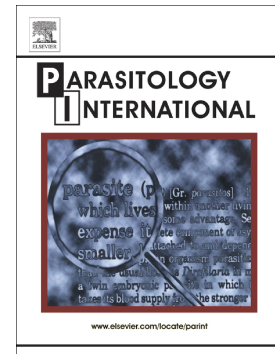


## Accepted Manuscript

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*Toxoplasma gondii* seroprevalence in goats, cats and humans in Russia

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## Abstract

Toxoplasmosis, a most common zoonosis, is caused by the protozoan parasite *Toxoplasma gondii*. However, there is little epidemiological information on *T. gondii* infections in humans and livestock animals in Russia. Therefore, in this study, the seroprevalence of *T. gondii* in goats in Russia was investigated. A total of 216 goats from 32 farms were investigated and 95 of them were seropositive for *T. gondii*. The difference in seroprevalence between the examined regions was not statistically significant. We next collected serum samples from 99 cats and 181 humans in Kazan city, the state capital of the Republic of Tatarstan, Russia, and examined their *T. gondii* seroprevalences. Thirty-nine of the 99 cat samples and 56 of the 181 human samples showed seropositivity. Logistical regression analysis revealed that the cat breeding history of the human subjects, but not their sex or age is a significant risk factor for *T. gondii* seropositivity. These findings suggest that the natural environment in Russia may be widely polluted with *T. gondii* oocysts shed by cats, and ingestion of these oocysts provides a major route for human infection with this parasite.

Keywords: Cats; Russia; Seroprevalence; *Toxoplasma gondii*

## Highlights:

- *Toxoplasma gondii* seroprevalence was examined in humans, cats and goats in Russia
- Antibodies to *T. gondii* were found in goats (43.9%), cats (39.9%) and humans (30.9%)
- Oocyst ingestion appears to be main route of human *T. gondii* infection.

*Toxoplasma gondii* is a zoonotic protozoan parasite that causes widespread infections in humans and other animals, including meat production animals. Although most infections in humans are asymptomatic, the parasite can cause severe complications in immunocompromised individuals and abortion when a mother is infected for the first time during pregnancy. Human toxoplasmosis is transmitted mainly through ingesting the tissue cysts present in *T. gondii* contaminated raw or undercooked meat, or through oral contact with the sporulated oocysts of this parasite when present in the feces of infected cats. It was reported that 30 to 63% of human infections are attributable to the consumption of undercooked meat in Europe [1]. An epidemiological study among more than 4,000 Japanese pregnant women identified a history of raw meat intake as a risk factor related to toxoplasmosis development, but cat ownership was not a significant factor [2]. In contrast, a nationwide representative cross-sectional survey in Germany showed that cat ownership was a risk factor for *T. gondii* seropositivity [3]. The relative importance of oocysts from cat feces or tissue cysts in meat products as sources of human *T. gondii* infections in each area is considered to be affected by the eating habits and cat rearing style of people in different countries. However, there is little information on the status of *T. gondii* infections among cats, domestic animals for meat production, and humans in Russia. In this study, we surveyed the seroprevalence of *T. gondii* in goats, cats and humans in Russia. All sampling and analysis procedures using human and all other animal derived samples were approved by the Ethical Committee of the Kazan State Medical Academy, Kazan, Russia (Permit no. 2/2002) and by the Animal Research Committee of Gifu University (Permit no. 17060). Permission to use the samples from humans was also granted by the Ministry of Health of the Russian Federation.

190 goat serum samples were obtained across seven states (oblast, city or republic) in Russia (Moscow oblast, Moscow city, Ryazan oblast, Nizhny Novgorod oblast, Samara oblast, Rostov oblast and the Republic of Tatarstan) between October and December 2013. 26 additional serum samples were also collected from different individual goats at Farm D in the Verkhneuslonsky District of the Republic of Tatarstan between February and April 2015 (Fig.1 and Table 1). *T. gondii* antibody detection from the total of 216 goat serum samples was conducted using a commercially available diagnostic kit for humans (Toxotest-MT; Eiken Kagaku, Tokyo, Japan); this system detects anti-*Toxoplasma* antibodies using a latex agglutination assay. Although this diagnostic system has not been approved for use in animals by the manufacturer, it was also confirmed that the same cut-off value (1:32) used for determining seropositivity in human diagnosis is applicable to goats [4]. As shown in Table 1, more than 30 % of goats

showed seropositivity in all of the study states. Considering that cyst infection is unlikely to happen to herbivorous animals and that endogenous-trans placental infection with *T. gondii* is not common among them [5], it is likely that the majority of the infected goats identified in this study had ingested oocysts shed by cats. The relatively high seroprevalence among goats across a wide area of Russia suggests that the Russian environment is widely and highly polluted with oocysts. Therefore, we examined 99 cat serum samples obtained in a veterinary clinic in Kazan city using Toxotest-MT and found that 39 individuals were positive for *T. gondii* (95% CI: 29.7-50.0 %). This speculation, high level pollution of Russian environment with *T. gondii* oocysts, is consistent with the high seroprevalence of *T. gondii* among these cats study. However, the seroprevalence in goats varied among the farms we examined (Table 1). The presence of cats on farms has been reported to be a risk factor for infection with *T. gondii* in goats in Myanmar [6]. Therefore, the differences in goat seroprevalence we noted between Russian farms may depend on the presence of cats in this country also.

We next examined a total of 181 human serum samples collected in four hospitals in Kazan city comprised the following: 49 from the examinees of routine health checks without any particular symptoms; 74 from the examinees in a specialized rheumatology hospital; 22 from the examinees of routine medical checks during pregnancy; and 36 from patients with active tuberculosis. Age and sex data of some of the examinees and patients were provided according to the ethical statement. In the specialized rheumatology hospital, we asked the 74 examinees about their cat breeding histories and obtained answers from 70 of them. To detect anti-*T. gondii* IgG, the human *Toxoplasma gondii* IgG (Toxo IgG) ELISA Test Kit (DIAGNOSTIC AUTOMATION / CORTEZ DIAGNOSTICS, INC. Woodland Hills, USA) was used according to manufacturer's instructions. We found that fifty-six of the 181 samples showed seropositivity to *T. gondii* (95 % CI: 24.3 – 38.2 %). And then logistic regression was performed using the statistical software R to assess age, sex, cat breeding history and the sampling hospitals as risk factors for seropositivity [7]. The variance inflation factor (VIF) was calculated using the "DAAG" package [8] and confirmed that none of the VIF values were < 10 and the mean VIF of the model was < 6. Multivariate analysis showed that only a cat breeding history was significantly associated with *T. gondii* seropositivity (odds ratio = 22.1,  $p < 0.01$ ). It has been reported that high frequency contact with cats or a history of cat breeding are not significant risk factors for contracting human *T. gondii* infections in Japan [2] However, in the present study, our data revealed that a

history of cat breeding is a high risk factor for *T. gondii* infection among the patients of a specialized hospital for rheumatology in Kazan city. Age and sex were not considered to be significant risk factors among these patients. Although we do not know whether these findings might apply to the Russian population generally, a history of cat breeding may be a risk factor in this country, or at least for a particular population within it. Among these patients, an age dependent increase in seroprevalence was not observed, suggesting that the incidence of *T. gondii* infection of a particular subpopulation is higher than others. In Russia, it is not common to serve rare steak: stewing is the most common way to cook meat here. The lower frequency of food-derived *T. gondii* infections might highlight the risk posed by oocyst-shedding cats. Indeed, it has been reported that *T. gondii* seroprevalence in pet cats with outdoors access was higher than those without such access [9]. In Russia, pet cats with free outdoors access are common even in rural areas. Therefore, raising cats outdoors may affect the prevalence of *T. gondii* among humans and other animals in Russia.

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Conflicts of interest: None

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Figure legend

Fig. 1 Locations of the study areas for *T. gondii* seroprevalence in goats in Russia. Although Moscow city is administratively independent from Moscow oblast, it was considered to be the capital of Moscow oblast in this study.

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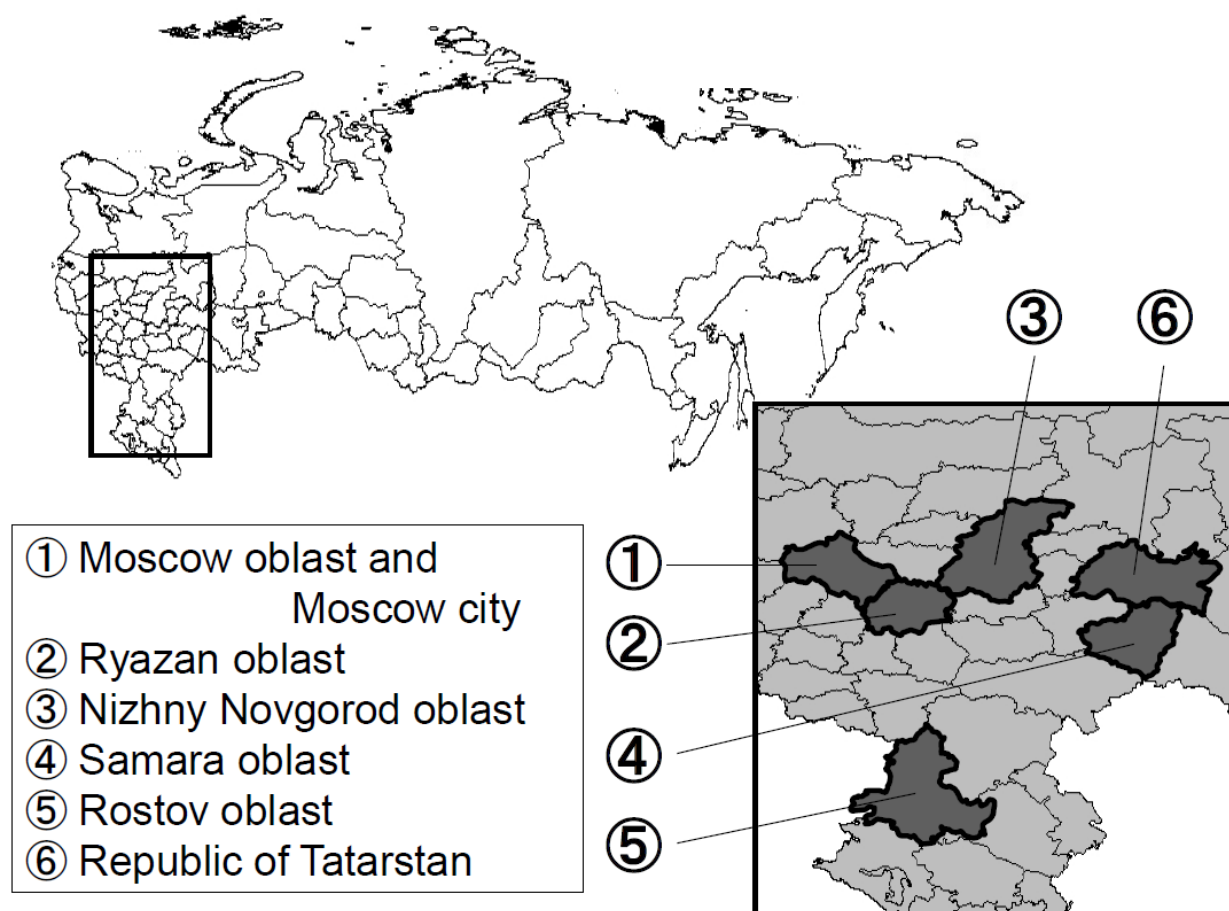


Fig. 1

Table1 Anti-*T. gondii* antibody seroprevalence among goats in Russia

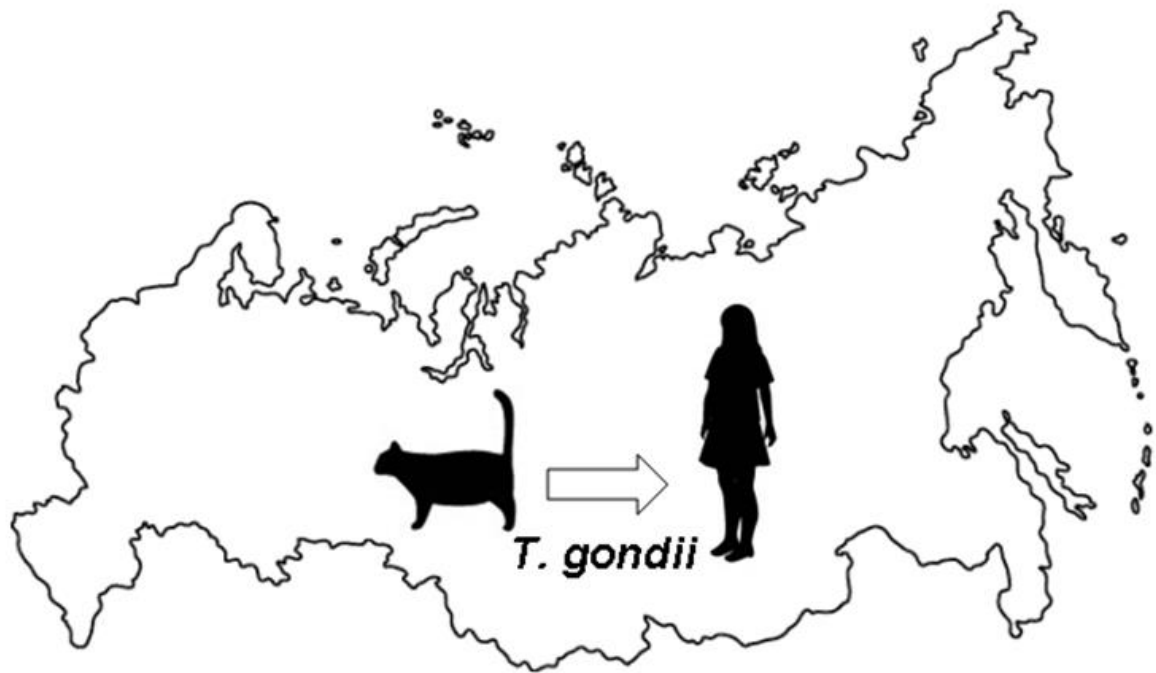
| State                         | Area*2               | Farm | Antibody Titer *1 |    |    |     |            | Seroprevalence (%) (95% CI) |                    |   |
|-------------------------------|----------------------|------|-------------------|----|----|-----|------------|-----------------------------|--------------------|---|
|                               |                      |      | $\leq 16$         | 32 | 64 | 128 | $\geq 512$ |                             |                    |   |
| Moscow Oblast and Moscow City | Serpkhov City        | A    | 3                 |    |    | 2   | 1          | 47.0 (34.6 – 59.7)          |                    |   |
|                               |                      | B    | 3                 |    |    |     |            |                             |                    |   |
|                               | Domodedovo City      | C    | 5                 | 2  |    |     | 1          |                             | 1                  |   |
|                               |                      | D    |                   |    |    |     | 1          |                             | 1                  |   |
|                               | Zaraysky District    | E    | 2                 | 1  | 1  | 2   | 1          |                             | 1                  |   |
|                               | Klinsky District     | F    | 1                 |    |    | 1   | 3          |                             | 1                  |   |
|                               |                      | G    | 1                 |    |    |     |            |                             |                    |   |
|                               |                      | H    | 3                 |    | 1  | 1   | 1          |                             | 1                  |   |
|                               | Moscow City          | I    |                   | 2  |    |     |            |                             | 1                  | 1 |
|                               |                      | J    | 7                 | 1  |    |     |            |                             |                    |   |
| K                             |                      | 10   |                   |    | 1  |     | 1          |                             |                    |   |
| Ryazan Oblast                 | Rybnovsky District   | A    | 9                 | 1  | 1  |     | 2          | 2                           | 40.0 (16.3 – 67.7) |   |
| Nizhny Novgorod Oblast        | Bogorodsky District  | A    | 3                 | 1  | 1  |     | 2          | 2                           | 55.8 (39.9 – 70.9) |   |
|                               |                      | B    | 3                 |    | 1  |     | 2          |                             |                    |   |
|                               |                      | C    |                   |    | 1  |     |            |                             |                    |   |
|                               | Semyonovsky City     | D    | 1                 |    |    | 1   |            |                             |                    |   |
|                               | Kstovsky District    | E    |                   |    |    | 1   | 2          |                             |                    |   |
|                               |                      | F    | 3                 | 2  | 2  | 2   |            |                             |                    |   |
|                               |                      | G    | 2                 |    |    | 1   | 1          |                             |                    |   |
|                               | Nizhny Novgorod City | H    | 7                 | 1  |    |     |            | 1                           |                    |   |
| Samara Oblast                 | Tolyatti City        | A    |                   |    |    | 1   |            |                             | 57.7 (36.9 – 76.6) |   |
|                               |                      | B    | 3                 |    |    |     |            | 2                           |                    |   |
|                               |                      | C    | 2                 |    |    |     |            | 4                           |                    |   |
|                               | Syzran City          | D    | 3                 |    |    |     |            | 3                           |                    |   |
|                               | Syzransky District   | E    | 2                 | 2  |    |     | 2          |                             |                    |   |
|                               |                      | F    | 1                 |    |    | 1   |            |                             |                    |   |

|                       |                          |    |    |   |   |   |   |    |                    |
|-----------------------|--------------------------|----|----|---|---|---|---|----|--------------------|
| Rostov Oblast         |                          | A  | 2  |   |   |   |   | 1  | 33.3 (8.4 – 90.6)  |
| Republic of Tatarstan | Leishevsky District      | A  | 1  |   | 1 | 1 | 1 |    | 28.6 (17.9 – 41.3) |
|                       | Verkhneuslonsky District | B  | 6  |   |   |   |   |    |                    |
|                       |                          | C  | 5  |   |   |   |   | 1  |                    |
|                       |                          | D* | 11 | 4 | 2 |   |   | 1  |                    |
|                       |                          | 3  | 19 | 2 | 1 |   | 3 | 1  |                    |
| <b>Kazan City</b>     | E                        | 3  |    |   |   |   |   |    |                    |
| Total                 |                          |    | 12 | 1 | 1 | 1 | 2 | 25 | 43.9 (37.3 – 50.9) |
|                       |                          |    | 1  | 9 | 2 | 5 | 4 |    |                    |

\*1 cut-off value (1:32) was used for determining seropositivity

\*2 Bold-faced type indicates capital city of state.

\*3 Serum samples were corrected twice in the farm from other individuals. Upper: 2013 Oct.–Dec. Lower: 2015 Feb.–Apr.



Graphical abstract

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