

## Current situation of *Giardia* infection in Spain: Implications for public health

David Carmena, Guillermo A Cardona, Luisa P Sánchez-Serrano

David Carmena, MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, Hammersmith Hospital Campus, London W12 0NN, United Kingdom

Guillermo A Cardona, Livestock Laboratory, Regional Government of Álava, 01520 Vitoria-Gasteiz, Spain

Luisa P Sánchez-Serrano, National Centre of Epidemiology CIBERESP, Instituto de Salud Carlos III, 28029 Madrid, Spain

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Correspondence to: David Carmena, PhD, MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, United Kingdom. [d.carmena@imperial.ac.uk](mailto:d.carmena@imperial.ac.uk)

Telephone: +44-20-83833014 Fax: +44-20-83838337

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species/assemblages and subassemblages involved is essential for accurately identifying the parasite and assessing zoonotic transmission. The public health significance of these findings has also been thoroughly discussed.

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### Abstract

*Giardia* is an enteric protozoan that infects a wide range of vertebrate hosts, being considered a major causative agent of gastrointestinal disease in humans in both developing and developed countries. *Giardia* infection has also a significant impact on livestock health, causing diarrhoea and resulting in significant economic loss. Transmission is either direct, through the faecal-oral route, or indirect, through ingestion of contaminated water or food. In this article, we review current knowledge about the epidemiology of giardiasis in different populations in Spain, including humans, livestock, companion animals, and wildlife. Environmental contamination of surface waters and raw foods by *Giardia* cysts is also addressed. Special relevance has been given to the data available on the molecular characterization of the *Giardia* isolates obtained from clinical and environmental samples, as determining the

### INTRODUCTION

Members of the genus *Giardia* (subphylum Sarcomastigophora, class Zoomastigophora) are flagellated, binucleated protozoa that affect the intestinal tract of a wide range of vertebrate hosts, including mammals, birds, reptiles and amphibians. *G. duodenalis* (syn. *G. intestinalis*, *G. lamblia*) is the only species isolated from humans so far, being the causative agent of endemic and epidemic diarrhea and malabsorption syndrome. Prevalence of human giardiasis has been reported to range from 2% to 7% in developed countries and 20%-30% in the developing countries, with more than 200 million people being affected every year<sup>[1]</sup>. Despite being known since the seventeenth century work by Antony van Leeuwenhoek, *Giardia* has only become recognized as a major public

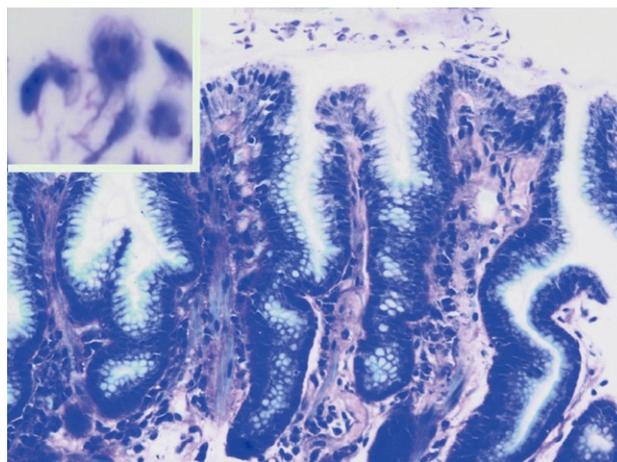
health concern in the last three decades after its identification in community outbreaks, travelers to endemic areas and immune-compromised individuals.

*Giardia* infection is often asymptomatic: as many as 50% to 75% of infected individuals may not develop any symptoms<sup>[2]</sup>. Common clinical manifestations associated to the infection include abdominal pain, steatorrhea, bloating, fatigue, flatulence and weight loss. Although not completely elucidated, the pathogenesis of giardiasis seems to involve damage to the intestinal brush border and mucosa (Figure 1), triggering of host immune responses, impairment in pancreatic function and alteration of duodenal flora<sup>[3]</sup>. In immune-competent individuals giardiasis resolves spontaneously in 1-4 wk, while immune-compromised individuals typically experience more severe and prolonged disease, lasting even for months if treatment is not provided.

Transmission of *Giardia* is *via* the faecal-oral route, either indirectly through contaminated water or food, or directly from person-to-person or animal-to-person contact. The life cycle of the parasite is direct comprising the infectious and environmentally resistant cysts (8-14 µm long) that are passed in the faeces, and the rapidly multiplying non-invasive trophozoites (9-21 µm long), which colonize the intestinal epithelium of the host and cause disease. Based on pheno- and genotypic differences and range of host specificities, the genus *Giardia* is currently classified into six species, with at least six additional variants or assemblages within *G. duodenalis*, which are likely to represent different species<sup>[4,5]</sup> (Table 1).

From a public health perspective, the understanding of the epidemiology of giardiasis and the transmission pathways that contribute to the disease burden is essential for (1) assessing the zoonotic potential and pathogenesis of *Giardia*; (2) identifying animal populations that can serve as a reservoir of disease for humans and domestic animals; and (3) evaluating risk factors potentially associated to the infection and implementing effective control measures. For these purposes molecular tools aiming to unravel the *Giardia* genotypes and subtypes have been proven very useful in recent years<sup>[6]</sup>. Therefore, the epidemiology and transmission dynamics of *Giardia* have been investigated in different scenarios including the environmental medium<sup>[7,8]</sup>, human populations<sup>[9]</sup>, production<sup>[10,11]</sup> and domestic<sup>[12]</sup> animals, and wildlife<sup>[13]</sup>.

Spain, located in southwestern Europe, is a developed country with a population of more than 46 million and an important agricultural industry including pigs (25.3 million), sheep (19.7 million), cattle (6.1 million), goats (2.9 million) and horses (0.2 million)<sup>[14]</sup>. In addition, pet populations including dogs and cats are also considerable, although not accurate census figures are currently available. The aim of this paper is to provide a comprehensive picture of the epidemiology of *Giardia* in Spain since 1990. Special attention has been paid to the genotyping data reported in the literature, in order to investigate potential cross-species transmission and the public health significance of these findings.



**Figure 1** Histological examination of a gastric lesion from an infected patient showing multiple *Giardia intestinalis* trophozoites (10-20 µm × 5-15 µm) adhering to the surface epithelium (magnification x 200, Giemsa stain). Magnification × 1000 (inset) reveals the typical tear-drop shape with twin nuclei of this parasite. Image reproduced with kind permission from Georg Thieme Verlag KG, Stuttgart, Germany.

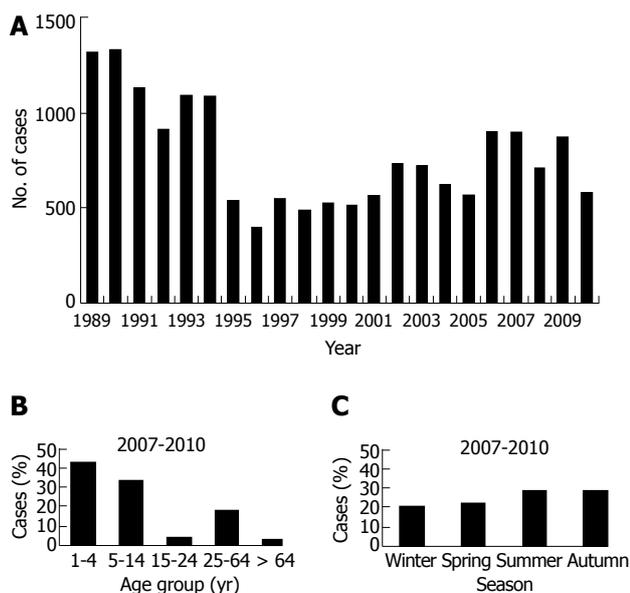
**Table 1** *Giardia* species and assemblages (adapted from<sup>[4,5]</sup>)

Species	Main hosts	Reported in human infections	Reported in surface waters
<i>G. duodenalis</i>	Mammals	Yes	Yes
<i>G. agilis</i>	Amphibians	No	No
<i>G. ardeae</i>	Birds	No	No
<i>G. microti</i>	Rodents	No	No
<i>G. muris</i>	Rodents	No	No
<i>G. psittaci</i>	Birds	No	No
<i>Assemblages</i>			
A (= <i>G. duodenalis</i> )	Mammals	Yes	Yes
B (= <i>G. enterica</i> <sup>1</sup> )	Mammals	Yes	Yes
C/D (= <i>G. canis</i> <sup>1</sup> )	Canines	Yes <sup>2</sup>	No
E (= <i>G. bovis</i> <sup>1</sup> )	Domestic ruminants, pigs	Yes <sup>2</sup>	No
F (= <i>G. cati</i> <sup>1</sup> )	Cats	Yes <sup>2</sup>	No
G (= <i>G. simondi</i> <sup>1</sup> )	Mice, rats	No	No
H	Seals	No	No

<sup>1</sup>Species names recently proposed; <sup>2</sup>To be confirmed with further molecular characterization.

## HUMAN INFECTION

Human giardiasis is not a compulsory notifiable disease in Spain, so the actual disease burden in the country is difficult to assess. However, cases are voluntarily notified to the national epidemiological surveillance network through the microbiological information (MI) system, which is based on reporting of confirmed microbiological diagnoses of individual cases provided by a network of parasitological laboratories, mainly from hospitals. Because the MI system only covers approximately 25% of the Spanish population, the figures provided are underestimations that can only give a glimpse into the disease trend rather than informing about its actual inci-



**Figure 2** Voluntary reporting of human giardiasis to the Spanish Microbiological Information system. A: Annual totals of notified cases of *Giardia* infections in Spain between 1989 and 2010 ( $n = 16\,974$ ); B: Age distribution of *Giardia* infections in Spain for the period 2007-2010 ( $n = 3077$ ); C: Seasonal distribution of *Giardia* infections in Spain for the period 2007-2010 ( $n = 3077$ ).

dence. Thus, a total of 16 974 cases of *Giardia* infections were notified to the MI system between 1989 and 2010 (Figure 2A). For the period 2007-2010, the male/female ratio was 1.27, with children under the age of 5 year old accounting for 42.7% of the reported cases (Figure 2B). The highest numbers of infections occurred during the summer and autumn seasons (Figure 2C). In addition, 15 outbreaks of human giardiasis have also been reported between 2003 and 2010, with a total of 199 people affected (Table 2). Most episodes took place in children educational centers involving direct person-to-person transmission, although waterborne transmission was also documented. An additional person-to-person outbreak of giardiasis has been recently described in a maternal infant unit<sup>[15]</sup>. A total of 16/42 individuals (12 infants and 4 mothers) were found positive for *Giardia* infection, with children aged 2-3 years showing the highest infection rates. None of the above mentioned outbreaks of giardiasis were investigated by genotyping, so the species/assemblages involved and the contamination source remained unresolved.

The prevalence of human giardiasis in Spain has been mainly studied in paediatric populations, but also in hospital outpatients and innate and immigrant subjects (Table 3). Typical infection rates of giardiasis ranged from 3%-7% and 13%-25% for asymptomatic and symptomatic individuals, respectively. These data are in agreement with those reported in other European countries including Portugal<sup>[16]</sup>, Italy<sup>[17]</sup>, Belgium<sup>[18]</sup> or The Netherlands<sup>[19]</sup>. None of the epidemiological studies carried out in Spain could demonstrate significant sex differences in the *Giardia* infection rates found. Young individuals were usually more likely to be affected by the disease than

**Table 2** *Giardia* outbreaks notified to the Spanish epidemiological surveillance network between 2003 and 2010

Year	Confirmed cases	Hospitalized cases	Location	Transmission
2003	6	0	School	Direct person-to-person
	38	0	School	Direct person-to-person
	3	0	School	Direct person-to-person
	26	0	School	Direct person-to-person
2004	30	0	Public premises	Drinking water
	4	0	School	Direct person-to-person
	14	1	Public premises	Direct person-to-person
2005	8	0	School	Drinking water
2006 <sup>1</sup>	-	-	-	-
2007	17	0	School	Unknown
	6	0	Public premises	Unknown
	3	0	School	Direct person
2008	5	0	Private premises	Unknown
	4	0	School	Unknown
	3	0	School	Direct person-to-person
	32	0	Swimming pool	Water
2009 <sup>1</sup>	-	-	-	-
2010 <sup>1</sup>	-	-	-	-

<sup>1</sup>No outbreaks were reported in 2006, 2009 and 2010.

older subjects<sup>[20,21]</sup>, with diarrhea being the most common clinical manifestation<sup>[22,23]</sup>. Two studies found that the occurrence of human giardiasis was more frequently detected in the autumn season<sup>[23,24]</sup>. *G. duodenalis* has been described as the second most common intestinal parasite after *Blastocystis hominis* in a hospital outpatient service in Catalonia<sup>[24]</sup>. The infection is also frequently reported in immigrant populations seeking medical assistance, often in asymptomatic individuals<sup>[25,26]</sup>. Because of the poor socioeconomic conditions and difficulty in accessing medical care, this collective may remain largely untreated and act as carriers of the disease in the host community. Therefore, screening for the infection and prompt treatment in immigrants from low-income countries is highly desirable in order to prevent or minimize public health hazard. To the best of our knowledge no seroepidemiological surveys investigating the prevalence of *Giardia* in human populations have been carried out in Spain in the last 20 years, so the general exposure to the parasite is unknown. Although *Giardia* is not considered an opportunistic pathogen, studies aiming to investigate the prevalence of the disease in immune-compromised (including AIDS) patients are also lacking for this period of time.

## LIVESTOCK INFECTION

There is increasing evidence suggesting that *Giardia* is a primary pathogen in production animals worldwide<sup>[10,11]</sup>. Global *Giardia* infection rates have been reported in the range of 3%-58% for cattle, 25% for water buffaloes, 1%-56% for sheep, 12%-36% for goats, 17%-31% for pigs and 5% for alpacas<sup>[5]</sup>. Similarly to human giardiasis, the pathogenesis of *Giardia* in animals is also a multifactorial process involving both parasite and host factors. The disease causes alteration of the small intestinal

**Table 3** Detection of *Giardia spp.* in human stool samples in Spain (1990 onwards)

Population	Province/region	Period	No. samples	Technique(s)	Infection rate (%)	Ref.
Mixed <sup>1</sup>	Madrid	NS	405	CM	7.4	[20]
Urban paediatric <sup>1,2</sup>	Salamanca	1987-1989	354	CC + CM	13.0	[21]
Rural paediatric <sup>1</sup>	Ávila	1992-1993	318	CC + CM	4.4	[87]
Innate <sup>1,2</sup>	Madrid	1991-1993	1281	CC + CM	21.0	[22]
Urban paediatric <sup>1</sup>	Cuenca	NS	891	CC + CM	36.4	[88]
Day care centre <sup>1,2</sup>	Salamanca	1990-1991	523	CC + CM + IFA	25.3	[23]
Urban and rural paediatric <sup>1</sup>	Andalusia	1994-1996	1917	CC + CM	5.0	[89]
Immigrants <sup>1,2</sup>	Madrid	1989-1999	NS	CC + CM	5.4	[25]
Urban paediatric <sup>1</sup>	Valencia	2005	270	CC + CM	4.3	[90]
Immigrants <sup>1,2</sup>	Barcelona	2001-2004	2464	NS	5.4	[26]
Outpatients <sup>2</sup>	Catalonia	1999-2005	13 913	CM	3.9	[24]
Rural paediatric <sup>1</sup>	Álava	2008-2009	328	CpAg-ELISA + ICT	3.5	[29]

CC: Concentration techniques; CM: Conventional microscopy; CpAg-ELISA: Enzyme-linked immunosorbent assay for the detection of copro-antigens; ICT: Immunochromatography assay; IFA: Immunofluorescence microscopy; NS: Not specified. <sup>1</sup>Asymptomatic; <sup>2</sup>Symptomatic (defined as the presence of diarrhoea and/or vomiting).

**Table 4** Detection of *Giardia spp.* faecal samples from farmed animals in Spain (1990 onwards)

Specie	Province/region	Period	No. samples	Technique(s)	Infection rate (%)	Ref.
Cattle <sup>1,2</sup>	Aragón	1990-1993	554	CC + CM	11.7	[30]
Cattle <sup>1</sup>	Granada	NS	592	CC + CM	0.2	[37]
Cattle <sup>1</sup>	Galicia	2005	734	IFA	30.1	[31]
Cattle <sup>1</sup>	Galicia	NS	379	IFA	26.6	[32]
Cattle <sup>1,2</sup>	Galicia	2007	1316	IFA	4.9-28.5 <sup>4</sup>	[34]
Cattle <sup>1</sup>	Galicia	2008-2009	649	CC + IFA	21-38 <sup>3</sup>	[33]
Cattle <sup>1</sup>	Álava	2008-2009	227	CpAg-ELISA + ICT	1.4	[29]
Sheep <sup>1</sup>	Granada	NS	1165	CC + CM	6.3	[37]
Sheep <sup>1,2</sup>	Zaragoza	1995-1997	583	CC + CM	2.7	[91]
Sheep <sup>1</sup>	Galicia	NS	575	IFA	32.7	[36]
Sheep <sup>1</sup>	Galicia	NS	446	IFA	19.2	[32]
Sheep <sup>1</sup>	Valencia	2005-2007	386	IFA + PCR	42.0	[35]
Sheep <sup>1</sup>	Galicia	2008-2009	377	CC + IFA	26-44 <sup>3</sup>	[33]
Goats <sup>1</sup>	Granada	NS	574	CC + CM	4.0	[37]
Goats <sup>1</sup>	Galicia	NS	116	IFA	19.8	[32]
Goats <sup>1,2</sup>	Canary Islands	2006-2007	315	IFA	42.2	[75]
Ostriches/rheas	Various	1997-2000	177	Nec. + CC + CM	< 1	[92]

CC: Concentration techniques; CM: Conventional microscopy; IFA: Immunofluorescence microscopy; CpAg-ELISA: Enzyme-linked immunosorbent assay for the detection of copro-antigens; ICT: Immunochromatography assay; Nec.: Necropsy; NS: Not specified; PCR: Polymerase chain reaction. <sup>1</sup>Asymptomatic; <sup>2</sup>Symptomatic (defined as the presence of diarrhoea); <sup>3</sup>Depending on age and geographical location; <sup>4</sup>Depending on age and sampling season.

brush border, affecting the epithelial permeability and impairing intake of water, electrolytes and nutrients. The combination of these factors results in malabsorptive diarrhoea and lower weight gain<sup>[27]</sup>. As a consequence, giardiasis has a significant economical impact derived from the lower productivity or death of the affected animals. Age is one of the most important risk factors associated with giardiasis, with young animals being more susceptible to infection than adults. Development of host protective response has been proposed as a mechanism to explain the decrease of prevalence rates and severity of the infection with the animal age<sup>[28]</sup>, although this hypothesis still remains controversial<sup>[29]</sup>.

In Spain *Giardia* infections have been identified in cattle, sheep, goats, pigs and farmed ostriches, with most epidemiological studies being carried out in the autonomous regions of Galicia and Aragón (Table 4). The prevalence of *Giardia* found in cattle were within the range

of 0.2%-38%, with most data based on cross-sectional point prevalence studies conducted with asymptomatic, apparently healthy animals. The wide variability observed may be partially explained by differences in management practices, environmental parameters, performance of the diagnostic testes used, and study designs. These remarks can be largely applied also to other animal production species. *Giardia* infection in cattle seems very spread, affecting between 53% to 97% of the sampled herds/farms<sup>[30-32]</sup>. Intensity of infection has been reported in the range from 7 to 15 000 cysts per gram (cpg) of faeces<sup>[31,33]</sup>. Because cattle may shed up to 40 kg of faeces a day and cyst excretion last for seven or more weeks in infected animals with chronic diarrhoea, these figures illustrate the huge contribution of cattle on the load of infective *Giardia* to the environment, including watersheds. Newborn calves have been found not only to harbour the highest *Giardia* prevalence rates (up to 58%)<sup>[32]</sup>

**Table 5** Detection of *Giardia* spp. in faecal samples from pets in Spain (1990 onwards)

Specie	Province/region	Period	No. samples	Technique(s)	Infection rate (%)	Ref.
Dogs <sup>1,2</sup>	Zaragoza	1992	81	CC + CM	4.9	[41]
Dogs <sup>1,2</sup>	Granada	NS	912	CC + CM	12.1	[37]
Dogs <sup>1,2</sup>	Murcia	2001-2004	48	Nec. + CC + CM	10.0	[42]
Dogs <sup>1,2</sup>	Córdoba	NS	1500	CC + CM + Nec.	1.0	[45]
Dogs <sup>1,2</sup>	Madrid	NS	1161	CC + CM	7.1	[44]
Dogs <sup>2</sup>	Various	2005-2006	441	SNAP Giardia test <sup>3</sup>	25.1	[43]
Dogs + cats	Madrid	NS	79	CC + CM	17.7	[46]
Cats <sup>2</sup>	Various	2005-2006	54	SNAP Giardia test <sup>3</sup>	14.6	[43]

CC: Concentration techniques; CM: Conventional microscopy; Nec.: Necropsy; NS: Not specified. <sup>1</sup>Asymptomatic; <sup>2</sup>Symptomatic (defined as the presence of diarrhoea and/or vomiting); <sup>3</sup>Idexx Laboratories, Westbrook, ME.

but also to shed considerably more cysts than adult animals<sup>[33]</sup>. In line with these findings diarrhea was primarily associated to suckling (< 1.5-mo-old) and weaning (1.5- to 4-mo-old) calves<sup>[30,31]</sup>, although concomitant viral or bacterial infections may also contribute to this condition. In some studies the seasonal prevalence of infection by *Giardia* has been found to be significantly higher in spring than in winter in dairy cattle<sup>[34]</sup>. Adult animals with low parasite burdens acting as asymptomatic carriers have been proposed as source of infection for younger animals<sup>[33]</sup>.

*Giardia* infections in sheep are also frequent in Spain. Reported prevalences varied markedly from 3% to 44% (Table 4), with lambs 1- to 3-mo-old bearing both the highest infection rates<sup>[35]</sup> and intensities of infection<sup>[33]</sup>. Giardiasis were found in 8%-98% of the flocks analyzed<sup>[32,35,36]</sup>, and the intensity of the disease ranged from 3 to 4319 cpg of faeces in apparently healthy animals. When studied, neither sex nor seasonal variation were significantly associated to giardiasis in sheep<sup>[37]</sup>. For goats, *Giardia* infection rates have been reported in the range from 4% to 20% (Table 4), with up to 90% of the flocks being affected by the disease<sup>[32,37]</sup>. Intensities of infection were similar to those observed in sheep, ranging from 15 to 1845 cpg of faeces<sup>[32]</sup>.

Importantly, *Giardia* cysts have also been detected at high densities ( $3 \times 10^3$  cysts/L) in pig slurries from swine farms in the autonomous region of Castilla and León<sup>[38]</sup>. Because animal slurry is commonly used as manure fertilizer for pasture and crops, the removal or inactivation of *Giardia* cysts is essential to minimize the risk of transmission through infected water and/or food.

## COMPANION ANIMAL INFECTION

*Giardia* is one of the most common parasites of cats and dogs around the world. The prevalence of *Giardia* infections in these hosts is typically high, in the range of 0.2%-19% and 1%-54% for cats and dogs, respectively<sup>[5,39]</sup>. Infection rates obtained by enzyme-linked immunosorbent assay are usually higher than those obtained by conventional microscopy. Infection is most commonly reported in kittens and puppies and also in kennel/shelter and stray populations. Cats and dogs parasitized by *Giardia* can be asymptomatic or, less frequently, can

have diarrhea due to maldigestion, malabsorption and increased motility<sup>[40]</sup>.

Few surveys have aimed to investigate the prevalence of feline and canine giardiasis in Spain. Studied populations included dogs attending veterinary clinics<sup>[41-43]</sup>, dogs kept in shelters<sup>[41,42,44,45]</sup> and stray dogs<sup>[42]</sup>. Faecal (presumably from dogs and cats) and soil samples from public parks and gardens have also been analyzed for the presence of *Giardia* cysts<sup>[45,46]</sup>. The infection rates found varied from 1% to 25% in dogs and 15% in cats (Table 5). Soil contaminated with *Giardia* cysts was detected in 19.4% of the samples analyzed in Madrid<sup>[46]</sup>, whereas no positive samples were reported in a similar survey conducted in the city of Córdoba<sup>[45]</sup>. As expected, the prevalence of *Giardia* was significantly higher in homeless animals (1.2%) than in housed (0.6%) animals<sup>[45]</sup>. When studied, neither sex nor habitat were significantly associated with *Giardia* infection<sup>[45]</sup>.

## WILD AND CAPTIVE ANIMAL INFECTION

Because of its ubiquitousness it is now clear that *Giardia* is able to infect a broad range of mammalian, avian and reptilian wildlife<sup>[13]</sup> with different degrees of host specificity. This situation has raised the important question of whether wildlife populations can serve as reservoirs of disease for human and domestic animals, particularly when located near or within water catchments or agricultural exploitations. For instance, beavers have been implicated (but very often not convincingly demonstrated) as the source of the contamination of water used for human consumption in several waterborne outbreaks of giardiasis in North America<sup>[13]</sup>. In Spain the only studies investigating the prevalence of *Giardia* infections in wild animal populations have been carried out so far in the autonomous region of Galicia (Table 6). Thus, *Giardia* cysts have been found in 6.8% of faecal samples from otters, a mammal that live in aquatic environments<sup>[47]</sup>. Although the intensity of infection reported was low (1-10 cysts per 20 µL of faecal concentrate), this finding demonstrates that otters may contribute to the pool of parasites within watercourses and stream banks. *Giardia* infection rates ranging from 5%-14% and 1%-5% have been found in deer and wild boars, respectively, shot in game preserves during hunting season<sup>[33,48]</sup>. Intensity of

**Table 6** Detection of *Giardia* spp. in faecal samples from wild and captive animals in Spain (1990 onwards)

Specie	Province/region	Period	No. samples	Technique(s)	Infection rate (%)	Ref.
Deers	Galicia	2008-2009	181	CC + IFA	6-14 <sup>2</sup>	[33]
Roe deers	Galicia	2008-2009	224	CC + IFA	5.3	[48]
Wild boars	Galicia	2008-2009	279	CC + IFA	3-5	[33]
Wild boars	Galicia	2008-2009	381	CC + IFA	1.3	[48]
Otters	Galicia	2004-2005	437	CC + IFA	6.8	[47]
Nonhuman primates <sup>1</sup>	Madrid and Valencia	NS	20	CC + CM + PCR	70.0	[49]

CC: Concentration techniques; CM: Conventional microscopy; IFA: Immunofluorescence microscopy; Nec.: Necropsy; NS: Not specified; PCR: Polymerase chain reaction. <sup>1</sup>Captive animals from zoological gardens; <sup>2</sup>Depending on geographical location.

infections was again low, varying from 5-47 and 5-120 cpg of faeces in deer and wild boars, respectively. Because of the roaming habits of these species, contamination of surface waters, agricultural land and pastures are highly expected. Unfortunately the lack of molecular data in these studies does not allow assessing neither the direction of transmission among species nor its zoonotic potential. Indeed humans and domestic animals may be the source of environmental contamination from which wildlife contract the infection.

*Giardia* infections have also been investigated in non-human, captive primates (including lemurs, monkeys and great apes) from zoological gardens in Madrid and Valencia<sup>[49]</sup>, where 70% of the studied samples were positive for *Giardia* cysts.

## FOOD AND SEAFOOD CONTAMINATION

Fresh produce, particularly when receiving minimal washing and heat treatment, is a potential vehicle of transmission for *Giardia* cysts and cause foodborne outbreaks of giardiasis<sup>[8]</sup>. Sources of cyst contamination include agricultural run-off and/or contamination of water courses used for crop irrigation and wash waters at packing houses<sup>[50]</sup>. In addition, freshwater and marine shellfish (e.g., mussels, clams and oysters) can concentrate *Giardia* cysts through filtering large volumes of contaminated water with livestock and slurry discharges or human sewage effluents.

In Spain a survey investigating the *Giardia* contamination of salad products, including lettuces and Chinese cabbage, from an agricultural area in Valencia reported the presence of cysts in 52.6% of the tested samples ( $n = 19$ )<sup>[51]</sup>. Not surprisingly, *Giardia* cysts were also detected in the water from the irrigation canal in concentrations ranging from 20 to 50 cysts/L. The authors proposed the use of manure as fertilizer for the crops as the most likely source of cyst contamination, although this hypothesis was not further verified.

Shellfish industry is an important economic activity in Galicia. Therefore, a number of studies aiming to determine the prevalence of *Giardia* in shellfish-farming areas along the Galician coast have been undertaken in the last decade. As a consequence, *Giardia* cysts have been detected in 16.7% of oysters<sup>[52]</sup> and in 41% of mussels destined for human consumption<sup>[53,54]</sup>. Considerable lev-

els of cysts were also found in water samples from rivers (1-20 cysts/L) and effluents from waste water treatment plants (233-1131 cysts/L, Table 7) in the region, a fact that highlights the importance of these waters in contaminating marine estuaries. Potential sources of water contamination included agricultural run-off and untreated human sewage<sup>[55]</sup>.

## WATER CONTAMINATION

Because *Giardia* infections are widespread in human and animal populations, contamination of the aquatic environment is inevitable. A number of events may influence the environmental load of *Giardia* cysts in surface waters, including agricultural practices, sewage contamination, heavy rainfall or snow/ice melting<sup>[7]</sup>. Taking into account the unpredictability of most of these factors, spatial and temporal variations in the cysts distribution in water bodies are highly expected, with cysts contents fluctuating in a spiking pattern. This important feature, together with the limitations of the current detection methods with respect to efficiency of recovery and viability or infectivity of the detected cysts, should be taken into consideration when evaluating the significance of occurrence data. *Giardia* cysts have been isolated from rivers, lakes or reservoirs used as source waters for human consumption or recreation, and also from treated drinking water supplies and recreational waters facilities, including swimming pools and water parks<sup>[7]</sup>. Reported cyst concentrations are in the range of 0.01-150 per litre, but concentrations up to 25 000 cysts per litre have been found in agricultural run-off and urban wastewater effluents<sup>[55]</sup>. This great ubiquitousness relies on the remarkable features of *Giardia* cysts that make them particularly suited for waterborne transmission. These include the vast numbers in which cysts are shed by infected humans or animals, their long survival rate in natural surface waters<sup>[8]</sup>, their small size and resilience to chlorine-based disinfectants (which allows them to pass through most of the industrial process water treatments, even when operated under optimum conditions)<sup>[55]</sup> and low infective doses<sup>[56]</sup>. At least 132 waterborne outbreaks of giardiasis have been reported worldwide since 1954<sup>[57]</sup>, the most important occurring in Norway in 2004 and affecting more than 1500 people<sup>[58]</sup>. *Giardia* has also been the most commonly identified pathogen in waterborne outbreaks

**Table 7** Detection of *Giardia* spp. in surface waters in Spain (1990 onwards)

Type water	Province/region	Period	No. samples	Positive samples (%)	Concentration (cysts/L)	Ref.	
Rivers	Álava	2000-2002	52	92.3	65	[59]	
	Galicia	2004	7	85.7	1-20	[53]	
	Galicia	2007	116	67.2	2-722	[34]	
	Galicia	2009	28	60.7	1-160	[60]	
Reservoirs	Álava	2000-2002	36	55.5	0.6	[59]	
	DWTP influent	Álava	2000-2002	57	26.9-45.2	0.05-0.5	[59]
		Galicia	2007	64	100.0	1-13	[61]
DWTP influent	Galicia	2008-2009	12	25-58	1.3-1.4	[33]	
	Galicia	2009	52	42.3	1-7	[60]	
	DWTP effluent <sup>1</sup>	Álava	2000-2002	57	0-19.2	0-0.01	[59]
Galicia		2007	64	100.0	0.5-4	[61]	
Galicia		2008-2009	12	25-33	1-1.3	[33]	
Galicia		2009	52	36.5	1-5	[60]	
Tap water <sup>2</sup>	Álava	2000-2002	82	26.8	0.02	[59]	
WTP influent	Galicia	2007	48	100.0	89-8305	[62]	
	Galicia	2004	11	90.9	29-1433	[53]	
	Galicia	2008-2009	12	100.0	415-2752	[33]	
	Galicia	2009	50	98.0	2-14 400	[60]	
	WTP effluent <sup>1</sup>	Galicia	2007	48	100.0	79-2469	[62]
Galicia		2004	16	87.5	233-1131	[54]	
Galicia		2008-2009	12	100.0	138-560	[33]	
Galicia		2009	50	96.0	2-6000	[60]	

DWTP: Drinking water treatment plant; WTP: Wastewater treatment plant. <sup>1</sup>Water treatment facilities with different physical, biological and/or chemical treatment processes in place; <sup>2</sup>Tap water with chlorination treatment only.

reported in the United States, with almost 28 000 cases during the period 1965 to 1996<sup>[55]</sup>.

In Spain, the detection of *Giardia* cysts in water samples has only been attempted in few surveys carried out in the autonomous region of Galicia and in the province of Álava (Table 7). *Giardia* cysts have been reported in 61%-92% of river samples (range 1-722 cysts/L)<sup>[34,53,59,60]</sup>, 55% of reservoir samples (mean concentration < 1 cyst/L)<sup>[59]</sup>, influent (25%-100% of samples; range: 1-13 cysts/L)<sup>[33,59,61]</sup> and effluent (0%-100% of samples; range: 0-5 cysts/L)<sup>[33,59,61]</sup> samples from drinking water treatment facilities (DWTF), and influent (91%-100% of samples; range: 2-14 400 cysts/L)<sup>[33,53,60,62]</sup> and effluent (87%-100% of samples; range: 2-6000 cysts/L)<sup>[33,53,60,62]</sup> samples from waste treatment facilities. In addition, *Giardia* cysts have also been consistently found in 27% of tap water samples (mean concentration: < 1 cyst/L) from municipalities with chlorination treatment only on their drinking water source<sup>[59]</sup>. The viability of the isolated cysts have been estimated to be in the range of 90% to 95%<sup>[34,60]</sup>. Interestingly, important differences in the removal efficiencies of the DWTF were found. For instance, three orders of magnitude removal for *Giardia* cysts were achieved by conventional DWTF in the province of Álava<sup>[59]</sup>, whereas much poorer performances were reported in DWTF in the autonomous region of Galicia<sup>[60,61]</sup>. These data clearly demonstrate that maintaining the DWTF in good operating order is essential to minimize the presence of *Giardia* cysts in finished water, and, in consequence, to reduce the potential risk of waterborne giardiasis outbreaks.

The presence of *Giardia* cysts in surface waters was consistently detected throughout the year in all the epi-

demiological surveys, with seasonal peaks occurring in spring/summer<sup>[34,61,62]</sup> or autumn<sup>[59]</sup>. This variation in the seasonal pattern may be explained, at least partially, by differences in agricultural (lambling, calving, grazing, muck spreading) or environmental (runoff from rainfall events) factors. In addition, no large-scale waterborne outbreaks of human giardiasis have been reported in Spain to date.

## MOLECULAR GENOTYPING DATA, CONSIDERATIONS FOR PUBLIC HEALTH

The advent and continuous development of molecular technology in recent years has significantly broaden our understanding of the epidemiology of *Giardia*, particularly those aspects related to the host range of different *Giardia* species and genotypes, the potential for cross-species transmission, and the environmental factors involved in the exposure to the pathogen<sup>[5,6]</sup>. *G. duodenalis* is the only species found in humans, although it can also infect a wide range of mammals, including livestock and pet animals. The current trend is to consider *G. duodenalis* as a multispecies complex with little morphological variation among its members, who can be assigned to eight genotypes/assemblages (A-H, Table 1). Typically, only assemblages A and B are associated with human infections. The two most common subtypes of assemblage A (AI and AII) differ significantly in host preference. Therefore, animals are mostly infected with subassemblage AI whereas humans are mostly infected with subassemblage AII<sup>[63]</sup>. Assemblages C and D have been isolated from dogs, cats, and wild canids; assemblage

**Table 8** Identification of *Giardia* isolates in Spain spp. from different origins

Origin	No. samples genotyped	Loci tested	Genotypes (assemblages, %)	Ref.
Human	108	<i>tpi</i>	AII (39.8); AII + B (3.7); B (56.5)	[63]
Human	211	<i>tpi</i>	AII + B (13.3); B (86.7)	[64]
Human	7	<i>tpi</i>	AII (28.6); AII + B (14.3); B (57.1)	[29]
Farm animals				
Cattle	4	$\beta$ -giardin + <i>gdh</i>	E (100.0)	[32]
Cattle	50	$\beta$ -giardin	AI (36.0); E (64.0)	[33]
Sheep		$\beta$ -giardin	E (100.0)	[36]
Sheep	12	$\beta$ -giardin + <i>gdh</i>	B (8.3); E (91.7)	[32]
Sheep	75	$\beta$ -giardin	AI (1.3); E (98.7)	[35]
Sheep	31	$\beta$ -giardin	B (35.5); E (64.5)	[33]
Goats	1	$\beta$ -giardin + <i>gdh</i>	E (100.0)	[32]
Goats	39	<i>tpi</i> + $\beta$ -giardin	E (100.0)	[75]
Captive animals				
Nonhuman primates	14	<i>SSrRNA</i> , <i>gdh</i> , <i>tpi</i> , $\beta$ -giardin	A (64.3); B (35.7)	[49]
Food				
Oysters	1	<i>tpi</i>	B (100%)	[52]
Water				
Rivers	NS	$\beta$ -giardin	AI (NS); AI + E (NS); AII (NS); AII + E (NS); E (NS)	[34]
DWTP influent	NS	$\beta$ -giardin	AI + E (NS); AII (NS); AII + E (NS)	[61]
DWTP effluent	NS	$\beta$ -giardin	AI + E (NS); AII (NS)	[61]
WTP influent	NS	$\beta$ -giardin	AI (NS); AI + E (NS); AII (NS); AII + E (NS)	[62]
WTP influent	10	$\beta$ -giardin	AII (50.0); AI + AII (10.0); AI + E (20.0); AII + E (20.0)	[33]
WTP effluent	NS	$\beta$ -giardin	AI (NS); AI + E (NS); AII (NS); AII + E (NS)	[62]
WTP effluent	12	$\beta$ -giardin	AI (8.3); AII (50.0); AI + E (16.7); AII + E (16.7); E (8.3)	[33]

DWTP: Drinking water treatment plant; NS: Not specified; WTP: Wastewater treatment plant.

E from hoofed animals including cattle, sheep, goats, and pigs; assemblage F from cats, assemblage G from rodents, and assemblage H from marine vertebrates. Assemblages A and B pose the broadest host range (particularly the former) and can be transmitted zoonotically, whereas assemblages C, D, E, F, G, and H have strong host specificities and therefore appears to be restricted in host range<sup>[4,6]</sup>. In addition, it is now well recognized that only the occurrence of the same *Giardia* species/genotype in human and animal populations living in the same household or geographic area can be considered indicative of zoonotic transmission.

Data on *G. duodenalis* genotypes and subtypes from human populations in Spain are limited, with only 326 isolates characterized by sequence analyses of the *tpi* gene in three independent molecular epidemiological studies (Table 8). The human populations analyzed included hospital patients<sup>[64,65]</sup> and asymptomatic school children<sup>[29]</sup>. Only assemblages A and B were detected, with assemblage B being most prevalent and accounting for 56%-87% of the infections, while subassemblage AII accounted for 29%-40% of the infections. Mixed infections of subassemblage AII + assemblage B were identified in 4%-14% of the isolates, while subassemblage AI has not been reported so far. These data are in agreement with those published elsewhere, where *G. duodenalis* assemblage B has been more commonly found than assemblage A in both developing and developed countries<sup>[5,6,9]</sup>. Interestingly, two of the Spanish studies found that assemblage B isolates were more often associated with asymptomatic infections, whilst subassemblage AII isolates were linked with the presence of clinical

manifestations<sup>[64,65]</sup>. Although similar observations have been obtained in surveys in United Kingdom<sup>[66]</sup> and Bangladesh<sup>[67]</sup>, the correlation between *G. duodenalis* genotypes and symptomatology remains controversial, as other studies have reported opposite results<sup>[19,68]</sup>. These disagreements may be influenced by factors including the developmental, nutritional and immunological status of the host, parasite-immune evasion mechanisms or synergistic effects due to *Giardia* mixed infections<sup>[3,69]</sup>. Taking together, these preliminary molecular data seem to indicate that human giardiasis in Spain is mainly the result of anthroponotic transmission, although the extent of this assessment must be corroborated in further molecular epidemiological surveys.

In Spain, molecular characterization studies of *Giardia* in farm animals have been carried out in cattle, sheep and goats, with some 210 isolates tested at the  $\beta$ -giardin gene, alone or in combination with the *gdb* or the *tpi* genes (Table 8). *G. duodenalis* assemblage E was the most prevalent genotype in all the species studied, ranging from 64% to 100% in cattle and sheep and 100% in goats. Assemblage B was identified only in 8%-35% of sheep, whereas subassemblage AI was found in 36% of cattle and 1% of goats. Subassemblage AII has not been reported in livestock in Spain to date, but isolates of this genotype have been found in cattle from the neighbour country of Portugal<sup>[70]</sup>. In cattle, genetic analyses for *G. duodenalis* have been performed in asymptomatic animals including neonatal calves, heifers and cows<sup>[32,33]</sup>. The finding that assemblage E is the most common genotype identified in herds is in line with the results reported in other surveys in Europe, North America and Australia.

lia<sup>[71-73]</sup>. Importantly, zoonotic genotype subassemblage AI was also identified in 36% of cattle. Although this genotype is less commonly detected in humans than subassemblage II, recent molecular data have suggested a potential zoonotic transmission involving AI isolates between cattle and human workers on dairy farms in India<sup>[74]</sup>. In another study, cattle have also been suspected to be a source of human giardiasis through contamination of drinking water, although this hypothesis could not be confirmed due to the lack of molecular evidence<sup>[29]</sup>. Assemblage E is also the genotype most frequently identified in sheep and goats in Spain<sup>[32,33,35,36,75]</sup>, in agreement with data published worldwide<sup>[5,71,76]</sup>. An interesting observation is the detection of zoonotic assemblage B in a considerable proportion of infected sheep, as this genotype is considered to be rare in sheep<sup>[5]</sup>. Zoonotic subassemblage AI has also been identified in few lambs. Although limited in number, current available genotyping data seem to indicate that healthy domestic ruminants may not be an important zoonotic reservoir for *G. duodenalis* in Spain, although these animals (particularly cattle and sheep) may harbour some *G. duodenalis* assemblages/genotypes that are infective to humans. These findings support the increasing evidence suggesting that the actual role of livestock as major reservoirs for *Giardia* infections in humans might be much less relevant than initially estimated<sup>[5,77,78]</sup>.

Despite the public health concerns raised about the role of dogs and cats in the epidemiology of human giardiasis, this issue is still object of intense debate. Recent molecular studies have shown that dogs and cats are most commonly infected with host-adapted genotypes including assemblages C, D and F<sup>[5]</sup>. However, a number of studies have shown that humans and dogs sharing the same living area/household can harbour isolates of *G. duodenalis* from the same assemblage, providing supporting evidence of zoonotic transmission between humans and domestic dogs<sup>[79-84]</sup>. To date, there are no data on genotypes of *G. duodenalis* in pet animals in Spain, although an epidemiological study has revealed that ownership of a dog or a cat tended to increase the prevalence odds of human giardiasis<sup>[29]</sup>.

Free-living animals including nonhuman apes, monkeys, cervids, rodents, birds and marine mammals among others have been found to be commonly infected with *Giardia*<sup>[5,13]</sup>. Molecular data collected in early studies under natural, pristine conditions seemed to indicate that wildlife harbour host-adapted genotypes/species of *Giardia*<sup>[13,77]</sup>, although later research has revealed that wildlife can also be infected with zoonotic assemblages A and B<sup>[71,85]</sup>. As an explanation for the later finding, some authors have proposed that wildlife is more likely to have become infected with zoonotic genotypes from water contaminated with faecal material of human or livestock origin, serving to amplify the numbers of the originally contaminating isolate<sup>[9]</sup>. Whatever the case, free-living animal populations may potentially act as res-

ervoirs of zoonotic disease. To date no genotyping analyses have been performed in *Giardia* isolates from wildlife species in Spain, although otters, wild boars and deer have been proposed as sources of *Giardia* contamination of surface waters for human consumption<sup>[33,61]</sup>. Characterization of *Giardia* isolates in captive wildlife has been attempted only in nonhuman primates housed in zoological gardens in Valencia and Madrid<sup>[49]</sup>. Based on multi-locus sequence analysis, only zoonotic assemblages A and B were found, with subassemblages AI and BIV being also identified in some of the isolates.

*Giardia* is ubiquitously found in surface waters and the transmission of *G. duodenalis* through drinking and recreational waters is well documented<sup>[7,57]</sup>. Unfortunately, molecular typing data about *Giardia* isolates from water samples is restricted to a very limited number of epidemiological studies, mainly on urban waste waters<sup>[5,8,86]</sup>. In Spain, molecular characterization of *Giardia* isolates has been carried out in water samples from rivers<sup>[34]</sup>, DWTPs<sup>[61]</sup> and WTPs<sup>[33,62]</sup> by sequence analyses of the  $\beta$ -*giardin* gene. Subassemblages AI and AII and to a lesser extent assemblage E were the subgenotypes more frequently identified, alone or as part of different mixed infection combination among them (Table 8). Interestingly, assemblage B has not been reported in sample water isolates to date. These preliminary results are in agreement with those reported in Italy, where assemblage A was the genotype predominantly found (up to 75%), while assemblage B was identified only in 7% of the isolates, and mixed infections of assemblages A + B accounted for 16% of cases<sup>[17]</sup>. In Spain, the high proportion of *Giardia* isolates characterized as subassemblage AII (responsible for anthroponotic transmission) provides strong evidence of the role of humans as significant contributors to the overall environmental parasite burden, while livestock or wildlife are the most likely primary sources of water contamination by the zoonotic subassemblage AI. Because assemblage E is mainly restricted to ruminants, cats and rats, and is rarely found infecting humans, the zoonotic potential of this genotype is considered minimal. On the contrary, the presence of zoonotic and anthroponotic genotypes in the effluents of both DWTPs and WTPs is a serious public health concern, as these waters are used as supply for human consumption<sup>[61]</sup>, recreation<sup>[34,62]</sup> or agriculture irrigation<sup>[33,62]</sup>. This situation is further aggravated when considering that  $\geq 90\%$  of the cysts isolated from water samples were viable<sup>[34,60]</sup>.

Finally, a single *Giardia* isolate from an oyster has been characterized as assemblage B (zoonotic)<sup>[52]</sup>. It is well-known that bivalve mollusks are filter feeders that can accumulate a wide range of small particles including pathogens (i.e., viruses, bacteria, protozoa) and inorganic substances (i.e., heavy metals). This feature can be used for sanitary assessment of water quality<sup>[86]</sup>. The authors proposed a mammalian source of contamination by faecal material, possibly involving humans, dogs or rodents.

## CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In light of the epidemiological evidence compiled over the past 20 years, it is clear now that *Giardia* infections are common in human, livestock and pet animal populations and wildlife in Spain. In addition, viable *Giardia* cysts are also frequently found in environmental samples (including drinking, recreational, and irrigation waters) and raw foods. Although limited in number of surveys and number of isolates genetically identified, current molecular data seem to indicate that human giardiasis in Spain is largely characterized by an anthroponotic transmission cycle, with a relatively small contribution from zoonotic reservoirs in production animals. These preliminary results must, however, be confirmed in future surveys and extended to other geographical areas and new human and animal populations, including companion animals and wildlife. A priority task is the investigation of the genetic diversity of *Giardia* in order to complete our current (and partial) understanding of the host range, transmission dynamics and zoonotic potential of this pathogen. Mainly lacking is information about the genotypes and subgenotypes commonly present in pet animals including dogs and cats. Molecular data from certain farm animal populations (i.e., pigs) and wildlife (particularly those species in close contact with surface waters) are also completely absent. This information is essential to ascertain the role of those species as reservoirs of human disease, to identify environmental sources of contamination and to implement appropriate control measures to ensure the prevention of transmission. In addition, research aiming to demonstrate transmission of giardiasis between animals and humans (and vice versa) is highly needed. These surveys must be based on longitudinal follow-up and simultaneous detection of the same *Giardia* subgenotypes in both humans and animals sharing the same household or focus of endemicity.

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