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THE PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG PEDIATRIC PATIENTS IN KUT, IRAQ: A COMPARATIVE STUDY BY MEANS OF MICROSCOPY AND MULTIPLEX PCR

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Abstract: Intestinal parasitic infections (IPIs) continue as a substantial public health issue in evolving nations, with pediatric population's presence particularly susceptible. This study examines the prevalence of Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum among children in Kut, Iraq, using both conventional microscopy and Multiplex PCR methodologies. A total of 257 stool specimens were procured from children aged 0 to 14 years crossways various hospitals and healthcare institutions. The specimens underwent an initial macroscopic and microscopic evaluation, subsequently followed by DNA extraction and examination by Multiplex PCR to supplement the detection capabilities for parasitic pathogens. The collective infection rate was determined to be 42.41%, with a larger prevalence experiential in females (49.24%) when compared with males (35.20%). Age-group analysis indicated that children aged 12 to 14 years showed the highest infection rate (60.00%), while the lowest prevalence was renowned in children under three years of age (37.50%). A proportional analysis between urban and rural environments revealed a heightened infection prevalence in rural locales (55.17% vs. 41.18%). The diagnostic efficiency of microscopy and Multiplex PCR confirmed considerable concordance, with Pearson correlation coefficients above 0.99 for all three parasites, so representative a healthy contract between the two diagnostic approaches. Nevertheless, Multiplex PCR shown a slightly superior detection rate for Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum. These results emphasize the authoritative for the addition of molecular diagnostic practices into standard clinical repetition to improve the sensitivity and accurateness of IPI detection, particularly within resource-constrained environments

Keywords: Pediatric Patients, Microscopy, Cryptosporidium parvum, Multiplex PCR, Chi-Square

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Introduction

Intestinal parasitic infections (IPIs) establish a difficult global health anxiety, particularly in developing regions considered by inadequate hygiene, perilous water resources, and substandard hygiene practices. These infections, persuaded by protozoan and helminthic parasites, excessively trouble pediatric populations, resultant in malnutrition, undersized growth, and cognitive impairments. Intestinal parasitic infections (IPIs) are a important global health issue, chiefly in developing regions where insufficient sanitation, unsafe water, and poor hygiene practices prevail. These infections, mainly caused by protozoa such as Entamoeba histolytica and Giardia lamblia, principal to plain health consequences, especially among children, including malnutrition, stunted

growth, and cognitive impairments [1,2]. The prevalence of IPIs is aggravated by socio-economic factors, with studies representative high infection rates amongst schoolchildren in Yemen, where 48% were affected [2]. Also, the co-infection of IPIs with other diseases, such as tuberculosis, container aggravate health results by damaging immune responses and drug efficacy [3]. Actual prevention and control strategies, including better sanitation, health education, and advanced diagnostic techniques, are vital to mitigate the influence of these infections. The quick and exact identification of intestinal parasitic infections (IPIs) is key for effective therapeutic interferences and reducing transmission risks. Although conventional methods like microscopy have been extensively used, they repeatedly exhibition low sensitivity and specificity, possibly important to misdiagnosis or delayed treatment [4,5]. In difference, molecular diagnostic techniques, like Multiplex PCR, suggestively improve analytical accuracy by allowing the concurrent detection of multiple pathogens, thus rationalization the diagnostic process [6]. These progressive methods, including real-time PCR and isothermal amplification, not only reduce diagnostic times but also improve the capability to recognize specific species and measure resistance to treatments [4]. Also, the integration of molecular diagnostics into clinical practice can chief to better-quality patient outcomes and more actual public health strategies, lecturing the challenges modelled by IPIs in both developed and developing regions [4].

In this study, we pursue to assess the prevalence of intestinal parasites among pediatric patients in Kut, Iraq, and compare the diagnostic effectiveness of traditional microscopy with that of the more classy Multiplex PCR. We concentrate on three major parasites—Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum—due to their considerable public health implications. The outcomes of this research will enhance the understanding of the epidemiology of IPIs within the region and aid in the formulation of more effective diagnostic and public health strategies. n evaluating the prevalence of intestinal parasites among pediatric patients in Kut, Iraq, it is crucial to consider the significant findings from recent studies. For instance, a study in Kirkuk reported a 9.5% infection rate of Giardia lamblia among pediatric patients, with higher prevalence in rural areas and younger age groups [7]. Similarly, research in Duhok highlighted a 21.68% positivity rate for Entamoeba spp., with a notable prevalence of E. histolytica [8]. Furthermore, a study on waterborne parasites indicated the presence of Cryptosporidium spp. in various water sources, emphasizing the potential for waterborne transmission [9]. The comparative diagnostic efficacy of traditional microscopy and Multiplex PCR is essential, as PCR has shown higher sensitivity in detecting these parasites, which could lead to improved public health strategies and better epidemiological understanding of intestinal parasitic infections in the region [9].

Methods

A comprehensive survey was undertaken to assess the quality and prevalence of intestinal parasites. A total of 257 stool specimens were systematically collected and analyzed from pediatric patients ranging in age from one day to 14 years, sourced from individuals admitted for treatment or visiting Al-Batool Educational Hospital, Al-Zahra Hospital in the Kut center, as well as various health facilities across the governorate of Kut and its neighboring districts. In order to gather pertinent information regarding the subjects, a meticulously structured Questionnaire was developed for each participant, about the following details: date of examination, gender, age, educational attainment of the examiners, educational level of the subjects, sources of potable water, characteristics of the residential area, and residential address diagnosis.

A macroscopic examination was led on the stool samples previous to the microscopic assessment, where in the stool was qualitatively measured in a gross manner, directing on the quantity,

shape, texture, and coloration (. The identification of blood or mucus within the fecal matter was also recognized, as such comments may suggest the attendance of vegetative phases (Trophozoites) related with the histolytic distorted. Additionally, a microscopic inspection was performed on the samples using various methodologies, amongst which the direct mount technique was working. In this specific method, a drop of physiological solution (0.9% Normal Saline) was presented on one side of the glass slide, while a drop of aqueous iodine solution (Lugol's iodine) was positioned on the opposite side of the slide. A tiny quantity of stool specimen, about the size of a match head, was removed from multiple sites within the sample using a wooden stick and methodically mixed with a drop of physiological solution. Then, an extra portion of the same sample was joint with an aqueous iodine solution in an analogous manner. A glass slide cover was practical to both samples. The specimens were then inspected using a light microscope equipped with a 40x objective lens to determine the vegetative phases (Trophozoites) and cysts of protozoa

Multiplex PCR

The technique of Multiplex PCR was employed to identify Entamoeba histolytica, Cryptosporidium parvum, and giardia lambila from human fecal specimens, specifically targeting the small subunit ribosomal RNA gene. The methodology comprised the subsequent procedures:

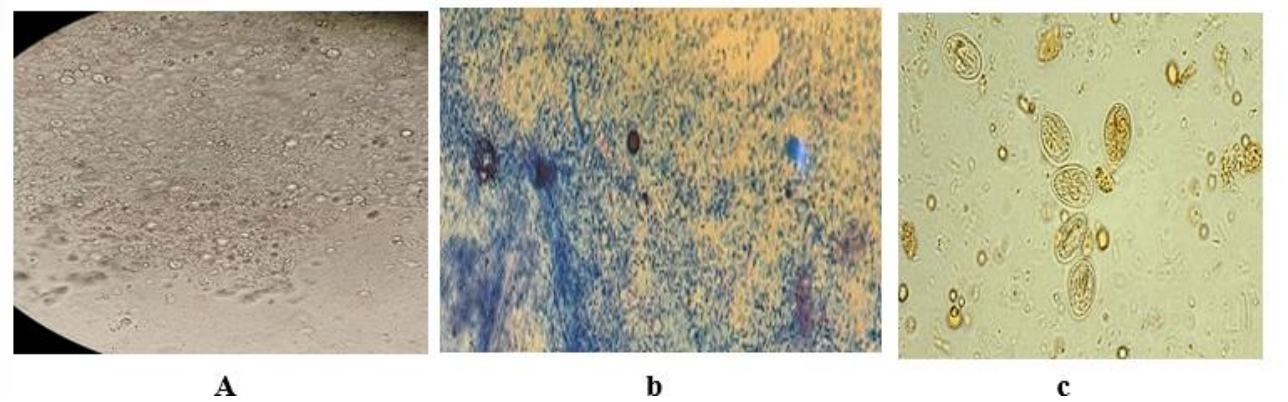
1. Stool DNA Extraction Sample lysis: A volume of 250µl of fecal matter was subjected to lysis utilizing ceramic beads and ST1 buffer, succeeded by incubation and centrifugation. DNA Binding: The addition of ST3 buffer facilitated the binding of DNA, which was subsequently filtered through a GD Column. Washing: The DNA was purified utilizing ST3 and a designated wash buffer. Elution: The elution of DNA was accomplished with a preheated buffer, resulting in purified DNA.
2. DNA Quantification The purity of the DNA was assessed employing a Nanodrop spectrophotometer at 260/280 nm to ascertain quality.
3. Multiplex PCR Preparation the PCR master mix was formulated utilizing GoTaq Green PCR Master Mix in accordance with the protocols established by the manufacturer.

Statistical analysis

In the statistical analysis of the results, The Chi-square (X2) method was used to statistically analyze the data according to the ready-made statistical program SPSS version xx (20. V). The significant differences between the study groups were compared using the least significant difference Test at a significant probability level of 0.05.

Results and Discussion

Microscopic analysis at a magnification of 100x elucidated the morphology of Cryptosporidium: Morphological characteristics are either spherical or ovoid in shape. Chromatic properties: the organism displays a pink or red coloration upon application of a modified Zell-Nielsen stain against a contrasting blue background. Entamoeba histolytica Morphology: exhibits a spherical configuration. Chromatic properties: during its active phase, the organism demonstrates a translucent appearance with a distinctly visible dark nucleus; in contrast, during its metamorphic phase, it assumes a more opaque structure characterized by a centrally located nucleus. Giardia lamblia Morphology: exhibits a resemblance to a pear or a smiley face. Chromatic properties: typically ranges from transparent to gray; nonetheless, through the application of specific staining methodologies, it may reveal light blue or purple pigmentation.



A b c

Figure 1: Microscopic Images (100x Magnification) of: (a) Entamoeba histolytica, (b) Giardia lamblia, and (c) Cryptosporidium parvum

Table 1: Infection Rate of intestinal parasites under Microscopic comparing Between Males and Females Number of Infected and Percentage Analysis.

sex	Number of Examined	Number of Infected	Infection Rate (%)
Male	125	44	35.20
Female	132	65	49.24
Total	257	109	42.41

The results of examination of a number of individuals divided by gender (male and female), and shows the relationship between the number of examined, the number of infected, and the percentage of infection. The table includes data on 257 people who were examined for a specific infection, the data show that females have a higher percentage of infection than males. 125 males were examined, of which 44 were infected, indicating an infection rate of 35.20%. This percentage means that less than half of the examined males were infected, which may indicate the presence of certain factors limiting the prevalence of infection among males. In contrast, 132 females were examined, of which 65 were infected, indicating an infection rate of 49.24%. This percentage is measured to be higher than that of males, meanwhile almost half of the examined females were infected. In total, 257 people were scrutinized, and the number of infected was 109, which resembles to a total infection rate of 42.41%. This total percentage designates that almost half of the studied group agonizes after the injury. This bring into line with findings from several studies that highpoint the prevalent nature of IPIs, chiefly in emerging regions. For example, a study in Somalia stated a high prevalence of Giardia lamblia (60.84%) and Entamoeba histolytica (33.07%) amongst patients, highlighting the public health trial modelled by these parasites [4]. Likewise, study in Iran found that 6.5% of patients tested positive for many intestinal parasites, with Blastocystis spp. being the most common [5]. The progressive infection rate amongst females (49.24%) compared to males (35.20%) may advise sex-related factors influencing exposure or vulnerability, a marvel also observed in other studies where demographic factors played a role in infection rates [3,2]. General, these results underline the need for beleaguered interventions to address the load of IPIs in affected communities. The inconsistency in the incidence ratios between males and females can be clarified by several factors. It is likely that biological factors are accountable for this alteration, as physical differences may affect individuals ' susceptibility or resistance to infections.

People who living in surroundings that lack proper health amenities may be more susceptible

to infection, irrespective of sex. This means that a profounder investigation is needed to find out whether these factors production a role in the feast of infection between males and females. The total percentage of infection, which amounted to 42.41%, is measured relatively high and designates a wide spread of infection in the studied group. Like a high percentage requires a careful study of the factors that donated to the spread of infection, be it related to sex, environmental and social factors. In particular, the high occurrence amongst females requires deeper study to understand the reason behindhand this disparity, and it may be essential to reflect the associated biological, social, and economic factors.

Table 2:.Infection Rate of Intestinal Parasites by Age Group – Number of Infected and Percentage Analysis

Age Group	Number of Examined	Uninfected (Number)	Uninfected (%)	Infected (Number)	Infected (%)
< 3 years	80	50	62.50	30	37.50
3-5	60	30	50.00	30	50.00
6-8	45	25	55.56	20	44.44
9-11	37	20	54.05	17	45.95
12-14	35	14	40.00	21	60.00
Total	257	139	54.09	109	45.91

For a table that displays data related to the distribution of infection incidence among different age groups of the examined children. Individuals are divided into five age categories, namely: under 3 years old, 3-5 years old, 6-8 years old, 9-11 years old, and 12-14 years old. In the age group under 3 years, 80 children were examined, of which 50 were uninfected (equivalent to 62.50%), and while 30 were infected (37.50%). For the age group of 3-5 years, 60 children were examined, of which 30 were uninfected (50.00%), and another 30 were infected (50.00%). showed in Baquba City, it was originate that children aged 1-5 years had the maximum prevalence of infections, with a distinguished number of cases connecting common parasites such as Entamoeba histolytica and Giardia lamblia [8]. Likewise, study in Nepal designated that children under five years old likewise faced significant risks, with Giardia lamblia existence the most widespread [9]. In the Dir Lower district of Pakistan, the uppermost frequency of intestinal parasitic infections was experiential in children under four years, importance the susceptibility of younger age groups [10]. General, these results underscore the dangerous need for embattled public health interferences to address the high rates of intestinal parasitic infections amongst young children in Iraq and alike regions [11, 12]. In the age group of 6-8 years, 45 children were examined of which 25 were uninfected (55.56%) while 20 were infected (44.44%). In the age group of 9-11 years, 37 children were inspected, of which 20 were uninfected (54.05%), and 17 were infected (45.95%). The 12-14 age group included 35 children of whom 14 were not infected (40.00%), and 21 were infected (60.00%). as showed by numerous studies. In the 6-8 years age group, 44.44% of children were infected, while in the 9-11 years group, the infection rate was slightly higher at 45.95%. Notably, the 12-14 years age group exhibited the highest infection rate at 60.00% [13, 14]. This trend aligns with findings from a systematic review indicating that older children are generally at a greater risk of intestinal parasitic infections, particularly in developing regions where sanitation and healthcare access are limited[15,16]. The most common parasites identified in similar studies include Entamoeba histolytica and Giardia lamblia, which are prevalent

in school environments where close contact facilitates transmission [13, 14]. Addressing these infections requires targeted interventions, improved sanitation, and health education to mitigate their impact on children's health and development [16]. In total, 257 children of all age groups were examined, of which 139 were uninfected (54.09%), and 109 were infected (45.91%). These data show that the incidence varies between age groups, as the older age group (12-14 years old) seems to have a higher incidence (60.00%) compared to other age groups, while the younger age group (less than 3 years old) shows the lowest incidence (37.50%).

Table 3: Infection Distribution by Area with Statistical Analysis (Chi-Square and Pearson Correlation)

Area	Number of Examined	Uninfected (Number)	Uninfected (%)	Infected (Number)	Infected (%)	Expected (Uninfected, Infected)	Chi2 Statistic	P-value (Chi2)	Pearson Correlation	P-value (Pearson)
Urban	170	100	58.82	70	41.18	91.95, 78.05	3.993401	0.045679	1.0	1.0
Rural	87	39	44.83	48	55.17	47.05, 39.95	3.993401	0.045679	1.0	1.0
Total	257	139	54.09	109	45.91	N/A	N/A	N/A	N/A	N/A

The table provides a comparison of urban and rural areas in terms of the number of individuals examined and the status of parasite infestation. In urban areas, 170 individuals were examined, of which 100 were uninfected by 58.82% and 70 were infected by 41.18%. The forecast was for the number of uninjured to be around 91.95 and the number of injured to be 78.05. The Chi-square statistic was 3.993401, and the P-value was 0.045679, which indicates a significant difference between the forecast and the actual results. The Pearson correlation was 1.0 which that means full compatibility among the variables. Moreover the urban surroundings often favor direct transmission of parasites owing to dense host populations, while rural areas may provision trophic transmission owing to healthier obtainability of middle hosts [17,18]. The important Chi-square statistic (3.993401) and P-value (0.045679) advise that the experiential variances in infection rates are not owing to chance, aligning with results that urbanization influences parasite community composition [19,20]. This highlights the need for beleaguered public health plans in urban areas to address the increasing prevalence of parasitic infections.

In rural areas 87 individuals were scrutinized, of which 39 were uninfected by 44.83% and 48 infected by 55.17%. The prediction was for the number of uninfected to be 47.05 and the number of infected to be 39.95 and The Chi-square statistic also touched 3.993401 through the same P-value of 0.045679, which designates that the alterations between rural and urban areas in terms of parasite infestation are statistically significant. The Pearson correlation was likewise 1.0, which means there is a complete correlation between the expected results and the actual results .For instance, a study in Quetta, Pakistan, described a 23% prevalence of intestinal parasitic infections in rural populations, with factors such as poor hygiene and low socio-economic status causative to this trend [21]. Correspondingly, investigation indicates that children in urban areas also face significant risks, with prevalence rates reaching from 4.8% to 48.9%, prejudiced by factors like hygiene and socio-economic conditions [22]. The Chi-square statistic of 3.993401 and a P-value of 0.045679 propose that the changes in parasite plague between rural and urban areas are statistically significant, strengthening the need for targeted interventions in both settings to address these public health anxieties [23]. The complete Pearson correlation of 1.0 designates a strong relationship between predictable and actual infection rates, highlighting the dependability of the data [24].

In total, 257 individuals were examined, where the number of non-infected was 139 by 54.09%, and the number of infected was 109 by 45.91%. This analysis indicates that there are significant differences between urban and rural areas regarding parasite infestation, with higher rates of infection in rural areas compared to urban, and this may be due to environmental and health factors associated with the countryside.

Table 4: Comparison of Infection Detection by Microscope and PCR with Statistical Analysis (Chi-Square and Pearson Correlation)

Parasite Type	Number of Infections (Microscope)	Percentage (%) (Microscope)	Number of Infections (PCR)	Percentage (%) (PCR)	Expected (Microscope, PCR)	Chi2 Statistic	P-value (Chi2)	Pearson Correlation	P-value (Pearson)
Entamoeba histolytica	44	40.37	50	38.76	43.05, 50.95	0.134492	0.934965	0.995587	0.059832
Giardia lamblia	36	33.03	42	32.56	35.72, 42.28	0.134492	0.934965	0.995587	0.059832
Cryptosporidium parvum	29	26.61	37	28.68	30.23, 35.77	0.134492	0.934965	0.995587	0.059832

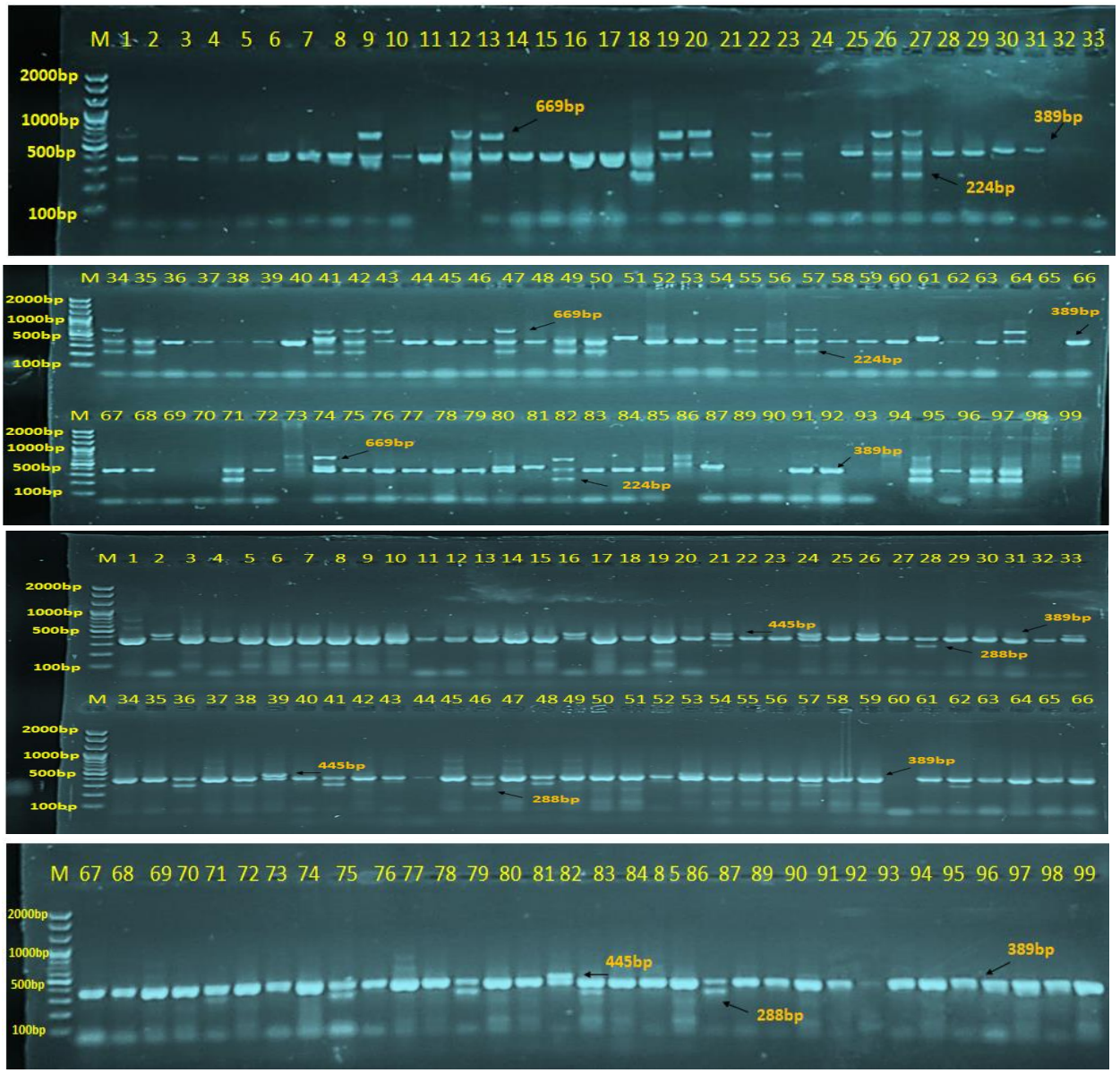


Figure (2): Agarose gel electrophoresis image that showed the Multiplex PCR product analysis of small subunit ribosomal gene to detect Entamoeba histolytica, Cryptosporidium sp and Giardia lamblia from Human stool samples. Where, the Lane (M): DNA marker ladder (2000-100bp) and the Lane (1-99) showed positive Entamoeba histolytica, Cryptosporidium sp, and Cyclospora sp at (389bp, 669bp, and 224bp PCR product size) respectively.

The table presents a comparison of the results of infection with parasites Entamoeba histolytica, Giardia lamblia and Cryptosporidium parvum using two diagnostic methods: microscopy and multiplex PCR. For entamoeba histolytica, 44 infections were recorded using microscopy with a percentage of 40.37%, and 50 infections using Multiplex PCR with a percentage of 38.76%. the limitations of microscopy in accurately diagnosing amebiasis due to its low sensitivity and difficulty in distinguishing E. histolytica from non-pathogenic species [25] [26]. In contrast, multiplex PCR assays have shown higher diagnostic performance, with sensitivity values of 0.96 for E. histolytica, indicating its effectiveness in detecting multiple pathogens simultaneously [27]. Furthermore, the PCR assays confirmed high specificity and positive prognostic values for both Giardia and Cryptosporidium that creation them greater to traditional methods [27]. The multiplex PCR is suggested for more accurate and rapid diagnosis of these intestinal protozoan infections. The expected values were 43.05 for microscopic examination injuries and 50.95 for PCR examination. The Chi-square statistic was 0.134492, and the P-value was 0.934965, which indicates that there is no significant difference between the two methods of detecting the parasite. As for the Pearson correlation, it was very strong with a value of 0.995587, which indicates a high agreement between the results of microscopy and PCR, with a probability value of 0.059832.

For Giardia lamblia, 36 infections were logged by microscopic inspection with a percentage of 33.03%, and 42 infections by means of Multiplex PCR with a percentage of 32.56%. The predictable values were 35.72 for microscope damages and 42.28 for PCR examination. The Chi-square statistic and its probabilistic value were also alike to those of entamoeba histolytica, which designates that there was no significant difference between the two methods in diagnosing this parasite either. The Pearson correlation value was too 0.995587, shiny a very strong correlation between the two methods. The results obtainable concerning the diagnostic performance of microscopic examination and Multiplex PCR for Giardia lamblia bring into line with current works that highlights the rank of both methods in noticing this parasite. A systematic review designated that immunoassays, including PCR, can effectively identify Giardia lamblia, with varying sensitivity and specificity depending on the method used [28]. Moreover, the comparative presentation of diverse diagnostic techniques, including PCR, has been shown to yield important results, with PCR frequently if higher sensitivity in noticing infections [29, 30]. The strong Person correlation value of 0.995587 you mentioned suggests a robust agreement between the two approaches which is reliable with studies that climax the reliability of both microscopic and molecular techniques in diagnosing Giardia infections [30]. So, in conclusion about the lack of significant difference between the two methods is supported by the literature, strengthening their complementary roles in clinical diagnostics.

For Cryptosporidium parvum, 29 infections were logged by microscopic inspection with a percentage of 26.61%, and 37 infections by means of Multiplex PCR with a percentage of 28.68%. The probable values were 30.23 for microscope injuries and 35.77 for PCR inspection. Alike to other parasites, and The Chi-square statistic did not demonstration significant alterations between the two methods with a P-value of 0.934965. The Pearson correlation value was actual high, representative a strong contract amid the consequences of the two inspections. The discovery of Cryptosporidium

parvum infections can differ meaningfully contingent on the diagnostic method working. In a study comparing conventional microscopy and molecular techniques similar Multiplex PCR and it was found that while microscopy logged 29 infections (26.61%), Multiplex PCR identified 37 infections (28.68%) with predictable values of 30.23 and 35.77, correspondingly. Notwithstanding these variances, the Chi-square statistic specified no significant differences between the two methods (P-value = 0.934965), that suggesting a strong agreement in results, as reinforced by a high Pearson correlation value. This agree with results from other studies, which demonstrate that though PCR methods, such as those developed for Cryptosporidium discovery frequently show higher compassion and specificity compared to microscopy, together methods can yield comparable results in certain contexts [31,32,33]. Furthermore, the expansion of well-organized DNA removal protocols further improves the reliability of molecular diagnostics for Cryptosporidium [34].

In general, the results indicate that there is a significant compatibility between the results of microscopy and Multiplex PCR examination, since there are no significant differences between the two methods in detecting any of the three parasites, which is confirmed by very high correlation values. the congruence between microscopy and multiplex PCR for the identification of intestinal parasites has emerged as a focal point of scholarly inquiry, yielding diverse outcomes across various investigations. While a subset of studies posits that multiplex PCR markedly improves detection rates in comparison to microscopy, evidenced by findings wherein PCR discerned 51.2% of samples as positive for intestinal protozoa, juxtaposed with a mere 6.2% identified by microscopy [35], other research indicates that both methodologies may produce analogous results for specific parasites. For example, an evaluative study of multiplex PCR assays revealed elevated sensitivity and specificity, with aggregate values ranging from 93.2% to 96.5% across different assays [36]. Moreover, the appraisal of real-time PCR assays has showcased substantial diagnostic accuracy for particular protozoa, suggesting that although PCR may uncover a greater number of cases, the interrelation between the two techniques remains significant for certain parasitic entities [27] [36]. Consequently, while PCR may provide superior detection capabilities, the compatibility of outcomes between the two approaches warrants attention [37].

Conclusion

This study clarifies the substantial load posed by intestinal parasitic infections within pediatric demographics in Kut, Iraq, as shown by the fact that closely fifty percent of the evaluated children tested positive for parasitic pathogens. The prevalence rates were remarkably elevated amongst female subjects and older children, with rural locales showing a higher occurrence of infection comparative to urban environments, probable attributable to differences in hygiene practices and water quality. The proportional analysis of microscopy and Multiplex PCR exposed that although both methodologies are capable in the diagnosis of intestinal parasitic infections, the Multiplex PCR validates superior sensitivity and owns the competence of concurrently detecting multiple pathogens. Assumed the qualities of molecular techniques, which include improved diagnostic exactness and working efficiency, it is supported that Multiplex PCR be combined into standard diagnostic performs in regions with endemic prevalence. These results emphasize the authoritative for enhanced public health initiatives, particularly in rural and marginalized communities, aimed at lessening the occurrence of intestinal parasitic infections. Future plans should include the encouragement of hygiene protocols, the delivery of drinkable water, and targeted deworming creativities. Also, the acceptance of progressive diagnostic modalities such as Multiplex PCR will be essential in refining the early documentation and treatment of these infections, thus easing their long-term health

consequences.

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