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Review: Equine cryptosporidiosis – cosmopolitan occurrence?

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Abstract

Cryptosporidium parasites infecting horses are distributed worldwide. The reported prevalence of equine cryptosporidiosis is quite variable, ranging from 0% to 37.8%. According to the literature, horses can become infected with various *Cryptosporidium* species, including *Cryptosporidium parvum*, *Cryptosporidium hominis*, *Cryptosporidium muris*, *Cryptosporidium* horse genotype, *Cryptosporidium tyzzeri*, and *Cryptosporidium andersoni*. Here we review the literature on equine cryptosporidiosis dating back to 1978. Because of the possibility of the human parasite *Cryptosporidium hominis* infecting horses, we examined the phylogeny of the *Cryptosporidium* small subunit ribosomal RNA and GP60 genes isolated from horses and found evidence of equine cryptosporidiosis caused by this species which is commonly assumed to be restricted to humans.

Introduction

Cryptosporidiosis is a globally distributed infection causing sporadic gastroenteritis, abdominal pain, vomiting and fever. Several zoonotic species have been identified in humans, but most cases of human cryptosporidiosis are caused by *C. parvum* and *C. hominis* [1]. Cryptosporidiosis has long been recognized as a serious veterinary problem in neonatal ruminants [2]. *C. parvum* is the main species found in goats [3], lambs, calves[4] and foals [5].

The observation that equines may be infected with *Cryptosporidium* species similar to those infecting humans [6-11], prompted to focus this review on the global occurrence of *Cryptosporidium* spp. in equines and review the literature describing *Cryptosporidium* species found in horses.

Taxonomy

The genus *Cryptosporidium* is classified in the phylum Apicomplexa, class Sporozoae, subclass Coccidia, order Eucoccidiida, suborder Eimeriina and family Cryptosporididae [12]. The taxonomic classification of the genus is still being debated, as recent phylogenetic studies have shown proximity to the subclass Cryptogregaria, which is part of the Gregarines in the phylum Apicomplexa, class Gregarinomorphea. This proposed classification is based on reports that oocysts can multiply outside the host, on the presence of a specialized feeding organelle and the absence of the apicoplast [13-19]. Recent studies using DNA sequencing have led to the currently proposed taxonomy of the genus *Cryptosporidium* comprising 31 species and more than 70 genotypes [20], including the newly described species *Cryptosporidium proliferans* [21] and *Cryptosporidium avium* [22]. The taxonomic classification of *Cryptosporidium* isolated from equines based exclusively on genetic data is uncertain, particularly in the absence of characteristic morphological traits of the oocysts and a lack of data on host range.

Epidemiology of equine cryptosporidiosis

Cryptosporidium in equines is distributed worldwide an on all continents. Most studies reviewed here are from American and European countries 38.4%, and 35.9% respectively, while studies from Asia, Oceania and Africa are 12.8%, 7.7% and 5.1%, respectively. Most studies reporting on *Cryptosoporidium* in equines are from North America, only three reports described equine infections from South Americans, whereas literature on *Cryptosporidium* in equines from Central America no was identified in our search (Table 1).

Between 2003 and today, many researchers have used PCR to identify *Cryptosporidium* species in equine samples, advancing our understanding of the occurrence of this parasite and the role of horses and foals in the epidemiology of cryptosporidiosis. Table 1 shows that horses can become infected with *C. parvum*, *C. hominis*, *C. muris*, *C. tyzzeri*, *C. andersoni, C. erinacei* and what has been named *Cryptosporidium* horse genotype [23]. The first two species are infectious to humans, and are responsible for approximately 90% of human cryptosporidiosis cases [1,24]. The susceptibility of horses to

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Table 1. Global occurrence of *Cryptosporidium*, species and GP60 genotype in equines according to diagnostic methodology

Continent	Country	Total Horses	Positives	Species	GP60 Genotype	Diagnostic Method	Reference
Africa	Algeria	*138*	*4*	*Cryptosporidium* hedgehog	----------	PCR	[52]
	Algeria	*219*	*5*	*Cryptosporidium parvum*	IIaA16G1R1	PCR	[6]
				Cryptosporidium hominis	IkA15G1		
				Cryptosporidium muris	RN66		
America	Brazil	*396*	*3*	*Cryptosporidium* spp.	----------	CF/SMB	[53]
	Brazil	*196*	*39*	*Cryptosporidium* spp.	----------	MKT	[54]
	Brazil	*92*		*Cryptosporidium parvum*	IIaA18G3R1	PCR	[11]
			20	*Cryptosporidium parvum*	IIaA15G2R1		
				Cryptosporidium hominis	IkA20G1		
	Canada	*2*	*2*	*Cryptosporidium* spp.	----------	SF/EM	[55]
	Canada	*35*	*8*	*Cryptosporidium* spp.		FS/IM	[41]
	United States of America	*29*	*5*	*Cryptosporidium* spp.	----------	HE/W-G/EM	[39]
	United States of America	*14*	*-*	----------	----------	SC	[25]
	United States of America	*22*	*22*	*Cryptosporidium* spp.	----------	FF	[56]
	United States of America	*91*	*--*	----------	----------	DFA/LC	[26]
	United States of America	*366*	*13*	----------	----------	AF/IFA/FC	[57]
	United States of America	*1*	*1*	*Cryptosporidium* spp.	----------	IM	[58]
	United States of America	*223*	*3*	*Cryptosporidium* spp.	----------	MAF	[59]
	United States of America	*349*	*16*	*Cryptosporidium* horse genotype	VIaA14G2	DFA/PCR	[34]
	United States of America	*88*	*18*	*Cryptosporidium*	----------	RT- PCR	[40]
	United States of America	*84*		*Cryptosporidium parvum*	IIaA13G2R2	PCR	[60]
			28	*Cryptosporidium parvum*	IIaA15G2R1		
				Cryptosporidium parvum	IIaA17G2R1		
Asia	Jordan	*74*	*6*	*Cryptosporidium parvum*	----------	qPCR	[61]
	China	*262*	*7*	*Cryptosporidium* horse genotype	VIaA15G4	PCR	[36]
	China	*29*	*2*	*Cryptosporidium andersoni*	MLST	SSFT/PCR	[7]
	China	---	*5*	*Cryptosporidium parvum*	IIdA19G1	PCR	[9]
				Cryptosporidium hominis	IkA16G1		
	China	*333*		*Cryptosporidium andersoni*	IdA15	PCR	[10]
			6	*Cryptosporidium hominis*			
Europe	Czech Republic	*2*	*11*	*Cryptosporidium* horse genotype	VIaA11G3	PCR	[23]
				Cryptosporidium parvum			
	Czech Republic	*3*	*3*	*Cryptosporidium parvum*	----------	PCR	[62]
	Czech Republic/Poland	*352*	*12*	*Cryptosporidium parvum*	IIaA15G2R1	ACMVSM, PCR	[8]
				Cryptosporidium tyzzeri	IXbA22R9		
				Cryptosporidium muris			
				Cryptosporidium horse genotype	VIaA15G4		
	Czech Republic/Poland	*352*	*12*	*Cryptosporidium parvum*	IIaA15G2R1	ACMVSM, PCR	[8]
				Cryptosporidium tyzzeri	IXbA22R9		
				Cryptosporidium muris			
				Cryptosporidium horse genotype	VIaA15G4		
	England	*644*	*17*	*Cryptosporidium* spp.	----------	IF	[63]
	England	*80*	*40*	*Cryptosporidium parvum*	----------	EPM /ZN	[64]
	England	*52*	*2*	*Cryptosporidium parvum*	----------	DI/EM/PCR	[47]
	England and Wales	*18*	*6*	*Cryptosporidium parvum*	----------	IS/IM/PCR	[65]
	Greece/Belgium/Netherlands/ Germany	*398*	*8*	*Cryptosporidium* horse genotype	----------	IFA/PCR	[37]
	Italy	*150*	*12*	*Cryptosporidium parvum*	----------	ZN/CF/LS/SF / DFA/PCR	[66]
	Italy	*74*	*2*	*Cryptosporidium parvum*	----------	ELISA/PCR	[67]
	Italy	*37*	*14*	*Cryptosporidium parvum*	VIaA15G4	PCR	[35]
				Cryptosporidium horse genotype	VIaA15G4		
	Italy	*73*	*14*	*Cryptosporidium parvum*	IIdA21G0	ZN/PCR	[27]
				Cryptosporidium parvum	IIdA22G1		

				Cryptosporidium horse genotype	VIaA15G4		
				Cryptosporidium parvum	IIaA23GR1		
	Italy	*64*	*2*	*Cryptosporidium parvum*	IIdA23G1	ZN/PCR	[44]
	Switzerland	*1*	*1*	*Cryptosporidium parvum*	----------	ZN/F/S/PCR	[42]
Oceania	New Zealand	*17*	*5*	*Cryptosporidium parvum*	----------	HE/PAS/G/AF/ PCR	[28]
	New Zealand	*9*	*9*	*Cryptosporidium parvum*	IIaA18G3R1	PCR	[5]
	New Zealand	*131*	*67*	*Cryptosporidium parvum*	----------	PCR	[38]

Subtitle: Tissues Stained with Hematoxylin and Eosin (HE), Wolbach-Giemsa (W-G), Electronic Microscopy (EM), Sucrose Centrifugation (SC), Sucrose Flotation (SF), Fecal Flotation (FF), Immunoflorescence (IF), Direct Fluorescent Antibody (DFA), Levitation Centrifugation Tests (LC), Sheather Fotation (SF), Immunofluorescent Microscopy (IM), Acid-Fast (AF), Immunofluorescence Test (IFA), Flow Cytometry (FC), Polymerase Chain Reaction (PCR), Epifluorescence Microscopy (EPM), Ziehl-Neelsen (ZN), Periodic Acid-Schiff (PAS), Giemsa (G), Direct Immunofluorescence (DI), Flotation (F), Sedimentation (S), Centrifugation and Flotation (CF), Safranine–Methylene-Blue (SMB), Modified Acid-Fast (MAF), Lugol Staining (LS), Imunogenetic Separation (IS), Enzyme-Linked Immunosorbent Assay (ELISA), Modified Kinyoun Technique (MKT), Real-time-PCR – RT-PCR, Aniline-Carbol-Methyl Violet Staining Method (ACMVSM), Sheather's Sugar Floatation Technique (SSFT

the same species infecting humans indicates that horses may play a role in the zoonotic transmission of these parasites.

It is important to note that the reported prevalence of *Cryptosporidium* in equines samples is quite variable, ranging from 0% [25,26] to 37.8% [27]. In our literature survey, equines of different age groups, genders and breeds were included, which may account for the wide range in reported prevalence. Thus, in Table 1, the actual prevalence may differ, in part due to the use of different diagnostic techniques, which makes it difficult to compare results from different surveys.

In New Zealand, a first-ever study was conducted on an outbreak of cryptosporidiosis in nine purebreed foals. Epidemiological, clinical, pathological and genetic data were compiled, and *C. parvum* isolates identified based on restriction fragment polymorphism and on the sequence of the 18S rRNA gene as belonging to what was then named "cattle" genotype of *C. parvum* [28] and later renamed *C. parvum*.

The first description of the infection of equines by *Cryptosporidium* hedgehog genotype (renamed *C. erinacei* [29], is from Algeria [30]. *Cryptosporidium* DNA belonging to this species was identified using PCR targeting the 18S rRNA gene and the gp60 gene. This observation indicates that the horses may participate as hosts or mechanical carriers in *C. erinacei* life cycle [30]. *C. hominis* DNA was amplified from an equine fecal sample from Algeria using PCR targeting the Heat shock Protein 70 (HSP70), the *Cryptosporidium* Oocyst Wall Protein (COWP) and TSP-related adhesive protein of *Cryptosporidium*-1(TRAP-C1) genes. *C. muris* was described for the first time in equine samples from Algeria by sequencing the 18S rRNA and GP60 genes [6]. These findings reinforces the hypothesis of a new species of *Cryptosporidium* infecting equines because until recently, few species of *Cryptosporidium* were found in horses, however, with the reports of *C. muris* and *C. tyzzeri* increase susceptibility to infection in horses [8]. As there are a few reports of equine infected with *C. hominis* [6,10,11], Laatamna's report of a horse in Algeria infected with this species is intriguing, due to the fact *C. hominis* is usually described infecting humans. Similar observations were reported from China [9] and from Brazil [11]. Interestingly, these studies found similar GP60 genotypes, designated IkA15G1, IkA16G1 and IkA20G1, respectively. Although the GP60 genotypes identified in these studies are not identical, the genotypes of additional genetic markers, such as the 18S rRNA, actin and HSP70, are consistent with horses being susceptible to *C. hominis* or a genotypically similar species (Figure 1). Although a human case of cryptosporidiosis caused by an isolate with GP60 genotype Ik was apparently identified (NCBI nucleotide accession number KU727290), Ik alleles are not frequently observed in humans. On the other hand, similar GP60 alleles were found in *C. cuniculus* [31], a species infecting rabbits and humans which is closely related to *C. hominis*. The significance of equine infections with *C. hominis*, or *C. hominis*-like parasites, for zoonotic transmission of this species is unknown.

The occurrence of *Cryptosporidium* spp. is often associated with urban areas and particularly with the contamination of water supplies with human fecal material [32,33]. In Brazil, *C. hominis* GP60 genotype IkA20G1 and *C. parvum* genotypes IIaA18G3R1 and IIaA15G2R1 were collected from foals that drank water from a river that receives untreated urban wastewater [11]. The putative presence of *C. hominis* in horses raises public health concerns [10], since is distributed in all continents of the world. The identification in recent studies of *C. hominis*, or *C. hominis*-like parasites, in horses [6,10,11] suggests that contact between humans and horses may favor the transmission of this species, which is not commonly associated with zoonotic transmission.

In the Czech Republic, equines infected with *C. parvum* and with the horse genotype were identified using PCR targeting the 18S rDNA and HSP-70 genetic markers. This work showed that the horse genotype is more closely related to *Cryptosporidium wrairi*, there being a 98.7% similarity for 18S and 99% similarity for HSP-70 [23] between these taxa. *C. parvum* and the *Cryptosporidium* horse genotype have been reported in several other studies from different countries, such as the Czech Republic [8], the USA [34], Italy [27,35], China [36], and Belgium [37]. Based on these observations, we conclude that horses worldwide may be infected with *Cryptosporidium* parasites. Published evidence suggests that *C. parvum*/*Cryptosporidium* horse genotype and *C. hominis*, are the most common agent of equine infections followed by *C. andersoni* and *C. muris, C. hedgehog (erinacei*) and *C. tyzzeri*.

PCR allowed *Cryptosporidium* species detection in equine population, including zoonotic ones. These findings are important and have generated worries for public health.

Pathogenesis and clinical manifestations

The incidence of clinical *C. parvum* infections in newborn foals may be underestimated, perhaps accounting for cases empirically diagnosed as foal heat diarrhea [38]. In one of the earliest reports on equine cryptosporidiosis, the susceptibility of immunodeficient foals to this infection was described [39]. Immunocompetent foals where also found to develop diarrhea while eliminating *Cryptosporidium* oocysts [25], potentially causing economic loss to owners. Thus, it is often unclear whether the primary cause of symptoms is cryptosporidiosis or a viral agent. In most cases, other infectious agents that can also causes diarrhea were not considered or not diagnosed. In Kentucky, *Cryptosporidium* infections are most commonly observed in foals affected by other infections, caused by bacteria and viruses [25,40]. Symptoms are poorly described in equines [41]. More researches

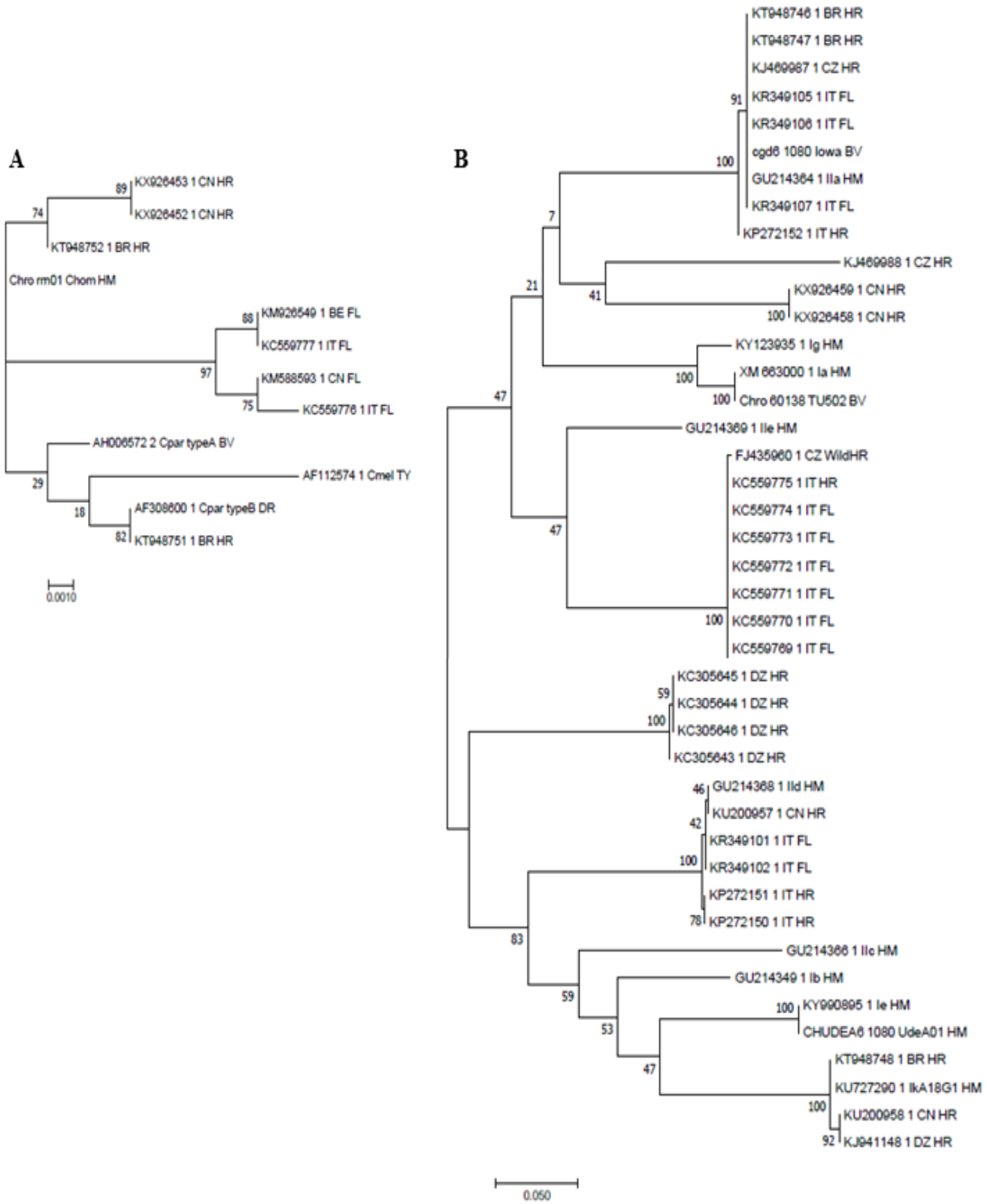

Figure 1. Phylogenetic trees based on the partial sequence of the 18S rRNA and GP60 genes show the phylogenetic context of *Cryptosporidium* sequences isolated from horses. A) Trees were generated using a 583-nt fragment of the 18S gene from equine *Cryptosporidium* isolates and *C. hominis, C. parvum* and *C. meleagridis* reference sequences. B) Phylogenetic tree based on approximately 100-nt fragment of the GP60 gene. Scale bars indicate 0.0010 and 0.050, respectively. BV, bovine, HM, human; DY donkey; HR/FL/WildHR, equine (horse, foal, wild horse, respectively

need to be performed to elucidate equine cryptosporidiosis symptomatology and pathogenicity of *Cryptosporidium* species.

In New Zealand, in post-mortem examinations of three foals, it was found that their intestines were full of fluid, were dilated, swollen and thin-walled, with no inflammation in the abdominal cavity. Microscopic examination of hematoxylin eosin stained sections of intestinal tissue revealed the presence in epithelial cells of numerous round organisms approximately two to five microns in size in much of the duodenum. These lesions were found to be consistent with cryptosporidiosis [28]. In Switzerland, a 9-day-old foal with diarrhea, fever and feces with fetid odor was also found to be infected with *C. parvum* [42].

Cryptosporidium caused inflammation and atrophy of the intestinal microvillous region with loss of absorptive surface, imbalance in the transport of nutrients and impairment in animal productivity [43].

Zoonotic potential

Currently, there is only one description of zoonotic transmission in equines. A study conducted in Italy it was shown that six veterinary students and hospitalized foals had symptoms consistent with cryptosporidiosis. *C. parvum* with GP60 genotype IIdA23G1 [44] was found in humans and animals. Students could have been infected by being in contact with foals infected with *Cryptosporidium* and because oocysts are highly resistant to environmental conditions and disinfectants, remaining viable for a long period of time [45].

An observation consitent with transmission between horses and other livestock species is a report of a horse infected with *C. andersoni*, a species commonly found in the abomasum of adult ruminants. The possible transmission between cattle and horses has also been reported from China [7]. Further evidence of horses being susceptible to *C. parvum* was reported from the UK, confirming that equines can potentially be a source of zoonotic infection of humans [46,47]. The extent of genetic diversity of Cryptosporidium isolated from equines was studied by sequencing polymorphic regions of the GP60 glycoprotein and heat shock protein HSP70 gene. These analyses revealed species and genotypes of *Cryptosporidium* genetically closely resembling those found in humans and bovines [5].

Cryptosporidium horse genotype was initially described in Przewalski's wild horse foal [23], being considered as specific genotype of horses, as found in New York, in foals and their mares has been reported [34]. However, the *Cryptosporidium* horse genotype was also found in an immuno-compromised woman in England [48], suggesting a risk to human health.

Many *Cryptosporidium* species were found in farm animals samples [41,49]. Generally, these animals drink untreated water from rivers passing through the farm and wells. This water may be contaminated with oocysts of zoonotic and zooantroponotic species of *Cryptosporidium.* Once they ingest oocysts, animals can be infected or act as mechanical carriers, shedding *Cryptosporidium* oocysts and contaminating pasture. Most of farm animals are herbivors, so there are two ways they can be infected: drinking contaminated water or eating contaminated grass. Oocysts in the environment can be also carried by rain to watercourses allowing another susceptible living being infection.

C. parvum has been found in faecal samples of livestock [32,50]. *C. parvum* was also found in faecal samples of wild mustangs and Chincoteague ponies. These animals have minimal contact with humans; however, they ingest water and graze in the same places as cattle and wildlife such as deer and elk [51].

Conclusion

We verified that the genus *Cryptosporidium* with its species is distributed worldwide in the equine population. We also observed that most pathogenic species for humans detected in equines are *C. parvum* and *C. hominis*, evidencing a public health problem.

Competing interest

The authors declare that they have no competing interests.

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