OPEN ACCESS

J. Adv. Vet. Anim. Res., 1(1): 8-10, March 2014
http://bdvets.org/JAVAR/javar-index.html

JAVAR Journal of Advanced Veterinary and Animal Research

RESEARCH ARTICLE

Direct ELISA aided coprological diagnosis of *Cryptosporidium parvum* infection in diarrheic neonatal calves in Mosul city, Iraq

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ABSTRACT

This study was conducted for the detection of *Cryptosporidium* (*C.*) *parvum* infection in neonatal calves. A total of 220 fecal samples (diarrheic 110 and non-diarrheic 110) of neonatal calves were collected from Mosul city, Iraq over a period of 16 months from November 2010 to March 2012. The age of the calves ranged from 1 to 30 days. All the fecal samples were analyzed by capture direct ELISA. The infection was found in 29.0% (n=32/110) of the diarrheic calves. The infection was mostly prevalent (p<0.001) in the calves of three weeks of age. No *C. parvum* infection could be detected in the non-diarrheic animals.

Keywords:

Calf, coprological diagnosis, *Cryptosporidium parvum*, diarrhea, direct ELISA

Received : 19 January 2014, **Revised**: 1 February 2014,
Accepted : 3 February 2014, **Published Online**: 15 February 2014.

INTRODUCTION

Neonatal calf diarrhea is considered as one of the most important diseases of neonatal calves that causes huge economic losses in the cattle industry (Millemann, 2009). The causative agents responsible for diarrhea in the calves are bacteria (mainly *Escherichia coli*), parasites (essentially *Cryptosporidia*) and some viruses (particularly rotaviruses and coronaviruses) (Radostits et al., 2007).

Cryptosporidium spp. is an apicomplexan intestinal protozoan parasite with worldwide geographical distribution. These are found in both wild and domestic animals. There are currently 20 recognized species of *Cryptosporidium*, which have been isolated from a large variety of hosts, including all amphibians, fishes, reptiles, birds, mammals (Chalmers et al., 2009). Four species of *Cryptosporidium* have been identified which infect cattle and these include *C. parvum*, *C. bovis, C. ryanae* and *C. andersoni* (Fayer et al., 2007). *C. parvum* may infect a wide range of hosts such as man, ruminants and many other animals (Morgan-Ryan et al., 2002). Diagnosis of this organism is usually based on the detection of oocysts in the feces by a number of different diagnostic techniques, for example, flotation of oocysts and modified acid-fast staining of oocysts (Cole et al., 1999). Oocysts have also been detected by using an immunofluorescence assay and flow cytometry (Cole et al., 1999), enzyme linked immuno-srobent assay (ELISA) (Werner et al., 2004), polymerase chain reaction (PCR) and loop-mediated isothermal amplification of DNA (Bakheit et al., 2008).

In Iraq, *Cryptosporidium* oocysts have been detected previously from fecal samples of several animals including goats (Al-Bakray, 2002), horses (Butty, 2011) and calves (Finish and Tawfeek, 2013). However, the data presented in all these studies were based on crude diagnostic techniques, for example, flotation of oocysts

Table 1. Occurrence of *C. parvum* infection in diarrheic neonatal calves in Mosul city, Iraq

Calf Type	Capture Direct ELISA Positive (%)				Total Positive Cases (%)	*p* value (χ2 test)
	Age of calf in week(s)					
	1st (n=27)	2nd (n=27)	3rd (n=28)	4th (n=28)		
Diarrheic calves (n=110)	9/27 (28.1)	0/27 (0.0)	**23/28 (71.9)**	0/28 (0.0)	**32**/110 **(29.0)**	**0.000***** (*p*<0.001)
Clinically healthy calves (n=110)	0/27 (0.0)	0/27 (0.0)	0/28 (0.0)	0/28 (0.0)	0/110 (0.0)	-

***Occurrence of *Cryptosporidium parvum* infection is significantly higher in the diarrheic calves of 3-week of age group (p<0.001).

and modified acid-fast staining. The purpose of this study was to diagnose the *C. parvum* in neonatal calves in Mosul city, Iraq by using a more precise diagnostic technique i.e., direct capture ELISA.

MATERIALS AND METHODS

Sampling: A total of 220 fecal samples were collected from neonatal calves of local breeds of both sexes. The sampling was done for a period of 16 months starting from November 2010 to March 2012. The animals selected for sampling ranged from 1 to 30 days in age. Out of 220 samples, 110 were collected from the calves suffering from diarrhea and the remaining 110 samples were collected from non-diarrheic (clinically healthy) animals of similar description from the same localities. All the collected samples were brought to the Veterinary Teaching Hospital of College of Veterinary Medicine, Mosul University, Mosul (a northern city in Iraq located about 400 km northwest of Baghdad).

Scr**eening by ELISA:** A commercial direct ELISA kit (Bio-x Diagnostics, Belgium) was used for the detection of *C. parvum* antigen in the fecal samples. All the samples were tested according to the manufacturer's instructions. The optical density was measured at a wavelength of 450 nm using microplate reader. The net optical density of each sample was calculated by subtracting the value of each sample-well from the corresponding negative control.

Statistical analysis: The prevalence rate among the calves was analyzed using SPSS-version 10.1. Significant difference among the variables was calculated using Pearson's Chi-square test. *P* value less than 0.05 was considered as significant

RESULTS AND DISCUSSION

Results indicated that 29.0% (n=32/110) diarrheic animals were positive for *C. parvum* (Table 1). No *C. parvum* infection was detected in the non-diarrheic animals. Several different pathogenic agents have been identified in the feces and intestinal tracts of diarrheic lambs, foals and kids. In case of calves, it is difficult to diagnose the responsible organism (e.g., bacteria, virus or protozoa) involved in diarrhea (Radostits et al., 2007). However, *Cryptosporidium spp*. infections have been reported in cattle and calves in many countries such as in France (Lefay et al., 2001), Sweden (Bjorkman et al., 2003), Turkey (Değerli et al., 2005), and Nigeria (Ayinmode and Fagbemi, 2010).

In Iraq, *Cryptosporidium spp*. has been reported previously in different species, with a prevalence rate ranging from 32 to 43.5% (Finish and Tawfeek, 2013; Al-Alousi and Mahmood, 2012). The discrepancy between our results and those of the studies done by Finish and Tawfeek, (2013) and Al-Alousi and Mahmood (2012) could be attributed to geographical differences and differences in timing of sample collection and methods of the laboratory diagnosis. In our study, diagnosis of *C. parvum* infection in neonatal calves was based on the results of direct capture ELISA.

Till today, a variety of methods have been developed to detect *Cryptosporidium spp*. infection in feces, such as flotation of oocysts and modified acid-fast stain (Cole et al., 1999), immunofluorescence assay and flow cytometry (Cole et al., 1999), ELISA (Werner et al., 2004), PCR and loop-mediated isothermal amplification of DNA (Bakheit et al., 2008). Among these, ELISA is considered as a highly sensitive and specific method for the detection of *Cryptosporidium spp*. antigens (Sevinç et al., 2003). In our study, the infection was detected in 28.1% (n=9/32) and 71.9% (n=23/32; *p*<0.001) samples of the diarrheic calves in 1st and 3rd weeks postnatally, respectively (Table 1). These findings are in support of several other findings where Cryptosporidiosis was found to be associated mainly with age (less than 1 month) and immune status of the calves (Lefay et al., 2001; Radostits et al., 2007).

However, in contrast to our results, using comparative studies, Lefay et al. (2001) and Sevinç et al. (2003) showed that the *C. parvum* infection was most commonly prevalent in the calves of 1-10 days of age; whereas, similar results were reported by Arsalan et al. (2001) who showed that the *C. parvum* infection rate was mostly prevalent in the calves between 1 to 3 weeks of age.

CONCLUSION

Through these findings, *C. parvum* was recognized as a serious source to cause neonatal calf scour in Mosul city, Iraq. Appropriate strategies should be taken to maintain the health status of calves.

AKNOWLEDGEMENT

This study was financially supported by the College of Veterinary Medicine, Mosul University, Mosul, Iraq.

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