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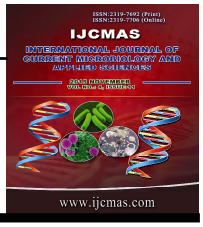

Original Research Article

Prevalence of *Cryptosporidium parvum* among Iraqi displaced people in Kirkuk city using direct microscopy, flotation technique and ELISA-copro antigen test

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Keywords

Cryptosporidium parvus, Giardia, Entamoeba, Blastocystis, ELISA-copro-antigen

A B S T R A C T

Poverty, famine and disasters had great role in causing outbreaks of infectious agents, particularly intestinal protozoon parasites. Continues of several wars upon Iraq from 1991 till to recent time has had potential impact for public health. So the current study was conducted to estimate prevalence of *Cryptosporidium pavrum* and other intestinal parasites among Iraqi displaced people (IDPs). For this purpose 780 stool samples from both gender and ages from below one year to 78 years were collected from people in 15 districts in Kirkuk Province, whom they live under poor hygienic condition and low level of sanitation. Three laboratory methods where performed on each collected stool sample; namely direct double wet preparation of 0.85% of NaCl solution and 1% Lugol's iodine for detecting parasitic rate .While modified Ziehl-Neelsen MZN hot technique and ELISA-copro antigen tests were used specifically for detecting the oocycts of *Cryptosporidium pavrum*. The overall rate of intestinal parasitic infection was 37.56%, this rate was divided in to 37.05% for 11 protozoon parasite found in current study versus to 0.51% for intestinal helminthes, $P<0.05$. High rates of protozoan parasites involve: *Cryptosporidium parvum, Entamoeba histolytica and Giardia lamblia,* the rates were 16.28%, 10.12% and 7.05% respectively. Meanwhile lower rates 1.66%, 0.64%, 0.51% and 0.25% were recorded for *Blastocystis hominis, Retromonas intestinalis, Cyclospora cayetanensis* and *Enterocytzo anbieneusi* respectively. On the other hand 0.12 % was recorded for each of: *Dientamoe bafragilis, Iodomoebabuts chilli,* and *Isospora belli*. Considering intestinal helminthes only 0.38 % for *Hymenolepis nana* and 0.12% for *Ancylostoma duodenali* were recorded. Statistically relationship between cryptosporidiosis and patients residency, types of lab methods, gender was not significant, $P>0.05$. Collectively statistical analyses reveal strongly relationship between occurrences of cryptosporidiosis and patient age particularly young aged patients and types of lab methods especially ELISA technique which reveal high efficacy in demonstrating the oocysts of *Cryptosporidium* than other three lab methods used in current study, while relationship between cryptosporidiosis and patients gender was not significant, $P<0.05$. The overall rate of intestinal parasites particularly *Cryptosporidium parvus* was high among young aged IDPs. ELISA-copro antigen test had value for detecting Cryptosporidiosis in stool samples than other laboratory methods.

Introduction

Cryptosporidiosis is a zoonotic disease caused by intestina apicomplexian protozoon parasite called *Cryptosporidium parvum* (Salman and Salih, 2013). The initial reports of *Cryptosporidium* infection in mice were published by Tyzzer in 1907. In 1955, Slavin (1955) described the parasite as a potential cause of diarrhea in turkeys. Cryptosporidiosis in calves was subsequently recognized in the 1970s. But it was not until *Cryptosporidium* infections were reported as a cause of death in AIDS patients in the 1980s that the protozoan parasite became accepted as a significant zoonotic pathogen warranting scientific research (Current *et al.,* 1983). *Cryptosporidium* sparked great public health interest after the large human waterborne outbreak in Milwaukee in 1993 and rapidly became recognized as one of the most serious and difficult to control waterborne pathogens to date. Subsequent reports have demonstrated its worldwide distribution and zoonotic potential (MacKenzie *et al.,* 1995; Current and Garcia, 1991). Contaminated food or water with infective oocysts and indirect contact with infected animal or humans can cause acute gastroenteritis and diarrhea in healthy people, but in immunocompromised patients, especially those with AIDS and malnourished children *Cryptosporidium* parasites can exert a chronic and life threatening disease (Mosier and Oberst, 2000; Cook, 1987). The aerialist study concerning *Cryptosporidium* in Kirkuk Province was done by Othman, (2000), who found 12.62% of cryptosporidiosis among infants. Also in the same province; Kadir *et al.* (2004) found 4.36% of cryptosporidiosis among children using modified Ziehl-Neelsen stain (hot technique). After 2010, *Cryptosporidium* was studied in Kirkuk by Salman and Ali (2013), Salman (2014) and Salman and Mustafa (2013) whom, they record the following rates: 14.47 %, 7.60 and6.45 % respectively. The recent crisis and violence led to massive population movement, and presence of over 1,500,000 internal displaced in Iraq (Xiao *et al.,* 1999). The displacement of populations from different communities often brings people into proximity either due to increased concentrations of displaced populations and also increased density in terms of the living environment. If one group is a carrier of illness, disease outbreaks reflecting endemic pathogens circulating within the community may occur (Watson *et al.,* 2007). Because of the internally displaced persons are at high risk for emerging parasitic infections, since in most cases they have a history of poor utilization of medical care and vaccination, living conditions of low socioeconomic status and a high possibility to be carrying symptomless diseases, so it is important to carry out an assessment for diarrheal risk factors particularly intestinal parasites with an emphasis on *Cryptosporidium* and to avoid any health risks. Therefore this study was conduct to investigate cryptosporidiosis rate in this particularly category.

Materials and Methods

Time and location

From 1st of August 2014 to 31th of May of 2015, cross sectional study was conducted in laboratory department of dentistry College Kirkuk University, and in Ibn-Nafies private medical laboratory.

Source of samples

A total of 780 stool samples were chosen from internal displaced persons living in schools, houses under construction and rented accommodations and houses in over 15 residential districts in Kirkuk city with

variant economical and hygienic levels and those people are originated from different cities and villages like Anbar, Ninewah, Salah-Eldin, Diyala, Fallujah and other areas which severely affected from conflicts and crisis in Iraq. Sample size was validated by applying the equation of sample size determination in unknown population. Patients are segregated into age groups for both males and females subjects by applying Yule method (Danielm, 1985). Also sample chooses were involve two sources: 711 stool samples for peoples living under low level of sanitations, low income and poor hygienic condition. Whereas 69 stool samples for people living in normal good level of sanitation, high income and good level of hygiene were chosen.

Stool samples collection

Prior to sampling a special questionnaire was filled for each patients consisting of essential information. Disposable container with wide screw lid was given to each patient to bring stool samples. The container label contain: name, date, address and number of container. Immediately about 3-5 ml of Potassium chromate solution was added to each container for preservation (Salman, 2015). Stool samples were kept in ice box and transferred directly to laboratory department for processing.

Sample processing

After samples arrival to laboratory, each sample was examined for detecting *Cryptosporidium* oocysts and other intestinal parasites stages using direct double wet preparation of 0.85% of NaCl and 1% of lugolsiodine (Salman, 2015). Modified Ziehl-Neelsen technique was performed to all fecal smears according to Othman (2000). Briefly fecal smear slide was flooded by strong carbolfuschin stain, beneath the slide was carefully heated till to fine steam appearance from upper surface of the slide. The slide has been left for 5 minutes then stain was discarded and washed with distilled water till to appearance of purple color. Decolorizing solution of 5 % of H_2SO_4 was added on slide and agitated for long of one minute; then an excess of acid was removed by rinsing with D.W. Then after counter stain "methylene blue was covered the smear for one minute. Finally the stain has been discarded and washed by D.W. air dried and examined under microscope using 100x via which, *Cryptosporidium* oocysts appears as pinkish round versus to blue background of slide. The third procedure involves ELISA corpo—antigen test, which has been done according to manufactured company instruction**.** Briefly the kit is consist of micro-plates of 96 wells, each well was solid phase coated with immobilized monoclonal antibodies specific for *Cryptosporidium parvum.* The first step involves removing an excess of potassium dichromate by three times washing with phosphate saline buffer (Pbs), till the supernatant becomes clear. Then after in case formed stool approximately 0.1 to 0.15 gm of specimen (about the size of small pea) was transferred in to 400µl of sample diluent. In case the stool was liquid. 150 µl of the specimen has been transferred in to 400 µl of sample diluent. The mixtures were thoroughly mixed (Vortex). The mixture was left at room temperature for about 30 minutes or centrifuged for 5 minutes at 3000 rpm to obtain good supernatant. Second step involve transferring 100 µl of the supernatant of each stool sample, positive, negative controls (duplicates) and cut-off if supported with the kit in to separate wells; the first well A1 was left without adding any reagent as blank. Gently shacked for about 30 seconds. ELISA plate was covered and incubated for 1 hour at 37 C at 100 %

humidity. Third step involve washing the wells by working wash solution (20 ml of concentrated wash solution in to 980 ml of deionized distilled water) by adding 300µl of wash solution, gentility agitated and discarded. This step was repeated for three times. After that the strips were dried and framed by gently tapping them over clean absorbent paper. Fourth step involve adding 100µl of substrate (TMB) solution for each well involving A1 well. The plate was incubated in dark place for 15 minutes. Fifth step was by adding 100µl of stop solution (1M of H2SO4) into each well. Color intensity was determined by using ELISA reader machine after adjusting the wave length on450/629 nm. The absorbance is proportional to the number of *Cryptosporidium parvum.* Samples that exhibit absorbance values higher than the cut-off should be considered positive. In current study the estimated and applied cut-off was.0.600 I.U/ml. This procedure was applied according the leaflet of manufactured company (Savyon Diagnostics Ltd.) Netherlands.

Statistical analysis: all obtained data were arranged in tables. Pearson Chi (X2) was used as a test of significance. Differences were recorded as significant whenever the probability (P) was less than 0.05 and 0.01. Two-Sample t-student test and CI were applied to estimate variance in mean standard deviation and standard mean. Also One-way ANOVA was performed to determine analysis of variance in techniques used in study.

Results and Discussion

IDPs populations were scanned for Cryptosporidium infection by Elisa technique, Modified ziehl-Neelsen method and zinc sulfate 33% flotation technique respectively and figure 1 shows Kirkuk a geographic information system (GIS) picture provided by coordinates number of all residential regions included in this study. Current study included different categories of IDPs so the life style and level of residency of them varied by their financials power and socioeconomically state (Table 1).

From the examining of 780 stool samples by using three laboratory methods the sum of overall rate of cryptosporidiosis was 22.68%. This rate involves the following rates 28.07 %, 21.92 % and 18.07 using Elisa technique, MZN method and zinc sulfate 33% Flotation technique respectively, P>0.05. In spite of high rates of *Cryptosporidium* records among IDPs in crowded areas with poor hygienic condition 711(91.15%) subjects from poor hygienic residence 214(30.09%), 167(23.48%), 137(19.26%) reveal positive result for cryptosporidiosis by using Elisa technique, MZN method and zinc sulfate 33% Flotation technique respectively. Versus to 7.24%, 5.79% and 5.79% for good quality life of IDPs in good hygienic residence using the same techniques respectively, but statistically relationship between cryptosporidiosis and patients residency, types of lab methods was not significant, P>0.05 (Table 2).

Table 3 is showing relationship between cryptosporidiosis and patients ages, gender. Via which no difference was found between males 23.55% and females 21.88 % in getting cryptosporidiosis, P>0.05. Meanwhile, according to type of laboratory methods employee, ELISA technique exert high rates 29.6% and 26.66% of *Cryptosporidium* in stool samples for males and females respectively. Controversy to 22.66%, 21.23% by using MZN method followed by 18.4% and 17.77% using flotation technique in males and females

respectively, P<0.05. On the other hand regarding patients ages, high percentage of *Cryptosporidium* infection were recorded among males aging below one year to 6 years and from 6 to 12 years, the rates were 42 (25.45%), 50 (30.3%), 56 (35.89) and 40 (35.39), 25 (22.12%), 20(17.69%) by Elisa technique, MZN method and zinc sulfate 33% flotation technique respectively. More than one statistical equations were applied to detect the relationship between *Cryptosporidium* and patients age, gender, type of lab methods. Collectively analyses reveal strongly relationship between occurrences of cryptosporidiosis and patient age particularly young aged patients and types of lab methods especially ELISA technique. While relationship between cryptosporidiosis and patients gender was not significant, P<0.05

Table 4 showing the overall rate 37.56 % of intestinal parasites among IDPs versus to 62.44% of stool samples negative for parasitic infection. Eleven protozoan parasites were recorded contributing 37.05 % from the all rate compare to 0.51 % of intestinal helminthes, P<0.05. High rates of protozoan parasites involve: *Cryptosporidium parvum, Entamoeba histolytica* and *Giardia lamblia*, the rates were 16.28%, 10.12 % and 7.05% respectively. Meanwhile lower rates 1.66%, 0-64 %, 0.51 % and 0.25% were recorded for *Blastocystis hominis, Retromonas intestinalis, Cyclospora cayetanensis* and *Enterocytzo anbieneusi* respectively. Onthe other hand 0.12 % was recorded for each of *Dientamoeba fragilis, Iodomoebabuts chilli, and Isospora belli.* Considering intestinal helminthes only 0.38 % for *Hymenolepis nana* and0.12% for *Ancylostoma duodenali* were recorded.

Co-existence phenomenon in current study was seen with protozoan parasites only, contributing all rate 4.86% which divided into 4.35% for double infection and 0.51% for triple infection, P<0.05. Regarding double existence of *Cryptosporidium* with other protozoan parasites involve the following rates: 2.30%, 1.14%, 0.25% for *Cryptosporiduim + Entaoemba histolytica, Cryptosporiduim + Giardia lamblia and Cryptosporiduim + Blastosycthominis* respectively compare to 0.126 for each of the followings: *Cryptosporiduim + Cyclospora cayetanensis, Entaoemba histolytica + Giardia lamblia* and *Blastosyct hominis + Giardia lamblia*. While triple existence were recorded as 0.126% for each of the followings: (*Cryptosporidium +Giardia lamblia + Cyclospora cayetanensis*), (*Cryptosporiduim + Giardia lamblia + Entaoemba histolytica),* (*Cryptosporiduim + Diaentamoeba fragilis + Entaoemba histolytica) and (Iodamoebabuts chilli +Giardia lamblia + Entaoemba histolytica*) shown in table 5.

Cryptosporidiosis is self-limiting in adult immune-competent people, but in children below 8 years old can cause severe illness due to loss of high amount of fluid from intestinal walls (Cook, 1987). On the other hand this type of parasitic infection is nosocomial, especially in Pediatric Hospital, medical care centers for primary public health and even in crowded communities living under standard level and poor hygienic condition, low level of sanitation (MacKenzie *et al.*, 1995). The main cause to cryptosporidiosis is water quality, supply and rout of transmission specially oral-feco rout. In current study the all rate of cryptosporidiosis 22.68 % was high among IDPs, which reflects the degree of contamination with parasitic phases particularly with oocysts of *Cryptosporidium*. The reason to this high rate most often related to nature of water consumption. It has been known, that a big

construction for improving of roads and infra-structure in Kirkuk province from 2008 to 2014was carried on. This action lead to breakdown of water pipes underground, because all of these pipes are very old, this lead to continue of water supply interruption in this Province. The second reason to this high rate of is high rate might be attributed to uncontrolled migration and inhabitation of IDPs to old buildings, in complete building, old schools. Moreover 4 to 5 families live in one house (highly crowded). All of these factors have had role in increasing the rate of cryptosporidiosis in current study. This finding was not agree with those recorded in the Province by Karyaghdi (2012), Al-Baiti (2011) and Salman and Mustafa (2013) whom they record the following rates: 10.8 %, 20.6% and 6.45 % respectively. It is not clear how much these differences may be explained by differences in study design, geographical location, population group, sensitivity of laboratory methods, or stage of disease. In spite of the result of statistical analysis that reveal no differences among three laboratory methods employed, but the efficacy of ELISA-copro antigen test as exerting 28.07 was superior to other methods 21,93 %, 18.07% and 16,28 % using MZN, flotation ZnSO4 technique and direct double wet preparations respectively. High efficacy ELISA-copro antigen test might be attributed to strong link between immobilized specific *Cryptosporidium* antibodies with added stool specimen containing the parasite (antigen) (Salman, 2014). While in MZN some technical errors such as over or un proper heating, type of decolorizing solution, washing may had role in demonstrating the oocysts under microscope (Pohlenz, 1981; Markell *et al.*, 1999; Garcia *et al.*, 1983). While in flotation technique, centrifugation, washing steps may have role in parasite destruction and discharging so low rate outcome by this method. Meanwhile low rate of Cryptosporidiosis might be due some technical errors such as thick smear preparation which had role in missing the parasitic stages due to overlapping of stool particles with parasites or due to 1% of Lugol's iodine solution use. This percentage may not color the oocyst of *Cryptosporidium* properly to be more visualized under microscope (Morgan *et al.*, 1998). Collectively ELISA-copro-antigen test in current study is high sensitive, specific, reliable, low time consuming compared to microscopy methods.

Regarding the age of IDPs and its role in rate of cryptosporidiosis, the current study revealing high rates of infection among young aged people particularly among age group below one year to 6 years (165 positive cases) and among children aging from 6 to 12 years (113 positive cases) compare to other age groups. This elevated rate highlighting the shadow on young age people in Iraqi communities particularly IDPs in getting infectious agents including *Cryptosporidium.* As it was known that cryptosporidiosis is self-limit in immune-competent, but it becomes more serious when other infectious agents co-existed as opportunistic protozoan parasite "*Cryptosporidium*" (Striepen, 2013). Till to preparing this article, Iraqi people condition was un-stable, continue of wars, improper water supply and even the quality of water not sterile, no attention towered to parasites investigations specially waterborne parasites such as *Giardia* Species, *Entamoeba histolytica, Cryptosporidium, Cyclospora cyetanensis* (Salman, 2015). Malnutrition, due to poverty, which leads to immune diminishing had role in increasing the susceptibility of acquiescing infectious agents including *Cryptosporidium*. Furthermore young aged people in their nature mostly have outdoor activities higher

than elderly people. They are more exposure to environmental factors for getting contamination in general (Striepen, 2013). All of these factors can explain high rate of cryptosporidiosis among young aged people in current study. This finding was in agreement with that recorded by Othman (2000) in Kirkuk city and with that reported in Pakistan by Iqbal *et al.* (1999). On the other hand regarding the role of laboratory methods in demonstrating the oocyst of *Cryptosporidium* with the age and gender of patients, it is obvious that ELISA-copro-antigen test was superior in its sensitivity, specificity on the other employed methods in spite of statistical analysis revealing non-significant as general comparisons with ages and gender in detecting the oocysts of *Cryptosporidium*, particularly in young aged patients for both genders. Considering 13 intestinal parasites detection in current study (11 protozoa and 2 helminthes) with new records of *Enterocytzoan bieneusi*, this reflects high degree of environmental contaminations of water and soil in Kirkuk Province (Salman *et al.*, 2015). Also this study referred the necessity of using more than routine and specific laboratory methods for stool samples for detecting intestinal parasite as with those methods applied in current study. Also these findings can encourage and emphasis scientific worker in this Province for caring out studies in order to draw maps for infectious agents showing the common parasites, their intensity according to districts in this Province. The overall rate of intestinal parasite 37.56 % in current study particularly 37.05 % of protozoon infection was high. This finding was attaching public health because high rates 16.28 %, 10.12 % and 7.05 % were with respectively. The medical importance here is that all high rates with 3 protozoan parasites are waterborne, so this finding reflects water contamination with parasites and water is an agent for increasing the rate of infection in this province if the water quality was not improved in general and particular for IDPs in this province (Watson *et al.*, 2007; Karyaghdi, 2012). The chance of occurrence of malabsorption due to giardiasis, extra-intestinal intestinal amoebiasis and severe dehydration due to cryptosporidiosis among young aged IDPs was probable in high percentages if the health status of this group of population was ignored by the government of Kirkuk Province. Low rate 0.51 % and only two helminthes record in current study among IDPs might be attributes to single stool sample collecting and type of laboratory method i.e. direct double wet preparation only in calculating the overall rate of parasitic infection in current study (WHO, 1991). Further studies using stool concentration method (both sedimentation and flotation), Bearman method for *Strongyloides* and hookworms should be carried on in future to exert comprehensive rate for intestinal helminthes in this Province. This finding was not agreed with that record among displaced people in Sudan by Mohammed *et al.* (2009) whom they found four species of infective parasites identified from individuals in all areas (displaced areas). These were: *Giardia lamblia* (12.3%), Haemophilus. nana (4.9%), *Entamoeba. histolytica* (0.4%) and *Trichuris. trichiura* (0.2%). Exerting high rates of protozoan and helminthic parasites in stool samples among IDPs in current study was critical, it reflects the degree of environment contamination in Kirkuk city with parasitic forms. The initial source of contamination of the urban area in Kirkuk Province is still unknown. Possibilities include the soil, use of contaminated water for irrigation and pesticide dilution, and the poor sanitary facilities available to seasonal field workers (Shield and Olson, 2003).

Table.1 Distribution of study population in residential districts in Kirkuk city

Residence Quality	Residential district names	Coordinates No. Of Residential district on Kirkuk GPS map	No.of individual (%)	Gender No.(%)	
				Male No.(%)	Female No.(%)
Poor hygeinic residences (building under construction, schools, halls, shells, shops and garages and camps)	Aloruba	13,14	35(4.48%)	18(51.42%)	17(48.57%)
	Hay Al-olamaa	8,9	58(7.43%)	27(46.55%)	31(53.44%)
	Hay Al-hussain	3,4,5	64(8.20%)	31(48.43%)	33(51.56%)
	1-Huzayran	22,23,24,25, 26	102 (13.07%)	47(46.07%)	55(53.92%)
	Alsayada	27	34(4.35%)	13(38.23%)	21(61.76%)
	Hay Al-hujaj	10,11,12	123(15.76%)	69(56.09%)	54(43.90%)
	Hay Al-baath	15	31(3.97%)	14(45.16%)	17(54.83%)
	Hay Al-rasheed	28,29,30	73(9.35%)	34(46.57%)	39(53.42%)
	Hamzalii	17	25(3.20%)	17(68.00%)	8(32.00%)
	Hay Al-wastii	16,19,20,21	113 (14.48%)	56(49.55%)	57(50.44%)
	Tissin	6,7	53 (6.79%)	22 (41.50%))	31(58.49%)
Well hygienic residence (rented house and departments)	Rahimawa	1	43(5.51%)	17(39.53%)	26(60.46%)
	Hay Al-islahziraii	18	17(2.17%)	8(47.05%)	9(52.94%)
	Almas	2	9(1.15%)	2(22.22%)	7(77.77%)
	Total		780	375 (48.07%)	405 (51.92%)
Total No.exam=780					

Table.2 Distribution of *Cryptosporidium* infection between internally displaced people in Kirkuk city according to residency and hygienic levels

Residency levels	Coordinates No. Of Residntal district on Kirkuk GPS map	Exam Samples No. and %	Number of positive samples and %			P value
			Elisa technique	Modified zeihl-Neelsen method	zinc sulfate 33% Flotation	
poor hygienic residence	3-17,19-30	711 (91.15%)	214 (30.09%)	167 (23.48%)	137 (19.26%)	** F-Value= 0.2 P-Value= 0.816
good hygienic residence	1,2,18	69 (8.84%)	5 (7.24%)	4 (5.79%)	4 (5.79%)	
Total		780	219 (28.07%)	171 (21.92%)	141 (18.07%)	Total % 22.68 %
Total No. exam = 780		Chi-sq == 0.119 ns (p>0.05)		P-Value = 0.942		

Table.3 Distribution of *Cryptosporidium* infection between internally displaced people in Kirkuk city according to age groups and gender

Age groups years	Gender							
	Males				**Females**			
	Exam No. and (%)	No. of positive sample and (%)			No. Of samples	No. of positive sample And (%)		
		Elisa technique	Modified Ziehl-Neelsen method	zinc sulfate 33% Flotation		Elisa technique	Modified Ziehl-Neelsen method	zinc sulfate 33% Flotation
Below 1 year to6	165 (44%)	56 (35.89%)	50 (30.3%)	42 (25.45%)	168 (41.48%)	54 (32.14%)	40 (23.80%)	39 (23.21%)
6....12	113 (30.13%)	40 (35.39%)	25 (22.12%)	20 (17.69%)	75 (18.51%)	33 (44%)	29 (38.66%)	19 (25.33%)
12..18	18 (4.80%)	3 (14.44%)	3 (16.66%)	3 (16.66%)	19 (4.69%)	2 (10.52%)	0 (0%)	0 (0%)
18...24	7 (1.86%)	0 (0%)	0 (0%)	0 (0%)	19 (4.69%)	2 (10.52%)	2 (10.52%)	2 (10.52%)
24...30	9 (2.40%)	1 (11.1%)	1 (11.1%)	1 (11.1%)	27 (6.66%)	4 (14.81%)	4 (14.81%)	3 (11.11%)
30...36	17 (4.53%)	1 (5.88%)	1 (5.88%)	1 (5.88%)	39 (9.62%)	8 (20.51%)	6 (15.38%)	4 (10.25%)
36...42	11 (2.93%)	6 (54.5%)	1 (9.59%)	0 (0%)	24 (5.92%)	4 (14.81%)	4 (14.81%)	4 (14.81%)
42...48	20 (0.53%)	3 (15.7%)	3 (15%)	1 (5%)	17 (4.19%)	0 (0%)	0 (0%)	0 (0%)
48...54	3 (0.8%)	1 (33.33%)	1 (33.33%)	1 (33.33%)	2 (0.19%)	0 (0%)	0 (0%)	0 (0%)
54...60	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (1.23%)	0 (0%)	0 (0%)	0 (0%)
60...66	61 (1.6%)	0 (0%)	0(0%)	0(0%)	6 (1.48%)	1 (16.66%)	1 (16.66%)	1 (16.66%)
66...72	5 (1.33%)	0 (0%)	0 (0%)	0 (0%)	2 (0.49%)	0 (0%)	0 (0%)	0 (0%)
72...78	1 (0.26%)	0 (0%)	0 (0%)	0 (0%)	2 (0.49%)	0 (0%)	0 (0%)	0 (0%)
Total	375 (48.07%)	111 (29.6%)	85 (22.66%)	69 (18.4%)	405 (51.92%)	108 (26.66%)	86 (21.23%)	72 (17.77%)
Total exam= 780		Sum positive rate of males=23.55% Chi-Sq=9.003**, p-value =0.0016				Sum positive rate of females=21.88 Chi-Sq =4.993 ns p-value =0.241		

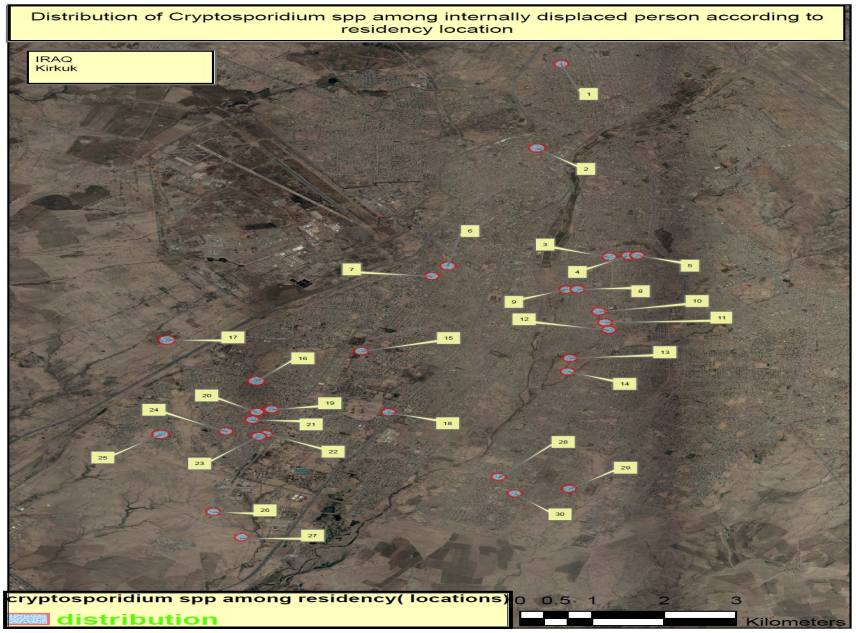
Table.4 Frequency of *Cryptosporidium parvum* and other intestinal parasites positive and negative percentages among displaced people in Kirkuk city using direct double wet preparation technique.

Name of parasites **Intestinal protozoan parasites**	**Number positive**	**Percentage positive**	**Number negative**	**Percentage Negative**	**Two-Sample T-Test and CI: +VE; -VE**
Cryptosporiduim spp	127	16.28%	653	83.71%	
Entaoemba histolytica	79	10.12%	701	89.87%	T-Test of mean difference = 0 (vs ≠ 0):
Giardia lamblia	55	7.05%	725	92.94%	
Blastosycthominis	13	1.66%	767	98.33%	
Cyclospora cayetanensis	4	0.51%	776	98.20%	N Mean StDev SE
Isospora belli	1	0.126%	779	99.87%	+VE 12 24.3 41.0 11.8
Chilomastix mesnelii	1	0.126%	779	99.87%	
Retortamonas intestinalis	5	0.645	775	99.35%	-VE 12 755.7 41.0 11.8
Enterocytzo anbieneusi	2	0.25%	778	99.74%	T-Value = -30.87
Diaentamoeba fragilis	1	0.126%	779	99.87%	P-Value = 0.00076
Iodamoebabuts chilli	1	0.126%	779	99.87%	
Total of Inestinal protozoa parasites	289	37.05%	491	62.95%	
Helmithic parasites					
Ancylostoma duodenale	1	0.12%	779	99.87%	
Hymenolepis nana	3	0.38%	777	99.61%	
Total of Helmithic parasites	4	0.51%	776	98.20%	
All total of Intestinal parasites	293	37.56%	487	62.44%	
Total no.exam =780					

Table.5 Distribution of *Cryptosporidium* spp and co-infection with other intestinal parasites between internal displaced people in kirkuk city

Type of Intestinal parasitic co-infection	NO positive	Percentage positive	Number negative	Percentage Negative	Two-Sample T-Test and CI: +VE; -VE
Double Intestinal parasitic co-infection					T-Test of difference = 0 (vs ≠):
Cryptosporiduim + Entaoemba histolytica	18	2.30%	762	97.69%	
Cryptosporiduim + Giardia lamblia	11	1.14%	769	98.58%	
Cryptosporiduim +Blastosycthominis	2	0.25%	778	99.74%	N Mean StDev SE Mean
Cryptosporiduim +Cyclospora cayetanensis	1	0.126%	779	99.87%	
Entaoemba histolytica +Giardia lamblia	1	0.126%	779	99.87%	+VE 10 3.80 5.88 1.9
Blastosycthominis+ Giardia lamblia	1	0.126%	779	99.87%	-VE 10 776.20 5.88 1.9
Total of double Intestinal parasitic co-infection	34	4.35%	746	95.64%	
Triple Intestinal parasitic co- infection					
Cryptosporiduim + Giardia lamblia +Cyclosporacayetanensis	1	0.126%	779	99.87%	T-Value = -293.53
Cryptosporiduim + Giardia lamblia +Entaoemba histolytica	1	0.126%	779	99.87%	P-Value = 0.000052
Cryptosporiduim+Diaentamoeba fragilis +Entaoemba histolytica	1	0.126%	779	99.87%	
Iodamoebabuts chilli +Giardia lamblia +Entaoemba histolytica	1	0.126%	779	99.87%	The overall rate of protozoan infection=37.05 %
Total of triple Intestinal parasitic co-infection	4	0.51%	776	98.20%	
Total no.exam =780	*Co-existence all rate=4.86%*				

Fig.1 Kirkuk a geographic information system (GIS)



Coordinate system: WGS1984UTM Zone 38N, Projection: Transvers mercator.
Datum: WGS 1984, False casting: 500.000.0000, False northing: 0.0000
Central meridian: 45.0000, Scale factor: 0.9996, Latitude of origin: 0.0000
Uints: kilometer, Scale: 1 Centimeter = 1 kilometer

Furthermore it can be explained by an excess crowding due to attending of displaced people from neighboring Provinces due to war. In addition to continues electric and water interruption in Kirkuk city which can accelerate stored food disturbance in houses, vegetables washing and even personal cleaning and water consumption (Döller *et al.*, 2002).

Cryptosporidium co-existence overall rate 4.86% in current study was vital from medical view specially double co-existence of *Cryptosporidium* with *Entamoeba histolytica* 2.30% in 18 stool samples followed by *Giardia lamblia+ Cryptosporidium* in 11 stool samples with the rate 1.14 %. Also triple existence of *Cryptosporidium + Entamoeba + Giardia* as 0.126 % was critical for public health of Kirkuk community in general and particularly for IDPs. Because these types of infections can lead to sever changes in parasites habitation inside the body of the host such as severe dehydration causes by *Cryptosporidium parvum*, smoothness of brush border by *Giardia* irritation to upper parts of small intestine of the host, which mostly terminated by malabsorption (Markell *et al.*, 1999). In addition to extra-intestinal amoebiasis (Liver, lung and brain) which impact to public health particularly among young aged people it considered as life threading parasitic infection (Salman, 2002).

Intestinal infection rate among IDPs was high particularly among young aged in both gender. ELISA-copro antigen test had value for detecting cryptosporidiosis in stool samples than other laboratory methods. Co-existence of *Cryptosporidium* oocysts with *Giardia lamblia* and *Entamoeba histolytica* should be taken in consider during examination of stool samples in Kirkuk Province using more specific laboratory methods for detecting infectious agent including intestinal parasites. Water supply and its quality in Kirkuk Province should be improved to reduce the rate of waterborne parasites.

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