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Molecular Detection of Protozoal Infection in Diarrheic Children in Wasit Province

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ABSTRACT

This cross-sectional study was conducted in Wasit province. collected 296 stool sample diarrheic children less than 16 years, selected 96 Randomly from some patients in hospitals and public health centers in Al-kut city and private clinics (from 1st December 2023 to 1st May, 2024 were included in the study. Method Finding the form of the parasite through direct smear microscopy to confirm the presence of an intestinal parasite infection, and diagnosis this parasite by molecular method polymerase chain reaction technique (PCR). The results showed that the cases were matched by age, gender, and where the person lived. The diarrhea was looked at using direct smear microscopy, which showed that *Entamoeba histolytica*, *Giardia.lamblia*, and *Cryptosporidium parvum* were found 23(24.0%), 5(5.2%) and 4(4.2%) respectively. While, Diagnosis by polymerase chain reaction(PCR). This result was 64(66.67%), 11(11.46%), 23(23.96%), respectively. In conclusion, PCR assay of molecular detection to protozoal infection in the present study is more accuracy than traditional methods like direct smear.

Introduction

Intestinal parasite infections (IPIs) are among the most neglected tropical diseases, resulting in significant morbidity and over 3.5 billion infections in approximately 450 million individuals [1]. Parasitic infection is a more prevalent and widespread issue today as a result of poor sanitation and impacts all individuals in most regions of the world, depending on conditions, shoe-wearing habits, lack of appropriate latrines, and other socioeconomic and geographic factors [2]. Children who are in school have a propensity of playing on polluted dirt, poor personal hygiene, and a less developed immune system. Because of this, they have a higher chance of getting gut parasite diseases [3]. Protozoa parasite diseases, like Giardia lamblia, Entamoeba histolytica, and Cryptosporidium, are the main reason babies get sick in the intestines in developing countries like the Middle East [4]. In this case, age, sex, poor cleanliness, the source of potable water, personal hygiene, location, touch with animals, and changes in the seasons are the most common things that put people at risk for protozoal parasitic infections [5]. In poor countries, these infections are a big problem for public health because they cause a lot of sickness and death in preschool and school-age kids [6]. A parasite protozoan called Entamoeba histolytica is the main cause of amoebiasis in people. It is very common for people to get amoebiasis, which kills about 67,900

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people every year. It has a big effect on the illness and death rates of children [7]. In severe instances, the infection may disseminate from the intestinal region to other organs, including the brain, lungs, or liver. All of these are substantially more hazardous and are more likely to lead to mortality[8]. *Giardia.lamblia*, a flagellated protozoan parasite, is present in the small intestines of numerous humans and displays symptoms of diarrhoea and abdominal pain [9]. Cryptosporediasis is one of the causes of protozoan intestinal infections in both humans and animals. Diarrhoea is the primary symptom, and in severe cases, it may be red. While the majority of coccidiosis cases are asymptomatic, young and immunocompromised patients may experience severe symptoms and eventual mortality [10]. They are transmitted through food, water, blood transfusion, and organ transplantation. *Entamoeba histolytica*, *Cryptosporidium parvum*, and *Giardia lamblia* are subgroups of tropical parasites that cause debilitating and fatal human maladies [11]. The objective of this investigation was to detection of intestinal parasite infection and diagnosis by molecular method polymerase chain reaction (18ss rRNA) ascertain the prevalence and the factors (age, sex, resident) that contribute to the prevalence of intestinal parasites in Wasit province.

Method

In Wasit county, this cross-sectional study was done. The study looked at 96 stool samples from children with diarrhoea aged 1 day to 16 years old who were patients at Al-Zahra Teaching Hospital, Al-Karama Teaching Hospital, Al-Kut Hospital for pediatrics and gynae obstetrics) and public health centers in Al-kut city from December 1, 2023, to May 1, 2024. The samples were chosen at random. used a pre-test formulation to find people who had an illness with an intestinal parasite. The cases were paired based on age, gender, and where the people living had diarrhoea. This was done using direct smear microscopy, and polymerase chain reaction were used to diagnose and find intestinal parasite infections.

Sample Collection

Using clean containers, the faeces samples were collected and sent right away to the hospitals' labs for further testing. The samples of feces were split into two groups. To look at the parasites under a microscope, the first part was used. The other 200 mg was kept at -20°C for genetic analysis using standard PCR [12].

Direct wet

A tiny drop of regular saline (0.9%) or an iodine stain was put on the glass slide for the direct smear. A wooden stick was used to mix a small amount of faeces with the water or iodine. Following this, a cover slide was put on top of the mixture. The prepared slide was then looked at through a 40X microscope. This made it possible to see and study the parasite's shape and how it moved [13]. The Z-N Technique is a modified Acid-Fast colouring method. This is how the method works [14].

Molecular assay

Conventional PCR. The PCR method, which uses the small subunit ribosomal (ssrRNA) gene, was used to find Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum in stool samples from people. The process described as "DNA extraction" was used to carry it out [15].

DNA extraction

Before the DNA was extracted, each sample of positive faeces was placed in a 2-ml microcentrifuge tube and rinsed three times with MilliQ H2O buffer. The ethanol was subsequently removed from the samples by centrifuging them at 2000 g for 5 minutes. Each sample of faeces (SRC Scientific, manufactured in Iraq) was treated with the lysate buffer of the QIAamp Fast DNA Stool Mini Extraction Kit. Subsequently, the manufacturer's recommended DNA extraction procedure was implemented on each of the 96 fecal-positive samples. (For use with the Faeces Mini Extraction Kit from SRC Scientific). PCR-ssrRNA gene *Entamoeba*

histolytic: ATTGGAGGGCAAGTCTGGTG, R: GCCTTGTGACCATACTCCCC, *Giardia lamblia* F: GGGCTAGAAGGCGATCAGAC, R: GGCGCCTACAAGACATTCCT, *Cryptosporidium parvum* F: ATCTAAGGAAGGCAGCAGGC, R: CCCCCAGAACCCAAAGACTT. The DNA was isolated and stored at -20°C for future use. The DNA electrophoresis technique was employed to analyse the purified DNA samples, which separates DNA fragments according to their size [16].

Statistical analysis

The statistical package for social sciences (SPSS) version 26 was used to enter and analyse the data. The data were shown as a frequency and a percentage. Chi-square and Fisher's Exact tests were used to find out how categorical factors were related to each other. Using the Cohen's Kappa test for agreement and McNemar test for disagreement. Considering a P-value less than 0.05.

Results

The cases were matched by demographic parameters such as age, sex and place of residence of diarrhea was examined by direct smear microscopy, this result to *E. histolytica, G. lamblea and C. parvum* for male 22.0%, 6.8%, 5.1% respectively. Then female 27.0%, 2.7%, 2.7% respectively. And distribution for age group more infection pre-school age 17.0%, 5.2%, 3.4% respectively. Place of residence effect to infction intestinal parasite in urban 23.3%, 2.7%, 4.1% respectively than rural 26.1%, 13.0%, 4.3% respectively too. Was The diarrhea was looked at using direct smear microscopy, which showed these parasites were found 23(24.0%),5(5.2%) and 4(4.2%) respectively While Diagnosis by polymerase chain reaction (PCR). This method results *E. histolytica, G. lamblia, C. parvum*, follow 64(66.67%), 11(11.46%), 23(23.96%), respectively. In conclusion, our work showed that PCR assays are better than direct microscopy at finding intestinal parasites because they are more sensitive and specific.

96 children were asked to take part in this study, and their answers were used to come up with the results. Table 1 shows the sociodemographic information about the people who took part in the study, as well as a list of any related diseases they had in the past. It was found that males represented around two-thirds of the sample with a percentage of 61.5%. Most of the sample were below the age of 4 years old (60.4%) and (76%) of them were living in urban places.

In Table 1, the association between sex and the microscopic exam of *E. histolytica, G. lamblia* and *C. parvum* was demonstrated. There was no significant association with a P-value more than 0.05 (0.577), (0.646) and (1.000) respectively.

Table 1: Association of sex with the presence of *E. histolytica*, *G. lamblia* and *C. parvum* by microscopy

Sex	***E. histolytica by Microscopy***		***G. lamblia by Microscopy***		***C. parvum by Microscopy***	
	Positive	***Negative***	***Positive***	***Negative***	***Positive***	***Negative***
Male	13 (22.0%)	46 (78.0%)	4 (6.8%)	55 (93.2%)	3 (5.1%)	56 (94.9%)
Female	10 (27.0%)	27 (73.0%)	1 (2.7%)	36 (97.3%)	1 (2.7%)	36 (97.3%)
P-value	0.577		0.646			1.000

The highest percentage of infection (42.9%) was present among patients belonging to the (9-12) years old age group followed by (33.3%) for those in the age group (5-8) years old while for *G. lamblia* highest percentage of infection (16.7%) was present among patients belonging to the(13-16) years old age group followed by (5.2%) present among patients less than 4 years .As for *C. parvum* The highest percentage of infection similar (8.3%)was present in(5-8 and 13-16) years old group followed by (3.4%)was present in group less than 4 years. Table 2 also found a non-

significant association between the age groups and the presence of the infection with *E. histolytica*, *G. lamblia* and *C. parvum* with a P-value equal to 0.182, 0.319 and 0.352 respectively.

Table 2: Association of age groups with the presence of *E. histolytica*, *G. lamblia* and *C. parvum* by microscopy

Age group (years)	*E. histolytica* by Microscopy		*G. lamblia* By Microscopy		*C. parvum* By Microscopy	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
≤4	10 (17.2%)	48 (82.8%)	3 (5.2%)	55 (94.8%)	2 (3.4%)	56 (96.6%)
5-8	4 (33.3%)	8 (66.7%)	0 (0.0%)	12 (100%)	1 (8.3%)	11 (91.7%)
9-12	6 (42.9%)	8 (57.1%)	0 (0.0%)	14 (100%)	0 (0.0%)	14 (100%)
13-16	3 (25.0%)	9 (75.0%)	2 (16.7%)	10 (83.3%)	1 (8.3%)	11 (91.7%)
P-value	0.182		0.319		0.352	

A slightly similar percentage of patients who were microscopically diagnosed with *E. histolytica*, *G. lamblia* and *C. parvum* between those who lived in urban and rural areas with a non-significant P-value (0.784), (0.087) and (1.000) respectively .as Table 4 demonstrates.

Table 3: Association of residency with the presence of *E. histolytica*, *G. lamblia* and *C. parvum* by microscopy.

Residence	*E. histolytica* by Microscopy		*G. lamblia* By Microscopy		*C. parvum* By microscopy	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Urban	17 (23.3%)	56 (76.7%)	2 (2.7%)	71 (97.3%)	3 (4.1%)	70 (95.9%)
Rural	6 (26.1%)	17 (73.9%)	3 (13.0%)	20 (87.0%)	1 (4.3%)	22 (95.7%)
P-value	0.784		0.087		1.000	

Polymerase chain reaction PCR Result

In this study conventional PCR is diagnosis parasitic infection *E. histolytica, G. lamblia, C. parvum.*

Table 4: Result of number infection three parasite diagnosis by Conventional PCR

Type of parasite	PCR positive		PCR negative		Total
	No.	**%**	**No.**	**%**	
E. histolytica	64	66.67	32	32.33	96
G. lamblea	11	11.46	87	87.90	96
C. parvum	24	23.96	73	73.76	96

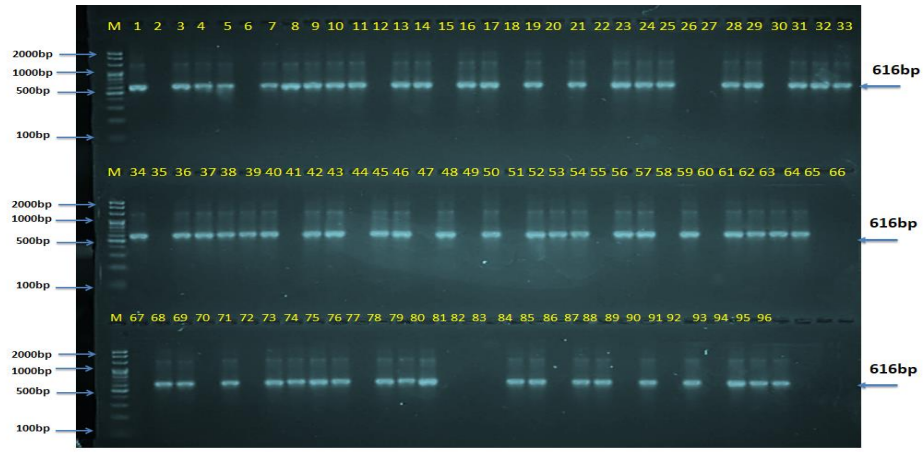

Fig. 1. The small subunit ribosomal (ssrRNA) gene's PCR product analysis was shown in an agarose gel electrophoresis picture to find Entamoeba histolytica in stool samples from people. Entamoeba histolytica was found at a PCR product size of 616bp in Lane (M): DNA marker ladder (2000-100bp) and Lane (1-96).

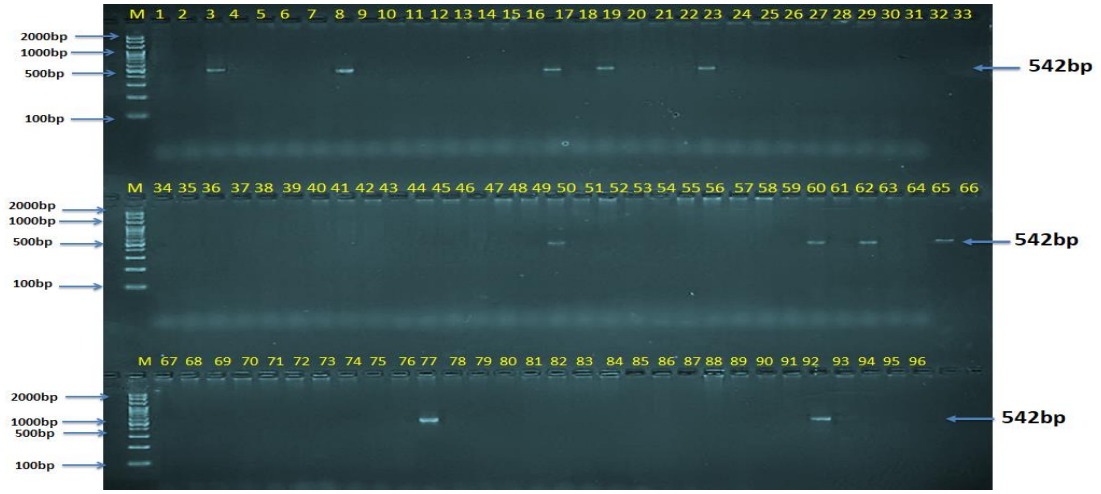

Fig. 2. Agarose gel electrophoresis image that showed the PCR product analysis of small subunit ribosomal(ssrRNA) gene for detection of Giardia *lamblia* from Human stool samples positive in (3,8,16,18,22,49,60,62,65,77,92). Where, the Lane (M): DNA marker ladder (2000-100bp) and the Lane (1-96) were showed positive Giardia *lamblia* at (542bp) PCR product size.

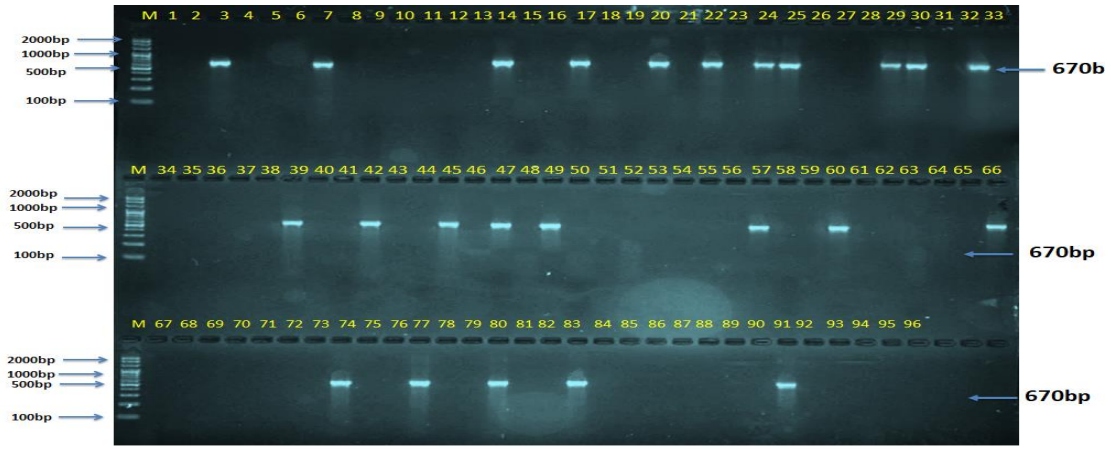

Fig. 3. This picture shows the PCR product analysis of the small subunit ribosomal (ssrRNA) gene for finding Cryptosporidium parvum in human stool samples that were positive in (3, 7, 14, 17, 20, 22, 24, 25, 28, 29, 32, 39, 42, 45, 47, 49, 57, 60, 66, 74, 77, 80, 83, 91). The DNA marker

ladder (2000–100bp) in Lane (M) and Lane (1–96) showed that Cryptosporidium parvum was present at a PCR product size of 670bp.

Discussion

Most of the time, intestinal parasite diseases are spread by dirty habits, like eating contaminated food and water or ingesting ova or cysts from unwashed hands and cuticles, or the larval stage penetrating the skin in dirty places [3]. In this study, the main gut protozoan diseases were found to be caused by Entamoeba histolytica, Giardia lamblia, and cryptosporidium parvum. A microscopic analysis showed that the number of good cases was 22.0%, 5.2%, and 4.2%, in that order. A study done in Zako City, Kurdistan, Iraq, found 395 cases of intestine protozoan illnesses, which fits with this result. Most of these infections were caused by Entamoeba histolytica; 271 (68.61%) of the infections were found by looking at them under a microscope. Giardia lamblia was found in 100 (25.31%) [17]. Along with the results of the Mosul City study, these results are also from this probe. Lab tests on 371 samples showed that 212 had parasites on them, with a 78% infection rate for the Entamoeba histolytica parasite and a 22% infection rate for the Giardia lamblia parasite, which could be seen under a microscope [18]. To *Cryptosporidium. parvum* study agree with study in Babylon province examined the feces of 987 patients 16 percentage [19]. In other study disagreed in Ankara collected 683 sample microscopic forms compatible, *E. histolytica*/ cysts, and *G. lamblia*, *Cryptosporidium parvum*, Oocysts were detected in stool samples of 18 (2.7%), 6 (0.9%), and 1 (0.1%), respectively [20]. It is thought that changes in the number of people infected with parasites are caused by differences in the study group, the method used, the location, the sensitivity and specificity of the laboratory methods, or the stage of the disease [13]. The result showd agreed with study in Al-Anbar [21]. E. histolytica and Giardia lamblia were most common in men (29.4% and 4.3%, respectively), while they were less common in women (27.3% and 3.2%). One in four people in Karbla were sick. Of those people, one in four were women (11.7% of the total population) [22]. But disagree with study in Arbil city in Iraq the higher percentage of protozoa infection was observed, among females (12.87%) than males (7.79%), and the difference was statistically significant [23]. Also disagree C. parvum in study in Kirkuk city was 58.75% and 65.35 % for males and females, respectively [24].

The women are more likely to be exposed to contaminated soil diseases or water than men, as they are more involved in the following activities caring for children, cleaning the home, processing raw fruits and vegetables, and scrubbing and washing home gardens. The maximum infection rate was recorded in the age group of 6-7 in Thi-Qar city, with a rate of 16.92%. This result is consistent with the distribution according to age group. Conversely, the age group less one year exhibited the lowest infection rate, with a rate of 15.40% [12]. The high incidence of intestinal parasites in general may be attributed to the lack of attention to general and personal hygiene, such as the failure to wash hands thoroughly and the neglect of washing vegetables and fruits, which is a result of the varying climatic conditions and geographical characteristics of different regions [25]. The age of the participants in Athupia varied from five to eighteen years, with fifty-nine percent of them falling within the grades of one to four [26]. Parasitic infections were reported to be more prevalent among patients residing in rural areas (22.6%) than in urban areas (13.4%) in Kurdistan Sulaimaniyah city. ($p>0.05$) [27]. In Babylon city, Iraq, the incidence of *C. parvum* infection was found to be greater among suburban inhabitants (87.4%) compared to urban residents (75.4%) causes direct contact with farm animals [28]. In molecular detection by conventional PCR this result parasitic to *E. histolytica* 64, prevalence 66.67%, *G. lamblia* 11 prevalence11.46% and *C. parvum* 23 prevalence 23.96%. This method more specificity and sensitivity PCR assay, *E. histolytica* was identified molecularly in 6% in Iraq [29]. 0.14% in Iran [30]. 14.7% in Egypt [31]. The present study collected 132 samples from Tikrit, of which (17) were positive cases of *Giardia lamblia* infection in the PCR method. A total of (47) samples were diagnosed microscopically for paediatric patients [14]. The maximum positive samples were detected in Qatar by PCR, 36/205 (17.5%), as opposed to direct microscopy, which detected 12 (6.0%) [32]. There are numerous benefits to using these PCR assays in comparison to conventional procedures. When first tested, they show a high level of selectivity and sensitivity, especially in groups with few parasites. The accuracy of their tests is 98.3% and the sensitivity is 96.5% [33].

Conclusion

The current study found that PCR tests are better than direct imaging at finding intestinal parasites because they are more sensitive and specific. It's also showed that children younger than five years old were more likely to have gut parasite diseases. The number of infections in men was higher than in girls. Illnesses are more common in rural places than in cities, because of worse health and animals that come into close touch.

Ethical considerations

Before sampling, all participants who took part in the study were (already informed about the aim of the study, agreed, and verbal consent was obtained from all). The study was conducted following the principles of the Declaration of Helsinki.

Ethical Issues

This study was approved by the ethical Committee of the Council of College of Medicine /University of Wasit in July 2023 and by the "Wasit Health Directorate (1201)" on 24/7/2023. All individuals in this study gave written consent to participate in the Rheumatoid arthritis questionnaire.

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الكشف الجزيئي عن عدوى الطفيليات الاولية في الاطفال المصابين بالاسهال في محافظة واسط

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الكلمات المفتاحية

الاسهال عند الاطفال,الطفيليات المعوية,PCRالتقليدي.

الملخص

أجريت هذه الدراسة المقطعية في محافظة واسط. وبشكل عشوائي، تم جمع ٩٦ عينة براز من الأطفال المصابين بالإسهال من يوم واحد إلى ١٦ سنة، من بعض المرضى في المستشفيات: (مستشفى الزهراء التعليمي، مستشفى الكرامة التعليمي، مستشفى الكوت للولادة والأطفال) ومراكز الصحة العامة في مدينة الكوت، من ١، ديسامبر ٢٠٢٣ إلى ١، مايو، ٢٠٢٤ شملت. الكشف عن شكل الطفيلي عن طريق الفحص المجهري اللطاخة المباشرة وجود عدوى الطفيليات المعوية. *E. histolytica* و *G.lamblea* و *C. parvum* أشارت النتائج إلى أن الحالات كانت متطابقة مع المعايير الديموغرافية مثل العمر والجنس ومكان الإقامة للإسهال تم فحصها بواسطة الفحص المجهري المباشر ، وهذه النتيجة إلى للذكور ٢٢,٠٪ ، ٦,٨٪ ، ٥,١٪ على التوالي. من الإناث ٢٧,٠٪ ، ٢,٧٪ ، ٢,٧٪ على. مكان الإقامة تأثير على الطفيليات المعوية في المناطق الحضرية ٢٣,٣ ٪ ، ٢,٧ ٪ ، ٤,١ ٪ على التوالي من المناطق الريفية ٢٦,١٪ ، ١٣,٠٪ ، ٤,٣٪ على التوالي أيضا. التشخيص عن طريق تفاعل البلمرة المتسلسل (PCR التقليدي). نتيجة هذه الطريقة *E. histolytica* ،*G. lamblia* ،*C. parvum* كانت ٦٤ (٦٦,٦٧٪) ، ١١ (١١,٤٦٪) ، ٢٣ (٢٣,٩٦٪) ، على التوالي. في الختام ، سلطت دراستنا الضوء على تفوق مقايسات تفاعل البوليميراز المتسلسل من حيث الحساسية والنوعية للكشف عن الطفيليات المعوية التي تحتوي على الفحص المجهري المباشر. أظهرت الدراسة الحالية أيضا أن معدلات الإصابة بالطفيليات المعوية كانت أعلى لدى الأطفال دون سن الخامسة. علاوة على ذلك ، تم اكتشاف معدلات إصابة أعلى بين الذكور مقارنة بالإناث. بالإضافة إلى ذلك ، هناك زيادة في حدوث العدوى في المناطق الريفية أقل صحة الاتصال المباشر.

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