Original Article

Molecular Detection of *Cryptosporidium parvum* by Specific Gene (Heat Shock Protein) in Children at Babylon Center

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Abstract

Background: *Cryptosporidium* is a diarrhea-causing single-celled parasite that infects the gastrointestinal tract of humans, livestock, birds, and wildlife populations. **Objectives:** Molecular detection of *C. parvum* by specific genes in children. **Materials and Methods:** A total of 300 stool samples were collected from patients aged 17 days to 10 years, (26 females and 49 males) and were represented using a specific gene [Heat shock protein gene (HSP70)] for the diagnosis of *C. parvum*. **Results:** The results indicated that from 300 stool samples, 75 (25%) were found to be positive for *C. parvum*. Additionally, polymerase chain reaction product analysis of the HSP70 gene in *C. parvum* reported that fifty of the 75 (66.66%) samples observed carried the HSP70. Also, the results indicated that the highest infection rate was recorded in patients of the 17-day age group (27, 36%, and the lowest rate was recorded in the patients of the 9–10 year age group (2, 2.7%) with a significant value. According to gender, the results indicated that the highest percentage was found in males (65.3%), and the lowest percentage was found in females (34.7%). Additionally, the highest infection rate was found in rural areas (62.5%), while the lowest infection was found in urban areas (37.5%). **Conclusion:** HSP70 could be a good diagnostic tool for the detection of *C. parvum* among pediatric patients as well as for the monitoring of water resources, in which low concentrations of parasites are usually observed.

Keywords: *Cryptosporidium*, diarrhea, heat shock protein gene (HSP70), PCR

Introduction

In outbreaks of waterborne gastroenteritis, such as the one that occurred in Wisconsin in 1993, resulting in over 400,000 cases of infection and sickness, *Cryptosporidium* is a primary causal agent. Both *Cryptosporidium hominis* and *Cryptosporidium parvum* are the main causal agents of *Cryptosporidium* diarrhea in humans.[1] Consuming food or water adulterated with the parasite's oocysts is the most common way for humans to become infected with *C. hominis*. The parasite damages the small intestine after infecting the digestive tract and produces severe diarrhea.[2] Additionally, the zoonotic protozoan *C. parvum*, which most usually affects animals, can also infect humans and is regarded as such.[3] When some species of Cryptosporidium infect susceptible hosts, cryptosporidiosis is caused, which results in weight loss, excessive watery diarrhea, and decreased appetite. Other clinical manifestations, such as vomiting and nausea in some humans infected with *C. hominis*, may be detected depending on the parasite and host species. Several authors[4,5] reported information on *Cryptosporidium* species and subtypes, including their prevalence. The eight chromosomes in the genomes of *Cryptosporidium* range in size from 0.945 to 2.2 Mb on average, with a total haploid genome size of roughly 9.2 Mb. Additionally, two small extrachromosomal cytoplasmic double-stranded ribonucleic acids (RNAs) are present in *C. parvum*. Each of the RNAs has an open reading frame, which allows for the encoding of RNA-dependent RNA polymerase and proteins that have constrained structural and functional

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similarity to mammalian protein kinases. The size of the small ribosomal RNA (rRNA) subunit of the *C. parvum* rRNA gene is 1.7 kb, the size of the large rRNA subunit is 3.6 kb, and the size of the 5.8S rRNA subunit is 151 bp. Researchers discovered that cryptosporidiosis mostly infected young children (5 years old) and young people in the late summer.

Materials and Methods

Collection, isolation, and identification of samples

A total of 300 clinical specimens (blood and stool) were collected from males and females suspected of parasitic infection after showing clinical manifestations. Whole blood and stool samples were collected from 180 males and 120 females suffering from diarrhea at Babylon Teaching Hospital for Maternity and Children, with ages ranging from 17 days to 10 years, from October 2022 to April 2023.

Polymerase chain reaction

A polymerase chain reaction (PCR) assay was performed to detect the HSP70 gene in *C. parvum* isolated from patients. The PCR primers with their sequences, amplicon sizes, and PCR conditions are illustrated in Tables 1–3.

Table 1: The polymerase chain reaction primers with their sequence and amplicon size

Primers		Sequence	Product size
HSP70	F	GGGTCGCCAAATTAAGAACG	180 bp
	R	ACATTTCTCTCGCCAGTTCC	

Table 2: Polymerase chain reaction (PCR) thermocycler conditions

PCR step	Temp. (°C)	Time	Repeat
Initial denaturation	95	5 min	1
Denaturation	95	30 s	30 cycle
Annealing	58	30 s	
Extension	72	1 min	
Final extension	72	5 min	1
Hold	4	Forever	–

Table 3: Polymerase chain reaction (PCR) Master Mix preparation

PCR master mix	Volume (μL)
DNA template 5–50 ng	5
Forward primer (10 pmol)	1
Reverse primer (10 pmol)	1
PCR water	13
Total volume	20

Ethical approval

The study protocol was approved by the Ethical Committee at Babylon Medical College and the relevant ethical committee in the health directorate.

Statistical analyses

The statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 26.0 (SPSS, IBM Company, Chicago, IL 60606, USA)

Results

Microscopic diagnosis of stool samples

The stool samples were detected microscopically using the modified Ziehl–Neelsen acid-fast staining method in which *C. parvum,* the oocyst, appears as pink-to-red round bodies against a blue background. From 300 stool samples, 75 (25%) were found to be positive for diarrhea-causing protozoan parasites *C. parvum*, while 225 (75%) samples tested negative. These results are shown in Figures 1 and 2.

Molecular diagnosis of parasites in stool samples

To determine if the parasite isolates were *C. parvum*, we characterized the isolates on the basis of the presence of heat shock protein genes (HSP70) by using the PCR technique. Figure 3 shows an agarose gel electrophoresis image that shows the PCR product analysis of the HSP70 gene in *C. parvum* isolated from stool samples of diarrheal patients. Fifty of the 75 (66.66%) samples from the patients observed carried the HSP70.

Detection of *C. parvum* by the immuno chromatography method

Figure 4 and Table 4 show the detection of *C. parvum* parasites using the chromatographic immunoassay

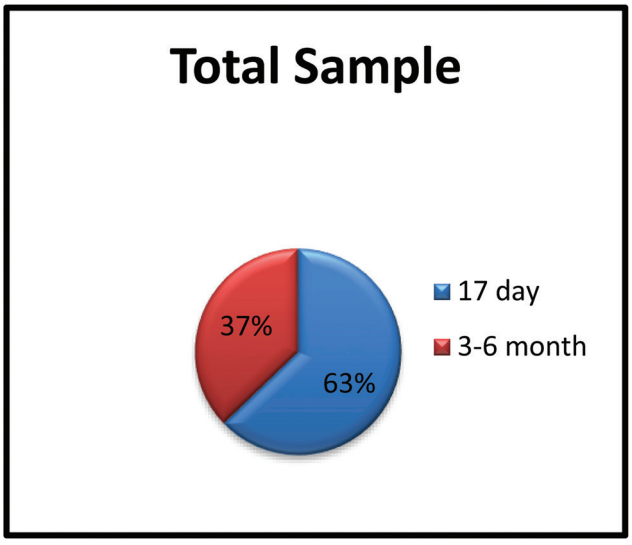

Figure 1: The prevalence of *Cryptosporidium parvum* among pediatric patients

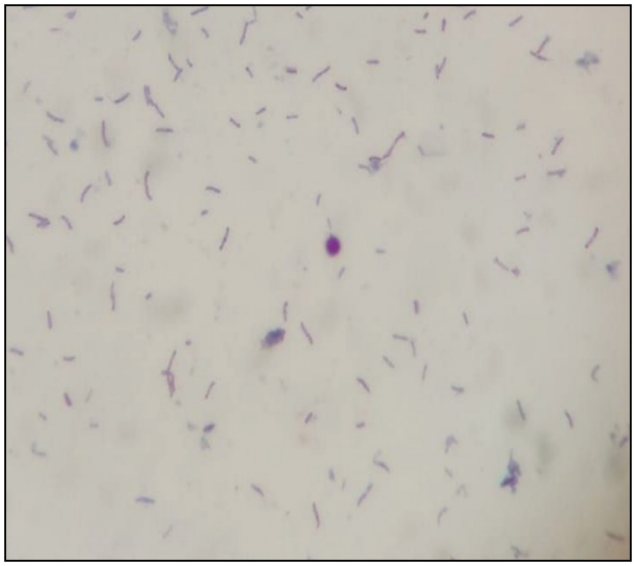

Figure 2: Microscopic image of oocyst of *Cryptosporidium parvum*

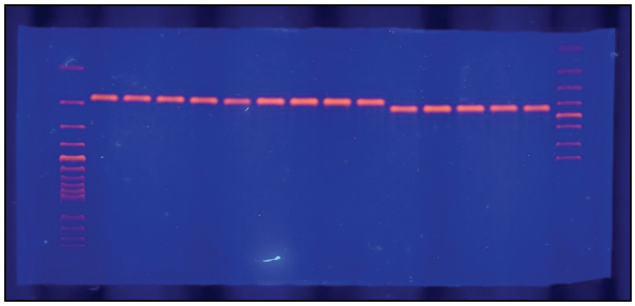

Figure 3: Agarose gel electrophoresis image that shows the polymerase chain reaction product analysis of heat shock protein genes (180 bp) among *Cryptosporidium parvum* from the stool samples of the patients. M = DNA molecular marker. All isolates are positive for the heat shock protein genes

method, where the presence of the red line indicates a positive result, while the absence of the red line indicates a negative result. The results of the present study showed the presence of three species of parasitic diarrhea agents: *E. histolytica* (9.33%), *G. lamblia* (16%), and *C. parvum* (16%).

Distribution of parasites according to age

From 75 positive samples, the highest infection rate was recorded in patients aged 17 days [27 (36%)], followed by 16 (21.3%) in patients aged 3–6 months, and the lowest rate of infection with *C.parvum* was recorded in patients, which was 2 (2.7%) in the age group of 9–10 years with a significant value. These results are shown in Figure 5.

Distribution of parasites according to gender

Figure 6 explains the distribution of *C. parvum* according to gender, with the highest percentage found in males (65.3%), and the lowest percentage found in females (34.7%).

Distribution of parasites according to residence

In this study, it was found that there was a highest infection rate in rural areas (62.5%), while the lowest infection rate was observed in urban areas (37.5%) in Al-Hilla city in the case of infection by the parasites microscopically. These results are shown in Figure 7.

Table 4: Detection of different species of parasites among the stool samples of the patients using immunochromatography kits

Type of parasite	No. of infections	%
E. histolytica	7	1.75
G. lamblia	12	30
C. parvum	12	30
Mixed	9	22.5

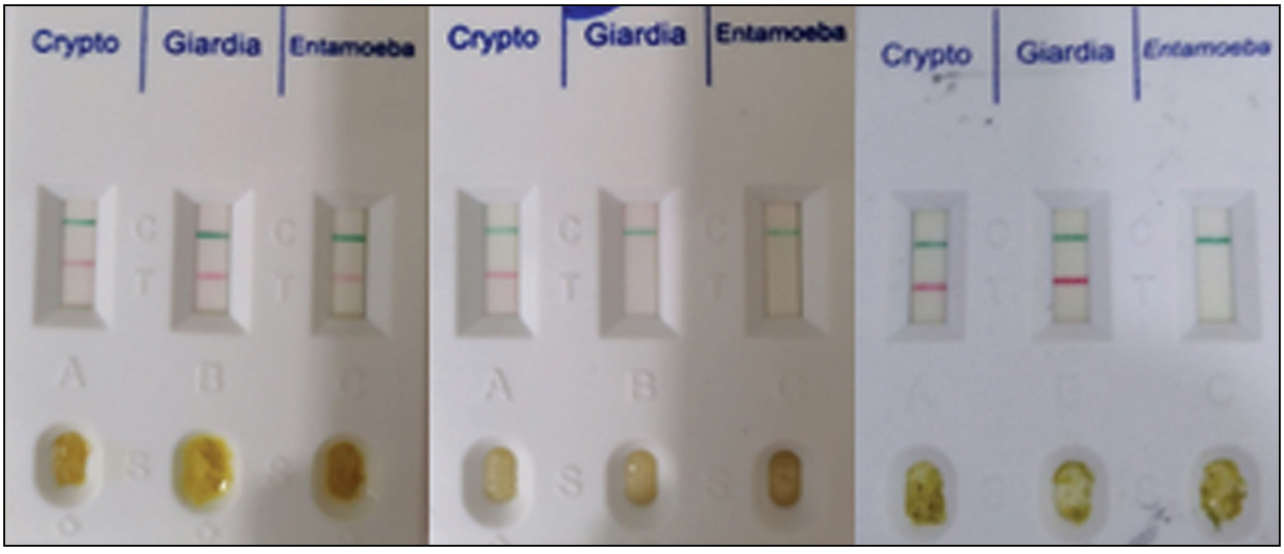

Figure 4: Immunochromatography kits for the detection of *Cryptosporidium parvum* from the stool samples of the patients

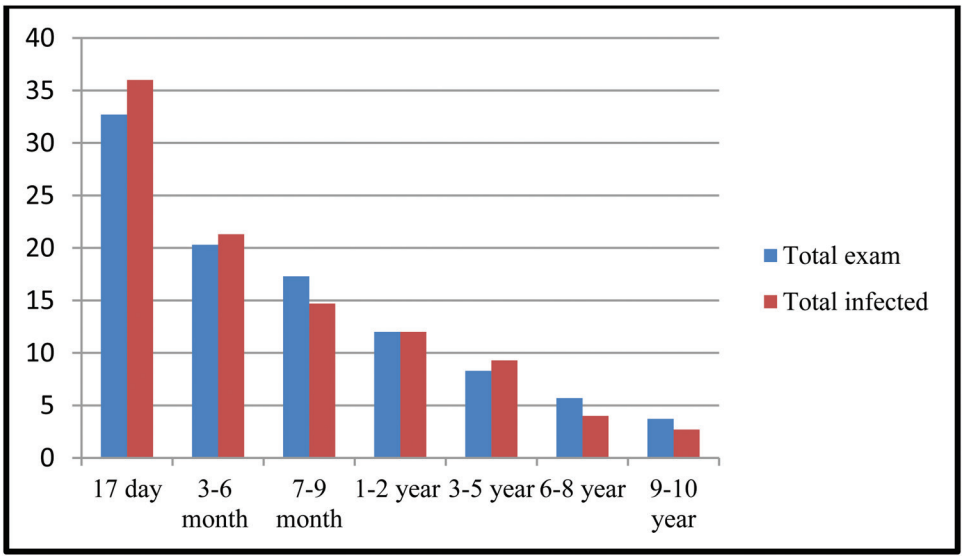

Figure 5: Distribution of *Cryptosporidium parvum* parasites according to age groups

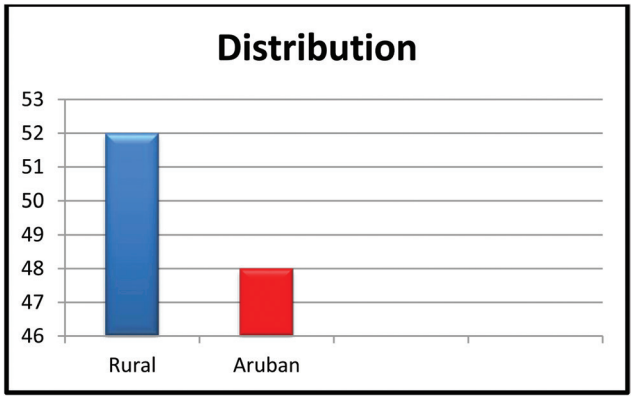

Figure 6: Distribution of *Cryptosporidium parvum* parasites according to the gender of patients

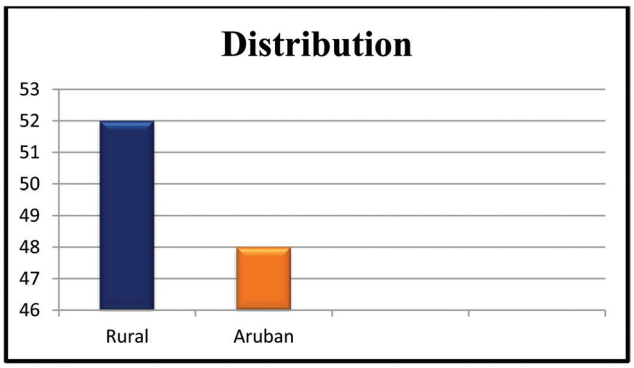

Figure 7: Distribution of *Cryptosporidium parvum* parasites according to the residence of patients

Discussion

Various staining methods have been used to assist in distinguishing *Cryptosporidium* oocysts from other environmental or fecal detritus. The most often used direct stain for staining *Cryptosporidium* oocysts in clinical microbiology laboratories is the Ziehl–Neelsen stain, also referred to as the acid-fast stain.[6,7] discovered that the rate of positive results in Basra was 23.8%, and[8] discovered that the rate of positive results in Diwaniyah cities was 29.29%. *C. parvum* was recorded in the southwest of Iran, and the positive rate there was (70.38%), which corresponded with our results and (70%) in Saudi Arabia.[9-11] Although it recorded 29.88% in Pakistan, other studies in Iraq, recording either higher or lower rates than this study, disagreed. Infection rates were greater in the provinces of Al-Najaf Al-Ashraf and Baghdad, where[12] recorded 58% and 47.33% of positive results, respectively.

The current investigation identified three parasite species that caused diarrhea in patients in the province of Babylon.[13,14] recorded 54.2%, 36.2%, and 7.9% for *E. histolytica*, *G. lamblia*, and *Cryptosporidium* spp., respectively, as the highest percentages of infection. Two parasite species were discovered in the Ramadi province: *G. lamblia* (34.5%) and *E. histolytica* (8.08%). Additionally,[15] discovered two parasite species in the province of Baghdad: *G. lamblia*, which has a 45.54% infection rate, and *E. histolytica*, which has a 33.44% infection rate[16] noted that the prevalence of *Cryptosporidium* infection was lower in Kirkuk than in the current study, which had a 16.28% prevalence. In accordance with those of earlier studies, the findings indicate a much higher frequency of infection in children under the age of 5. This is attributed to developing digestive tract mucosal immunity, unsanitary behavior with increased exposure, and incorrect handwashing at this age.[17]

The study of[18] observed that the major pathogen linked with death in toddlers (ages 12–23 months) and the second leading pathogen associated with moderate-to-severe diarrhea in children under 2 years of age was

Cryptosporidium spp. The analysis of[19] in the age range (1–5 months), documented rates of (10.77%) in Erbil City, concur with studies of [20] and our study. In Duhok, the age groups (1–5 months), (15–20 months), and (20 months) showed, respectively, (11.11%), (3.13%), and (16%), in agreement with our findings. The location of the sampling, the variety of animals in the area, the season, the climatic conditions, the volume of the sample, the population of the animals in the area, the rainy seasons, etc. are only a few of the numerous variables determining the prevalence of opposing interests.

Therefore, *C. parvum* transmission in emerging countries differs from that in developed countries. Microscopic analysis revealed that males had the highest infection rates and females had the lowest infections stratified by age group and year. This finding was consistent with that of a previous study[21] conducted in Baghdad, where males had the highest infection rates (58.5%) and female infections had the lowest (41.5%) and was incompatible with the study in Al-Sowera, which did not record any significant differences between the genders. The study in Al-Diwaniya province recorded the highest rate of infection for males (6.12%), while the lowest infection for females (5.11%). The difference in the rate of infection between males and females may be due to the fact that males are more mobile and active and their contact with the external environment factors at play and of being the working group in the communities; this is what makes them more relevant to getting infected by pathogens. Females as well as males eat well and drink in public places or from street vendors, in addition to the nature of anarchism and a lack of attention to personal hygiene and washing of hands, which increases the chances of being infected with Giardia, and the absence of significant differences between males and females could be due to mobility and having the same opportunity to infection in both genders due to intestinal parasites.[22] The lack of availability to clean drinking water, reliance on river water directly as a source of water, lack of guidance and counseling by the relevant authorities, lower health and cultural levels of the rural population, lack of hospitals and health centers in those areas, the occasional use of animal waste and human feces as an antiseptic, and other factors all contribute to the high incidence of infection in rural areas. So, infection was less common in families with private sanitation facilities compared to community sanitation.[23] The significant level of variability scattered over the whole sequence of the *Cryptosporidium* HSP70 gene made it seem like a potential target for genotyping. The term "Cryptosporidium HSP70 gene" refers to a particular gene in the *Cryptosporidium* parasite that codes for the heat shock protein 70 (HSP70). Heat shock proteins are essential for shielding cells from environmental stresses like heat, cold, toxins, and other types of cellular damage.[24]

Conclusion

HSP70 could be a good diagnostic tool for the detection of *C. parvum* among pediatric patients as well as for the monitoring of water resources with low concentrations of parasites.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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