Detection of Some Microbial Infectious Agents among Children Aging Below Two Years in Kirkuk City.

*Yahya Jirjees Salman, **Luqman Ali Salih

* Department of medical microbiology, College of Medicine, Kirkuk University.

**Public Health Laboratory, Kirkuk Health Office.

Abstract:

Background: The diagnosis of infectious agents of diarrhea among children is a challenging clinical problem in daily practice.

Aim: This study was undertaken to detect some microbial agents among children suffuring from diarrhea below two years in Kirkuk city.

Sitting and design: Clinical blocked controlled study on 221 children in Kirkuk city.

Materials and methods: by Stool examination using different methods including routine stool examination for bacteria and parasites and some special tests for Rota viruses and other pathogens. Statistical analysis was performed using Chi-square test.

Results: From examination of 221 stool samples, only 145(65.59%) samples were positive for microbial agents, which included:35.29%,23.07% and 7.33 % for protozoan, rotavirus and bacterial infections respectively P<0.05.The common protozoa involve *Entamoeba histolytica* 19.09 %,*Cryptosporidium* oocysts 14.47 % and *Giardia lamblia* 0.90 % P<0.05.Realationship between the distribution of microbial infection in regard of gender and child age was significant in which protozoan infections in males 20.34% was higher than females14.91 %, while it was not significant in regard of Rota-virus and bacterial infections .Also intestinal infections rate 11.73 % was higher among children aging from 19 to 24 months than other age groups reversely to 8.13 % of rotavirus infection that recorded among children aging from one month to 6 months. High rate of rotavirus infection 15.38 % was recorded among children depending on bottle feed comparing to breast and mixed mode of feed P<0.05.While mixed modes of feeding both bottle & breast feeds reveal high rates of intestinal protozoa and bacterial infections, the rates were 14.47 % and 3.7 % respectively P<0.05.The association of microorganism distributions with family member numbers and child family residency were statistically significant P<0.05. Relationships between microbial distributions and socio-economic state of children families, stool consistency, pH and number of feces discharge per day were not significant statistically.

Conclusion: microbial infectious agents rates among children below two years old in Kirkuk city were high specially *Entamoeba histolytica* ,*Cryptosporidium parvum* and rotavirus.

Key words: Cryptosporidium, Entamoeba histolytica, Rotavirus, Children

Introduction:

Diarrhea remains one of the most common illnesses of children and one of the major causes of infant and childhood mortality in developing countries [(1)].

Diarrhea in children account for approximately 5000000 deaths per year in the developing world [(2)], and about 150 million episodes of acute diarrhea occur annually in the Eastern Mediterranean Region (EMR) among children under five years of age, [(3)].Causative agents of infectious diarrhea include bacteria, viruses, protozoa and helminthes [(4)]. Among them viral infection is the most common cause of diarrhea in children between 6

and 18 months with a higher prevalence during the cooler months of the year [5] .Four types of viruses cause most viral gastroenteritis: Rotavirus , Adenovirus, Caliciviruses and Astrovirus.Rotavirus is the leading cause among children aging from 3 to 15 months [6].The most widely used method of detection of viral gastroenteritis is the enzyme-Linked immuno-sorbent assay (ELISA)and other detections such as Electron microscopy and recently developed technique of poly acryl amide gel-electrophoresis (PAGE) of RNA[7].

Intestinal parasites are the common cause of human diarrhea, worldwide give rises in morbidity and mortality, particularly in developing countries [8]. The most common infectious protozoa that transmitthrough food and water contamination with infective stages of *Entamoeba histolytica* , *Giardia lamblia* and *Cryptosporidium parvum* [9,10].The diagnosis of intestinal parasites includes using different laboratory methods such as direct wet preparation or by concentration techniques , in addition to some serological tests such as ELISA and non-serological methods such as Polymerase chain reaction PCR[11].

Considering bacteria causing diarrhea or bacillary dysentery are variable, especially children below two years, the common bacteria include: *Escherichia coli* (different serotypes), Shigella different species and serotypes, Salmonella species, and micro-aerophilic bacteria the Campylobacter species. Diagnoses of these bacteria requires selective media &biochemical tests and use of different anti-sera to reach the final identification of isolated bacteria [12].

Material & methods:

This study was carried out in Kirkuk public health laboratory, Kirkuk Pediatric Hospital and Kirkuk General Hospital from first of August 2010 to thirty first of December 2010.

The total number of patients enrolled in the study were (221) patients, including (127) males and (94) females. From each child fresh stool sample was collected in sterile disposable plastic containers. The following data sample number, date name of patient, age and sex, were labeled on each container. Preservative solution was added an adequate amount 2ml of 2.5% potassium dichromate solution to each specimen. Samples were directly processed for bacteriology and examined within half an hour for parasites and viruses.

Laboratory diagnosis for protozoa parasites includes macroscopic examination and microscopic that involves double wet preparations, modified Ziehl-Neelsen technique and wet preparations of buffered methylene blue according to[13,14].For rotavirus detection , latex agglutination test and rapid lateral-immuno-chromatography assay using stool sample were applied for each specimen according to that used by [15].Isolation of Enter pathogenic bacteria ,all stool specimen were inoculated directly on to MacConkey agar and cultured on Tetrathionate broth for (18 hours at 37c°) then transferred on MacConkey and XLD plates after incubation for (24) hours, suspected colonies were identified using standard microbiological methods [17].The Escherichia coli isolates were preceded for serotype using a slide agglutination method by antiserum for *E.coli* to identify *enteropathogenic E. coli (EPEC)* strains using EPEC (O and K) poly I, III (plasmatic antisera Ltd) batch

No. A5521, A5537.Standard Operating Procedure (SOP) was applied for isolation and identification of Shigella four species: *Shigella dysenteriae, Shigella boydii, Shigella flexneri* and *Shigella sonnei*. By using *Shigella sonnei* polyvalent anti sera (by Bio-rad Ltd) batch No.(6D2032) and *Shigella dysenteriae* monovalent anti sera (6H2027) and *Shigella flexneri* polyvalent anti sera (5L 3026) [18]
Statistical analysis:-All data were distributed in to tables and the differences of variation were tested by using Chi-square test of the probability value $p< 0.05$.

Results:

The rate of microbial infections was 65.59 % which divided into 35.29 % for protozoan infections ,followed by23.07% and 7.33 %for cryptosporidium oocysts and bacterial infections respectively $P<0.05$, also the same table shows microbial distribution regained to sexes, through with males were highly infected 12.94% than females 8.89% (table 1).
The high rate of protozoan infection was 19.91 % for *Entamoeba histolytica*, and the lowest rate 0.90 % was with *Giardia lamblia* $P<0.05$ (table 2).
Regarding frequency of protozoan infection between gender, table (3) showing significant difference $P<0.05$, through which the rate of infection among males was 20.34 % compare to 14.91 % among females, while for rotavirus infection it wasn't significant $P >0.05$. Also the same table reveals differences between microorganisms distribution in relation to age, via which protozoan rate was 11.73 % among children aging from 19 months to 24 months in compare to other age groups. While frequency of rotavirus infection was highly recorded among children aging from one month to 6 months, the rate was 8.13 % (table 4).
Socio-economic state of children families number and defecation per day also were studied in relation to microorganisms distribution,rotavirus infection was high in families with more than 7 members $P<0.05$, among moderate economic state and children passing 5 - 8 times of feces per day, intestinal infection frequent was high 43 % , $P<0.05$. While bacterial infections were highly recorded among children families with 4 – 6 members, moderate economic state's more than 9 times and above of feces discharge daily. (table5).
Regarding microorganisms distribution in relation to stool: consistency ,color and pH results in table (6) show no significant differences $p> 0.05$,through which most of rotavirus infections were within liquid yellowish stool with pH between 6 -7, while parasitic infections were liquid- yellowish stool with pH between (3-5) and bacterial infections were liquid –yellowish with pH 5-7.

Protozoa morphology (Cryptosporidium) :

1-Lugal's iodine preparations demonstrate, round oval oocystic structure, contain dark Brown area in the center of the oocysts with yellow to brown color of the cytoplasm. Black dots in the brown area within sporocysts considered as Sporozoites (Figure 1).
2- In Modified Ziehl- Neelsen preparations, the oocysts also oval to spherical measures more than (4µm) pink to red in color with blue or green background according to type of counter stain, also dark dots inside sporocysts may consider sporozoites (Figure 2)

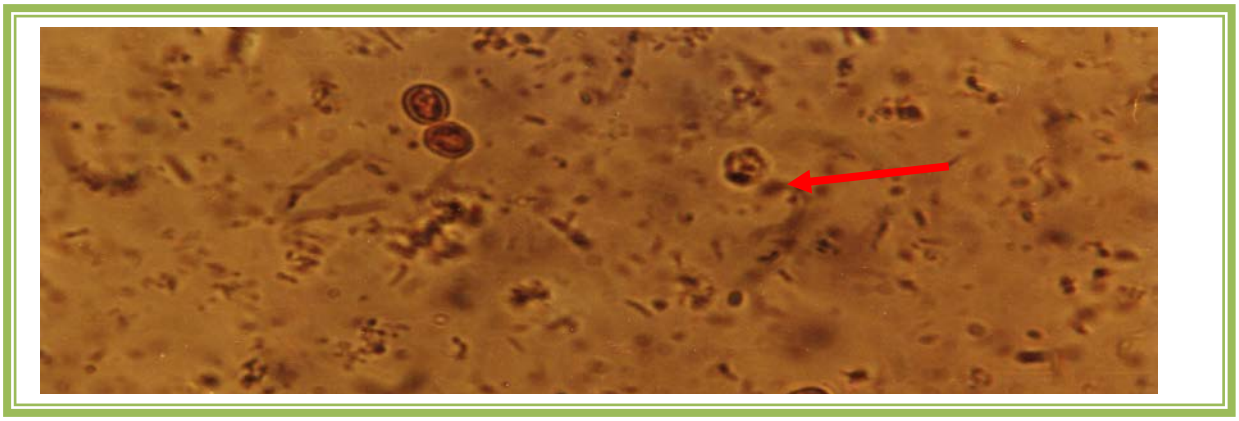

Figure (1): *Cryptosporidium* oocyst by using lugol's Iodine 5% (mounting). (X 100)

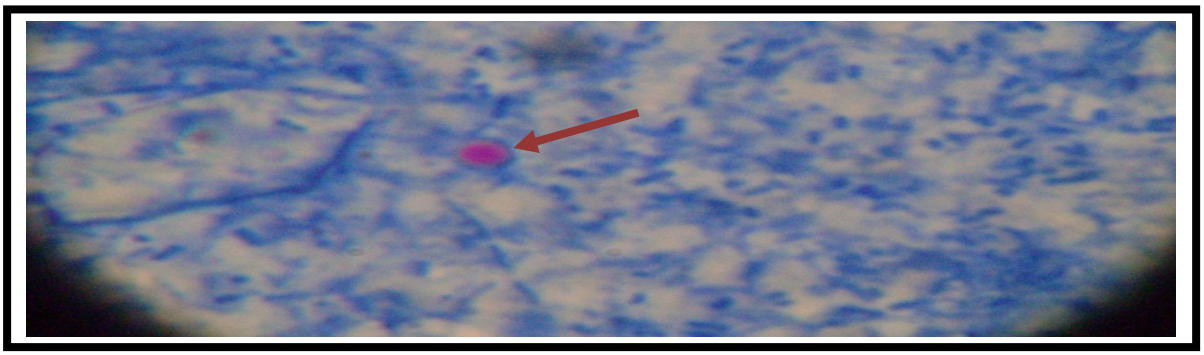

Figure (2): *Cryptosporidium oocyst* by using Modified Ziehl- Neelsen (hot method) M.B counter stain. (X 100).

Table (1): Distribution of Microbial causative agents according to gender.

Microbial Agents/Gender	Male		Female		TOTAL	
	No. Positive	Positive %	No. Positive	Positive %	No. Positive	Positive %
Protozoan Infection	45	20.36	33	14.93	78	35.29*
Viral Infection	30	13.57	21	9.5	51	23.07
Bacterial Infection	11	4.97	5	2.26	16	7.23
Total	86	12.9 *	59	8.89	145	65.59

Total number of stool exam =221

* *Chi-Sq. (Male) = 26.481 d.f =1 p< 0.05*
* *Chi-Sq. (Female) = 25.858 d.f =1 p< 0.05*

Table (2): Distribution of Intestinal Parasite according to the (positive & negative) rates.

Intestinal parasite (protozoan)	No. +ve sample	% + ve	No.-ve sample	% - ve	TOTAL
1- Entameba histolytica	44	19.91	177	80.09	100
2-Cryptosporidium spp.	32	14.48	189	85.52	100
3- Giardia lambilia	2	0.90	219	99.09	100
TOTAL	78	35.29	143	88.23	100

* Chi-Sq. = 7.980 d.f = 2*p< 0.05*

Table (3): Frequency of Intestinal parasite among males and females in different age group.

Age group	No. Exam			No. Positive %		
	Male	Female	Total	Male	Female	Total
(1-6)month	44	33	77	9(4.07)	7(3.16)	16(7.23)
(7-12)month	47	28	75	15(6.78)	9(4.07)	24(10.85)
(13-18)month	14	19	33	7(3.16)	5(2.26)	12(5.42)
(19- 24)month	22	14	36	14(6.33)	12(5.42)	26(11.73)
TOTAL	127	94	221	45(20.34)	33(14.91)	78(35.29)

*Chi-Sq. = 28.973 df=3 P<0, 05

Table (4): Frequency of rotavirus among males and females in different age groups.

Age group	No. Exam			No. Positive %		
	Male	Female	Total	M	F	T
(1-6)month	44	33	77	11(4.97)	7(3.16)	18(8.13)
(7-12)month	47	28	75	9(4.07)	8(3.61)	17(7.68)
(13-18)month	14	19	33	3(1.35)	3(1.35)	6(2.7) **
(19-24)month	22	14	36	7(3.16)	3(1.35)	10(4.51)
TOTAL	127	94	221	30(13.09)	21(9.47) *	51(23.07)

*Chi-Sq. = 0.904 d.f = 3 ** p> 0* *** p < 0.05*

Table (5): The association of Micro-organisms with family Members, economic state and number of defecation per day.

Microorganisms	*Family members* *			*Economic state* **			*NO. of defecation* *** *per day*		
	1 - 3	4 - 6	7 above	Poor	Moderate	Rich	2 - 4	5 - 8	9 above
Rotavirus	2	15	34	7	33	11	12	28	11
Intestinal parasite	17	43	18	14	54	10	19	43	16
Bacterial Infection	2	12	2	4	10	2	7	7	29
Total	21	70	54	25	97	23	38	78	56

*chi-Sq= 41.781 df=2 P<0.05 ** chi-Sq=2.7 df =2.7 P>0.05
***Chi-Sq=2.981 df=2 P> P>0.05

Table (6): Frequency of microorganisms in relation to consistency, PH and color of stool.

Microorganisms	Consistency*				pH**			Colour***		
	F	S	L	W	3 -5	6 -7	8 - 9	Yellow	Green	Brown
Rotavirus Infection	3	12	32	7	13	27	14	35	10	6
Parasite Infection	0	24	43	11	29	24	23	36	29	12
Bacterial Infection	0	7	8	2	5	5	7	9	6	2

*F = Formed, *S=Soft, * L =Loose, *W =Watery
* Chi--- Sq=6.664 d.f =4 *p > 0.05*
** Chi--- Sq =6.898 d.f =4 *p > 0.05*
** * Chi--- Sq=6.796 d.f =4 *p > 0.05*

Discussion:

The rate of microbial infections (65.59%) was very high, that reflecting the degree of environmental contamination in this province mostly the causes due to continuous lack of municipal water, lack of insecticides or defect in spraying program by the governorate, also it may be attributed to poor hygienic condition and low level of sanitation. This rate not agree with 30.03% that recorded by Al Bayati SH. [15]in Tuz town in Tikrit province. The rates of intestinal protozoan especially *Entameoba histolytica* was the most common enteric pathogen , 19.9%, this rate was low when it was compared to 54.9 %, 47.6% and 41.2 % ,33.8 % in Al Najaf province, and Kirkuk city respectively by [16, 17 and 18].While it was higher than those 16.7 %, 15.3%and8.6% in Nicaragua and Palestine, Turkey by [19, 20 and 21] respectively. Variations might be attributed to number of samples and to use of buffered methylene blue preparation in present study which consider selective method for detection of *Entamoeba histolytica*. Considering cryptosporidium infection, the rate 14.48% was compatible with that reported in Kirkuk 12.62% [22].While low rates were recorded in Basra 8.13% [23], 2.3% in Diyala[24]. Also higher rates than in present study were recorded in the following countries 32% and 70.3% in Jordan and Kuwait respectively [25, 26].The variances in the rates of cryptosporidium infection might be due to different factors such as : Geographical distribution, period of the study, Environmental factors, diagnostic techniques, seasonal variation and number of samples.

Giardia lamblia rate in the present study was very low (0.9%) in compare to those recorded in Al Najaf 28.1% [16]2.5%in Korea [27], 13.2% in Cameron [28] and (41) % in México [29]. This low rate can be explained by the facts that related to duration of study carry on autumn and winter and to use of double preparation samples. Rotavirus infection rate in the study 23.08% was higher than those recorded in Basra18.50% with peak among children aging from 7 to 12 months [23]. This observation was close compatible with that recorded in the Diyala [24].In contrast our rate was lower than the prevalent rate 37% of rotavirus diarrhea estimated in Irbil in 2006 [14], and in neighboring countries 40% , 63%in Turkey and Iran respectively [30,32]. This variation in the prevalence of rotavirus infection among different areas is probably due to the social habits of the population, e.g. personal hygiene, and or environmental variations that may be related to growth of rotavirus pathogens particularly in contaminated water.

Also the high rate of rotavirus infection among those with artificial feeding (bottle) as compared to babies depending on breast feeding, can be explained by using tap water without boiling, contamination of bottle milk with infectious agents from other child or animal contacts or mechanical transmission [33]. Breast feeding infants reveal low frequency of microbial infection, this reflects an important role of breast feeding to increase the immunity of children and protect them against the infection [32,33]. The infection rate with other enteropathogens(*E . coli* and Shigella species) were found as 7.23%, it exposure the light on high degree of

contamination specially among baby depending on bottle feeding or due to low level of mother education in milk preparation. In spite of low rate of bacterial infection in the present study comparing to Rotavirus and protozoa infections, but recording EPEC and Shigella Species refers to suitable percentage of bloody mucoid diarrhea attributed to bacterial infection, which requires good management of diarrhea especially from laboratory views in addition to clinical treatment[11]. The frequency of protozoa infection in male children 20.34% higher than in female children (14.91%) can be explained by the fact that the males are more active , mobile and integrated to the environment , more ever , it may involve gender specific immunological differences [8]. Regarding relationship between the prevalence of infection and family size it was shown that the rate of infection was lowest among the family with low members this reflect that chance or frequency of microbial infection can give rises in relation to more people contact to each other's, in crowded family sharing fomites, spoons, glasses than those in non-crowded families.

Conclusion:

Microbial infectious agents rates among children below two years old in Kirkuk city were high specially Entamoeba histolytica ,Cryptosporidium parvumand rotavirus, and using of modified Ziehl-Neelsen, buffered methylene blue preparations and lateral immune-chromatography assay reveal high efficacy in diagnosis of cryptosporidium, *Entamoeba histolytica* and rotavirus infections respectively.

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