Efficacy of some laboratory methods in detecting *giardia lamblia* and *cryptosporidium parvum*in stool samples

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Abstract

Background: *Giardia duodenalis* and *Cryptosporidium parvum* are the most prevalent intestinal parasites of human. It also infects a wide range of mammals .Two genotype of *G.duodenalis* (A and B) were commonly reported among humans with different frequency of distribution in different geographical locations. Methods :total of 434 stool samples were collected from peoples in kikrkuk city during October 2012toAugust 2013. Zinc sulphate flotation technique was applied on 226 positive stool, serological methods involve *Giardia* ELISA-corpo antigen, Direct fluorescent assay (DFA), and lateral immune-chromatography assay(Triage panel) .Extractions of Giardia genome DNA from stool samples were performed using QIAamp Stool Mini kit with a modified protocol. PCR- one step procedure was used to amplify a fragment of *Giardia lamblia* genome using k725 gene locus,(Mixture primers of human A and B assemblages) Results:The overall rate of intestinal parasitic infection was 52.07%,*Giardia lamblia* rate was 24.65%.Common isolated parasites were *Cryptosporidium parvum* ,*Blastocystishominis*, other intestinal protozoan parasites and nematode helminthes:7.60 & 5.76 %,7.37 and 6.68 % respectively. Sensitivity and specificity of PCR method and direct microscopy were higher than other four methods used for detecting giardiasis. Triage panel method exert high rate of giardiasis than revealing of *Cryptosporidium and Entamoeba histolytica*. From the application of five methods for *Cryptosporidium* diagnosis, DFA and modified Ziehl-Neelsen(MZN) methods show high sensitivity and specificity than other three methods. Application of PCR single step using mixture primers assemblages A and B, show high rate of sensitivity than other methods in detecting giardiasis.Amplified *Giardia* genome length extended from 220 to 750 bps with mean of 437.56 bps. Conclusions: PCR assay targeting *K725 gene* locus is a sensitive tool and discriminates mixed genotypes of *G.lamblia*. DFA and MZN are sensitive tools for detecting *Cryptosporidium parvum* in stool samples.

Keywords: *Giardia, Cryptosporidium* ,ELISA ,DFA,PCR,Sensitivity.

كفاءة بعض الطرق المختبرية في تحديد الجياردیا لامبليا وخفيات الأبواغ في نماذج البراز

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الخلاصة

المحتوى :طفيلي الجياردیا لامبليا وخفيات الأبواغ من اكثر الطفيليات المعوية انتشارا بين البشر.ويتسبب أيضاخمج العديد من الثديات.سجل نمطين جينين للجياردیا لامبليا(أ و ب) بين البشر بنسب تردد مختلفة في مناطق جغرافية مختلفة.
الطرق:جمعت 434 أنموذج برازمن الأشخاص في مدينة كركوك من الفترة تشرين الأول 2012 لغاية أب 2013. طبقت تقنية التطويف بكبريتات الخارصين على 22 أنموذج موجب للطفيليات.الطرق المصلية شملت الأليزا-مستضد البراز، طريقة الومضان المباشر وطريقة الترحيل الكروماتوكرافي المناعي الجانبي. تم أستخلاص الدنا لطفيلي الجياردیا بأستخدام عدة (كوي امب مني) المحورة. تم تضخيم الدنا بأستخدام عدة تفاعل الحلقي المتضاعف(القوالب كانت مزيجا من نمط جيني أ و ب) على الجين (ك 725).
النتائج: كانت نسبة الخمج الكلية بالطفيليات المعوية 52.07 %،نسبة الجياردیا لامبليا كانت 24.65 %. أكثر الطفيليات شيوعا كانت خفيات الأبواغ، الأريمة البشرية،أبتدائيات معوية أخرى وديدان خيطية معوية بالنسب الأتية: 7.60 %، 5.76 %، 7.37%و6.68% على التوالي. كانت حساسية وخصوصية طريقتي تفاعل الحلقي المتضاعف وطريقة المسحة الرطبة أعلى من الطرق الأربع الأخرى. أظهرت طريقة الترحيل الكروماتوكرافي كفاءة عالية في تحديد الجياردیا لامبليا مقارنة بأظهار خفيات الأبواغ و المتحولة النسيجية.من أستخدام خمس طرق مختبرية لتشخيص خفيات الأبواغ،أظهرت طريقة الترحيل الكروماتوكرافي وطريق زيل-نيلسين المحورة حساسية وخصوصية اعلى من الطرق الثلاثة الأخرى.تطبيق الخطوة الواحدة لتقنية تفاعل الحلقي المتضاعف بأستخدام قالب خليط مكون من النمط الجيني (أ و ب) أظهرت حساسية وخصوصية عالية في تحديد الجياردیا لامبليا. أمتدت طول الدنا المتضخم من 220 الى 750 زوج قاعدة مع وسيط مكون من 437.56 % زوج قاعدة.
الأستنتاجات:تعتبرتقنية تفاعل الحلقي المتضاعف المستهدف للموقع الجيني (ك 725) طريقة ذات حساسية عالية في أظهارالنمط الجيني المختلط . تعتبر طريقتي الترحيل الكروماتوكرافي وطريق زيل-نيلسين المحورة ذات حساسية عالية في تحديد خفيات الأبواغ في نماذج البراز.
الكلمات الدالة: جياردیا لامبليا،خفيات الأبواغ، الأليزا، الترحيل الكروماتوكرافي ،طريقة زيل-نيلسين المحورة.

Introduction

The initial reports of *Cryptosporidium* infection in micewere published by Tyzzer [1] in 1907. In 1955, Slavin [2]described the parasite as a potential cause of diarrhea inturkeys. Cryptosporidiosis in calves was subsequently recognizedin the 1970s [3]. But it was not until *Cryptosporidium*infections were reported as a cause of death in AIDS patientsin the 1980s that the protozoan parasite became accepted as asignificant zoonotic pathogen warranting scientific research [4].The first record of *Cryptosporidium* in Kirkuk city was in 2000 by Othman [5] who found the rate12.62 % of *Cryptosporidium parvum*oocysts in feces of 150 infants. A variety of tests have been developed for the diagnosis of *Cryptosporidium*. Most of them involve direct detection by microscopic examination of tissues or fecal material using staining techniques [6]. Many specialized staining procedureshave been described to facilitate the reliable detectionof oocysts. The modified acid-fast stain (AF) is widely usedbecause of its low cost and simple methodology; unfortunately, it displays relatively low sensitivity with feces [7].The sensitivity of the AF stain on fecal smears can be increased10- to 100-fold by examination of prepared slidesunder UV light with a rhodamine (540–560 nm) filter [8].Several immune-labeling techniques using poly-monoclonal antibodies have also been developed, but theseare more expensive than conventional staining, while theirsensitivity and specificity seem to be the same [9]. Newrapid immunoassays designed for simple diagnostic testingwith minimal training are commercially available (e.g., TheBeckton Dickinson ColorPAC, and The BIOSITE DiagnosticsTriage Parasite Panel), but their suitability for use inindividual laboratories depends on the balance between theassay cost, the reduced time and the number of specimensprocessed daily [10,11]. Although these tests do not replaceroutine diagnostic methods, their high sensitivity and specificity suggest that they may be useful to confirm *Cryptosporidium* infections in patients with low parasite numbers and to distinguish between *Cryptosporidium* and other waterborne parasites like *Giardia and Entamoeba*. Recently, developed PCR protocols have proven to bevery specific and highly sensitive. The application of these PCR protocols to detect *Cryptosporidium* species in environmental and clinical settings has been established [12]. *Giardia intestinalis* (also known as *G. lamblia, G. duodenalis*) is the most commonly diagnosed protozoan worldwide causing non-bacterial diarrhoea [13]. In recent years, genotypic classification has been applied for the identification of this parasite [14]. Diagnosis of *Giardia* by conventional microscopic methods following the application of fecal concentration techniques, especially Zinc sulphate flotation and centrifugation remains a relatively reliable indicator of infection [15].The detection of *Giardia* parasite in stool samples by microscopy or ELISA is of limited epidemiological value. The development of the rapid lateral immune-chromatography assay Triage panel improved the sensitivity of detecting and quantitating the fecal*Giardia* cysts and more accurate prevalence rate and cysts excretion intensities as compared to the conventional microscopy .There is need for a sensitive and specific diagnostic procedure for detecting the etiological agent of infectious disease, with *Giardia*, molecular techniques particularly PCR based procedures have greater sensitivity and specificity than the conventional diagnosis that are reliant on microscopy or immune-diagnosis [17]. One of major advantage of PCR based techniques is the ease of interpretation which usually involves the visualization of small number of bands on a gel [18]. DNA sequence analysis of the 16S rRNA gene revealed the presence of Assemblage A (2%) and Assemblage E (25%) in *G. duodenalis* infection [19]. The goals of this study, first is to evaluate the employee of six laboratory methods for detecting *Giardia lamblia* and *Cryptosporidium parvum* in stool samples in kirkuk city. The second aim is to extract *Giardia lamblia* DNA from stool samples and to detect purity and genomic mass of the *Giardia* parasite.

Materials and methods

A total of 434 human stools samples from patients suffering from enteritis have been testedin the Medical Research laboratory – Kirkuk College of Medicine to compare the sensitivity and specificity of six laboratory tests : Microscopy(direct wet preparation and zinc sulphate flotation technique),Modified Ziehl-Neelsen method, and copro-antigens using ELISA ,direct fluorescent assay(DFA),Lateral immune-chromatography assay(Triage panel)and amplification of *Giardia*DNA from stool samplesusing K725 gene loci(Mixture assemblages A and B)have been determined by conventional PCR method. Prior to processing complete informations were reported in a special questionnaire prepared for this purpose. One aliquot of each sample was immediately examined using direct wet preparations of lugols iodine 1% and 0.85 % of NaCl for detecting motility of *Giardia* stages and other intestinal protozoan parasites. The residue of each specimen was preserved by adding sufficient amount of 2.5 % of potassium dichromate for examination by other laboratory methods [20]. A second aliquot of each stool specimen was immediately frozen and stored at -20 C^0. Subsequently, the frozen aliquots were thawed and mixed thoroughly before testing with immune-chromatographic dipstick tests **(Triage Micro Parasite Panel)** whichis an enzyme immunoassay for the detection of *G. lamblia, E.histolytica/dispar and Cryptosporidium parvum* in fresh or fresh frozen, un-fixed human fecal specimens. The presence of the specific antigens was detected usually by the presence of a purple- black color bar next to the name printed and the test device. This procedure was done according to[21] .The *Giardia* CELISA (acapture enzyme immunoassay) kit is a qualitative *in vitro* enzyme immunoassay for the detection of *Giardia lamblia* cyst antigen in stool samples was used. The procedure was applied according to instructions of manufactured company and according to that used by[22].For direct fluorescent-antibody assay (DFA) (MeriFluor*Crypto and Giardia*; cell labs company), small amount of stool sample approximately 200 µl was placed on special microscopic slide supported with the kit. The specimen was completely air dried. Some drops of acetone were applied on the smear for five minutes. Fixation of the smear was done by adding 25µl of RR2 to smear and, control positive and covering well area. The slides were kept in a humid chamber and incubated at 37 C^0 for 30 minutes, with avoiding the dryness of the slides. The slides were gently rinsed with Phosphate Buffer (PBS) for one minute. Excess moisture around the well was gently drained by a piece of soft tissue. A drop of RMG(mounting reagent) was applied on the slide, and then covered with cover slip. Scanning the entire specimen using a fluorescence microscope initially at x200magnification, then at x400, and x1000 for confirmation .The slide either read immediately or stored at 2-8C^0 in dark place for 24 hours. Demonstration of *Giardia* parasite was assessed by using fluorescent microscope wave length 480nm and 550 nm[23].Genomic mass of *Giardia lamblia*was detected by using the four following steps: **First**: DNA extraction from stool samples, for that, the E.Z.N.A.® Stool DNA Kit was purchased from Omega bio kit company –German. **Second** step was DNA purity assessments: Total of 107 extracted DNA elutes in step one were checked for purity usingThermo Scientific Nano-Drop^{TM} 2000c spectrophotometer manual protocol ,that carried on by using a ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. [24]**. Third** step was, the amplification of each specimen that done by using conventional GeneAmp® PCR System 9700, Dual 384-Well Sample Block Module .While amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany was used to detect *Giardia lamblia*(in one step).It contains the following: Tube **A** forward primer, which consist of a mixture of assemblage A1, A2 and B).Tube **B** reverse primers for all assemblages. Positive control (tube D1) , negative Control (tube D2) ,DNA Marker (tube E) : (max 1000 bp): 100, 150, 200, 300, 400, 500, 600, 700, 800, 900,1000bp and Dye (tube F) . Thermo-cycler (Gene Amp® PCR System 9700Dual 384-Well Sample Block Module) was switch on for sample amplification process, and the

amplification was done according to manufactured company instruction which included the following cycles: 15 seconds at 95^0 C, 15 seconds at 60^0 Cand 15 seconds at 72^0 C .Each temperature degrees were repeated 35 cycles. Step **four**:Gel Electrophoresis which involve the following procedure; Gel agarose 2.0% in TAE (1x) buffer(agarose powder 2.0 gm was dissolved in 100 ml Tris –acid borate buffer which prepared by adding 10 ml of TAE 1x to 100ml of distilled water. Heated gently avoiding boiling, 50µl of ethidium bromide stain solution ((0.5µg/ml). was added to agarose solution then poured in to gel tang containing special chambers with standard coombs .After 5 to 10minutes and before the gel completely dry, the coombs were up stand hold to permit pores in the gel).About 200ml of 1x TAE buffer was added to gel chamber,2µl of dye (tube F) was added to each micro-tubes. Amount of 10µl of marker (tube E: 100bp) were inserted in to the first and the last lane of electrophoresis, while other lanes were inserted with amplified samples. The gel electrophoresis instrument was set for 60 min. at 120 Volt. After finishing the electrophoresis, the visualizing of giardia DNA bands were done with wearing UV goggles. The length of giardia genome was measured by using UV standard scale and confirmed with the length of marker bands at the first and last lanes to give out the length of giardia genome /bps. **Statistical analysis:** The following terms and equations were used for detecting the efficacy of laboratory methods in detecting *Giardia lamblia*; TP=True positive, TN=True negative, FP=false positive, FN=false negative, PPV=positive predictive value and NPV=negative predictive value.

Sensitivity=TP/(TP+FN).Specificity=TN/(TN+FP),Accuracy=(TN+TP)/(TN+TP+FN+FP),PPV=TP(TP+FP) and NPV=TN/(TN+FN) [25].All data in the present study were stored in Microsoft Excel program and arranged in tables. By using some statistical formulas such as: Chi-square, t-student test, Fisher test and sign test for medium were used to detect variances among parameters in the study at probability 0.05 and0.01.

Results: The overall rate of intestinal parasitic infections was52.07 % distributed in 226 stool samples; this rate involve high rate of giardiasis 24.65%.*Cryptosporidium* rate was7.60 %, followed by 5.76 for *Blastocystishominis*and 7.37 % for other intestinal parasites (*Entamoebahistolytica,Entamoebacoli,Endolimex nana* and one stool sample show *Balantidium coli*).Intestinal helminthic rate was 6.68 %*(Enterobiusvermicularis,Ancylostomaduodenali and Ascarislumbricoides).*P<0.05.

Table-1- Percentages of positive and negative rates of parasitic infections .

Parasites	No. positive +ve	Percentage +ve	No. Negative -ve	Percentage -ve
Giardia lamblia	107	24.65 *	327	75.35
Cryptosporidium parvum	33	7.60	401	92.40
Blastocystishominis	25	5.76	409	92.23
Other protozon parasites	32	7.37	402	92.63
Helminthes	29	6.68	405	93.32
Total	226	52 .07	208	47.93

Total number examined= 434 *P<0.05

According to lab. methods; PCR technique revealed high rate of Giardia infections 18.43 %,followed by 15.20 % for direct microscopy and 11.75 % for flotation technique. Serological methods exerted the following rates: 13.33 %, 12.44 % and 9.90 % using ELISA, DFA and Immuno-chromatography (Triage panel) respectively. Statistically, the sensitivity of the methods and PDV were significant especially PCR technique that showed74.76 % versus to 40.10 % sensitivity of Triage panel. While specificity, accuracy of methods and NDV exert no differences.Table-2.

Table-2- Distribution of *Giardia lamblia* according to laboratory methods.

Lab methods	No.+ve	% +ve	Sensitivity % *	Specificity %	Accuracy %	PDV	NDV	T-value and P
PCR	80	18.43 a*	74.76 b*	92.37 c*	88.93 d*	82.5 e*	92.37	a*T=19.25 P<0.05
Direct wet preparation	66	15.20	61.68	88.37	84.10	78.75	88.37	b*T=42.51 P<0.05
ELISA	57	13.13	53.27	86.73	80.81	75	96.00	C*t=110.48 P<0.05
DFA	54	12.44	50.46	86.05	80.37	73.75	95.61	D* T=68.86 P<0.05
Flotation	51	11.75	47.66	85.37	79.48	68.75	94.11	E*t=74.38 P<0.05
Triage	43	9.90	40.10	83.63	77.22	56.25	90.56	F*t=85.48 P<0.05

PDV=Positive predictive value NDV=Negative predictive value Total number examined=434

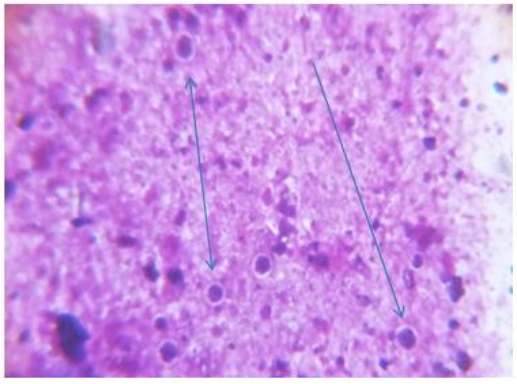
Table3 was summarizing the benefit of Triage panel in detecting *Giardia,Entamoeba* and *Cryptosporidium*, by which it has been shown that high rate of *Giardia lamblia* 9.90 % was recorded compare to low rates for other two parasites<0.05.Also direct wet preparation method showed the same findings .

Table - 3 –Correlation among three laboratory methods for detection of *Giardia lamblia.*

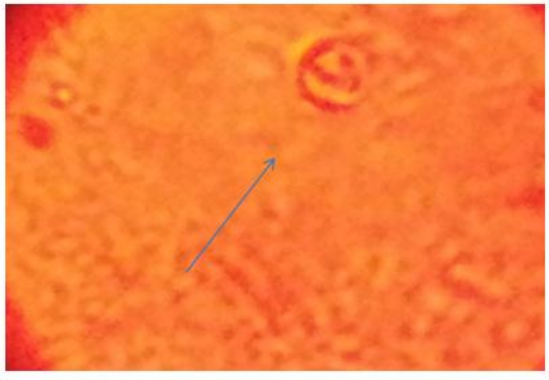
Parasites	Direct wet Preparation No, and % +ve	ELISA corpo antigen No, and % +ve	Triage Cassette No, and % +ve
Giardia lamblia	66 15.20	57 13.13	43 9.90 *a
Cryptosporidium	19 4.37	Not applied	14 3.22 *b
Entamoebahistolytica	8 1.84	Not applied	11 2.53 *c

*a P<0.05 , * b P>0.05 and * c P>0.05

Table 4 was clarifying the benefit of DFA use in demonstrating the oocysts of *Cryptosporidium parvum* by which high rate 6.91 % with 90.90%of sensitivity and99.25 % of specificity was recorded followed by modified Ziehl-Neelsen and Triage panel ,while positive rates and sensitivity were decreased by using Microscopy methods(Direct wet preparation and flotation),P<0.05.Figure(1) and (2).



Figure(1) Cryptosporidium parvum oocysts , modified Ziehl-Neelsen method.



Figure(2) Direct fluorescent assay demonstrating the oocysts of Cryptosporidium under 450 nm.

Table – 4.Comparison among five laboratory methods used for detection of *Cryptosporidium parvum*oocysts.

Lab methods	No.+ve	% +ve	Sensitivity %	Specificity %*
DFA	30	6.91 *	90.90**	99.25
Modified Ziehl-Neelsen	26	5.99	78.78	98.28
Triage Cassette	14	3.22	42.42	95.47
Direct wet preparation	8	1.84	24.24	94.13
Flotation ZnSo4	5	1.15	15.15	93.47

* P>0.05 **P<0.05 Total number examined =434

The molecular study of *Giardia lamblia* using the extract of DNA from 107 stool samples positive for *Giardia*(4 extract *Giardia* +helminthes were ignored), revealed 1.705 % of meangenome purity and 437.56 bps genomic mass or density in 80 extract. While the extract of *Giardia* positive with other protozoa; purity and mean genomic mass were1.56 % and 439.89 bps respectively.Statistical analysis exerts no significance among purity rates and genomic mass of *Giardia* parasite. The use of PCR kit, K725, the amplificated genomes reveal bands migration during electrophoresis process ranged from 220 bps to 750 bps, but the majority of *Giardia*gemones were detected between 350bps to 441bps.Table-5 .Figure (3).

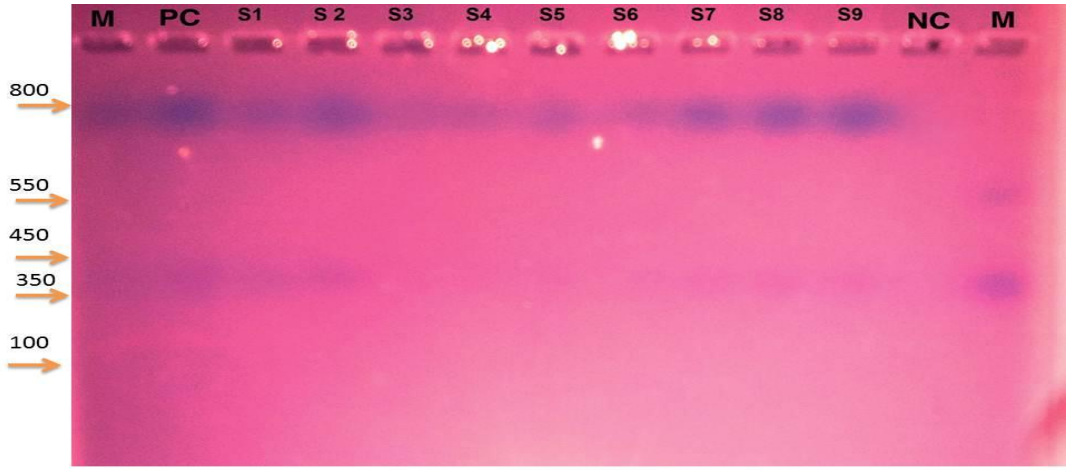


Fig.(3):PCR kit:k725- Giardia product on an ethidium bromid-stained 1% a garose gel.lane M,molecular weight marker(1000bp); lane NCnegative control; lane PC: positive control ; lanes S1 to S9 :PCR products from clinical sampls.

Table - 5 - Determination of parasites genome mass and genomes purity.

Parameters / Parasites	Genome mass /kbp *	Genome purity % *	Number
Pure *Giardia lamblia*	**437.56 * a**	**1.705**	**80**
Giardia+ Cryptosporidium pavrum	430.40	1.35	12
Giardia +Blastocystishominis	394.00	1.41	4
Giardia + Entamoeba coli	490.05	2.47	3
Giardia + Entamoebahistolytica	335.00 * b	1.35	1
Giardia+Crptosporidum+Entamoeba coli	550.00 * c	1.25	1
Total Giardia mixed with other protozoa	**439.89**	**1.56**	**23**
All total	**438.72**	**1.62**	**103****

Sign test of median value a*P>0.05 b and c* P<0.05 ** 4 samples of helminthes ignored

Discussion

The overall rate of intestinal infections52.07 % and *Giardia lamblia* rate 24.56 % in the present study were high when compared to those 0.90 %, 9.3 %,13.13,13.7 %,14.41and15.8 % In Kirkuk, Al-Kerbala ,Kirkuk, Al-Najaf,Kirkuk and Babylon recorded by[26,27,28,29,30 .31] . Also it was not agree with those of 11.4 % and 17.1 % recorded in Libya and Brazil respectively by [32 and 33]. The rate of *Giardia lamblia*24.56 % was lower than those 44.59%, 35.89 %, and 62.2 % recorded in Kirkuk, Erbil in Iraq and in Egypt respectively by [34, 35, and 36]. High prevalence of parasitic infection reflects: lower educational level to health hygiene among children, poor experience in toilet use, overcrowded families, water contamination with *Giardia* parasite, and lack of insecticides that had role in mechanical transmission of the infective stages of intestinal parasites. The variance of *Giardia* rates from one region to another might be due to nature of residence survey, level of personal hygiene and sanitation, safety of water consumption from water supplies. In addition to type of diagnostic techniques, size of samples. The rate of infection in males was higher than in females. This might be due to that males are mostly outside their houses and are mostly exposed to fecal transmitted parasites. This finding was not agreed with those reported in two studies done among different localities of Al-Tameem governorate [30 and 31] and with that recorded by Kadir and al-Barzanji in Arbil [34,37] and with that recorded by Al-Hanoon in Mosul [39] whom they did not find significant differences in the rate of infection between males and females. These differences were probably due to the differences in technique used, or could be due to socioeconomic status [34]. For diagnosis of *Giardia* infections; PCR single step detection of *Giardia* parasite provided the best result, with sensitivity of 74.76% In contrast, the Giardia-Triage panel that reveal low sensitivity 40.10 % .Our finding was agree with that recorded by Maraha,2000 and Sharpe, et.al.2001 [21,11] Lower sensitivity of triage panel might be due that three types of antigens were emmbolized on chromatography paper holding three types of specific antibodies [38] or due to high rate of *Giardia* co-infection in the present study .The sensitivity of ELISA test 53.27%was lower than 764.% that recorded in Duhok province by [40]. Similar results have been found in Egypt [41], the United States [42] and Germany [43]. The ELISA copro-antigen and Triage panel assays were less time-consuming and easier to perform, but were less sensitive than conventional microscopy methods. In spite of Triage employee reveal 9.90 % of giardiasis compare to lower rates for *Entamoeba histolytica* and *Cryptosporidium*. While PCR technique remain high sensitive , specific and accurate than other methods ,but it is not easy to performed and costly, it can be used in researches or incase when *Giardia* persist in patient in spite of treatment., Thus, these tests might be a useful addition to stool examination for parasites including *Giardia lamblia and Cryptosporidium* but not a substitute for microscopically methods in the diagnosis of giardiasis .The *Giardia* genome extraction in current study was accurate and precise ,because the genomic purity 1.705 % was close to standardized mean 1.6 to 1.8 %. Also *Giardia lamblia* mass mean 437.6bps was very close to 432 bps fragment recorded in Baghdad by Kader and baker,in2011[44] whom they use gdh gene locus amplified in the PCR using primers GDHiF and GDHiR .The extraction of DNA from cysts of the parasite eliminates the difficult stages of parasite cultivation and selective growth of parasite in culture media. The use of QIAamp DNA extraction Mini kit with a modified protocol in which glass beads have been used and freeze-thawing was performed. The protocol had a result of 100% successful DNA extraction[44] Genomic mass extension within study from 280 to 750bps was not agree with that recorded by [44] whom they were show *Giardia duodenalis* genomic extract from sewages ranged from 530 to 750 bps. The isolated giardia genomes in this study consisting of assemblages A and B which was compatible with that recorded in Iran[45] .The modified Z.N staining method that used in this study was sensitive, simple, rapid, and showed sufficient color contrast to permit screening

even at low magnification, but DFA method revealed high sensitivity 90.90% and 99.25 % of specificity in this study in spite of low number of cases 33. *Cryptosporidium* rate7.60% was lower than 10 % recorded in Kuwait [46] and with thatrecorded by Othman [5] in Kirkuk city.The infection rate observed in the present study could have been still higher if more than one stool specimen had been collected from each child, especially in children with watery diarrhea. As in most parasitic infections, the shedding of *Cryptosporidium* oocysts may be intermittent, even in those patients with massive diarrhea [46] and two or three fecal specimens may therefore be required to detect *Cryptosporidium* oocysts[47].

Conclusions

Giardia rate in kirkuk city was high compare to *Cryptosporidium* rate. PCR and Microscopy diagnosis has had high sensitivity and specificity than other method for detecting giardiasis while DFA was superior for other methods in detecting Cryptosporidium oocysts. For first time; human *Giardia* genome in Kirkuk city was extracted and PCR technique exert its consistence of mixture of **A** and **B** assemblages.

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