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Infectivity and pathogenicity of *Cryptosporidium andersoni* to a novel host, southern multimammate mouse (*Mastomys coucha*)

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Abstract

The infectivity and pathogenicity of *Cryptosporidium andersoni* (bovine isolate) for neonatal and adult southern multimammate mice (*Mastomys coucha*) was studied using transmission experiments. *C. andersoni* isolate used in this study was not infective for BALB/c mice, but experimental infection proved susceptibility of neonatal and adult *M. coucha* to the infection. The prepatent period was 20–24 days, the patent period varied between 46 and 59 days. No signs of clinical illness or macroscopic findings were detected in infected animals. *Cryptosporidium* developmental stages were detected only in the glandular part of the stomach of *M. coucha* in histological sections stained with Wolbach's modification of Giemsa and using immunofluorecence. Histopathological changes were characterized by dilatation and epithelial metaplasia of infected gastric glands without inflammatory response in the lamina propria. Neonatal *M. coucha* were more susceptible to *C. andersoni* infection than adults. *M. coucha* seems to be a useful laboratory model for study of *C. andersoni* infection.

Keywords: *Cryptosporidium andersoni*; *Mastomys coucha*; Infectivity; Pathogenicity; 18S rRNA gene

1. Introduction

Cryptosporidium andersoni has been described by Lindsay et al. (2000) as a new species of the genus *Cryptosporidium* isolated from cattle (*Bos taurus*) and was distinguished from *C. muris* on genetic and infectivity basis (Morgan et al., 2000). The isolates from cattle did not infect immunocompetent and immunodeficient mice (Lindsay et al., 2000; Morgan et al., 2000). Recently, however, a novel type of *C. andersoni* isolated from cattle was successfully transmitted to immunocompetent and severe combined immunodeficiency (SCID) mice (Satoh et al., 2003; Matsubayashi et al., 2004, 2005). *C. andersoni* also infects Bactrian camel (*Camelus bactrianus*), Bobak

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marmot (*Marmota bobac*), European wisnet (*Bison bonasus*) (Ryan et al., 2003) and Mongolian gerbils (*Meriones unguiculatus*) (Koudela et al., 1998). The infection has been also described in HIV-positive patients (Guyot et al., 2001).

The information on the biology of *C. andersoni* infection in host from order Rodentia is poorly known. In the present study, the patterns of oocysts shedding and pathological findings in a novel *C. andersoni* host, southern multimammate mouse (*Mastomys coucha*) from the family Murinae, are described.

2. Material and methods

Oocysts of *C. andersoni* (7.7 (6.9–8.7) μm × 6.2 (5.5–6.9) μm; $n = 100$) were obtained from naturally infected cattle in South Bohemia, Czech Republic and were purified from faecal samples using sucrose gradient and cesium chloride gradient centrifugation (Arrowood and Sterling, 1987; Kilani and Sekla, 1987) and measured using morphometrical analysis based on digital image analysis (software M.I.S. QuickPHOTO Pro, camera, Olympus Camedia C-5060WIDEZOOM, 5.1 pixels). Pure oocysts were stored in the dark at 4 °C in distilled water. Both original field bovine isolate and re-isolated oocysts from the faeces of infected *M. coucha* were identified using molecular markers. Total DNA was extracted from 50 μl suspensions cleaned of faecal debris after purification using DNeasy Tissue Kit (Quiangen, Valencia, California). To increase the quantity of recovered DNA, the nucleic acid was eluted in 100 μl of elution buffer included in the DNeasy Tissue Kit. Fragment of 18S rRNA (1207 bp) gene was amplified by PCR. Briefly, primer pair (5′AACTTTACGGATCGC-AACTTTACGGATCGCATCTCTGA3′ and 5′CCCAT-CCCATCACGATGCATACTCATAA3′) was used for amplification (Satoh et al., 2003). The PCR mixture contained 1× PCR buffer (Top-Bio, Praha, Czech Republic), 0.2 mM dNTP (Jena Bioscience GmbH, Jena, Germany), 1.0 U Taq (Top-Bio), and 1 μM of each forward and reverse primer in a 25 μl reaction volume. Two phase PCR protocol was used. First 5 cycles each consisting of 94 °C for 60 s, 44 °C for 90 s and 72 °C for 120 s, followed by 25 cycles consisting of 94 °C for 60 s, 48 °C for 90 s and 72 °C for 120 s were performed. An initial hot start at 95 °C for 5 min and final extension at 72 °C for 10 min were included. PCR products were detected on 1% agarose gels supplemented with ethidium-bromide and visualized by UV light. Purified products were sequenced in both directions using the same PCR primers in 15 μl reactions (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystem, Forest City, California) and an ABI3130 sequencer analyzer (Applied Biosystem). Sequences were aligned and completed using DNA SeqMan 5.06 program (DNASTAR Inc., Madison, Wisconsin). Obtained sequences were compared with sequences in GenBank.

A partial sequence of the 18S rRNA (1207 bp) of isolates was 100% similar with the *C. andersoni* sequence in GenBank (accession no. AB089285).

Ten 8-week-old (adult) and ten 7-day-old (neonatal) southern multimammate mice (*M. coucha*, kindly provided by D. Modrý, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic) and 10 8-week-old and ten 7-day-old BALB/c mice (ANLAB) were used for experimental infection. Adult animals and litters were housed separately in standard plastic cages and were fed with commercial rodent food and water ad libitum. Each animal was inoculated orally by a stomach tube with a dose of 1×10^6 oocysts. Faecal samples were obtained daily from 4th day post infection (DPI). Infection intensity was determined as a number of oocysts per gram (OPG). Briefly, each glass slides were weighted immediately after smearing (0.001 g accuracy), stained by aniline-carbol-methyl violet staining method (Miláček and Vítovec, 1985) and entire smears were microscopically examined by light microscopy at 1000-fold magnification. The OPG was estimated on the basis of number of oocysts counted.

Three *M. coucha* and three BALB/c mice were sacrificed on DPI 30 and the remaining animals on DPI 90. Samples of stomach (glandular and non-glandular part), small intestine (upper, middle and lower portions), caecum and colon were collected for histological examination, fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, with Wolbach's modification of Giemsa staining and with non-species-specific FITC-labeled antibodies against *Cryptosporidium* oocyst wall (*Cryptosporidium* IF Test, Crypto cel, Medac).

3. Results

Coprological examination of *M. coucha* revealed fully sporulated *C. andersoni* oocysts in all experimental animals. *C. andersoni* oocyst found were ovoid, 7.7 (6.9–8.5) μm × 6.2 (5.5–6.9) μm ($n = 300$). Oocysts were morphometrically and molecularly identical with those of the used *C. andersoni* bovine strain. No *Cryptosporidium* oocysts in faeces of BALB/c mice (both groups) were found during the experiment. The patterns of oocyst shedding in *M. coucha* are presented in Fig. 1. Oocysts were first-time detected in faeces on DPI 24 in neonatal and on DPI 20 in adult *M. coucha*. The patent period of *C.*

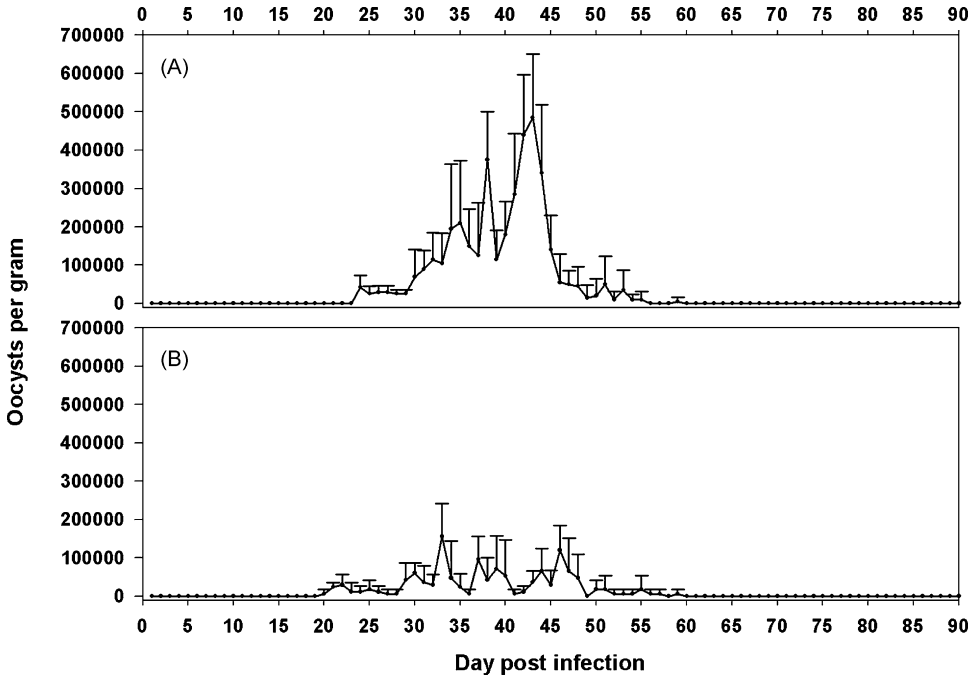

Fig. 1. Excretion of oocysts in gram of faeces in southern multimammate mice inoculated with 1×10^6 oocysts of *Cryptosporidium andersoni* (bovine isolate). Mean of 10 examined animals with standard errors: (A) neonatal mice (7-day-old) and (B) adult mice (8-week-old).

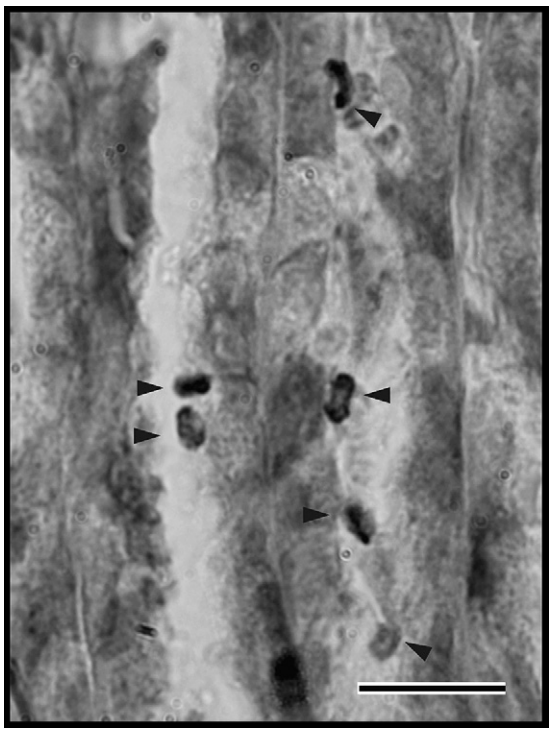

Fig. 2. Histological sections of the gastric mucosa (glandular part) of southern multimammate mouse. Cryptosporidial developmental stages (arrows) in gastric glands; 30 DPI; Wolbach's modification of Giemsa staining; bar = 2 μm.

andersoni infection in neonatal and adult *M. coucha* varied from DPI 48–59 and 46–59, respectively. The infection intensity levels were higher in neonatal *M. coucha* than in adult animals (Fig. 1). Three peaks of infection intensity during the patent period in both experimental groups were observed. In neonatal animals, the number of OPG gradually increased till 43 DPI (485,000 OPG) and in adult mice OPG culminated on 33 and 46 DPI (156,000 and 120,000 OPG).

No clinical signs and macroscopical findings of gastric cryptosporidiosis were observed in *M. coucha* autopsied at 30 DPI. Cryptosporidial infection in all examined *M. coucha* (at 30 DPI) was found in mucosal glandular epithelium of the glandular part of the stomach (Figs. 2 and 3). No cryptosporidial developmental stages and pathologic changes in non-glandular part of stomach were observed. Histopathological changes of gastric cryptosporidiosis were characterized by epithelial metaplasia and dilatation of gastric glands containing cryptosporidial developmental stages. These changes were more distinct in neonatal animals. In infected neonatal and adult *M. coucha*, several cryptosporidia were found in the middle and lower parts of the gastric mucosa epithelium. No cryptosporidial stages were found in the stomach or intestine of both groups of BALB/c mice and in both groups of *M. coucha* sacrificed on DPI 90.

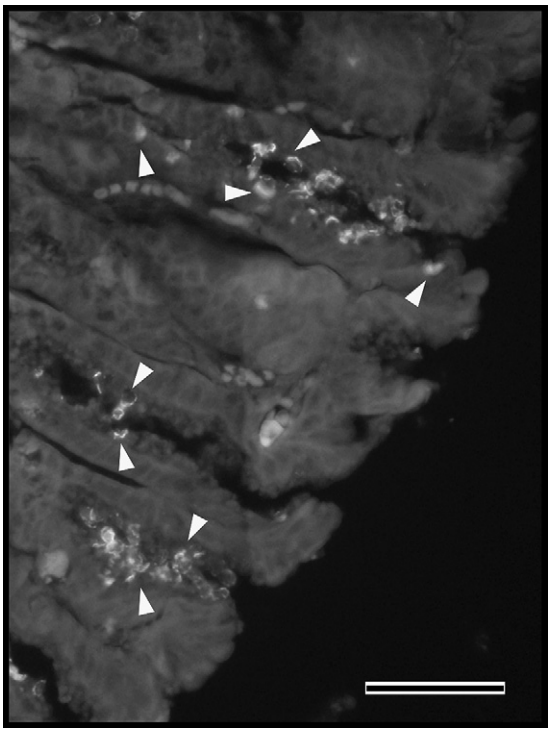

Fig. 3. Histological sections of the gastric mucosa (glandular part) of southern multimammate mouse. *Cryptosporidium* oocysts (arrows) in gastric glands; 30 DPI; immnunofluorescence staining using non-species-specific antibodies against *Cryptosporidium* oocysts wall (*Cryptosporidium* IF Test, Crypto cel, Medac); bar = 5 μm.

4. Discussion

The prepatent period of the infection (20–24 DPI) in experimentally infected animals corresponds approximately with that reported by other authors in other experimental hosts. Koudela et al. (1998) described the prepatent period of 15–19 DPI in 8-week-old Mongolian gerbils (used most probably *C. andersoni*; bovine isolate) and Enemark et al. (2002) reported the prepatent period of 25 DPI in the case of *C. andersoni* in 4-day-old calf. In contrast, Matsubayashi et al. (2005) described oocyst shedding of a novel type of *C. andersoni* (bovine isolate) in immunodeficient and immunocompetent mice from 6 DPI. It roughly corresponds with beginning of *C. muris* oocysts shedding in rodents, 6 DPI (Iseki et al., 1989; Rhee et al., 1991, 1995) and 10 DPI (Taylor et al., 1999). The patent period of *C. andersoni* in a typical host (cattle) can reach up to several years (Lindsay et al., 2000). In our experiments, 46–59 days lasting excretion of *C. andersoni* oocysts in new rodent hosts, *M. coucha*, was similar to that of *C. muris* infected SPF mice (Iseki et al., 1989). On the contrary, it was longer than 19 days patent period obtained in C.B.-17/Icr-+/+ mice infected with *C. andersoni*—novel type (Matsubayashi et al., 2005) and 18–36 days in *C. andersoni* infected Mongolian gerbils (Koudela et al., 1998). The differences in the prepatent and patent period probably depend on the experimental animals or *C. andersoni* isolates used.

Site of infection of *C. andersoni* in the gastrointestinal tract of *M. coucha* was similar to that reported in cattle and Mongolian gerbils and restricted in a glandular part of the stomach (Koudela et al., 1998; Lindsay et al., 2000; Kváč and Vítovec, 2003).

Gastric cryptosporidiosis does not induce any clinical symptoms and any macroscopic changes are not detectable. Histopathological changes are characterised by hypertrophy, atrophy and metaplasia of glandular epithelium and dilatation of infected glands while inflammatory infiltrates in the propria of the abomasal mucosa are usually absent (Anderson, 1987; Özkul and Aydin, 1994; Aydin and Özkul, 1996; Kváč and Vítovec, 2003). Taylor et al. (1999) described an inflammatory response in the gastric mucosa of outbred nude mice receiving 1×10^6 of *C. muris* oocysts and noticed that the changes may differ in fully immunocompetent mice. In the present study, histopathological changes in the stomach of *M. coucha* infected with *C. andersoni* were characterised by metaplasia and dilatation of gastric glands and no inflammatory infiltrates in the lamina propria of the abomasal mucosa in histological sections were found.

Results of the present study confirm the susceptibility of *M. coucha* to *C. andersoni* infection. This host, which is easy to maintain in the laboratory, may represent a suitable model host for studies of *C. andersoni* infection.

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