

## **CRYPTOSPORIDIUM INFECTION IN WEANERS, BULL CALVES AND POSTPARTURIENT COWS IN THE BELGRADE AREA**

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*This is the first study reporting on Cryptosporidium infection in weaners, bull calves and postparturient cows in Yugoslavia. The investigation was done on asymptomatic animals. The infection was diagnosed according to the stool examination, using Sheathers sugar flotation, a modified Ziehl-Neelsen technique and BBL TB Quick Stain. Cryptosporidium oocysts were detected in 31.0% weaners, 49.0% bull calves and 47.5% postparturient cows. These findings clearly demonstrate the presence of asymptomatic infection in animals in the Belgrade area and indicate the potential role of such cattle as reservoirs of parasites.*

*Key words: Cryptosporidium, weaners, bull calves, postparturient cows*

### INTRODUCTION

Cryptosporidiosis is caused by the coccidian protozoon, *Cryptosporidium* spp. Members of the genus *Cryptosporidium* are parasites of a wide range of vertebrates, including humans. Three species can be found in cattle. As the most common species, *Cryptosporidium parvum* infects epithelial surfaces, especially those along the gut. *Cryptosporidium muris* infects only the glands of the stomach (abomasum in cattle). The new species *Cryptosporidium andersoni* also infects the abomasum of cattle and is genetically distinct from *Cryptosporidium muris* (Lindsay *et al.*, 2000).

*Cryptosporidium parvum* is predominantly a parasite of neonatal calves. Older cattle generally develop a mild infection, even when not exposed previously to this parasite. However, studies are showing that many adult animals continue to produce low levels of *Cryptosporidium* spp. oocysts on a regular basis (Rhee *et al.*, 1991, Lorenzo *et al.*, 1993, Scott *et al.*, 1995, Esteban and Anderson, 1995, Fayer *et al.*, 2000). *Cryptosporidium* infection can be highly prevalent among adult cattle. Lorenzo *et al.* (1993) detected *Cryptosporidium* spp. among 71.75% of adult, asymptomatic cattle. Scott *et al.* (1995) detected *Cryptosporidium parvum* in 62.4% fecal samples from adult cattle. Fayer *et al.* (2000) detected *Cryptosporidium parvum* in 28.8% of the 7 to 9-month old cattle and 10.5% of the adult cows examined. *Cryptosporidium andersoni* was present in 12.5% adult cows in Maryland farms (Fayer *et al.*, 2000).

Adult cattle may contribute to the numbers of *Cryptosporidium* oocysts in the environment. This enhances the environmental load and serves as a source of infection for neonates, other animals and humans. In Yugoslavia this is the first study on the prevalence of *Cryptosporidium* infection among adult cattle.

#### MATERIALS AND METHODS

The prevalence of *Cryptosporidium* spp. in healthy, asymptomatic cattle was investigated in the Belgrade area.

A total of 160 animals was examined, including 29 weaners aged 3-6 months, 26 bull calves aged 6-9 months, 25 bull calves aged 9-12 months and 80 postparturient cows (31 aged 3-5 years, 41 aged 5-7 years and 8 aged 7-9 years). The cows were examined 1-10 days after partus.

Individual fecal samples were taken per rectum with plastic gloves and placed in technically sterile plastic containers. Specimens were stored in a refrigerator at +4 °C.

The infection was diagnosed according to the stool examination, using Sheathers sugar flotation, a modified Ziehl-Neelsen technique and BBL TB Quick Stain (a more rapid version of the cold stain method). Sheathers sugar flotation was used for concentration of the fecal specimens (Garcia et al., 1983). The modified Ziehl-Neelsen technique (Garcia and Bruckner, 1993) and BBL TB Quick Stain (Mišić et al., 2001) were used as specialized staining procedures, for confirmation.

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

#### RESULTS AND DISCUSSION

Positive samples were detected in 72 animals (45.0 %) out of the 160 examined cattle.

Measuring the oocysts using brightfield microscopy showed that most of the examined cattle were infected with *Cryptosporidium parvum*, because the mean size of the oocysts was around 4-5  $\mu$ m (Figure 1).

In three cases bigger oocysts were detected. They were probably oocysts of *Cryptosporidium muris* or *Cryptosporidium andersoni*, because the mean size was around 7-8 x 6  $\mu$ m. Infection with *Cryptosporidium muris* usually causes no overt illness, but retards acid production. Therefore, as concerns the dairy industry, protein digestion in the abomasum probably is retarded, and milk production in cows that are chronically afflicted with *Cryptosporidium muris* is reduced by about 13% (Anderson, 1998).

*Cryptosporidium* oocysts were detected in 34 (42.5%) out of 80 examined weaners and bull calves aged 3 to 12 months (Table 1). The highest prevalence was detected among bull calves aged 6-9 months (53.8%).

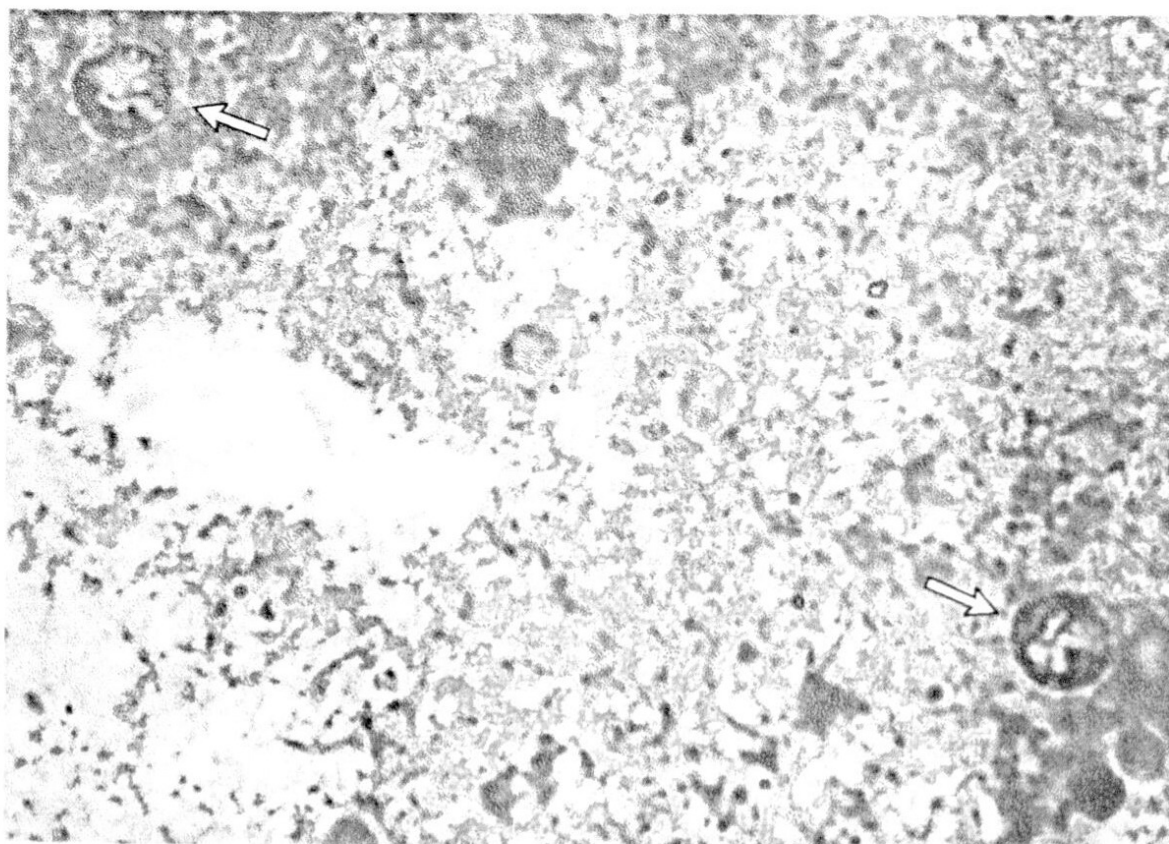


Figure 1. *Cryptosporidium parvum* oocysts (indicated by arrow), BBL TB Quick Stain (x 1000)

Table 1.

*Cryptosporidium* infection in weaners and bull calves aged 3 to 12 months

Age (months)	Examined		Positive	
	No		No	%
3-6	29		9	31.0
6-9	26		14	53.8
9-12	25		11	44.0
Total	80		34	42.5

Among asymptomatic, postparturient cows a high prevalence of *Cryptosporidium* infection was also detected. Thirty eight out of 80 examined 3-9 year-old postparturient cows excreted *Cryptosporidium* oocysts (Table 2).

The results of a previous study on *Cryptosporidium* infection in calves aged up to 3 months demonstrated that *Cryptosporidium parvum* is frequently involved in the aetiology of calf neonatal diarrhea in the Belgrade area (Mišić *et al.*, 2001). It can cause considerable direct and indirect economic losses.

The current study reveals a high prevalence of *Cryptosporidium* spp. among weaners, bull calves and postparturient cows in the Belgrade area. The high prevalence among these calves and adult cattle is probably due to the presence

Table 2.  
*Cryptosporidium* infection in postparturient cows

Age (years)	Examined		Positive
	No	No	%
3-5	31	15	48.4
5-7	41	21	51.2
7-9	8	2	25.0
Total	80	38	47.5

of animal carriers as well as to the physical features of some facilities on the farms where oocysts could remain viable and infectious.

Adult carriers seem to play an important role as a source of infection. Therefore, they should be kept apart from neonatal calves (Mišić et al., 2001).

At present, there is no totally effective therapy for eliminating *Cryptosporidium* other than a healthy immune system. Therefore, the control of cryptosporidiosis relies mainly on hygienic measures and good management. Strict control measures should be undertaken on dairy farms, including physical and chemical disinfection, and deratisation (Mišić et al., 2000).

Neonates can acquire the infection at birth mainly because of the high number of oocysts shed by the dams at parturition (Faubert and Litvinsky, 2000). Therefore, calves should be moved away from the dams 4 hr after birth. Dairy personnel should not use the same broom for cleaning calf hutches and the maternity pen, because this way they can cross-contaminate oocysts from infected dams to newborn calves. Careful attention to hygiene in the management of sick calves is also critical to minimize the spread of the parasite to other animals (Harp and Goff, 1998). This management practice could reduce the number of clinical cases because neonates would no longer be in contact with an environment that is highly contaminated. The total number of dairy cattle, total number of other species of agricultural animals on the farm, and the distance of the barn water source from the septic system is significantly associated with increased risk of *Cryptosporidium parvum* infection (Mohammed et al., 1999). Improved hygiene and management practices would minimize the exposure of calves to *Cryptosporidium* oocysts in the initial days of life. It would also lower the prevalence of cryptosporidial infection among asymptomatic adult cattle.

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#### INFEKCIJA KRIPTOSPORIDIJAMA KOD ZALUČENE TELADI, JUNADI I KRAVA POSLE POROĐAJA NA PODRUČJU BEOGRADA

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#### SADRŽAJ

U ovom radu se prvi put iznose podaci o infekciji kriptosporidijama kod zalučene teladi, junadi i krava posle porođaja u Jugoslaviji. Ispitivanje je sprovedeno kod životinja bez kliničkih simptoma a infekcija je dijagnostikovana koprološkim pretragama, upotrebom flotacione metode po Sheatheru, modifikovane Ziehl-Neelsenove tehnike i BBL TB Quick bojenja. *Cryptosporidium* oociste ustanovljene su kod 31,0 % zalučene teladi, 49,0 % junadi i kod 47,5 % krava posle porođaja. Ovi nalazi jasno pokazuju prisustvo asimptomatske infekcije kod ovih životinja na području Beograda i ukazuju na potencijalnu ulogu goveda kao rezervoara parazita.