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Investigation of *Cryptosporidium* and *Giardia lamblia* infection with trials of treatment in sheep and goats at the Al-Mashroa district, Babylon province

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Abstract : The current study was conducted during the period from September 2013 till August 2014 to investigate the percentage of infection for *Giardia lamblia* and *Cryptosporidium* sp. in sheep and goats and try to treat the project area in the province of Babylon . The number of samples examined a sample of 85 of the stool sheep and goats, some suffering diarrhea and other ostensibly intact and ages between 15 days to more than a year. Incidence of parasite *Cryptosporidium* sp. reached 13.33 % in sheep and goats in 12%, while the incidence of parasite *Giardia lamblia* to 3.33 % in sheep and goats in 8% Is not recognized trophozoites developed for *Giardia* in this study. Infection rate was highest in the age group from 15 days up to 3 months for the parasite *Cryptosporidium* sp. and more than 3 months to 6 months for the parasite *Giardia lamblia* in sheep and goats respectively. Examination showed that the highest prevalence of the disease in animals suffering from diarrhea compared with no infected outwardly and large . And that the infection rate was higher in the winter compared to the rest of the seasons of the year and that attempts to therapy conducted on animals infected *Cryptosporidium* sp. using some antibiotics explained that Alparomyomsin sulfate 100 mg / kg of body weight for a period of three to five days is the most effect comparing antibiotic Other, as well as Attempts by various anti-worms that were conducted on some infected animals with *Giardia* that Alfenbandasole 50 mg / kg of body weight for three to five days is the most effective compared to other antibiotics used in therapy. Where treatment has led to the disappearance of symptoms, And improve the health status of infected animals. This is the first study that sheds light on the extent of parasite *Giardia lamblia* and *Cryptosporidium* sp. presence in sheep and goats in the project area in the province of Babylon, where each of the parasite *Cryptosporidium* sp. of epidemic and *Giardia* important for lambs and young infected goats them naturally, and is likely to be lambs and young infected goats and pot infection rate of *Giardia lamblia* and *Cryptosporidium* sp. for other animals as well as human in this region.

Introduction:

The genera *Cryptosporidium* and *Giardia* have been recognized as important entropathogens in various species of domesticated animals and humans. Infection by these protozoans has been associated with economic losses from the occurrence of diarrhea, and more rarely, death of producing animals[1]. The protozoan diseases, Cryptosporidiosis and Giardiosis, are important enteric diseases of sheep and goats, resulting in diarrhea, inefficient weight gains, and occasionally death[2]. Cryptosporidiosis is a widespread, serious economic disease affecting animals who are preweaned, recently weaned, in unsanitary, stressful, or crowded conditions, as well as after entering feedlots[3]. The age of the animals is one of the most important risk factors associated with Giardiosis and Cryptosporidiosis with young animals being more susceptible to infection that adult ones[1]. Coccidian protozoon *Cryptosporidium* causes Cryptosporidiosis in a wide range of vertebrates, including

humans. *Cryptosporidium* is prevalent in sheep and goats and considered to be an important agent in the ethiology of neonatal diarrhea syndrome of lambs and goat kids[4]. It causes considerable direct and indirect economic losses, and morbidity can approach up to 100 % in less than six months of age goats and sheep[5]. *Cryptosporidium* is one of the main causes of morbidity and mortality in young livestock, being considered one of the major entropathogens associated with neonatal diarrhea in ruminants[6]. *Cryptosporidium sp.*is a protozoa that can cause diarrhea similar to that of rotavirus infection and can cause diarrhea in lambs and kids 5 to 10 days of age. Affected animals are often active, alert, and nursing. The diarrhea is usually very liquid and yellow[7]. Cryptosporidiosis in sheep and goats is a disease of economic importance characterized by diarrhea and occasional death as well as progressive weight loss in chronic infection[8]. Giardiosis is a chronic , intestinal protozoal infection that is seen worldwide in most domestic and wild mammals , many birds, and people. The number of defferent speciese and the zoonotic potential of *Giardia spp*. are controversial[9]. *Giardia* induced diarrhea is more commonly, but not limited to 2 to 4 week old lambs and kids . The diarrhea is usually transient, but infection animals can continue to shed cysts for many weeks, providing a source of infection for other animals and possibly human[10]. There is circumstantial evidence that *Giardia sp*. that infection domestic animals can infect people. It appears that some *Giardia sp* isolates are infective to a variety of mammals, while others are more speciese specific. Wild animals may also be reservoirs[11]. *Giardia duodenalis* is a well known enteric protozoan that affects a wide range of domestic and wild animals as well as human, causing acute, self-limiting, but often severe diarrhea, weight loss, lethargy, and poor condion in young animals and particulary in immunocompromised individuals[12]. *Giardia lamblia* has recently emerged as an important parasite in domestic ruminants due to the unexpectedly high levels of infection . In animals, the typical pattern of infection is a peak of faecal shedding of cysts between five and ten weeks of age[13, 14]. There is a close association between the prevalence of the *Cryptosporidium* and *Giardia* infection and age of the animal[15]. Various trials have been developed for treatment and control of *Cryptosporidium* and *Giardia* infection in sheep and goats. [16]treated Cryptosporidiosis, with most drugs normally used to treat coccidian in kids and lambs[17]. Studied the effects of halofuginone lactate (100 µg/kg B . W.) for treatment and prevention of Cryptosporidiosis in lambs. [18]studied the therapeutic efficacy of paromomycin aminoglycoside antibiotic on natural *Cryptosporidium parvum* infection in lambs and kids . [19] used azithromycin (a macrolide antibiotic) in the treatment of Cryptosporidiosis in immunosupressed lambs and kids. [20]found that fenbendazole is an effective and reduction in lambs.

The **aim** of this present study was design to carry out the prevalence, age, seasonal distribution of contamination of the zoonotic parasites in *Cryptosporidium* and *Giardia* infection among sheep and goat in north Hilla city in Babylon Province.

Materials and methods

Animals:

A total of 60 fecal samples from sheep and 25 from goats were collected, 20 and 9 from adult sheep and goats (over 12 months of age) and 40 and 16 from juvenile sheep and goats respectively (less than 12 months) (Table 2). This samples were carried out in rearing sheep and goats in the Al-Mashroa area in the north of Babylon Province. The juvenile animals were divided in to three groups by age, (group 1from 15 days to 1 month) ;(group 2, $> 1 \leq 3$ months) ; (group3, $>3 \leq 6$months). Animals from one year onward were classified as adults (group 4) (table2). The animals were released in the morning to browse in the shrub jungles and communal pasture for 6 – 8 hr. and housed in stilted sheds with wooden altered floors at night. The lambs and kids were housed with their dams until they were 1 – 2 months of age in half walled sheds which were overcrowded and in poor hygiene.

Sampling:

Faecal samples were collected from sheep and goats from September 2013 to Augest 2014, Fresh faecal samples were collected per rectum and placed in technically plastic containers. Specimens were stored in a refrigerator at 4 C° . The consistency of the samples was scored as diarrhoeic or non diarrhoeic. In animals with diarrhea, the date of sampling, origin, and age, were recorded for each animal.

Parasitological examination :

Cryptosporidium infection was diagnosed through faecal examination. Fresh faecal samples were collected directly from the rectum, and marked for identification. After collection, the samples were placed in an ice chest to conserve the material until processing in the laboratory. Faecal samples were concentrated using sheather's flotation technique in saturated sucrose solution[21]. The surface film from the top was transferred with disposable culture loop on to a microscope slide and covered with glass slip. The entire covered area was examined under high power (magnification X 40). The modified Ziehl – Neelsen technique was used for confirmation as it is specialized staining procedure. Fresh faeces and isotonic saline were mixed and spread out on the microscope slide to obtain a homogenous and transparent film. Slides were air dried, fixed in absolute methanol for 3 minutes, stained in cold carbol fuchsin for 10 minutes and decolorized in 3 % hydrochloric acid for 1 minute. Then 1 % methylene blue was applied for 30 seconds. Rinsed in tap water and air dried. After staining the faecal smears were observed under an optical microscope, initially at (magnification X40) and then at (magnification X100) for identification of oocysts of *Cryptosporidium sp*. [22] . Oocyst size was measured using bright field microscope with a calibrated eyepiece micrometer. Cysts of *Giardia sp*. Were found by examining the deposit of formol ether concentrate of a faecal preparation[23], or floating the sample in Zinc Sulfate, a solution which has been found superior in getting *Giardia* cysts to float [24] . Staining the sample with some sort of iodine under the microscope to make the *Giardia* show up easier. Cyst Size was measured using bright field microscopy with a calibrated eyepiece microscope.

Treals of treatment:

Twelve sheep and goats infected naturally with *Cryptosporidium parvum* were selected. The selected cases were randomly distributed according to their ages into four groups (3 animals per each), animals in 1, 2, 3, were medicated with Sulphadimidine (Sulphamethazine) (140 mg/kg of body wt. for 3 – 5 days orally), Nitrofurazone (25 mg/ kg of body wt. 3 – 5 days orally) and paromomycin sulfate (100 mg/kg of body wt . for 3 – 5 days orally) . respectvily, sheep and goats in group four unmediated . Drug efficacy was assessed by evaluating the presence of diarrhea, oocyst shedding and weight gain. Eight sheep and goats naturally infection with *Giardia duodenalis* were selected. The selected cases were randomly distributed according to their ages into four groups (2 animals per each) , animals in 1 , 2 , 3, were medicated with fenbendazole (5 mg/10 kg of body wt. 3 – 5 days per os), Albendazole 5 % (5mg/kg of body wt. 3 – 5 days per os) and Levamisole HCL (5 mg / kg of body wt. 3 – 5 days orally respectively . Animals in group four unmediated. Drug efficacy was assessed by evaluating the presence of diarrhea, cysts shedding and weight gain.

Result :

The examined of 60 and 25 faecal samples from sheep and goats 9 (15.0%) and 4 (16.0%) contained oocysts of *Cryptosporidium parvum* from sheep and goats respectively. and 3 (5.0 %) and 3 (12.0%) contained cysts of *Giardia duodenalis* from sheep and goats respectively.As for the monoinfection with *Cryptosporidium parvum* oocyst and *Giardia duodenalis* cysts (13.33 %) and (3.33%) of the sheep and (12.0%) and (8.0 %) in goats were affected (Table 1). All samples containing *Cryptosporidium parvum* oocysts came from juvenile animals. No oocysts were detected in faecal samples from adult animals. The juvenile animals infected with *Cryptosporidium parvum* belonged to groups (1 and 2), where the age range was from 15 days to 3 months, in comparing the overall group of juvenile animals (up to 6 months of age) with the adult animals (more than 1 year of age) , we found that juvenile animals more prone to infection by this protozoan (Table 2). *Giardia duodenalis* cysts were found in 5 (8.33%) and 2 (8.0 %) faecal samples in sheep and goats respectively. The infected animals belonged to groups (2 and 3) , these covering the age ranges from > 1 to ≤6 months. The group of juvenile animals was more affected than the adult groups Table 2 . Regarding the prevalence of *Cryptosporidium parvum* and *Giardia duodenalis* infection in diarrhoeic and non diarrhoeic this investigation revealed that infection were detected in (28.0%) and (16.0%) out of the examined diarrhoeic and (20.0%) and (4%) out of the non diarrhoeic sheep. Respectively and (0%) in adult sheep. In goats *Cryptosporidium parvum* and *Giardia duodenalis* were detected in (40.0%) and (20.0%) , out of the examined diarrhoeic , (33.33%) and (0%) out of the non diarrhoeic goats respectively and (0%) in adult goats (Table 3) . Seasonality and geography overall prevalence of *Cryptosporidium parvum* and *Giardia duodenalis* was higher in samples taken during winter than in samples taken during summer for both parasites in this region (Table 4 and 5) .

Therapeutic trials of treatment.

Concerning the efficacy of some antibiotic drugs against natural infection with *Cryptosporidium parvum* , the obtained results revealed reduction in Cryptosporidial oocyst output in three treatments groups in comparison to the unmediated group . On 2th day of treatment the difference was not celar , while in the 3th day of treatment all antibiotics showed low reduction in oocysts output . On 4th and 5th days Paromomycin sulfate caused the most rapid fall in the oocysts output compared with other treatment groups and this may refer the efficiency of paromomycin sulfate as the drug of choic. The efficacy of some anthelimintic drugs against natural infection with *Giardia duodenalis* , the obtained results revealed reduction in *Giardia duodenalis* cysts output in three treatment groups in comparison to the unmediated group. Fenbendazole was 100% effectivein eliminating *Giardia duodenalis* cysts from the feces within 3 – 5 days compared with the other treatment groups and this may refer the efficiency of Fenbendazole as the drug of choice , besides on hygienic measures , strict sanitation, good management and quarantine of sick animals .

Table (1) : Parasites detected in faecal samples of 214 sheep and 160 goats .

Infection rate (%)		**Number of animals infection**		**Parasites**
Goats	Sheep	Goats	Sheep	
12	13.33	3	8	*Cryptosporidium sp*
8	3.33	2	2	*Giardia sp.*
4	1.66	1	1	*Cryptosporidium sp + Giardia sp*,
24	18.33	6	11	Total

Table (2) : Prevalence of *Cryptosporidium sp*. And *Giardia sp*. Infections in sheep and goats according to the age .

Number of infected animals (%)				**No.of examined animals**		**Age group**
Goats		**Sheep**				
Giardia	*Cryptosporidium*	*Giardia*	*Cryptosporidium*	Goats	Sheep	
0	3(50)	0	5(50)	5	11	15 d-1 month
1(50)	2(33.33)	3(60)	3 (30)	5	13	>1≤3 month
1(50)	1(16.66)	2(40)	2(20)	6	16	>3≤6 month
0	0	0	0	9	20	>1 year
2(8)	6(24)	5(8.33)	10(16.66)	25	60	Total

Table (3) : Prevalence of *Cryptosporidium sp*. And *Giardia sp*. in examined sheep and goats according to animal status.

Number of infected animals (%)				**No.of examined animals**		**Animal status**
Goats		**Sheep**				
Giardia	*Cryptosporidium*	*Giardia*	*Cryptosporidium*	Goats	Sheep	
2(20)	4(40)	4(16)	7(28)	10	25	Diarrheic
0	2(33.33)	1(4)	3(20)	6	15	Non diarrheic
0	0	0	0	9	20	Adult
2(8)	6(24)	5(8.33)	10(16.66)	25	60	Total

Table (4) : Monthly prevalence of *Cryptosporidium* oocyst and *Giardia* cyst excretion by four groups of naturally infected sheep.

Group4				**Group3**				**Group2**				**Group 1**				**month**
%	***Giardia* positive animals**	%	***Crypto* positive animals**	%	***Giardia* positive animals**	%	***Crypto*. positive animals**	%	***Giardia* positive animals**	%	***Crypto*. positive animals**	%	***Giardia* positive animals**	%	***Crypto*. positive animals**	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Sept.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Octo.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nove..
0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.09	1	Dece.
0	0	0	0	0	0	9.09	1	33.33	2	18.18	2	0	0	18.18	[2]	Janu.
0	0	0	0	33.33	2	9.09	1	16.66	1	9.09	1	0	0	9.09	1	Febr.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.09	1	Marc.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Apri.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	May
0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	June
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	July
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Augu.
0	0	0	0	33.33	2	13.63	2	50	3	27.27	3	0	0	45.45	5	Total

Table (5) : Monthly prevalence of *Cryptosporidium* oocyst and *Giardia* cyst excretion by four groups of naturally infected goats.

Group4				**Group3**				**Group2**				**Group 1**				**month**
%	***Giardia* positive animals**	%	***Crypto*.positive animals**	%	***Giardia* positive animals**	%	***Crypto* .positive animals**	%	***Giardia* positive animals**	%	***Crypto*. positive animals**	%	***Giardia* positive animals**	%	***Crypto*. positive animals**	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Sept.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Octo.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nove..
0	0	0	0	0	0	9.09	1	0	0	9.09	0	0	0	0	0	Dece.
0	0	0	0	16.66	1	0	0	16.66	1	9.09	1	0	0	18.18	2	Janu.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.09	1	Febr.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Marc.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Apri.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	May
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	June
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	July
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Augu.
0	0	0	0	16.66	1	9.09	1	16.66	1	25	2	0	0	27.27	3	Total

Discussion:

The result of the study demonstrated that *Cryptosporidium parvum* and *Giardia lambelia* infection occurred only in some animals raised on both sheep and goats farms. Thus. This is the first report about *Cryptosporidium* and *Giardia* prevalence in sheep and goats in the Al-Mashroa area in Babylon Province, In the present study a close association between the prevalence of the *Cryptosporidium parvum* and *Giardia lambelia* infection and age of the animal was observed , juvenile sheep and goats were more parasitized by *Cryptosporidium parvum* and *Giardia lambelia* than adult. These results concur with studies by[25 , 26] and [27]. The prevalence of infection by *Cryptosporidium parvum* was (16.66%) and (24.0%) in sheep and goats less than 3 months of age and (8.22%) and (8.0%) for *Giardia lamblia* in sheep and goats respectively less than 6 months of age, [28] and [29] reported that Cryptosporidiosis , caused by *Cryptosporidium parvum* is primarily a disease of lambs and kids [8] . Found a strong correlation between the age and precence of the protozoan in sheep and goat and young animals being more suspitable that adult ones . This opservation . contrasts the findings reported by other workers who found that the infection was detected in a wide range of age groups extending from two week old to adult animals of more than one year [30] .The differences in the frequency of *Cryptosporidium* and *Giardia* prevalence in sheep and goats raised in different geographical regions can be the result of differences in contamination of the environment with oocysts and cysts of the parasites or different infectivity of *Cryptosporidium* and *Giardia* sp. populations[31]. It is also possible that the quality of zoohygienic conditions of animal husbandry and grazing practices may influence the exposure of animals to *Cryptosporidium* and *Giardia* infection. This can cause considerable direct and indirect economic losses . Our study revealed that both the shedding and intensity of shedding of oocysts and cysts were higher in diarrheic than in non diarrheic groups of animals, the lowest prevalence of the infection was opserved in adult animals (Table 3). These observations are in conformity to[26, 4, 32, 33] and[34]. The incidence of *Cryptosporidium* and *Giardia* in diarrheic young animals might be due to young age which severely affected by the parasites where as the young animals were immunologically immature and have greater prevalence of infection and experience more than adults [10] .Though the avidence is insufficient to conclude that this protozoan was the primary cause of diarrhea, especially bacteria and viruses were not examined. Mixed infections with *Cryptosporidium* and *Giardia sp*. in this study were recorded . These finding agree to large extent with those of [35, 36, 31] and[34, 37] reported that concomitant infections alternatively called mixed infections, are common in the nature, and often involve parasites. Many concomitant protozoal infections in humans and animals were reported. The results of this study confirm that *Cryptosporidium sp* and *Giardia sp*. exist in sheep and goats in this geographical area should be considered as one of the agents in the wthiology of neonatal diarrhea in lambs and kids similar to studies in other countries[38]. Our study showed that the highest prevalence of infection with *Cryptosporidium* and *Giardia* parasites was detected in winter seasons. This result agree with the study reported by[8] and [10] showed that there are substantial differences in prevalence of *Cryptosporidium* and *Giardia* between different years and between seasons, which illustrates the dangers of basing assessments on single years, or on parts of years, because of variations can be large.therefore, snapshots representing the size, morphology and morphometry of the oocysts of *Cryptosporidium* and cysts of *Giardia* were consistent with those mentioned for *C.parvum* and *G. lamblia* by other workers[39; 38 , 40]. On the other hand trophozoites of *Giardia lamblia* were not detected . these result conformity to[31 , 8],concerning the efficacy of some antibioticsand anthelmintic drugs, against natural infection with *Cryptosporidium parvum* and *Giardia lamblia* the obtained results revealed that paromomycin sulphate was the drug of choice in reducing both cryptosporidial oocyst output and severity of clinical signs compared with the other drugs used in treatment of *Cryptosporidium parvum*. These results agree with those reported by[16 ,18, 19]. Fenbendazole was 100% effective in eliminating *Giardia lamblia* cysts from the feces and proved successful in preventing natural disease in a controlled clinical field trial in sheep and goat, compared with other anthelmintic drugs used for treatment of *Giardia lamblia* . these finding agrees to large extent with those of [20] and[41 ,43]. Contrast [4] and with our results [8] reported that effective treatments are not available, but because the disease is usually mild and self- limited, supportive care, primarily hydration , is important. Control is strict sanitation and quarantine of sick animals. Disinfection of contamination housing with ammonia or formalin will kill the oocysts.[42,43,44]

Conclusion:

The results of the current study demonstrate that *Cryptosporidium parvum* and *Giardia lamblia* are involved in the ethiology of lambs and kids neonatal diarrhea and must be considered as a problem. Some

factors may be related to the overcrowding and the hygienic conditions of the lambs and kids areas. In view of the public health significance of cryptosporidiosis, and giardiosis further studies are needed.

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