An epidemiologic study on *Cryptosporidium spp.* in Kirkuk city with some trials for *in vitro* treating the parasite.

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Abstract:

This study was included the examination of 1836 stool samples from patients who attended the Pediatric General Hospital, Azadi Teaching Hospital, and a number of Primary Health Centers in Kirkuk city. The age of the patients were ranged from < 1 to > 60 years. The samples were collected from January to December 2009. *Cryptosporidium* incidence rate among human population in Kirkuk city was not high (0.98%). The incidence of the parasite was significantly higher in male with rate of 1.16% among 947 stool samples examined than female with rate of 0.79% among 889 stool samples examined. The most age group which was infected was <1 , 1-9 year and 50-59 year with rate of 1.02, 1.14, 2.38% for each age group, respectively. The infection was more severe in children than in adults. Only one case of double infection with *Trichomonas hominis* was seen among the positive cases. The seasonal prevalence of the parasite had not showed significant differences between the different months of the year. The parasite was more prevalent among tap water users and among people who had animals at residence. The results of the watery and alcoholic plant extracts in *in vitr*o tests of the chorion stem cell cultures showed that the *Curcuma longa* had the highest effect with inhibition rate of 42.7, 81.8% for each of watery and alcoholic plant extracts, respectively at 1000mg/kg. Very low effect were recorded for each of *Coriandrum sativum* and *Viscum album.* The effect of adding folic acid and potassium chloride alone or together to the watery and alcoholic plant extracts had no synergetic effect, they were decreased the inhibition rate of all extracts and azithromycin. Because there are little studies on *Cryptosporidium* prevalence in Kirkuk city, despite the huge efforts for treating the parasite there is no curative treatment, we tried to study the parasite epidemiology in this city and find out the *in vitro* effect of some medicinal plant extracts on the parasite.

Introduction:

Cryptosporidiosis caused by coccidian parasite of the genus *Cryptosporidium* represent a puplic health problem. The infection has been reported worldwide as a frequent cause of diarrhea. It cause a potentially life-threatening disease in people with AIDS and contributes significantly to morbidity among children in developing countries[1].The epidemiology of cryptosporidiosis is complex, involving both direct and indirect routes of transmission, from person to person and from animals to man. *Cryptosporidium spp*. cause acute gastroenteritis and diarrhea worldwide in both immunocoptent and immunocompromised persons with a rate of 0.3-32% [2].

The environmental contamination raises the possibility of aerosol transmission of *Cryptosporidium* from person to person, various routes of transmission such as aerosol infection is fairly likely, since *Cryptosporidium* oocysts are shed in large numbers during acute infection and are immediately infective to others [3]. Zoonotic infection by direct contact with mammalian livestock especially lambs and calves is common [4].The parasite can be transmitted indirectly through drinking water or eating food that has been contaminated with oocysts. Insects play a significant role in oocysts transmission. House flies, cockroaches, beetles and synanthropic flies can transmit the oocysts by their exoskeletons and in their digestive tracts too. [5, 6].

Cryptosporidiosis has a worldwide distribution and in most surveys is among the major pathogens causing diarrheal diseases. It has major public health implications because infection can result from exposure to low doses of oocysts .The oocysts are highly resistant and survive long periods in the environment [7]. In developing countries *Cryptosporidium* is responsible for 8-19% of cases of diarrheal disease. A review of several human population studies in developed and less developed countries reported prevalence ranging from 9% to 48% in Africa and Asia, 0.6-4.3% in North America, 3-20% in South America, 1-2% in Europe, , 4-10% in Australia[1] .The prevalence of *Cryptosporidium* is not constant among different countries and in a country from year to year. In many countries cryptosporidiosis incidence varies from year to year and the infection frequency appears to vary according to seasons of the year. There is evidence of seasonal peaks in several studies worldwide particularly in Spring and Autumn, which do not necessarily both occur in any one locality, nor recur year by year [8, 9].

Age group play an important role in *Cryptosporidium* infection, the pathogen can be found infecting all age groups but it is more common among children and elderly as most studies have indicated [8, 9].

One of the most biologically intriguing, and clinically frustrating features of cryptosporidiosis is its resistance to antimicrobial drugs. Unlike many of its relative (*Toxoplasma, Eimeria,* and *plasmodium*), there is no curative therapy for cryptosporidiosis, despite *in vitro* and *in vivo* testing of many compounds[10].There is no completely satisfactory treatment for cryptosporidial enteritis has been successfully developed, and a wide variety of

medications have been tested as possible treatments for the illness but there is currently no drug that can cure cryptosporidiosis therefore efforts are still needed to develop an effective drug [11, 12].

Materials and methods:

Population study:

This study was include the examination of 1836 stool samples (947 male, 889 female) from patients with different age groups who attended the parasite section in Azadi Teaching and Pediatric General Hospital and a number of Primary Health Centers for gastro interties disorders in Kirkuk city from January to December 2009. A questionnaire form was prepared to each patients which include the following items: Date, age, sex, source of drinking water, animals at residence (livestock, dogs, cats, birds), days of suffering from diarrhea, number of bowel motions per day. All patients were asked according to the information above by direct inter-viewing to the adults individuals, and with children parents for infants.

Stool samples collection:

Single or double stool samples were obtained from each patient in a tightly covered wide mouth plastic disposable container. The samples were labeled with sample number, date and name of patient. Each specimen was proceeded for parasitological examination.

Stool examination: Included both direct examining and indirect staining method.

Direct stool examination: Was included the macroscopic examination for checking the consistency, color, odor, and microscopic examination of saline wet mounts which was employed to demonstrate cysts, trophozoites, eggs, larvae.

Staining method:

The staining method used was modified Ziehl-Nelson method [13]. Fecal smears were made on a clean slide, air dried. The smears were fixed with 95% ethanol for 1-5 min. The prepared fixed smears were stained with carbol- fuchsine for 20 min. Rinsed in tap water, decolorized with 5% H_2SO_4 for 30 second, rinsed in tap water. Counter stained with malachite green for 2 min, finally examined with light microscope, using high power.The *Cryptosporidium* oocysts appeared as red-pink or white spherical bodies against green background. The stool samples which were positive for *Cryptosporidium* oocysts were prepared for oocysts isolation by sucrose flotation method.

Treatment experiments:

For trials to treat *Cryptosporidium* parasite three medicinal plants were chosen which have been used for treating intestinal disorders and diarrhea. The plants were *Curcuma longa* rhizomes, *Coriandrum sativum* and *Viscum album* fruits. The plant parts were obtained from local markets in Kirkuk city and the species were confirmed in Traditional Plant Center (T.P.C.) in College of Science / Tikrit University. For extracting the plant component Prabhakar [14] procedures were used with some modifications.

Alcoholic plant extractions: Medicinal herbs were grounded by rotary grinder to yield a fine powder,10gm of plant powder was weighted and placed in a maceration jar with 200ml of 95% ethanol mixed thoroughly. The jar was closed tightly to prevent evaporation of alcohol and was left in a dark place for 72hrs at room temperature, shacked tightly at least twice for 3 min each day. After 72hrs, the jar was opened, the extract was filtered with gauze then with filter paper (Whatmann No. 1).The solvent was evaporated in water bath at 50-60C°. the dry extract percentage yield was calculated and stored at 4C° until used.

Watery plant extractions**:** 10gm of powdered plant was weighted, added to pyrex beaker containing 200ml distilled water. The mixer was heated for 2hr on a hot plate at 60C°. Heat source was removed and the beaker cooled at room temperature. Solution was filtered, dried, stored as in alcoholic extracts above.

Placental stem cells isolation:

Human placenta were obtained after normal or cesarean delivery from healthy mothers (37-40 weeks gestation) and put in sterile saline 4C° in container and immediately transferred from hospital to the laboratory within (27-30 min).

The placental membranes (amnion, chorion) were mechanically peeled off from the cotyledon (deciduas) a small pieces of about 5cm² of each membrane was cut, the parts were washed several times with saline to remove blood ,after cleaning the parts were farther cut in to 1-1.5cm size by sharp scalpel size 11 and incubated at 37C° for 30-45min. with 0.05% trypsin containing 0.53 mM EDTA4Na, the cells from the first 10 minutes of digestion were discarded to exclude debris , incubation was continued for 60 min.. After incubation was completed the tissue was crushed with forcep to release individual cells. The large pieces of tissue were removed by using double layer gauze. The cells were sediment by low speed centrifuge (250 rpm for 5 min), washed twice with saline and distilled water , viability of cells was determined by applying trypan blue suspension (0.4%), cells were suspended in fresh medium (RPM 1640) counted with a neubar slide chamber for culturing[15].

Parasite preparation for culturing:

Stored oocysts in $K_2Cr_2O_7$ were washed several times with PBS (PH 7.2) until the $K_2Cr_2O_7$ was completely removed .The oocysts were then incubated with 10mMHCL for 10min.at 37C° then with 0.5% trypsin and 1.5% sodium taurocholate in PBS (PH 7.2) at 37C° for 45-60min. for excystation. The rate of excysted sporozoite was 97%. The sporozoites were counted by nubare chamber and suspended in RPM media containing 10% calf serum for culturing.

Culturing of *Cryptosporidium*:

The harvested placental chorion stem cells were inoculated in (PRM 1640) media supplemented with 25mM Hepes, 0.03 mg/ml L-Glutamine, 0.2 mg/ml sodium bicarbonate, 0.88 mg/ml ascorbic acid , inactivated fetal calf serum 10%, penicillin (5000 Iu/ml), streptomycin (5mg/ml), and amphotericin B (50µg/ml), in a 96 well micro titer plate champer at 37C° under CO_2 and humidity in candle jars.The cells were left for 12hrs. in incubator to allow the adhesion of the cells. Infection was then induced with the sporozoites (except in control wells) at a parasite : cell ratio of 1:1. After 6 hrs.the medium was replaced to remove the an adherent parasites outside cells and the incubation continued at 37C° in CO_2 atmosphere. After 24hr. the culture was exposed to the watery and alcoholic plant extracts at concentrations of (250, 750, 1000 µ/ml) in triplicate for each concentration. After 24 hr. of incubation with plant extracts cells were removed physically from the micro titer wells by rubber spatula on to clean slide, fixed in methanol and stained with giemsa for observation of *Cryptosporidium* stages by light microscope. Hundred cell were examined , the percentages of infected cells and the inhibition rate of each plants were calculated. The rates of three successive reading were done for all the treated wells[16]. Azithromycin (10µ/ml) was used in control wells, the synergism effect of folic acid (0.34µ/ml) and potassium chloride (5µ/ml) with each watery and alcoholic plant and with azithromycin were evaluated too.

Different statistical tests were used for analyzing the results according to the data's: Analysis of variance for factorial experiment with two factors and(F)tests. χ^2(chi-square) test in style of independent and in style of homogeneous.

Results:

Population study:

A total of 1836 stool samples from 947 males and 889 females in whom their ages were ranged from few days to > 60 years were examined in a trial for studding *Cryptosporidium* incidence in Kirkuk city center. Low incidence (0.98%) of the parasite was recorded among human population. Significant ($p<0.05$) difference was appeared between the parasite incidence and sex, with rate of 1.16 % for males and a rate of 0.79 % for females (table 1). The most age group which was significantly infected with the parasite was <1year,1-9 and 50-59 years with rates of 1.02, 1.14, 2.38 % for each age group, respectively (table 3).

The stool samples were watery with foul odor for all the infected patients and they were ranged from watery brown with no or little mucus for the adults to yellowish -green granulated very mucoid for the infants.The modified Ziehl- Nelson method was used for detecting the parasite in the stool samples in which the parasite was appeared as red-pink to white bodies against the green background (fig.1).

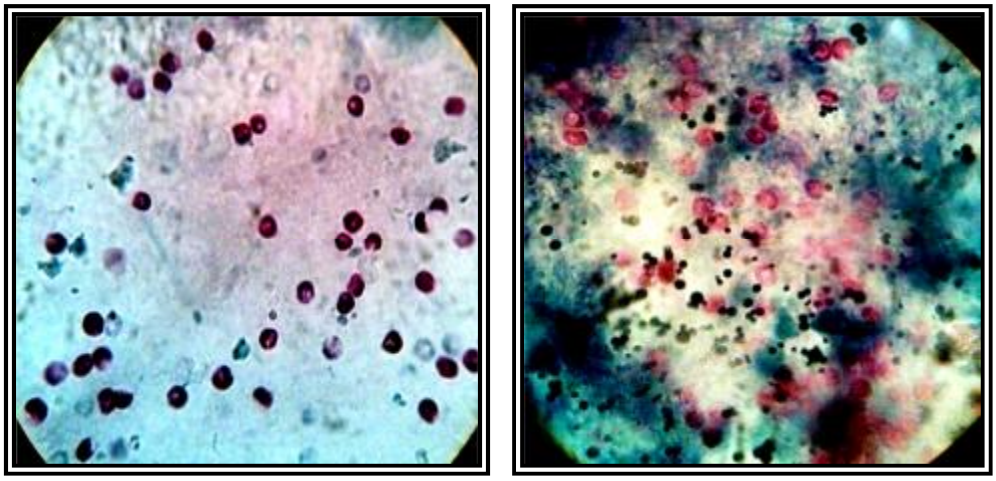

Fig.1: Red or pink C*ryptosporidium* ***oocysts in human isolates stained by M.Z.N.1000x.***

Table 1 : Numbers and percentages of *Cryptosporidium* infection according to age and sex.

Age group	Male			Female			Total number		
	Examined No.	Positive No.	Percentage %	Examined No.	Positive No.	Percentage %	Examined No.	Positive No.	Percentage %
< 1	351	4	1.14	433	4	0.92	784	8	1.02
1-9	372	5	1.35	240	2	0.84	612	7	1.14
10-19	95	1	1.05	79	0	0	174	1	0.57
20-29	10	0	0	8	0	0	18	0	0
30-39	51	0	0	46	0	0	97	0	0
40-49	28	0	0	32	1	3.13	60	1	1.67
50-59	19	1	5.2	23	0	0	42	1	2.38
> 60	21	0	0	28	0	0	49	0	0
Total number	947	11	1.16	889	7	0.79	1836	18	0.98

Mixed infection with other parasites was not seen except one case with the none pathogen *Trichomonas hominis*, the other parasites which were seen during samples examination were *E. histolytica*, *G. lambilia*, *E. coli*, *H. nana*, *Ascaris* ova's, and *E. virmicularis* cases. The bowel motions per day (fig.2) showed highest rate of 14/18 (77.7%) among infants which was more than six times per day followed by six and five times each with rate of 2/18 (11.1%). No one in the infected group had four or three bowel motions per day. The days of suffering from diarrhea (fig.3) were ranged between 2- more than 5 days with high incidence in more than 5 days group with rate of 12/18 (66.6%), and a rate of 1/18 (5.5%) for each of 3 and 2 days. The results of the relation between water source and *Cryptosporidium* positive samples (18/1836) (fig.4) showed high incidence among tap water users with rate of 16/18 (88.8%), followed by well water users with rate of 2/18 (11.1%). None of the positive samples were among the filtered, boiled and bottled water users. Cryptosporidiosis showed more frequency in patients with animals at residence 11/18 (61.1%) compared with those no animals at residence17/18 (38.8%) (fig. 5).

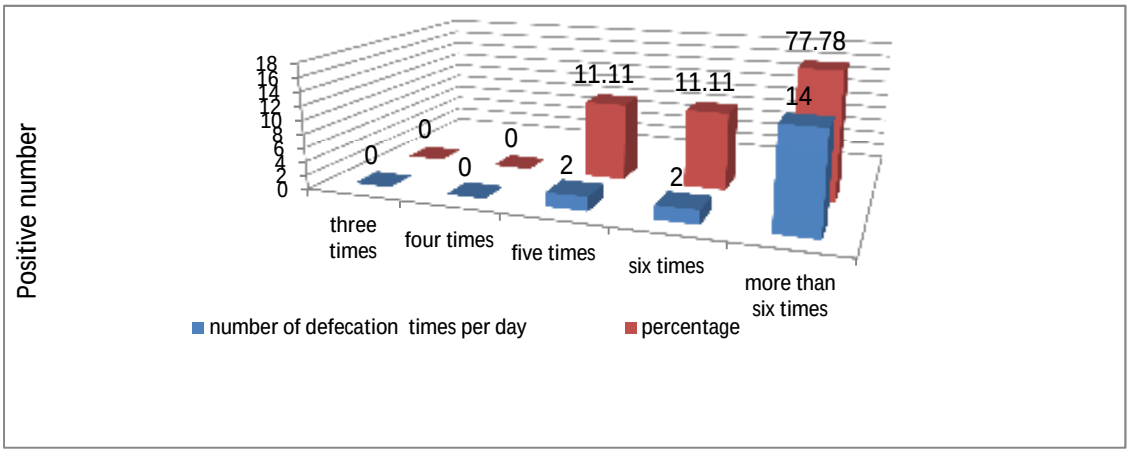

Fig. 2: *Cryptosporidium* in relation to bowel motions per day.

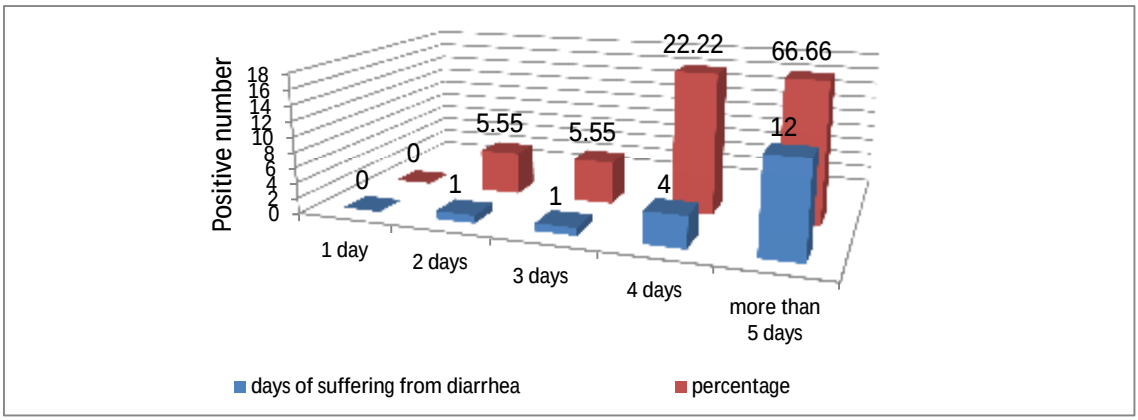

Fig. 3: *Cryptosporidium* in relation to days of suffering from diarrhea with its percentages.

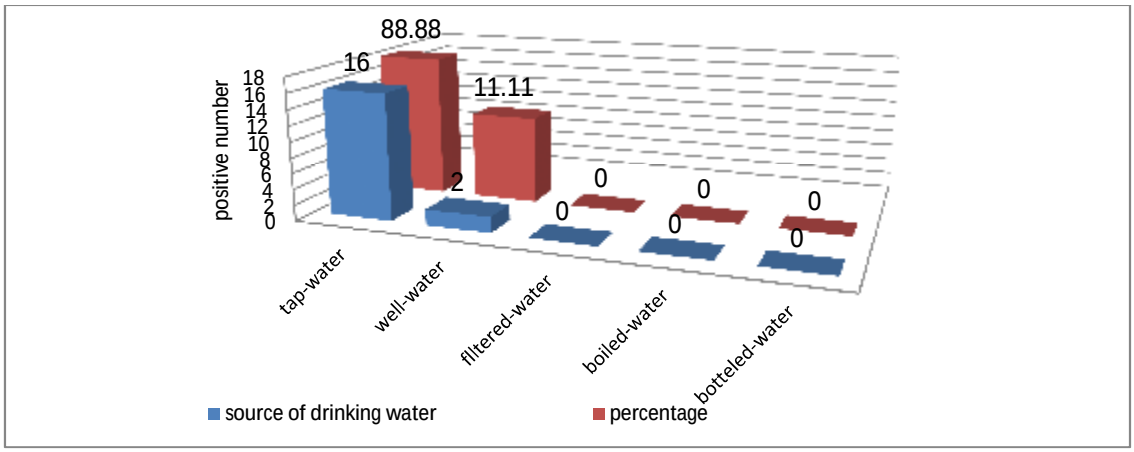

Fig. 4: *Cryptosporidium* in relation to drinking water with its percentages.

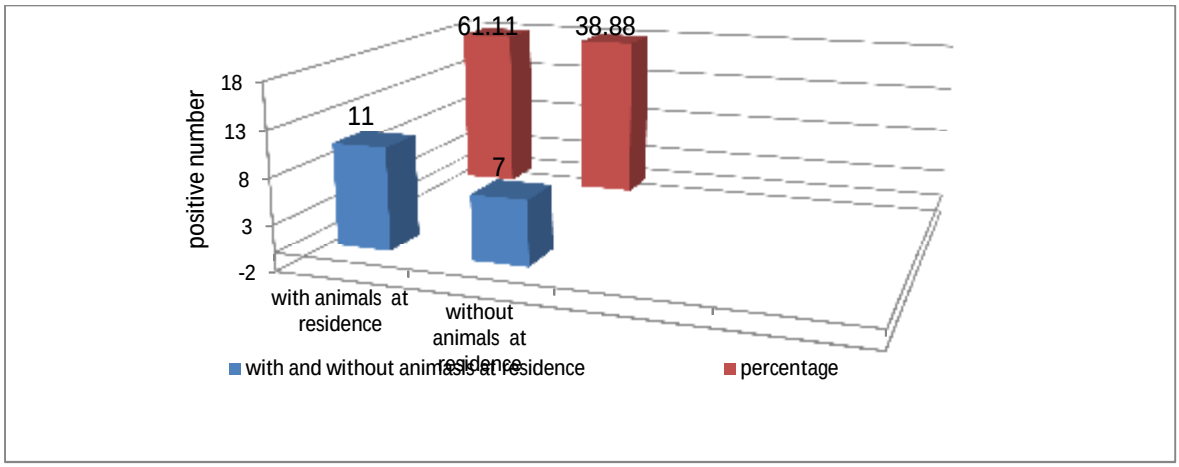

Fig. 5: *Cryptosporidium* in relation to animals at residence.

The seasonality prevalence of *Cryptosporidium:*
The seasonal prevalence of the parasite (table2) from January to December 2009 showed no significant ($p<0.05$) differences were seen between the parasite prevalence and the seasons, but the parasite was peaked in January with rate of 3.85%, August with rate of 2.26% and December with rate of 2.38%. No positive cases was recorded in each of May, October, and November.

Table 2: Seasonality prevalence of *Cryptosporidium* in relation to sex

Months	Male			Female			Total number		
	Examined No.	Positive No.	Percentage %	Examined No.	Positive No.	Percentage %	Examined No	Positive No.	Percentage %
January	42	2	4.8	36	1	2.8	78	3	3.85
February	37	1	2.7	51	0	0	88	1	1.14
March	59	0	0	61	1	1.64	120	1	0.84
April	64	0	0	53	1	1.9	117	1	0.86
May	51	0	0	46	0	0	97	0	0
June	88	0	0	65	1	1.54	153	1	0.65
July	146	0	0	141	1	0.7	287	1	0.17
August	105	5	4.8	116	0	0	221	5	2.26
September	115	3	2.6	89	0	0	204	3	1.47
October	71	0	0	91	0	0	162	0	0
November	77	0	0	53	0	0	130	0	0
December	48	0	0	36	2	5.6	84	2	2.38
Total number	947	11	14.9	889	7	13.48	1836	18	13.62

***In vitro* inhibition effect of watery plant extracts.**
C. longa had significantly highest inhibition effect on the infected cells and it was dose related (table 3). At 1000 µg/ml it had the highest effect with rate of 72.7% compared with azithromycin. Followed by 750mg/ml with rate of 59.1% and that was equal to the azithromycin effect. The lowest effect was at 250mg/ml with rate of 13.8%,which was significantly lower than the effect of azithromycin.
The *C. sativum* and the *V. album* had no significant effect on the infected cells compared with *C. longa* and azithromycin and the highest effect was at 1000 mg/ml with rate of 22.7% for each of the two plants.
Adding folic acid at concentration of 0.34µg/ml had reduced the inhibition rate of all plant extracts and azithromycin too. With folic acid adding again the *C. longa* had significantly the highest inhibition effect with rate of 63.6, 50, 13.8% for each of 250, 750, 1000 µg/ml, respectively.

The *in vitro* inhibition effect of alcoholic plant extracts
The alcoholic extracts effect(table 4) were not differed significantly from that of watery extracts and the *C. longa* was more effective than the other two plants (*C. sativum, V. album)* with rate of 22.7, 54.5, 81.8% in 250, 750, 1000, µg/ml, respectively, and it was higher than.

Table 3: The *in vitro* inhibition effect of watery plant extracts .

Plant types	Con. µg/ml	No.infected cells in watery extracts	Inh. Rate %	No.infected cells in watery extracts +folic acid	Inh. rate%	No.infected cells in watery extracts +potassium chloride	Inh. rate%	No.infected cells in watery extracts +folic acid+ potassium chloride	Inh. rate%
Coriandrum sativum	250	20	9.09	22	0	19	13.6	21	4.54
	750	20	9.09	21	4.54	20	9.09	21	4.54
	1000	17	22.7	20	9.09	18	18.2	20	9.09
Curcoma longa	250	15	13.8	17	22.7	16	27.3	19	13.6
	750	9	59.1	11	50.0	10	54.5	14	40.9
	1000	6	72.7	9	59.1	8	63.3	10	54.5
Viscum album	250	19	13.6	21	4.54	20	9.09	21	4.54
	750	20	9.09	20	9.09	18	18.2	21	4.54
	1000	17	22.7	19	13.6	19	13.6	21	4.54
Control (Azithromycin)	10	9	59.1	11	50.0	10	54.5	12	45.5
Control without treatment	-------	22		22		22		22	

Azithromycin (59.1%) in 1000 µg/ml. The *C. sativum* and *V. album* had no significant effect in all concentrations used comparing with *C. longa* and azithromycin. The folic acid adding had significantly reduced the inhibition rate of azithromycin and all plant extracts at all concentrations used , it reduced the inhibition rate of *C. longa* and azithromycin with rate of 13.8, 36.4, 68.2% in 250, 750, 1000 µg/ml of *C. longa* and 50% for azithromycin comparing with the *C. longa* and azithromycin alone.

Potassium chloride adding had significantly resulted in reducing the inhibition rate like folic acid, in *C. longa* with rate of 36.4, 63.6% for 750, 1000 µg/ml, respectively, and with azithromycin with rate of 54.5%, and it had no significant effect with the other two plant extracts. The using of the components (folic acid, potassium chloride) together with the plant extracts and azithromycin had resulted in significantly reducing the inhibition rate of all plant extracts and azithromycin, the inhibition rate of *C. longa* and azithromycin were 36.4, 59.1% for 750, 1000 µg/ml in *C. longa* and 45.5% in azithromycin comparing with effects of the plant extracts and azithromycin alone .

Table 4: The *in vitro* inhibition effect of alcoholic plant extracts

Plant types	Con. µg/ml	No.infected cells in alcohol-ic extract-s	Inh. rate%	No.infected cells in alcoholic extracts+folic acid	Inh. rate%	No.infected cells in alcoholic extracts+ potassium chloride	Inh. rate%	No. infected cells in alcoholic extracts+folic acid+potassium chloride	Inh. rate%
Coriandrum sativum	250	21	4.54	22	0	20	9.09	22	0
	750	19	13.6	20	9.09	19	13.6	20	9.09
	1000	19	13.6	20	9.09	18	18.2	19	13.6
Curcoma longa	250	16	27.3	17	22.7	17	22.7	19	13.6
	750	10	54.5	14	40.9	13	36.4	14	40.9
	1000	4	81.8	8	63.6	7	68.2	9	59.1
Viscum album	250	20	9.09	20	9.09	21	4.54	19	4.0
	750	18	18.2	20	9.09	17	22.7	19	13.6
	1000	16	27.3	17	22.7	17	22.7	18	18.2
Control (azithromycin)	10	9	59.1	11	50.0	10	54.5	12	45.5
Control without treatment	------	22		22		22		22	

Discussion:

population study.

From 1836 stool samples examined 18 samples were positive for *Cryptosporidium* infection with rate of 0.98%. This is low rate indicating that cryptosporidiosis is not prevalent in the area which may be due to high hygiene because of sanitary educations of people.This result in comparison with other studies is not in agreement with Al-Alousi [17] 9.42% in Tikrit, Othman[18] 12.62% in Kirkuk. But it is a little agree with 2.6% in Deyala[19],[20]1.5% and with worldwide prevalence of the parasite which was between 0.3-32% and with the parasite prevalence in north America(0.6-4.3%) [2].

The differences between this results and the other studies may be because of the technique used for the diagnosing or due that the parasite prevalence is not constant from year to year and from one area to another in the same country [9],or due to sample size differences or because most of studies was concentrated on one age group (infants) or children but in this study all age groups was contained .

The distribution of the parasite was significantly higher in male than in female, this reflects that males are more susceptible to the parasite than female, or a type of *Cryptosporidium* receptor specific antigen may present in the intestinal cells of males and absent in female, more researches about this subject is needed. This result was in agreement with [8, 9, 21]. In contrary to this, some studies found that females were more reliable to the infection[18, 22], and others found no significant differences between the male and female infections[17].There is relation between the parasite prevalence and age group in this study ,the most age group which is infected is less (2.38%). This result is similar to most other studies on the parasite prevalence, it agree with Mahgoub study [8] in Jordon who shows rate of 57% in 5-<7 year and 38% in 1-3 years and 17% in <1 year, highest rate of 18.18% was in 1 month -1 year in Othman study [18]. Al-Alousi [17] indicate high prevalence in age group below sex months and younger than 1 year, and not agree with Gatel [21] who found lowest rate among 4-5 year, but agree with Amin [9] who referred to high incidence in elderly person because of high risk of secondary person to person transmission.

The high prevalence of the parasite in this study in children is because of the high susceptibility in children population to the parasite due to their low IgG, IgM, IgA specific antibody against the parasite as indicated by Lee [23] who cleared low specific antibody titer in children which was increased with age increases then decreased, or due to the low care of their mothers, their feedings artificially, house hold transmission in young children to contact in playing groups and carelessness in day care or school in older children.

The elderly appear to be at risk to cryptosporidiosis because of their low immunity and the overall weakness of the body in this age[22]. The stool samples of infected patients was diahrric with no or little mucus, and the watery stool lasts for 2-3 days with defection number per day 5-6times for adults to very sever watery mucoid stool lasts for 4 to more than 5 days with defection number per day more than six times. The watery nature of the stool is because of the parasite pathogenesis which cause the intestinal cells to release soluble factors that increase intestinal secretion of water and chloride and also inhibit absorption. But the mucoid stool and the severity of infection which lasts for more days with more defections time per day in children is because of their low immunity to the parasite or because of the presences of some types of parasite receptors in the intestinal cells of children and which is absent in adults, as indicated by Upton[24] who says older animals generally develop poor infections, it is likely that one or more developmentally regulated antigens along the intestinal tract are responsible for neonates, rather than adults, developing severe cryptosporidiosis, he suggested that this may be a 57KDa antigen found on ileal cells of neonates that binds a 47KDa ligand at the apical end of sporozoites. humans, on the other hand, are the host that can be infected at any time in their lives, and only previous exposure to the parasite results in either full or partial immunity to challenge infections.

These data confirm observation by others [21,9] that *Cryptosporidium* cause diarrhea which is watery with mucus, and that the infections are more prevalent and more severe in younger children, especially in highly endemic regions.

Wet mount is not very efficient in the parasite diagnosing, because the oocysts are very small size and miss diagnosing them with fungus is very likely especially if they present in small numbers.

Mixed infection with other parasites are not seen except one case with none pathogen *Trichomonas hominis* which agreed with [25]when they record only one case of double infections, this may be because of some type of toxic compound which may be released by the parasite and effects certain types or species of other parasites, or due to low numbers of *Cryptosporidium* positive samples in this study which decrease the chance of double or mixed infection .

Cryptosporidium appear to be more prevalent among tap and well water users and among people with animals at residence.The presences of parasite oocysts in different source of waters and its responsibility for contaminating drinking water and several outbreaks of the parasite have been improved[26,17],because of the oocysts prolonged survival and the resistance to disinfectants treatments which can be attributed to the presence of a thick oocyst wall, the disinfectant like chlorination at the levels allowed which kill the other organisms dose not kill the *Cryptosporidium* oocysts,in addition to that no one of the *Cryptosporidium* positive cases was among the boiled water users group,(boiling the water will kill the parasite),these data was supported by data from other researches, Mahgoub [8] who found high rates of infection among well water and tap water users with rate of 48.4,35.3% respectively.

Knowing the fact that the *Cryptosporidium* is a zoonotic diseases will explain the high prevalence of the parasite among the group with animal at residence in this study which agreed with Mahgoub[8]study who record higher rate of infection among patient with animal at residence (45%) comparing with those no animals at residence 36.2%. which may be due to contact with these animals. Several animal species have been confirmed to infecting human, and that the human have to distinct transmission cycles, anthroponotic cycle caused by *C. hominis* and zoonotic cycle caused by *C. parvum* and other animal species [7, 9].

No one of drugs used by the patient have any effect for treating them from the infection. Similar results were obtained from other studies with no or little effect of the drugs used. Woods [27] was used more than 100 drugs, few number of them had little effect but most had no effect. One possible explanation for

this is that *Cryptosporidium* establishes a compartment within the host cell, this unique parasitophorous vacuole may somehow shelter the parasite from drugs as some researches indicated [10,27].

The seasonality prevalence of *Cryptosporidium*

Concerning with season highest rate of infection was in January (3.85%) followed by August (2.26%) and December (2.38%). This is similar to that found by Al-Alousi [17] in Tikrit who record 23.8% in Sep., 5.06% in Dec., 4.86% in Jan., 5% in Feb., he record a peak in March (12.98%) which is not in agreement with our result. In Jordon Mahjoub[8] was recorded 56% in Jan., 50% in Feb., 21% in Aug., but he did not record high rate in Sep. Gatel [21] in Kenya showed highest infection rates between Nov. to Feb. and lowest rate was in Jan.- Jul. which is somehow similar to our result. Amin [9] in USA. Observed high peaks in Sep. and Feb..

This variety of seasonality prevalence of the parasite in different areas may because of limited period of the studies which vary from one to another and not all research had studied the overall prevalence in the whole months of the year or may be because of the imbalance in the environmental conditions (temperature, humidity, desiccation) found between different areas which may effect on seasonal prevalence of the parasite, the imbalance of density of livestock between these areas may affect too.

The high winter months prevalence of the parasite may wash of *Cryptosporidium* from the land into rivers or lead to effluents by passing sewage treatment and being discharged directly into surface waters which subsequently used for public water supplies, and the summer peak of the parasite may associated with the hot and the needs for more water or drinks, or because of the presences of high numbers of insects like flies and cockroaches which may mechanically transmit the parasite and contaminate water and food stuff .

***Invitro* inhibition effect of watery plant extracts.**

The effect of the plant extracts was dose related, the highest dose had highest effect. The *C. longa* had significantly high inhibition rate comparing with the antibiotic and the two other plants. Extract and essential oil of *C. longa* had inhibit the growth of a variety of bacteria, parasites (*P. falciparum*, *Leishmania major*) and pathogenic fungi [28, 29]. It has been found that the infectivity of the sporozoites had been inhibited at least 60% in the presence of 200µm curcumin (substance in *C. longa*) but no significant reduction of viability of *C. parvum* oocysts had been recorded after incubating it with curcumin [30].

Thus the curcumin effect may be on the intracellular stages but not on the oocysts with thick wall. The *in vitro* effect of the curcumin may because of containing large amounts of phenolic and other components, which was confirmed by [31, 32] whom record the high effect of (blueberry, mekabufucoidan) two rich in poly phenolic substances on *C. parvum.* Curcumin, a natural polyphenolic compound, has been found to be active against a variety of diseases including anti -carcinogenic, antimicrobial and antiprotozoal effect [33, 30]. Curcumin inhibits cell proliferation and induces cell cycle changes in colon adinocarcinoma cell lines by a prostaglandin-independent path way and may inhibit or stop the parasite proliferation and development. Several studies had confirmed the effect of *C. longa*, A study of chicks infected with the *Eimera maxima* demonstrated that diets supplemented with 1% turmeric resulted in a reduction in small intestinal lesion and improved weight gain [34].

The effect of adding folic acid or potassium chloride alone or together had reduce the inhibition rate of all plant extracts, this may because they are vitamin and mineral which may enriching the media and may be they are essential for the parasite growth or increase the gliding motility of the sporozoite as Upton [35] indicated in their study on *C. parvum* development in HCT-8 cells in which they supplements their media by some vitamins, sugars, and hormones each one had resulted in increases in parasite numbers *in vitro.*

***In vitro* inhibition effect of alcoholic plant extracts.**

No significant differences has been noted between the watery and alcoholic plant extracts this may because very closely related compounds has been extracts by the two solvent or due to extraction method which include heating in watery extracts and only maceration in alcoholic extracts which may had lead to extracts the same active components for both solvents, this results was identical to the results showed by Eguale and Sinch [36, 37] whom showed no significant differences between the watery and alcoholic extracts of *C. sativum* and *C. long*. The *C. longa* was more effective than the other plants, and adding of folic acid or potassium chloride alone or together had resulted in reducing the inhibition rate of all plants and azithromycin this may because of the same reasons mentioned for watery extracts

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دراسة وبائية عن طفيلي داء البويغات الخبيئة في مدينة كركوك مع بعض المحاولات لعلاج الداء في الزجاج.

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الملخص

تضمنت الدراسة الحالية فحص 1836نموذج غائط من المرضى المراجعين لمستشفى الأطفال العام و مستشفى ازادي التعليمي و عدد من المراكز الصحيةالاولية في مركز مدينة كركوك و الذين تراوحت اعمارهم بين اقل من سنة الى اكبر من 60 سنة من الفترة من شهر كانون الثاني الى شهر كانون الأول لسنة2009 . نسبة انتشار الطفيلي لم تكن عالية (0,98٪) بين سكان المدينة و نسبة انتشاره كان معنويا اعلى في الذكور (11٪ من 947 نموذج غائط) منه في الاناث (7٪ من 889 نموذج غائط) .

الفئات العمرية التي ظهرت فيها الاصابات اكثر كان فئة اقل من سنة ،1–9 ، 50–59 سنة وبنسب 1,02، 1,14، 2,38 ٪ لكل فئة على التوالي. الاصابات كان اكثر حدة في الاطفال مقارنة بالبالغين . تم تسجيل حالة واحدة للاصابة المشتركة ضمن كل الحالات الموجبة . لم تكن لوفرة الطفيلي الموسمية اية اهمية معياريةغير ان العينات الموجبة تركزت في كانون الثاني واب و كانون الاول. وقد ظهر الطفيلي اكثر وفرة في عينات مستهلكي مستخدمي ماء الحنفية و مربوا الحيوانات في البيوت، كانت نتائج المستخلص الكحولي والمائي للنباتات في اختبارات الزجاج في مستنبت الخلايا الجذعية للحبل السري للانسان قد اظهرت ان مستخلص نبات الكركم كان اكثر تاثيرا في تثبيط نمو الطفيلي و بنسبة 42,7 و 81,8 ٪ لكل من المستخلص المائي و الكحول على التوالي عند تركيز 1000 مايكروغرام / كغم . تاثيرات قليلة جدا لوحظ لكل من نباتي الكزبرة و الدبق. لم يكن لاضافة حامض الفوليك و كلوريد البوتاسيوم لوحدهمااو معا للمستخلص الكحولي والمائي للنباتات تاثير تآزري بل قلل نسبة التثبيط لجميع المستخلصات و المضاد الحيوي أيضا . و بسبب قلة الدراسات حول الطفيلي في مدينة كركوك . وبالرغم من الجهود الكبيرة المبذولة لإيجاد علاج للطفيلي ولكن لا يوجدعلاج شافي ضد الطفيلي . لذلك كان الهدف في دراستنا الحالية هو دراسة وبائية الطفيلي في المدينة مع محاولة لأيجاد تأثير بعض النباتات الطبية على الطفيلي في الزجاج.