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Multimammate rat (*Mastomys natalensis*), Tristram's jird (*Meriones tristrami*) and Wagner's gerbil (*Gerbillus dasyurus*) as laboratory models of acute neosporosis

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Abstract

To test the different sensitivity of rodents of the subfamily Murinae and Gerbillinae, Wagner's gerbils (*Gerbillus dasyurus*), Tristram's jirds (*Meriones tristrami*) and multimammate rats (*Mastomys natalensis*) were inoculated with *Neospora caninum* tachyzoites. Clinical signs of neosporosis appeared in all inoculated animals. Histopathological examination confirmed the presence of tachyzoites in brains, lungs, skeletal muscle, myocardium, liver, in serosa of stomach and intestines, and in vesicular accessory genital glands. An examination of brains by PCR revealed presence of *N. caninum* DNA in all experimentally *N. caninum* infected rodents. The susceptibility of Wagner's gerbils and Tristram's jirds further proved the high sensitivity of gerbiline rodents to the *N. caninum* infection. The finding of *N. caninum* tachyzoites in the vesicular accessory genital glands of the infected gerbils suggests the usefulness of the rodent model for demonstration of *N. caninum* in the male reproductive system. Moreover, the multimammate rat was a susceptible experimental host to be the first immunocompetent rodent of the subfamily Murinae.

Keywords: *Neospora caninum*; Multimammate rat; Jird; Gerbil

1. Introduction

Neospora caninum (Apicomplexa: Sarcocystidae) is a protozoan pathogen of reproductive system of cattle responsible for an important economical loses in the cattle industry (Dubey, 2003). Mice are a common laboratory model for studying *N. caninum* infection, isolation of *N. caninum* stages from different hosts or development of vaccines (Sawada et al., 1997; Omata et al., 2004; Cannas et al., 2003). Because *N. caninum* is not pathogenic for outbred laboratory mice, immunodeficient or immunosuppressed mice are used (Rettigner et al., 2004; Lindsay and Dubey, 1989). The laboratory rat (*Rattus norvegicus*) was described as a resistant host (Lindsay and Dubey, 1990a), although an information about naturally infected rats (*R. norvegicus*) was published recently (Huang et al., 2004; Hughes et al., 2006). Mongolian gerbils (*Meriones unguiculatus*) are the most frequently used immunocompetent experimental hosts of *N. caninum* (Dubey and Lindsay, 2000; Ramamoorthy et al., 2005). Several additional rodent species were tested for susceptibility to *N. caninum* infections. Pipano et al. (2002) described a successful inoculation of Tristram's jirds (*Meriones tristrami*) and sand rats (*Psammomys obesus*). Uchida et al. (2003) informed about an experimental infection in Djungarian hamsters (*Phod-*

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opus sungorus). So far, rodents belonging to the subfamily Gerbillinae, genus *Meriones* and *Psammomys*, were documented to be the most sensitive laboratory models for experimental neosporosis.

In this study we tested that it is true also for Wagner's gerbils (*Gerbillus dasyurus*), another gerbiline rodent genus. Considering the differences in sensitivity of members of murine rodents (subfamily Murinae), we inoculated multimammate rats (*Mastomys natalensis*), which are commonly used laboratory rodents.

2. Materials and methods

2.1. Experimental rodents

Six adult outbred females of multimammate rat (*M. natalensis*), 3 adult outbred females of Tristram's jird (*M. tristrami*) and 3 adult outbred males of Wagner's gerbil (*G. dasyurus*) were used. All animals were conventionally bred in our laboratory. They were kept in standard plastic cages and provided with peleted food for laboratory rodents and water *ad libitum*.

The Tristram's jirds were used as a positive control.

2.2. Cultivation of N. caninum tachyzoites

Cultures of *N. caninum* tachyzoites (NC-1 isolate) were cultivated at 37 °C on Vero cells in RPMI 1640 medium with 5% bovine fetal serum and antibiotics (60 μg penicillin + 50 μg streptomycin/ml) (Sigma). The medium was changed every 2–3 days and at the same time the cultures were checked using an inverted microscope Nikon TMS. After 4 weeks of the cultivation, the tachyzoites were harvested and counted in Bürker's chamber.

2.3. Inoculation

Multimammate rats were inoculated intraperitoneally each with 1×10^6 live tachyzoites. Tristram's jirds and Wagner's gerbils were also inoculated intraperitoneally but each with dose 1×10^5 of live tachyzoites. The animals were checked daily for the presence of clinical signs of neosporosis. When they became apparently ill (strong apathy, emaciation, neurological signs), they were euthanised by a diethyl ether overdose for a necropsy.

The Ethical committee of Faculty of Veterinary Sciences, UVPS Brno, approved all procedures involving experiments with the rodent hosts.

2.4. Necropsy and histopathology

During the necropsy, part of a brain was compressed between two microscopic slides and examined under a light microscope. The brain (right hemisphere) and various organs (lungs, heart, muscle of hind leg, liver, stomach, duodenum, jejunum, ileum, caecum, colon, lien, uterus or vesicular accessory genital glands, kidney) were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). The stained sections were examined and photographed using Olympus AX 70 microscope. The left hemisphere of the brain was stored in −21 °C for subsequent PCR diagnosis.

2.5. DNA isolation

The frozen brain tissue of an individual experimental host was homogenised and 40 mg were used for isolation of total DNA by a commercial invisorb spin tissue mini kit (Invitek) according to manufacturer's instructions. A brain tissue of negative laboratory mouse and NC-1 tachyzoites served as controls for the DNA isolation.

2.6. PCR

Primers Np21-plus and Np6-plus annealing to Nc5 region of *N. caninum* genome were used for a PCR amplification (Müller et al., 1996). The PCR reaction was performed in 25 μl volume containing 1 μl of the total DNA, 12.5 μl of a commercial premix "PPP master mix" (Top-Bio s.r.o.), 1 μl of each primer (10 pmol/μl) (Generi Biotech) and 9.5 μl of PCR H_2O. The PCR was carried out in a PCR thermocycler Tpersonal (Biometra) with the following conditions: an initial denaturing at 94 °C for 5 min; followed by 40 cycles of denaturation (94 °C, 1 min), annealing (63 °C, 1 min) and an extension (74 °C, 3.5 min); with final extension of 74 °C for 10 min (Šlapeta et al., 2002). Negative and positive controls were also included in each reaction. The PCR products were separated by horizontal electrophoresis on 1.5% agarose gel stained with an ethidium bromide and viewed under a UV light. An expected molecular mass of the PCR products was 337 bp.

3. Results

3.1. Clinical signs and necropsy findings

3.1.1. Gerbillus dasyurus

Clinical signs of an acute neosporosis appeared 7 DPI (days post-infection) in all three animals. Gerbils were apathetic with roughened coat. A male No. 1 was euthanised at 7 DPI due to a paralysis of all extremities. An animal No. 2 was euthanised at 8 DPI with paresis of hind limbs. A male No. 3 was euthanised at 9 DPI, due to a poor condition and severe apathy. The necropsy revealed hepatomegaly and an exudate in the abdominal cavity in No 1. and No. 2. Moreover, a single *N. caninum* pseudocyst was found in the brain of male No. 1.

3.1.2. Meriones tristrami

Animal No. 4 was found dead at 11 DPI, at necropsy an exudate was found in the abdominal cavity. Females No. 5 and No. 6 were emaciated with roughened coat. Moreover,