*SHORT COMMUNICATION*

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# Risk factors involved in transmission of *Toxoplasma gondii* and *Neospora caninum* infection in rabbit farms in Northern Italy

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## Abstract

**Introduction and objective.** In Italy, rabbits are frequently reared for meat production. The aim of the study was to find the seroprevalence of *T. gondii* and *N. caninum* parasites, and risk factors of infection in rabbit farms.

**Material and methods.** Blood samples from 260 apparently healthy breeding rabbits were collected on 13 commercial farms in Northern Italy. Rabbits were divided into categories according to age, number of births, breed, province and size of farm. Samples were tested for antibodies to *T. gondii* and *N. caninum* using the indirect fluorescence antibody test (IFAT); samples with a titre ≥ 50 were considered positive.

**Results.** Antibodies to *T. gondii* and *N. caninum* were found in 38 (14.6 %) and 3 (1.2 %) rabbits, respectively. A statistically significant difference (p-value ≤ 0.05) was found only in *T. gondii* prevalence among different rabbit breeds and provinces.

**Conclusion.** Rabbits from Northern Italy are at risk of *T. gondii* and *N. caninum* infection; however, it is lower compared to seroprevalence noted in other animal species or in humans.

## Key words

Toxoplasmosis, neosporosis, rabbits, serological survey, risk factors

## INTRODUCTION AND OBJECTIVES

Toxoplasmosis is a disease that in sensitive hosts causes different clinical symptoms, affects their reproduction and can lead to death [1]. In livestock, it is also connected with economic losses because sheep, goats and rabbits belong to the animal species very susceptible to *Toxoplasma gondii* infection. In Europe, there are many serological studies focusing on detection of *T. gondii* antibodies in rabbits; however, to our knowledge there is no similar study from Italy. The only study from Italy was undertaken by Zanet et al. [2] who isolated the DNA of *T. gondii* from tissues of 2.1 % of 144 wild rabbits (*Sylvilagus floridanus*).

The consumption of raw or undercooked meat originated from meat-producing animals, including rabbits, is a very important source of *T. gondii* infection in humans. Since toxoplasmosis is a zoonosis [3], and rabbits are frequently used for meat consumption in Italy, it is important to know the risk factors of *T. gondii* infection from consumption of rabbit meat for humans, and the important factors for the transmission of *T. gondii* among rabbits. The rabbits could be infected with *T. gondii* by the ingestion of food or water contaminated with *T. gondii* oocysts from cat faeces [3], or by transmission of *T. gondii* to offspring through transplacental infection [4]. Rabbits are usually without clinical symptoms, therefore detection of antibodies is very important in epizootiological studies. Rabbits could be infected with *T. gondii* and simultaneously with similar parasite *N. caninum*. This is why the aim of the presented study was to detect both *T. gondii* and *N. caninum* antibodies in rabbits, and evaluate the risk factors of infection on rabbit farms.

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## MATERIALS AND METHODS

During 2009, blood samples were collected from the auricular vein of 260 apparently healthy breeding rabbits on 13 commercial rabbit farms in Northern Italy (Veneto Region). All farms were industrial-cycle type with breeding and growing units located in the same house, with the number of breeding and growing does ranging between 300–3,000 and between 2,000–14,000, respectively. Breeding does were individually housed in wire cages equipped with a nest box. Rabbits were fed *ad libitum* and drinking water was administered through nipple watering systems. Artificial insemination by using external semen was practiced on all farms.

All sampled doe rabbit showed no signs of clinical symptoms. Data, including age, breed, number of births and health conditions, were obtained through questionnaires at the farms. The rabbits were divided into five age categories: 2–5 months (n=59); ≥ 5–7 months (n=66); ≥ 7–13 months (n=74); ≥ 13–30 months (n=26); and rabbits with unknown age (n=35); five categories according to number of births: 0 birth (n=60); 1–2 births (n=71); 3–7 births (n=57); 8–15 births (n=37); and rabbits with an unknown number of births (n=35). Four commercial hybrid lines: W (n=40),

X (n=160), Y (n=40), and Z (crossbred of New Zealand White x Californian, n=20), marked similarly as in the study of Lonardi et al. [5], were included in the current study. Doe rabbits were also divided according to the provinces in which they were bred: Padova (n=20), Rovigo (n=20), Treviso (n=160) and Verona (n=60); and the size of the farms: small farms (<1,000 does, n=120) and large farms (≥ 1,000 does, n=140). Data are summarized in Table 1.

Each blood sample was centrifuged and removed serum was frozen at -20°C until testing. Antibodies to *T. gondii* and *N. caninum* were detected by indirect fluorescence antibody test (IFAT) using a commercially available *T. gondii* antigen and *N. caninum* antigen (VMRD, Pullman, USA), respectively, and anti-rabbit IgG FITC conjugate (Sigma Aldrich, Czech Republic). Positive and negative control sera from an experimental study of domestic rabbits with *T. gondii* oocysts [6] were included in the tests. Sera were diluted with PBS two-fold, starting at 1:50. In the present study, cut-off titre 50 was used for both parasites, which is higher than in experimental studies (cut-off titre 20), to minimize the risk of false positive samples due to cross-reactivity.

**Table 1.** Characteristics of rabbits and results of *Toxoplasma gondii and Neospora caninum* seroprevalence (IFAT)

| Characteristics | No. of rabbits tested | *T. gondii* Positive (%) | *N. caninum* Positive (%) |
|---|---|---|---|
| **Gender** | | | |
| Female (does) | 260 | 38 (14.6 %) | 3 (1.2 %) |
| **Age categories (months)** | | | |
| 2–5 | 59 | 4 (6.8 %) | 0 (0 %) |
| ≥ 5–7 | 66 | 8 (12.1 %) | 2 (3 %) |
| ≥ 7–13 | 74 | 10 (13.5 %) | 0 (0 %) |
| ≥ 13–30 | 26 | 3 (11.5 %) | 1 (3.8 %) |
| not known | 35 | 13 (37 %) | 0 (0 %) |
| **No of births** | | | |
| 0 | 60 | 4 (6.7 %) | 0 (0 %) |
| 1–2 | 71 | 6 (8.5 %) | 2 (2.8 %) |
| 3–7 | 57 | 9 (15.8 %) | 0 (0 %) |
| 8–15 | 37 | 6 (16.2 %) | 1 (2.7 %) |
| many but not known | 35 | 13 (37 %) | 0 (0 %) |
| **Breed** | | | |
| W | 40 | 3 (7.5 %) | 0 (0 %) |
| X | 160 | 23 (14.4 %) | 3 (1.9 %) |
| Y | 40 | 12 (30 %) | 0 (0 %) |
| Z | 20 | 0 (0 %) | 0 (0 %) |
| **Provincie** | | | |
| Padova | 20 | 0 (0 %) | 0 (0 %) |
| Rovigo | 20 | 3 (15 %) | 0 (0 %) |
| Treviso | 160 | 11 (6.9 %) | 3 (1.9 %) |
| Verona | 60 | 24 (40 %) | 0 (0 %) |
| **Size of farm** | | | |
| Small farms (< 1000 does) | 120 | 19 (15.8 %) | 1 (0.8 %) |
| Large farms (≥ 1000 does) | 140 | 19 (13.6 %) | 2 (1.4 %) |

In case of *T. gondii*, the results of chi-square test proved (with the risk of error not more than 5%) dependence on breed ($\chi2$=12.64, df=3, p-value=0.005) and on provinces ($\chi2$=42.08, df=3, p-value < 0.05). Multiple comparison method based on arcsine transformation proved that there is difference in prevalence between breeds X and Z and between Y and Z; and also between two provinces Padova and Rovigo, two provinces Padova and Verona and two provinces Treviso and Verona. In case of *N. caninum*, statistical analysis could not be done because of small number of positive samples.

Seroprevalence was statistically analysed, considering the variables of age, number of births, breeds, province and size of farms. Data analysis was performed by Chi-Square test for independence resp. Fisher exact test for Tables 2 × 2 using STATISTICA Cz 12 [7]. Dependence on age and seroprevalence was evaluated by Wilcoxon test. Null hypothesis was tested to ascertain if *T. gondii* seroprevalence depended on age, number of births, breeds, province and size of farms. Multiple comparison method based on arcsine transformation was used to compare *T. gondii* prevalence between breeds and provinces. The differences were considered statistically significant when p-value was ≤ 0.05.

## RESULTS AND DISCUSSION

In the presented study, antibodies to *T. gondii* and *N. caninum* were found in 38 (14.6%) and 3 (1.2%) doe breeding rabbits, respectively. No co-infection between *T. gondii* and *N. caninum* was found. Titres of *T. gondii* antibodies were at a low level (50–100). To the best of the authors' knowledge, this is the first serological study focused on *T. gondii* and *N. caninum* seroprevalence in rabbits from Northern Italy, and the first detection of *N. caninum* antibodies in domestic rabbits in Europe. It could be concluded that the does from Northern Italy are at risk of *T. gondii* and *N. caninum* infection; however the seroprevalence is lower compared to the seroprevalences noted in other animal species, or in humans.

Similar *T. gondii* prevalence was obtained by the different methods used. Antibodies to *T. gondii* were detected, e.g. by indirect haemagglutination test in 23.4 % (18/77) rabbits from China [8], by Enzyme-Linked Immuno Sorbent Assay (ELISA) in 15.5 % (103/1883) rabbits from the Czech Republic [9] and in 11 % (22/194) rabbits from Egypt [10], or by Modified Agglutination Test (MAT) in 16.3 % (70/429) rabbits from Mexico [11].

The results of the present study, based on different risk factors (age, number of births, breeds, provinces and size of farm), are summarized in Table 1. Lower *T. gondii* and *N. caninum* prevalence (6.8% and 0%, respectively) were found in rabbits until 5 months of age, while in older rabbits, prevalence increased to 13.5 % and 3.8 %, respectively (p-value > 0.05). These results are in contrast to a study by Alvarado-Esquivel et al. [11] from Mexico; they detected higher *T. gondii* seroprevalence 41.9% in young rabbits (age category: 0.3–1 month) compared to older ones. Similar to Alvarado-Esquivel et al. [11], high *T. gondii* seropositivity of young domestic rabbits was confirmed by Uhliková and Hübner [4]; they also discussed the possibility of transplacental transmission of *T. gondii* in rabbits. The seroprevalence of *T. gondii* increased with the number of births, without statistical significant difference (p-value=0.4141) however. This increasing trend of prevalence may be associated with post-natal infection through ingestion of food or water contaminated with *T. gondii* oocysts. The highest prevalence was found in hybrid line Y (30 % for *T. gondii*) and X (14.4 % and 1.9% for *T. gondii* and *N. caninum,* respectively), while Z hybrids were negative for both *T. gondii* and *N. caninum* antibodies. The difference in *T. gondii* prevalence in breeds was statistically significant in some of breeds (test statistics=12.64, df=3; p-value=0.005), which is in contrast with the results of Alvarado-Esquivel et al. [11], who did not find statistical differences in breeds. The

rabbits came from four provinces; the highest seroprevalence of *T. gondii* (40%) and *N. caninum* (1.9%) antibodies was found in Verona and Treviso provinces, respectively. In the case of *T. gondii,* the results were statistically different (test statistics=42.08, df=3; p-value < 0.05) between some of the provinces. Alvarado-Esquivel et al. [11] also found a statistically significant different *T. gondii* prevalence in rabbits from six municipalities in Mexico. This fact was explained by different climatic conditions. However, in the present study, there were no strong differences in climatic conditions of the six provinces, and the samples were collected on 13 farms. The highest *T. gondii* seroprevalence (55%) was found in rabbits from one large farm in Verona province; the highest prevalence of *N. caninum* antibodies (5%) was found on one small and two large farms in Treviso province. Nevertheless, there was no statistically significant difference (test statistics=0.26 resp. 0.20, df=1; p-value > 0.05) between either *T. gondii* and *N. caninum* seroprevalence and size of farms.

Two of 38 (5%) rabbit does positive for *T. gondii* antibodies had been treated for respiratory or enteric disorders, which could be connected with symptoms of *T. gondii* infection. No signs of disease were recorded in the other does.

## CONCLUSION

It is worth noting that rabbit does from Northern Italy are at risk of *T. gondii* and *N. caninum* infection which could be transmitted to their offspring that are mainly used for human consumption. That is why it is very important to implement good hygienic conditions on farms where the rabbits are bred. There was no data available about the presence of cats and dogs on the rabbit farms to study this risk factor. However, avoidance of the presence of cats and dogs on the farms is highly recommended to minimize the risk of contamination of the environment, food and water with *T. gondii* and *N. caninum* oocysts, respectively.

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