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PREVALENCE OF *CRYPTOSPORIDIUM spp.* IN CALVES AND DIFFERENT WATER RESOURCES

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ABSTRACT

The study was planned to show the prevalence of *Cryotosporidium spp.* in calves and water sources in Salahaddin governorate. The study included 137 faecal samples of calves in the villages and indoor husbandry from Al-Ishaky private farm, 87 males and 50 females for period from beginning of July 2000 to end of April 2001. The age of calves was ranging from one month to one year. Water samples were collected from tap water, panel of Tikrit city, pond water of rain, river water and sewage water. Detection of *Cryptosporidium* oocysts was done by modified Ziehl-Neelsen and Carbol Fuccin Dimethyl Sulfoxide Methods, Direct Immunofluorescent Test was also used in water samples. The rate of infection in calves was (37.95%); in males (40.2%) being higher than females (34%). The distribution of infection was highest among calves below one month (43.2%) of age. The highest rate of *Cryptosporidium* was in summer (50%) and the lowest was in winter (18.5%).

The number of oocysts per liter of filtrated tap water was (0.2 oocysts/ liter), which was lower than infiltrated water of Tikrit planet (3 oocysts/ liter), pond water (20 oocysts/liter), river water (6 oocysts/ liter) and farm water (40 oocysts/ liter). In sewage water, oocyst was isolated only in one sample (0.1 oocyst/ liter). There was a strong correlation between turbidity of water samples and number of oocysts detected; as water samples with highest turbidity contained greatest number of oocysts.
Key words: Cryptosporidium, calves, water.

انتشار أنواع داء البويغات الخبيئة (*Cryptosporidium spp.*) في العجول والمصادر المختلفة من المياه

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الخلاصة

صممت هذه الدراسة لإظهار وجود أنواع داء البويغات الخبيئة في العجول والمياه في محافظة صلاح الدين. وقد تضمنت هذه الدراسة نحو 137 عينة براز من العجول من القرى ومن داخل الدور ومن مزرعة الأسحاقي الأهلية وتضمنت 87 من الذكور و 50 من الإناث من بداية تموز 2000 إلى نهاية نيسان 2001, وقد تراوحت أعمار العجول بين شهر إلى سنة واحدة. لقد جمعت عينات المياه من مياه الشرب ومن مشروع الماء في تكريت وبركة ماء المتجمعة من مياه الأمطار وماء النهر ومياه المجاري. لقد أستعمل لغرض الكشف عن أكياس البيض لجنس (*Cryptosporidium*) طريقة زيل نلسن المحورة و طريقة كاربول فوكسين داي مثيل سلفوكسايد. استعملت طريقة الومضان غير المباشر (IFAT) للكشف عن أكياس البيض في المياه. وقد وجد أن معدل الخمج في العجول كان (37.97%) وهو أكثر في الذكور (40.2%) من الإناث (34%). وظهر أن أعلى توزيع الخمج كان في الفئة العمرية 1 شهر (43.2%) وكان أعلى معدل للخمج في الصيف (50%) وأدناه في الخريف(18.5%). أما عند فحص المياه فقد وجد أن أعداد أكياس البيضة في اللتر من مياه الشرب المرشحة (0.2%) كيس بيض/لتر وهو أقل من المياه غير المرشحة (3) كيس بيض/لتر. أما عدد أكياس البيض في اللتر في مياه البركة كان (20) كيس بيضة/لتر وعدد أكياس البيض في مياه النهر كان (6) كيس بيضة/لتر وقد وجد أن عدد الأكياس في مياه المزرعة (40) كيس بيضة / لتر وقد عزلت أكياس البيضة من عينة واحدة من مياه المجاري (0.1) كيس بيضة/لتر. لقد ظهر وجود أرتباط قوي بين العكورة وعدد أكياس البيض المكتشفة فقد أرتبط زيادة عدد أكياس البيوض مع درجة العكورة العالية.

INTRODUCTION

Cryptosporidium has been described in many hosts since its discovery in the early part of the 20th century, but it remained obscure until the recognition by veterinary workers in the 1970 of its importance as a cause of scours in young livestock animals (1). Particularly as a result of collaborative studies involving both medical and veterinary workers, *Cryptosporidium* was also recognized in man (2).

Detection of oocysts in animal's faecal smears has been demonstrated by veterinary workers and later in humans (3). Some cases, particularly among the immunocompetent, appeared to have an association with animals, particularly cattle, as their infection was obscured and generally referred to as an emergency opportunistic zoonosis (4). *Cryptosporidium spp.* can be easily transmitted from one mammalian species to another and its transmissions cause contamination of surface and ground water (5). The present study aimed to Show the prevalence of *Cryptosporidium spp.* in calves and different water resources in Salahaddin Governorate.

MATERIALS AND METHODS

Collection of faecal samples: The study was conducted on 137 calves, aged from one month to one year, from the beginning of July 2000 to end of April 2001. Rectal faecal smears were collected randomly from calves in the villages and indoor husbandry, 87 males and 50 females from Al-Ishaky private farm about 120 kilometer southern of Salahaddin Governorate.

Collection of water samples: For detecting of oocysts in water, the water samples were collected from the following sites: 1-Tap water 2-Panel of Tikrit city before filtration 3-Pondwater collection of rain water 4-River water from Ouinate region about 15 km southern Tikrit city 5-Sewage water 6-Farm water Al-Ishaki farm from the well in the farm and from Sewage of the stable.

Laboratory Examination: The laboratory examinations were conducted at laboratory of researches in College of Education for Woman, Department of Biology.

The modified Ziehl- Neelseen (acid fast) and Carbol- Fuchsin Dimethyl Sulfoxide (CF-DMS) were used for examination of rectal smears. Examinations of water samples were done after filtration with filter system (local made) under negative pressure using vaccume pump through membrane filter 293mm diameter & 1.2 u pore size with initial rate flow water of 15 L/minute: cellulose nitrate types of membrane filter paper. The sediment were suspended in equal volumes of Tween 80 0.1% (T80) and Sodium Dody Coel Sulphate (0.1%) SDS, centrifuged at 1200 G for 10 minutes. Smears were made from the sediment for staining by CF-DMS and Auramine Orange (AUO). Drops of the suspension was distributed on a glass slides for immunofluorescent technique (IFT) technique.

Statistical Analysis: Analysis of variance, the student t-test and correlation test were done to show the significant difference between the variables. The significant level $P<0.05$, <0.01 was regarded significant.

RESULTS

1- Animal study: The distribution of *Cryptosporidium* oocysts in cattle according to sex and age is shown in table (1). It is revealed that the rate of infection in females (34%) was lower than in males (40.2%), statistically there were significant differences between sexes (P<0.05). The distribution of infection was highest among cattle below one month of age (43.2%) followed by those among age group > year (38.8%) and from one month up to 6 months (30.76%), (P<0.05). Concerning the seasonal distribution of cryptosporidiosis, there was no significant differences in the rate of infection in different seasons in both male and female calves (Table 2). The highest rate of infection was in Summer (50%), followed by Autumn (43.8%), Spring (32.3%) and Winter (18.5%), respectively.

2- Water study: From filtrated tap water collected from the study area, an average of 0.2 oocysts per liter of water was detected; two oocysts were detected by IFA method, 1 by Auramin method and no oocysts were seen by DMS method. Statistically there was weak positive correlation between the number of oocysts and turbidity (r=2.66 %), (Table 3). In samples, which were collected from infiltrated water of Tikrit planet, a total of 3 oocysts/ liter of water was detected (Table 4). In infiltrated pond water samples, 20 oocysts per liter of sample were observed (Table 5). Statistically there was significant correlation between turbidity of samples and number of oocysts detected in the samples, the highest number of oocysts per liter of samples with highest turbidity (r=98.7). In filtrated river water samples 6 oocysts were detected per liter of sample (Table 6). Statistically there was strong correlation between turbidity of samples and number of oocysts (96.86). Table (7) reveals that the number of oocysts per liter of sewage water was 0.1, which detected in sewage water sample, with turbidity 30. In filtered farm water a total 40 oocysts were detected in (table 8). The correlation between oocysts and turbidity of samples was highly significant (r=59.30).

Table 1: Distribution of *Cryptosporidium spp.* oocysts in cattle in Salahaddin Governorate according to sex and age.

** Sex	Male			Female			Total		
*Age Group	Samples Examined	No. +Ve	positive %	Samples Examined	No. +Ve	Positive %	Samples Examined	No. +Ve	Positive %
< Month	25	13	52	19	6	31.6	44	19	43.2
6 Months	28	9	32.1	11	3	27.27	39	12	30.7
> Year	34	13	38.2	20	8	40	54	21	38. 9
Total	87	35	40.2	50	17	34	137	52	37.96

*Age $p<0.05$; **Sex $p<0.05$

Table 2: Distribution of *Cryptosporidium spp.* oocysts in cattle in Salahaddin Governorate according to seasons.

**Sex	Male			Female			Total		
*Season	Samples Examined	No. +Ve	Positive %	Samples Examined	No. +Ve	Positive %	Samples Examined	No. +Ve	positive %
Summer	25	15	60	15	7	46.7	44	22	50
Autumn	18	9	50	14	5	35.7	32	14	43.8
Winter	17	3	17.6	10	2	20	27	5	18.5
Spring	23	8	34.7	11	3	27.3	34	11	32.4
Total	87	35	40.2	50	17	34	137	52	37.96

*Season $p> 0.05$; **Sex $p> 0.05$.

Table 3: Prevalence of cryptosporidial oocysts in filtrated Tap water (T).

	Sample No.	Volume *(L)	Oocysts *No./L.	**DMS	# AuO	## IFAT
T1	60	500	0.1	-	-	+
T2	6	500	-	-	-	-
T3	56	500	0.1	-	+	+
Total	176	1500	0.2			

r = 2.666 %; *L = liter; **DMS =Dimethyl Sulfoxide Stain; # AuO = Auramin Orange; ## IFAT = Indirect Fluorescent Antibody Test.

Table 4: Prevalence of cryptosporidial oocysts in Tikrit planet before filtration (P).

Sample No.	Turbidity	Volume *(L)	Oocysts No. / L.	**DMS	# AuO	## IFAT
P1	75	36	2	+	-	-
P2	75	56	1	-	+	-
P3	75	57	-	-	-	-
Total	225	149	3			

R = 0; * L = liter.

Table 5: Prevalence of cryptosporidial oocysts in filtrated Pond water (PO).

Sample No.	Turbidity	Volume *(L)	Oocysts No. /L.	**DMS	#AuO	##IFAT
PO1	80	400	7	+	+	+
PO2	105	400	8	+	+	+
PO3	52	350	5	-	+	-
Total	237	1150	20			

R = 98.7%; *L = liter.

Table 6: Prevalence of cryptosporidial oocysts in infiltrated River water (R).

Sample No.	Turbidity	Volume *(L)	Oocysts No. /L.	**DMS	# AuO	## IFAT
R1	49	350	3	+	+	+
R2	67	300	1	+	+	+
R3	54	310	2	-	+	+
Total	170	960	6			

R = 96.86; *L = liter

Table 7: Prevalence of cryptosporidial oocysts in infiltrated Sewage water (S)

Sample No.	Turbidity	Volume (L)	Oocysts No. /L.	**DMS	# AuO	## IFAT
S1	6	50	-	-	-	-
S2	20	50	-	-	-	-
S3	30	50	0.1	-	+	-
Total	56	150	0.1			

R = 55.75%; *L = liter

Table 8. Prevalence of cryptosporidial oocysts in infiltrated Farm water (F)

Sample No.	Turbidity	Volume (L)	Oocysts No. / L.	**DMS	#AuO	##IFAT
? FW	39	25	10	+	+	+
FR	32	20	0	-	-	-
?? FS	48	15	30	+	+	+
Total	119	60	40			

R = 99.3%; *L = liter; ? : Well in the farm; ?? : Sewage in the stable.

DISCUSSION

In this study, the rate of cryptosporidiosis in dairy calves was high (37.9%) in Salahaddin Governorate. This might be related to management system and acquiring the infection from old animals which may act as carrier or reservoir of infection; shedding large number of oocysts without showing clinical symptoms. Anderson (6) indicated the peak of oocysts shedding reached during parturition season, which lead to environmental and water pollution. Ali (7) reported that the rate of infection in cattle and calves reached (59%) in Basrah province. Al-Zubaidy and Abdul-Latif (8) showed that (30%) of newly borne calves were infected with the cryptosporidiosis in West province. Regarding the age distribution, the highest rate of infection was seen in calves below one month of age. This might be due to incomplete immune system of young calves at this age and might be due to the fact that young calves acquired the infection from infected mother (9). As far as the seasonal distribution of infection is concerned, it was found in the present study that the highest rate of infection among calves in Summer months followed by Autumn and Spring and the lowest was during Winter months. The high rate of infection in summer and autumn might be due to environment being suitable for survival of *Cryptosporidium* oocysts and in accordance with parturition period of cattle. The high rate of infection was also found in several parts of Iraq, during autumn and spring months; Al-Taee (10) in Diyala Governorate and Khalil (11) in Mosul province. The high rate of infection might be due to shedding of oocysts in faeces from mothers in spring and autumn, hormonal effect, feeding and environmental factors.

The lowest rate of cryptosporidiosis in Winter months reflects, the effect of low temperature in winter, as temperature decreases to zero (0°C) and might lead to stop the sexual cycle of the parasite inside the host (12). Al-Zubaidy (13) found the highest rate of *Cryptosporidium* infection in born calves in August and the lowest in March in Baghdad Governorate, while Al-Taee (10), showed the lowest in January in Diyala Governorate, the variation in the distribution of infection in different provinces might be due to environmental variation (temperature, humidity, rainfall, ...etc) in different parts of the country. As far as the distribution of *Cryptosporidium spp.* in drinking water is concerned, the present study revealed the presence of *Cryptosporidium* oocysts in drinking water samples collected in the studied area. This is considered as one of public health problems in the region, which reflects the contamination of treated drinking water supply with this parasite (14). The finding of *Cryptosporidium* oocysts in municipality water supply is in agreement with that reported by Ali (7) who isolated the oocysts of the parasite in municipality water supply in Basrah Governorate. He supposed that it might be due to inefficiency of water planet of Basrah in disinfection and filtration of water supply. The finding of this study is not in agreement with that reported in Baghdad by Al-Gelany in 2003 (15) and in Mosul by Al-Jarjary in 2001 (16) who did not detect *Cryptosporidium* oocysts in municipal water supply. The difference between the finding of this study and that reported in Baghdad and Mosul, might be due to difference in the period of study or it might be due to water planet of Baghdad and Mosul is more efficient than that in Salahaddin Governorate. In addition to that it might be due to concentration of chlorine used during period of this study is 0.1 p.p.m. of water, which is not effective against the *Cryptosporidium* oocysts and the parasite can resist it. It is recommended to increase the concentration of chlorine to 2 p.p.m. in water supply to be able to kill the oocysts (17). Detection of 0.2 oocysts per liter of infiltrated and disinfected tap water is unacceptable for any pathogen to be present in municipality water supply. This reflects that the oocysts can resist the disinfectant used (0.1 p.p.m. chloride) in water supply. Oocysts of *Cryptosporidium* was also observed by Rush *et.al.*, (18), who showed the level of oocysts/ liter of infiltrated and disinfected water supply. Although oocysts were detected in chlorine treated and infiltrated water supply, but the necessity of disinfection of drinking water should not be neglected, as in present study, the number of oocysts in infiltrated tap water was 0.2 oocysts/ liter; in infiltrated water of Tikrit planet 3 oocysts/ liter; in infiltrated pond water 20 oocysts/ liter and in infiltrated farm water 40 oocysts/ liter (17). Detection of high number of *Cryptosporidium* oocysts per liter of pond and farm water might be due to contamination of water with animal parasite, as transmission of infection could occur by contamination of environment with faecal materials (18). The finding of *Cryptosporidium* oocysts in pond and farm water was also reported by Al-Gelany (15) in Baghdad region, she suggested that the source of contamination of pond and farm water by contribution of insects, migrating birds and swimming of children during summer season . In contrast to the finding of this study, Ali (7) did not find the oocysts of the parasite in lake water in Basrah province, this might be due to the different source of study he carried out on lake water away from housing and animal breeding. While Al-Jarjary (16) found the parasite in water sample collected from flow of river in Yarimja village in Mosul Governorate, which is in access of children and animals.

In this study it was found that there was a significant correlation between presence of oocysts of *Cryptosporidium spp.* and turbidity of water samples. *Cryptosporidium* oocysts have been detected in water, which was adequately treated to remove bacterial and viral pathogen (19). Chapman and Rush (20) indicated that oocysts of *Cryptosporidium* did not easily pass through the sand filter, and that some disintegration of oocysts may occur during filtration. Laboratory diagnosis of human cryptosporidiosis is based upon the demonstration of *C. parvum* oocysts in faecal specimens by a variety of non-specific staining methods such as Giemsa, Modified Ziehl-Neelsen, Safranin-Methylene Blue, and Phenol-Auramine (21). These methods have been developed for the detection of large numbers of oocysts present in stool specimens of patients with cryptosporidiosis. Recently Immunofluorescence Methods have provided enhanced sensitivity and specificity over conventional staining methods for the detection of low numbers of *C. parvum* oocysts in faecal specimens (22 and 23). Immunofluorescence techniques have also been employed for the detection of water born oocysts from a variety of environmental water and waste water samples (24).

From the results of this study, it was concluded that the prevalence of cryptosporidiosis was high in calves in Tikrit area and contamination of different water resources with oocysts of *Cryptosporidium spp.*, including the municipality water supply.

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