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Revisión de Literatura

Molecular characterization of Cryptosporidium parvum and Cryptosporidium hominis GP60 subtypes worldwide

Caracterización molecular de los subtipos de la GP60 de Cryptosporidium parvum y Cryptosporidium hominis alrededor del mundo

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Abstract:

Cryptosporidium is a zoonotic parasite very important in animal health as well as in public health. It is because this is one of the main causes of diarrhea in children, calves, lambs and other variety of youth mammalians in a lot of countries. The globalization has enabled the exchange of biological material in different regions worldwide, encouraging the spread of diseases and exposure to these biological agents to different environmental conditions, inducing adaptation through genetic changes. Based in the polymorphism of the gene for GP60, this review intended to present the distribution of Cryptosporidium parvum and Cryptosporidium hominis in humans and calves worldwide. The subtype that affects cattle more frequently corresponds to IIaA15G2R; while the subtype most frequently isolated from human samples is IaA19G2.

Keywords: Cryptosporidiosis, molecular epidemiology, public health.

Resumen:

Cryptosporidium es un parásito zoonótico muy importante en salud animal así como en salud pública. Esto se debe a que el parásito se constituye en una de las principales causas de diarrea en niños, terneros, corderos y una gran variedad de mamíferos jóvenes en una gran cantidad de países. Debido a que la globalización ha permitido el intercambio de material biológico en diferentes regiones alrededor del mundo, se ha favorecido la propagación de enfermedades y se han expuesto a los agentes biológicos a diferentes condiciones ambientales, induciendo así la adaptación a través de cambios genéticos. Con base en el polimorfismo del gen GP60, esta revisión pretende presentar la distribución de Cryptosporidium parvum y Cryptosporidium hominis en humanos y terneros alrededor del mundo. El subtipo que afecta con mayor frecuencia al ganado vacuno corresponde a IIaA15G2R1; en tanto que el subtipo aislado con mayor frecuencia a partir de las muestras humanas es IaA19G2.

Palabras clave: Criptosporidiosis, epidemiología molecular, salud pública.

Introduction

Cryptosporidium spp. is a ubiquitous protozoan that infects humans and a large variety of vertebrate animals around the world with significant implications for public health. The impact of this parasite on public health can be found in the high morbidity possible in children and immunocompromised people. In addition, Cryptosporidium spp. causes economic losses due to increases in the rates of morbidity and mortality in animals, and due to negative effects on the development of young animals. The route of infection is fecal-oral, nevertheless, the ingestion of oocysts can occur in several ways such as through person-to-person contact,

contact with household pets, farm animals or ingestion of contaminated food, drinking water or water contacted during recreation (1).

Cryptosporidiosis is a significant cause of death in calves, potentially producing economic losses for the farms of some countries, however this has still not been assessed. The most pathogenic species of Cryptosporidium is Cryptosporidium parvum, it is a result of the ability of C. parvum sporozoites to invade the intestinal epithelium, after excystation from the oocyst, producing shortening and destruction of the villi, reducing their absorptive capacity, and leading to negative effects on productive processes in the host, such as growth. C. parvum transmission is characterized by a low infectious dose. Following infection, clinical cases appear between 7 and 30 days after the birth of a calf as acute diarrhea, depression, anorexia, abdominal pain and death as a result of dehydration and cardiovascular failure (2).

Studies of the parasite at the morphological and phenotypic levels are unable to establish taxonomic differences (3); therefore epidemiological studies of the parasite, making use of molecular tools, have been carried out in the past two decades, generating information about the species, its genotypes and its subtypes (4) facilitating an understanding of its epidemiology, taxonomy and evolutionary genetics.

Molecular diagnostic

So long as the morphology of the oocysts does not permit differentiation between the species of Cryptosporidium spp., microscopic identification presents problems for the determination of the species that affects humans or animals and the role of these species in the disease or in its transmission. Furthermore, the majority of infections are subclinical and recognition requires more sensitive methods like the polymerase chain reaction (PCR), the current method of choice for diagnosis of the disease. Thus the identification and evaluation of the prevalence of different species of Cryptosporidium has been achieved using molecular tests (5). For this reason, molecular tools have become the key to identification of species (6) and are recognized as essential for the determination of the taxonomy of Cryptosporidium. These tools underlie the ability to understand the biology, epidemiology and relationship to health, identifying the various species of Cryptosporidium and their populations as genotypes that have not been recognized as distinct species (1). The advances in the techniques of molecular biology have significantly improved the diagnosis of cryptosporidiosis, as well as the genetic characterization of species of Cryptosporidium (7).

For the categorization of species, genotypes or subtypes of Cryptosporidium, PCR based techniques are used, employing primers for the selective amplification of one or more genetic loci (markers) followed by an enzymatic cleavage or sequencing (7). PCR has permitted the identification and subtyping of Cryptosporidium spp., facilitating identification of the routes of transmission between animals and humans (8).This technique also has deepened investigations in the field of molecular epidemiology of the parasite, facilitating the phylogenetic reconstruction and evolutionary analysis of it, specifically identifying genotypes and species, and others with a wide range of possibilities (9).

In general, for the determination of species, regions of low or moderate variability have been used. Approaches to species determination have been described based on differences encountered in the sequences of the small subunit of rRNA, the gen for actin and the heat shock protein, and in the identification of subtypes of glycoprotein GP60 (4). The direct sequencing of DNA continues to be the best approach for the detection of variations or genetic polymorphisms. Included among the genes of low variability used in these studies are, for example, the gene for the small subunit of rRNA (18S rDNA,) the Cryptosporidium oocyst wall protein (COWP) the 70KDa heat shock protein (HSP-70) or the gene for actin. Within the regions of moderate variability, the genes for β -tubulin, TRAP (C1, C2 and C4) or the intergenic regions ITS-1 and ITS-2 have been used. These genes are used as well in taxonomic studies such as diagnostics or epidemiology; however these regions uniquely identify the species and some genotypes (10). For example, the small subunit of ribosomal RNA (SSUrRNA) is used to genotype Cryptosporidium in human and animal tests and in tests of water. This is due to a natural multicopy gene and the presence of semiconserved and hypervariable regions that facilitate the design of type-specific primers (11).

The analysis of the GP60 gene is frequently used in the subtyping of Cryptosporidium because of the heterogeneity of the sequence and its relevance to the biology of the parasite (11). Therefore, in the identification of genotypes, subtypes or lineages, highly polymorphic regions are used (6), like the GP60 gene and mini or microsatellite regions like ML1 and ML2 (12).

Making use of nested PCR, sequence analysis of the gene for GP60 has found that its sequence is similar to a microsatellite, having repeats of a serine codon (TCA, TCG or TCT) at the extreme 5' terminus of the gene (6,11), finding a high degree of polymorphism in the sequence isolates from C. hominis, C. parvum, and C. meleagridis permitted determination of the genotype and the subtype. A Roman numeral and small case letter identify the subtype. Both represent the genotype of Cryptosporidium spp. For example, Ia and Ib are subtypes of C. hominis, while IIa and IIb are subtypes that correspond to C. parvum (13). Various groups of subtypes have been identified in these two species: 7 groups of subtypes in C. hominis (Ia-Ig), 6 groups of subtypes in C. meleagridis and 11 subtypes of families in C. parvum (IIa-III) (4); the subtypes of the families IIa and IId have been recognized as zoonotics (14). Within each group of subtype, various subgenotypes exist principally based on the number of trinucleotide serine repeats (4). The name of the GP60 subtypes begins with the subtype of the designated family (Ia, Ib, Id, Ie, If, etc for C. hominis, and IIa, IIb, IIc, IId, etc for C. parvum) followed by the number of repetitions of TCA (represented by the letter A), TCG (represented by the letter G) or TCT (represented by the letter T). In the subtype of the family of C. parvum IIa, there are a few genotypes that possess two copies of the sequence ACATCA just before the trinucleotide repeat. These genotypes are represented as “R2” (R1 represents many subtypes) (11). For example, the subtype IIaA15G2R1 is a subtype of C. parvum (IIa) with 15 repeats of TCA (A) 2 TCG repeats (G) and one ACATCA (13). In humans, as well as, in farms animals, specially in cattle the subtype of the most prevalent family corresponds to the family IIa, specifically IIaA15G2R1 (11).

Due to the need in epidemiology and public health to characterize the populations and subtypes within the distinct species of the genera Cryptosporidium, the analysis of various hypervariables loci are frequently used (MLT, multilocus typing) that increase the precision of the genotyping. In this way, a few patterns of MLT are created depending on the genotype combinations for each loci analyzed. These studies can be performed by detection of differences in length of the amplified fragments (MLFT) on agarose gels, or by sequencing (MLST), permitting the use of markers with single nucleotide polymorphisms (SNPs) (12). Satellites are characterized by allelic variability, and are used to explore the genetic structure of a population such as in the analysis of lineage and in the construction of a genetic map. Micro and minisatellites have been frequently used in work with other parasites such as Plasmodium spp. and Trypanosoma spp. With the information generated, it has been possible to increase the knowledge of, or to understand the epidemiology of the genetic structure in the population of these parasites (10). Similar markers also have been important tools in the understanding of the structure of the population of C. parvum (15), and have been successfully used to study the population dynamics of Cryptosporidium, evaluating their routes of transmission and their zoonotic potential. In a recent review it was shown that to study the existing variation between C. parvum and C. hominis using multilocus analysis, 55 markers in various combinations have been used over different platforms (16). The markers most used are 5B12, CP47, GP60, hsp70, ML1, ML2, MS5-Mallon, MS9-Mallon, MSB, MSC 6-7 and TP14 (17).

Thanks to tools that permit us to obtain biological and genetic data, some genotypes have been recognized as unique and different, taking the name and the status of species. For example, the canine genotype has begun to be called C. canis; the porcine genotype C. suis; the bovine genotype B C. bovis; and the deer-like genotype C. ryanae (1). Furthermore, the specific diagnosis of cryptosporidiosis through molecular tests allows precision in the identification and characterization of species of Cryptosporidium, a central condition for the control of this disease and the comprehension of the complexities of its epidemiology (10).

Molecular epidemiology

The tools of molecular biology have not only helped to resolve the taxonomy of Cryptosporidium, but also have made a valuable contribution in understanding the range of hosts of different species and genotypes (18). Additionally the molecular characterization of the circulating parasites can permit the evaluation of the distribution and zoonotic potential of species and subtypes as well as their routes of transmission to humans and animals under different epidemiological situations (2).

At the start of the HIV/AIDS pandemic the reports of pathogenic opportunists focused attention on cryptosporidiosis in humans. A summary of the literature at that time found reports of 159 cases of cryptosporidiosis in immunocompetent patients and 71 cases in immunocompromised patients. In 26 cases a clear association was established between the bovine infection and humans, however the transmission from animals to humans was not confirmed in any of the 71 cases of immunodeficient patients. Additionally, the dissemination from person to person had been reported. The reports of urban transmission without evidence of zoonotic transmission provided support for the hypothesis of Casemore and Jackson, which indicated that the infection in humans was not necessarily zoonotic, leading to the recognition of two independent cycles of transmission. The molecular studies then have provided evidence that these two routes of infection for human were related through two genotypes - the “human genotype” transmitted from human to human and the “bovine genotype” transmitted from animals to humans with the bovine sources as principle reservoirs (1).

The investigations at the epidemiological level required techniques with a greater power of discrimination, that could differentiate an intraspecific level (19). The implementation of subtyping with the GP60 gene has permitted the identification of geographic and temporal differences in the transmission of Cryptosporidium spp., and a better appreciation of the implication of the parasite in public health (4).

In a review including databases from Elsevier, Scielo, PubMed, SpringerLink and Wiley Library, the reports related to subtyping based on GP60 of the parasite in 29 countries, 28 cities that reported 163 subtypes of C. parvum. Concordant with the report by Couto et al. (14) and Feng et al (20), it was found that around the world the family of C. parvum that appeared with the greatest frequency is IIa (41/163), followed by the family IId (17/163), two families that have been recognized for their zoonotic implications. The subtype reported with the greatest frequency and that as well is present on every continent, with the exception of Oceania, is IIaA15G2R1 (18/28), followed by IIaA16G1R1 and IIaA18G2R1 which have been reported in 9 of 28 (Table 1, Figure1).

TABLE 1

Table 1: Families and subtypes of C. parvum reported in cattle and humans.

Country	Family	Subtype	Host	Reference
Germany	IIa	IIaA15G2R1* IIaA14G2R1 IIaA17G2R1 IIaA18G2R1 IIaA21G0R1 IIaA22G1R1 IIaA16G1R1	Cattle	(25)
	IId	IIdA22G1	Cattle	(25)
Argentina	IIa	IIaA21G1R1	Cattle	(26)
	IIa	IIaA17G1R1 IIaA18G1R1 IIaA20G1R1* IIaA21G1R1 IIaA22G1R1 IIaA23G1R1	Cattle	(2)
	IIa	IIaA18G1R1 IIaA20G1R1* IIaA21G1R1 IIaA22G1R1 IIaA23G1R1 IIaA16G1R1 IIaA19G1R1	Cattle	(24)
Australia	IIa	IIaA15G2R1 IIaA17G2R1 IIaA18G3R1* IIaA19G2R1 IIaA19G3R1	Human	(27)
	IIc	IIcA5G3a	Human	(27)
	IIa	IIa15G2R1 IIaA18G3R1 IIaA17G2R1* IIaA18G3R1 IIaA20G3R1	Cattle	(27) (28)
Brazil	IIa	IIaA20G2R1 IIaA20G2R2 IIaA19G2R1 IIaA19G2R2 IIaA18G1R1 IIaA18G2R2 IIaA16G3R2 IIaA14G2R2	Cattle	(14)
	IIa	IIaA15G2R1*	Cattle	(29)
Egypt	IIa	IIaA15G1R1 IIaA15G2R1	Human & Cattle. Human	(8)
	IId	IIdA20G1*	Human & Cattle.	(8,29)
Spain	IIa	IIaA15G2R1* IIaA13G1R1 IIaA15G1R1 IIaA16G2R1 IIaA16G3R1 IIaA17G2R1 IIaA18G3R1	Cattle Human Cattle Cattle Cattle Cattle Cattle	(12,30)
	IIa	IIaA19G3R1 IIaA15G2R1* IIaA13G1R1	Cattle Cattle Cattle	(30)
	IIc	IIcA5G3R2	Human	(12)
	IId	IIdA22G2R1 IIdA23G1	Human & Cattle	(12)
United States	IIa	IIaA15G2R1*	Bovine	(31,32)
	IIa	IIaA15G2R2 IIaA11G2R1 IIaA17G2R1 IIaA18G2R1 IIaA19G2R1	Cattle	(32)
Ethiopia	IIa	IIaA15G2R1* IIaA16G2R1 IIaA16G1R1	Human	(33)
France	IIa	IIaA15G2R1* IIaA18G1R1*	Cattle	(34)
Hungary	IIa	IIaA16G1R1* IIaA17G1R1 IIaA18G1R1	Cattle	(35)
	IId	IIdA22G1 IIdA19G1	Cattle	(35)
India	IIa	IIaA15G2R1* IIaA13G2R2 IIaA14G2R1a IIaA14G2R1b	Cattle	(31)
	IIc	IIcA5G3*	Human	(6)
	IId	IIdA14G1 IIdA15G1	Human	(6)
	IIe	IIeA7G1	Human	(6)
England and Wales	IIa	IIaA16G3R1 IIaA14G2R1 IIaA19G1R1 IIaA18G1R1	Cattle	(19)
	IIa	IIaA20G3R1 IIaA19G3R1 IIaA15G2R1 IIaA17G1R1	Cattle	(36)
Iran	IIa	IIaA15G2R1* IIAA16G3R1	Human & Cattle	(37)
	IId	IIdA15G1 IIdA18G1 IIdA20G1a IIdA21G1a IIdA26G1	Human & Cattle	(37)
Ireland	IIa	IIaA18G3R1* IIaA15G2R1* IIaA17G2R1 IIaA19G4R1 IIaA20G3R1 IIaA19G3R1 IIaA17G3R1 IIaA20G5R1 IIaA18G2R1 IIaA20G2R1	Cattle	(38)
Jamaica	IIc	IIcA5G3d	Human	(39)
Japan	IIa	IIaA15G2R1*	Cattle	(40)
Jordan	IIa	IIaA15G1R1 IIaA20G3R1	Human	(41)
	IIc	IIcA5G3a	Human	(41)
	IId	IIdA14G1 IIdA20G1* IIdA24G1 IIdA29G1	Human	(41)
Kuwait	IIa	IIaA15G1R1 IIaA15G2R1	Human	(3)
	IIc	IIcA5G3a	Human	(3)
	IId	IIdA20G1* IIdA18G1	Human	(3)
	IIf	IIfA6	Human	(3)
Malasya	IId	IIdA15G2R1	Human	(42)
Mexico	IIa	IIaA15G2R1 IIaA16G1R1	Human	(43)
Nigeria	IIc	IIcA5G3h	Human	(44)
	IIe	IIeA10G1	Human	(45)
Netherlands	IIa	IIaA15G2R1* IIaA17G1R1	Human & Cattle.	(9)
	IId	IIdA15G1 IIdA16G1 IIdA18G1	Human	(9)
	IIa	IIaA16G3R1 IIaA13G2R1 IIaA14G2R1 IIaA17G2R1 IIaA18G4R1 IIaA18R1 IIaA19G2R1 IIaA11G2R1 IIaA16G1R1 IIaA19G1R1 IIaA18G1R1 IIaA16G2R1 IIaA18G3R1 IIaA21G3R1 IIaA12G2R1	Cattle	(9)
	IIj	IIjA24R2	Cattle	(9)
Peru	IIc	IIcA5G3a* IIcA5G3b IIcA5G3c	Human	(46)
Portugal	IIa	IIaA15G2R1* IIaA16G2R1	Human & Cattle	(47)
	IIb	IIbA14	Human	(47)
	IIc	IIcA5G3	Human	(47)
	IId	IIdA17G1 IIdA19G1 IIdA21G1 IIdA22G1	Human & Cattle Human Human Human	(47)
Czech Replubic	IIa	IIaA15G2R1* IIaA16G1R1 IIaA22G1R1 IIaA15G1R1 IIaA18G1R1	Cattle	(48)
Romania	IIa	IIaA15G2R1* IIaA16G1R1	Cattle	(49)
Switzerland	IIa	IIaA18G3R1	Human	(27)
	IId	IIdA22G1	Human	(27)
United States of Serbia and Montenegro	IIa	IIaA16G1R1* IIaA18G1R1 IIaA20G1R1	Cattle	(50)
	IId	IIdA18G1b	Cattle	(50)
	IIj	IIjA16R2 IIjA17R2	Cattle	(50)

*Subtype of C. parvum found in the highest frequency in the study.

Figure 1. Distribution of subtypes of *C. parvum* and *C. hominis* found in the highest frequency in different parts of the world.

FIGURE1
Figure1

In 18 of 29 countries, 6 families and 67 subgenotypes of C. hominis were reported. With relation to this species, the most frequent found in the articles consulted were family Ib (28/89), followed by the family Ia (24/89), the subtype IbA10G2 being most frequently reported in the countries (9/18), followed by the subtype IbA9G3 (Table 2, Figure 1).

TABLE 2
Table 2: Families and subtypes of C. hominis reported in humans.

Country	Family	Subtype	Reference
Australia	Ia	IaA23	(27)
	Ib	IbA5G2T3 IbA9G2 IbA9G2T1 IbA10G2*	(27)
	Id	IdA15G1 IdA16 IdA25	(27)
	If	IfA11G1T1 IfA12G1	(27)
China	Ia	IaA9R3	(51)
	Ib	IbA16G2 IbA19G2 IbA20G2*	(51)
	Id	IdA21	(51)
Spain	Ia	IaA21G1R1	(12)
	Ib	IbA10G2R2	(12)
Ethiopia	Ib	IbA9G3	(33)
India	Id	IdA15G1 (Cattle)	(31)
	Ia	IaA18R3 IaA19R3 IaA21R3 IaA26R3 IaA27R3 IaA29G1T3R3	(6)
	Ib	IbA9G3	(6)
	Id	IdA14G1 IdA15G11 IdA16G1	(6)
	Ie	IeA11G3T2 IeA11G3T3*	(6)
	If	IfA13G1	(6)
Iran	Id	IdA20	(37)
	If	IfIA22G1	(37)
Jamaica	Ib	IbA10G2*	(39)
	Ie	IeA12G3T3	(39)
Jordan	Ib	IbA6G3 IbA9G3 IbA10G2 IbA20G2	(41)
	Id	IdA21 IdA24*	(41)
Kuwait	Ib	IbA9G3 IbA10G2	(3)
	Id	IdA14	(3)
	Ie	IeA11G3T3	(3)
Malasya	Ia	IaA14R1	(42)
	Ib	IbA10G2R2	(42)
	Id	IdA15R2	(42)
	Ie	IeA11G2T3R1	(42)
	If	IfA11G1R2	(42)
Mexico	Ia	IaA15R3 IaA14R3*	(43)
	Ib	IbA10G2	(43)
	Id	IdA17	(43)
	Ie	IeA11G3T3*	(43)
Nigeria	Ia	IaA14R3 IaA16R3 IaA24R3 IaA25R3 IaA23R3 IaA25R3	(44)(45)
	Ib	IbA13G3	(44)
	Ie	IeA11T3G3	(44)
Netherlands	Ib	IbA10G2*	(9)
	Id	IdA17 IdA14	(9)
	Ic	IcA5G3R2	(9)
Perú	Ia	IaA11R4 IaA12R4 IaA13R4 IaA13R7 IaA14R6 IaA15R3	(46)
	Ib	IbA10G2*	(46)
	Id	IdA10 IdA15 IdA20	(46)
	Ie	IeA11G3T3	(46)
Portugal	Ia	IaA19R3	(47)
	Ib	IbA10G2*	(47)
	Id	IdA15	(47)
	Ie	IeA11G3T3	(47)
	If	IfA14G1	(47)
Switzerland	Ib	IbA10G2	(27)
	Id	IdA15G1	(27)
United Kingdom	Ib	IbA10G2 IbA9G3 IbA12G3T3 IbA10G2	(27)

*Subtype of C. hominis found in the highest frequency in the study.

Evolutionary genetics

Cryptosporidium spp. belongs to the phylum Apicomplexa, class Sporozoae, subclase Coccidia, order Eucoccidiida, suborder Eimeriina, family Cryptosporidiidae (4).

In 2003 the complete genome of C. parvum as well as C. hominis was published in CryptoDB ® demonstrating a high degree of similarity ranging between 95 y 97% . The genome of Cryptosporidium parvum is about 9 million base pairs in 8 chromosomes (17).

The organisms belonging to the phylum Apicomplexa, like the majority of the protists, diverged relatively early in the eukaryotic lineage and have many biological characteristics that are not shared with the principle models of eukaryotic systems (intracellular parasitism for example, or the possession of secondary plastids). Several large-scale sequencing efforts have drastically increased the number of genes known from the Apicomplexa. However, assigning biological function to many of these genes continues to be a major challenge. The generation of loss-of-function mutants is greatly facilitated by the fact that the parasites have a haploid genome over the majority of their life cycles (21).

Similar to other parasites in the phylum Apicomplexa, the life cycle of Cryptosporidium spp. has a sexual phase during which recombination between genetically different strains facilitated not only the evolution and appearance of subtypes but also the adaptation of Cryptosporidium spp. (5) and generation of genetic variation between different populations in agreement with the ecological demands and epidemiological conditions of a region (22).

The species of C. parvum that infect humans and some animals could undergo meiotic recombination between different lineages. This could play an important role in the evolution of virulent subtypes (5). Genetic recombination appears to be associated with the high frequency of polymorphism in the gene for GP60. For this, standardized association indices have been used, measured between alleles, which is zero in panmitic populations and with positive values in non-panmitic populations, alternating these behaviors due to the presence of different genotypes and their subsequent recombination generating impact on the genetic equilibrium (22). Markers like the actin gene, heat shock gene and the small ribosomal subunit have been used for phylogenetic investigations and the construction of the current classification (10) however the comparison has been criticized for the lack of a system of genotypic standardization (22).

Recent studies have suggested that the telomeric/subtelomeric regions are highly polymorphic and could carry putative virulence factors. When a locus shows extraordinary levels of genetic differentiation in the population, compared with other loci, it could be interpreted as evidence of positive selection (23). The identification of the grade of intraspecies variation using multilocus methods depends on three factors: the characteristics of the sampling, the types of techniques and the structure of the local population of the parasite (17). Cacciò et al (22) analyzed different geographic zones evaluating the relation between those zones and GP60 polymorphisms. This study produced no evidence consistent with geographic isolation and the presence of mutations (22). However Del Coco et al (24) found an association between subtypes and the location, possibly indicating a geographic segregation, concluding that is was necessary to do more studies to evaluate the degree of association of subtypes and pathogenicity, including the postulate that more genes may be associated with this condition, suggesting the evaluation of the relationship between different geographical situations where Cryptosporidum spp. is present (24).

Conclusion

Molecular diagnosis of the parasite allows the design of strategies to avoid contamination of the environment, human and animal populations of the studied region. This type of diagnosis can be the most successful tool in the preventive management of cryptosporidiosis.

The phylogenetic studies allow to know the distribution, zoonotic potential and the genetic variation of Cryptosporidium species and subtypes. The variations among the different subtypes of the GP60 gene often occur as a consequence of synonymous or silent mutations in the microsatellite region, where they do not affect the coding capacity of the codon for serine. These mutations must be the end result of a purifying process of negative, positive, or neutral selection and may or may not be related to the virulence of the parasite.

Geographic area and its specific environmental conditions could affect the genetic composition of parasite, acting as an inductor agent of mutations and differentiating subtypes worldwide.

Conflict of interest

The authors of this paper declare no conflict of interest that may jeopardize its validity.

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