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Review Article

Cryptosporidium hominis infections in non-human animal species: Revisiting the concept of host specificity

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Abstract

Parasites in the genus *Cryptosporidium*, phylum Apicomplexa, are found worldwide in the intestinal tract of many vertebrate species and in the environment. Driven by sensitive PCR methods, and the availability of abundant sequence data and reference genomes, the taxonomic complexity of the genus has steadily increased; 38 species have been named to date. Due to its public health importance, *Cryptosporidium hominis* has long attracted the interest of the research community. This species was initially described as infectious to humans only. This perception has persisted in spite of an increasing number of observations of natural and experimental infections of animals with this species. Here we summarize and discuss this literature published since 2000 and conclude that the host range of *C. hominis* is broader than originally described. The evolving definition of the *C. hominis* host range raises interesting questions about host specificity and the evolution of *Cryptosporidium* parasites.

Keywords: *Cryptosporidium hominis*; Host specificity; Speciation; Experimental infection; Natural infection; Transmission

1. Introduction

The genus *Cryptosporidium* belongs to the phylum Apicomplexa and comprises an unknown number of species of parasitic protozoa. Infection with *Cryptosporidium* parasites is known as cryptosporidiosis. It ranks among the most important causes of infectious diarrhea, particularly in infants living in low-income regions of the world (Kotloff et al., 2013). According to the National Center for Biotechnology Information's Taxonomy Browser, 38 *Cryptosporidium* spp. have been named to date. The number and taxonomy of the genus is likely to change. Species are typically defined on the basis on host range and information obtained from sequencing polymorphic genetic loci. With the continuous fall of DNA sequencing costs, more genomes will no doubt be sequenced, more genetic variation will be uncovered, and additional species will likely be named. The trend for naming species was initially rooted in the observation that different host species tend to be infected with genotypically distinct *Cryptosporidium* parasites. Examples of this approach are *Cryptosporidium canis*, *Cryptosporidium muris*, *Cryptosporidium felis* or *Cryptosporidium erinacei*. In the absence of easily observable morphological traits or other observable phenotypes, host specificity has played a fundamental role in the definition of new species. The biological definition of species, i.e., a reproductively isolated population, has not been taken into consideration when proposing new species because live oocysts are rarely available and non-overlapping host ranges make it difficult to perform crossing experiments in mixed infections to test reproductive compatibility. The rationale for defining *Cryptosporidium* spp. is consistent with the observed segregation of genotypic polymorphisms according to host species. The partial correspondence between host range and genotype has logically been interpreted as the outcome of a speciation process resulting from the reproductive isolation of *Cryptosporidium* genotypes infecting different host species

(Feng et al., 2018). As is often the case in biological research, every model has exceptions. In the world of *Cryptosporidium* speciation, such exceptions are genetically defined populations which infect a taxonomically diverse range of hosts. Prime examples of such populations are the human zoonotic pathogens *Cryptosporidium parvum* and *Cryptosporidium meleagridis*. In terms of host range, the latter species is unique for its broad host range which includes birds (Slavin, 1955), humans and other mammals (Chappell et al., 2011). Putative genetic determinants of host range have been described (Li et al., 2017), but due to a lack of suitable research tools, *Cryptosporidium* "host specificity genes" remain to be discovered, assuming they exist.

Since its naming in 2002 (Morgan-Ryan et al., 2002), *C. hominis* has been considered a species capable of infecting only humans. Sporadic reports of livestock infected with *Cryptosporidium hominis* (Table 1) have not been sufficiently numerous to detract from the established notion of *C. hominis* exclusively parasitizing humans. From an evolutionary perspective, *C. hominis* raises interesting questions. Akiyoshi et al. (2003) reported that in germ-free neonatal pigs co-infected with *C. hominis* and *C. parvum*, the latter species was more virulent, eventually “displacing” *C. hominis* in mixed infections (Akiyoshi et al., 2003). Assuming *C. hominis* would be at a similar competitive disadvantage in natural human infections, and assuming *C. hominis* infects no other species than humans and taxonomically related primates, *C. hominis* would be at risk of being displaced from the human population, the only species it is believed to be capable of infecting, and could logically be assumed at risk of becoming extinct. Clearly this is not the case. The epidemiology of the *C. hominis* model needs revising. With this aim, here we review the literature for evidence of *C. hominis* having a broader host range than was originally assumed (Morgan-Ryan et al., 2002).

2. Search strategy

An electronic search of the database PubMed (https://www.ncbi.nlm.nih.gov/pubmed) was conducted without restrictions in language of publication. The results were limited to articles published from January 2000 to December 2019. The search terms used included (*Cryptosporidium hominis* OR *C. hominis*) AND (livestock OR cattle OR sheep OR goat OR pig OR companion OR domestic OR dog OR cat OR wildlife OR free-living OR non-human primate OR monkey OR captive OR zoo OR experimental infection). To be eligible for inclusion, studies had to report experimental infections, cross-sectional or longitudinal studies, molecular epidemiological studies, or case reports. Regarding the diagnosis strategy, only studies based on molecular (PCR and Sanger sequencing) methods were considered. A detailed list with all non-human animal species and their taxonomic classification where *C. hominis* DNA has been detected can be found at Mendeley Data (https://data.mendeley.com/, DOI: 10.17632/kytr45wyvc.2).

3. Experimental infections with *Cryptosporidium hominis* oocysts in animal models

The susceptibility of the neonatal germ-free pig to *C. hominis* was first observed in 2000 (Widmer et al., 2000). Prior to 2002, *C. hominis* was referred to in the literature as *C. parvum* genotype H (Widmer et al., 1998) and later genotype 1 (Sestak et al., 2002). The change in nomenclature and the elevation of genotype H (genotype 1) to species (*C. hominis*), makes the early literature on *C. hominis* difficult to navigate. Although the infrastructure to work with germ-free piglets is not typically available in research laboratories, the pig model has been an important tool to study *C. hominis*, its interaction with the host (Pereira et al., 2002), with other *Cryptosporidium* spp.

(Sheoran et al., 2018), for drug development (Lee et al., 2017, 2018, 2019), and to maintain isolate TU502, the only laboratory-propagated line of *C. hominis*. Ebeid and colleagues (Ebeid et al., 2003) also reported that conventional, colostrum-deprived new-born piglets were susceptible to *C. hominis* (Table 2). The fact that in their experiments only one of two animals developed cryptosporidiosis following infection with 1–2 million oocysts is worth noting.

The report that Mongolian gerbils (*Meriones unguiculatus*) immunosuppressed with dexamethasone were susceptible to *C. hominis* (Baishanbo et al., 2005) came as a surprise, as rodents were until then assumed to be resistant to this species (Akiyoshi et al. 2002; Morgan-Ryan et al., 2002). Since the publication of gerbil infectivity experiments by Baishanbo et al. in 2005, no other laboratory appears to have adopted the model. Experience with this model by one of the current authors (G. Widmer) showed that the gerbil is difficult to work with, primarily due to the narrow range for useful and safe dexamethasone treatment. Insufficiently immunosuppressed animals do not excrete oocysts, whereas gerbils can quickly succumb if exposed to high dexamethasone doses. Since the publication of observations by Baishanbo et al. in 2005, the report has been cited only six times and none of the citing references describe original research using the gerbil model of *C. hominis* infection. Interestingly, neonatal gerbils were reported to be resistant to *C. hominis* (Akiyoshi et al., 2002).

Experimental infections of ruminants with *C. hominis* are few but nonetheless significant in the context of this review (Table 2). Giles et al. (2001) were able to infect one of two colostrum-deprived lambs following the inoculation of 2×10^5 *C. hominis* oocysts originating from a symptomatic human patient (Giles et al., 2001). The infection progressed slowly, reaching its peak 15 days p.i. The lamb shed relatively few oocysts. The susceptibility of lambs to *C. hominis* was later confirmed (Ebeid et al.,

2003). It is revealing that in this study only one of six lambs became infected despite the very large dose (1–2 million) of oocysts administered. A third instance of successful experimental infection of a ruminant with *C. hominis* was reported by Akiyoshi et al. (2002). This observation had practical implications; sequencing of the genome of the *C. hominis* (Xu et al., 2004) was made possible by oocysts produced in a calf. In both instances, neonatal calves derived by vaginal birth were recovered using stringent conditions to avoid transmission of *C. parvum* from the cow at birth (Akiyoshi et al., 2002). Calves were orally infected on day 0 or 1 p.i. with 2 million oocysts, resulting in a brief infection and the abundant production of oocysts. DNA from these oocysts was genotyped to ensure that the calf was excreting *C. hominis* and subsequently used to sequence the genome (Xu et al., 2004). Akiyoshi et al. (2002) reported that they were unable to infect interferon-γ knock-out mice (Akiyoshi et al., 2002), which are highly susceptible to *C. parvum* (Griffiths et al., 1998).

A review of experimental infections with *C. hominis* would not be complete without mentioning research with human volunteers. Based on the intentional infection of 21 healthy adult volunteers with doses of TU502 oocysts ranging from 10–500, the 50% infectious dose was estimated to range between 10 and 83 oocysts, depending on the definition of infection (Chappell et al., 2006). The very low infectious dose contrasts with the large number of oocysts used in the animal infections cited above. These observations raise the possibility that the host range of *C. hominis* is to some extent a function of the infectious dose.

4. Transmission of *Cryptosporidium hominis* in production and companion animal species

Production animals including cattle, sheep, goats, horses, donkeys, and Bactrian camels have all been demonstrated to be competent hosts for *C. hominis* globally (Fig. 1 and Table 1). Bovine cryptosporidiosis is primarily caused by four *Cryptosporidium* spp. with a marked age-related prevalence: *C. parvum* predominantly infects pre-weaned calves, whereas *C. bovis* and *C. ryanae* are preferentially detected in asymptomatic cattle aged between 1 and 6 months, and *C. andersoni* in older animals (Santin et al., 2008). Known to be present as a minor sub-population during serial passage in experimentally infected calves (Tanriverdi et al., 2003), the first natural bovine infection by *C. hominis* was documented in clinically ill cattle (a calf and an adult cow) during a survey of the occurrence of cryptosporidiosis in livestock in Scotland, UK (Smith et al., 2005). Over the following few years, the parasite was sporadically reported in domestic cattle in South Korea (Park et al., 2007), a post-weaned calf and a cow with diarrhoea in India (Feng et al., 2007; Rajendran et al., 2011), three diarrheic cows in Malawi (Banda et al., 2009), three cows in Australia (Ng et al., 2011; Waldron et al., 2011), and a calf in England (Ghaffari et al., 2014). These studies showed that *C. hominis* was able to primarily cause light to moderate ($<2 \times 10^4$ oocysts/mL of faeces) infections in cattle of all ages, with and without clinical symptoms (diarrhoea). Epidemiological studies comprising larger sample sizes confirmed that *C. hominis* was sporadically present at low frequency rates in cattle herds from countries from three different continents including Australia (Zahedi et al., 2018), China (Chen et al., 2012), France (Razakandrainibe et al., 2015), Kenya (Kang´ethe et al., 2012), New Zealand (Abeywardena et al., 2012), and Uganda (Witto, S.G., Kankya, C., Kazibwe, A.J.N., Akurut, G., Ochwo, S., 2019. Occurrence of *Cryptosporidium hominis* in cattle bordering the Lake Mburo National Park in Kiruhura district, Western Uganda. bioRxiv 562793). Interestingly, *C. hominis* was the dominant

188 species of *Cryptosporidium* in calves in New Zealand (Abeywardena et al., 2012).
189 Although the origin of these infections was unclear, dispersal of wastewater effluent on
190 farm pastures (Abeywardena et al., 2012), sewage spill-over events in water catchment
191 areas (Zahedi et al., 2018), and waterborne transmission (Razakandrainibe et al., 2015)
192 were proposed as the most likely sources of anthroponotic *C. hominis* infections of
193 cattle.

194 Available molecular data on the frequency of *C. hominis* genotypes in bovines
195 are revealing. Cattle are primarily infected by genetic variants belonging to the 60 kDa
196 glycoprotein (*gp60*) genotype family Ib (predominantly IbA10G2), whereas the
197 IdA15G1 genotype has been described infecting cattle in Australia and France (Fig. 1
198 and Table 1). Remarkably, IbA10G2 is the most prevalent *C. hominis gp60* genotype
199 causing human cryptosporidiosis in Africa, Australia, and Europe (Waldron et al., 2009;
200 Cacciò and Chalmers, 2016; Squire and Ryan, 2017), whereas IdA15G1 has been
201 detected in patients with gastrointestinal disorders in Australia and it is also the
202 dominant genetic variant infecting Aboriginal people in that country (Koehler et al.,
203 2013; Ng-Hublin et al., 2017). Taken together, these data suggest that bovine
204 cryptosporidiosis caused by *C. hominis* is mainly of an anthroponotic nature.

205 Similar to what has been documented in cattle, *C. hominis* infections have also
206 been occasionally reported in ovine and caprine animals (Fig. 1 and Table 1). The first
207 description of a naturally occurring *C. hominis* infection in sheep was reported from
208 Australia (Ryan et al., 2005). Subsequent investigations reported the presence of the
209 parasite in a pygmy goat shedding a moderate amount of oocysts (5×10^5 oocysts/g of
210 faeces), and in a diarrhoeic 10 days old lamb from the UK, respectively (Giles et al.,
211 2009). Remarkably, *C. hominis* was the only *Cryptosporidium* sp. identified in domestic
212 goats in South Korea (Park et al., 2006), and the second most prevalent (after

213 *Cryptosporidium xiaoi*) in a large molecular epidemiological study conducted on a
214 geographically isolated herd of Scottish sheep (Connelly et al., 2013). All *C. hominis*
215 genotypes identified to date in sheep and goats have been assigned to IbA10G2 (Table
216 1), reinforcing the anthroponotic origin of these infections.

217 Host species susceptible to *C. hominis* include equines (horses and donkeys) and
218 camelids (Bactrian camels) (Fig. 1 and Table 1). Studies investigating the occurrence
219 and molecular diversity of *Cryptosporidium* spp. in these hosts tell us a very different
220 story than the one previously depicted in bovines, sheep and goats. Indeed, *C. hominis*
221 infections in equines and camelids appear to be specifically caused by the host-adapted
222 *gp60* genotype Ik. There is no evidence, so far, of infections caused by other *C. hominis*
223 genetic variants. Initially identified in a horse in Algeria (Laatamna et al., 2015), the
224 *gp60* genotype Ik has been further confirmed in two foals in the state of São Paulo,
225 Brazil (Inácio et al., 2017) and in an adult horse in China (Jian et al., 2016). In those
226 studies the genotype was confirmed with different genetic markers including actin,
227 *Cryptosporidium* oocyst wall protein (*cowp*), 70 kDa heat shock protein (*hsp70*), *gp60*,
228 and small ribosomal subunit (18S rRNA). Of interest, donkeys seem particularly suited
229 to harbouring *C. hominis* Ik infections, as demonstrated in two molecular surveys
230 conducted in China (Fig. 1 and Table 1). In the first survey, a *gp60* genotype belonging
231 to the family Ik (IkA16 and IkA16G1) was identified in 73% of the 82 microscopically
232 *Cryptosporidium*-positive donkey samples from five provinces/autonomous regions in
233 China (Jian et al., 2016). In the second survey, 75 out of 551 apparently healthy
234 donkeys from the Xinjiang Uygur Autonomous Region and Inner Mongolia harboured
235 *C. hominis* infections with a IkA16G1 genotype, accounting for the vast majority (94%,
236 75/80) of the cases of cryptosporidiosis detected in this host (Li et al., 2019). Of note,
237 *gp60* genotype family Ik effectively infects some species of non-human primates

(NHP), but reported human cases infected by this genetic variant of *C. hominis* are very rare (see section 6).

Finally, *C. hominis* has been identified based on the 18S rDNA (but not the *gp60*) sequence in a stray dog in northern Spain (Gil et al., 2017). To date, this is the only description of this species in companion animals globally. If confirmed in future studies, this finding may have important public health consequences due to the strong bond and close relationship between dogs and their owners.

5. *Cryptosporidium hominis* in wildlife other than primates

Cryptosporidium hominis oocysts have been molecularly detected in faecal samples from migratory birds of the family Anatidae (Canada geese), in ungulates (deer), marsupials (kangaroo, wallaby), marine mammals (dugong), mesocarnivores (badger, dingo, fox), frugivorous bats (flying fox), and rodents (field mouse) (Fig. 1 and Table 3). Although they do not represent true infections, *C. hominis* has also been identified in freshwater (Graczyk et al., 2001) and in marine bivalve molluscs cultivated for human consumption (Gómez-Couso et al., 2004).

Most of the reports describing the presence of *C. hominis* in wildlife species describe a very low number (1–4) of positive animals (Table 3), raising doubts whether these findings represent true infections (i.e. occurrence of endogenous developmental stages of the parasite) or are just the consequence of passive carriage of ingested oocysts (Zhou et al., 2004; Mateo et al., 2017). Ideally, suspected active infections should be confirmed by histological examination of the intestinal mucosa (Morgan et al., 2000), but this is not a practical option as most cases are identified a posteriori in epidemiological surveys of faecal DNA. Molecular and sequencing studies based on markers other than the 18S rDNA gene can also be useful to identify unexpected or rare

C. hominis genotypes. Interestingly, most of the *C. hominis*-positive samples detected by 18S-PCR in free-living animal species failed to yield amplicons at other loci such as *gp60* (Ng et al., 2011; Koehler et al., 2016). These results are indicative of light infections as, in contrast to the multi-copy 18S rDNA gene, other markers are single-copy and therefore more difficult to amplify from field samples. These observations suggest that *C. hominis* may cause lighter infections in non-human hosts. This hypothesis is consistent with the finding that faecal specimens from wildlife shedding *C. hominis* oocysts are typically well formed, suggestive of subclinical infections.

Australia is by far the geographical region where the epidemiology of *C. hominis* in wildlife has been investigated in greater detail (Fig. 1 and Table 3). As a result of a large monitoring program initiated in 2009, deer, dingoes, kangaroos, and wallabies living in the main drinking water catchment areas (some of them with little anthropogenic activity) have all been demonstrated to act as sources/carriers of *C. hominis* oocysts (Ng et al., 2011; Koehler et al., 2016; Zahedi et al., 2018). These data indicate that wildlife may play a not yet fully elucidated role in the contamination of watersheds with human-infectious oocysts, a possibility with some implications for the management of drinking water sources. DNA sequences generated in those surveys, although restricted to a limited number of samples, revealed that *gp60* genotypes IbA10G2 and IdA15G1 were circulating among kangaroo and wallaby populations (Koehler et al., 2016; Zahedi et al., 2018). Interestingly, Aboriginal people in Australia have been shown to be mainly infected with *C. hominis gp60* IdA15G1, whereas non-Aboriginal people were predominantly infected with the IbA10G2 *gp60* genotype (Ng-Hublin et al., 2017). Furthermore, alleles IbA10G2 and IdA15G1 have been found to be the most common *C. hominis* genotypes involved in waterborne cryptosporidiosis outbreaks in Australia during the period 2007–2011 (Ng-Hublin et al., 2018). As a

result of these outbreaks investigations, a clustering of cases with the IdA15G1 *gp60* genotype was detected in Aboriginal people living in remote areas, affecting primarily children under 4 years of age. Furthermore, the *C. hominis* IbA9G2 *gp60* genotype has been identified in a captive flying fox from the east coast of Australia (Schiller et al., 2016). The IbA9G2 genotype has been previously found circulating at low frequency in human clinical cases in the same geographical area (Waldron et al., 2009). Taken together, these data strongly suggest a spill-over of anthroponotic *C. hominis* from humans to wildlife, even in remote and pristine areas, very likely through contaminated water. However, the possibility that these *C. hominis* genotypes are endemic cannot be completely ruled out. Therefore, it is reasonable to think that at least some free-living animal species including kangaroos and wallabies can act as competent hosts for anthroponotic *C. hominis* and transmit it through sylvatic cycles. In this regard it should be noted that the reverse process, often overlooked, is also possible; human-borne *C. hominis* which moved into wildlife may be transmitted to humans (Kelly et al., 2009).

Additional evidence of *C. hominis* transmission from humans to wildlife comes from different European studies reporting the sporadic occurrence of the IbA10G2 genotype of the parasite in hedgehogs in the Netherlands and of striped field mice (n = 2) in Slovakia (Krawczyk et al., 2015; Danišová et al., 2017; Čondlová et al., 2018) (Fig. 1 and Table 3). Importantly, IbA10G2 is the most dominant *Cryptosporidium* genotype infecting humans in Europe (Chalmers et al., 2008). *Cryptosporidium hominis* of unknown genotype has also been identified in Spanish red foxes (Montoya A, Checa R, Rodríguez E, López AM, Carmena D, Fidalgo L, Gálvez R, Marino V, Fuentes I, Miró G. El zorro rojo (*Vulpes vulpes*), potencial reservorio natural de *Cryptosporidium hominis* en el noroeste de España. XXI Congress of the Spanish Society of Parasitology, 3-5 July, 2019, Pontevedra, Spain. 2019, p84). This host has already been demonstrated

313 to harbour human-infective *Cryptosporidium* spp. including *C. parvum* and
314 *Cryptosporidium ubiquitum* (Mateo et al., 2017). This finding can be explained by the
315 high mobility and increasing presence of foxes in urban and peri-urban settings, where
316 they scavenge and feed on human food waste.
317
318 **6. *Cryptosporidium hominis* in captive and wild primates**
319 Captive and free-living NHP are common hosts of *Cryptosporidium* spp. (Ryan
320 et al., 2014). Because humans and NHP (particularly great apes) are genetically closely
321 related, it is highly expected that they all are vulnerable to infections by very similar (or
322 even the same) set of pathogens including *C. hominis*. Indeed, there is strong molecular
323 epidemiological evidence demonstrating that NHP including baboons, chimpanzees,
324 squirrel monkeys, langurs, and different species of macaques can act as suitable hosts of
325 *C. hominis* (Fig. 1 and Table 4). Due to the relative ease of access, most of these studies
326 were conducted on captive NHP housed in zoological gardens (Liu et al., 2015),
327 monkey breeding farms (Karim et al., 2014) and research facilities (Spano et al., 1998;
328 Ye et al., 2014). Surveys investigating the occurrence of *Cryptosporidium* parasites in
329 free-living NHP are scarcer and have been reported only from protected areas (Karim et
330 al., 2014), from recently captured animals (Li et al., 2011) or free-range monkey
331 populations living close to humans (Ye et al., 2012).
332 Available sequence data on the diversity and frequency of *C. hominis* genotypes
333 in NHP are particularly interesting. Compared with production and companion animals
334 and other wildlife species, NHP are susceptible to infections by a remarkably high
335 variety of *C. hominis gp60* genotypes including members of the families Ia, Ib, Id, Ie, If,
336 Ii, Ik, Im, and In (Fig. 1 and Table 4). Whereas *gp60* genotype families Ia-If have been
337 consistently reported in human populations at variable frequency rates in different

338 geographical areas (Squire and Ryan, 2017; Feng et al., 2018), genotypes belonging to
339 the *gp60* Ii, Ik, Im, and In families seem animal-adapted and are very rarely or not at all
340 seen in humans. To date the monkey genotype IiA17 has been identified in two patients
341 in Scotland (Mallon et al., 2003), and in two patients (a father and son) in Sweden with
342 a travel history to Thailand where they visited a monkey farm (Lebbad et al., 2018).
343 Two additional Swedish patients with no relation between them were found infected
344 with allele IkA18G1 and very likely represented autochthonous cases (Lebbad et al.,
345 2018). Sequence analyses at the actin, *cowp*, *hsp70*, thrombospondin-related adhesive
346 protein of *Cryptosporidium*-1 (*trap-c1*), and *gp60* loci show that *gp60* genotype families
347 Ii, Ik, Im, and In are closely related, but genetically divergent from human-adapted *C.*
348 *hominis* as well as from *Cryptosporidium cuniculus*, providing genetic evidence
349 supporting their potential infectivity to humans and NHP (Laatamna et al., 2015;
350 Lebbad et al., 2018; Chen et al., 2019). More research is clearly needed to ascertain the
351 extent and frequency of zoonotic transmission of genotypes with the Ii, Ik, Im, and In
352 *gp60* alleles. The genetic relationships among *gp60* gene sequences from non-human
353 species reported globally, as inferred by a neighbor-joining analysis, are shown in Fig.
354 2.

355 Regarding geographic origin, most of the studies reporting the presence of *C.*
356 *hominis* in NHP have been carried out in China, particularly in zoological gardens and
357 breeding farms (Karim et al., 2014; Liu et al., 2015). These surveys demonstrated that
358 captive animals were primarily infected by monkey-adapted genotypes of the parasite
359 (Table 4). Remarkably, free-ranging rhesus monkeys living in a large urban park in
360 Guiyang, the capital city of Guizhou province in southwestern China, where found
361 infected by six different genotypes belonging to four (Ia, Id, Ie, and If) *C. hominis gp60*
362 families. Because these animals were in close contact with visitors, the authors

363 suspected that these infections were acquired from humans, but also highlighted that
364 infected animals can also act as natural reservoirs of human-pathogenic
365 *Cryptosporidium* (Ye et al., 2012).

366 In Africa, *Cryptosporidium* is largely absent from forest-dwelling NHP with
367 little or no contact with humans, but prevalent in NHP living in disturbed forest areas
368 outside protected national parks (Salzer et al., 2007; van Zijll Langhout et al., 2010;
369 Salyer et al., 2012). These data seem to suggest that human-driven activities (e.g.
370 climate change, deforestation, unsustainable agriculture, trafficking and poaching,
371 tourism) very likely represent a risk factor for the transmission of anthroponotic
372 enteropathogens to free-living NHP, and represent a serious concern for endangered
373 species living in natural and protected areas (Wallis and Lee, 1999; Muehlenbein et al.,
374 2010). Indeed, this view is supported by two molecular surveys conducted in Kenya and
375 Tanzania. In the first one, recently captured baboons were shown to be primarily
376 infected by anthroponotic alleles IbA9G3 and IfA12G2, in addition to the less
377 prevalent, host-adapted allele IiA14 (Li et al., 2011). In the second one, free-ranging
378 baboons and chimpanzees living in the Gombe ecosystem were found infected by *C.*
379 *hominis gp60* IfA12G2, the very same genetic variant circulating in humans in that
380 geographical area (Parsons et al., 2015). Considering that alleles IbA9G3 and IfA12G2
381 are relatively common findings in faecal specimens of human origin in many African
382 countries including Kenya and Tanzania (reviewed in Squire and Ryan, 2017), these
383 data clearly illustrate the role of humans as a source of *Cryptosporidium* infections to
384 wild NHP.

385

386 **7. Conclusions and outlook**

Cryptosporidium hominis can successfully infect a wide range of mammal species other than human and non-human primates. Animal infections by human-adapted *gp60* families Ia-If are primarily the direct consequence of pathogen transmission between humans, livestock and wildlife. Waterborne transmission is the most probable route of transmission. Because animal infections with *C. hominis* are typically light and asymptomatic, the health risk to threatened or critically endangered wildlife species is unknown. The prevalence and epidemiological importance of mixed *C. parvum* - *C. hominis* infections also remains to be elucidated. The role of animals harbouring *C. hominis* as reservoirs for human infections is unknown. Specific *C. hominis gp60* genotypes seem to be adapted to NHP (Ii) and to equine/camelid (Ik) species. These genotypes rarely cause infections in humans, perhaps representing cryptic species emerging as a result of reproductive isolation. The epidemiology of *C. hominis* appears to be more complex than when the species was named (Morgan-Ryan et al., 2002), involving not fully elucidated transmission cycles between humans, livestock, companion animals, wildlife, and the environment. Research on genetic mechanisms responsible for host specificity is clearly warranted. Of particular interest is the possibility that host specificity is a quantitative (e.g. a function of infective oocyst dose) rather than a categorical trait. Given the ability of *Cryptosporidium* parasites to undergo genetic recombination (Tanriverdi et al., 2007), host specificity inferred on the basis of a single genetic marker such as *gp60* needs to be substantiated with multi-locus methods or genome sequencing.

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Legends to Figures

Fig. 1. Global distribution of *Cryptosporidium hominis* infections in non-human animal species. *Gp60* genotype families are indicated when known. Host species are coloured as follows: black silhouettes represent livestock animal species; dark green silhouettes indicate free-living animal species other than non-human primates; dark red silhouettes represent captive and wild non-human primates.

Fig. 2. Phylogenetic relationships among *Cryptosporidium hominis* genotypes identified in non-human species globally, as inferred by a neighbor-joining analysis of the *gp60* gene sequence. Genetic distances were calculated using the Kimura two-parameter model. Red open circles represent sequences from livestock animal species. Red open triangles represent sequences from wildlife species other than non-human primates. Red open diamonds represent sequences from non-human primate species. Black filled dots represent reference sequences. Bootstrap values lower than 75% are not displayed. *Cryptosporidium parvum* was used as outgroup taxon to root the tree.

728 **Table 1**. Reports describing the occurrence and molecular diversity of *Cryptosporidium hominis* in production and domestic animal species

729 globally. GenBank accession numbers of 18S rRNA and *gp60* sequences are shown when available.

Host species	**Country**	**Period**	**No. samples tested**	**No. samples positive to *C. hominis***	**GenBank accession No. (18S rRNA locus)**	***gp60* genotype (No.)**	**GenBank accession No. (*gp60* locus)**	**Reference**
Cattle	Australia	NS	70	1	–	Unknown	–	Ng et al. (2011)
	Australia	2010	205	3	–	IbA10G2 (1)	JF727780-JF727782	Waldron et al. (2011)
	Australia	2013-15	1111	14	MG516756, MG516758	IbA10G2 (11), IdA15G1 (3)	MG516779, MG516781	Zahedi et al. (2018)
	Australia	–	–	–	–	IbA10G2	KX375349	Unpublished
	China	2007-09	2056	24	GU070731-GU070732	Unknown	–	Chen et al. (2012)
	France	2015	412	15	–	IbA9G2 (1), IbA9G3 (8), IbA13G3 (1), IbA14G2 (1)	MG676495-MG676505	Razakandrainibe et al. (2018)
	India	2004-05	12	1	–	IdA15G1 (1)	DQ871347	Feng et al. (2007)
	India	2007-08	589	1	HM627527	Unknown	–	Rajendran et al. (2011)
	Kenya	NS	1734	1	HQ259584	Unknown	–	Kang´ethe et al. (2012)
	Malawi	2001-02	1346	3	–	Unknown	–	Banda et al. (2009)
	New Zealand	2009-09	180	12	–	IbA10G2R2 (12)	JQ837920, JQ837922, JQ837926-JQ837929	Abeywardena et al. (2012)
	Scotland	1999-00	411	2	–	Unknown	–	Smith et al. (2005)
	South Korea	2000	17	6	DQ054818	Unknown	–	Park et al. (2006)
	Uganda	2014	363	20	KY586953-KY586963	Unknown	–	Witto et al. (2019)[c]
	UK	2003	52	1	–	IbA10G2 (1)	KF537685	Ghaffari et al. (2014)
	USA	1994-03	6[a]	6	–	Unknown	–	Tanriverdi et al.

								(2003)
Sheep	Australia	2002-03	1647	1	–	Unknown	–	Ryan et al. (2005)
	Scotland	2004-06	255	11	–	IbA10G2 (11)	KC679684-KC679695	Connelly et al. (2013)
	UK	NS	1	1	–	IbA10G2 (1)	EU186152	Giles et al. (2009)
Goat	South Korea	2000	7	3	DQ054819	Unknown	–	Park et al. (2006)
	UK	NS	1	1	EU186156	Unknown	–	Giles et al. (2009)
Horse	Algeria	2011-13	219	1	–	IkA15G1 (1)	KJ941148	Laatamna et al.(2015)
	Brazil	2010-11	92	2	KT948752	IkA20G1 (2)	KT948748	Inácio et al. (2017)
	China	2008-13	5	1	KU200955	IkA16G1 (1)	–	Jian et al. (2016)
	China	–	–	–	–	IkA16G1	KU200958	Unpublished
Donkey	China	2008-13	82	60	KU200955	IkA16 (2), IkA16G1 (58)	KU200962, KU200963	Jian et al. (2016)
	China	2015-19	551	75	MK775038-MK775039, MK775043-MK775044	IkA16G1 (72)	MK761058-MK761063[b]	Li et al. (2019)
	China	–	–	–	–	IkA16	KU200962	Unpublished
Bactrian camel	China	–	–	–	–	IkA19G1	MH442995	Unpublished
Dog	Spain	2013-16	194	1	KX774313	Unknown	–	Gil et al. (2017)

730 NS: Not specified.

731 [a] Laboratory isolates from experimentally infected animals.

732 [b] Sequences not yet released at the time of publication.

733 [c] Witto, S.G., Kankya, C., Kazibwe, A.J.N., Akurut, G., Ochwo, S., 2019. Occurrence of *Cryptosporidium hominis* in cattle bordering the Lake

734 Mburo National Park in Kiruhura district, Western Uganda. bioRxiv 562793.

735 **Table 2**. Experimental infections with *Cryptosporidium hominis* oocysts.

Host	Immunosuppression	Parasite isolate	Inoculation of oocysts	Via	Detection post-inoculation (days)	Peak of shedding (days)	Oocyst score at the peak of the infection	Symptoms	Duration of shedding (days)	Reference
Calf	No	TU502	NS	Oral	NS	NS	NS	NS	20	Akiyoshi et al. (2002)
	No	SNU-H1	$2x10^7$	Oral	4	5–9	NS	Diarrhoea	NS	Guk et al. (2004)
Gerbil	Yes	Cp/H4	10^2–$2x10^5$	Oral	NS	14	20[b]	NS	21	Baishanbo et al. (2005)
Lamb	No	T5	$5x10^5$	Oral	10	15	10^6 opg	None	25	Giles et al. (2001)
	No	Crypto-26	10^6–$2x10^6$	Gastric probe	5	NS	NS	No	10	Ebeid et al. (2003)
Mouse	Yes	SNU-H1[a]	$1x10^6$	Oral	2–3	12	$6x10^5$ opm	NS	27	Guk et al. (2004)
	Yes	SNU-H1[a]	$1x10^6$	Oral	2–3	10	$2x10^5$ opm	NS	27	Guk et al. (2004)
	Yes	SNU-H1[a]	$1x10^6$	Oral	2–3	4	$7x10^5$ opm	NS	27	Guk et al. (2004)
Piglet	No	NEMC1	10^5–10^6	Oral	9	NS	3-5	None to mild	NS	Widmer et al. (2000)
	No	TU502	NS	Oral	NS	NS	NS	NS	20	Akiyoshi et al. (2002)
	No	GCH1, OH, H2576, H3438	10^3–10^5	Gastric probe	NS	NS	NS	NS	NS	Morgan-Ryan et al. (2002)
	No	HuG1	10^3–10^5	Oral	9	NS	NS	None to mild	17	Pereira et al. (2002)
	No	TU502	$1x10^5$	Oral	3–4	NS	NS	Mild	6	Akiyoshi et al. (2003)
	No	Crypto-11, Crypto-26	10^6–$2x10^6$	Gastric probe	4–5	NS	NS	Diarrhoea	4–9	Ebeid et al. (2003)
	No	TU502	$1x10^6$–10^7	Oral	4–5	5–7	NS	NS	10–18	Sheoran et al. (2012)

736 Opg, number of oocysts per gram of faecal material; Opm, number of oocysts per mL of faecal supernatant; NS, not specified.

737 [a] Mixture of *C. hominis* + *Cryptosporidium parvum*.

738 [b] Number of oocysts detected in 10 microscopic fields.

739 **Table 3**. Reports describing the occurrence and molecular diversity of *Cryptosporidium hominis* in wildlife (excluding non-human primates)

740 animal species globally. Accession numbers of sequences at the 18S rRNA and the *gp60* loci deposited in GenBank are shown when available.

Host species	**Country**	**Period**	**No. samples tested**	**No. samples positive to *C. hominis***	**GenBank accession No. (18S rRNA locus)**	***gp60* genotype (No.)**	**GenBank accession No. (*gp60* locus)**	**Reference**
Badger	Spain	2003-16	70	1	KY052170	Unknown	–	Mateo et al. (2017)
Canada Goose	USA	1999-02	209	2	–	Unknown	–	Zhou et al. (2004)
Deer	Australia	2011-15	944	2	KU531663	Unknown	–	Koehler et al. (2016)
Dingo	Australia	NS	44	1[a]	–	Unknown	–	Ng et al. (2011)
Dugong	Australia	1997	1	1	–	Unknown	–	Morgan et al. (2000)
Flying fox	Australia	2015	281	2	–	IbA9G2	–	Schiller et al. (2016)
Hedgehog	Netherlands	2013	90	NS	–	IbA10G2	–	Krawczyk et al. (2015)
Kangaroo	Australia	NS	160	18	–	Unknown	–	Ng et al. (2011)
	Australia	2013-15	3228	2	MG516755, MG516757	IdA15G1 (2)	MG516780, MG516782	Zahedi et al. (2018)
	Australia	–	–	–	–	IbA10G2	KX375348	Unpublished
Wallaby	Australia	2011-15	74	1	–	IbA10G2 (1)	KU531699	Koehler et al. (2016)
Red fox	Spain	2016	82	4	MK770261-MK770264	Unknown	–	Montoya et al. (2019)[b]
Striped field mouse	Slovakia	2012-13	107	1	–	IbA10G2 (1)	KU311668	Danišová et al. (2017)
Striped field mouse	Slovakia	2012-13	71	1	–	IbA10G2 (1)	Identical to KU311668	Čondlová et al., (2018)

741 NS, not specified.

742 [a] *Cryptosporidium hominis*-like.

[b] Montoya A, Checa R, Rodríguez E, López AM, Carmena D, Fidalgo L, Gálvez R, Marino V, Fuentes I, Miró G. El zorro rojo (*Vulpes vulpes*), potencial reservorio natural de *Cryptosporidium hominis* en el noroeste de España. XXI Congress of the Spanish Society of Parasitology, 3-5 July, 2019, Pontevedra, Spain. 2019, p84.

746 **Table 4**. Reports describing the occurrence and molecular diversity of *Cryptosporidium hominis* in captive and free-living non-human primates

747 globally. Accession numbers of sequences at the 18S rRNA and the *gp60* loci deposited in GenBank are shown when available.

Host species	**Country**	**Period**	**No. samples tested**	**No. samples positive to *C. hominis***	**GenBank accession No. (18S rRNA locus)**	***gp60* genotype (No.)**	**GenBank accession No. (*gp60* locus)**	**Reference**
Baboon	Kenya	2006-08	235	6	–	IbA9G3 (2), IfA12G2 (2), IiA14 (1)	JF681172-JF681174	Li et al. (2011)
	Tanzania	2010-11	47	5	–	IfA12G2 (3)	–	Parsons et al. (2015)
Chimpanzee	Tanzania		26	4	–	IfA12G2 (3)	–	Parsons et al. (2015)
Cynomolgus monkey	China	2006-13	778	4	GU319778-GU319780	IiA17 (1)	KF679724	Karim et al. (2014)
Francois´ leaf monkey	China	2006-13	15	1	GU319778-GU319780	Unknown	–	Karim et al. (2014)
Macaque	China	2011	205	1	–	IdA14	–	Ye et al. (2014)
Macaque	China	2016-18	1452	86	MG952704	ImA18 (38), In (1), InA14 (6), InA17 (1), InA26 (6), IiA17 (3)	MG952706, MG952710-MG952714,	Chen et al. (2019)
Rhesus monkey	China	2006-13	1316	9	GU319778-GU319780, KF679722, KF679723	IbA12G3 (7)	KF679725	Karim et al. (2014)
Rhesus monkey	China	2010	411	39	–	IaA13R7 (2), IaA13R8 (8), IaA14R7 (2), IdA20 (13), IeA11G3T3 (13), IfA16G2 (1)	JX000568-JX000570	Ye et al. (2012)
Rhesus monkey	USA	NS	1	1	–	Unknown	–	Spano et al. (1998)

Rhesus monkey	USA	NS	1	1	–	IiA17 (1)	HM234173	Feng et al. (2011)
Squirrel monkey	China	2014	1	1	KP314259	IkA7G4	KP314263	Liu et al. (2015)

748 NS, not specified.