Prevalence Of Cryptosporidium Sp. And Treatment By Using Some Plants Extracts In Al-Hilla City\Babylon Province

Fadhil Hassan Alwan Al-Dulaimi
Local environmental research center Babylon Univ.
Ahmed Khudhair Aubbies Al-Hamairy
Science college of women Babylon Univ.
Abbass Hussain Mughier
Basic education Babylon Univ

Abstact :

The study was conducted to investigat the prevalence of *Cryptosporidium* sp. in children who were attended to laboratories maternity and pediatrics general hospital in Al-Hilla city of Babylon province for the period from Septmber 2010 to May 2011. The age groups of children was ranged from less than 6 months to 5 years old and to show the effect of six plants extracts on the parasite that injected in Balb\c mice. The study was carried out on 605 fecal samples of children, 318 males and 287 females. Detection of *Cryptosporidium* oocysts was done by modified Ziehl-Neelsen methods.The results show the rate of *Cryptosporidium* infection was (9.42 %), it did not vary between males and females. The highest rate of infection was among age group less than 6 months to less than one year old. The distribution of infection was highest in September (23.75 %) followed by March (13.75 %) and November (10.3 %) and the lowest was in January (4.54 %). The effect of six plant extracts on the shedding of oocysts in experimental mice, decrease the shedding of *Cryptosporidium* oocysts in mice by using 250 mg / kg body weight, route it was shown that the efficacy of *Peganum harmala* (65.9 %) followed by *Artemesia herba alba* (56.5 %), *Ricinus communis* (34.3 %).and lowest was *Thymus vulgaris* (19.1 %). It was also shown that the efficacy of plants extracts (500 mg / kg body weight) was higher than (250 mg / kg B.W.) in shedding of the parasite oocysts.

الخلاصة :

اجريت الدراسة الحالية للتحري عن البويغيات الخبيئة *Cryptosporidium* sp. في الأطفال المراجعين لمختبرات مستشفى الولادة والاطفال في مدينة الحلة / محافظة بابل للمدة من ايلول ٢٠١٠ إلى ايار ٢٠١١ تراوحت مجاميع أعمارهم بين اقل من ستة اشهر الى خمس سنوات، وكذلك استخدام ستة مستخلصات نباتية وملاحظة تأثيرها على البويغات المحقونة في الفئران المختبرية . ونفذ التحري من خلال فحص ٦٠٥ عينة غائط من الأطفال المراجعين(٣١٨ من الذكور و ٢٨٧ من الإناث). لقد استعمل لغرض الكشف عن أكياس البيضة لجنس *Cryptosporidium* طريقة زيل نلسن المحورة . أجريت الدراسة في الفئران البيضاء المختبرية لبيان تأثير فعالية مستخلصات نباتية مختلفة على طرح أكياس البيضة للطفيلي وهي : الزعتر *Thymus vulgaris*، الشيح *Artemisea herba-alba* ، الخروع *Ricinus communis* ، الزيتون *Olea europea* ، الحرمل *Peganum harmala* ، الثوم *Allium sativum* بينت نتائج الدراسة ان معدل انتشار الطفيلي كان (٩.٤٢ %) مع عدم وجود فروق بين الذكور والإناث وكان أعلى معدل في الفئة العمرية(أصغر من ستة أشهر) إلى (أصغر من سنة واحدة). سجل اعلى نسبة للاصابة في أيلول (٢٣.٧٥ %) وتلاه (١٣.٧٥ %) في آذار ثم (١٠.٣%) في تشرين الثاني بينما اقل نسبة للاصابة كانت في كانون الثاني(٤.٥٤%) . وقد وجد أن تأثير المستخلصات النباتية على طرح أكياس البيضة في الفئران المختبرية المصابة تجريبيا بالطفيلي قد أدى إلى تقليل أعداد الأكياس المطروحة في براز الفئران. إن استعمال ٢٥٠ ملغم / كغم من وزن الجسم من مستخلص الحرمل *Peganum harmala* هو أعلى فعالية في الكفاءة (65.9 %) وأعقبه الشيح *Artemisea herba-alba* (٥٦.٥%) ثم الخروع *Ricinus communis* (٣٤.٣ %) وأقلها كان للزعتر *Thymus vulgaris* (١٩.١ %) . كما ظهر أن الجرعة ٥٠٠ ملغم / كغم من وزن الجسم كان أكثر كفاءة علاجية في التأثير على أعداد الأكياس المطروحة.

Introduction :

Cryptosporidium zoonotic agents which frequently infected human as well as domestic and wild animals it is waterborne parasites , transmitted via fecal contamination (Oyibo *et al.* ,2011).

particularly as a result of comparative studies involving both medical and veterinary workers, *Cryptosporidium* was also recognized in man many of the early reports of

the infection in humans were in immunocompromised subjects, particularly those suffering from AIDS (Hassanpour *et al*., 2011)

The disease is associated with self-limited, mild to sever diarrheas lasting for several days among healthy individuals and profuse-cholera like diarrhea often becomes irreversible among immune deficient ones, that may contrast sharply, causing prolonged life threatening disease a symptomatic form of *Cryptosporidium* infection is common and constitutes a source of infection for many hosts (Syed & Javid . 2011) . In symptomatic type of infection there may be excessive loss of fluid, varies from 3-6 to 17 liters of watery diarrhea/day in human (Mannheimer & Soave ,1994).

Feacal-oral mode of transmission may be very important between domestic animals and man (Mackenzie *et al*.,1994). In human, however, the contact between person to person spreads the infection that has been implicated in many outbreaks in all parts of the world (Mahammad *et al*., 2011) .

The parasite can be easily transmitted from one mammalian species to another, All these transmissions of the parasite cause contamination of surface and ground water (Mancassola ,1997). The first case of human cryptosporidiosis was reported from contamination of drinking water (Schmidt & Roberts, 2000). As cryptosporidiosis is self-limited illness in immunocompetent patients, therefore only supportive management is required while in immunocompromised patients, it is life threatening and may lead to death (Michael *et al*., 2011).

Subsequently, particularly as a result of collaborative studies involving both medical and veterinary workers, cryptosporidiosis was also recognized in man (Markell *et al*. ,1999). In the past, many reports assumed the parasite to be host specific and several species of *Cryptosporidium* were named on the basis of host occurrence. It has been demonstrated in preliminary cross-reaction studies that infections were readily transmittable between various hosts (Tzipori *et al*.,1980). Furthermore the several studies indicated that some degree of host specificity might occur between vertebrates (Abdel – Wahab & Abdel – Moagood , 2011) . Members of the genus *Cryptosporidium* are placed taxonomically within the phylum Apicomplexa the classification of the genus *Cryptosporidium* (Diego & Mirna, 2008).

The aim of the study are the prevalence of *Cryptosporidium* sp. in who were attended to laboratories maternity and pediatrics general hospital in Hilla city of Babylon province, and Isolating *Cryptosporidium* sp. from human and conducting trials of treatment of the parasitic infection and primary searching effective of six plants extracts on parasite .

Material & Methods :

The study was conducted on 605 random samples of feacal rectal smears of children attained to lab. Maternity & pediatrics general hospital in Hilla city, age ranged from less than 6 months to 5 years started from September 2010 to May 2011.

The information for each sample in this work were registered. The lab. examinations were conducted at lab. of researches in college science of women, department of biology.

The modified Ziehl- Neelseen stain M ZN-ST (acid fast) was used for examination of feacal smears as following (Henriksen & Pohlenz ,1981).

smear was fixed with Methanol alcohol for 5 minutes and allowed to dry at room temperature.Then the dried smears were stained for an hour in carbol fucshin prepared by dissolving 15% carbol fucshin in methanol (stock solution). ziehl fucshin 10 ml added to 90 ml 5% phenol.Then rinsed in tap water.Then differntiate in 2% H_2SO_4 solution for 20 seconds with agitating the slide.Rinsed in tap water.Then

smear was stained with 5% malachite green solution for 5 minutes and washed in tap water and followed until dried.Then examined by using 40x and 100x oil immersion objectives.

Oocysts were obtained from naturally infected children with acute diarrhea and proved to be infected with *Cryptosporidium* by M ZN-ST (acid fast) .

Isolation of the oocysts from children faeces (Arrowood & Sterling ,1987)**:** as follows: The faeces was suspended in PBS of pH 7.2 in ratio of 20-80 and centrifuged at 500g for 10 minutes. The supernatant was discarded and the sediment was suspended in 15ml centrifuge tubes of PBS pH 7.2, and 3-5ml of ether were added and mixed vigorously.Then centrifuged at 500 g for 1 minute was made and four layers were differentiated from bottom to the top:- 1-Sediment, 2-PBS, 3-Debris and 4-Solvent. Layers were discarded and the sediment layer that consisted of 75% of oocysts was used for purification of the oocysts. These oocysts were prepared in 2.5% $K_2Cr_2O_7$ until used.

The mice were infected with *Cryptosporidium* sp. oocysts that isolated from human faeces using a dose of 10^3 oocysts. This dose was determined after trail infections of three doses: 10^2, $5x10^2$, and 10^3 oocysts to each mouse per os, in order to achieve the infection that give the suitable number of oocysts sheds from infected mice. Collection of the oocysts of *Cryptosporidium* sp. from infected mice (Blagburn *et al*., 1998)**:**

Intestine of infected mice were cut in small pieces, was added water and homogenized in plastic tube with sputa sol 25 and incubated at ٤C°. and homogenate was transferred in room temperature 90-120 minutes and homogenized thoroughly. The homogenate was centrifuged at 1000 g for 10 minutes.Then the sediment was washed two times with cold tween 20 (0.1%) in distilled water. The sediment was stored in 2.5% $K_2Cr_2O_7$.

White mice of Balb\ c strain were reared in lab-animal room and managed in standard plastic cages bedding with wood shaving, and they were cleaned 1-2 times weekly according to the number of mice. They were fed with concentrated diet .

Six groups of mice each of 3 mice 2 weeks age were infected orally by stomach tube per os with 10 oocysts . The infected mice and control examined for 2 weeks by fecal floatation Sugar sheathers.Six species of plant were used in this stud were collected from Al-Hilla herbs shops:

Thymus vulgaris , Artemisea herba-alba , Ricinus communis , Peganum harmala , Olea europea , Allium sativum .

The leaves were the only part that was used in this study and collected parts of plants were dried in the air in shade and milled in mortar and pistol. The milled substances were sieved by using fine mesh sieve and the powder material adjusted in dissector containing $CaCl_2$ in order to complete dryness. All the powders were extracted in ethyl alcohol 96% according to Harboren (1973). The dried plant powder was extracted in ethyl alcohol (96%) in ratio 1-10 (W/V) mixture was shacked continuously up to 24 hours under 4°C.

The solution filtrations under high pressure in buchner funnel for expression of the filtration for 15 minutes. The filtrate was collected and evaporated in vaccum. Rotary evaporator under less than 60° C. The rest of the extracted substance (chlorophylic extracts) and water was filtrated by using whatman filter paper no. 4. The filtrate was returned to evaporator to remove the water. The extracted substances (in dark bottle) were sent to college of medicine in Al-Qadesyia University for lyophilization. The lyophilized material was stored at 4°C until used.

Experimental studies for the lyophilized plants extracts:

The effect of lyophilized extracts on the oocysts of *Cryptosporidium* sp. was carry out in two lines *in vivo*.

A-Six groups of 3 white mice Balb\c each, 1 week old were infected with 10^3 oocysts of *Cryptosporidium* sp. collected from naturally infected human and purified in ether per os using stomach tube. Faeces of infected mice were examined daily for oocysts and counted. After 3 days post infection (P.I) when the maximum number of the oocysts was reached, the mice groups were treated with the Lyophilized extract in dose equivalent to 250 mg / kg body weight per os using stomach tube. at the sixth day post infection (P.I)the oocysts were numerated.

B- A group of 3 mice were infected with 10^4 oocysts and a group kept without treatment as a control group. The treated and control groups of mice were sacrificed and the intestine were separated for collection and enumeration of the total number of oocysts .

C-The lyophilized extract of higher effect on shedding of oocyst were chosen to another run of treatment with dose of 500 mg / kg body weight; as in A.

The efficiency of the extract was done according to Egerton (1963) by using the following formula:

$$\text{Efficacy \%} = \frac{\text{Rate No. of the control group - Rate No. of the treated group}}{\text{Rate No. of the control group}} \times 100$$

The different statistical tests were used for analysis the results according to the type of the data(F- test. ANOVA) The significance level $p < 0.05$, 0.01 was regarded significant (Snedcor & Cochran, 1978).

Results :

Table(1) show the distribution of cryptosporidiosis was done according to age and sex. A total of 605 stool samples were examined from children, 318 were males and 287 were females. It was found that the rate of cryptosporidiosis in males (7.55 %) did not vary significantly from females (9.75%) .

Regarding the age distribution, statistically there was no significant difference between the age groups. It can be seen in table (1), that the rate of *Cryptosporidium* positive cases was highest among age groups from less than 6 months to more than one year (10.95 % & 10.76 %), followed by those below less than 6 months (6.87 %) and the lowest rate was among more than 5 years of age (2.5 %). Table (2) shows that the distribution of *Cryptosporidium* oocysts in children in rural areas (5.24 %) was significantly ($P < 0.05$) lower than urban areas (12.72 %). Although statistically there was no significant difference in the rate of *Cryptosporidium* oocysts in children in different months of the year, but it was shown that the highest rate of oocysts infected occussed in September (23.75 %), followed by March (13.75 %), November (10.3%), December (5.20%), February (4.65 %), January (4.54%) and April (4.08%) respectively.

The effect of plants extracts on the shedding of oocysts in mice is indicated in (Table 3), that 250 mg / kg body weight of different plant extracts decrease the shedding of *Cryptosporidium* oocysts in infected mice, with significant difference in the number of oocysts between pre and post treatment ($P < 0.05$).

Table (4) indicates that the efficacy of 500 mg/ kg body weight of plants extract on the shedding of oocysts in mice. The efficacy of *Peganum harmala* was highest

(69.7 %) followed by *Allium sativum* (65.6%) and the lowest was *Thymus vulgaris* (20 %).

It is clearly observed that the efficacy of 500 mg/ kg body weight of different plants extract on the total number of *Cryptosporidium* oocysts in the intestine of infected mice was greater than those treated with 250 mg / kg body weight of the plants extract

Table (1): Distribution of *Cryptosporidium* sp. oocysts in children According to sex and age .

*Sex	Male			Female			Total		
**Age Group	Exam. No.	Infec. No.	(%)	Exam. No.	Infec. No.	(%)	Exam. No.	Infec. No.	(%)
< 6 Months	51	3	5.88	65	6	9.23	131	4	6.87
>6 Months	80	9	11.25	60	4	6.6	146	16	10.95
>One Year	141	11	7.80	143	18	12.6	288	31	10.76
<5 Years	46	1	2.17	19	-	-	40	1	2.5
Total	318	24	7.55	287	28	9.75	605	57	9.42

*Sex p> 0.05

**Age p> 0.05

Table (2): Distribution of *Cryptosporidium* sp. oocysts in children according to Living area and months.

Region	*Rural			*Urban			Total		
**Month	Exam. No.	Infec. No.	(%)	Exam. No.	Infec. No.	(%)	Exam. No.	Infec. No.	(%)
September	34	3	8.82	46	16	34.78	80	19	23.75
November	40	3	7.5	57	7	12.3	97	10	10.3
December	41	1	2.43	55	3	5.45	96	5	5.20
January	37	1	2.7	51	3	5.88	88	4	4.54
February	40	1	2.5	46	4	8.7	86	4	4.65
March	33	3	9.1	48	8	16.6	80	11	13.75
April	42	2	4.76	35	2	5.71	98	4	4.08
Total	267	14	5.24	338	43	12.72	605	57	9.42

*Living area p < 0.05

**Month p > 0.05

Table (3): Effect of plant extracts 250 mg / kg body weight on the shedding of *Cryptosporidium* oocysts in infected mice.

Plant Extracts	No. Oocysts ($X10^3$) *Prt	6^{th} day **post	Efficacy %	Control group
Peganum harmala	3.5	1.1	65.9	3
Artemisea herba-alba	2.5	1.0	56.5	
Ricinus communis	3.8	2.4	34.3	
Olea europea	3.6	1.5	32.1	
Allium sativum	4.3	3.5	31.6	
Thymus vulgaris	3.4	2.7	19.1	

*Prt= Pre-treatment

**Post= Post-treatment

P < 0.05

Table (4): Effect of some chosen plant extracts in dose 500 mg / kg body weight on the shedding oocysts in infected mice.

Plant Extracts.	Oocysts No. ($X10^3$) Prt.	Pot.	Efficacy %	Control group
Peganum harmala	3.5	1.1	69.7	3.5
Allium sativum	3.3	1.2	65.6	
Artemisea herba-alba	3.2	1.4	50.3	
Ricinus communis	2.5	1.3	44.3	
Olea europea	2.7	1.6	33.9	
Thymus vulgaris	3.4	2.7	20	

*Prt=Pre-treatment
**Post=Post-treatment
$P < 0.05$

Disscussion :

From 605 fecal smears from children examined 57 were positive with the rate of infection being (9.42%). This is high, which indicates that cryptosporidiosis is one of significant cause of diarrhea among children in Hilla city. The high rate of infection in this study might be related to poor hygiene, contamination of food or water supply and close contact with infected persons or animals specially dogs and cats (Radostitis *et al*. ,1994).

Comparing the finding of this study with that reported by other studies carried out in the country. It is lower than that found in Baghdad (14.6%), (Al-Gelany,1998) . in Mosul (14.3%), (Al-Alousi *et al*.,2003); (20.52%), (Khalil,2000) , and in Kirkuk (12.62%), (Al-Moula,1999). almost identical to that found in Basrah (8.82%), (Nadham,1996) but higher than in Diyala (2.8%), (Al-Taie,1997).

The difference between the finding of this study and other provinces might be related to period of study, environmental factors, sample size, and the techniques used for identification of the infection(Schmidt & Roberts ,2000).

It is also lower than that reported in other countries e.g. (12.7%), (Gerba *et al*., 1996) in Sudan; (67%), (Nimiri and Hijazi,1994) in Jordan; (14%), (Chen *et al*., 1991) in China.

The distribution of cryptosporidiosis does not differ significantly between sexes, this reflects that both male and female children are equally susceptible. This finding is not in agreement with that shown in Kirkuk, (Al-Moula,1999);in Baghdad, (Al-Gelany,1998); in Basrah, (Nadham,1996); and in China, Chen *et al*.,1991) who found that the distribution of infection in males was higher than females but not in agreement with that reported by Al-Moula (1999) in Kirkuk, who showed the rate of infection in females higher than males.

Concerning the age distribution, it seems from the result of this study that the distribution of cryptosporidiosis is not related with the age of children, as there is no significant difference in the rate of infection among different age groups. The highest rate of infection in the studied groups is among those below 6 months to below one year of age. This might be due to low care and attention given to children from their mothers such as using unsterilizing feeding bottles and unboiled water, (Ungar, 2000 ; Maryam *et al* ., 2010). This finding goes with finding of Al-Moula (1999) who found the highest rate of infection in infants aging from 1 month to 1

year, Iqbal *et al.* (2001) and Nimiri and Hijazi (1994), which reveals that cryptosporidiosis is common among infants and young children suffering from diarrhea, especially within the first two years of age (Mohammad *et al* ., 2011).

As far the residency concern, it is shown that children in rural areas have lower rate of infection than urban ones. This might be related to type of feeding, as most children in urban areas have fed on artificial diet, which have more chance to get infection them those fed on breast feeding similar finding by was reported in Kirkuk province (Al-Moula, 1999 ; Othman,2000), but against the finding in Korea (Chai *et al*., 2001) who reported higher rate of infection in rural (14%) than urban area (3.7%).

The lowest rate of infection in January might be due to oocysts of parasites destroyed in low temperature (4˚C) statistically there was no significant difference in the rate of infection between different months. The present study was not carried out throughout a year; therefore seasonal comparison of rate of infection is impossible. It was found the highest rate of infection in September and the lowest in January (Sultan *et al* ., 2011) .

Shedding light on the study of other workers, it is shown that rate of infection is hight summer and spring seasons and low in winter months (Othman,2000), in Kirkuk and (Al-Yassin,1999) in Tikrit. It has been shown that *Cryptosporidium* oocyst can be found during warm and humid months, (Syed and Javid,2011).

In the present study six plants extracts were tried (*Peganum harmala*, *Artemisea herba-alba*, *Ricinus communis* , *Olea europea*, *Allium sativum* , and *Thymus vulgaris*), as alternatives of chemical drugs due to their toxicity and unavailability in the country. The medical plant extracts efficacy was tried in mice as alternative of human and economically important live stocks (Maryam *et al* ., 2010) .

Using 250 mg/kg body weight of plant extracts, the highest efficient plant extract was *Peganum harmala* (65.9%), followed by *Artemisea herba-alba* (56.5%), *Ricinus communis* (34.3 %),*Olea europea* (32.1%) , *Allium sativum* (31.6%) and *Thymus vulgaris* (19.1%).

Peganum harmala is a medical plant, its chemical contents are glucosindate and prosporing and it is used as antidysentric (Majeed & Mahmood ,1988). The second effective drug was *Artemisea herba- alba*, its efficacy might be due to its contents of artemisin and santonin. It was used against *Ascaris* parasite (Al-Khazraji, 1991) and was found to be actives against hydatidosis in laboratory mice (Al-Nakeeb ,2004). The anti-parasitic action of *Allium sativum,* is also reported and suggested that might be due to its chemical contents of glucose and fructose. The anticryptosporidial activity of sugar has been found by Harp (1999), who found that sucrose and to a lesser extent isomaltose reduced intestinal colonization of *C. parvum* in neonatal mice (Syed & Javid . 2011) .

The efficacy of *Ricinus communis* was also high, which also included glucose in its content in addition coumarine, volatile oil, sulphur, isothiocyanate, and glucosinolate and it acted as vermifuge and intestinal carminative (PDR,1998).

Al-Musaui (2000) studied the effect of alcoholic extract of *Artemisea herba- alba* plant extract on *Hymenolepis nana*, and, she observed 10-14 compounds in both the dry and fresh parts of plants, among the compounds determined was *Esculetin* as a coumarine compound. He concluded that the dry and wet extracts of *Artemisea herba-alba* were effective on infertility, egg development, and average eggs output of *H. nana*. He also found the efficacy of alcoholic extract of *Artemisea herba- alba* on *Hymenolepis_nana* was (100%), on days fifth and sixth post-infection using 500 and 250 mg/kg body weight respectively.

Regarding the efficacy of *Olea europea*, it was (32.1%), which contents include Mannite, glucose, oleosteral, resine, olivine, and resine . It has an active antiparasitic effect (Al-Aubaidy ,1999).

It is also reported that the efficacy of *Thymus vulgaris* was (19.1%), which is also reported to be parasite killer. Its chemical contents include beta caryophyllena, (Maryam *et al*., 2010).

Increasing the dose of plant extracts from 250 to 500 mg/kg body weight to show the effect of five plants (*Artemisea herba-alba*, *Olea europea*, *Peganum harmala*, *Allium sativum*) on *Cryptosporidium* oocysts. It was clearly indicated that the efficacy of plant extracts was increased with increasing the doses of plant extracts as shown in table (4).

from the results of this study, it is concluded the followings:

The prevalence of cryptosporidiosis is high among children at Hilla city and the plant extract of *Peganum harmala* have an effect on *Cryptosporidium* sp. infection in Balb/c mice.Freshly isolated oocysts from man are more infective to laboratory mice.

References :

Abdel-Wahab, A. and Abdel-Moagood, S. (2011) . Identification of Cryptosporidium Species infecting Camels (Camelus dromedaries) in Egypt. J. America Sci., 7(2): 385-388 .

Al-Alousi, T. I.; Al-Ani A. J. and Al-Rawi S. A.(2003). Cryptosporidiosis in children and calves in Mosul, Iraq, Med. J. Tikrit Univ . 119 pp .

Al-Aubaidy, S. S. H.(1999). Experimentally studies on the effects of alcoholic extract of fresh and dry Cardaria draba. L. (Desv.) on some intestinal parasitic helminths. M. Sc. thesis, College of Education for women, Tikrit Univ. 121pp. (inArabic).

Al-Gelany, B. A. A.(1998) . Study of epidemiology of cryptosporidiosis in Baghdad.M. Sc. Thesis, Coll. of Vet. Med., Baghdad Univ. 127 pp (in Arabic).

Al-Khazraji, S. M. (1991). Biopharmacological study of Artemsia herba-alba. M. Sc. Thesis, Coll. Pharm., Univ.Baghdad: 124 pp.

Al-Moula, Y. H. A.(1999). Epidemiology of acute Cryptosporidiosis and rotaviral gastroenteritis among infant and young children in Al-Tameem province. M.Sc. Thesis, Col. Med., Tikrit Univ.114 pp (in Arabic).

Al-Musaui.A.M. (2000).Effect of Artemisia herba – alba plant extract on Hymenolepis nana in white mice Balb/c . M.Sc. thesis, Coll. Sci., BabylonUniv:98 pp. (in Arabic).

Al-Nakeeb, S. A. R.(2004). Seroepidemiological and therapeutic study on hydatid cyst infection in Kirkuk and Tikrit provinces. M. Sc. thesis, Coll. Me., Tikrit Univ.128 pp. (in Arabic).

Al-Taie, M. H.(1997). Epidemiological study of cryptosporidiosis in Diyala province, M. Sc. Thesis, Coll. Vet. Med. Univ. Baghdad.117 pp. (in Arabic).

Al-Yassin, S. T. A.(1999). study of Cryptosporidiosis in immunocompromised patients M. Sc. Thesis, Col. Med. Tikrit Univ.112 pp (in Arabic).

Arrowood, M. J. and Sterling, C. R.(1987). Isolation of Cryptosporidium oocysts and sporozoites using discontinuous sucrose and isopycnic Percoll gradients. J. Parasitol., 73, Pp: 314-319.

Blagburn, B. L.; Drain, K. L.; Land, T. M. (1998) . Comparative efficacy evaluation of dicationic carbazole compound, Nitazoxanide, and paromycin against C. parvum infections in neonatal mouse model. Antimicrob Agent Chemother, 42, Pp: 2877-2882.

Chai, J.; Guk, S.and Lee, S.H.(2001).An epidemiological survey of Cryptosporidium, 30, Pp: 1252-1258.

Chen Y.G.; Yao, F. B.; Li, H. S. and Lu, M.(1991). Epidemiological studies on human cryptosporidiosis in rural and urban areas of Jiangsu. J. Chin. Microbiol.,30, Pp: 492-494.

Diego, A. G. L.and Mirna, A. P. (2008) . First report of Cryptosporidium Sp . oocysts in oysters (Crassostrea rhizophorae) and Cockles (Tivela mactroides) in Brazil. J. wat. and Heal. 6(4) : 27-32 .

Egerton.(1963) . Evaluation of antihelminthic in the laboratory and their application to field condition. Proc. Inter. Conf. Wld. Vet. Parasitole., 1, Pp: 46- 53.

Gerba, C. P.,; Rose, J. B. and Hass, C.N.(1996). Sensitive populations who is at the greatest risk Int. J. Food. Microbiol.30, Pp: 113-123.

Harborn, J. B.(1973) . Phytochemical methods. A guide to modern techniques ofplant analysis. Chapmen and Hall, New York, Pp: 1-80.

Harp, J. A.(1999) . Oral dosing of neonatal mice with sucrose reduces infection with Cryptosporidium parvum. J. Parasitol. 85 (5), Pp: 952-955.

Hassanpour, A. ; Mashayekhi, M. and Safarmashaei. S. (2011) . Prevalence of Cryptosporidiosis in foals and humans to be in contact them in Tabriz Area in Iran. Advances in Environmental Biology, 5(6): 1070-1072 .

Henriksen, S. A. & Pohlenz, J.(1981) . Staining of cryptosporidia by a modified Zeihl-Neelseen technique. Acta Vet. Scand. 22, Pp: 594-596.

Iqbal, J., Hira, P. R.; Al-Ali, F. and Philip, R.(2001) . Cryptosporidiosis in Kuwait children: seasonality and endemicity. Clin. Microbiol. Inf.7, Pp: 261- 266.

Khalil, L., Y.(2000) . Compromised for efficacy of some diagnostic tests for cryptosporidiosis in children and lambs in Nineva province. M. Sc. Thesis, Coll. of Vet. Med. Univ. Mosul.115 pp.

Majeed, S. H.; and Mahmood, M. J. (1998) . Iraqi plants and herbs between public medicine and scientific search, Counsel of scientific research. Researches of biology center, 1st edition, Dar Al- Thawrah for journalism and distribution Chemother, 42, Pp: 2877-2882.

Mackenzie, W. R.; Hoxie, N. J.; Proctor, M. E.; Gradus, M. S.; Blaire, K. A.; Peterson, D. E.; Kazmierczak, J. J.; Addis, D. G.; Fox, K. R.; Roes, J. B. and Davis, J. P. A.(1994). massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. N. Eng. J. Med.331, (3), Pp:161-167.

Mancassola, R.; Richard, A. and Naciri, M.(1997) . Evaluation of decoquinate to treat experimental cryptosporidiosis in kids. Vet. Parasitol. 69, Pp: 31-37

Mannheimer, S. B. and Soave, R.(1994). Protozoan infection in patients with AIDS: Cryptosporidiosis, isosporiasis, cyclosporiasis and microsporidiosis. Infectious Disease Clinics of North America, 8, p: 2.

Markell, E. K.; John, D. T. and Lrotoski, W. A. (1999). Medical Parasitology. W. B. Saunders Company, U.S.A.589-592.

Maryam, F. ; Javid, S. and Fatemah, G. (2010) . Effects of two anticoccidial drugs, Monensin, Toltrazuril and the mixture of them on Cryptosporidium parvum In vitro . Jundishapur J Microbiol., 4(2): 71-4.

Michael, J. B. ; Srilakshmi, P. ; Sara, M. L. ; Kruthi, M. and Tyler, J. C. (2011) . Parasite metogen-activated protein kinases as drug discovery targets to treat Human protozoan pathogens .J. Sig. Trans.Vol , 16 Art. doi:10.

Mohammad, H. R. ; Saeid, F. ; Ehsan, N. A. ; Mohammad, m. d. and Hadi, R. S. (2011) . A survey of parasites of Domestic Pigons (Columba livia domestica) in South Khorasan, Iran .Med. J.Vet. Res., 4(1): 18-23

Nadham, K.M.; Al-Sadoon, I. A. and Adel, T.(1996). First report of cryptosporidiosis among Iraqi children. Eastern Mediterranean Health J.,2, Pp:115-119.

Nimiri, L. F. and Hijazi, S. S.(1994) . Cryptosporidium a cause of gastroenteritis in preschool children in Jordan,J. Clin. Gastroenteol.,19, Pp:288-291.

Othman, N. F.(2000) . Comparison between different laboratory methods for diagnosis of Cryptosporidium sp. A diploma in PCLI Dissertation, Coll. Med. Tikrit Univ. (in Arabic)**.**

Oyibo, W. A. ; Okangba, C. C. ; Obi, R. K. ; Nwanebu, F. C. and Ojuromi, T. (2011) .

Diagnosis of intestinal Criptosporidiosis in Africa : Prospects and challenges . PDR

.(1998) . For herbal medicines, 1st edition, medical economics company, Montvale, New Jersy, J. Appl. Bio.Sci. 40: 2659 - 2667 .

Radostitis, O. M.; Blood, D. C. and Gay, C. C. (Eds.).(1994) . Cryptosporidiosis, Textbook of Medicine Disease of Mammals, 8th edit.,Pp:1195-1199.

Schmidt, G. D. and Roberts, L. S. (2000). Foundations of Parasitology, 6th. edn. McGraw-Hill International Editions :338-342.

Snedcor, G. W. and Cochran, W. G. (1978) .Statistical methods 6th edit., the lowa

state university press, U. S. A.

Sultan, A. ; Samaullah, K. ; Shahid, N. K. ; Farzana, B. ; Sumaira, S. and Masood, A. (2011) . Prevalence of zoonotic parasites in Drinking water of three districts of Khayber Pakhtunkhwa Province, Pakistan. Pak. J. life soc. Sci., 9(1): 1 – 3 .

Syed, M. M.-Q. and Javid, S. (2011) . An examination of Cryptosporidium pavum Infection in neonate Balb/c mice and rats . Jundishapur J Microbiol. 4(3): 185-190.Tzipori, K. W. Angus, I. Campbell, and E. W. Gray.(1980) . Cryptosporidium: Evidence for a singlespeacies genus. Infection and Immunity, , 30, Pp: 884-886.

Ungar, B. L.(2000) . Infectious diseases and their etiologic agents. In: “Principles and practice of infectious diseases 5th. Edit. Mandell, G.L. Bennett, J. E. and Dolin, R.(Eds.). Churchill Livingstone, Philadelphia,Pp: 2903-2914.