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Cryptosporidiosis in Cattle and Sheep in Duhok City / Kurdistan Region / Iraq

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Abstract

The study was performed to detect cryptosporidiosis in animals in Duhok city /Kurdistan region of Iraq by examining 348 fecal samples of cattle and 280 of sheep using Modified Ziehl Neelsen Method (MZNM) to detect the oocysts of *Cryptosporidium*. A total of 233 fecal samples (129 cattle, 104 sheep) were randomly chosen to detect coproantigen of *Cryptosporidium* by Enzyme Linked Immunosorbent Assay (ELISA).

By MZNM, the total rate of cryptosporidiosis was 26.15% , 11.07% in cattle and sheep respectively. The infection rates were 28.99%, 24.29% in diarrheic and non-diarrheic groups of cattle respectively. While in sheep; the infection rates were 23.26% and 5.67% in diarrheic and non-diarrheic groups, respectively.

Out of 129 fecal samples of cattle, 31(24.03%), and 37 (28.68%) were positive by MZNM, and ELISA respectively. A total of 104 fecal samples of sheep, 23(22.12%), and 24(23.08%) were positive by MZNM, and ELISA, respectively.

The highest infection rate by MZNM, and ELISA was among the age group 1-2 months in diarrheic cattle and sheep, while in non-diarrheic cattle and sheep, it was among 3-6 months age group. This study indicated that infection was associated with diarrhea in all ages and it decreased with increasing of age.

Key Words: Cryptosporidiosis, Cattle, Sheep, ELISA, Duhok

الابواغ الخبيئة في الابقار والاغنام في دهوك اقليم كوردستان/ العراق

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الخلاصة:

تهدف هذه الدراسة الى كشف الاكياس البيضية لطفيلي الحوينات الخبيئة في الابقار والاغنام في دهوك / اقليم كوردستان/العراق، بفحص 348عينة براز من الابقار و280 عينة براز من الاغنام خلال الفترة من تشرين الثاني 2010 ولغاية تشرين الاول 2013باستخدام صبغة نلسون المحورة. ركزت جميع العينات بطريقة الترسيب بالايثر والفورمالين قبل الصبغ. تم اختيار 233 عينة براز بشكل عشوائي من مجموع هذه العينات المصبوغة لكشف الاكياس البيضية للطفيلي بواسطة الاليزا (ELISA) وشملت 129 عينة براز من الابقار و104 عينة براز من الاغنام.

اوضحت صبغة نلسون المحورة ان انتشار اكياس البيض كان بنسبة 26.15% في الابقار و 11.07% في الاغنام.

كان معدل الانتشار في الابقار المفحوصة 28.99% في المجموعة المصابة بالاسهال و 24.98% في المجموعة غير المصابة بالاسهال. في الاغنام ظهر الانتشار بنسبة 23.26% في الحيوانات المصابة بالاسهال و 5.67% في الاغنام غير المصابة بالاسهال.
بلغت نسبة الانتشار لاكياس البيض في عينات الابقار (29) عند فحصها باختبارات صبغة زل نلسون المحورة والاليزا 31(24.03%) و37(28.68%) على التوالي. من مجموع 104 عينة براز من الاغنام، كان عدد العينات الموجبة 23(22.12%) و 24(23.08%) باختبار صبغة نلسون المحورة واختبار الاليزا على التوالي.
اعلى نسبة انتشار في الابقار والاغنام المصابة بالاسهال ظهرت في الفئة العمرية 1-2 شهر بالاختبارات الثلاث (صبغة نلسون المحورة والاليزا . بينما ظهرت اعلى نسبة للانتشار في الابقار والاغنام غير المصابة بالاسهال في الفئة العمرية 3-6 شهور بالاختبارات. اظهرت هذه الدراسة بان الاصابة بالحيوانات المفحوصة قلت بازياد العمر ووجد ارتباط وثيق بين حدوث الاسهال والاصابة وكذلك شيوع الاصابة دون السريرية.

Introduction

Cryptosporidium species are monoxenous eukaryotic obligate intracellular protozoan parasites (Phylum: Apicomplexa) infects humans, ruminants, carnivores, birds, reptiles, amphibians and fish and equines (1,2) causing gastrointestinal tract infections. *Cryptosporidium* invades the epithelial cells of the intestine of human and animals, causing mild diarrhea in immunocompetent individuals and severe, life-threatening diarrhea in immunocompromised individuals, *Cryptosporidium* commonly seen in children, young calves, and immunocompromised individual such as AIDS patients (3). The veterinary importance of *Cryptosporidium* comes from it is effects on newborn and young animals causing diarrhea (4, 5).

Genus *Cryptosporidium* has many species with many genotypes, a complex life cycle, including both sexual and asexual reproduction, an auto infectious cycle for immunocompromised individual, and the ability to complete its development within a single host (6).

Cryptosporidiosis has been reported in zoo and wild animals, representing an important reservoir of the disease in nature (7). Outbreaks in animal and man result from contamination of water and/or food sources by oocysts from infected mammals or from sewage that may pollute the water supplies (8). Oocycts of *Cryptosporidium* resist the hard environmental conditions and water treatment processes facilitates its transmission to animals (2, 7).

The importance of cryptosporidiosis in farm animals especially in calves focused on the results of diarrhea caused by this parasite alone which is one of the main causes of diarrhea in newborn ruminants, or it occurs with other pathogens like *Entamoeba*, *Rota* virus, *Salmonella* and *Escherichia coli*, that included loss of weight, dehydration, abdominal pain, retardation in growth, morbidity and economic losses in production of animals and it is very difficult to control (9).

Researches throughout Iraq have recorded and documented the presence of *Cryptosporidium* and its distribution in farm animals and human, as well as its importance as one of the diarrhea causative agents (10-15). Due to the seriousness of this disease in farm animals, and the absence of studies on the disease in Duhok /Kurdistan region, the present study was carried out to study the infection with *Cryptosporidium* in cattle and sheep in Duhok Governorate.

Materials and Methods

The current study was carried out at the College of Veterinary Medicine, Duhok University; Kurdistan Region during the period from November 2010 to October 2013. A total of 348 and 280 fecal samples were collected from cattle and sheep respectively from different regions throughout Duhok Governorate for the diagnosis of the *Cryptosporidium* infection by using MZNM (16).

A total of 233 fecal samples (129 cattle, 104 sheep) were selected randomly for examining by copro-antigen ELISA kit (17).

Fecal samples of cattle and sheep were collected from different flocks distributed among villages within

Duhok governorate: Duhok, Sumel, Zakho, Amedi, Shekhan and Aqra from diarrheic and non-diarrheic animals of both sexes and all ages.

ELISA was done for the determination of *Cryptosporidium* antigen in feces, by using *Cryptosporidium* fecal antigen detecting kit produced by DRG Instruments Company, Germany, EIA- 3467.

The Chi-square test and Fisher test were used to analyze the association between rate of infection and the explanatory variables including age, sex, diarrhea and clinical signs, for all groups and species under study and for all techniques. In all cases, the values were considered to be statistically significant when the *P*-value was less than 0.05.

Notes for expression of significations of values in tables and figures:

- Different superscript small letter within each column indicate significant differences among values.
- Similar superscript small letters within each column indicate no significant differences among values.
- Different superscript capital letters within each row mean significant differences among values.
- Similar superscript capital letters within each row indicate no significant differences among values.
- Absent of superscript capital letters within rows and absentof superscript small letter within each column mean there are no significant differences among values.

Results

Out of 348 fecal samples of cattle; 91(26.15%) were positive whereas in 280 fecal samples of sheep; 31(11.07%) were positive with *Cryptosporidium* oocysts by MZNM (Table 1).

Table (1): The total infection rate of cryptosporidiosis among Cattle and sheep examined by MZNM.

Species of Animals	Number of Fecal samples Examined	No. of positive results	%
Cattle	348	91[a]	26.15
Sheep	280	31[a,b]	11.07
Total	628	122	19.42

Oocysts of *Cryptosporidium* spp. appear as red bright corpuscles with varying degree of staining on blue background (Figure 1).

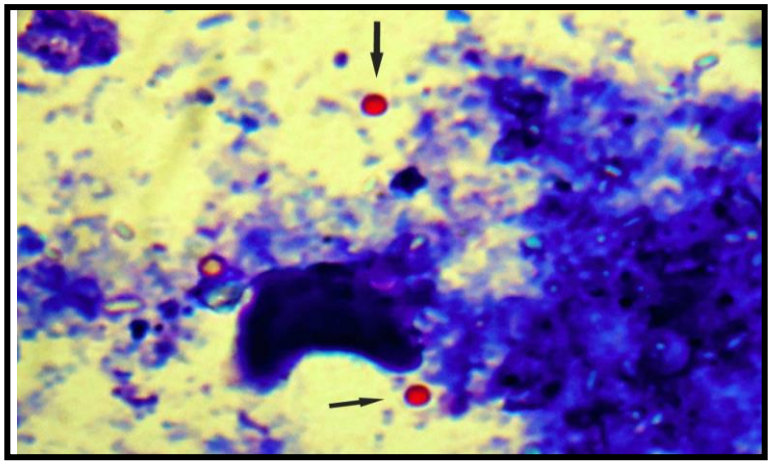

Figure (1): the oocysts in fecal sample of cattle stained by MZNM (1000X).

It was clear from (Table 2), the highest rate of infection was found among 1-2 months of age, 34/104(32.69%) were positive, followed by the age of 3-6 months were 22/81(27.16%) positive, while the lowest rate was in 7-10 months of age were 14/67(20.90%) positive, there was no significant differences in the infection rate among age groups.

Table (2): The infection rate of cryptosporidiosis among cattle by MZNM according to age.

Age groups (months)	Number of fecal samples Examined	No. of positive samples	%
1-2	104	34	32.69
3-6	81	22	27.16
7-10	67	14	20.90
>10	96	21	21.88
Total	348	91	26.15

Age wise the infection rate of cryptosporidiosis in diarrheic and non-diarrheic cattle (Table 3) reveals that the highest infection rate in diarrheic cattle was among 1-2 month of age were 26/61 (42.62%) positive; followed by 3-6 months of age were 7/27 (25.93%) positive; whereas the lowest rate was among 7-10 months of age 3/26 (11.54%) were positive. Significant difference was found among diarrheic age groups between the first group and the third group, and it indicated that the infection rate was significantly concentrated in 1-2 months of age followed by the 3-6 months of age. In non-diarrheic group, the highest rate of infection was found among 3-6 month of age as it was 15/54 (27.78%) positive; whereas, the lowest rate of infection was among 1-2 months age in which 8/43 (18.6%) were positive. The significant relation among these values was absent.

Significant differences between diarrheic and non-diarrheic groups regarding to the age was found only between the first age group meaning that the high rate of infection between diarrheic and non-diarrheic groups was significantly in the diarrheic group between 1-2 months of age. The infection rate of the total number of diarrheic group (28.99%) is non-significantly higher than the non-diarrheic group (24.29%).

Table (3): The infection rate of Cryptosporidiosis among diarrheic and non-diarrheic cattle by MZNM according to age.

Age groups (months)	Diarrheic Cattle			Non-Diarrheic Cattle		
	No. of fecal samples.	No. of positive samples	%	No. of fecal samples	No. of positivee samples	%
1-2	61	$26^{A,a}$	42.62	43	8^{B}	18.6
3-6	27	$7^{a,b,c}$	25.93	54	15	27.78
7-10	26	$3^{b,c}$	11.54	41	11	26.83
>10	24	4^{c}	16.67	72	17	23.61
Total	138	40	28.99	210	51	24.29

In table (4), the total infection rate of cryptosporidiosis oocysts in male was 44/ 171(25.73%) positive; while in female it was 47/ 177(26.55) positive; without significant differences between them.

Table (4): The infection rate of Cryptosporidiosis among cattle by MZNM according to sex.

Sex	Number of fecal samples Examined	No. of Positive samples	%
Male	171	44	25.73
Female	177	47	26.55
Total	348	91	26.15

In (Table 5), *Cryptosporidium* oocysts appeared among age of 1-2 months which was 18/ 82 (21.95%) positive; as the highest infection rate. Infection with oocysts was 6/63(9.52%) among age of 3-6 months and was the lowest rate among the more than 10 months of age which was 3/76 (3.95%) positive. High significant differences were present between first age group with the third and the fourth age groups indicating that the infection rate was significantly among 1-2 months age followed by 3-6 age.

Table (5): The infection rate of Cryptosporidiosis among sheep by MZNM according to age.

Age group (months)	Number of samples Examined	No. of positive samples	%
1-2	82	18[a]	21.95
3-6	63	6 [a,b,c]	9.52
7-10	59	4[b,c]	6.78
>10	76	3[c]	3.95
Total	280	31	11.07

Among different ages when infection is distributed according to diarrheic and non-diarrheic sheep (Table 6); the highest infection rate of *Cryptosporidium* appeared as 11/30(36.67%) positive among the diarrheic age groups and as 7/52 (13.46%) positive among the non-diarrheic age groups, both results were among the age of 1-2 months, the difference between both results among the 1-2 months of age diarrheic and non-diarrheic was highly significant indicating that the highest infection rate was among the diarrheic group. The second age 3-6 months included 5/16 (31.25%) among the diarrheic groups and 1/47(2.13%) positive among the non-diarrheic groups, the significant differences between the two results indicated that the infection with *Cryptosporidium* in the diarrheic group was high than the non-diarrheic one in this age group.

The age group more than 10 months appeared to have the lowest infection rate among both diarrheic age groups which was 2/23(8.7%) positive, and the non-diarrheic age groups which was 1/53 (1.89%) positive without any significant differences between both results. Significant differences within each group diarrheic and non-diarrheic; were present only among diarrheic age groups between the 1-2 months of age with the more than 10 months of age. In general the total infection rate among the diarrheic group was significantly higher than the non-diarrheic regardless of age groups.

Table (6): The infection rate of Cryptosporidiosis among diarrheic and non-diarrheic Sheep by MZNM according to age.

Age group (months)	Diarrheic sheep			Non-Diarrheic sheep		
	Number of samples	No. of positive samples	%	Number of samples	No. of positive samples	%
1-2	30	$11^{A,a}$	36.67	52	7^{B}	13.46
3-6	16	$5^{C,a,b}$	31.25	47	1^{D}	2.13
7-10	17	$2^{a,b}$	11.76	42	2	4.76
>10	23	2^{b}	8.7	53	1	1.89
Total	86	20^{E}	23.26	194	11^{F}	5.67

As shown in Table (7), the infection rate with *Cryptosporidium* among the male age groups was 11/39 (28.21%) positive among the 1-2 months age group as highest infection rate, while it was 1/18(5.56%) positive among the 7-10 months age group as lowest infection rate without any significant differences among age groups. The infection rate among female age group was as follows: out of 43 fecal samples 7(16.28%) were positive among 1-2 months age as highest infection rate, the lowest rate was among more than 10 months of age were1/54(1.85%) positive. There were no significant differences among males and females regarding age groups but regardless of age groups, the male total infection rate was significantly higher than the females.

Table (7): The infection rate of Cryptosporidiosis among sheep by MZNM according to Sex.

Age group (months)	Male			Female		
	Number of samples	No. positive samples	%	Number of samples	No. positive samples	%
1-2	39	11^{a}	28.21	43	7^{a}	16.28
3-6	27	$4^{a,b}$	14.81	36	$2^{a,b}$	5.56
7-10	18	$1^{a,b}$	5.56	41	$3^{a,b}$	7.32
>10	22	$2^{a,b}$	9.1	54	1^{b}	1.85
Total	106	18^{A}	16.98	174	13^{B}	7.47

Table (8) shows a comparison between the two techniques (MZNM and ELISA). Among cattle samples, it is clear that the highest rate of positive samples was with ELISA (28.68%) and least (24.03%) with MZNM. The same patterns were obtained for sheep as indicated in table (8), the differences in rates of positively obtained by two tests for cattle, and sheep samples were statistically significant.

Table (8): The results of fecal samples examined by MZNM and ELISA among cattle and sheep.

Species	No. of samples	Tests			
		MZNM +	%	ELISA +	%
Cattle	129	31^{A}	24.03	37^{A}	28.68
Sheep	104	23^{A}	22.12	24^{A}	23.08
Total	332	54^{A}	16.26	61^{A}	18.37

Discussion

This is the first work in Duhok / Kurdistan region on the cryptosporidiosis in animals. MZNM of a fecal smear for detecting *Cryptosporidium* oocysts in fecal samples, is easy, inexpensive and allows the detection of other parasites (*Isospora* and *Cyclospora*), whereas, the disadvantages of the concentration and staining procedures are time-consuming and also require an experienced examiners to read the slides (18).

In the present study, the total infection rate in cattle was 26.15%, which was lower than the result obtained by (19) who found a rate of 40% among calves in Mousl city the nearest region to Duhok with similar breeding conditions, because breeding of cattle is more common in Mosul than Duhok due to local economic and traditional factors that makes breeding of animals an important income source for the families inhabiting around the city.

The results of the present study are close to the results of the study carried out on calves in Erzurum province in Turkey in which a prevalence rate of 22.8% was reported among calves (20). In Iran, lower prevalence rates were reported by (21) who reported a rate of 6.2% among cattle of Asfahan, while (22) reported a rate of 18.9% in Kerman. Frequency of infection among cattle has been reported from different parts of the world with nearly 13.24% in Norway (23), 40% in Germany, 45.5% incidences in USA, 20% of calves in Canada, (24, 25). The infection rate of *Cryptosporidium* among livestock in Japan was 12% (26), Vietnam 33.5% (27), Malaysia 36% (28) and Australia 48% (29).

Infection rate of *Cryptosporidium* among sheep in this study was 11.07%, this result did not agree with the result obtained by (30), who found a prevalence rate of 26.66% by examining 180 fecal samples of sheep in Mosul by using the modified Ziehl-Neelsen stain. The result of the current study was approach the result obtained by (31), who recorded a total rate of 15.8% cryptosporidiosis among neonatal lambs and

sheep in Baghdad. A study of (32) recorded 13.8% prevalence rate with *Cryptosporidium* in sheep of Iran, while (33) reported 2.5% among sheep in Hamadan district. In Turkey (34) reported a rate of 13.17% among sheep which is close to the result of the current study.

Infection rate in the current study was differs among of different ages groups, the highest rate was among 1-2 months age group, while the lowest rate was found among 7-10 months age group without significant differences among age groups, that means all age groups of cattle were susceptible to infection, therefore the correlation between infection and age of calves was regarded to be symptomatic infection which is characterized by diarrhea as similar mentioned by (35).

Diarrhea is the main symptom for *Cryptosporidium* infection and the significant association between infection with *Cryptosporidium* and diarrhea was obtained by many researchers (36-38). In this study, the total result of infection rate among the diarrheic group (28.99%) was non-significantly higher than the non-diarrheic group (24.29%). This result disagreed with the results of (13) in Mosul and by (39) in Al–Diwania where the rate of infection among diarrheic calves 44% was higher than apparently healthy calves 12%. It also disagreed with Jafari *et al.* (33) in Iran who found infection was higher in diarrheic calves than non-diarrheic, and with (20) in Turkey who found diarrheic infectious rate was 30.3% while the non-diarrheic was 10%. Result of this study differ from the result of other researches due to the high rang of age used in this study for cattle and concentrated a symptomatic infection in young calves, while asymptomatic infection appeared in older calves (40), therefore the total result of prevalence between diarrheic and non-diarrheic cattle appeared non-significantly related.

The present study reveals that the significant highest infectious rate among diarrheic calves was among 1-2 month age group followed by 3-6 months age group, whereas, the lowest rate was among 7-10 months age group, also the significance relation between the diarrheic and non-diarrheic group regarding age was found only between the first age group 1-2 months which means the high rate of infection between diarrheic and non-diarrheic groups is concentrated significantly among the diarrheic group between 1-2 months of age. These results indicate that highest infection rate was among diarrheic group in 1-2 months of age and the 3-6 months of age as well as the infection decreased with age increase. These results agreed with the result obtained by (13) who found high prevalence rate (50%) among the age group 1day -2 months among diarrheic calves, and infection was age dependent which was decreased by age increase, and it agreed with (41) who found newborn and young calves were more susceptible to infection in Mashhad/Iran and infection usually occurred symptomatically . The result of this study also agreed with (42), in Turkey who recorded 22.14% infections rate among calves in Hakkari city with high prevalence among 3-6 months and the lowest rate among 12-36 months of age. The result of current study is similar to that reported by Singh *et al.* (43) who demonstrated that *Cryptosporidium* was commonly found among 30 day's old dairy calves. Studies have shown a direct association between age of animals and the risk of *Cryptosporidium* infection, most of them occurred between the first and fourth week of age (44). A study of (45) found the only significant risk factor for *Cryptosporidium* infection in dairy cows was age. The oocysts were not found among animals younger than 4 days old (46, 47) and was only detected after 7 days of age (48). The highest reported infection rates were among calves between 7 and 14 days (47), or 8 and 21 days old (48). Infection of young calves and the opposite relation between age and infection were mentioned by many researchers, which is due to underdeveloped immunity system of young animals and infection occurred by ingestion of oocysts directly from the adults (38,41), calves were housed in such away that direct contact between the animals was possible. It has been reported that management of animals in which calves are housed with their mothers and without contact with other calves tends to

decrease the prevalence of *Cryptosporidium* infections (47). Intensive dairy cattle production, overcrowding, poor hygiene and inadequate management measures are risk factors for diarrheic diseases in farms (49). Other important risk factors of cryptosporidiosis spreading in newborn ruminants are the method and frequency of pet cleaning and the floor type of the pet, infection risk decreases when calves are housed individually in pets with a cement floor previously disinfected, and daily washing with water under pressure drag is used (50, 51). Malnutrition has negative effects on cellular immunity which leads to persistent infection in the intestine (52), the continuous infection with many species and subspecies along life, with malnutrition, both factors insured continuous shedding of oocysts in older ages with or without symptoms where sub clinical infection is common in animals older than two months of age (40), however, a general trend was observed in many studies: the prevalence of *Cryptosporidium* declined with the increase of age (53, 54).

In this study, there is no significant difference in infection rate between male and female regarding or regardless to age, this agree with (55), they stated that sex had no effect on the prevalence rate because both genders bred in a way that both of these were subjected to the same environmental and contaminated factors.

Regarding to the age, also the highest rate of infection with *Cryptosporidium* occurred among age group 1-2 months which was significantly higher, followed by 3-6 age group, without significant differences between both values. It was found significantly as the lowest rate among more than 10 months age group and the prevalence decreased with the increase of age. This result agreed partially with results obtained by (31) who found higher rate of infection (34.95 %) among lambs less than one month ,then 1-2 months while the lowest rate 2.77 % was recorded among animals at 6 to 7 month old. The result of this study agreed with a study carried out by (36) in Baghdad who revealed age dependent infection in sheep with high prevalence among neonatal and young sheep. But the rate of prevalence among young sheep in this study is less than the rate obtained by (31) and (56) who recorded infectious rate 58.33% among young sheep, also the result of this study differs from the result recorded by (57) who recorded (38.8%) infection rate among lambs 1-2 months in Turkey. The highest infectious rate of young lambs are mainly due to direct contact lambs with dams and ground as well as insufficient immunity system of lambs that needs proper time after birth to be completely effective and protective (31). In this study, the prevalence decreased with the increase of lambs age and this agree with the results obtained by (58- 60).

There were no significant differences among males and females regarding age group but regardless to age group; the total infection rate in male was significantly higher than in female, this result agrees with the result obtained by (61) who reported differences in infection rate between males and females in Al-Diwania city where females were higher than males, and disagreed with the result obtained by (31) who mentioned no differences between males and females infection rate, these differences might be due to sample size and the original number of males and females in stocks because the parasite has the same ability for infecting males and females, so *Cryptosporidium* infection is sex independent (55).

Out of 129 fecal samples of cattle, the positive samples by MZNM and ELISA were 31(24.03%), 37(28.68%) respectively. Among sheep, out of 104 fecal samples, the positive samples by MZNM and ELISA were 23 (22.12%), 24(23.08%) respectively. According to above; the positive results obtained by ELISA were higher than MZNM for selected samples in cattle and sheep without significant differences between them.

The differences among results of the two test in this study were due to differences in sensitivity and specificity among tests, were ELISA was more sensitive than MZNM, the similar results were obtained by (62- 63).

The age of 1-2 months of diarrheic group appeared to have the highest infection rate with *Cryptosporidium* by the two tests used in this study (MZNM and ELISA); 3-6 month of age in non-diarrheic group appeared as the highest infective group by the two tests, this agreed with (45) who found calves less than 2 months old were more frequently infected with *Cryptosporidium* than others.

The results of the present study showed that the prevalence of *Cryptosporidium* sp. in diarrheic animals was higher than that in non-diarrheic animals in Kurdistan Region – Iraq and ELISA seems to be more sensitive and specific than MZNM.

References

1- Fayer, R. (2010). Taxonomy and species delimitation in *Cryptosporidium*. Exp. Parasitol., 124: 90–97.

2- Jex, A. R. and Gasser, R. B. (2010). Genetic richness and diversity in *Cryotpsporidium homonis* and *Cryptosporidium parvum* reveals major knowledge gaps and need for application of next Generation technologies-Research review. J. Biotecnolo. Advanc. 28: 17-26.

3- Fayer, R.; Santin, M.; Trout, J. M. and Greiner, E. (2006a). Prevalence of species and genotypes of *Cryptosporidium* found in 1–2-year-ol dairy cattle in the eastern United States. Vet. Parasitol., 135: 105–112.

4- Fayer, R.; Santin, M.; Trout, J. M. and Greiner, E. (2006a). Prevalence of species and genotypes of *Cryptosporidium* found in 1–2-year-ol dairy cattle in the eastern United States. Vet. Parasitol., 135: 105–112.

5- Fayer, R.; Santin, M.; Trout, J. M. and Du ey, J. P. (2006b). Detection of *Cryptosporidium felis* and *Giardia duodenalis* assemblage F in a cat colony. Vet Parasitol., 140: 44-53.

6- Rosileia, M.; De Quadros, S. M. T.; Marques, C. R.; Amendoeir, A.; Larissa, A.; De Souza, P. R. and Amendoeira-Carla, C. C. (2006). Detection of *Cryptosporidium* oocysts V.R. Simpson, Wild animals as reservoirs of infectious diseases. Vet. J., 163: 128–146.

7- Ziegler, P. E.; Wade, S. E.; Schaaf, S. L. and Chang,Yung-Fu, M. H. O. (2007). *Cryptosporidium* spp. From small mammals in the New York City watershed. J. Wildlife Dise., 43(4): 586–596.

8- Karanis, P.; Kourenti, C. and Smith, H. (2007a).Waterborne outbreaks of protozoan parasites, a worldwide review of outbreaks and lessons learnt. J. Water Hlth., 5(1): 1-38.

9- Fayer, R.; Santin, M. and Xiao, L. (2005). *Cryptosporidium bovis* sp. (Apicomplexa: Cryptosporidiidae) in cattle (Bos taurus). J. Parasitol. 91: 624-629.

10- Abdulla, H. M. (2010). Prevalence of *Cryptosporidium* species and some intestinal protozoa in children suffering from diarrhea in Erbil governorate.Thesis in Medical Microbiology. Medical College of Medicine, Hawler Medical University.

11- Khalil, M. M.; Al- Zubaidi, M. Th. S. and Kadhim, F. S. (2011). Prevalence of Cryptosporidiosis in calves and efficiency of ELISA in detection of the infection compared with some traditional Methods. Iraqi J. Vet. Med., 53(2) 2155: 533 – 543.

12- Al-Baytee, A. J.; Shatha Q. J. and Hadi S. M. (2012). Prevalence of *Cryptosporidium* oocysts in different types of water in Al-Mansoria Diala province. J. Al-Nahrien Univ., 1(15): 103-107.

13- AL-Alousi, T. I. and Mahmood, M. I. (2012). Detection of *Cryptosporidium* oocycts in choldren in Mousl. Iraq. Proceeding of the eleventh veterinary scientific conference; 2012: 280-285.

14- Suleiman E. G.; Butty, E. T. and AL-Abadi, A. A. (2012). Detection of oocysts of *Cryptosporidium* spp. in gall bladder of sheep slaughtered in Mosul, Iraq. Iraqi journal for Veterinary Science, 26 (1): 41-44.

15- Rahi, A. A. (2013).Comparison among Modified Acid-Fast Stain and Some Immunological Methods in Diagnosis of *Cryptosporidium parvum* in Kut City. Wasite University, college of science.

16- Smith, H. (2008). Diagnosis.In; Fayer, R. and Xiao, L. (Eds). *Cryptosporidium* and Cryptosporidiosis. CRC Press. Tayler and Francis Group: 173-07.

17- Kuzehkanan, A. B.; Rezaeianl, M.; Zeraati, H.;Mohebali, M.; Meamar, A. R.; Babaei, Z.; Kashi1, L.; Heydarnezhadi, M. and Rezaie, S. (2011). A Sensitive and Specific PCR Based Method for Identification of *Cryptosporidium* Spp. Using New Primers from 18S Ribosomal RNA. Iranian J. Parasitol., 4(6): 1-7.

18- Huang, D. B.; Chappell, C. and Okhuysen, P. C. (2004). Cryptosporidiosis in children. Semin Pediatr, Infect. Dis., 15: 253–259.

19- Bakr, M. H. H. (2005). The study of epidemiology, experimental immunity and transmission for cryptosporidiosis in Nineveh governorate. Ph.D. Thesis in Parasitology. College of Vetrinary Medicine, Mousl University.

20- Sari, B.; Akta, M. S. and Arslan, M. O. (2008a).The prevalence of *Cryptosporidium* spp. in calves in Erzurum province. Turkiye Parazitolojii Dergisi, 32(2): 116-119 (in Turkish with English abstract).

21- Azami, M. (2007). Prevalence of *Cryptosporidium* infection in cattle in Isfahan, Iran. J. Eukaryot. Microbiol., 54(1):100-2.

22- Fotouhi, A. R.; Fasihi, H. M.; Solayman, B. S.; Kamyabi, H.; Atapour, M. and Sharifi, I. (2008). Epidemiology of *Cryptosporidium* infection of cattle in Kerman/Iran and Molecular Genotyping of some Isolates. J. Kerman Univ. Med. Scien.; 15(4): 313-20.

23- Hamnes, I. S.; Gjerde, B. and Robertson, L. (2006). Prevalance of Giardia and *Cryptosporidium* in dairy calves in three areas of Norway. Vet. Parasitol., 140: 204-216.

24- Santin´n, M.; Trout, J. M.; Xiao, L.; Zhou, L.; Greiner, E. and Fayer, R.(2004). Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet. Parasitol., 122: 103 117.

25- Kumar, D.; Sreekrishnan, R. and DAS, S. S. (2005). Cryprosporidiosis: an emerging disease of zoonotic importance. Proc. Nat. Acad. Scien. India., 75: 160-172.

26- Sakai, H.; Tsushima, Y. and Nagasawa, H. (2003).*Cryptosporidium* infection of cattle in theTokachi District, Hokkaido. J. Vet. Med. Scien.; 65: 125-7.

27- Nguyen, S. T.; Nguyen, D. T. and Le, D. Q. (2007). Prevalenceand first genetic identification of *Cryptosporidium* spp in cattle in centralViet Nam. Vet. Parasitol., 150: 357-61.

28- Halim, N. A.; Plutzer, J.; Bakheit, M. A. and Karanis, P. (2008). First report of *Cryptosporidium* deer-likegenotype in Malaysian cattle. Vet. Parasitol., 152: 325-9.

29- Becher, K. A.; Robertson, I. D.; Fraser, D. M.; Palmer, D. G. and Thompson, R. C. (2004). Molecular epidemiology of *Giardia* and *Cryptosporidium* infections in dairy calves originating from three sources in Western Australia. Vet. Parasitol., 123:1-9.

30- Abdullah, I. A. (2005). Prevalence of *Cryptosporidium* in Sheep in different localities of Ninevah province, Iraq. J. Mesopot. Scien., 7(16): 93-101.

31- Yakoob, A. Y. and Kathim, T. A. (2009). Epidemiological study of cryptosporidiosis in sheep of Baghdad province. Iraqi J. Veterinary Med.; 2(33).

32- Harandi, F.M.and Ardakani, F. R. (2008) *Cryptosporidium* infection of sheep and goats in Kerman: Epidemiology and risk factor analysis.J.Vet .Res., 63(1): 47-51.

33- Jafari, R.; Maghsood, A. H. and Fallah, M. (2013). Prevalence of *Cryptosporidium* infection among livestock and humans in contact with livestock in Hamadan District, Iran. J. Res. Hlth. Scien. (13) (Abstract).

34- Cicek, M.; korkoca, H. and Gul, A. (2008). Investigation of *Cryptosporidium* spp. workers of the Van municipality slaughterhouse and slaughtered animals. Acta. Parasitologica Turkia, 32(1): 8-11.

35- Ghalehbin, B. M.; Arzanlou, M.; Fallah, E.; Kazemi, A. and Asgharzadeh, M. (2008). Molecular identification of Cryptosporidium sp. in the cattle samples in Ardabil city, Northwetern Iran. J. Anim. Vet. Adv., 7(3): 246-250.

36- Al-Azawi, M. W. (2003). Study in epidemiology of cryptosporidiosis with isolation of parasitic antigen with using of some extracts from plants for treatment. Ph.D. Thesis. College of Veterinary Medicine, University of Baghdad.

37- Ulutas, B. and Voyvoda, H. (2004). Cryptosporidiosis in diarrhoeic lambs on sheep farm.Turkiye parasitol Derg., 28: 15-17.

38- Sevinc, F.; Usla, V. and Derinbay, O. (2005). The prevalence of *Cryptosporidium parvum* in lambs around Konya. Turk J. Vet. Anim. Scien., 29: 1191 – 1194.

39- Abdul Razak, A. M and Jasm, Gh. A. A. (2011). Prevalence of cryptosporidiosis in calves and child In Al Diwania city. Iraq. J. Vet. Med., 35 (1):190 – 194.

40- Gul, A.; Mutalip, Ç. and Özlem, K. (2008). Pr evalence of Eimeria spp., *Cryptosporidium spp*. and *Giardia spp*. in Calves in the Van Province. Türkiye Parazitoloji Dergisi, 32(3): 202 – 2.

41- Safavi, E. A.; Gholam, R. M. and Abolghasem, N. (2011). Prevalence of *Cryptosporidium* spp. infection in some dairy herds of Mashhad (Iran) and its association with diarrhea in newborn calves. comparative clinical pathology., 2 (20): 103-107.

42- Göz, Y.; Gül, A. and Aydın, A. (2007). Prevalence of *Cryptosporidium* spp. in cattle in Hakkari region. Yüzüncü yıl Üniversitesi Veteriner Fakültesi Dergisi., 2(18): 37-40.

43- Singh, B. B.; Sharma, R.; Kumar, H.; Banga, H. S.; Aulakh, R. S.; Gill, J. P. S. and Sharma, J. K. (2006). Prevalence of *Cryptosporidium parvum* infection in Punjab (India) and its association with diarrhea in neonatal dairy calves. Vet. Parasitol., 140, Cockwill, C.; McAllister, T. A.; Jelinski, M.D.; Morck 162–165.

44- Castro-Hermida, J.; Gonzlez-Losada, Y.; Mezo-Menéndez, M. and Ares-Mazs, E. (2002b). A study of cryptosporidiosis in a cohort of neonatal calves. Vet. Parasitol., 106: 11–17.

45- Inpankaew, T.; Jiyipong, T.; Pinyopanuwat, N.; Chimnoi, A.; Thompson, A. and Jittapalapong, S. (2010). Prevalence and genotyping of *Cryptosporidium* spp. from dailry cow fecal samples in western Thailand. Southeast Asian J. Trop. Med. Public Health, 4(41).

46- Sturdee, A. P.; Bodley-Tickel, A. T.; Archer, A. and Chalmers, R. M. (2003). Longterm study of

Cryptosporidium prevalence on a lowland farm in the United Kingdom. Vet. Parasitol., 116: 97–113.

47- Kvac, M.; Kouba, M. and Vtovec, J. (2006). Age-related and housingdependence of *Cryptosporidium* infection of calves from dairy and beef herds in South Bohemia, Czech Republic. Vet. Parasitol., 137: 202–209.

48- Del Coco, V. F.; Cordoba, M. A. and Basualdo, J. A. (2008). *Cryptosporidium* infection in calves from a rural area of Buenos Aires, Argentina. Vet. Parasitol., 158: 31–35.

49- Arslan, M. Ö.; Sar, B.; Kara, M.; Tasci, G. T.; Ekinci, A. I. and Gündüz, N. (2012). Research on the prevalence of *Eimeria* and *Cryptosporidium* species in cows in periparturient period in Kars region. J. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, Supplement A (18): 65-70.

50- Castro-Hermida, J.; GonzJlez-Losada, Y. and Ares-Mazs, E. (2002a). Prevalence of and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain). Vet. Parasitol., 106: 1–10.

51- Castro-Hermida, J.; Pors, I.; Méndez-Hermida, F.; Ares-Mazs, E. and Chartier, C. (2006). Evaluation of two commercial disinfectants on the viability and infectivity of *Cryptosporidium parvum* oocysts. Vet. J., 171: 340–345.

52- Al-Shahery, M. N. and Hassan, M. H. (2007). Immunological and experimental study for cryptospordiosis. Iraqi J. Vet. Scien., 21(1): 129- 140.

53- Langkjaer, R. B.; Vigre, H.; Enemark, H. L. and Maddox-Hyttel, C. (2006). Molecular and phylogenetic characterization of *Cryptosporidium* and *Giardia* from pigs and cattle in Denmark. Parasitol., 89(11): 1-12.

54- Khan, S. M. (2010). Molecular characterization and assessment of zoonotic transmission of *Cryptosporidium* from dairy cattle in West Bengal, India. Vet. Parasitol., 171: 41–47.

55- Abbass, E. T. (2006). Detection of *Cryptosporidium* in bile of gall bladder of goats in Ninevah province. Iraq J. Vet. Scien., 9 (1): 149-152.

56- Al-Kelani, B. A. (2003). Epidemiological and diagnostic study for cryptospordiosis in animals and man in the village of Al-Dahab Al- Abyad. Ph. D. Thesis. College of Vetrinary Medicine, Baghdad University, (Abstract).

57- Sari, B.; Arslan, M. Ö.; Gicik, Y.; Kara, M. and Taşçi, G. T. (2008b). The prevalence of *Cryptosporidium* species in diarrhoeic lambs in Kars province and potential risk factors. Trop. Anim. Health. Prod., DOI 10.1007/s11250-008-9260-0.

58- Noordeen, F.; Faizal, A. C.; Rajapakse, R. P.; Horadagoda, N. U. and Arulkanthan, A. (2001) Excretion of *Cryptosporidium* oocysts by goats in relation to age and season in the dry zone of Sri Lanka. Vet. Parasitol., 99: 79–85.

59- Alonso-Fresán, M. U.; García- Álvarez, A.; Salazar-García, F.; Vázquez-Chagoyán, J.C.; Pescador-Salas, N. and Saltijeral-Oaxaca, J. (2005). Prevalence of *Cryptosporidium* spp. in asymptomatic sheep in family flocks from México State. J. Vet. Med. B., 52: 482–483.

60- Castro-Hermida, J. A.; González-Warleta, M. and Mezo, M. (2007). Natural infection by *Cryptosporidium parvum* and *Giardia duodenalis* in sheep and goats in Galicia (NW Spain). Small Rumin Res., 72: 96–100.

61- Al-Qaaby, S. R. (2005). Epidemiological study for cryptosporidosis and diarrhea causative agents in Al- Dewania governorate. Master Thesis. College of Vetrinary Medicine. Al-Qadsiya University.

62- Sevinc, F.; Irmak, K. and Sevinc, M. (2003). The prevalence of *Cryptosporidium parvum* infection in the diarrhoiec and non- diarrhoeic calves. Revue . Méd. Vét., 5(154): 357-361.

63- Yilmaz, H.; Tas-Cengiz, Z. and Cicek, K. (2008). Investigation of cryptosporidiosis by enzyme-linked immunosorbent assay and microscopy in children with diarrhea. Saudi. Med. J., 4(29): 526-529.