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Prevalence of *Toxoplasma gondii* in common moles (*Talpa europaea*)

Inge M Krijger[1], Jan BWJ Cornelissen[2], Henk J Wisselink[2] and Bastiaan G Meerburg[1*]

Abstract

Background: The prevalence of *Toxoplasma gondii* in common moles, *Talpa europaea*, was investigated in order to determine whether moles can serve as an indicator species for *T. gondii* infections in livestock.

Findings: In total, 86 moles were caught from 25 different sites in the Netherlands. Five different trapping habitats were distinguished: pasture, garden, forest, roadside, and recreation area. No positive samples (brain cysts) were found during microscopic detection (n = 70). Using the Latex Agglutination Test (LAT), sera of 70 moles were examined, whereby no sample reacted with *T. gondii* antigen. Real Time-PCR tests on brain tissue showed 2 positive samples (2.3%).

Conclusions: Because of the low number of positives in our study, the use of the common mole as an indicator species for livestock infections is currently not recommended.

Keywords: Parasite, Moles, Reservoir, Food safety, Outdoor farming, Wildlife, Indicator

Findings

Toxoplasma gondii is a zoonotic pathogen, which can infect a wide range of intermediate hosts [1–3]. The disease burden of clinical toxoplasmosis is high [1,4–8] and the risk for human infection is considered substantial [9]. In farm environments [10,11] and especially in animal production systems with outdoor access for livestock a major risk emerges because of the coincidental uptake of oocysts or intermediate hosts [12]. If cats (definitive parasite hosts) that were not previously exposed to *T. gondii* consume infected intermediate hosts tissue (e.g. birds or rodents), they can start shedding oocysts which may disperse into the soil by precipitation [13] and be taken up by earthworms [14]. Earthworms carry infectious *T. gondii* [15–17], although from these studies it is unclear whether the parasite originated from the earthworms or the soil associated with them. Here, we test whether the common mole (*Talpa europea*), whose staple food consists of earthworms [18], can be an indicator species for *T. gondii.* Use of an indicator species would be beneficial as this reduces the need to obtain blood samples from livestock to measure *T. gondii* prevalence. Moreover, moles are considered a pest and are regularly trapped because of their destructive behaviour. To our knowledge, only two studies have previously investigated *T. gondii* prevalence in common moles. In the first study, a case-report of a dead mole that carried *T. gondii* was presented [19]. In the second study, 7 of 18 common moles examined were found to be positive using a Modified Agglutination Test (MAT) [20].

In March 2013, 25 different sites in the Netherlands were surveyed using lethal mole traps. As the common mole is considered as a nuisance species, these animals had to be eliminated and in such cases no approval of the animal experimental ethics committee (DEC) is required according to Dutch law & regulations. Trapping sites were distributed over 4 provinces: Zuid-Holland, Gelderland, Utrecht, and Overijssel (Figure 1). Five trapping habitats were distinguished: pasture, garden, forest, roadside, or recreation area. The origin of each mole was registered and the gender noted, except for the first samples (gender 'unknown'). Mole traps were checked daily and upon capture, moles were transported to the Central Veterinary Institute in Lelystad under cool conditions (4°C) and dissected within 48 h in order to collect blood samples from the heart and brain samples. If moles could not arrive in the laboratory within 48 h after capture, they were frozen

* Correspondence: Bastiaan.Meerburg@wur.nl
[1]Wageningen UR (University & Research centre), Livestock Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
Full list of author information is available at the end of the article

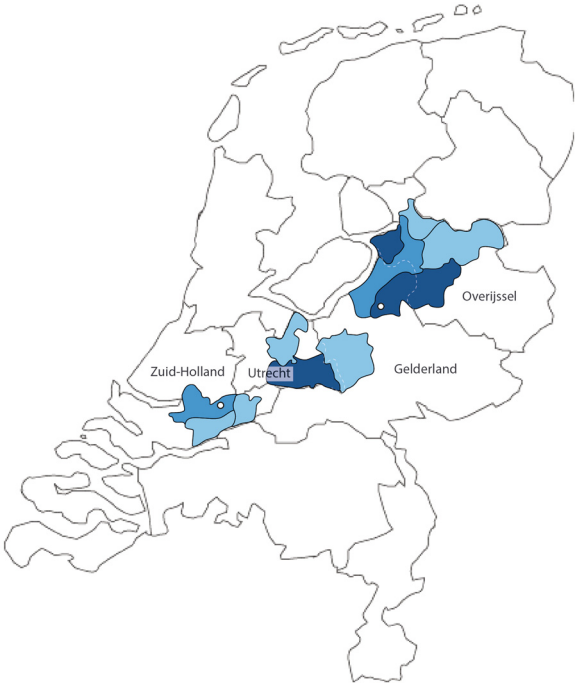

Figure 1 Trapping sites of common moles in the Netherlands classified to the postal code. Dark blue: >10 trapped moles, medium blue: >5 trapped moles, light blue: <5 trapped moles, white: no moles trapped. Circles (o): positive moles.

at −18°C (n = 16) until they were dissected. The night before dissection, frozen moles were thawed at 4°C.

Half of the brain of each non-frozen mole (n = 70) was homogenised after dissection using a cell strainer (0.2 μM) and plunger with addition of 5 ml PBS (phosphate buffered saline, 8.2 g NaCl, 0.20 g KCL, 1.15 g Na_2HPO_4, 0.38 g KH_2PO_4 per litre). In order to enrich the *T. gondii* cysts, 5 ml Percoll (Sigma Aldrich, Zwijndrecht, the Netherlands) 30% gradient was added underneath the homogenised brain sample. After centrifuging this mixture (1200 *g* for 15 min at 4°C), resuspended in PBS, a 25 μl pellet was analysed using a light microscope (20 ×), (Floating Technique [21]), according a modified protocol [22]. Frozen moles were not included in this analysis. DNA was extracted from homogenised brain tissue (the frozen samples were homogenised by using an ultra turrax) with the DNeasy Blood & Tissue kit (Qiagen GMBH, Hilden, Germany), using a slightly adjusted protocol: glass homogeniser beads were added to the sample mixed by a vortex during 60 s to facilitate lysis, after which lysis buffer was added. Samples were then incubated at 56°C for 2.5 h. Hereafter, the protocol of the test manufacturer was followed. The extracted DNA samples were stored at −20°C until tested by Real-Time PCR. For serologic detection of both *T. gondii* IgG and IgM antibodies by direct agglutination, blood obtained from non-frozen mole hearts (n = 70) was tested with

Table 1 Overview of the trapping numbers and habitat types

Habitat type	#Trapped (positives between brackets)	Male	Female	Unknown gender
Forest	4	3	1	0
Garden	24 (1)	14	10	0
Recreation area	24 (1)	15	9	0
Pasture	32	20	8	4
Roadside	2	0	2	0

Toxo-reagent kit RST701 (Mast Diagnostics Ltd, Bootle, UK), according to manufacturer's instructions. The test was performed in microtiter plates with U-shaped wells (Greiner Bio-One GmbH, Frickenhausen, Germany). Control and diagnostic sera were diluted 1:8, and titrated on the plates to a dilution 1:1024. In addition to control sera from the Toxo-reagent kit, internal positive cat and pig samples were used. The plates were analysed at normal daylight against a dark background. A positive reaction was noted when *T. gondii* agglutinated in the well until a dilution of 1:64 (1:32 will be regarded as doubtfull, <32 as negative) while a negative reaction was noted by sunken latex on the bottom of the well.

DNA samples were tested for *T. gondii* by a Real-Time PCR, oriented at target AF146527 [23]. Results were obtained using amplification primers Tox-9 and Tox-11 [24] as forward and reverse primers, respectively. Reactions were set up to a final volume of 25 μl containing 1 μl of DNA sample, 4 μl H_2O, and 20 μl SYBR Green PCR Master Mix (Applied Biosystems, Paisley, UK). The Real-Time SYBR Green PCR reaction products were analysed with an ABI 7500 Real-Time PCR system with SDS Software Version 2.0.1 (Applied Biosystems, Paisley, UK) by a first cycle of 10 min of activation at 95°C. The subsequent dissociation step consisted of: 95°C/15 s; 60°C/1 min; 95°C/15 s for 40 cycles and the dissociation was measured stepwise, every 0.5°C.

In total, 86 common moles were trapped (Tables 1 and 2). Samples were not evenly distributed over different trapping habitats (Figure 1). No positive samples (brain cysts) were found during the microscopic detection of the 70 non-frozen moles. Using the Latex Agglutination Test (LAT), sera of 70 moles were examined, whereby

Table 2 Results of the LAT and real time-PCR detection

Test	Serology				DNA			
	♂	♀	Unknown gender	Subtotal	♂	♀	Unknown gender	Subtotal
+	0	0	0	0	2	0	0	2
-	44	22	4	70	50	30	4	84
Total	44	22	4	70*	52	30	4	86*

*Fresh, non-frozen moles were serologically tested. All moles (including the frozen) were DNA tested.

only 1 sample (1.4%) reacted with *T. gondii* antigen, at a 1:16 dilution (internal positive control samples reacted up to a 1:128 dilution). Using a cut off value of a dilution of 1:32 as questionable and a >1:64 dilution as positive, this sample is considered as negative. The Real-Time PCR test on tissue samples revealed that 2 male moles were positive (Ct 36.61 and 36.57, respectively) for *T. gondii* (2/86 = 2.3%). These moles were trapped in a garden and a recreation area.

In this study, *T. gondii* presence in moles was studied by different tests. Our LAT showed no sample that reacted with *T. gondii* antigen. For the Real-Time PCR brain tissue was used as this is the target organ in mice and it is also used for human prenatal diagnostics. Ct-values were high for the two moles testing positive with Real-Time PCR (36.61 and 36.57, respectively), which means that little *T. gondii* DNA was present in the brain samples. Such high values could indicate false positives. However, in an earlier study, it was found that the Ct-values of experimentally infected mice were around 20, whereas in naturally infected mice it varied between 35 and38 [10,11]. Therefore, we assume that the two samples found positive using Real-Time PCR were indeed positive. The positive samples from the Real Time-PCR were not positive using the LAT. A reason can be that it takes time for the animal to produce antibodies against *T. gondii*, whilst parasite DNA is already present.

In previous studies from France and Germany common moles were identified as possible intermediate hosts for *T. gondii* [19,20]. However, infection rates in our study (0–2.3%, depending on the testing method) were significantly lower than the prevalence in the French study, where 39% of the moles from North-Eastern France tested positive [20].

Generally, animal age is positively related to the risk of *T. gondii* infection. In our study, the average age of trapped moles may have been lower, as most of them originated from habitats where moles are trapped regularly and so mainly juveniles are present. However, the age of the trapped moles was not determined. Because of the low number of positive samples in our study, no relation could be found between the prevalence of *T. gondii* in common moles, their gender or habitat type of the trapping location. Consequently, it must be concluded that the use of common moles as an indicator species is not yet feasible. Concerning habitat, we assumed beforehand that in areas where generally more cats are present (gardens and pastures) the infection rate would be the highest. However, due to the low number of positive specimens, it was not possible to prove this hypothesis statistically. In order to acquire more insight into this relationship, it would be necessary to increase the number of trapping locations and to test larger numbers of common moles. A comparison of *T. gondii* incidence in moles between countries that apply mainly indoor animal production systems (such as The Netherlands) and countries that apply outdoor animal production systems might be useful as well, as the low infection incidence could also be related to the type of production system. In conclusion, the use of common moles as an indicator species for *T. gondii* should be the subject of further study, preferably linked to detection of this pathogen in its diet (earthworms) and in near-by livestock.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
IK carried out the fieldwork, participated in the dissections, participated in the laboratory work and drafted the manuscript. JC carried out the immunoassays. HW participated in the design of the study. BM conceived of the study, and participated in its design and coordination and finalized the manuscript. All authors read and approved the final manuscript.

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Author details
[1]Wageningen UR (University & Research centre), Livestock Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands. [2]Wageningen UR (University & Research centre), Central Veterinary Institute, P.O. Box 65, 8200 AB Lelystad, The Netherlands.

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