Veterinary Parasitology 184 (2012) 317–320

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Neospora caninum and *Toxoplasma gondii* in brain tissue of feral rodents and insectivores caught on farms in the Netherlands

B.G. Meerburg[a,b,*,1], S. De Craeye[c,1], K. Dierick[c], A. Kijlstra[a,d]

[a] *Wageningen University & Research Centre, Livestock Research, P.O. Box 65, 8200 AB Lelystad, The Netherlands*
[b] *Wageningen University & Research Centre, Plant Research International B.V., Agrosystems Research, P.O. Box 616, 6700 AP Wageningen, The Netherlands*
[c] *Scientific Institute of Public Health, Communicable and Infectious Diseases, National Reference Center for Toxoplasmosis, Engelandstraat 642, B1180 Brussels, Belgium*
[d] *University Hospital Maastricht, Department of Ophthalmology, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands*

ARTICLE INFO

Article history:
Received 25 July 2011
Received in revised form 31 August 2011
Accepted 6 September 2011

Keywords:
Neospora caninum
Toxoplasma gondii
Rodents
Livestock
Farms
Insectivores

ABSTRACT

We investigated the presence of both *Neospora caninum* and *Toxoplasma gondii* in 250 brain tissue samples from 9 species of feral rodents and insectivores caught on 10 organic farms in the Netherlands in 2004. Collected samples were conserved in 4% paraformaldehyde solution and analysed by real-time PCR. For *N. caninum*, 31 samples originating from 6 species tested positive (12.4%): common shrews (33.3%), wood mice (17.6%), harvest mice (16.7%), house mice (15.4%), white-toothed shrews (10.8%) and common voles (4.2%). For *T. gondii*, the overall contamination level was 4%, and only three species were found to be positive: house mice (9.0%), common voles (4.2%) and white-toothed shrews (2.0%). Most *N. caninum* infected samples (27/31; 87%) were found on farms where dogs were present. Due to the observation that rodents and insectivores can contract both parasites, they might function as indicator species for the parasitic load on farms.

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1. Introduction

On farms, the emergence of protozoan parasites such as *Neospora caninum* (*N. caninum*) and *Toxoplasma gondii* (*T. gondii*) may have severe consequences. *N. caninum* can be a major cause of abortion in cattle and this parasite can either be vertically transmitted in utero or horizontally by uptake of oocysts excreted by canids (either domestic or feral). Earlier studies have already suggested that rodents may play a role in the dissemination of *N. caninum* throughout the environment (Huang et al., 2004; Hughes et al., 2006; Jenkins et al., 2007).

As felids are the definitive hosts for *T. gondii*, they may excrete oocysts contaminating the farm environment. *T. gondii* is a major cause of abortion and foetal abnormalities in sheep (Hide et al., 2009), but even more importantly, when taken up by livestock (e.g. pigs) this parasite may be transmitted to humans through the consumption of raw or undercooked meat (Kijlstra and Jongert, 2008), leading to significant health problems.

Rodents can transfer many pathogens either directly or indirectly (Meerburg, 2010; Meerburg et al., 2009), and can be considered as one of the main pathways of *T. gondii* transmission to livestock. First, some farm animals may accidentally eat rodents. Pigs are omnivorous animals that are able to catch and eat rodents. They might even consume dead animals, thus ingesting tissue cysts. Moreover, congenital transmission rates in a natural population of rodents were recently found to be 75% (Hide et al., 2009). Another study demonstrated that strict rodent control on three pig farms had a direct influence on the *Toxoplasma*

* Corresponding author at: Wageningen University & Research Centre, Livestock Research, P.O. Box 65, 8200 AB Lelystad, The Netherlands. Tel.: +31 317 481375; fax: +31 320 238050.
E-mail address: Bastiaan.Meerburg@wur.nl (B.G. Meerburg).
[1] These authors contributed equally to this work.

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doi:10.1016/j.vetpar.2011.09.001

Table 1
Overview of collected animal material and their capture location.

	Gender		Capture location					
Species	# Males	# Females[a]	Feeding passage	Storage	Outdoor area	Pasture	Nature	Unknown
House mouse	43	35 (9)	35	19	14	4	4	2
Common vole	15	9 (2)	1	2	2	12	7	0
Wood mouse	10	7 (0)	0	1	0	3	13	0
Brown rat	4	4 (0)	0	0	0	0	0	8
Harvest mouse	4	2 (0)	0	0	0	0	6	0
Bank vole	2	1 (0)	0	0	0	2	1	0
Field vole	1	2 (1)	0	0	0	2	1	0
Common shrew	6	3 (0)	0	0	0	2	7	0
White-toothed shrew	38	64 (5)	3	16	24	21	37	0
Total	123	127 (18)	39	38	40	46	76	10

[a] # of pregnant females between brackets.

seroprevalence of the resident pigs: significant less pigs were *T. gondii* seropositive when an active rodent control campaign was instated, showing the importance of rodent control to acquire safer meat (Kijlstra et al., 2008).

Here, we investigate the presence of both *T. gondii* and *N. caninum* in brain tissue samples of feral rodents and insectivores caught on organic farms in the Netherlands in order to better understand the potential role of these small mammals in the transmission of both parasites. We also focus on the locations on the farms where these animals were caught in order to facilitate future risk assessment studies.

2. Materials and methods

2.1. Samples

A total of 250 feral rodents and insectivores were trapped in live-traps on 10 organic farms (9 pig farms, 1 poultry farm) in the Netherlands in 2004 (Meerburg et al., 2006). The animals were humanely euthanized using CO_2. Their heads (without skin) were stored in 4% PBS buffered paraformaldehyde solution until tested. For each sampled animal, the location of the farm where it was captured was recorded: feeding passage (near the feed trough), storage (inside the stable), outdoor area (next to the stable, solid/slatted concrete floor, sometimes roofed), pasture, and “nature area” (further from the stable, e.g. wild vegetation). For some animals the location was not recorded and those samples were classified as unknown. The presence of dogs or cats on the farm was also noted.

2.2. DNA extraction

Half the brain from each animal was homogenized in a Potter Homogenizer in the presence of phosphate buffered saline at pH 7.4. After centrifugation at 21,500 × *g* during 5 min, the pellet was washed by resuspension in PBS and recentrifuged at 21,500 × *g* during 5 min. DNA extraction was performed using the Qiagen DNA Minikit (Qiagen, Venlo, The Netherlands), using a slightly modified protocol: to the washed pellet, 20 μl of proteinase K solution (from the kit) and 400 μl ATL lysis buffer was added. After homogenisation with a micropipette, the samples were incubated at 56 °C overnight or until complete lysis. Then, 400 μl AL buffer was added and after mixing and an incubation of 10 min at 70 °C, the samples were centrifuged at 21,500 × *g* for 5 min to remove unlysed debris. To precipitate the DNA, 400 μl of 95+% ethanol at −20 °C was added to each supernatant. After brief mixing, each supernatant was then loaded on a DNA extraction column and from here on the manufacturer's instructions were followed. Briefly: the columns were washed once with 500 μl AW1 buffer and once with 500 μl AW2 buffer, the DNA was eluted with 200 μl of AE buffer. The DNA samples were stored at −20 °C until tested by real-time PCR.

2.3. Real-time PCR

The extracted DNA samples were tested for both parasites by triplex quantitative real-time PCR as described before (De Craeye et al., 2011). Briefly, the detection was performed with dual labelled probes on the following targets: AF146527 to detect *T. gondii* (Homan et al., 2000), X84238 for *N. caninum* (Yamage et al., 1996) and cellular r18S to check for PCR inhibition and DNA quality. Two slight modifications were made to the previously published protocol: the reactions were carried out in a BioRad CFX 96 real-time PCR thermocycler (Bio-Rad, Hercules, United States of America) and the following dual labelled probe for the detection of *N. caninum* DNA was used: 5′-Hex-CCTTCTGAG-Zen-TCGGGTTGTGTTTGGC-BHQ2-3′ (Integrated DNA technologies, Leuven, Belgium).

2.4. Statistics

A Pearson chi-square test was used to see whether there were differences in contamination level between samples of both sexes.

3. Results

In total 250 brain samples were collected and analysed, representing 7 species of rodents captured on various locations on or around the farm: house mouse (*Mus musculus*), common vole (*Microtus arvalis*), wood mouse (*Apodemus sylvaticus*), brown rat (*Rattus norvegicus*), harvest mouse (*Micromys minitus*), bank vole (*Myodes glareolus*), field vole (*Microtus agrestis*); and 2 species of insectivores: common shrew (*Sorex araneus*) and white-toothed shrew (*Crocidura russula*). Those samples originated from 123 male and

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