



Diarrea infecciosa en niños cubanos: Apuntes de estudios moleculares desarrollados para la detección de parásitos intestinales



Luis Enrique Jerez Puebla, Fidel Ángel Núñez Fernández, Lucia Ayllón Valdés, Iredys Cruz Rodríguez, Madelaine Miranda Machado, Irais Atencio Millán, Annia Fong González, Raúl Atilano Cordovi Prado, Alicia Martínez Izquierdo, Ainadys Garciga Mesa.



- Rotavirus
- Norovirus
- *Salmonella*
- *E. coli*
- *Campylobacter*

Current Infectious Disease Reports (2020) 22:4
<https://doi.org/10.1007/s11908-020-0713-6>

PEDIATRIC INFECTIOUS DISEASES (I BROOK, SECTION EDITOR)

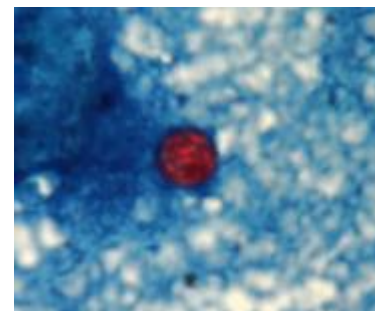
Acute Infectious Diarrhea and Gastroenteritis in Children

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Multicenter Study > PLoS Negl Trop Dis. 2016 May 24;10(5):e0004729.

doi: 10.1371/journal.pntd.0004729. eCollection 2016 May.

The Burden of Cryptosporidium Diarrheal Disease among Children < 24 Months of Age in Moderate/High Mortality Regions of Sub-Saharan Africa and South Asia, Utilizing Data from the Global Enteric Multicenter Study (GEMS)



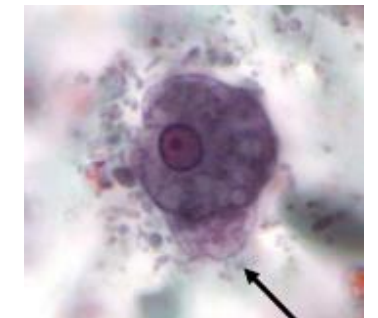
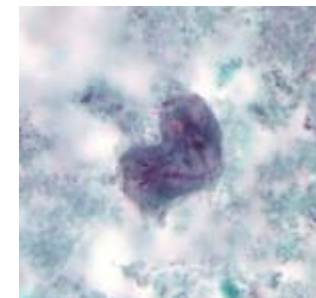
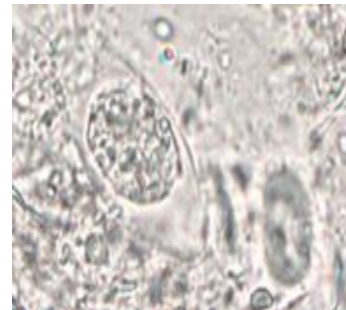
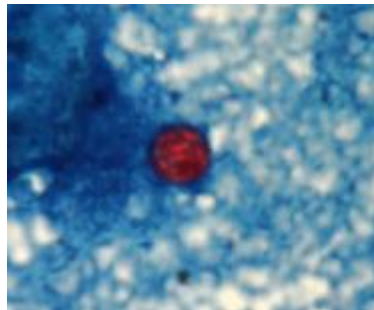
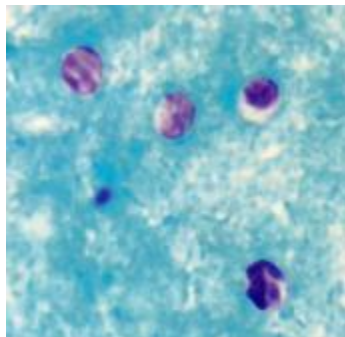


Diarrea provocada por parásitos

Protozoos:

- ➡ *Cryptosporidium* spp
- ➡ *Cyclospora cayetanensis*
- ➡ *Cystoisospora belli*
- ➡ *Giardia lamblia*
- ➡ *Entamoeba histolytica*

Coccidios





> Mem Inst Oswaldo Cruz. 2003 Jun;98(4):539-42. doi: 10.1590/s0074-02762003000400021.
Epub 2003 Aug 18.

Intestinal coccidia in Cuban pediatric patients with diarrhea

F A Núñez¹, O M González, I González, A A Escobedo, R A Cordoví

Affiliations + expand

PMID: 12937770 DOI: 10.1590/s0074-02762003000400021

> Ann Trop Med Parasitol. 2011 Jan;105(1):47-56. doi: 10.1179/136485911X12899838413420.

Hospitalization of Cuban children for giardiasis: a retrospective study in a paediatric hospital in Havana

A A Escobedo¹, P Almirall, M Alfonso, Y Salazar, I Avila, S Cimerman, F A Núñez, I V Dawkins

Affiliations + expand

PMID: 21294948 PMCID: PMC4089796 DOI: 10.1179/136485911X12899838413420

Parasitosis Humana 2025 | CUBA



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Cryptosporidium en niños en la provincia Ciego de Ávila

Palabras clave: Cryptosporidium, niños.

Key words: Cryptosporidium, children.

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Recibido: 22/09/2010

Aceptado: 18/11/2010

> Bol Chil Parasitol. 1997 Jul-Dec;52(3-4):50-4.

[Cryptosporidiosis in children with acute diarrhea from Ciego de Avila province, Cuba]

[Article in Spanish]

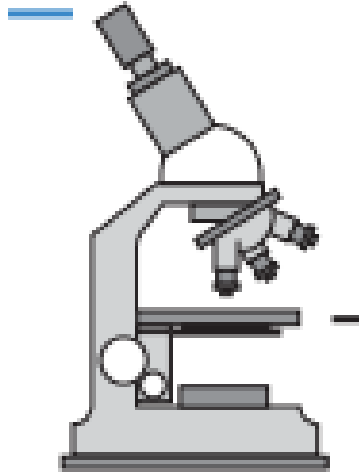
M Suárez Hernández¹, M Díaz González, J Bustelo Aguila, A Sánchez Sibello, I Vidal García

> Rev Cubana Med Trop. 2003 Jan-Apr;55(1):14-8.

[Diagnosis of coccidia and microspores in specimens of diarrheal feces from Cuban HIV seropositive patients: first report of microspores in Cuba]

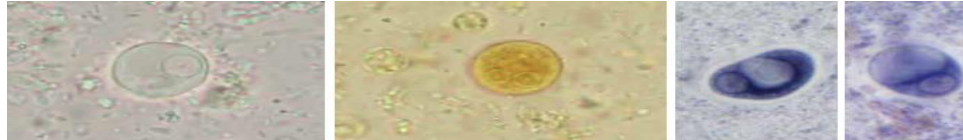
[Article in Spanish]

Virginia Capó de Paz¹, Marta Barrero Brínguez, Beltrán Velázquez Viamonte, Caridad Luzardo Suárez, Alina Martínez Rodríguez, Zaida Alujas Martiñex



Laboratorio de Parasitología

Regla de Oro en el diagnóstico Parasitológico



JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2011, p. 257–262
0095-1137/11/\$12.00 doi:10.1128/JCM.01796-10
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Vol. 49, No. 1

Evaluation of Multiplex Tandem Real-Time PCR for Detection of *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia intestinalis* in Clinical Stool Samples[∇]

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J. Harkness,^{1,2} and J. T. Ellis²



Diagnosis of intestinal protozoan infections in patients in Cuba by microscopy and molecular methods: advantages and disadvantages

Luis Enrique Jerez Puebla^a, Fidel A. Núñez-Fernández^{a,b}, Jorge Fraga Nodarse^a, Irais Atencio Millán^a, Iredys Cruz Rodríguez^a, Isabel Martínez Silva^c, Lucía Ayllón Valdés^c, Lucy J. Robertson^{d,*}

Table 1

Comparison of identification of intestinal parasites by microscopy-based and molecular-based methods among 133 patients with gastrointestinal symptoms.

Intestinal parasites identified ^a	Number of positive cases identified			Proportion infected (95% CI)
	By microscopy	By PCR	In total (microscopy and/or PCR)	
<i>Giardia duodenalis</i>	17	21	21	15.8 (10.5–23.0)
<i>Cryptosporidium</i> spp.	3	5	5	3.8 (1.4–8.7)
<i>Entamoeba histolytica</i> / <i>E. dispar</i>	4	0 ^e	4	3.0 (0.9–7.7)
<i>Blastocystis</i> spp. ^b	12	0	12	9.0 (5.1–15.2)
<i>Cyclospora</i>	1	0	1	0.8 (<0.01–4.6)
<i>Cyclospora cayetanensis</i> ^{a, b, c}				12.0 (7.4–18.8)
<i>Dientamoeba fragilis</i>	0	16	16	31.6 (24.3–39.9)
Total infected with at least one intestinal protozoan parasite^{ad}	29	38	42	

^a Modified Ziehl-Neelsen (particularly relevant for identifying *Cryptosporidium* spp. and *Cyclospora cayetanensis*, was used only on diarrheic samples ($n = 31$), but all samples were examined by other light microscopy approaches and by PCR ($N = 133$).

^b *Blastocystis* spp. and *Cyclospora cayetanensis* not investigated by PCR.

^c *Cyclospora cayetanensis* only investigated by modified Ziehl-Neelsen in diarrheic samples ($n = 31$).

^d Due to co-infections (see text), total infected with at least one intestinal protozoan parasite does not equal sum of number of positive cases identified.

^e PCR only detects *Entamoeba histolytica*.

PCR Múltiple para *Giardia/Cryptosporidium* /*Entamoeba histolytica*

133 pacientes pediátricos atendidos en HPD-WS e IPK (periodo enero-diciembre 2020)

- ❖ *G. lamblia* ($\kappa = 0.88$)
- Cryptosporidium* spp. ($\kappa = 0.74$)



Diagnosis of intestinal protozoan infections in patients in Cuba by microscopy and molecular methods: advantages and disadvantages

Luis Enrique Jerez Puebla^a, Fidel A. Núñez-Fernández^{a,b}, Jorge Fraga Nodarse^a, Irais Atencio Millán^a, Iredys Cruz Rodríguez^a, Isabel Martínez Silva^c, Lucía Ayllón Valdés^c, Lucy J. Robertson^{d,*}

^a L.E. Jerez Puebla et al.

Journal of Microbiological Methods 179 (2020) 106102

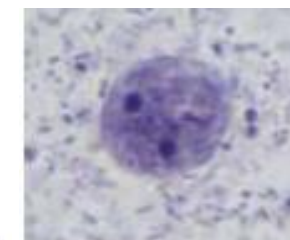
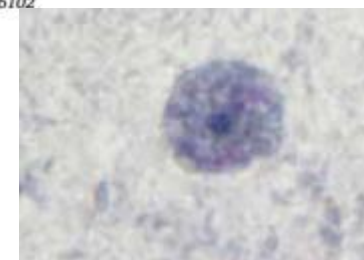
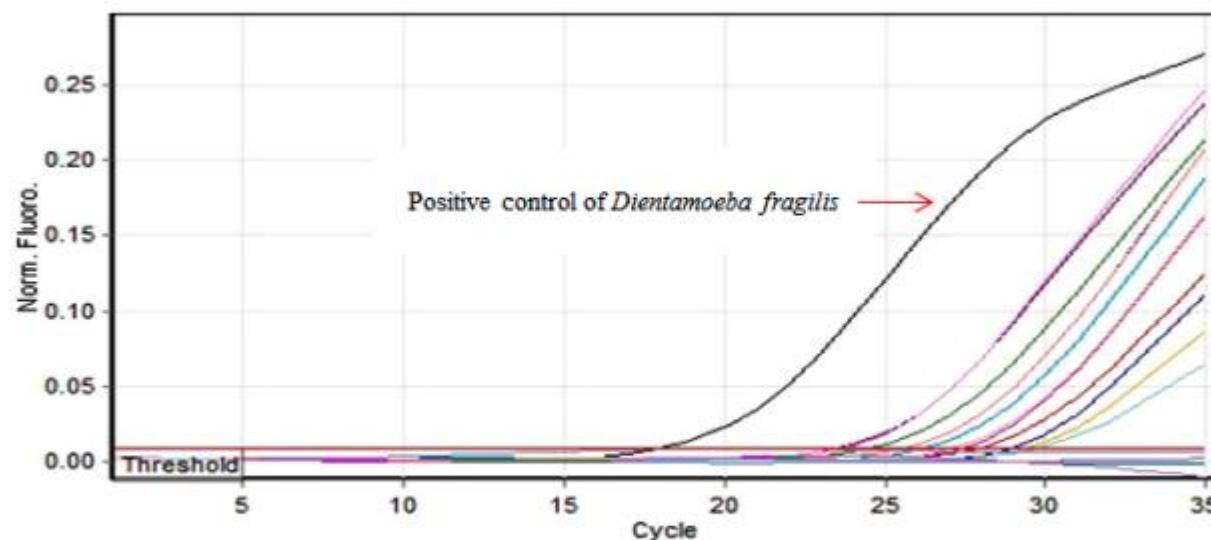


Fig. 1. Detection of *D. fragilis* in stool samples by real-time PCR following the protocol of Stark et al., 2006. Positive control (black curve) and representation of positive samples identified. The negative control did not amplify and is running below the red threshold (cut-off) line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

❖ Primera vez que se detecta *D. fragilis* por métodos moleculares



Parasitology

Sporadic cyclosporiasis in symptomatic Cuban patients: Confirmation of positive results from conventional diagnostic methods by molecular assay

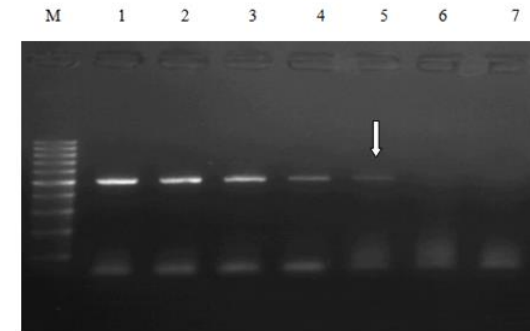
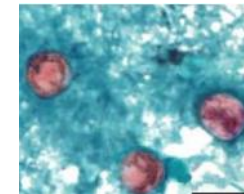
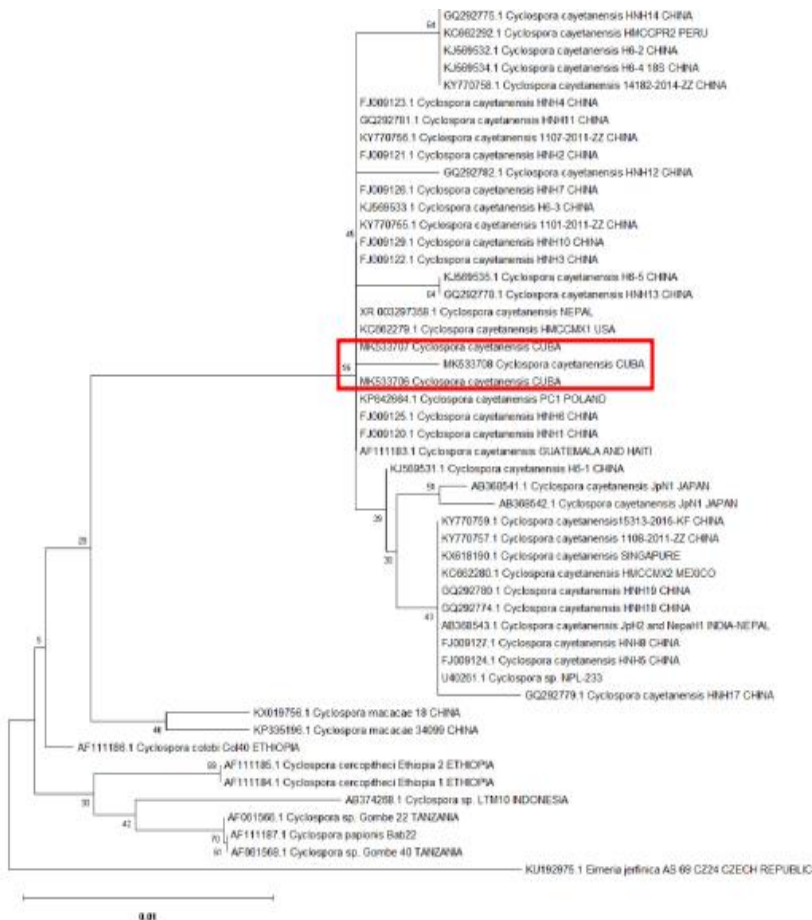
Luis Enrique Jerez Puebla ^a, Fidel A. Núñez Fernández ^{a,b}, Jorge Fraga Nodarse ^a, Iraís Atencio Millán ^a, Iredys Cruz Rodríguez ^a, Lisette Pérez Santos ^c, Yanet Pintos Saavedra ^c, Laura Rodríguez Moreno ^a, Lucy J. Robertson ^{d,*}



Cyclospora cayetanensis

❖ 7 muestras positivas (0.56%)

❖ Se secuenciaron por primera vez en Cuba
aislamientos de *Cyclospora cayetanensis*





La prevalencia de infección por parásitos intestinales fue de 38,7%. Las especies más representativas fueron *G. duodenalis* (15,8 %), *Blastocystis* spp. (13,7 %) y *Cryptosporidium* spp. (13,0 %).

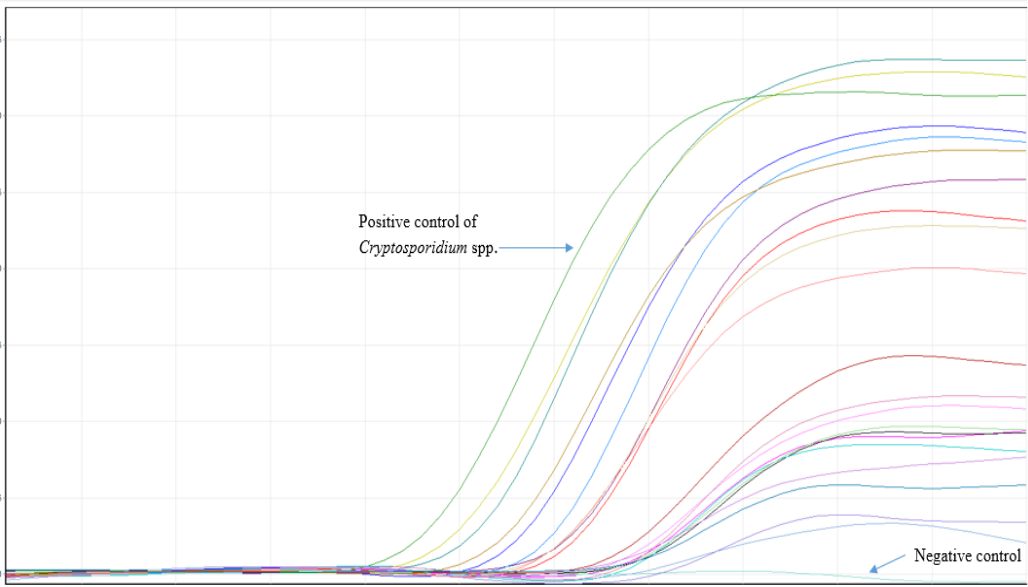
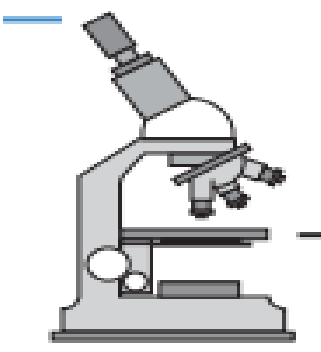


Tabla 2. Evaluation of the performance of the parasitological and molecular test, and Concordance index (kappa), for the diagnosis of *Cryptosporidium* spp. in children admitted with diarrhea at two Pediatric Hospitals in Havana, 2023.

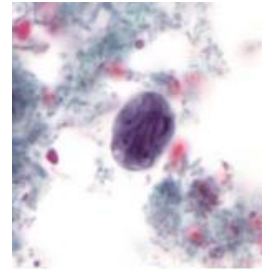
Techniques	Conventional techniques for <i>Giardia</i>	
	(Reference technique)	
	Positive	Negative
Positive to <i>Cryptosporidium</i> spp.	6	9
PCR for <i>Cryptosporidium</i> spp.		
Negative to <i>Cryptosporidium</i> spp.	2	96
Parameters	Percentage (95% CI)	
Sensitivity:	75.0 % (38.7-100)	
Specificity:	91.4 % (85.6-97.3)	
Positive Predictive value:	40.0 % (11.9-68.1)	
Negative Predictive value:	97.8 (94.7-100)	
Kappa index:	0.47 (95% CI: 0.43-0.79)	

Tabla 3. Evaluation of the performance of the parasitological and molecular test, and Concordance index (kappa), for the diagnosis of *Giardia* in children admitted with diarrhea at two Pediatric Hospitals in Havana, 2023.

Techniques	Conventional techniques for <i>Giardia</i>	
	(Reference technique)	
	Positive	Negative
Positive to <i>Giardia</i>	15	11
PCR for <i>G. duodenalis</i>		
Negative a <i>Giardia</i>	3	84
Parameters	Percentage (95% CI)	
Sensitivity:	83.3 % (63.3-100)	
Specificity:	88.4 % (81.5-91.4)	
Positive Predictive value:	57.7 % (36.8-78.6)	
Negative Predictive value	96.6 (92.1-100)	
Kappa index:	0.61 (95% CI: 0.43-0.79)	



- **12,8%**

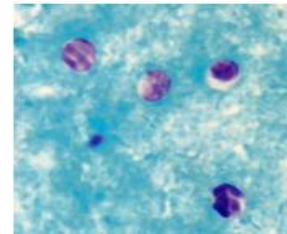


16% ↑



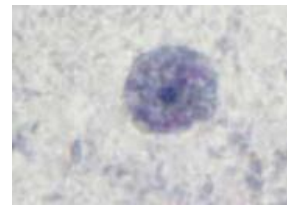
Entamoeba dispar como especie infectante

2,1%



4,9% ↑

0 %



12 % ↑

Impacto



Beneficio científico

- ❖ Se evidenció la utilidad de la PCR en tiempo real en el diagnóstico de especies del complejo *Entamoeba histolytica/dispar*, y de *Cryptosporidium* spp. que infectan al humano.
- ❖ Se secuenciaron por primera vez aislamientos cubanos de *Cyclospora cayetanensis* los que demuestran una alta homología con aislamientos de esta coccidia intestinal en otras regiones del mundo.
- ❖ Se identificó por primera vez en Cuba, mediante técnicas moleculares, a la especie *Dientamoeba fragilis* implicada en casos de niños con sintomatología gastrointestinal.
- ❖ Se introdujo en el LNRP del IPK técnicas moleculares para el diagnóstico de especies parasitarias asociadas con diarreas crónicas en pacientes inmunodeprimidos.



GRACIAS