

# Tracking *Toxoplasma gondii* in freshwater ecosystems: interaction of the parasite with the exotic mustelid American mink (*Neovison vison*) in Spain

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## INTRODUCTION

*Toxoplasma gondii* is a zoonotic protozoan parasite with a worldwide distribution. Felids are the definitive hosts, excreting oocysts in faeces to the environment. Humans and virtually all warm-blooded species can become infected by several routes of transmission. Water-borne transmission may play an important role in the epidemiology of this parasite. The objective of this study was to assess the importance of this way of transmission analyzing the infection of *T. gondii* in the American mink (*Neovison vison*), a widely distributed invasive species in freshwater ecosystems in Spain.

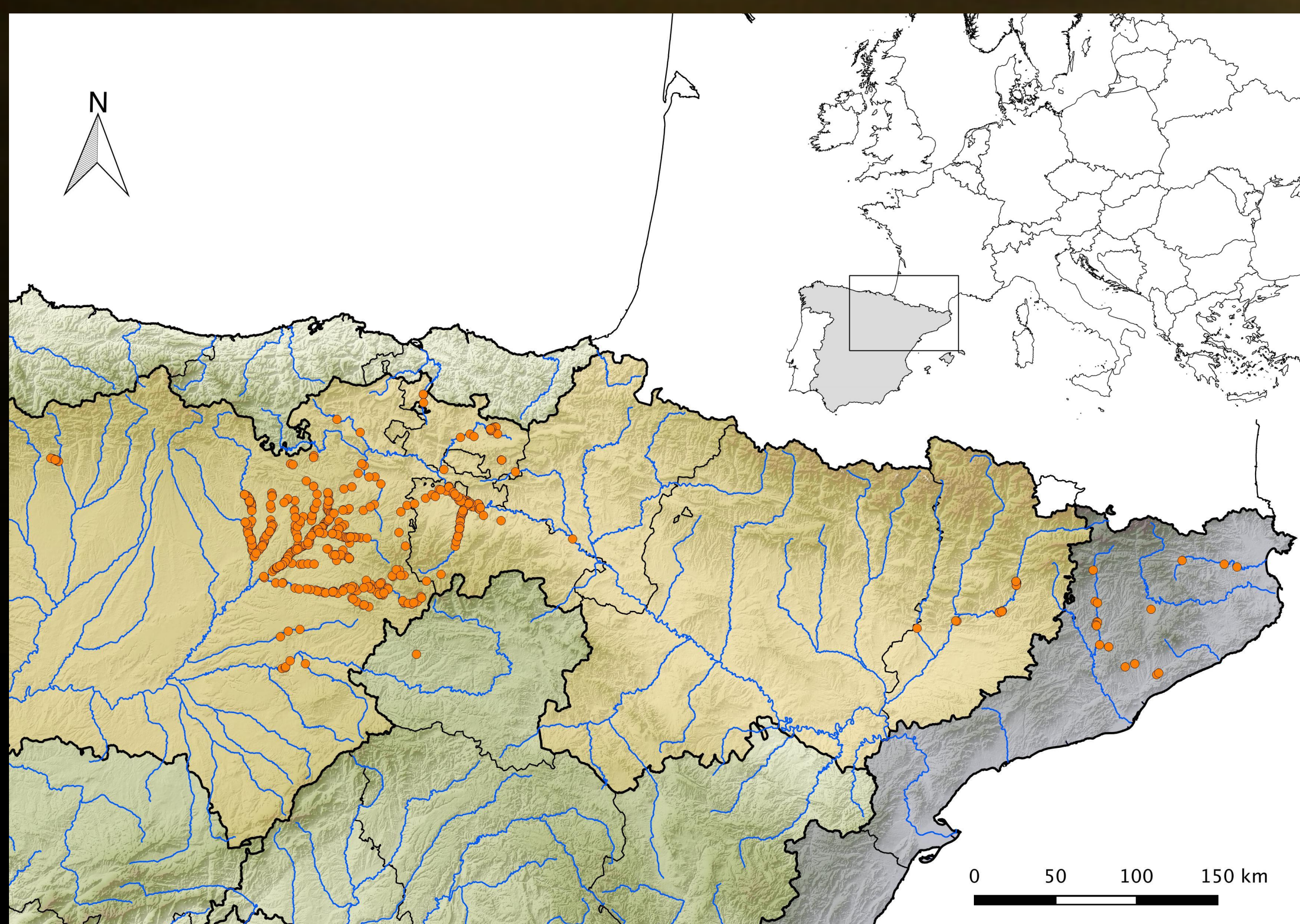


Figure 1. Map showing the location of the serum samples (orange spots). Bio-regions 2 (Northern plateau) and 5 (East and South coast) are colored in yellow and blue respectively.

### Primers and probe

Toxo-SE	5'-AGGCGAGGGTGAGGATGA
Toxo-AS	5'-TCGTCTCGTCTGGATCGCAT
Toxotaqman	5'-6FAM-CGACGAGAGTCCGAGAGGGAGAAGATGT—BHQ1

Table 1. Primers and probe used to perform the rtPCR

## MATERIALS AND METHODS

Serum samples from 678 American minks (AM) were collected from 4 Spanish regions that correspond with two bio-region and two river basins (Figure 1; Table 2). The AM were captured during the 2010-2015 national eradication campaigns. Antibodies against *T. gondii* were assayed by the modified agglutination test (MAT titers  $\geq 1:25$ ). Additionally, 120 samples from brain tissue were used to perform a specific real time PCR (rtPCR) (Table 1).

Associations between serological results and independent variables were analyzed using a Pearson's chi-square test.

Table 2. Seroprevalence of *Toxoplasma gondii* (MAT  $\geq 1:25$ ) by categories. Statistically significant differences when different letters (a,b) within categories ( $p < 0.05$ ).

Category	Positive / Examined (%)	
Study area	Catalonia	38/46 (82.6)
	Castilla-León	403/510 (79.0)
	La Rioja	48/67 (71.6)
	Pais Vasco	45/55 (81.8)
Bio-region	2	510/653 (78.1) <sup>a</sup>
	5	22/23 (95.7) <sup>b</sup>
River Basin	Duero	360/460 (78.3)
	Ebre	119/161 (73.9)
Sex	Female	289/370 (78.1)
	Male	182/239 (76.2)
Age Group	Juvenile	103/150 (68.7) <sup>a</sup>
	Adult	352/444 (79.3) <sup>b</sup>
	Elderly	33/37 (89.2) <sup>b</sup>
Total	534/678 (78.8; CI <sub>95%</sub> 75.5–81.8)	

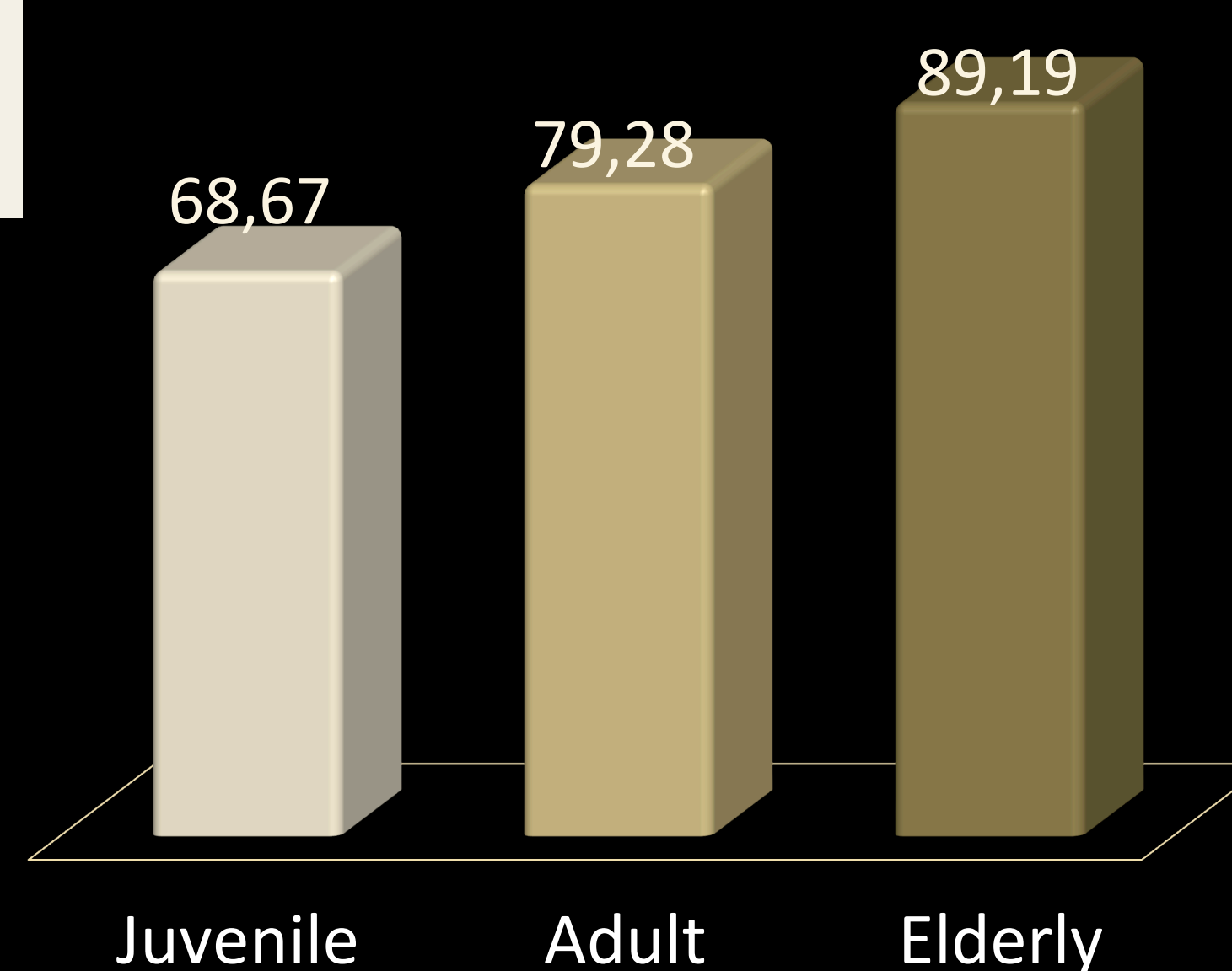


Figure 2. Seroprevalence of *Toxoplasma gondii* related to age.

## RESULTS

Titers of 1:25 or higher were found in 534 of 678 American minks. An overall 78.8% seroprevalence was detected with titers of 1:25 (11.2%), 1:50 (7.3%), 1:100 (32.6%) and 1:500 (48.9%). Statistical significant differences were observed by age groups (Figure 2) and bio-regions. (Table 2). DNA was detected in 11 out of 120 brain samples (9,2%) by the rtPCR.

## DISCUSSION

The seroprevalence observed in the AM was higher than previous data on terrestrial mammals in the Iberian Peninsula and Europe. These results highlight the importance of the aquatic environment in the epidemiology of *T. gondii*. Differences observed between age groups reflect a higher probability of life time exposure associated to horizontal transmission of the parasite. AM diet is mainly composed by aquatic invertebrates and fish which may act as transporters of oocysts. Another source for horizontal transmission could be through direct ingestion of contaminated water. Differences among bio-regions could be explained by ecological traits such as definitive host densities. Domestic cats in highly populated areas, such as bio-region 5, might be an important source of oocyst contamination through freshwater runoff. Molecular characterization of the parasite would help to identify the routes and mechanisms of land to sea transmission.

## CONCLUSIONS

There is a high and widespread exposure of American minks to *T. gondii* in freshwater ecosystems in Spain.

Water-borne transmission of oocysts may be an important route of transmission for *T. gondii*.

The AM is a good sentinel specie for *T. gondii* contamination in aquatic habitats.

Differences among age groups reflect the predominance of the horizontal transmission.

Differences in bio-regions highlight the need of more complex spatial analyses.

The rtPCR could be a useful tool for future studies in molecular epidemiology.