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CRYPTOSPORIDIUM INFECTION IN CALVES AGED UP TO THREE MONTHS

MIŠIĆ ZORANA, KATIĆ-RADIVOJEVIĆ SOFIJA and KULIŠIĆ Z

Department of Parasitology Faculty of Veterinary Medicine, Bul. JNA 18, Belgrade, Yugoslavia

The prevalence of Cryptosporidium infection among calves aged up to three months in the Belgrade district was examined. The animals were grouped according to age, i.e., up to 15 days old, 16 to 30 days, 31 to 60 days and 61 to 90 days old. The infection was diagnosed using Sheathers sugar flotation and three staining procedures: modified Ziehl-Neelsen's, modified Kinyoun's and BBL TB Quick Stain techniques.

Among the examined calves 54.0% excreted Cryptosporidium oocysts. The majority of positive results was detected among calves aged up to 15 days (63.1%). Most of the positive animals, aged up to one month, had diarrhea. The highest number of positive cases was detected using the flotation method according to Sheather (54.0%), followed by modified the Ziehl-Neelsen method (53.2%), while the modified method according to Kinyoun and the BBL TB Quick Stain method detected a smaller number of positive animals (50.0%).

Key words: Cryptosporidium, calves, diarrhea, diagnostic methods

INTRODUCTION

Since the 1980's, *Cryptosporidium parvum* has been recognized as an important enteropathogen in many species of mammals, including human beings. Cryptosporidiosis has been accepted as a life-threatening infection mainly in young ruminants and immuno-deficient patients. Two *Cryptosporidium* species have been identified in mammals: *Cryptosporidium parvum* and *Cryptosporidium muris*. *Cryptosporidium parvum* causes a severe disease in humans and ruminants, while infection with *C. muris* is asymptomatic and so far has not been identified in humans (O'Donoghue, 1995). Experimental infection indicates a primary etiological role for cryptosporidia in the neonatal diarrhea of calves (Tzipori *et al.*, 1983; Heine *et al.*, 1984). On cattle farms the incidence of diarrhea varies considerably from none (Myers *et al.*, 1984) to more than 59.00% (Garber *et al.*, 1994). In farm animals diarrhea is frequently a multifactoral problem. In young calves *Cryptosporidum* spp. may act in connection with other agents, especially *Rotavirus*, *Coronavirus* and K99+ *Escherichia coli* (Reynolds *et al.*, 1986; Bellinzoni *et al*, 1990; McDonough *et al.*, 1994; Fagan *et al.*, 1995). In Yugoslavia, little information is available on the clinical significance and epizootiology of *Cryptosporidium* infection in calves. Prevalence varied from 25-44.2% (Cvetković and Dimitrijević, 1988) to 58.0% (Mišić *et al.*, 1999). Many

cases of diarrhea of unknown etiology in calves may be indicative of more frequent occurrence of cryptosporidial infection.

The aim of this study was to examine the prevalence of *Cryptosporidium* spp. among different age groups of calves, up to three months old, in the Belgrade district and to compare some direct methods for detection of *Cryptosporidium* oocysts in calf feces.

MATERIALS AND METHODS

Fresh stool specimens were collected from calves kept on public and private farms in the Belgrade area. Specimens were stored in a refrigerator at $+4^{o}$C.

A total of 126 calves was examined, including 65 calves aged up to 15 days, 24 calves aged 16 to 30 days, 15 aged 31 to 60 days and 22 aged 61 to 90 days.

The infection was diagnosed according to the stool examination, while the diagnostic values of one method of oocyst concentration using flotation and three staining methods were simultaneously evaluated. Sheather's sugar flotation was used for concentration of the fecal specimens (Garcia *et al.*, 1983). Three specialized staining procedures were used: Ziehl-Neelsen's and a modification of Kinyoun's acid-fast technique (Garcia and Bruckner, 1993) as well as BBL TB Quick Stain (a more rapid version of the cold stain method). A major characteristic of *Cryptosporidium* oocysts is that they are acid-fast. Once stained with the aniline dye, basic fuchsin, they are difficult to decolorize, and retain the red color even when treated with a mixture of acid and alcohol. In the Ziehl-Neelsen technique, methylene blue is the counterstain. In the modified Kinyouns acid-fast technique, brilliant green provides the necessary contrast. In the TB Quick Stain, methylene blue is used as the counterstain, but a separate decolorization step is not required, since the decolorizer/counterstain Reagent B decolorizes and counterstains the smear in one step.

The entire coverslip area of Sheathers sugar flotation was examined under high power (total magnification of x 400), whereas the stained smears were examined under oil immersion (x 1000). Oocyst size was measured using a calibrated eyepiece micrometer.

RESULTS AND DISCUSSION

Cryptosporidium oocysts were found in 68 out of 126 fecal samples.

The morphometric data derived from the measurement of oocysts indicated that the animals were infected with *Cryptosporidium parvum* because the mean oocyst sizes were between 4-5μm (Figure 1).

The majority of positive cases was detected among the calves aged up to 15 days (63.1%). In the second age group 50.0% of the calves excreted oocysts. In the fourth age group oocyst excretion was detected in 40.9%, while in the third age group 40.0% of the examined calves were positive (Table 1).

The prevalence of *Cryptosporidium spp.* varies among cattle farms and geographic locations, and is probably present in every domestic cattle herd worldwide. Comparison of the results obtained with those from similar examinations (Henriksen and Krogh, 1985; Ongerth and Stibbs, 1989; Nikitin and Pavlasek, 1990; Fagan *et al.*, 1995), indicated that *Cryptosporidium parvum* is most

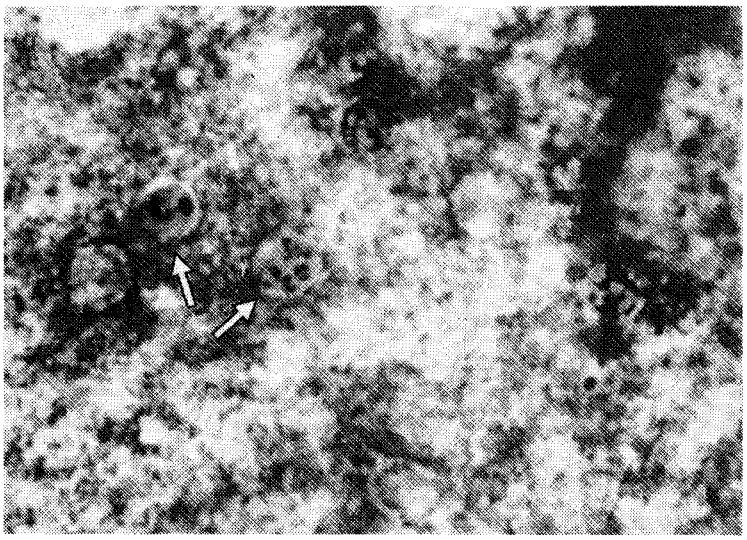

Figure 1. *Cryptosporidium* oocysts (indicated by arrow), modified Ziehl-Neelsen's technique (x 1000)

Table 1. Prevalence of *Cryptosporidium parvum* among calves aged up to three months, divided into age groups

Age group	Days	Examined	Positive	
		No	No	%
I	1 - 15	65	41	63.1
II	16 - 30	24	12	50.0
III	31 - 60	15	6	40.0
IV	61 - 90	22	9	40.9
Σ	1 - 90	126	68	54.0

often detected in calves aged up to 30 days with the peak incidence in calves aged up to 15 days.

Among the calves positive for oocyst excretion 82.4% had diarrhea. In the youngest age group the majority of positive calves also had diarrhea (97.6%). In the second age group 91.7% of positive animals had diarrhea. In the third age group there was the same percentage of positive calves with and without diarrhea, while in the fourth age group 77.8% of positive animals were without diarrhea (Table 2).

Table 2. - Prevalence of diarrhea among calves positive for *Cryptosporidium parvum*

Age group	Days	Positive with diarrhea		Positive without diarrhea		Total positive	
		No	%	No	%	No	%
I	1 - 15	40	97.6	1	2.4	41	100.00
II	16 - 30	11	91.7	1	8.3	12	100.00
III	31 - 60	3	50.0	3	50.0	6	100.00
IV	61 - 90	2	22.2	7	77.8	9	100.00
Σ	1 - 90	56	82.4	12	17.6	68	100.00

Most of the positive calves aged up to 30 days also had diarrhea. Older calves are also frequently infected, but the infection is usually asymptomatic. In most parasitized calves without diarrhea, the intensity of infection was light and none of the animals had any apparent symptoms characteristic for cryptosporidiosis. However, those animals are excreting oocysts and present an important source from which neonatal calves can become infected at a very young age. Therefore, those animals should be kept away from neonatal calves and strict control measures should be undertaken to avoid infection of calves at an age when they are clinically susceptible to *Cryptosporidium* infection (Mišić *et al.*, 2000).

Among the calves aged up to three months most positive cases were detected using the flotation method according to Sheather (54.0%), followed by the modified Ziehl-Neelsen's method (53.2%), while the modified method according to Kinyoun and the BBL TB Quick Stain method detected a smaller number of positive animals (50.0%) (Figure 2).

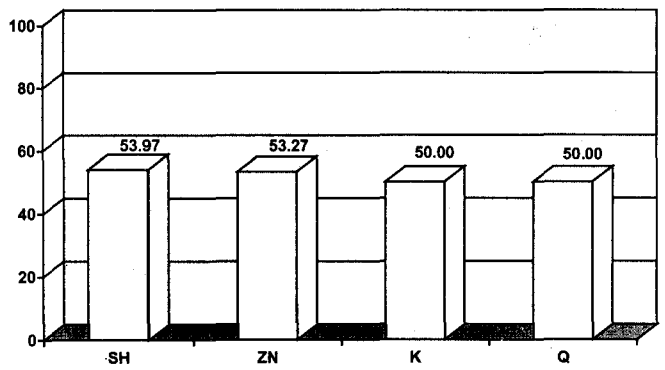

Figure 2. Comparison of the diagnostic value of four techniques used for diagnosis of *Cryptosporidium* infection in calves

The best diagnostic results were obtained using Sheather's sugar flotation and the modified Ziehl-Neelsens technique which is similar to those obtained by other authors (Moodley *et al.*, 1991; MacPherson and McQueen, 1993). All four techniques were similarly useful for the detection of *Cryptosporidium* oocysts in the feces of calves with diarrhea. In the feces of calves without diarrhea, where a smaller number of oocysts is present, the modified Kinyoun's technique and BBL TB Quick Stain were less useful.

Two techniques should be used in parallel for the diagnosis of cryptosporidiosis, as recommended by other authors (Casemore *et al.*, 1985; Lazar and Radulescu, 1989). Sheather's sugar flotation can be used for the detection and the modified Ziehl-Neelsen's technique for the confirmation of *Cryptosporidium* infection in calves.

REFERENCES

1. *Casemore DP, Armstrong M, Sands RL,* 1985, Laboratory diagnosis of cryptosporidiosis, *J Clin Pathol*, 38 (12), 1337-1341.
2. *Cvetković Lj, Dimitrijević S*, 1988, Kriptosporidioza životinja i ljudi, *Veterinarski glasnik*, 42 (6-7), 381-388.
3. *Fagan JG, Dwyer PJ, Quinlan JG,* 1995, Factors that may affect the occurrence of enteropathogens in the feces of diarrheic calves in Ireland, *Irish Veterinary Journal*, 48 (1), 17-21.
4. *Garber LP, Salman M, Hurd HS, Keefe T, Schlater JL,* 1994, Potential risk factors for *Cryptosporidium* infections in dairy calves, *Journal of the American Veterinary Medical Association,* 205, 86-89.
5. *Garcia LS, Bruckner DA, Brewer TC, Shimizu RY,* 1983, Techniques for the recovery and identification of *Cryptosporidium* oocysts from stool specimens, *J Clin Microbiol*, 18 (1), 185-190.
6. *Garcia LS, Bruckner DA,* 1993, Diagnostic medical parasitology, *American Society for Microbiology, Washington D.C.*, 528-531.
7. *Heine J, Pohlenz JF, Moon HW, Woode GN,* 1984, Enteric lesions and diarrhea in gnotobiotic calves monoinfected with *Cryptosporidium* species, *J Infect Dis*, 150 (5), 768-775.

8.Henriksen SA, Krogh HV, 1985, Bovine cryptosporidiosis in Denmark. 1. Prevalence, age distribution, and seasonal variation, *Nord Vet Med*, 37 (1), 34-41.

9.Lazar L, Radulescu S, 1989, A comparative study of coprological methods for identifying *Cryptosporidium* spp. oocysts, *Rev Ig Bacteriol,* 34 (4), 361-368.

10.MacPherson DW, McQueen R, 1993, Cryptosporidiosis: multiattribute evaluation of six diagnostic methods, *J Clin Microbiol*, 31 (2), 198-202.

11.Mišić Z, Katić-Radivojević S, Kulišić Z, 1999, Nalaz *Cryptosporidium* spp. kod goveda različite starosti, *Prvi simpozijum iz oblasti veterinarske nauke i prakse, Zlatibor, Zbornik kratkih sadržaja*, 54.

12.Mišić Z, Katić-Radivojević S, Kulišić Z, 2000, Primena zoohigijenskih mera u suzbijanju i preveniranju kriptosporidioze kod teladi, *XI Savetovanje DDD u zaštiti životne sredine, Tara, Zbornik radova*, 161-164.

13.Moodley D, Jackson TF, Gathiram V, van den Ende J, 1991, A comparative assessment of commonly employed staining procedures for the diagnosis of cryptosporidiosis, *S Afr Med J*, 79 (6), 314-317.

14.Myers LL, Firehammer BD, Border MM, Shoop DS, 1984, Prevalence of enteric pathogens in the feces of healthy beef calves, *American Journal of Veterinary Research*, 45, 1544-1549.

15.Nikitin VF, Pavlásek I, 1990, The most important parasitic intestinal infection in calves and their role in diarrhea diseases, *Vet Med (Praha)*, 35 (4), 201-206.

16.O'Donoghue PJ, 1995, *Cryptosporidium* and cryptosporidiosis in man and animals, *Inter Parasitol,* 25, 139-195.

17.Ongerth JE, Stibbs HH, 1989, Prevalence of *Cryptosporidium* infection in dairy calves in western Washington, *Am J Vet Res*, 50 (7), 1069-1070.

18.Tzipori S, Smith M, Halpin C, Angus KW, Sherwood D, Campbell I, 1983, Experimental cryptosporidiosis in calves: clinical manifestations and pathological findings, *Vet Rec*, 112 (6), 116-120.

INFEKCIJA KRIPTOSPORIDIJAMA KOD TELADI STARE DO TRI MESECA

MIŠIĆ ZORANA, KATIĆ-RADIVOJEVIĆ SOFIJA i KULIŠIĆ Z

SADRŽAJ

Ispitivana je prevalencija infekcije sa *Cryptosporidium* spp. kod teladi do tri meseca starosti na području Beograda. Istraživanje je sprovedeno prema starosnim grupama i to: kod životinja starih do 15 dana, 16 do 30 dana, 31 do 60 dana i 61 do 90 dana. Infekcija je dijagnostikovana primenom metode flotacije po Sheather-u i tri metode bojenja: modifikovane Ziehl-Neelsen-ove metode, modifikovane Kinyoun-ove metode i BBL TB Quick bojenja.

Oociste *Cryptosporidium parvum* ustanovljene su kod 54,0% ispitane teladi. Najveći broj pozitivnih grla ustanovljen je kod teladi stare do 15 dana (63,1%). Većina životinja starih do mesec dana imala je dijareju. Najveći broj pozitivnih grla otkriven je primenom metode flotacije po Sheather-u (54,0%), zatim primenom modifikovane Ziehl-Neelsen-ove metode (53,2%), dok je primenom modifikovane Kinyoun-ove metode i BBL TB Quick bojenja otkriven manji broj pozitivnih životinja (50,0%).