EVALUATION OF *CRYPTOSPORIDIUM PARVUM* INFECTION IN CALVES UNDER ONE YEAR WITH SPECIAL EMPHASIS TO AGE AND GENDER IN BAQUBAH-DIYALA PROVINCE, IRAQ

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ABSTRACT

The aim was to evaluate distribution of cryptosporidium parvum (*C. parvum*) among calves under one year with special emphasis to age and gender in Baqubah-Diyala province, Iraq. Fifty calves suffering from diarrhea were included. Stool samples were collected for diagnosis of *C. parvum* by Ziehl-Neelsen (hot and cold) staining techniques. A total of 66% of diarrheic calves was infected with *C. parvum*. Significant difference as well as correlation regarding the *C. parvum* oocysts scoring was reported between ZN cold technique and ZN hot staining procedure (P value=0.000). Good agreement (kappa =0.780, p value= 0.000), was reported between ZN hot staining procedure and ZN cold technique for diagnosis of *C. parvum* oocysts in calves. The main affected age group of calves was (1-2) month, in which (46%), was infected with *C. parvum*. Female was infected more than males, (34%). Males have a risk of getting infection at (1.948) time than female. No significant correlation was reported between positivity of *C. parvum*, age group and gender of calves.

In conclusion, the prevalence of *C. parvum* appear to be elevated. Although males were infected approximately twice time than females, the susceptibility of calve for *C. parvum* infection was independent from age and gender.

Key words: cryptosporidium parvum, Calves, prevalence, Iraq.

INTRODUCTION

Cryptosporidium is intracellular protozoan parasite that live in the gastrointestinal tract of mankind and many other vertebrates animals including mammals, reptiles, birds and fish (Da'as, 2010). Cryptosporidiosis is one of the most common causes of calf scour (The Moredun Foundation, 2014). It is caused by a parasite called Cryptosporidium and is usually seen in young calves less than six weeks old (Avendaño *et al.*, 2018). Symptoms include diarrhoea, dehydration, loss of appetite, fever and abdominal pain. There are four species of the Cryptosporidium para-

site which infect cattle, however *Cryptosporidium parvum* (*C. parvum*) is the most common species detected in calves less than six weeks of age (Xiao and Cama, 2018). This species of Cryptosporidium can also infect humans. Calves become infected with Cryptosporidium when they ingest *C. parvum* oocysts (eggs). These oocysts reside in the environment in bedding, pasture, soil and drinking water. They are very infectious, with only ten oocysts required to cause disease in susceptible calves (Xiao and Cama, 2018). Considering an infected calf can spread billions of eggs, it is easy to see why the disease spreads so quickly on farm. It is not only young calves with clinical signs of the disease, such as diarrhoea, that are shedding these oocysts into the environment. Infected calves at six to seven weeks of age may show no clinical signs of infection even though they can still produce oocysts. Adult cattle can also act as potential reservoirs for the *C. parvum* parasite, they can also shed oocysts but do not necessarily show any symptoms of the disease (Xiao *et al.*, 2015).

Cryptosporidiosis is a diarrheal disease caused by microscopic parasites of the genus cryptosporidium (Grothen *et al.*, 2017). The parasite is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very resistant to chlorine disinfectants. Both the disease and the parasite are commonly known as "Crypto (Suler *et al.*, 2016). Current evidence indicates that ruminants are a reservoir of zoonotic Cryptosporidium from where humans get infected by contaminated food and water or through direct contact with livestock, for example animal handlers (Robertson *et al.*, 2010 ; Grothen *et al.*, 2017).

This study was designed to investigate the distribution of cryptosporidium in diarrheic calves in Baqubah-Diyala province; evaluate the agreement between ZN hot and cold techniques for diagnosis of cryptosporidium in diarrheic children and calves and finally, evaluate the possible effect of age and gender as a possible risk factors for cryptosporidiosis in children and calves.

SUBJECTS AND METHODS

Study area and study population

This study was conducted on 50 calves less than one year suffering from gastrointestinal illness, living in Baqubah city-Diyala province. This study was conducted according to the principles of the Helsinki declaration. Duly filled consent forms were obtained from all owners participating in the study. Approval of the

ethical review committee of the college of veterinary medicine, Diyala University, Iraq was obtained before initiating the study.

Stool samples From Calves

The stool samples were collected from November 2016 to June 2017. Samples were labeled with details of age, sex, place and date of collection. Animal was sampled once only and the collected samples were transported to the laboratory for further processing. The inclusion criterion was diarrhoea, defined as passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual (Adamu, 2010). The sample was mixed again, and the specimen containers were sealed well. All samples were reinforced with parafilm, the container was inserted in a plastic bag, and samples were stored at 4 ºC in the clinical pathology laboratory at college of veterinary medicine, Diyala university.

Diagnosis of *C. parvum* in stool samples

Two staining modalities; Ziehl–Neelsen staining (ZN), (hot and cold) techniques was applied (Rekha *et al.,* 2016).

Data Analysis

Demography and cross tabulation were calculated by Statistical analysis using SPSS for windows TM version 17.0. Chi square was used to verify possible association between infection and exposure with different factors. Values were considered to be statistically significant when the p-value obtained was less than 0.05. The concordance of the Zn hot and by cold staining techniques was studied using the Cohen's kappa index of agreement. The level of confidence limits was 0.095 and here is one possible interpretation of Kappa value 15. Poor agreement= Kappa value less than 0.20 (b) Fair agreement= Kappa value 0.20 to 0.40; Moderate agreement= Kappa value 0.40 to 0.60 (d) Good agreement= Kappa value 0.60 to 0.80; Very good agreement= Kappa value 0.80 to 1.00 (Al-Ezzy, 2016).

RESULTS AND DISCUSSION

Current study revealed that the frequency of *C. parvum* infection in calves suffered from diarrhea was 33/50, (66%), while the negative infection among diarrheic calves was 17/50, (34%) as shown in table 1 and illustrated in Figures 1 and 2.

Regarding the prevalence in calves, current study reported that *C. parvum* was recovered from (66%) of diarrheic calves which come closely to that reported by Bhat *et al.* (2012), 65.71%. Current results lower than that reported in Tunisia

(86%) (Soltane *et al.*, 2007), Canada (78%) and come closely to the prevalence in Argentina (67%) (Trotz-Williams *et al*., 2007 ; Garro *et al*., 2016); In Egypt was 65.7-74.2% (Sim *et al.*, 2015). The prevalence in Myanmar was 56% among calves of less than 6 months (Bawm *et al.*, 2014). High prevalence rates were also reported in Iran (100%). In Sweden, the prevalence was 52% in calves (Silverlås *et al.*, 2009); In Canada, (32%) of calves were infected (Budu-Amoako *et al.*, 2012); Germany (41.3%) (Gillhuber *et al.*, 2014); Nigeria (33%) (Faleke *et al.*, 2014), Tanzania (35 %) (Swai and Schoonman, 2010) and Brazil (45 %) (Almeida *et al.*, 2010). In Egypt, the prevalence of human infection was 49.1%, while in ruminant 32.2% (Helmy *et al.*, 2013) while in south Indian dairy calves was 40.75% (Venu *et al*., 2013).

The difference in rate of infection may attributed to attributed to differences in stocking rate as well as the husbandry system of livestock production in these countries, the difference in climatic condition and seasonal; breed and age variation also play a role as well (Fikre *et al.*, 2017).

As shown in Table 2, both of ZN cold score technique and ZN hot staining were identical in diagnosis of negative *C. parvum* among diarrhea cases of calves, 17/50, (34%). A total of 10/50, (20%), of cases were recorded as positive with low and 11/50, (22%) moderate scores in both techniques and 4/50, (8%) were recorded as positive with heavy score in both techniques. A total of 3/50, (6%) of cases were reported as having moderate score of *C. parvum* oocysts using ZN cold technique and have heavy oocysts score using ZN hot staining procedure.

A total of 2/50, (4%) of cases were reported as have low score of *C. parvum* oocysts using ZN cold technique, while they have moderate score in ZN hot staining procedure. on the other hand, 1/50, (2%) have low score of *C. parvum* oocysts using ZN cold technique and have heavy oocysts score using ZN hot staining procedure. A total of 2/50 of cases reported with moderate score in ZN cold staining procedure have low score (1/50, 2%) and heavy score (1/50, 2%) when screening via ZN hot staining technique. A total of 3/50 of cases reported with moderate score in ZN cold staining procedure have low score (2%) and moderate score (4%) when screening via ZN hot staining technique.

Significant difference as well as correlation regarding the *C. parvum oocysts* scoring was reported between ZN cold technique and ZN hot staining procedure (P value=0.000). Good agreement (kappa= 0.780, p value= 0.000), was reported be-

tween ZN hot staining procedure and ZN cold technique for diagnosis of *C. parvum* oocysts.

In current study, significant correlation regarding the detection and scoring of *C. parvum* oocysts among calves was reported between ZN cold and ZN hot staining procedure, with obvious differences in scores which come in contrary with previous report by (Abdel-Rady and Sayed, 2008), who found significant difference in rate of detection between them without utilizing of any scoring system. Current result reveal good agreement (kappa=0.780, p value=0.000) between ZN hot staining procedure and ZN cold for diagnosis of *C. parvum* oocysts in calves. This come in line with (Abdel-Rady and Sayed, 2008), reported that ZN hot and cold techniques was reliable, easy and relatively simple with low cost and appropriate for detection of oocysts with low numbers. The discrepancy between ZN hot and cold techniques in scores may be attributed to the length of sample treatment and processing, presence of fecal debris that hidden some of the oocysts beside the effect of heating which facilitate the penetration of the stain to the oocysts wall.

Cold ZN is simple and easy to learn and practice, it is economical and less cumbersome, is suitable under field conditions, and can be practiced even in remote areas and at periphery where laboratory facilities are limited. There are more practical advantages of cold ZN like, no need of heating in the staining procedure and eliminated the need for separate decolorizing step (requires only two reagents in the staining procedure) (Weldu *et al*., 2013).

As shown in table 3, the minimum age of calves presented with diarrhea was (1 month), while the maximum age was (8 month). The main affected age group was (1-2) month, in which, (46%), was infected with *C. parvum*, followed by (3-4) month, in which, (18%) was infected with *C. parvum*. The third age group was (7-8), in which, (2%) was infected. Neither significant difference (p value=0.241), nor significant correlation was reported between positivity of *C. parvum* and age group of calves (p value=0.917).

Current study revealed that (18%) of calves of age (3-4) month infected with *C. parvum* which lesser than that reported by Swai and Schoonman (2010), at the same age group (27%) and (Akinkuotu *et al.*, 2014) reported that 78.1% of infected calves at this age group. The third age group was (7-8) month, with (2%) was infected which is obviously lower than that reported in Iran (Ranjbar and Fattahi, 2017) revealed that 11.3% of calves with 61-180 day of live were infected with *C. parvum* and Akinkuotu *et al.* (2014) reported that 36.4% of infected calves at

age group of more than three months to one year. In Nigeria, Ayinmode and Fagbemi (2010) reported 27.4% at the age of less than 6 months. In general these results come closely to that reported by Bawm *et al*. (2014), mentioned that *Cryptosporidium spp.* was detected in (76.8%) in calves less than 6 months old.

In current study, No significant correlation was reported between positivity of *C. parvum* and age group of calves (p value=0.917) which come in line with (Fikre *et al.,* 2017). This come in contrary with that reported by Ayinmode and Fagbemi (2010) and Bawm *et al.* (2014), who found that calves of less than 6 months were susceptible for infection with *Cryptosporidium spp.* two times than older age. Calves may become infected directly after birth from infected post parturient dam or from contaminated floor due to heavy load of excreted oocysts from individual calves or cow in the cowshed; indicating a heavy environmental contamination in the calving area (Tiranti *et al.*, 2011). Infected calves play a major role in environmental contamination in 1-12 days after birth due to heavy load of oocysts shedding. Infection usually peaked at one to two weeks of age, with a shedding duration of one to two weeks (Tiranti *et al.*, 2011).

As shown in table 4, Male presented with diarrhea was 27/50, (54%), in which (32%) was infected with *C. parvum*. Females represent (46%) of children presented with diarrhea, in which, (34%), was infected with *C. parvum*. Neither significant difference (p value=0.276), nor significant correlation was reported between positivity of *C. parvum* and gender of calves (p value=0.285). Males have a risk of getting infection at (1.948) time than female.

Among calves, male presented with diarrhea was (54%), in which (32%) was infected with *C. parvum*. Females represent (46%) of calves presented with diarrhea, in which (34%), was infected with *C. parvum.* Current results were higher than that reported in Iran by (Ranjbar and Fattahi, 2017) revealed that 14.8% of male calves were infected with *C. parvum* compared with (16.9%) for females. This finding come in accordance with (Fikre *et al.*, 2017), reported cryptosporidium infection in 26.7% among females compared with 20.8% of female calves. In India Mallinath *et al.* (2009) reported 6% infection among females calves compared with 3.67% among males calves. In south western Nigeria, Ayinmode and Fagbemi (2010), reported that reported 38.1% infection among females calves compared with 17.1% among males calves.

No significant correlation was reported between positivity of *C. parvum* and gender of calves (p value=0.285) which come in line withSoltane *et al.* 2007, Fikre

et al. (2017) and Ranjbar and Fattahi (2017). Males have a risk of getting infection at (1.948) time than female. These results come closely to that reported by Almeida *et al.* (2010) and Bawm *et al.* (2014).

In conclusion, the prevalence of *C. parvum* appear to be elevated. Although males were infected approximately twice time tan females, the susceptibility of calve for *C. parvum* infection was independent from age and gender. As good agreement between them, both hot and cold techniques can be used alternatively for diagnosis of *C. parvum* and give accurate results.

Table 1. Frequency of *C. parvum* infection in calves

Total No. of Examined calves	*C. parvum* infection	Frequency
50	Negative	17(34%)
	Positive	33(66%)

Table 2. Agreement between ZN cold score and ZN hot staining technique for detection of *C. parvum* infection in calves

ZN cold Score	ZN hot score in calves				
	Negative	Low	Moderate	Heavy	Total
Negative	17(34%)	0(0%)	0(0%)	0(0%)	17(34%)
Low	0(0%)	10(20%)	2(4%)	1(2%)	13(26%)
Moderate	0(0%)	1(2%)	11(22%)	1(2%)	13(26%)
Heavy	0(0%)	1(2%)	2(4%)	4(8%)	7(14%)
Total	17(34%)	12(24%)	15(30%)	6(12%)	50(100%)
$\chi 2$	87.253				
P value	.000				
R	0.873				
P value	.000				
Kappa	0.780				
P value	.000				

Table 3. Age as a possible risk factor associated with *C. parvum* infection in calves

Age of Calves (months)		
Minimum	1	
Maximum	8	
Mean ±Std. Deviation	2.38 ±1.29	
Age (months)	Cryptosporidium infection In calves	
	No. of Examined Calves	positive
1-2	35(70%)	23(46%)
3-4	13(26%)	9(18%)
5-6	1(2%)	0(0%)
7-8	1(2%)	1(2%)
Total	50(100%)	33(66%)
χ^2	6.732	
P value	0.241	
R	0.015	
P value	0.917	

Table 4. Gender as a possible risk factor associated with *C. parvum* infection in calves

Gender	Cryptosporidium Infection In Calves Total		
	No. of Examined Calves	Positive	
Male	27(54%)	16(32%)	
Female	23(46%)	17(34%)	
Total	50(100%)	33(66%)	
χ^2	1.188		
P Value	0.276		
R	0.154		
P Value	0.285		
Risk Estimate	95% Confidence Interval		
Odds Ratio For Gender (Male / Female)	Value	Lower	Upper
	1.948	0.583	6.509

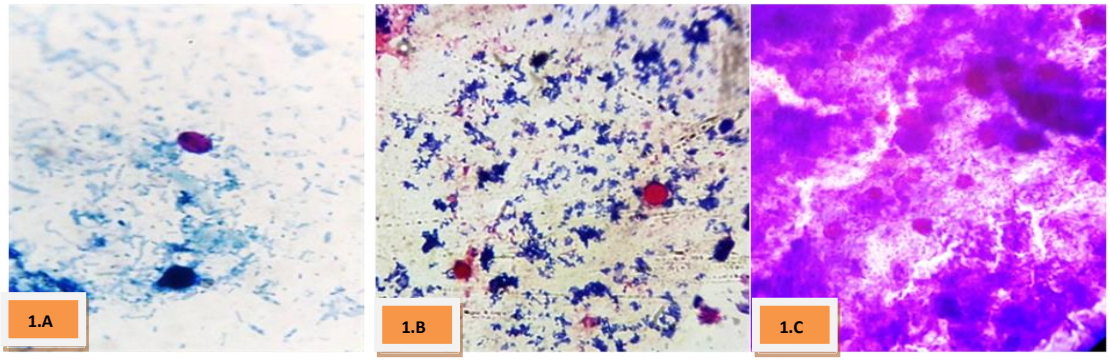

Figure 1. *C. parvum* oocysts detected in diarrheic stool by ZN hot staining technique In calves: A. low score, B. moderate score, C. heavy score (100x)

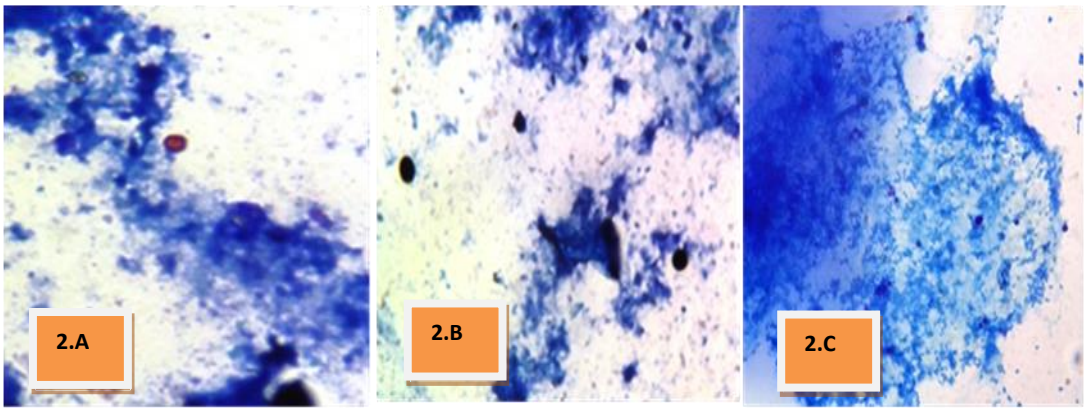

Figure 2. *C. parvum* oocysts detected in diarrheic stool by ZN cold staining technique, in calves: A. low score, B. moderate score, C. heavy score (100x)

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تقييم الإصابة بالكربتوسبوريديم في العجول تحت سنة من العمر مع التركيز بشكل خاص على العمر والجنس في بعقوبة – محافظة ديالى - العراق

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المستخلص

يهدف البحث الى تقييم انتشار *C. parvum* بين العجول تحت عمر السنة المصابه بالاسهال مع التركيز على العمر والجنس في بعقوبة - محافظة ديالى، فضلا عن تقييم التوافق بين طريقة الصبغ ZN البارد والحار. تضمنت الدراسة 50 عجلا مصابا بالاسهال اخذت منها عينات البراز لغرض تشخيص *C. parvum* وكانت نسبة الاصابة 66%. سجل اختلاف معنوي واضح وعلاقة معنوية بين طريقتي الصبغ البارد والحار من حيث درجة الاصابة (P value=0.000)،وسجلت الدراسة توافقا جيداً بين طريقتي الصبغ البارد والحار من حيث تحديد درجة الاصابة (kappa =0.780).

كانت العجول بعمر 2-1 شهر هي الاكثر تاثرا إذ كانت نسبة الاصابة (46%)، وكانت نسبة الاصابة بين الاناث اكثر من الذكور (34%) في حين ان الذكور اكثر عرضه للاصابة من الاناث (1.948). لا توجد علاقة معنوية بين الاصابة بـ *C. parvum*، وعمر العجول وجنسها.

تستنتج هذه الدراسة ان الاصابة بـ *C. parvum* سجلت ارتفاعا ملحوظا على الرغم من ان الذكور اكثر عرضه للاصابه بالضعف من الاناث الا ان قابليه الاصابه ليس لها علاقه بعمر العجول أو جنسها.

كلمات مفتاحيه: الكربتوسبوريديم بارفم، انتشار، العجول، العراق.