

STUDY THE PREVALENCE OF GIARDIASIS AND CRYPTOSPORIDIOSIS AMONG CHILDREN AT AL-RESSAFA SIDE OF BAGHDAD BY COMPARISON BETWEEN THE EFFICIENCY OF SOME DIAGNOSTIC METHODS ⁺

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Abstract:

The present study is a cross sectional study conducted on children suffered from watery diarrhea at different ages attended to some special pediatric hospitals and primary health care centers in Al-Ressafa side of Baghdad , Stool samples were collected from 166 child during the period (from the 1st.of Nov. 2012, till the end of Jan. 2013) and examined by Direct method, then they were examined using different laboratory diagnostic methods including ELISA technique ,Concentrated method by flotation technique ,and Zehil-Nelssen staining methods .ELISA technique was used to detect *Giardia lamblia* and *Cryptosporidium* spp. which is the first survey conducted in Iraq to investigate the both parasites by the same kit .

The results revealed that the infection rate was (6.63%) by using direct exam compared with (10.24%) *Giardia lamblia* using concentrated method by flotation technique and (37.35%) with *Cryptosporidium* spp. by Modified Zehil-Nelssen (M.Z.N.) method, and the infection rate was (39.76%) by using ELISA technique for the both parasites.

The sensitivity and specificity of ELISA technique in this study was (100%) and (64.51%) respectively when compared with direct microscopic exam, and (100%) sensitivity with (96.15%) specificity of ELISA compared with M.Z.N. method .

The high infection rate was among children (≤ 5 years) old, especially at the age (≤ 1 year) which was (72.7%) and (36.36%) respectively .

دراسة أنتشار داء الجيارديات وداء الأبواغ الخبيثة بين الأطفال في جانب الرصافة من مدينة بغداد
بالمقارنة بين كفاءة بعض الطرق التشخيصية

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المستخلص:

الدراسة الحالية تمثل دراسة مقطعية تناولت الأطفال المصابين بالإسهال المائي بفئات عمرية مختلفة ممن يراجعون بعض مستشفيات الأطفال التخصصية ومراكز الرعاية الأولية في مناطق مختلفة من جانب الرصافة من بغداد ، للحصول على الرعاية الطبية والعلاج .

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تم جمع (166) نموذج براز خلال الفترة من (بداية شهر تشرين الثاني 2012 ولغاية نهاية شهر كانون الثاني 2013) وفحصت بطريقة الفحص المباشر ثم تم إجراء الدراسة عليها بفحصها باستخدام عدد من طرق التشخيص المختبرية منها طريقة الفحص التركيز للعينة وطريقة صبغة زيل نيلسن المحورة واختبار الأدمصاص المناعي (الأليزا) للتحري عن طفيلي الجيارديا لامبليا وطفيلي الأبواغ الخبيثة وهذا أول فحص من نوعه يجرى في العراق للتحري عن طفيلي الجيارديا لامبليا وطفيلي الأبواغ الخبيثة بطريقة الأليزا لكلا الطفيليين معا".

أظهرت النتائج نسبة إصابة (6.63 %) بطريقة المسحة المباشرة مقارنة بطريقة تركيز العينة باستخدام تقنية التطويف و بنسبة (10.24%) للجيارديا لامبليا ،بينما بلغت نسبة الإصابة (37.35%) بطريقة صبغة زيل نيلسن المحورة لطفيلي الأبواغ الخبيثة مقارنة مع طريقة الأليزا التي أعطت نسبة إصابة كلية بالطفيليين بلغت (39.76 %). تبين أن فحص الأليزا بدقة (100%) و بكفاءة (64.51%) مقارنة مع فحص المسحة المباشر، وبدقة (100%) و بكفاءة (96.15 %) مقارنة مع طريقة صبغة زيل نيلسن المحورة .

كما أظهرت الفئات العمرية الأقل من خمس سنوات نسبة إصابة عالية بكلا الطفيليين وأعلى نسبة إصابة للفئة العمرية دون السنة الواحدة و بنسبة إصابة بلغت (72.7 %) و(36.36%) على التوالي .

Introduction:

Giardia lamblia and *Cryptosporidium* spp. are ubiquitous enteric protozoan pathogens that infect humans, in the small intestine causes watery diarrhea in undistinguished cause . Direct child to child fecal oral route is the main possible way for transmission of infection in endemic situation [1].

Also animal hosts like calves, lambs, goats and pets are important source of human infection [2]. They are also significant waterborne pathogens. In developing regions of the world, *Giardia lamblia* and *Cryptosporidium* spp. constitute part of the complex group of parasitic, bacterial and viral diseases that impair the ability to achieve full potential and impair development and socio-economic improvements. All diseases included in the WHO Neglected Diseases Initiative have a common link with poverty and, as the current view is to take a comprehensive approach to all these diseases, both *Giardia lamblia* and *Cryptosporidium* spp. were included in 2004 [3].

OBJECTIVES: cross sectional study was conducted to detect the percent of infection with Giardiasis and Cryptosporidiosis also to compare between some methods used for detection of *Cryptosporidium* spp. and *Giardia lamblia* in fecal samples and revealed the best method to apply in laboratories .

Material and Methods:

Cross- sectional study include 166 diarrheic stool samples were collected from Children & 20 normal stool samples as a control , with ages ranging from less than one year to 12 years from some places at different sites in Al Ressaafa side of Baghdad governorate who attend to Welfare hospital, Al-Sadder hospital, and Al-Zaffaranih hospitals ,and JeserDiala health care center at the period from (1st. of November 2012, to end of January 2013).

Direct examined by Microscope and direct wet mount , iodine stain and M.Z.N. stain for concentration method by flotation technique for detection *Giardia lamblia* cyst and *Cryptosporidium* spp. oocyst .[4], also samples preserved using 10% formalin and examined using ELISA technique (Crypto \Giardia Ag Combo(Fecal) ELISA, DIAGNOSTIC AUTOMATION,INC.,USA. Cat # 8310-3) for detection the presence of surface (oo)cyst

antigen in stool. A positive reaction indicates that the patient is shedding detectable amounts of either antigen of both *Giardia lamblia* and *Cryptosporidium spp.*

Results and Discussion:

Regarding the laboratory diagnosis used in this study (table 1) showed that General Stool Examination method (G.S.E.) revealed that 6.63% of patient are infected with *Giardia lamblia* . This method is the conventional method used in diagnosis in all laboratories which can show *Giardia lamblia* only and failed to diagnose *Cryptosporidium spp.* by routine method while *Cryptosporidium spp.* need a special request form [5].

Table(1): The infection rate of *Giardia lamblia* by using GSE

GSE	Gender					
	females		males		Total	
	N0	%	N0	%	N0	%
Neg. samples	65	94.20	90	92.78	155	93.37
Pos. <i>G.lamblia</i>	4	5.80	7	7.22	11	6.63
Total	69	100.00	97	100.00	166	100.00

$\chi^2=0.131$ $df= 1$ $p= 0.717$
 pos.=positive
 Neg.=Negative

The infection rate of Giardiasis is higher than results of other studies conducted in Al-Karkh side of Baghdad ,where Giardiasis was 1.77% [6] and lower than Giardiasis that was mentioned in AL-Mahmoudyia area in Baghdad ,which was 34% [7]; in Al-Ressafa side of Baghdad province 33% [8]; in Sulimanya province was 17.7% and 16.37% [9,10]; in Al-Ressafa side of Baghdad 11.9% [11]; in Wassit province 11% [12] and in Karbala province 8.3% [13].

In this study the percentage of Giardiasis using direct method was low when compared with the above mentioned study who use the same method which may be due to season factor , when Giardiasis records the low rate in winter and the high rate in summer [8] and this study took place in autumn and winter seasons which agreed with Ichhpujani`s study, where these parasite were more likely in hot (Tropical area) rather than cold[14].Variation in results may also be due to difference in the size of samples , socioeconomic status and geographic distribution of samples .

By using ELISA techniques, the total infection rate with Giardiasis and Cryptosporidiosis was 39.76% , (table 2) where there was only 6.63% positive samples by routine method .

Table (2) : Infection rate of Giardiasis and Cryptosporidiosis using ELISA techniques

	ELISA		
	Neg. samples	Pos. samples	Total
No.	100	66	166
%	60.24%	39.76%	100.00

While G.S.E. is not sufficient method in diagnosis , ELISA technique can show positive result which can limit the diagnosis of infection when suspected with *Giardia lamblia* and *Cryptosporidium* spp. and exclude other confused or misdiagnosis diarrheic cases and by this method we can limit the distribution of these parasite.

When Comparing ELISA with G.S.E. statistically from aspect of Sensitivity and Specificity, the ELISA test showed high sensitivity 100% with specificity 64.5% as shown in Fig.(1)

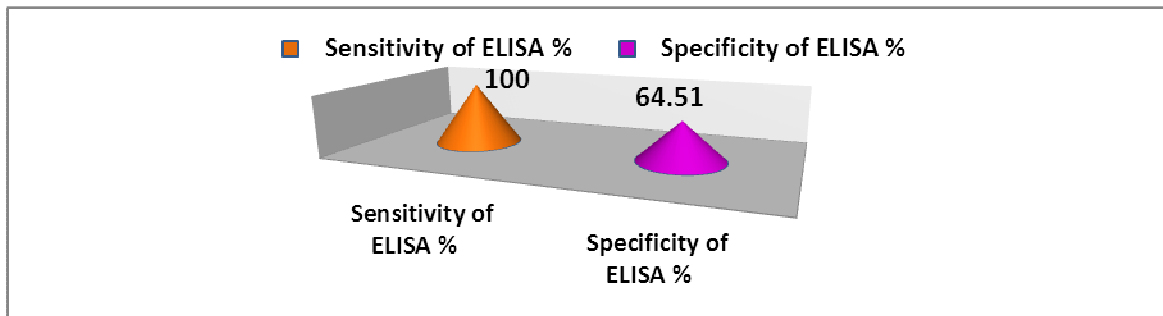


Fig.(1):Sensitivity and Specificity of ELISA compared with GSE

These results disagree with results of [15] who used ELISA test for detection Giardiasis and found that the sensitivity 76.4% and specificity 100% , this difference may due to differ in sample size or due to the fact that their kit for ELISA test contained single antibodies (Anti- *Giardia lamblia*) only; While the kit of ELISA used in this study contain both (Anti-*Giardia lamblia* and Anti-*Cryptosporidium* spp.).

Particularly ELISA shown to be nearly two times more sensitive than conventional method, agree in that with other studies conducted in Iraq and United State [16-18]. It is the first trail to survey Giardiasis and Cryptosporidiosis using same ELISA kit by explore two antigens in stool sample where as other studies explained *Giardia lamblia* alone .

Giardia/Cryptosporidium ELISA kit is an immunoassay developed to allow the simultaneous qualitative detection of *Giardia lamblia* and *Cryptosporidium* (oo)cyst antigens at stool specimens in which positive results of the test signify the presence of either *Giardia* or *Cryptosporidium* or the presence of both pathogens. Further testing is required to identify the parasite(s) present within positive specimens. The tests are designed as an efficient and cost effective method of research screening specimens and endemic areas for the presence of *Giardia lamblia* and *Cryptosporidium* spp. in situations where a large number of specimens are routinely found to be negative .

By using concentrated method (flotation technique using Sheather's solution without staining and by using iodine stain) (fig.2) , It gave a higher percentage 10.24% Giardiasis and 4.22% Cryptosporidiosis. This technique considered as routine method for detection of (oo)cyst for *Giardia lamblia* and *Cryptosporidium* spp. in most scientific laboratories (research lab.).

Giardiasis infection rate appear in the present study appear to be similar to other studies done in Iraq like that in Nenivah where Giardiasis was 15.8% [19]; in Al-Karkh side of Baghdad 12% and 10.72% [20,21] and lower than results recorded in Thi- Qar 23 .7% [22] . This result of Giardiasis is higher than that revealed in Diwaniah where Giardiasis was 9.8% [23] ,where all these studies made on children using concentration methods for samples.

The infection rate recorded by this study consider a high than other studies may be due to the pollution of drinking water in Baghdad city ,contamination of water and food as

Giardia lamblia cyst remain live and infective for human more than two month in water and resistant for chlorination[24]; loss host specificity of *Giardia lamblia* [25].

The fluctuation and difference in the results of Giardiasis may be due to difference in time and duration when these studies carried out, in addition to other factors such as environmental, nutritional, socio-economic, geographical conditions, demographic and health-related behavior as well as number of patients samples in screening study and diagnostic method used[21].

By using concentrated method by flotation technique *Cryptosporidium* show positive result as 2.4% by direct exam without staining and 4.2% using iodine stain fig(2).

However, the results declared that iodine solution gave better results in flotation technique compared with normal saline especially for detection of *Cryptosporidiosis* , and revealed more details that cannot be seen in the unstained preparation.

By using modified Zehil Nelseen staining technique,It was found that among 166 samples there were 62 samples positive at 37.3% infection rate with *Cryptosporidium* spp.fig.(2).

Those results of *Cryptosporidiosis* were lower than that recorded in Wassit governorate and in Al-Anbar which were 50% and 39.13% [26,27];and in Egypt *Cryptosporidiosis* 33.3% [28]. However, it was higher than results recorded in Kirkuk which was in range of 21% [29];in Mosul 18.9% [30]; in Al-Basrah was 9.2% [31] who used the same technique of M.Z.N. stain for concentrated samples.

The high infection rate with *Cryptosporidiosis* revealed in this study are supported to other studies who reported *Cryptosporidiosis* in different animals close related to human, house fly and in many foodstuff [32-36].

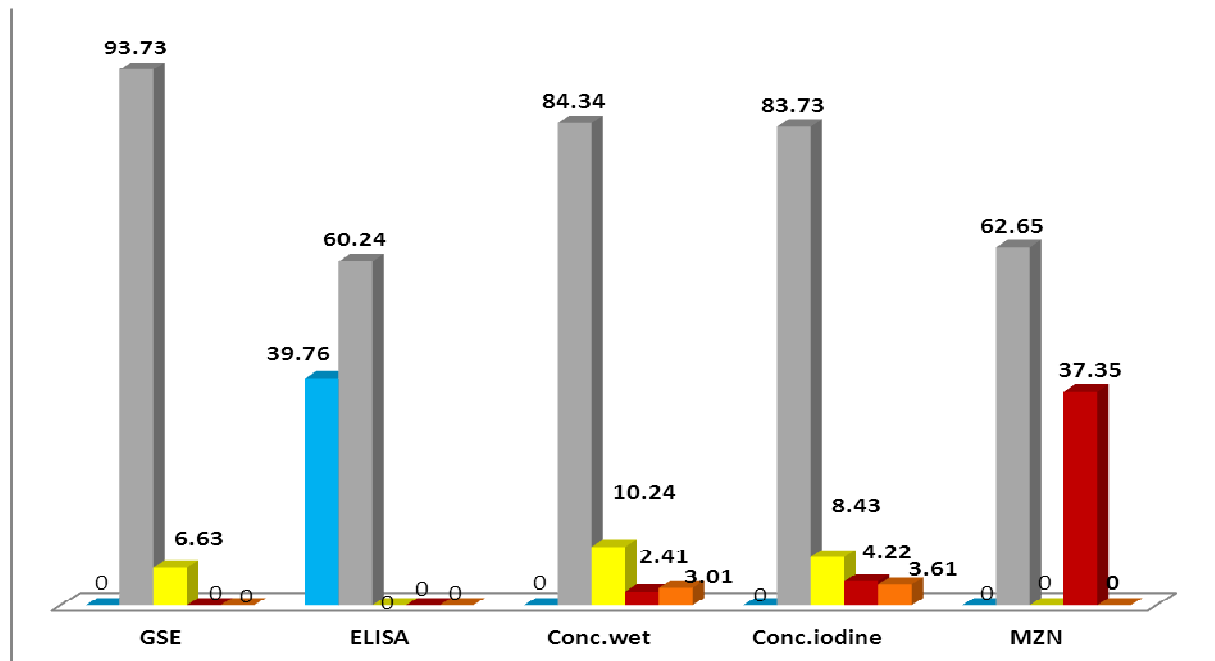


Fig.(2): Infection rate of Giardiasis and Cryptosporidiosis according to methods used

*GSE=General stool examination

*M.Z.N. stain=Modified Zehil Nelseen stain. For conc.samples

Pos.=Positive samples for Giardiasis and Cryptosporidiosis at all

Neg.=Negative samples for the both parasites.

Conc.wet=concentrated method without staining

Conc .iodine=concentrated and iodine

Pos.G= positive samples for *Giardia lamblia*

Pos.C= Positive samples for *Cryptosporidium* spp.

Pos.G&C=Mixed infection with the both parasites.

Regarding the specificity and sensitivity of both ELISA and M.Z.N. methods, Fig.(3) , and Fig.(4) showed that ELISA technique showed 100% sensitivity ,and 96% specificity comparing with 93% sensitivity ,and 100% specificity by M.Z.N. respectively.

Moreover, the results indicate that M.Z.N. method is highly sensitive and specific for Cryptosporidiosis in stool sample for diarrheic human.

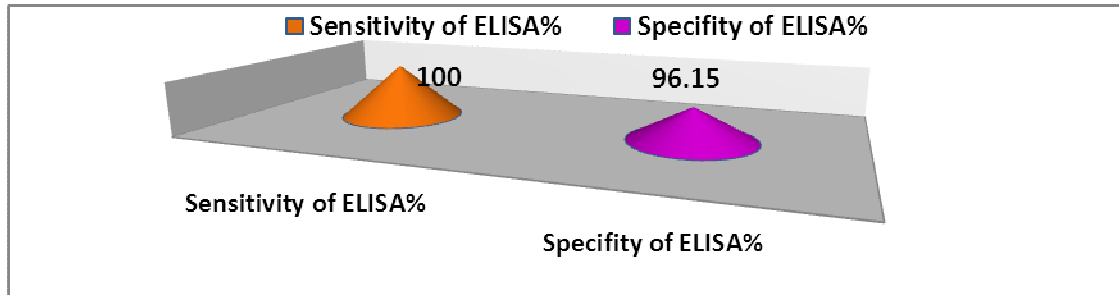


Fig. (3): Sensitivity and Specificity of ELISA compared with M.Z.N. stain

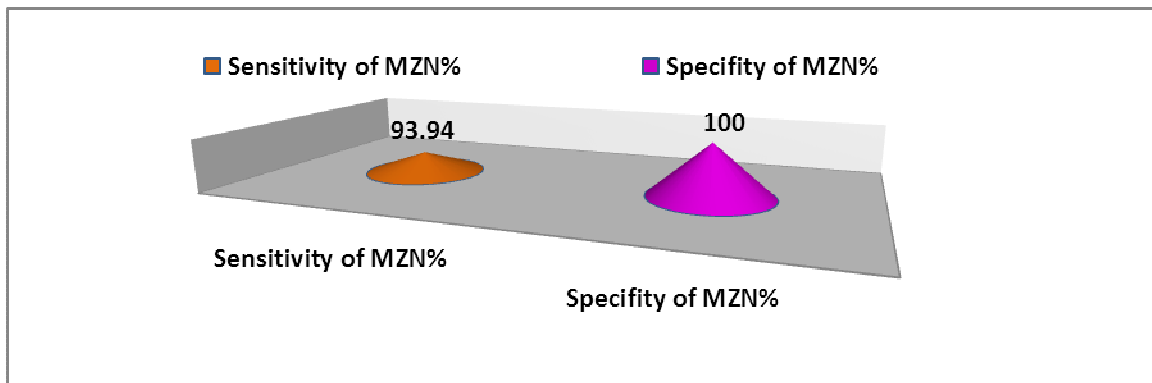


Fig.(4):Sensitivity and Specificity of M.Z.N. stain compared with ELISA

Generally speaking, M.Z.N. staining method declare a highly sensitivity and specificity in diagnosis of Cryptosporidiosis, this result disagree with those who showed that iodine method is preferred than M.Z.N. stain [37&38].

When we shed a light on the effect of some socio-demographic factors affect the infection rate with Giardiasis and Cryptosporidiosis , fig.(5) showed the distribution of Giardiasis and Cryptosporidiosis according to the residence of patient ,where there no significant difference in related with infection at all.

However, there was an association between residence and Cryptosporidiosis among patient at (P. value=0.049) and the highest infection rate 35.4% was among children attended to Jeser-diala health care center as shown in Fig.(6) .

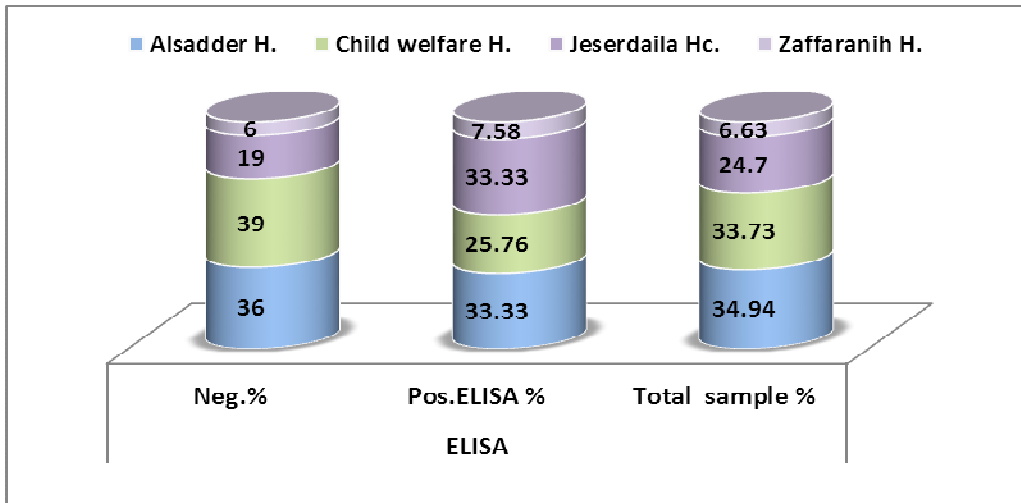


Fig. (5) :Distribution of infection rate with Giardiasis and Cryptosporidiosis in different hospitals according to ELISA results

H.= Hospitals
HC=Health care centers

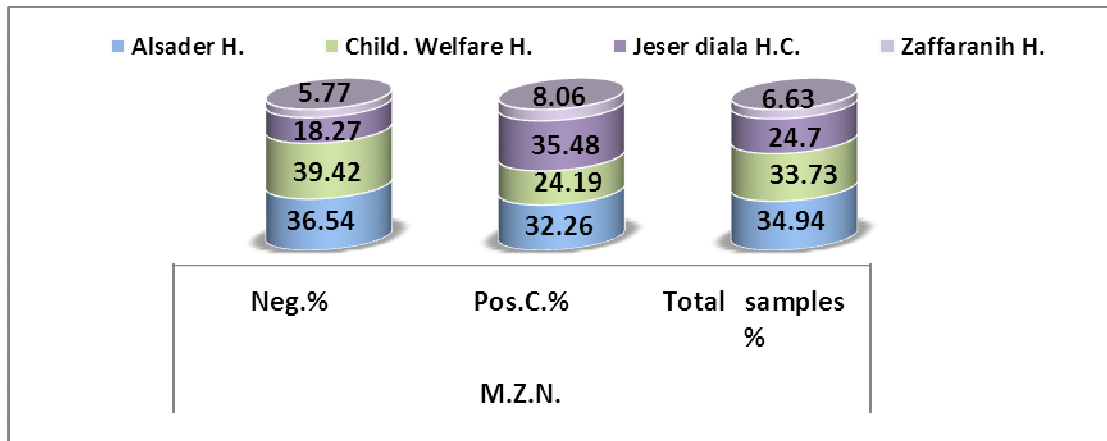


Fig. (6): Infection rate with Cryptosporidiosis in different hospitals according to M.Z.N. stain results. Pos. C.=Positive *Cryptosporidium* spp.

In concerning to the residence of patients where they attend to some hospitals and health care centers , the difference was mainly in relation with low socioeconomic status and health care behavior .

This result agree with Reports in Arabian countries showed that the infection rate with *Cryptosporidium* spp. among pediatric in rural and semi urban area is higher than urban area as mentioned by [39& 40], but this results disagree with [41] who reported no significant difference between urban and rural infection rates.

The infection rate among children in Jeser-diala might be expected to differentially facilitate *Cryptosporidium* transmission, because of disparities in animal exposure, access to safe water and sanitation, and population density. *Cryptosporidium*-associated diarrhea occurs mainly in younger children and inversely correlates with age, being more prevalent in children aged 1 year or less, particularly in rural and suburban regions [42&43] .

Regarding the effect of age group on the infection rate with *Giardia lamblia* and *Cryptosporidium* spp., Fig.(7) showed the high rate of infection was recorded in age ≤ 5 years which showed that more than 70% of infected children were included in this study .

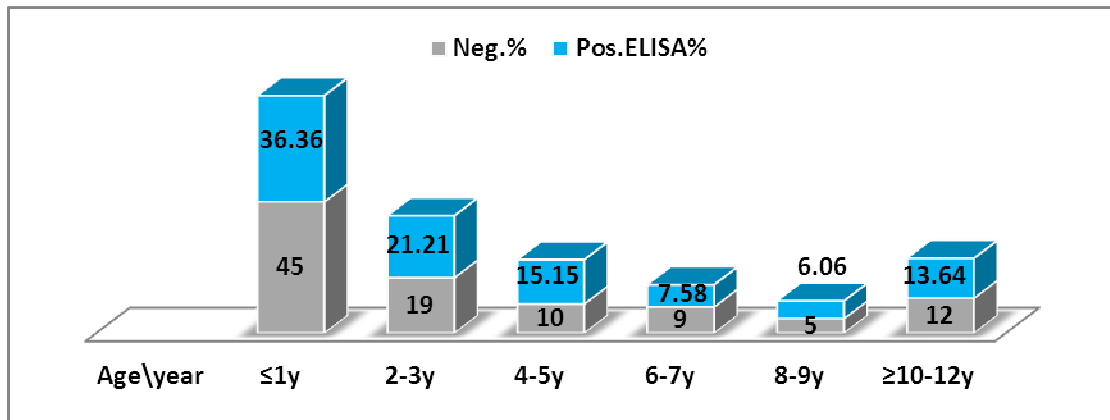


Fig. (7): Distribution of Giardiasis and Cryptosporidiosis among age group according to ELISA result

This result was agree with that recorded by [12;29;34] in Iraq and [28] in neiboring country as most cases of Cryptosporidiosis occurred among children less than 7 years of age, and particularly in the first two years of life due to their immune system are not well developed.

Children are usually exposed to protozoan parasite within a few weeks after birth, and with maternal protection through breastfeeding (and the prenatal placenta transferred immunity), symptomatic infections sometimes are delayed until 6-24 months of age [50].

on the other hand, which will provide a relatively protecting immunity level for the child, in spite of the increasing risk factor of exposure to different types of parasites with different strain, which correlates with the age increasing of the child ,and results showed that age (10 years) was more likely to infection with these intestinal parasites this may be due to social and economical level as well as living in crowded area , related with low level of education in health and their contact into crowded, play in group , sharing the same water cycle, pipe which enhance the chance for high infection rate [51].

Results revealed that the high rate of Giardiasis and Cryptosporidiosis was among female 43.4% rather than male 37.1% in (table 3) ;but statistically it was not significant.

Table (3): Infection rate of Giardiasis and Cryptosporidiosis according to gender by using ELISA techniques.

ELISA	Gender					
	females		males		Total	
	No.	%	No.	%	No.	%
Neg. samples	39	56.52	61	62.89	100	60.24
Pos. samples	30	43.48	36	37.11	66	39.76
Total	69	100.00	97	100.00	166	100.00

$\chi^2=0.682$

df= 1

p= 0.409

This result is quiet similar to these mentioned by [12,29,34] in Iraq where female was higher than male, but [22]found that infected male lower than female, although all these studies mentioned than no significant association between gender and rate of infection.

Although clinical studies of humans and field studies of non-human animals are suggestive, several factors, including exposure rates, social behavior, habitat, and diet cannot be held constant and could contribute to the observed differences between male and female in parasite infection [21&52]. Direct child to child fecal oral route is the main possible way for the transmission of infection in endemic situation [53].

These differences in prevalence rate of infection between these studies and ours is likely due to: date, Sampling size and technique, change in sanitary conditions, economic practice, personal hygiene and environmental factor responsible for such rate. Shortage of drinking water in schools and contamination of waters from pipes could be other factors.

This study revealed that there were a high infection rate with Giardiasis and Cryptosporidiosis among children which mostly misdiagnosed by conventional methods and there is a need to improve the routine method and support its results by using other methods like concentrating and staining methods specially for detection Cryptosporidiosis when failed to diagnose it by routine method, and to use such ELISA kit in scientific research and in survey on endemic area with the parasites to limit the transmission of these parasites among children .

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