Seroepidemiology of Toxoplasma gondii Infection in Blood Donors from Western Romania

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Abstract: Background: Toxoplasma gondii is estimated to infect 33% of blood donors worldwide, and seroprevalence varies widely between countries. We aimed to evaluate, for the first time, the seroprevalence and risk factors associated with T. gondii in blood donors from Western Romania. Methods: Serologic testing to demonstrate the presence of T. gondii antibodies in blood donors was conducted in 1347 healthy blood donors. Risk factors for T. gondii infection were assessed through an epidemiological questionnaire. Results: The overall prevalence of T. gondii antibodies was 45.9%, with a significant age-associated increase (p < 0.001) from 32.6% in age group 18–25 years to 67.6% in age group 56–63 years. T. gondii seroprevalence decreased with increasing level of education, from 64.3% in individuals who graduated from elementary/middle school to 40.4% in those who graduated from University (p < 0.001). The multiple logistic regression analysis revealed that age, level of education and having pets (cats and/or dogs) were significantly associated with T. gondii infection. Conclusions: This study brings new and valuable data regarding the seroepidemiology of T. gondii infection in Romania. Our findings indicate a high prevalence of T. gondii antibodies in blood donors and may serve as a starting point for further epidemiological studies that should lead to implementation of prevention programs for toxoplasmosis.

Keywords: Toxoplasma gondii; antibodies; seroprevalence; risk factors; epidemiology; blood donors; Romania

1. Introduction

Toxoplasmosis is a zoonotic infection caused by an obligate intracellular parasite called Toxoplasma gondii. This protozoan has a worldwide distribution [1,2]: between 10.0% and 97.4% of the adult population is estimated to be infected [3]. T. gondii transmission may involve all three life-cycle stages of the parasite: oocysts (from water, vegetables, or soil), tissue cysts with bradyzoites (from raw or undercooked meat/primary offal), and tachyzoites (from blood products, tissue transplants, or unpasteurized milk) [3,4]. T. gondii may be also transmitted from the mother to the fetus by tachyzoites, which can cross the placenta [4]. Most often, the specific route of transmission cannot be established because the vast majority become infected inadvertently [2].
In most immunocompetent patients, the acute phase of the infection is asymptomatic, but clinical signs may appear in congenitally infected children and immunocompromised individuals [2]. Blood donors can impose risk of *T. gondii* infection for the susceptible recipients, such as immunosuppressed individuals and pregnant women [5,6]. Moreover, assessing the seroprevalence of *T. gondii* in blood donors offers important data regarding the prevalence of infection in apparently healthy individuals [7,8] and may indicate the spreading of infection in the general population.

Foroutan-Rad et al. estimated that approximately 33% of blood donors worldwide are infected with *T. gondii*, with the highest global rates on the African continent (46%) and the lowest in Asia (29%) [6]. Seroprevalence varies between countries: 6.26% in China [9], 9.3% in Taiwan [10], 19.66% in India [11], 20.5% in Serbia [7], 25.6% Turkey [12], 36% in Portugal [13], 48.1% in Brasil [14], and 67.92% in Côte d’Ivoire [15].

Previous reports from Western Romania showed a 55.8% prevalence of *T. gondii* antibodies in pregnant women [16], 57.6% among women of childbearing age [17] and 64.8% in the adult population [18]. There is no information regarding the magnitude of infection with *T. gondii* in Romanian blood donors. Therefore, in this study we aimed to evaluate the seroprevalence and risk factors associated with *T. gondii* in blood donors from Western Romania.

2. Materials and Methods

2.1. Study Design and Study Population

We enrolled 1347 consecutive volunteer healthy blood donors, in the order they presented to the Regional Blood Transfusion Center in Timisoara between 19 November–21 December 2018. Blood donors had to comply with the donation eligibility criteria set by the Romanian Ministry of Health [19]. Individuals with chronic hepatitis, liver cirrhosis, cancer, schizophrenia, epilepsy, diabetes or anemia were excluded from blood donation following the official blood donation procedure [19].

The software used in the Blood Center provided subjects’ demographic data (age, gender, area of residence) using a code, without their identification. An epidemiological questionnaire was carried out to obtain information regarding the risk factors associated with *T. gondii* infection: level of education (elementary/middle school, high school, University), consumption of raw and/or undercooked meat, contact with soil (gardening and/or agriculture activities), having cat(s) and having dog(s). Study participants were grouped according to their age in 5 age groups: 18–25 years, 26–35 years, 36–45 years, 46–55 years and 56–63 years.

2.2. Serological Testing

Serum samples were tested at the Center for Diagnosis and Study of Parasitic Diseases, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania. Pastorex Toxo kit (Bio-Rad, Marnes-la-Coquette, France), a latex particle agglutination assay, was used for simultaneously detection of immunoglobulin G (IgG) and/or immunoglobulin M (IgM) antibodies to *T. gondii*. Pastorex Toxo demonstrated an excellent ability to detect *T. gondii* antibodies in patients with acute and chronic toxoplasmosis [17,18,20]. Testing, quality controls and interpretation of results were based on manufacturer’s criteria.

2.3. Statistical Analyses

Statistical analyses were performed using Epi Info Version 7.2 (CDC, Atlanta, GA, USA) and MedCalc for Windows, version 19.4 (MedCalc Software, Ostend, Belgium). Data are presented as number (percentage), mean ± standard deviation (SD), and odds ratio (OR) with 95% confidence interval (CI). For comparison between *T. gondii* positive and negative blood donors, we used 2-tailed Fisher exact test and logistic regression (Wald forward stepwise method) with a *p*-value of <0.05 to represent statistical significance. Multiple logistic regression was performed for those variables that were found to be significantly associated with *T. gondii* infection in univariate analyses.
2.4. Ethics and Informed Consent

This study was approved by Victor Babes University of Medicine and Pharmacy Timisoara Ethics Committee (06/16.03.2018). All study participants provided written informed consent.

3. Results

The 1347 blood donors enrolled in the study were aged between 18 and 63 years (mean age = 33.6 ± 10.9 years), 755 (56.1%) were males and 979 (72.7%) were residents of urban areas (Table 1).

Table 1. Seroprevalence of *Toxoplasma gondii* infection in blood donors from Western Romania according to age, area of residence and gender.

| Variables               | No. Tested | Prevalence of *T. gondii* Infection | Univariate Analysis |
|-------------------------|------------|-----------------------------------|---------------------|
|                         |            | N (%)                             | OR (95% CI)         | p-Value          |
| Age groups (years)      |            |                                   |                     |
| 18–25                   | 411        | 134 (32.6)                        | 1 (Ref.)            | -                |
| 26–35                   | 407        | 173 (42.5)                        | 1.5 (1.15–2.03)     | 0.003            |
| 36–45                   | 282        | 158 (56.1)                        | 2.6 (1.93–3.60)     | <0.001           |
| 46–55                   | 211        | 129 (61.1)                        | 3.2 (2.30–4.59)     | <0.001           |
| 56–63                   | 36         | 24 (67.6)                         | 4.1 (2.01–8.52)     | <0.001           |
| Area of residence       |            |                                   |                     |
| Urban                   | 979        | 430 (43.9)                        | 1 (Ref.)            | -                |
| Rural                   | 368        | 188 (51.1)                        | 1.3 (1.05–1.70)     | 0.020            |
| Gender                  |            |                                   |                     |
| Male                    | 755        | 342 (45.3)                        | 1 (Ref.)            | -                |
| Female                  | 592        | 276 (46.6)                        | 0.9 (0.76–1.18)     | 0.628            |
| Total                   | 1347       | 618 (45.9)                        | -                   | -                |

N, number of *T. gondii* seropositive individuals; OR, odds ratio; CI, confidence interval; Ref., reference.

The overall seroprevalence of *T. gondii* antibodies was 45.9% (95% CI: 43.23–48.55) and tended to increase with age from 32.6% in age group 18–25 years to 67.6% in age group 56–63 years, showing a significant age-associated increase (*p* < 0.001; OR = 1.5; 95% CI: 1.36–1.65) (Table 1).

Compared to blood donors aged 18–25 years, the seroprevalence was significantly higher in individuals aged 26–35 years (42.5%), 36–45 years (56.1%), 46–55 years (61.1%) and 56–63 years (67.6%) (Table 1).

When data were analyzed according to the area of residence, a significantly higher seroprevalence was found in blood donors residing in rural areas (51.1%) compared to those from urban areas (43.9%) (*p* = 0.020) (Table 1).

Both age (*p* < 0.001; OR = 1.5; 95% CI: 1.36–1.65) and area of residence (*p* = 0.036; OR = 1.3; 95% CI: 1.02–1.66) remained statistically significant when they were evaluated using a logistic regression model.

No statistically significant difference was found between rates of infection in females and males (*p* = 0.628) (Table 1).

Of the 592 females included in the study, 493 (83.3%) were aged between 18–45 years (mean age = 28.9 ± 8.2 years) and 72.4% (357/493) of these were residents of urban areas (Table 2). *T. gondii* antibodies were detected in 43.2% (213/493) females of childbearing age, with no significant difference according to area of residence (*p* = 1.00; OR = 0.9; 95% CI: 0.66–1.47) (Table 2). The prevalence of *T. gondii* infection was significantly higher in women aged 46–63 years (63.6%, 63/99) compared to those of childbearing age (*p* < 0.001; OR = 2.3; 95% CI: 1.47–3.60).
Table 2. Seroprevalence of *Toxoplasma gondii* infection in female blood donors from Western Romania aged 18–45 years, according to area of residence.

| Variables                  | No. Tested | Prevalence of *T. gondii* Infection | Univariate Analysis |
|---------------------------|------------|-------------------------------------|---------------------|
|                           |            | N (%) | OR (95% CI) | p-Value |
| Area of residence         |            |       |             |         |
| Urban                     | 357        | 154 (43.1) | 1 (Ref.) | -       |
| Rural                     | 136        | 59 (42.0)  | 0.9 (0.66–1.47) | 1.00 |
| Total                     | 493        | 213 (43.2) | - | -       |

N, number of *T. gondii* seropositive individuals; OR, odds ratio; CI, confidence interval; Ref., reference.

*T. gondii* seroprevalence decreased with increasing level of education, from 64.3% in blood donors who graduated from elementary/middle school to 40.4% in those with university (*p* < 0.001; OR = 0.6; 95% CI: 0.55–0.79). Seroprevalence was significantly higher in individuals with elementary/middle school (64.3%, 54/84) compared to those with high school (48.9%, 306/625) (*p* = 0.010; OR = 1.8; 95% CI: 1.17–3.01) and to those with university (40.4%, 258/638) (*p* < 0.001; OR = 2.6; 95% CI: 1.65–4.26) (Table 3).

Table 3. Risk factors for *Toxoplasma gondii* infection in blood donors from Western Romania.

| Risk Factors                        | No. Tested | Prevalence of *T. gondii* Infection | Univariate Analysis |
|-------------------------------------|------------|-------------------------------------|---------------------|
|                                     |            | N (%) | OR (95% CI) | p-Value |
| Level of education                  |            |       |             |         |
| Elementary/Middle school            | 84         | 54 (64.3) | 1 (Ref.) | -       |
| High school                         | 625        | 306 (48.9) | 1.8 (1.17–3.01) | 0.010 |
| University                          | 638        | 258 (40.4) | 2.6 (1.65–4.26) | <0.001 |
| Consumption of raw and/or uncooked meat |   |      |             |         |
| no                                  | 778        | 371 (47.7) | 1 (Ref.) | -       |
| yes                                 | 569        | 247 (43.4) | 1.1 (0.96–1.48) | 0.121 |
| Contact with soil                   |            |       |             |         |
| no                                  | 949        | 414 (43.6) | 1 (Ref.) | -       |
| yes                                 | 398        | 204 (51.3) | 1.3 (1.07–1.72) | 0.011 |
| Own cat(s)                          |            |       |             |         |
| no                                  | 1132       | 501 (44.3) | 1 (Ref.) | -       |
| yes                                 | 215        | 117 (54.4) | 1.5 (1.12–2.02) | 0.007 |
| Own dog(s)                          |            |       |             |         |
| no                                  | 1020       | 442 (43.3) | 1 (Ref.) | -       |
| yes                                 | 327        | 176 (53.8) | 1.5 (1.19–1.97) | <0.001 |
| Own any pet: cat(s) and/or dog(s)   |            |       |             |         |
| no                                  | 989        | 424 (42.9) | 1 (Ref.) | -       |
| yes                                 | 358        | 194 (54.2) | 1.5 (1.24–2.01) | <0.001 |

N, number of *T. gondii* seropositive individuals; OR, odds ratio; CI, confidence interval; Ref., reference.

Consumption of raw and/or undercooked meat was not found to be a risk factor for *T. gondii* infection in blood donors (Table 3). However, individuals who reported contact with soil throughout gardening and/or agriculture activities had higher *T. gondii* seropositivity compared to those who did not confirm having contact with soil (*p* = 0.011; OR = 1.3; 95% CI: 1.07–1.72) (Table 3).

Cat owners (*p* = 0.007; OR = 1.5; 95% CI: 1.12–2.02) and dog owners (*p* < 0.001; OR = 1.5; 95% CI: 1.19–1.97) were both associated with *T. gondii* infection (Table 3). Among those reporting to have any pet (cat and/or dog) *T. gondii* seroprevalence (54.2%, 194/358) was significantly higher compared to those who did not report having any pet (42.9%, 424/989) (*p* < 0.001, OR = 1.5; 95% CI: 1.24–2.01) (Table 3). However, when cat owners and dog owners were combined in a logistic regression model, cat owners were not found to be
associated with exposure to *T. gondii* (*p* = 0.386; OR = 1.1; 95% CI: 0.81–1.71), but dog owners remained associated with seropositivity (*p* = 0.040; OR = 1.4; 95% CI: 1.01–1.92).

When age, area of residence, level of education, contact with soil, having cats, having dogs and having any pet (cats and/or dogs) (identified as risk factors for *T. gondii* infection in the univariate analyses), were evaluated using a multiple logistic regression model, only age, level of education and having any pet were found associated with *T. gondii* seropositivity (Table 4).

### Table 4. Risk factors for *Toxoplasma gondii* infection in multiple logistic regression.

| Variables                        | OR (95% CI) | p-Value |
|----------------------------------|-------------|---------|
| **Age groups (years)**           |             |         |
| 18–25                            | 1 (Ref.)    |         |
| 26–35                            | 1.7 (1.27–2.28) | <0.001 |
| 36–45                            | 2.5 (1.87–3.53) | <0.001 |
| 46–55                            | 3.1 (2.15–4.32) | <0.001 |
| 56–63                            | 3.8 (1.85–8.00) | <0.001 |
| **Level of education**           |             |         |
| Elementary/Middle school         | 1 (Ref.)    |         |
| High school                      | 0.5 (0.36–0.95) | 0.031  |
| University                       | 0.4 (0.27–0.72) | 0.001  |
| **Own any pet: cat(s) and/or dog(s)** |             |         |
| no                               | 1 (Ref.)    |         |
| yes                              | 1.5 (1.17–1.94) | 0.001  |

OR, odds ratio; CI, confidence interval; Ref., reference.

4. Discussion

This is the first report on the seroprevalence and risk factors of *T. gondii* infection in blood donors from Western Romania. The 45.9% seroprevalence found in our study is higher than the 20.5%, 30.6%, 32.1%, 36% and 38.1% seroprevalence reported by European investigators in blood donors from Serbia [7], Bosnia and Herzegovina [21], Czech Republic [22], United Kingdom [23] and Portugal [13], respectively. These differences between countries may be explained by different sample size and various sampling strategies with the study group [7], environmental conditions [3], eating and hygiene habits [2,24] and different assays (with different sensitivities and/or specificities) [6] used to identify the presence of *T. gondii* antibodies.

As previously shown by former authors, we noticed that the prevalence of *T. gondii* antibodies increased with age, and this is a result of a prolonged length of exposure to the parasite [7,12,21,25].

Gender was not associated with *T. gondii* infection in our survey, and this is in accordance with results of previous studies [7,8,10,25].

Our findings showed the presence of *T. gondii* antibodies in serum samples of 43.2% of female blood donors of childbearing age. The seroprevalence was higher than the 7.9% reported in Bosnia and Herzegovina [21] and 22.3% in Taiwan [10]. Sociocultural habits influence the transmission routes of *T. gondii* in a population [21] and may explain the differences observed. Older women were found to be more infected with *T. gondii*, suggesting that females of childbearing age are at greater risk to become infected [7]. However, in Western Romania, we noticed a decrease in *T. gondii* seroprevalence in women of childbearing age from 57.6% in 2008 [17] to 43.2% in 2018. Although in 2008 the study was not conducted in blood donors, the difference in *T. gondii* seropositivity observed after 10 years suggests a possible decline in seroprevalence. This downward trend was recently observed in Serbia (from 85% to 31%) [7] and Italy (from 41.1% to 12.4%) [26], and may be explained by better awareness of *T. gondii* infection especially due to internet sources [7], socioeconomic level increase, improvement in quality of life, and changes in nutritional habits [26].
Educational level has previously been shown as an important risk factor for the occurrence of *T. gondii* infection in pregnant women from Western Romania [16]. In the present study, seropositivity for *T. gondii* decreased with increasing level of education, similar to other reports [8,10,13]. Higher level of education is linked with more knowledge about this infection and its methods of prevention, and a low risk of exposure [16].

Consumption of raw/undercooked meat was not found to be a risk factor for *T. gondii* infection in Romanian blood donors. This confirms our previous findings in Romanian pregnant women [16] and is in agreement with the results recently published by other authors [7,25]. Introduction of modern systems and better hygiene conditions in animal farms [7,26], improved hygiene practices during meat processing [26], increasing use of frozen meat and industrially processed meat products [7] may explain the outcome.

Similar to other reports [10,27], contact with cats was found to be associated with *T. gondii* seropositivity when using an univariate analysis in our study group. However, when logistic regression was performed, contact with cats was no longer identified as a risk factor for *T. gondii*, and this is in agreement with previous findings [7,13]. Interestingly, contact with dogs may increase the risk for toxoplasmosis in our study group. This observation is similar to those reported in previous publications [16,28,29]. Dogs could play an important role in transmission of *T. gondii*, acting as possible mechanical carriers by contaminating their fur with oocysts [28–31]. In addition, dogs, through feeding on cat feces, can defecate *T. gondii* oocysts, probably after passive gastrointestinal transport [29–33]. In Romanian dogs, *T. gondii* seroprevalence varied from 25% in Central Romania (Cluj-Napoca) [34], to 63% in Southern Romania [35]. Although having pets (cats and/or dogs) was associated with *T. gondii* seropositivity (in univariate analyses) multiple logistic regression analysis did not show having dogs to be an independent risk factor for toxoplasmosis. Having pets (cats and/or dogs) at home was recently found to be a risk factor in Romanian pregnant women [16] and in Chinese pet owners [36]. Therefore, cats and dogs (the most popular pet animals worldwide) may serve as potential sources of infection with *T. gondii* in humans, due to close contact with their owner [37].

Results of our survey indicate that increasing age and lower educational level were also significantly associated with *T. gondii* infection in a multiple logistic regression analysis. Similar findings were previously reported by investigators working in the field. For instance, age persisted as a predictor for *T. gondii* infection in studies conducted in Serbia and Mexico [7,8], and level of education was identified as a risk factor for toxoplasmosis in Portugal when using multivariate regression analyses [13].

Blood donors are usually healthy individuals, with a limited range of age, and prevalence of *T. gondii* infection in blood donors does not reflect the seroprevalence in general population [8]. However, *T. gondii* can be transmitted by blood transfusion from asymptomatic seropositive individuals in early stages of acute infection, adding an extra burden on the global population [6,10]. Moreover, it has been documented the transmission of *T. gondii* by transfusion of leukocytes or platelets, and the possibility of survival of the parasite in citrated blood at 5 °C for more than 50 days [6].

5. Conclusions

The present study brings new and valuable data regarding the seroprevalence and basic demographic risk factors for *T. gondii* infection in Romanian blood donors. Our results indicate that the prevalence of *T. gondii* antibodies in this population group is among the highest in Europe. Age, level of education and having pets (cats and/or dogs) were found to be significantly associated with *T. gondii* infection in a multiple logistic regression analysis.

Public health authorities should promote information regarding the epidemiology of *T. gondii* in order to reduce transmission. Our data may serve as a starting point for further studies that should lead to implementation of prevention programs for toxoplasmosis.
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References

1. Robert-Gangneux, F.; Dardé, M.-L. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin. Microbiol. Rev. 2012, 25, 264–296. [CrossRef] [PubMed]
2. Montoya, J.G.; Liesenfeld, O. Toxoplasmosis. Lancet 2004, 363, 1965–1976. [CrossRef]
3. Pinto-Ferreira, F.; Caldart, E.T.; Pasquali, A.K.S.; Mitsuka-Bregnano, R.; Freire, R.L.; Navarro, I.T. Patterns of Transmission and Sources of Infection in Outbreaks of Human Toxoplasmosis. Emerg. Infect. Dis. 2019, 25, 2177–2182. [CrossRef] [PubMed]
4. Tenter, A.M.; Heckeroth, A.R.; Weiss, L.M. Toxoplasma gondii: From animals to humans. Int. J. Parasitol. 2000, 30, 1217–1258. [CrossRef]
5. Singh, G.; Sehgal, R. Transfusion-transmitted parasitic infections. Asian J. Transfus. Sci. 2010, 4, 73–77. [CrossRef]
6. Foroutan-Rad, M.; Majidiani, H.; Dalvand, S.; Daryani, A.; Kooti, W.; Saki, J.; Hedayati-Rad, F.; Ahmadpour, E. Toxoplasmosis in Blood Donors: A Systematic Review and Meta-Analysis. Transfus. Med. Rev. 2016, 30, 116–122. [CrossRef]
7. Stopić, M.; Štajner, T.; Marković-Denić, L.; Nikolić, V.; Djilas, I.; Srszentić, S.J.; Djurković-Djaković, O.; Bobić, B. Epidemiology of Toxoplasmosis in SERBIA: A Cross-Sectional Study on Blood Donors. Microorganisms 2022, 10, 492. [CrossRef]
8. Alvarado-Esquivel, C.; Rascon-Careaga, A.; Hernández-Tinoco, J.; Corella-Madueño, M.A.; Sánchez-Anguiano, L.F.; Aldana-Madrid, M.L.; Velasquez-Vega, E.; Quizán-Plata, T.; Navarro-Henze, J.L.; Badell-Luzardo, J.A.; et al. Seroprevalence and Associated Risk Factors for Toxoplasma gondii Infection in Healthy Blood Donors: A Cross-Sectional Study in Sonora, Mexico. BioMed Res. Int. 2016, 2016, 9597276. [CrossRef]
9. Wang, T.; Han, Y.; Pan, Z.; Wang, H.; Yuan, M.; Lin, H. Seroprevalence of Toxoplasma gondii infection in blood donors in mainland China: A systematic review and meta-analysis. Parasite 2018, 25, 36. [CrossRef]
10. Chiang, T.Y.; Hsieh, H.H.; Kuo, M.C.; Chiu, K.T.; Lin, W.C.; Fan, C.K.; Fang, C.T.; Ji, D.D. Seroprevalence of Toxoplasma gondii Infection among healthy blood donors in Taiwan. PLoS ONE 2012, 7, e48139. [CrossRef]
11. Stephen, S.; Pradeep, J.; Anitharaj, V.; Janarthanam, V. Seroprevalence of toxoplasmosis in voluntary blood donors of Puducherry and surrounding districts of Tamil Nadu. J. Parasit. Dis. 2017, 41, 1158–1161. [CrossRef] [PubMed]
12. Yılmaz, A.; Yazıcı, E.; Turk, C. Assessment of seroprevalence of Toxoplasma gondii in blood donors applied to the blood center of Gazi university hospital. Iran. J. Microbiol. 2021, 13, 243–247. [CrossRef] [PubMed]
13. Rodrigues, F.T.; Sousa, A.P.; Escovaõ, M.A.; Condeco, J.; Cardoso, L.; Lopes, A.P. Seroprevalence of Toxoplasma gondii in blood donors in Portugal. Transfus. Apher. Sci. 2020, 59, 102777. [CrossRef] [PubMed]
14. Nakashima, F.; Pardo, V.S.; Miola, M.P.; Murata, F.; Paduan, N.; Longo, S.M.; Brandão de Mattos, C.C.; Pereira-Chioccola, V.L.; Ricci, O., Jr.; de Mattos, L.C. Serum IgG Anti-Toxoplasma gondii Antibody Concentrations Do Not Correlate Nested PCR Results in Blood Donors. Front. Cell. Infect. Microbiol. 2020, 9, 461. [CrossRef]
15. Siransy, L.; Dasse, S.R.; Dou Gonat, S.P.; Legbedji, A.; N’guessan, K.; Kouacoua, P.A.; Yeboah, R.; Menan, H. Immunity Status of Blood Donors Regarding Toxoplasma gondii Infection in a Low-Income District of Abidjan, Côte d’Ivoire, West Africa. J. Immunol. Res. 2016, 2016, 6830895. [CrossRef]
16. Olariu, T.R.; Uronsu, S.; Hotea, I.; Dumitrascu, V.; Anastasiu, D.; Lupu, M.A. Seroprevalence and Risk Factors of Toxoplasma gondii Infection in Pregnant Women from Western Romania. Vector Borne Zoonotic Dis. 2020, 20, 763–767. [CrossRef]
17. Olariu, T.R.; Darabus, G.; Cretu, O.; Jurovits, O.; Giura, E.; Erdelean, V.; Marincu, I.; Iacobiciu, I.; Petrescu, C.; Koreck, A. Prevalence of Toxoplasma gondii antibodies among sexual women of childbearing age in Timis County. Lcruc. Stiintifice Med. Vet. Timis. 2008, 41, 367–371.
18. Olariu, T.R.; Petrescu, C.; Darabus, G.; Lighezan, R.; Maziliu, O. Seroprevalence of Toxoplasma gondii in Western Romania. Infect. Dis. 2015, 47, 580–583. [CrossRef]
19. Ministerul Sănătății Publice din România (Romanian Ministry of Public Health). ORDIN Nr. 1193 Din 7 Iulie 2007 Pentru Aprobarea Normelor Privind Informatiile Care Trebuie Comunicate de Către Donatori la Fiecare Donare și Admisibilitatea Donatorilor de Sângie și de Componente Sanguine Umane (ORDER Nr. 1193 of 7 July 2007 for the Approval of the Rules on the Information to be Provided to Donors of Blood and Blood Components of Human Origin, as well as the Information to be Communicated by Donors at Each Donation and the Admissibility of Donors of Human Blood and Blood Components). Available online: http://www.lexmed.ro/doc/Ordin_1193_2007.pdf (accessed on 14 March 2022). (In Romanian)

20. Villard, J.; Cimon, B.; Franck, J.; Fricker-Hidalgo, H.; Godineau, N.; Houze, S.; Paris, L.; Pelloux, H.; Villena, I.; Candolfi, E.; et al. Evaluation of the usefulness of six commercial agglutination assays for serologic diagnosis of toxoplasmosis. Diagn. Microbiol. Infect. Dis. 2012, 73, 231–235. [CrossRef]

21. Bobić, B.; Milosavić, M.; Guzijan, G.; Djurković-Djaković, O. First Report on Toxoplasma infection in Bosnia and Herzegovina: Study in Blood Donors. Vector Borne Zoonotic Dis. 2016, 16, 807–809. [CrossRef]

22. Svobodová, V.; Literák, I. Prevalence of IgM and IgG antibodies to Toxoplasma gondii in blood donors in the Czech Republic. Eur. J. Epidemiol. 1998, 14, 803–805. [CrossRef] [PubMed]

23. McDonald, C.P.; Barbara, J.A.; Contreras, M.; Brown, S. Provision of a panel of anti-Toxoplasma-negative blood donors. Vox Sang. 1989, 57, 55–58. [CrossRef] [PubMed]

24. Dubey, J.P.; Jones, J.L. Toxoplasma gondii infection in humans and animals in the United States. Int. J. Parasitol. 2008, 38, 1257–1278. [CrossRef]

25. Kalantari, N.; Sheikhansari, M.R.; Ghaffari, S.; Alipour, J.; Gorgani-Firouzjaee, T.; Tamadoni, A.; Bayani, M. Seroprevalence and molecular detection of Toxoplasma gondii in young healthy blood donors in Northern Iran. Trop. Biomed. 2018, 35, 1017–1027. [PubMed]

26. Fanigliulo, D.; Marchi, S.; Montomoli, E.; Trombetta, C.M. Toxoplasma gondii in women of childbearing age and during pregnancy: Seroprevalence study in Central and Southern Italy from 2013 to 2017. Parasite 2020, 27, 2. [CrossRef]

27. Mahmoudvand, H.; Saedi Dezaki, E.; Soleimani, S.; Baneshi, M.R.; Kheirandish, F.; Ezatpour, B.; Zia-Ali, N. Seroprevalence and risk factors of Toxoplasma gondii infection among healthy blood donors in south-east of Iran. Parasite Immunol. 2015, 37, 362–367. [CrossRef]

28. Frenkel, J.K.; Lindsay, D.S.; Parker, B.B.; Dobesh, M. Dogs as possible mechanical carriers of toxoplasma, and their fur as a source of infection of young children. Int. J. Infect. Dis. 2003, 7, 292–293. [CrossRef]

29. Etheredge, G.D.; Michael, G.; Muehlenbein, M.P.; Frenkel, J.K. The roles of cats and dogs in the transmission of Toxoplasma infection in Kuna and Embera children in eastern Panama. Pan. Am. J. Public Health 2004, 16, 176–186. [CrossRef]

30. Fábrega, L.; Restrepo, C.M.; Torres, A.; Smith, D.; Chan, P.; Pérez, D.; Cumbra, A.; Caballero, E.Z. Frequency of Toxoplasma gondii and Risk Factors Associated with the Infection in Stray Dogs and Cats of Panama. Microorganisms 2020, 8, 927. [CrossRef]

31. Gebremedhin, E.Z.; Sarba, E.J.; Tola, G.K.; Endalew, S.S.; Marami, L.M.; Melkamsew, A.T.; Presti, V.; Vitale, M. Prevalence and risk factors of Toxoplasma gondii and Leishmania spp. infections in apparently healthy dogs in west Shewa zone, Oromia, Ethiopia. BMC Vet. Res. 2015, 21, 284. [CrossRef]

32. Lindsay, D.S.; Dubey, J.P.; Butler, J.M.; Blagburn, B.L. Mechanical transmission of Toxoplasma gondii oocysts by dogs. Vet. Parasitol. 1997, 73, 27–33. [CrossRef]

33. Shapiro, K.; Bahia-Oliveira, L.; Dixon, B.; Dumètre, A.; de Wit, L.A.; VanWormer, E.; Villena, I. Environmental transmission of Toxoplasma gondii: Oocysts in water, soil and food. Food Waterborne Parasitol. 2019, 15, e0049. [CrossRef] [PubMed]

34. Cozma, V.; Ţuteu, O.; Titilincu, A.; Osztian, R.M. Seroprevalence of Toxoplasma gondii antibodies in dogs from Cluj Napoca. Rev. Romana Paraziţă 2007, 17, 23–26.

35. Fernoaş, C.; Codreanu, M.D.; Cornilă, M.; Nae, R.T.; Ionita, M.; Mitrea, I.L. Clinical follow-up of dogs with neurological disorders and positive for antibodies against Toxoplasma gondii. Sci. Works C Ser. Vet. Med. 2015, 61, 131–134.

36. Cong, W.; Elsheikha, H.M.; Zhou, N.; Peng, P.; Qin, S.Y.; Meng, Q.F.; Qian, A.D. Prevalence of antibodies against Toxoplasma gondii in pets and their owners in Shandong province, Eastern China. BMC Infect. Dis. 2018, 18, 430. [CrossRef]

37. Oi, M.; Yoshikawa, S.; Maruyama, S.; Nogami, S. Comparison of Toxoplasma gondii Seroprevalence in Shelter Cats and Dogs during 1999–2001 and 2009–2011 in Tokyo, Japan. PLoS ONE 2015, 10, e0135956. [CrossRef]