are usually utilised to detect *Giardia* in environmental and faecal samples (see Cacciò et al. 2005 and Smith et al. 2006), and the choice of a specific molecular typing method mainly depends on the purpose of the study. However, analysis should be carried out on the basis of PCR targets that have been used successfully in epidemiological or taxonomic studies and for which the sequences are multicentre validated.

In Italy, for molecular identification of *Giardia* cysts and *Cryptosporidium* oocysts from human/animals stools and environmental samples, most of the common loci have been used (Tables 5 and 7) with different assays; a PCR-RFLP targeting the β-giardin gene (Cacciò et al. 2002b) was developed for the rapid discrimination of Assemblages and more recently a fluorescence resonance energy transfer real-time PCR has been developed to detect and quantify *G. duodenalis* cysts (Berrilli et al. 2006c). *Cryptosporidium* oocyst wall protein (COWP) has been the most targeted gene for *Cryptosporidium* and a semi-nested PCR has been performed (Traversa et al. 2004; Giangaspero et al. 2005a) as well as mini- and microsatellites for subgenotyping (Cacciò et al. 2000, 2001).

When two loci have been used simultaneously for *Giardia* (Brandonisio et al. 2007), the contradictory results obtained open up serious discussion about the reliability of genotyping based on only one gene target.

A total of 353 *Giardia* isolates of various origin have been genotyped in Italy, and zoonotic Assemblage A seems to be the most widespread—with the highest levels in humans and livestock (50%), followed by Assemblage B (14%) found especially in humans but also in cattle, while mixed Assemblages were present in 9%.

The wider distribution in Italy of Assemblage A in humans seems to be in contrast with the results reported from other countries, where Assemblage B appears more common, as reviewed by (Cacciò et al. 2005), who have listed genotyping of 1,438 human faecal samples from 14 different countries and reported that 69% of those samples belong to Assemblage B and 26% to Assemblage A. However, although consistent dominance of Assemblage B seems to be unequivocal with regard to England and Wales—where most of the samples (1206) come from—in all other surveyed countries, such high Assemblage B prevalence appears to be strongly biased by undersampling. In fact, in Uganda, Korea and Ohio, 100% Assemblage A is reported, but the analyses were conducted on only 3, 5 and 14 isolates, respectively.

Data from Italy are based on 120 human isolates tested until now, so that data on the higher prevalence of Assemblage A over Assemblage B appear to be more significant and reliable. Considering that the greatest zoonotic risk is from Assemblage A and that Assemblage B represents a lower risk because it appears to be mainly human transmitted, we assume the existence of different transmission cycles within diverse geographical regions. In animals, both zoonotic and non-zoonotic Assemblages were found to be associated with *Giardia* infections. Interestingly, while dogs harbour more host specific Assemblages, other species such as cats and cattle harbour a greater number of zoonotic Assemblages, thus acting as a potential source of infection for humans.

Subgenotypes of *Giardia* Assemblages A and B have also been detected in humans and animals in Italy; however, the epidemiological significance of these subgenotypes is still obscure (see Hunter and Thompson 2005).

Regarding *Cryptosporidium*, 112 human, animal and environmental samples have been genotyped and the zoonotic species *C. parvum* was the most frequently detected (85.7%), mainly in livestock but also in dogs, water, wastewater and shellfish. This widespread presence of *C. parvum* poses an unquestionably high level of risk of disease for humans from these reservoirs in Italy.

Conclusion and future perspectives

Since 2002, after a worldwide shared agreement to map the epidemiological situation and the risks related to *Giardia* and *Cryptosporidium* infections, Italian research has added more information about prevalence and molecular data from different origins and areas of the country and contributed to the development of reliable molecular assays.

Data available in Italy indicate widespread faecal contamination by *Giardia* and *Cryptosporidium* from land to sea. *Giardia* and/or *Cryptosporidium* have been detected in humans, companion animals, sheep, cattle and water buffaloes, in wastewater, surface water, and also in vegetables and shellfish. Interestingly, many species of animals harbour more zoonotic species/genotypes (Assemblage A *G. duodenalis* and *C. parvum*) than species-specific ones.

It seems that in Italy the role of farm animals can be significant for human infection, due to the higher circulation of Assemblage A of *Giardia* and *C. parvum* among humans and animals, and among pet cats.

For cryptosporidiosis, analysis of outbreaks and case-control studies coupled with genotyping data in other geographical areas have confirmed the public health significance of zoonotic transmission, especially from cattle to humans through direct contact with infected cattle or transmission through drinking water (Hunter and Thompson 2005).

For *Giardia*, even if some genotypes can circulate between humans and dogs, the higher prevalence of host specific genotypes in Italy may suggest that zoonotic transmission is unlikely to occur, except in particular socio-cultural conditions such as those registered in a localised endemic focus in India (Traub et al. 2004).

In addition, although still referred to a limited number of collecting sites, the constant occurrence of zoonotic species/Assemblage in wastewaster and surface water suggests a high circulation of *Giardia* and *Cryptosporidium* in the environment and the potential risk associated with the reuse of wastewater.

In Italy, while the prevalence of sporadic human giardiosis and cryptosporidiosis is similar to other industrialised countries, only one outbreak of waterborne cryptosporidiosis has been documented. This could be because, in comparison to other countries such as England and Wales, Italy uses more water drawn from aquifers than surface water as a source for drinking water treatment plants, and another reason may be the very high consumption of mineral water (among the highest in Europe). However, the possibility that small outbreaks have not been detected because of failures in the surveillance system cannot be ruled out.

It will be possible to draw conclusions in terms of public health when more consistent data on molecular epidemiology (i.e. prevalence studies on a larger number of animal species coupled with genotyping, especially from localised transmission foci) become available in our country. In addition, the quality of current genotyping tools—mostly based on analysis of single genetic loci—should be further improved. At least two loci should be investigated to obtain the sufficiently reliable information, and one locus should be 18S ribosomal DNA for both parasites (Smith et al. 2006). A multi-locus approach to characterization of *Giardia* and *Cryptosporidium* isolates appears essential to provide a broad consensus on their taxonomic status. However, caution is necessary in describing species and intraspecific intragenotype variants or genotypes; this represents an important field for further investigation.

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