Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 148 (2007) 346–349
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

Evidence of *Neospora caninum* DNA in wild rodents

E. Ferroglio [a,*], M. Pasino [a], A. Romano [a], D. Grande [a], P. Pregel [b], A. Trisciuoglio [a]

[a] *Dipartimento di Produzioni Animali, Epidemiologia ed Ecologia, Università di Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy*
[b] *Dipartimento di Patologia Animale, Università di Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy*

Received 7 May 2007; received in revised form 20 June 2007; accepted 21 June 2007

Abstract

Seventy-five house mice (*Mus musculus*), 103 rats (*Rattus norvegicus*) and 55 field mice (*Apodemus sylvaticus*) from North-West Italy were PCR analysed for *Neospora caninum* infection. Brain, kidney and muscle tissues collected from the above mentioned animals were tested by PCR using Np6 and Np21 primers. The brain tissue from 2 house mice and 2 rats, the kidney from 4 rats, 1 house mouse and 1 field mouse and muscle from 10 rats, 8 house mice and 1 field mouse were tested positive for *N. caninum*. Sequencing showed a 96–97% identity of PCR products with *N. caninum* NC1 sequence. Our findings support previous report on house mouse and rat, and for the first time, provides the evidence of the infection also in field mice. Based on our data, it could be hypothesized that mice can act as a reservoir of *N. caninum*, and they can play a role in maintaining/spreading *N. caninum* infection also in the sylvatic cycle. The possibility that dogs could be infected by eating infected house mice suggests new opportunities for *N. caninum* prophylaxis and control.

Keywords: *Neospora caninum*; House mouse; Rat; Field mouse; PCR; Intermediate host

Neospora caninum is a protozoan parasite of dog and livestock recognised for the first time in 1988 (Dubey et al., 1988). In cattle *N. caninum* infection was recognized for the first time in 1989 (Thilsted and Dubey, 1989) and it is actually reported as a primary abortion pathogen in cattle herds worldwide (Dubey, 2003). The cycle has been only recently clarified with dog (McAllister et al., 2000; Lindsay et al., 1999) and coyote (*Canis latrans*) (Gondim et al., 2004) as known definitive host, and cattle or other species as the intermediate host. Cattle can be vertically infected, but also horizontal, point-source transmission, due to oocystis elimination by dogs, can occur (McAllister et al., 2000). Dogs eliminate oocystis in faeces after ingestion of experimentally infected mice, but apart from vertical transmission, the main source of infection for dogs has been considered aborted foetal fluids or placental material (Dijskstra et al., 2002). The life cycle of protozoa (family Sarcocystidae) closely related to *N. caninum* is a predator-prey type, with carnivorous that shed oocysts in the faeces after consumption of tissue cysts in intermediate host, which became infected by ingestion of oocysts (Current et al., 1990). The possibility that small mammals could act as a potential reservoir of infection has been suggested by Wouda et al. (1999), and has been recently demonstrated in rat (*Rattus norvegicus*) and house mice (*Mus musculus*) from Taiwan (Huang et al., 2004), U.K. (Huges et al., 2006) and United States (Jenkins et al., 2007). Considering that no data are available on the presence of *N. caninum* in feral rodents in continental Europe and

* Corresponding author. Tel.: +39 011 6709002; fax: +39 011 6709000.
E-mail address: ezio.ferroglio@unito.it (E. Ferroglio).

0304-4017/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2007.06.031

in field mice (*Apodemus sylvaticus*), we deemed it interesting to evaluate, by means of PCR, the presence of infection in house mice, field mice and rats from Piedmont (North-West Italy). In this region previous studies have evidenced the presence of *N. caninum* infection in both domestic and wild animals (Ferroglio and Rossi, 2001; Ferroglio et al., 2001, 2005, 2007; Ferroglio and Trisciuoglio, 2003).

Seventy-five house mice (*M. musculus*), 103 rats (*R. norvegicus*) and 55 field mice (*A. sylvaticus*) were captured with snap traps as part of pest control program around cattle farms in Piedmont with a previous history of abortion due to *N. caninum* (North-West Italy, 44.5°N; 7.5°E). Captured rodents were dissected under clean laboratory conditions with sterile scalpels, one for each animal and tissue, in order to avoid cross contaminations. Liddel et al. (1999) reported that the predominant site of infection in experimentally infected mice was the brain and previous reports on wild rodents (Huang et al., 2004; Huges et al., 2006; Jenkins et al., 2007) exanimate only the brain of captured animals. However, a lot of reports showed *N. caninum* presence in other tissues such as lung, skeletal muscles, heart and kidney (Barber et al., 1996; Ho et al., 1997; Wyss et al., 2000; Peters et al., 2000, 2001; Dubey et al., 2004; Basso et al., 2005; McInnes et al., 2006). So, we collected brain, kidney and skeletal muscle (gluteal muscle) from captured rodents and samples were immediately frozen in single vials at −20 °C. DNA extraction was performed from 25 mg of brain, kidney and gluteal muscle tissue of sampled animals, using the Gen Elute™ Mammalian genomic DNA extraction kit (Sigma–Aldrich, St. Louis, MO, USA) according to manufacturer's protocol. DNA extracted from cultured *N. caninum* (NC1) tachyzoites was used as a positive control for the PCR reaction.

The used primers were Np6 plus (5′CTCGCCAG-TCAACCTACGTCTTCT3′) and Np21 plus (5′CCC-AGTGCGTCCAATCCTGTAAC3′), as suggested by Muller et al. (1996). DNA amplification was performed in 50 μl reaction mix, containing 8 μl of DNA extract (approximately 50 ng of DNA), 0.5 μM each primer and 25 μl of RedTaq™ Ready Mix™ PCR Reaction Mix (Sigma–Aldrich, St. Louis, MO, USA). Amplifications were carried out in a Bio-Rad iCycler thermal cycler. Samples were initially denaturated at 94 °C for 1 min, then submitted to 40 amplification cycles at a denaturing temperature of 94 °C for 1 min, an annealing temperature of 63 °C for 1 min, and an extension temperature of 74 °C for 3 min. A final extension step of 72 °C for 10 min was performed. After amplification, 10 μl aliquots from each reaction were analysed by electrophoresis on 2% agarose gel, in comparison with molecular weight markers (DNA Molecular Weight Marker V, Roche Diagnostics, Mannheim, Germany; PCR 100 bp Low Ladder, Sigma–Aldrich, St. Louis, MO, USA). Gels were stained with MegaFluor kit (Euroclone, Milano, Italy) under the conditions suggested by the manufacturer and photographed on a 254 nm UV transilluminator using a CCD Camera (Gel-Doc Bio-Rad). Positive controls (DNA extracted from *N. caninum* NC1 culture) and negative controls (distilled water) were included in each step (extraction, amplification and electrophoresis run). Samples were considered positive when a 337 bp specific Np6–Np21 product (Muller et al., 1996) was present (Fig. 1).

Fourteen rats (13.6%), nine house mice (13.8%) and two field mice (3.6%) were positive for *N. caninum*. In detail, PCR positives were found from the brain tissue of 2 house mice and 2 rats, from the kidney of 4 rats, 1 house mouse and 1 field mouse and from skeletal muscle of 10 rats, 8 house mice and 1 field mouse. The two brain positive mice were positive also in muscle, but negative in kidney, while the brain samples from the two positive rat were negative in both kidney and muscle tissues. Fifteen animals tested positive only in muscle (eight rats and five mice), four only in kidney (two rats, one house and one field mouse) and two rats were positive in both muscle and kidney tissues.

Two amplicons from rats, one from house mouse and one from field mouse were cloned using the Quiagen PCR CloningPlus kit and sequenced by BMR Genomics, University of Padova (Accession nos. are: EF202081, EF202080, EF202082 and EF202079,

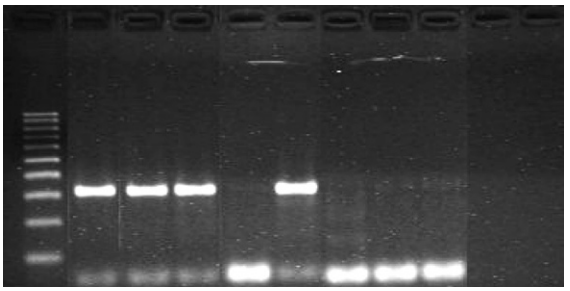

Fig. 1. Agarose gel electrophoresis of PCR products (Np6 plus and Np21 plus primers) from muscle in a rat captured from farm with a previous history of abortion attributable to *Neospora caninum* (lane 2), from brain in a house mouse (lane 3) and from kidney in a field mouse (lane 4). Positive control DNA prepared from cultured *N. caninum* NC1 (lane 6) and negative control (distilled water, lane 9 and reagents used for DNA extraction, lane 8). Lane 1 represents PCR 100 bp Low Ladder marker (Sigma–Aldrich, St. Louis, MO, USA). Lanes 5 and 7 represent negative samples. Note the specific amplification products of 337 bp in lanes 2–4 and 6, corresponding to muscle of a rat, brain of a house mouse, kidney of a field mouse and to the positive control.