

## RESEARCH NOTE

FAILED ATTEMPT OF *CRYPTOSPORIDIUM ANDERSONI* INFECTION IN LAMBSMartin Kváč<sup>1,2</sup>, Oleg Ditrich<sup>1</sup>, Martin Kouba<sup>2</sup>, Bohumil Sak<sup>1</sup>, Jiří Vítové<sup>2</sup> and Dana Květoňová<sup>1</sup><sup>1</sup>Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic;<sup>2</sup>Department of Anatomy and Physiology of Farm Animals, Faculty of Agriculture, University of South Bohemia, Studentská 13, 370 05 České Budějovice, Czech Republic

**Abstract.** Four lambs 4 months old were inoculated with *Cryptosporidium andersoni* oocysts (bovine isolate) in a dose of  $5 \times 10^6$  per animal followed later by  $1 \times 10^7$  oocysts per animal. No animal shed *Cryptosporidium* oocysts during 56 days post infection, no lamb showed clinical symptoms of cryptosporidiosis and no macroscopic changes were detected in the abomasum immediately after autopsy. Histological examinations did not demonstrate the occurrence of *Cryptosporidium* in the abomasum and other selected organs.

*Cryptosporidium andersoni* (Apicomplexa: Cryptosporidiidae) was described in 2000 by Lindsay et al. (2000). The description was based on molecular differences that were found between murine and bovine isolates of cryptosporidia in the stomach of mouse, respective cattle. The name *C. muris* remained for the murine isolates, while a new species, *C. andersoni*, was described from so-called *C. muris*-like bovine isolates. The typical hosts of *C. andersoni* are cattle (*Bos taurus*) but *C. andersoni* can also infect the Bactrian camel (*Camelus bactrianus*), bobak marmot (*Marmota bobac*) and European wisent (*Bison bonasus*) (Ryan et al. 2003). Koudela et al. (1998) successfully infected Mongolian gerbils (*Meriones unguiculatus*) with *C. muris* (bovine isolate, probably *C. andersoni*) and Pospischil et al. (1987) described abomasal cryptosporidiosis in four mountain gazelles (*Gazella gazella cuvieri*).

*Cryptosporidium andersoni* infection has also been described in HIV positive patients (Guyot et al. 2001). Anderson (1991) experimentally verified that oocysts morphologically similar to *C. muris* isolated from the faeces of a Bactrian camel were infective for mice, although oocysts designated as *C. muris* from cattle faeces were not and Fayer et al. (1991) have previously described isolates of *C. muris* from a Bactrian camel which did not infect calves. *Cryptosporidium andersoni* and *C. muris* (bovine isolate) are not found to be infective for laboratory outbred, inbred, immunocompetent and immunodeficient mice and rats and are also non-infective for common and bank voles, rabbits, goats and guinea pigs (Anderson 1991, Koudela et al. 1998, Lindsay et al. 2000, Sréter et al. 2000).

The spontaneous infection of *C. andersoni* in sheep has not been described previously. The infection of sheep with *C. parvum* was first reported by Meutin et al. (1974). Chalmers et al. (2002) described a novel isolate of *Cryptosporidium* from sheep which had different *Cryptosporidium* oocyst wall protein (COWP) PCR product sequences in comparison with *C. andersoni* (difference in 20.85 bases [bp] per 100 bp) and with *C. parvum* (genotype 2) (in 8.40 bp per 100 bp). An attempt to infect lambs with bovine isolate of *C. andersoni*

and evaluation of these animals as possible model hosts of this parasite are described in the present study.

The oocysts of *C. andersoni* were obtained from a Holstein bullock 146 days old. The bullock, one of a group, was kept in a stall on deep straw bedding. The partial 18S rRNA sequence (434 bp) of our *Cryptosporidium* isolate was 99% similar to the *C. andersoni* sequence (AB089285) (Satoh et al. 2003) from the GenBank. Six Merinolandschaft × Charollais cross-bred 3 1/2 months old lambs purchased from a commercial farm (1. Jihoceská zemědělská společnost a.s., Czech Republic) were used in all experiments. The experimental group consisted of 4 lambs and the control group consisted of 2 lambs. Both groups were housed in stalls of the University of South Bohemia in České Budějovice, Czech Republic. Viability of cryptosporidium oocysts was tested by propidium iodide staining (Dowd and Pillai 1997). After 2 weeks of daily monitoring for the presence of *Cryptosporidium* oocysts with negative result, the experimental group were inoculated with a dose of  $5 \times 10^6$  *C. andersoni* oocysts in 10 ml of sterile water and the control group with 10 ml of sterile water. Since no animals shed *Cryptosporidium* oocysts by day post infection (DPI) 28, the experimental group was re-inoculated with  $10 \times 10^6$  *C. andersoni* oocysts per 10 ml/animal while the control group received 10 ml of sterile water. All animals were examined for the presence of oocysts in faeces using the modified Ziehl-Neelsen (ZN) staining method (Henriksen and Pohlenz 1981) and modified Miláček-Vítové staining (MV) (Miláček and Vítové 1985) each day. All experimental animals were euthanized with a thiopental overdose on DPI 56 (primary infection) and autopsied. Immediately after autopsy, tissue samples were prepared for histology and scanning electron microscopy (SEM). Purified oocyst samples were prepared and examined for the presence of *C. andersoni* using histological, SEM and molecular methods as previously described by Kváč and Vítové (2003).

Animals from both groups were negative for *Cryptosporidium* oocysts in the 2-week-period before inoculation, although faeces of all animals were positive for *Giardia intestinalis* cysts and *Eimeria* spp. oocysts (natural infections). No lambs shed *Cryptosporidium* oocysts by DPI 28 (primary infection), which was longer than the prepatent period (25 days) experimentally verified in a calf (Enemark et al. 2002). All experimental lambs were reinfected with *Cryptosporidium* oocysts but all faecal staining proved negative during the length of the experiment.

No lambs showed clinical symptoms of cryptosporidiosis in subsequent autopsy, no macroscopic changes were detected in the abomasum or other examined organs (oesophagus, rumen, reticulum, omasum, duodenum, proximal, central and distal jejunum, ileum, caecum, colon, rectum, large biliary ducts at the *porta hepatis*, *ductus choledochus*, gall bladder, liver, pancreas, lungs and spleen), and histological exami-

**Address for correspondence:** M. Kváč, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic. Phone: +420 387 775 419; Fax: +420 385 310 388; E-mail: kvac@centrum.cz

nation proved negative for the occurrence of *Cryptosporidium* coccidia in selected organs and the abomasum where life-cycle stages would be expected during an infection (Pospischil et al. 1987, Anderson 1987, Kváč and Vítová 2003).

Although in the present study a limited number of animals was tested, it can be concluded that the isolate used was non-infective for lambs at 4 months of age.

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