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Multiplex PCR for detection of intestinal parasites in patients at Wasit province

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Abstract---Intestinal parasitic infestation still represents an economic and public health problem in the world particularly in the developing countries including the Middle East .To estimate the current prevalence of intestinal parasitic infection among people living in Wasit province/ middle of Iraq .100 fecal samples were collected from males and females of different ages attending AL-Karama Teaching Hospital suffering of gastroenteritis symptoms during the period from August to November 202, only 45 stool sample were positive for intestinal parasite. The total infectivity rate by intestinal parasites was (45%).This study indicated high occurrence of gastro-intestinal parasites in Wasit province, Iraq. The overall infection rate by intestinal protozoa was significantly higher than intestinal helminth infection. In the present study prevalence of intestinal parasitic infection was *Entamoeba histolytica* 15(33.3%). the percent of the infection among males were 9(20%) while the females were 6(13.3%). According the residence in urban areas were reported 5(11.1%) and rural areas were 10(22.2%). The Co-infection appears in 19 samples with percent of infection (42.2%) in males infection were predominance 11(24.4%) than the females 8(17.7%). According to the residence urban areas were reported 7 (15.5%) and rural areas were 12(26.6%). According to *Blastocystis hominis* infection was reported 7(15.5%) and the male was 2(4.4%) while the female was 5(11.1%). the residence urban areas were reported 5 (11.1%) and rural areas were 2(4.4%). *Cryptosporidium parvum* and *Giardia lamblia* were reported 2(4.4%)infection in rural areas in male.

Keywords---intestinal parasite, human, multiplex PCR, feces.

Introduction

Intestinal parasitic infections are present all around the world, especially in developing countries. These infections are mostly associated with poverty, poor sanitation facilities, low hygienic level and low quality of food and water supply. The parasitic infections can be a good index for hygienic and sanitary level of the society (1-3) . Intestinal parasitic infections are caused either by protozoan and helminthes parasites or both and main clinical manifestation of the disease caused by these parasites is diarrhea (4).

Parasites made up of protozoa such as (*Entameoba histolytica*, *Giardia lamblia*) and helminthes (*Ascaris lumbricoides*, *Ancylostoma duodenale*, *Trichuris trichiura*) are the major virulence parasites in the world leading to serious infection and death (5). an important cause of deaths among children less than 5 years old (6).and cause morbidity and mortality in human immunodeficiency virus (HIV)-positive individuals word wide (7).

In developed countries, protozoan parasites more commonly cause gastrointestinal infections compared to helminthes (8). According to the World Health Organization (WHO), approximately 500 million people worldwide suffer from amoebiasis, with an annual mortality between 40000 and 110 000 (9). The most common clinical picture of intestinal protozoan pathogen infection is diarrhea (10) and nutritional disorders that may lead to iron deficiency anemia, malnutrition and they may have a negative impact on growth and cognitive development of a child (11,12).

Symptoms often start with fever, nausea, and vomiting, followed by abdominal cramps and frequent watery diarrhea, which may last for 3-8 days, the infected children may also have a cough and runny nose. (13) Several recent diagnostic tests are now available which allow the microscopic detection of the parasite and facilitate accurate diagnosis. These tests include Enzyme linked immunosorbant assay (ELISA) and polymerase chain reaction (PCR) (14). The combination of microscopy and molecular technology allows the identification of pathogenic and non-pathogenic species, and provides basic knowledge of genotype which is very useful for understanding the transmission and clinical characteristics of the infections (15).

Materials and Methods

Sample collection

Samples were collected from patients who attended to the AL-Karama Teaching Hospital in Wasit province. The samples were classified according to the gender and age of patients. Each container was labeled with the patient's number and name. Stool samples were divided into two parts: one part used for microscopic diagnosis, and the other part of the stool sample was retained for Multiplex PCR as frozen (-22°C) (16).

Primers

The multiplex PCR primers for detection *Entamoeba histolytica*, *Blastocystis hominis*, *Cryptosporidium parvum*, *Giardia lamblia*, *Enterobius vermicularis*, and *Ascaris lumbricoides* based on small subunit ribosomal rRNA genes were designed in this study using NCBI-Genbank and primer 3 plus design. These primers were provided from Scientific Researcher. Co. Ltd.

Multiplex PCR

Multiplex technique was performed for detection *Entamoeba histolytica*, *Blastocystis hominis*, *Cryptosporidium parvum*, *Giardia lamblia*, *Enterobius vermicularis*, and *Ascaris lumbricoides* based on small subunit ribosomal rRNA genes from Human stool samples. This method was carried out according to following steps:

Stool DNA Extraction

Stool DNA from stool samples were extracted by using Presto™ Stool DNA Extraction Kit and done according to company instructions.

Genomic DNA estimation

The extracted genomic DNA from stool samples was checked by using Nanodrop spectrophotometer (THERMO.USA) that checked and measured the purity of DNA through reading the absorbance in at (260 /280 nm).

Multiplex PCR master mix preparation

The mPCR master mix was prepared by using (Go taq Green PCR Master Kit) and this master mix done according to company instructions.

PCR Thermocycler Conditions

Initial Denaturation (95°C for 5 min), Denaturation (95 °C for 30 sec) Annealing (58°C for 30 sec) Extension (72 °C for 1 min), Final extension (72 °C for 5 min) Hold (4 C Forever).

mPCR product analysis

The mPCR products of was analyzed by agarose gel electrophoresis.

Results and Discussion

After examining 100 stool samples of outpatients in Al-Karama Teaching hospital at Wasit province. The results appeared that 45(45%) of feces samples were positive for intestinal parasite among diarrheal cases that examined by microscope using direct method and 0.0% among control group. This agreed with study (17) in Wasit infection percentage (48.67 %). The study also provides close

outcomes to that of (18) in Babylon province 48.8%. While (19) recorded 70.4% in Nineveh province.

Table 1: Result of infection with intestinal parasite according to the residence

| Parasite | No (%) | Rural | Urban | Total |
|----------------------|-----------|-----------|----------|-----------|
| <i>E.histolytica</i> | 15(33.3%) | 10(22.2%) | 5(11.1%) | 15(33.3%) |
| Co-infection | 19(42.2%) | 12(26.6%) | 7(15.5%) | 19(42.2%) |
| <i>G.lambliia</i> | 2(4.4%) | 2(4.4%) | 0(0%) | 2(4.4%) |
| <i>B.hominis</i> | 7(15.5%) | 5(11.1%) | 2(4.4%) | 7(15.5%) |
| <i>C.parvum</i> | 2(4.4%) | 2(4.4%) | 0(0%) | 2(4.4%) |

The present study revealed that the overall prevalence rate of intestinal parasite was higher among families who live in rural areas than those in urban areas, this result agreed with (20) and at Wasit Province. Another study conducted among children in Kassala City, east of Sudan revealed that the rate of parasitic infections was higher in children from rural areas than urban areas (21) and (22), but contradicts with (23) who found that the prevalence of *C. parvum* infection in urban and rural children was similar.

Table 2: Disstribution of intestinal parasite infection according to the gender

| Intestinal parasite | Infection in male | Infection in female | Total |
|----------------------|-------------------|---------------------|-------------|
| <i>E.histolytica</i> | 9(20%) | 6(13.3%) | 15(33.3.5%) |
| Co-infection | 11(24.4%) | 8(17.7%) | 19(42.2%) |
| <i>G.lambliia</i> | 2(4.4%) | 0(0%) | 2(4.4%) |
| <i>B.hominis</i> | 2(4.4%) | 5(11.1%) | 7(15.5%) |
| <i>C.parvum</i> | 2(4.4%) | 0(0%) | 2(4.4%) |

The distribution of intestinal parasitic infections according to gender, generally infection percentage in male were higher than female The higher rates of infection in male than in female with parasites like *Entamoeba histolytica*, *Giardia lamblia*, *Enterobius vermicularis*, *Ascaris lumbricoides*, were recorded by some researchers (24). On the other hand, researchers (25) recorded higher infection rate with intestinal parasites in female than in male and others found an equality between both genders (26, 27).

Table 3: Distribution of intestinal parasite according age groups

| Parasite/Age | 1-15 years | 16-30 years | 31-45 years | 46-60 years | 61-75 years | Total |
|----------------------|------------|-------------|-------------|-------------|-------------|-----------|
| <i>E.histolytica</i> | 6(13.3%) | 2(4.4%) | 3(6.66%) | 2(4.4%) | 2(4.4%) | 15(33.3%) |
| Co-infection | 9(20%) | 2(4.4%) | 4(8.8) | 2(4.4%) | 2(4.4%) | 19(42.2%) |
| <i>G.lambliia</i> | 0(0%) | 1(2.2%) | 0(0%) | 0(0%) | 1(2.2%) | 2(4.4%) |
| <i>B.hominis</i> | 4(8.8%) | / | 2(4.4%) | 1(2.2%) | / | 7(15.5%) |
| <i>C.parvum</i> | 1(2.2%) | / | / | / | 1(2.2%) | 2(4.4%) |

The results found the highest prevalence of parasites in the age group (1-15) years. This result was an agreement with (28) who found that the highest rate of

parasitic infection in the age group (1-15) years old, but disagreed with (29) and (30) who illustrated that there were no significant differences between age groups.

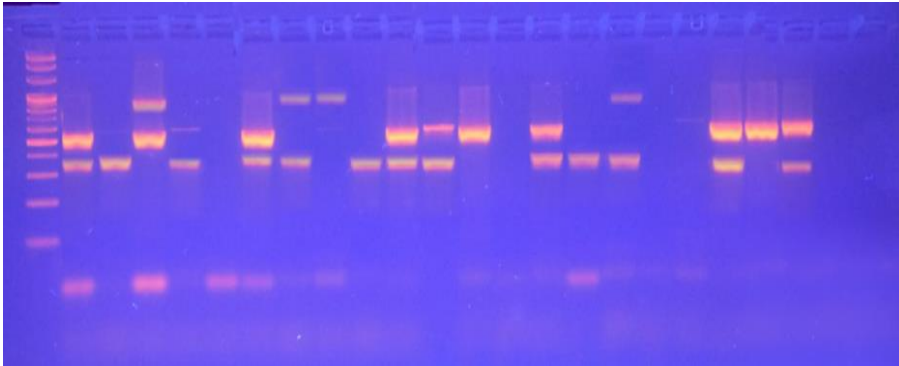


Figure: Agarose gel electrophoresis image that showed PCR product analysis for intestinal parasites from stool samples.

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