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The Current Molecular Epidemiological Scenario of Cryptosporidium, Giardia and Blastocystis in Spain. Implication for Public Health

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1 **The current molecular epidemiological scenario of *Cryptosporidium*, *Giardia* and**

2 ***Blastocystis* in Spain. Implication for public health**

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Abstract

The enteric protozoan parasites *Cryptosporidium* spp. and *Giardia duodenalis* are major

contributors to the burden of gastrointestinal diseases globally. Both pathogens

primarily affect children living in resource-poor settings with limited or no access to

clean water and sanitation facilities, but are also significant public health threats in

developed countries. Additionally, *Cryptosporidium* spp. and *G. duodenalis* are

common causes of waterborne and foodborne outbreaks of gastrointestinal disease

globally. Besides, the Stramenopile *Blastocystis* sp. is the most common eukaryotic

organism reported in the human gut. Although its pathogenicity is a topic of debate,

there is increasing evidence demonstrating that this protist can be associated with

gastrointestinal disorders (diarrhoea, irritable bowel syndrome) and extra-intestinal

manifestations, including urticaria.

Because *Cryptosporidium* spp., *G. duodenalis* and *Blastocystis* sp. share the

same transmission (faecal-oral) route, are able to infect a wide range of animal species

other than humans with variable host specificities, and their infective forms are

environmentally resilient, the study of these pathogens should be ideally approached

under the One Health umbrella. In this context, molecular-based methods including

PCR and sequencing provide powerful tools to investigate the epidemiology and

transmission of these parasites.

In Spain, cryptosporidiosis and giardiosis, but not blastocystosis, are notifiable

diseases. However, the true incidence of these infections remain largely unknown

because underdiagnosing and underreporting. Symptomatic cryptosporidiosis and

giardiosis disproportionally affect children under four years of age, but we know now

that subclinical infections are also common in apparently healthy individuals of all age

groups. However, molecular data regarding the frequency and diversity of these

pathogens are limited and spatially and temporally discontinuous. This chapter aims to

provide, from a public veterinary health perspective, an updated account on the

epidemiology of *Cryptosporidium*, *G. duodenalis* and *Blastocystis* in Spain, with an

emphasis on the description of the species/genotypes circulating in symptomatic and

asymptomatic human populations. Current knowledge on the presence of these

pathogens in production (livestock), companion (dogs and cats) and wildlife animal

species is also discussed, including their potential role as natural reservoirs of human

infections, and the available evidence of zoonotic (and anthroponotic) transmission

events.

1. Introduction

The enteric protozoan parasites *Cryptosporidium* spp. (Apicomplexa: Cryptosporidiidae), and *Giardia duodenalis* (Metamonada: Hexamitidae) are among the most important causes of protozoan-diarrheal illness in humans and animals globally. Clinical manifestations vary from asymptomatic carriage to acute diarrhoea and chronic disease. Transmission of giardiosis and cryptosporidiosis is through the faecal-oral route, either indirectly via accidental ingestion of contaminated water or food, or directly via human-to-human or animal-to-human contact. Approximately 200 million people in Asia, Africa and Latin America present symptomatic giardiosis with some 500,000 new cases reported each year (WHO, 1996). Cryptosporidiosis is a leading cause of diarrhoeal death in children younger than 5 years in sub-Saharan Africa and Southeast Asia, only second after rotaviral enteritis (GBD, 2016). Both giardiosis and cryptosporidiosis primarily affect young children living in low-income countries and are strongly linked with malnutrition, growth faltering, and cognitive impairment (Guerrant et al., 1999; Halliez et al., 2013). These very same disorders, although to a much lower extent, have been described in some Spanish paediatric populations (Azcona-Gutiérrez et al., 2017). The Stramenopile *Blastocystis* sp. is one of the most common enteric parasites found in human stool samples. It is estimated that up to 1 billion people would be colonized/infected with this protozoan species globally (Clark et al., 2013). The clinical and public health relevance of *Blastocystis* sp. remains controversial, as it is commonly found in both apparently healthy individuals and patients suffering from intestinal (diarrhoea, irritable bowel syndrome) and extra-intestinal (urticaria) manifestations (Scanlan et al., 2014). Hence, it is currently not possible to distinguish between colonization and infection.

At least 38 *Cryptosporidium* species have been named today, of which *C. hominis* and *C. parvum* account for ~90% of human cryptosporidiosis cases globally (Ryan et al., 2014). The only *Giardia* species that is pathogenic to humans, *G. duodenalis*, is indeed a species complex consisting of eight (A to H) assemblages; assemblages A and B, which commonly infect humans and other mammals, assemblages C and D in dogs and other canids, assemblage E in hoofed animals, assemblage F in cats, assemblage G in rodents and assemblage H in pinnipeds (Cacciò et al., 2018). Extensive genetic diversity has also been demonstrated within *Blastocystis* sp., allowing the differentiation of at least 17 subtypes (ST), of which STs 1–9 and 12 infect humans, among other mammal species (Stensvold et al., 2007; Ramírez et al., 2016). *Blastocystis* ST1–4 are the subtypes more frequently identified in humans. Because *Cryptosporidium* spp., *G. duodenalis*, and *Blastocystis* sp. genetic variants have marked differences in host range and specificity, molecular epidemiological surveys are essential not only to determine the frequency and diversity of species/genotypes in a given population or geographical region, but also to characterize transmission pathways, to identify the range of host species able to harbour these pathogens, and to assess zoonotic (or anthroponotic) potential.

2. The Spanish epidemiological scenario

The epidemiology of *G. duodenalis* and *Cryptosporidium* spp. in Spain has been the subject of previous reviews (Navarro-i-Martinez et al., 2011; Carmena et al., 2012), so the interested reader is referred to them for in-depth information. Regarding human infections, most of the available data comes from observational, transversal studies conducted in schoolchildren populations by conventional (microscopy) diagnostic methods. In brief, reported prevalence rates of human giardiosis ranged from 3–7% in

asymptomatic individuals to 13–25% in symptomatic individuals. *Giardia duodenalis*

infections have also been identified in ovine (3–44%), bovine (5–30%), canine (5–

20%), feline (14%), and wildlife (1–16%) animal populations (Table 1). Similarly,

Cryptosporidium infections were more frequently found in individuals declaring

gastrointestinal complaints (5–30%) than in asymptomatic subjects (1–5%).

Cryptosporidium spp. infections were a common cause of diarrhoea in young calves (4–

60%) and lambs (5–90%). In contrast, *Cryptosporidium* spp. infections in domestic

dogs (7–15%) and cats (5–10%) were mostly reported in asymptomatic animals (Table

1).

Importantly, *Giardia* cysts and *Cryptosporidium* oocysts have been found in up

to 100% of the surface waters intender for human or agricultural consumption (Carmena

et al., 2007; Castro-Hermida et al., 2009), including recreational river areas (Castro-

Hermida et al., 2010). When the viability of the (oo)cysts was assessed with vital dyes,

infective forms of *Giardia* and *Cryptosporidium* were detected in 57–61% of the

samples analysed (Castro-Hermida et al., 2010). (Oo)cysts of both pathogens were also

a frequent finding (16–60%) in packed salads and other fresh produce (e.g. Amorós et

al., 2010). The fact that infective *G. duodenalis* cysts and *Cryptosporidium* oocysts are

regularly found in drinking waters and ready-to-eat, fresh meals explain why these

pathogens are common causes of waterborne and foodborne outbreaks of diarrhoeal

illness globally (Efstratiou et al., 2017; Ryan et al., 2018, 2019).

In addition, *Giardia* cysts and *Crytosporidium* oocysts have been isolated from

soil samples in Spanish public parks (Martínez-Moreno et al, 2007; Dado et al., 2012),

Although these studies did not assess neither the viability/infectivity of the parasite

forms nor their species/genotypes, environmental contamination with faecal material

from companion animals or even humans can be a source of infection to people in close

129   contact with contaminated soil, particularly children playing in sand pits on public

130   grounds.

131   Comparatively far lesser epidemiological information is currently available on

132   *Blastocystis* carriage/infection in Spain (reviewed in Paulos et al., 2018). As in the case

133   of *G. duodenalis* and *Cryptosporidium* infections, most of the research conducted on

134   *Blastocystis* sp has been generated in microscopy-based prospective (Del Aguila et al.,

135   1997; Paulos et al, 2018) or clinical retrospective (González-Moreno et al., 2011;

136   Salvador et al., 2016) studies (Table 1). *Blastocystis* sp. has also been identified in

137   intensively reared pigs (Navarro et al., 2008) and farmed ostriches and rheas (Ponce

138   Gordo et al., 2002).

139

140   **3.   Current surveillance and research limitations**

141   Cryptosporidiosis and giardiosis, but not blastocystosis, are compulsory notifiable

142   diseases in Spain since March 2015 (Table 1). Based on official data, a total of 1,627

143   and 646 confirmed cases of giardiosis and cryptosporidiosis were reported in Spain in

144   2015 (ECDC 2018a,b). Of note, these figures represent a fraction of the true number of

145   cases. Variable health care seeking behaviour by patients, poor awareness among

146   primary care physicians, underdiagnosing, and underreporting are all important

147   contributing factors to this problem. When uncertainties associated to those factors are

148   considered, the true number of cases are expected to be 2–3 order of magnitude higher

149   than the declared number of cases (Cacciò and Chalmers, 2016). Information regarding

150   the situation of human blastocystosis is even scarcer. In practical terms this general lack

151   of information means that the seasonal distribution of these enteric parasites along the

152   year are largely unknown. Historical case series at national scale are also lacking.

153   Seasonal patterns have been investigated at regional level during relatively short periods

154   of time. These studies have evidenced that *Cryptosporidium* cases peak during late

155   summer and early autumn (Abal-Fabeiro et al., 2015; Azcona-Gutiérrez et al., 2017).

156   The very same temporal trend has been previously reported in other European countries

157   including Germany, The Netherlands, and UK and has been associated to increased

158   travel and exposure to recreational water (Chalmers et al., 2009; Fournet et al., 2013).

159   Importantly, molecular studies investigating the occurrence and genetic

160   variability of *Cryptosporidium* spp., *G. duodenalis* and *Blastocystis* sp. infections in

161   human and animal populations and in environmental (water, soil) samples in Spain are

162   limited (reviewed in Navarro-i-Martinez et al., 2011; Carmena et al., 2012; Paulos et al,

163   2018). Active molecular-based epidemiological surveillance has been demonstrated as a

164   very useful tool in outbreak investigations (e.g. Fuentes et al., 2015) and to identify

165   novel or rare species/genotypes of enteric parasites in clinical samples (Martínez-Ruiz

166   et al., 2016; Merino et al., 2019; Millán et al., 2019).

167   No routine surveillance or monitoring programs to detect the presence of the

168   infective stages of *Cryptosporidium* spp., *G. duodenalis*, and *Blastocystis* sp. in drinking

169   water and fresh produce are currently in place in Spain. Indeed, current legislation does

170   not specify the maximum concentration levels of these pathogens in water and food

171   matrices.

172

173   **4.   Molecular epidemiological research in humans**

174   The molecular diversity of *G. duodenalis*, *Cryptosporidium* spp., and *Blastocystis* sp. in

175   human infections in Spain has been mainly assessed in clinical populations with or

176   without clinical manifestations. In some instances, these parasites were accidentally

177   identified during routine testing at clinical laboratories. This is particularly true for

178   *Blastocystis* sp. Available genotyping data indicate that *Giardia duodenalis* assemblage

179   B is more prevalent than assemblage A in most of the surveys conducted (Sahagún et

180   al., 2008; de Lucio et al., 2015; Gabín-García et al., 2017), although both genetic

181   variants have been detected at similar frequencies in other studies (Azcona-Gutiérrez et

182   al., 2017). At the sub-genotype level BIV is the most common sub-assemblage

183   circulating in those clinical patient populations. Similarly, sub-assemblage BIV has

184   been the predominant genotype found in children attending day care centres (Mateo et

185   al., 2014), in schoolchildren (Cardona et al., 2011), and in community surveys (de Lucio

186   et al., 2017). These data suggest that, besides parasite genotype, other determinants (e.g.

187   co-infections, host immune status, microbiome) may play a role in the progression from

188   asymptomatic carriage to symptomatic infection. A recent study investigating *G.*

189   *duodenalis* infections in individuals of all ages with and without clinical symptoms

190   revealed that children were more commonly infected by assemblage B, whereas

191   asymptomatic infection was more common in patients with assemblage A than in those

192   with assemblage B (Wang et al, 2019). Importantly, asymptomatic carriage of *G.*

193   *duodenalis* has been demonstrated by PCR in a larger than expected proportion of

194   apparently healthy schoolchildren attending primary and secondary schools in Madrid

195   (Reh et al., 2019). This finding may have important consequences as unnoticed school

196   and household transmission events could represent a public health issue for at-risk

197   populations such as young children, the elderly and immunocompromised individuals.

198   Regarding cryptosporidiosis, human cases in Spain are primarily caused by *C.*

199   *hominis* (80–90%) and *C. parvum* (10–18%) (de Lucio et al., 2015; Segura et al., 2015;

200   Azcona-Gutiérrez et al., 2017), but these frequencies may vary depending on the region

201   considered. For instance, in the Autonomous region of Galicia (northwest Spain), it was

202   *C. parvum* and not *C. hominis* the main *Cryptosporidium* species detected in summer

203   peaks during the period 2000–2008, particularly in rural areas (Abal-Fabeiro et al.,

204 2015). Considering that this Spanish region breeds and feeds large populations of dairy

205 and beef cattle, the finding mentioned above has been interpreted as the consequence of

206 zoonotic *C. parvum* transmission. Infections by less common *Cryptosporidium* species

207 including *C. meleagridis* (Abal Fabeiro et al., 2014; Segura et al., 2015), *C. canis* (Abal-

208 Fabeiro et al., 2014; de Lucio et al., 2016), and *C. cuniculus* (Martínez-Ruiz et al.,

209 2016) have also been reported sporadically, some of them reflecting zoonotic events.

210 These surveys revealed that IbA10G2 and IIdA15G2 were the sub-genotypes more

211 prevalent within *C. hominis* and *C. parvum*, respectively. Taken together, available

212 molecular data seem to indicate that human giardiosis and cryptosporidiosis in Spain are

213 primarily of anthroponotic origin, with a comparatively smaller contribution from

214 zoonotic reservoirs in production and companion animals (Abal-Fabeiro et al., 2014,

215 2015; Azcona-Gutiérrez et al., 2017).

216   In Spain, ST4 has been demonstrated as the most prevalent (94%) *Blastocystis*

217 subtype circulating in symptomatic, mono-infected patients (Dominguez-Marquez et al.,

218 2009). Interestingly, ST4 has been detected at considerable lower rates than ST1–3 in

219 general (Paulos et al., 2018) and schoolchildren (Reh et al., 2019) populations in

220 different Spanish geographical regions, suggesting that ST4 may be more pathogenic

221 than other *Blastocystis* subtypes commonly seen in humans.

222

223 **5.   Molecular epidemiological research in livestock**

224 In Spain, molecular genotyping studies have been conducted in *G. duodenalis*-positive

225 samples from cattle, sheep, and goats; these surveys revealed that assemblage E was the

226 most prevalent genotype in all the species studied, ranging from 64% to 100% in cattle

227 and sheep and 100% in goats (Castro-Hermida et al., 2006, 2007, 2011a; Cardona et al.,

228 2015). Zoonotic assemblage B was identified in 8–35% of sheep, whereas assemblage

229   A sub-assemblage AI was found in 1% of goats. In cattle, genetic analyses for *G.*

230   *duodenalis* have been conducted in asymptomatic animals including neonatal calves,

231   heifers, and cows (Castro-Hermida et al., 2007, 2011a). Besides assemblage E, sub-

232   assemblage AI was also identified in 36% of cattle.

233   *Cryptosporidium* infections in cattle have a marked age-related pattern; thus, *C.*

234   *parvum* is more prevalent in pre-weaned calves, whereas *C. bovis*, *C. andersoni*, and *C*

235   *ryanae* are more frequently found in post-weaned calves, heifers, and adult animals

236   (Santín et al., 2008). Molecular epidemiological data from Spanish herds confirm this

237   trend (Castro-Hermida et al., 2007, 2011a; Quilez et al., 2008b; Cardona et al., 2015).

238   Calves, lambs, and goats infected with *C. parvum* have been demonstrated to harbour

239   zoonotic sub-genotypes (e.g. IIaA15G2) of the parasite and should, therefore,

240   considered as natural reservoirs of human cryptosporidiosis (Quílez et al., 2008a; Díaz

241   et al., 2010). Intriguingly, feline-specific *G. duodenalis* assemblage G and *C. felis* have

242   been detected in cattle in northern Spain (Cardona et al., 2015). This finding reveals that

243   inter-species transmission of apparently host-specific species/genotypes is possible

244   when environmental conditions are favourable.

245   Overall, molecular epidemiological data from Spain were in line with those

246   suggesting that the actual role of livestock as major reservoirs for *G. duodenalis* and

247   *Cryptosporidium* spp. infections to humans might be much less relevant than initially

248   anticipated (O'Handley, 2007; Feng and Xiao, 2011). The molecular diversity of

249   *Blastocystis* sp. carriage/infections in livestock is unknown at present.

250

251   **6.   Molecular epidemiological research in companion animals**

252   Molecular epidemiological studies investigating the genetic diversity of *G. duodenalis*,

253   *Cryptosporidium* spp., and *Blastocystis* sp. in domestic dogs and cats in Spain are

254 particularly scarce. These studies (conducted in stray, sheltered, and owned animals)

255 showed that both *G. duodenalis* and *Cryptosporidium* infections were primarily caused

256 by canine-specific (e.g. *G. duodenalis* assemblages C and D, *C. canis*) or feline-specific

257 (e.g. *G. duodenalis* assemblage F, *C. felis*) genetic variants of these parasites (Ortuño et

258 al., 2014; de Lucio et al., 2017). However, other surveys have demonstrated that dogs

259 can harbour infections by potentially zoonotic *G. duodenalis* sub-assemblages AII, BIII,

260 and BIV. However, most of these genetic variants contained single nucleotide

261 polymorphisms that were not present in sequences of human origin belonging to the

262 same sub-assemblages. Taken together, these facts seem to suggest that sub-

263 assemblages AII, BIII, and BIV may be naturally circulating in canine populations (Gil

264 et al., 2017; Adell-Aledón et al., 2018; Sanchez-Thevenet et al., 2019). Zoonotic

265 transmission of giardiasis and cryptosporidiosis could not been demonstrated between

266 pet dogs and cats and their owners in northern Spain, indicating that this kind of events

267 should be relatively infrequent. Intriguingly, *C. hominis* of unknown sub-genotype was

268 detected in a sheltered dog in this very same geographical region (Gil et al., 2017).

269 Whether this finding represents a true infection or an accidental acquisition and

270 mechanical carriage of *C. hominis* oocysts of anthroponotic origin via environmental

271 contamination remains to be clarified in further molecular studies. Neither companion

272 dogs nor cats seem suitable hosts for *Blastocystis* sp. (Paulos et al., 2018).

273

274 **7.   Molecular epidemiological research in wildlife**

275 Previous epidemiological studies have reported the occurrence of *Giardia duodenalis*

276 and *Cryptosporidium* spp. infections in small rodents and insectivores (Torres et al.,

277 2000), birds (Reboredo-Fernández et al., 2015; Cano et al., 2016), ungulates including

278 roe deer and wild boars (Castro-Hermida et al., 2011a,b), and several carnivore species

279   including badgers, red foxes, genets, beech martens, otters, and wolves (Méndez-

280   Hermida et al., 2007; Mateo et al., 2017). Available molecular data revealed that

281   zoonotic *G. duodenalis* assemblage B was present in buzzards, quails, and magpies

282   (Reboredo-Fernández et al., 2015). The finding of BIV (the *G. duodenalis* sub-

283   assemblage more prevalent in Spanish human populations) in waterfowl may represent

284   a public health concern, as these birds commonly nest, breed, and feed in source waters

285   intended for human consumption (Cano et al., 2016).

286   Since its naming in 2002 (Morgan-Ryan et al., 2002), *C. hominis* has been

287   largely recognized as a human-specific species. However, this notion has been

288   challenged by recent experimental and molecular epidemiological data demonstrating

289   that *C. hominis* is able to successfully infect a wide range of mammal species other than

290   human and non-human primates including cattle (e.g. Razakandrainibe et al., 2018),

291   sheep (e.g. Connelly et al., 2013), donkeys (Jian et al., 2016), kangaroos (Zahedi et al.,

292   2018), and field mice (Čondlová et al., 2018), among others. In Spain, no evidence of *C.*

293   *hominis* infections circulating in livestock has been described yet, but *C. hominis*

294   oocysts have been identified in the intestinal tract of a domestic dog (Gil et al., 2017), a

295   badger (Mateo et al., 2017) and four red foxes (Montoya et al., 2019). These findings

296   may be just the consequence of passive carriage of ingested oocysts but may also

297   represent true infections as consequence of spill-over events of anthroponotic *C.*

298   *hominis* from humans to companion and wildlife (Figure 1).

299

300   **8.   Molecular epidemiological research in surface waters**

301   *Giardia* cysts and *Cryptosporidium* oocysts have been reported in up to 100% of the

302   surface waters intender for human consumption, and in 27% of tap water from

303   municipalities in northern Spain with chlorination treatment only (Carmena et al.,

304   2007). Both parasites have also been detected in the final effluent of drinking water

305   treatment plants at concentrations of 0–4 (oo)cysts per litre in northwest Spain (Castro-

306   Hermida et al., 2008, 2015), and at concentrations <1 (oo)cyst per litre in north-eastern

307   Spain (Ramo et al., 2017). Vital staining dyes revealed that up to 95% of the (oo)cysts

308   identified were viable (Castro-Hermida et al., 2015). Sequence analyses allowed the

309   identification of a wide range of species/genotypes, including *C. hominis*, *C. parvum*, *C.*

310   *andersoni*, *C. ubiquitum*, and *C. muris* within *Cryptosporidium*, and assemblages A

311   (including sub-assemblages AI and AII) and E within *G. duodenalis* (Galván et al.,

312   2014; Castro-Hermida et al., 2015). In addition, *Cryptosporidium* oocysts have also

313   been detected in 19% of public swimming pools with a maximum concentration of 13

314   oocysts per litre (Gracenea et al, 2018). Considered together, these results demonstrate

315   that both *Cryptosporidium* spp. and *G. duodenalis* are ubiquitous in environmental and

316   recreative waters in Spain. This fact, together with the ineffectiveness of treatments in

317   drinking water treatment plants in eliminating/inactivating the (oo)cysts of both

318   protozoan species represent a perceptible risk for waterborne outbreaks of giardiosis

319   and/or cryptosporidiosis, and a serious concern for the water industry and the public

320   health authorities responsible for assuring the safety and quality of drinking water.

321

322   **9.   Concluding remarks**

323   Despite the unquestionable progress achieved in the last two decades, the epidemiology

324   of the diarrhoea-causing enteric parasites *G. duodenalis*, *Cryptosporidium* spp., and

325   *Blastocystis* sp. in Spain remain insufficiently understood. Current molecular data

326   demonstrate that the transmission of these pathogens and the factors that determine their

327   pathogenicity are more intricate than initially anticipated and involve interconnected

328   human, animal, and environmental reservoirs. The species/genotypes involved in the

14

329   infections are an important factor in determining the pathogenicity of the infection, but

330   it is not the only determinant in the equation. Other variables including co-infections,

331   host age and immune status, and microbiota abundance and diversity are also key actors

332   in tipping the balance between health and disease.

333

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335   None declared.

336

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343

344   **References**

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611

Tables

Table 1. Main surveillance, epidemiological, and research features of *Giardia duodenalis*, *Cryptosporidium* spp., and *Blastocystis* sp. in Spain.

	Giardia duodenalis	*Cryptosporidium* spp.	*Blastocystis* sp.
Surveillance			
Notifiable disease?	Yes[3]	Yes[3]	No
Official number of cases[1]	1,627	646	Unknown
Time series?	No	No	No
Outbreak investigation	Limited	Limited	No
Prevalence[2] (%)			
Asymptomatic humans	3–7	1–5	3–22
Symptomatic humans	13–25	5–30	8–23
Ovine	3–44	5–90	Unknown
Bovine	5–30	4–60	2
Dogs	5–20	7–15	0
Cats	14	5–10	0
Wildlife	1–16	1–27	2
Surface waters	25–100	15–90	Unknown
Fresh produce	16–50	20–60	Unknown
Research			
Genotyping studies?	Limited	Limited	Limited
Transmission studies?	Limited	Limited	Limited

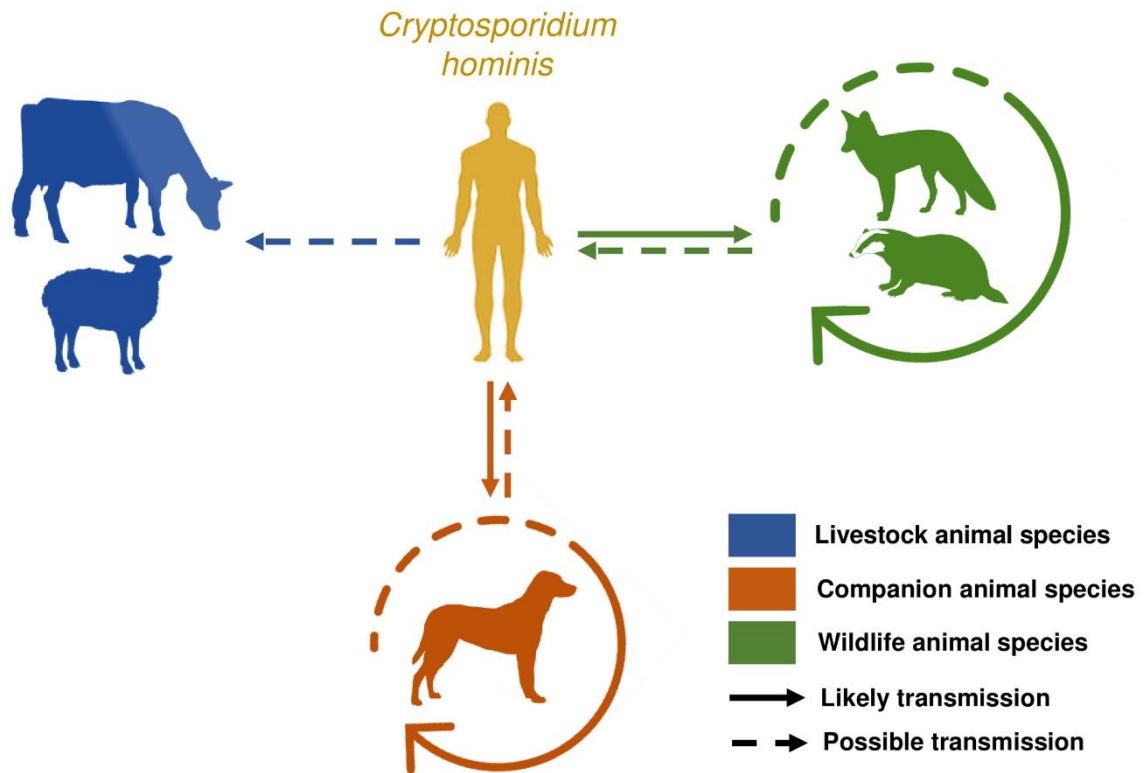614   [1] Confirmed cases reported to the European Centre for Disease Prevention and Control in 2015.

615   [2] Composite data from references Carmena et al. World J Clin Infect Dis. 2012;2:1–12; Navarro-i-Martinez et al. Enferm Infecc Microbiol Clin.

616   2011;29:135–143; and Paulos et al., Zoonoses Public Health. 2018;65(8):993–1002.

617   [3] Since 2015.

618

619 **Legends to Figures**

620 **Figure 1.** Potential transmission pathways of *Cryptosporidium hominis* among human,

621 livestock, companion, and wildlife animal species in Spain according to current molecular

622 epidemiological data.

623