Study of *Cryptosporidium parvum* infection in calves in Thi-Qar province using mZN stain and ELISA methods

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Abstract

Cryptosporidium parvum is a zoonotic protozoan parasite with a wide range of vertebrate hosts. The present study was conducted to determine the incidence of *Cryptosporidium parvum* in diarrheatic and non diarrheatic calves in Thi -Qar province in south of Iraq.Tow hundred fecal samples were collected from suspected calves between November 2011 and February 2012 and examined by mZN stain and 94 sample were selected and examined by direct ELISA .The studied calves were divided according to sex (male and female),age (<1 months, 1- 6 months,6-12months) and clinical signs (diarrheatic non diarrheatic) .32 (16%) out of 200 and 26 (27.65%) out of 94 suspected samples were given positive result by using mZN stain and ELISA respectively. According to mZN and ELISA the infection was more prevalent (27.5% and 38.9%) and (18.6% and 31.9%) with significant differences ($p< 0.05$) in age group < 1 month and diarrheatic calves respectively, in addition to that it was highly (13.63% and 26.3%) in female but with no significant differences($P>0.05$).This study showed that *Cryptosporidium parvum* can be a risk factor, particularly for those with diarrhea syndrome and under one month age.

Introduction

Cryptosporidiosis is caused by protozoan parasites of the genus *Cryptosporidium*, in which there are 18 'valid' species. In livestock, *C. parvum, C. andersoni, C. baileyi, C. meleagridis and C. galli* have been reported to cause morbidity and outbreaks of disease (11). *Cryptosporidium parvum* causes scour in young, un weaned mammalian livestock, however, weaned and adult animals can also become infected. Signs range from a mild in apparent infection to severe scouring, and the young, old or immuno-compromised are most susceptible(14**)***Cryptosporidium parvum* is an apicomplexan parasite that causes the diarrheal disease cryptosporidiosis in humans and economically important food animals throughout the world (12). It has, become a major economic concern for livestock producers and public health officials (5). Cryptosporidiosis in ruminants is said to be on the increase (6) and it is now considered a major disease and one of the main causes of morbidity and mortality in newborn livestock (7,25). The disease in calves, children, lambs and goat kids is characterized by diarrhea, anorexia, abdominal pain, apathy and depression. It has been suggested that destruction of the intestinal epithelia by the parasite may also increase susceptibility to other enteric pathogens (7,19).The disease is transmitted by the thick walled oocyst form which is remarkably resistant to common disinfectants and routine chlorination of drinking water. Person to person transmission specially among children is common , *Cryptosporidium* oocysts are prevalent in surface waters, extremely resistant to commonly used disinfectants, and generally survive for several months in aquatic environments (24). *Cryptosporidium* has little or no host specificity and animals such as rodents, cattle, and domestic pets serve as a reserve for zoonotic transmission to human , Such transmission occur either by direct contact or by contamination of water supplies with fecal matter (7) There was no previous study conducted to demonstrate the *Cryptosporidium parvum* infection in calves in Thi-Qar province specially by

using ELISA method, so the aims of study were:

- Using of ELISA method in comparing with mZN stain to detect the incidence of infection of *Cryptosporidium parvum* in calves ,in addation to determine the effect of sex, age and clinical signs on this incidence.

Materials and Methods.

Sample collection.

Fecal samples were collected from 200 calves in Thi-Qar province in south of Iraq , of different sexes (88 males and 112 females) and ages (<1 months, 1- 6 months,6-12months) during the period from November 2011 to February 2012.

5-10 gm of each fecal sample were collected in sterile disposable plastic containers, and the data were recorded in special questionnaires sheet for each sample including ,age, sex, clinical signs, color and smell of feces ,date, and district. All samples were transported under cold conditions to laboratory and each sample was divided into two parts (one part in 10% formal-saline for ELISA method and other without preservative for examination by mZN stain).

Modified Zeil Nelson examination.

The samples were examined by mZN stain as described previously (28) that briefly, moderately thick smear was prepared, the slide was immersed in cold strong carbol-fuchsin and it was stained for 15 minutes, rinsed in tap water and then the slide decolorized in 1% acid methanol for 10–15 seconds with over detained must be avoided, rinsed again, and counterstained with 0.4% malachite green or methylene blue (two smears were prepared) for 30 seconds, rinsed and air-dried the slide. Microscopical examination was done using a 100X magnification.

ELISA examination.

94 out of 200 fecal samples were subjected to ELISA examination to detect the *Cryptosporidium parvum* antigens by used a commercial ELISA kit (Eruoclone S.P.A.code L11113.Italia). these samples were processed according to the manufacturer's recommendations.

Statistical analysis. Chi-Square (X2) was used for detect statistical difference of data prevalence of disease and effect of other factors .Results considered significant when the p-value was less than 0.05.

Results

1- Cryptosporidiosis according to mZN stain

A- Incidence rate of *C. parvum* in calves by using mZN stain method.

32(16%) out of 200 calves' fecal samples were positive according to examination with modified Zeil Nelson stain distributed in different region of south ,middle and north of Thi-Qar province .

B- Incidence of *C. parvum* according to the sex:

Ult Highly positive result of infection were seen among females 20(17.85%) when compare with males 12(13.63%) but with no significant differences (p>0.05) (Table 1).

Table(1): Incidence of *C. parvum* according to the sex

Sample	Sex	Total No.	No of positive	Percentage(%)
Calves	Male	88	12	13.63
	Female	112	20	17.85
	Total	200	32	16

C- Incidence rate of *C. parvum* in different age groups of diarrheatic and non diarrheatic calves .

According to symptom of diarrhea the infection of Cryptosporidiosis was detected in 28(18.66%) and 4 (8%) of 150 diarrheatic and 50 non diarrhoeic calves

respectively ,the intensity of oocysts was higher in diarrheatic animals than in non diarrheatic with significant differences ($p<0.05$).The infection was given highest percentage (27.5%) in calves under(<1 month) age group when compared with other age groups. (Table2)

Table(2): Incidence rate of *C. parvum* in diffe.rent age groups of diarrheatic and non diarrheatic calves

Age groups (month)	No.of examined cases	No.&% of positive cases	Diarrheatic calves		Non diarrheatic calves	
			Examined	Positive %	Examined	Positive %
<1	80	22 (27.5%)	60	20 (33.33%)	20	2 (10%)
1 -6	60	6 (10%)	50	5 (10%)	10	1 (10%)
6-12	60	4 (6.66%)	40	3 (7.5%)	20	1 (5%)
Total	200	32 (16%)	150	28 (18.66%)	50	4 (8%)

2-Cryptosporidiosis according to ELISA method.

A- Incidence of *C. parvum* in calves by using ELISA method

26(27.65%) out of 94 fecal samples were given a positive result according to ELISA method.

B- Incidence of *C. parvum* according to the sex using ELISA method.

As same as of mZN stain the females recorded a high percentage 16 (28.57%) in contrast with males 10(26.31 %) with no significant difference ($p<0.05$) (Table 3).

Table (3): Incidence of *C. parvum* according to the sex using ELISA method

Sex	Total No.	No. of positive	Percentage (%)
Male	38	10	26.31
Female	56	16	28.57
Total	94	26	27.65

C- Incidence of *C. parvum* infection in different age groups of diarrheatic and non diarrheatic calves.

C. parvum infection was detected in 23 (31.94.%) and 3 (13.63%) of 72 diarrhoeic and 22 non diarrhoeic calves respectively ,the intensity of oocysts was higher in diarrhoeic animals than in non diarrheic, statistically, an association between present of diarrhea sing and the infection with *C. parvum* at level $p<0.05$. In connection to the age groups the rate of infection was highest 16(38.09%) in under one month age group when compared with other studied groups with significant differences ($p<0.05$). (Table 4)

Table (4): Incidence of *C. parvum* infection in different age groups of diarrheatic and non diarrheatic calves.

Age groups (month)	No. of examined cases	No.&% of positive cases	Diarrheatic calves		Non diarrheatic calves	
			Examined	Positive %	Examined	Positive %
<1	42	16 (38.09%)	32	14 (43.75%)	10	2 (20%)
1 -6	28	6 (21.42%)	20	5 (25%)	8	1 (12.5%)
6-12	24	4 (16.66%)	20	4 (20%)	4	0
Total	94	26 (27.65%)	72	23 (31.94%)	22	3 (13.63%)

C -Evaluation of Sensitivity and Specificity of mZN stain and ELISA methods

Out of 94 fecal sample were tested, 26 sample were positive by ELISA ,only 12 sample were positive by modified zeil nelson stain. On other hand out of 81 negative by mZN stain 14 sample were positive by ELISA(Table 5).

Table (5): correlation between mZN stain & ELISA methods

Test		M zn stain test		Total
		Positive	Negative	
ELISA	Positive	12 True positive	14 False positive	26
	Negative	1 False negative	67 True negative	68
Total		13	81	94

Sensitivity =12/(12+1)X100=92%
Specificity=67/(67+14)X100=94%

Discussion

Cryptosporidium parvum is an intestinal protozoan parasite and primary pathogen causing acute diarrhoea in calves and children (15)The most evident symptom of *Cryptosporidiosis* is diarrhoea ,and furthermore may be non specific signs such as dehydration, fever, anorexia, weakness and progressive loss of condition .Diarrhoea is usually selflimiting in immunocompetent animals, however it can be life threatening in young (1-4 weeks old) and immunocompromised animals. Infected immunocompetent animals are usually asymptomatic, and are potential reservoirs for the infection of other farmed animals and humans (13).

Prevalence of *Cryptosporidium parvum* with mZN stain in calves .

This is the first study in Thi-Qar province about the prevalence of *Cryptosporidium* in calves of under one year old, the results of these study revealed that 16% of the calves were infected with *Cryptosporidium parvum*,that completely similar to result which reported by (2) in Al Qadissia province but lower than the result of (3)

who reported that 34 % of calves in Baghdad were infected with Cryptosporidiosis.Studies carried out in other countries report different percentages of prevalence. Paroxysmal Similar values to those found in the present study were reported in Tanazania ,(22) record 16.5% , in Canada (29), found a prevalence of 20%,in Argentina (38) found a prevalence of 17% , and in France, (18), reported a prevalence of 17.9%. Lower percentages have been observed by (39) in the United States (17) in Norway and (23) in Canada when they showing prevalences of 0.9%, 12%, and 13%, respectively.Higher values were observed by (10) in Tanazania ,(30) in Kawait, (35) in India, (6) in Spain, and (37) in Japan ,they were showed prevalences of 35%, 38.3% ,38.1%,, 47.9% and 93%, respectively.These discrepancies in the prevalence could be attributed to the criteria used in the selection of the study population, and different between geographical localities and the reports may reflect differences in the level of calves management practices employed a farm level, housing-related facters, single housed calves, or cleanness of the calf sleeping places,In the present study, infection with *Cryptosporidium parvum* in neonatal calves was prevalent and was also associated with clinical diarrhea and intensity of oocysts was significantly higher in diarrheatic calves than in non diarrheatic .Thus there is a significant association between *Cyrptosporidium parvum* infection and diarrhea, these result in agreement with many other studies like (20,31,34) the detection rate of Cryptosporidiosis. in newborn diarrheic calves in this study was 28 (18.66%), that which lower than the result (51.8%) recorded by (9) in Iran(Mashhad) , and higher than those found in neonatal diarrheic calves in other parts of Iran(Kerman) 14.1% (32).The high negative percentage in diarrhoeic calves suggest the presence of other enteropathogens that could cause acute diarrhea in calves(15)The results of this study showed relationship between age and infection in which present in 27.5% under 1 month age . These results are agreement with those reported by (6,8,17,21,31,35) they reported the highest prevalence of Cryptosporidiosis in calves between one month old. It could, therefore, be concluded that infection took place immediately after birth. The initial exposition to infective oocysts seems to take place in the maternity pen, or immediately after being transferred to the cowshed. It indicates a heavy environmental contamination in calves area.The study referred to that ,there is no significant association between gender and *Cryptosporidium* infection at ($p<0.05$), this result is in agreement with another studies such(36,40) ,which may be explained by the fact that the both sex are exposed to the similar source of infection, but (3) in Baghdad pointed to that there is significant differences in infection between males and females.

Prevalence of *Cryptosporidium parvum* with ELISA in calves .

the result of this study showed that the cryptosporidium corpoantigen was found in 27.65% (26/94) of calves samples were tested ,these suggested of the sheding of cryptosporidium oocycts in environment from bovine infection and possibility of human infection in thi qar province this result similar to (27.3%) by (34) in turkey,higher values recorded in other contries in calves ,54% by (31) and 38% by (27) in Tanazania,(42.8%) by (16) in Zambia, and (4) record prevalence of 32.3%in calves in Nigeria.Lower results record 2.4% (39) and5.3% (22)in Uganda ,these differences attributed to environmental conditions ,size of samples and season of studies. The present study showed association between clinical diarrhea and cryptosporidium infection c parvum was detected in 23(31.94%) and 3(13.63%) of 72 diarrhoic and 22 non diarrhoiec calves, respectively,the intensity of oocysts was significantly higher in diarrhoeic calves tha n in non diarrhoeic calves,in this study the magirity of positive were less than one month age

(43.75%).Several other studies also observed ahigher prevalence of c *parvum* among diarrhoeic calves as compared with non diarrhoeic calves (6,18,26,37) this results supported by (8) which found that this parasite is the primary cause of acute diarrhea in newborn calves .this results disagree with (4) in Nigeria who showed no significant difference between diarrhoeic and non diarrheic calves. Cryptosporidiosis is associated with age that agreement with another studies (20,33,30) which this may attributed to the immature immunity,factors related to variation in age groups examind, or sufficient feed with colstrum (34) this finding is in contrast with (1)in Malisia who showed no statistical difference in ages of calves with infection. This study showed no significant differences between sex and this indicates both sex live in same conditions(4).

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دراسة الأصابه بطفيلي الـ *Cryptosporidium parvum* في العجول في محافظة ذي قار بأستخدام طريقتي صبغة زيل نلسون المحورة والأليزا

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الخلاصة

يعد طفيلي الـ *Cryptosporidium parvum* من الطفيليات الابتدائية المشتركة الإصابة بين الحيوان والإنسان بحيث تصيب طيف واسع من المضائف الفقرية.أجريت هذه الدراسة لتحديد مدى حدوث الإصابة بطفيلي الـ *C. parvum* في العجول في محافظة ذي قار في العراق.تم جمع 200 نموذج براز من عجول مشكوك بإصابتها خلال الفترة من بداية شهر تشرين الثاني 2011 وحتى نهاية شباط 2012، حيث فحصت أولا باستخدام صبغة زيل نلسون المحورة (mZN)، بعدها اختير 94 نموذج براز وتم فحصه بطريقة الاليزا (ELISA).تم تقسيم العجول المدروسة حسب الجنس (ذكور وأناث) وحسب العمر(أقل من شهر ومن شهر الى 6 اشهر ومن 6 اشهر الى 12 شهر) وحسب العلامات السريريه (وجود الاسهال وعدم وجود الاسهال).ظهر ان 32(16%) من أصل 200 و 26(27.65 %) من أصل 94 من النماذج المفحوصة قد أعطت نتائج موجبة للإصابة باستخدام صبغة زيل نلسون المحوره والاليزا حسب التوالي.وطبقا لطريقتي صبغة زيل نلسون المحوره والاليزا فقد كانت الاصابة اكثر حدوثا (27.5 % و 38.9%) و (18.6% و 31.9%) وبفرق مهم احصائيا ($p<0.05$) في العجول بعمر اقل من شهر واحد والتي أظهرت علامات الإسهال وحسب الترتيب ، كما ان الإصابة في الإناث (13.63% و26.3%) أعلى مما علية في الذكور ولكن بدون فرق معنوي ($P>0.05$).لقد بينت الدراسة الحالية إن الإصابة بطفيلي الـ *C. parvum* قد تكون عامل خطورة خاصة في العجول التي تقل أعمارها عن الشهر والتي ظهرت فيها علامات الإسهال