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COMENTARIO

The confused taxonomy of *Cryptospoidium*

La confusa taxonomía de *Cryptosporidium*

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Resumen: Los últimos descubrimientos en la biología y filogenética de *Cryptosporidium* refuerzan la necesidad de una exhaustiva revisión del ciclo de vida y la taxonomía de este parásito. Tanto futuros estudios de cultivo in vitro e in vivo así como estudios moleculares y genéticos permitirán avanzar en el profundo conocimiento de este interesante parásito.

Palabras clave: *Cryptosporidium*, biología, filogenética, taxonomía, ciclo de vida.

Abstract: The last discoveries in the *Cryptosporidium* biology and phylogenetics reinforce the necessity of a exhaustive review of both life cycle and taxonomy of this parasite. Besides in vitro and in vivo studies, further molecular and genetic studies will allow to advance in the thorough knowledge of this interesting parasite.

Keywords: *Cryptosporidium*, biology, phylogenetics, taxonomy, life cycle.

Many aspects of the biology and taxonomy of *Cryptosporidium* species remain confused, in spite its first report appeared practically one century ago, and it is an obligate intracelular enteropathogen parasite of humans and many vertebrate animals, mainly mammals.

Scarcely considered for decades due to its apparently non-pathogenic nature, *Cryptosporidium* has been studied very actively over the last 20 years after its medical relevance as a dangerous opportunistic parasite and widespread water contaminant was fully recognized (Fayer and Ungar, 1986). Despite the lack of an efficient in vitro culture system and appropriate animals models, significant advances have been made in a short period of time allowing a larger understanding of the biology, immunology, genetics and epidemiology of this parasite.

Among the aspects of its biology that remain confused, in the last years its phylogenetic relation with several microorganisms has become main investigation line for many researchers because of interesting and unexpected results that offered first studies of this Apicomplexa.

Currently, species of *Plasmodium* causing malaria in humans are classified in the same order (Eucoccidiorida) but in different suborder (Haemosporina) than species of *Cryptosporidium*. More closely related to *Cryptosporidium* spp. are the other true coccidia (suborden Eimeriorina), *Isospora belli*, *Sarcocystis* spp., and *Toxoplasma gondii*, which infect human beings, and *Eimeria* spp., which infect mammals and birds.

Cryptosporidium was originally classified as a coccidian based on its life cycle features (Levine, 1988). However, *Cryptosporidium* demonstrates several peculiarities that separate it from any other coccidian. These include: the location of *Cryptosporidium* within the host cell where the endogenous developmental stages are confined to the apical surfaces of epithelial cells (intracellular but extracytoplasmic); the attachment of the parasite to the host cell where a multi-membranous attachment or feeder organelle is formed at the base of the parasitophourous vacuole to facilitate the uptake of nutrients from the host cell; the presence of two morpho-functional types of oocyst, thick-walled and thin-walled, with the latter responsible for the initiation of the auto-infective cycle in the infected host; which lacks morphological structures such as sporocyst, micropyle, and polar granules; and finally the insensitivity of *Cryptosporidium* to all anticoccidial agents tested so far (O'Donoghue, 1995; Fayer et al., 1997; Carreno et al., 1999). These unique biological and morphological features have been completed by additional molecular studies, which strongly grouping *Cryptosporidium* as a separate clade of the coccidia taxa (Relman et al., 1996; Barta, 1997; Morrison and Ellis, 1997; Lopez et al., 1999).

A study by Carreno et al. (1998) based of SSrRNA secuenciation showed that *Cryptosporidium* and gregarines formed a separate clade from the Apicomplexa clade containing the coccidia. Subsequent studies about the *Cryptosporidium* development in both *in vitro* and *in vivo* culture showed the presence of extracellular stages in the *Cryptosporidium* life cycle. Hijjawi et al. (2002, 2004) described the presence of extracellular gamont-like stages in syzygy during some stages, even though they indicated that ultrastructural confirmation would be needed. Last year, this ultrastructural confirmation was possible thanks to in vitro culture studies where by using different line cells it has been possible to describing all the development stages described in *Cryptosporidium* life cycle further several extra cellular stages in the supernatant of all infected cultures, which were described in detail by using optic, normaski and transmission electron microscopy images (Rosales et al., 2005).

The trophozoite/gamont-like stage resembles a similar stage found in gregarines (Levine, 1985; Macwell, 1977). Another stages founded were spores with eight sporozoites, also resembling those in gregarines. These spores could be self-infective and cause cyclic development as occurs with thin-walled oocysts of which there is only one ultra structural image (Current and Reese, 1986). The origin of extra cellular stages of *C. parvum* is not known, but it is possible that when the culture is infected with sporozoites some penetrated into the host cell and developed extra cellular motile trophozoite stages with capacity to associate with each other taking place a division process and sporogenesis.

On the other hand and under ultra structural point of view efforts to detect a mitochondrion-like organelle in so far the different *C. parvum* stages have been unsuccessful. Nevertheless, a structure resembling the mitochondrion of other apicomplexas has been observed in the merozoites of *C. muris*. But also, electron microscopy observations seem to support the

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presence in *C. parvum* sporozoites of a membrane-bound organelle (Coombs et al., 1997), whose perinuclear location and morphology are compatible with it being the plastidia-like organelle found in *Toxoplasma* and *Plasmodium*.

Several genetic studies carried out in the last years have contributed data that also strengthen an exhaustive revision of the *Cryptospordium* taxonomy. The analysis of the *Cryptosporidium* species gene sequences has revealed a lack of introns interrupting the conding protein regions. The absence of introns as a general feature of the *Cryptosporidium* genome was confirmed by additional molecular studies. Char et al. (1996) published a study in which the codon usage *C. parvum* genes was compared with that determined for other members of the phylum Apicomplexa. Surprisingly, this analysis failed to show any close relationship between *C. parvum* and two other Eimeriorina especies (*T. gondii* and *E. tenella*) but rather a higher similarity to *P. Falciparum*, *Babesia bovis* and *Theileria parva* (Haemosporida spp.) and to *Entamoeba histolytica.*

The predominantly introless structure of *C. parvum* genes and the distintive codon usage add to the many peculiar traits of this parasite strengthen the hypothesis that *Cryptosporidium* have an evolutionary history that is distinct from that of related members of the phylum Apicomplexa (Spano and Crisanti, 2000).

Thus, after a century since the discovery of *Cryptosporidium*, important questions concerning to many aspects of the parasite biology, immunology and epidemiology remain unanswered. Our inability to develop adequate models for the study of this parasite is a great obstacle to advance in the *Cryptosporidium* knowledge, in fact, most of what we know about *Cryptosporidium* is thanks to an important contribution provided by molecular biology studies. In spite of many *Cryptosporidium* research groups hardly work to get a continue and productive *Cryptospordium* in vitro culture, a wonderful achievement, further molecular studies will be necessary to clarify the taxonomy of this surprising parasite.

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