

Determining if host serum IgG titer is associated with *Toxoplasma gondii* virulence

***An Honors Thesis Submitted to
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in partial fulfillment of the Honors requirements***

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**by
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ABSTRACT

Toxoplasma gondii is a protozoan parasite that causes the disease toxoplasmosis in animals and humans. This parasite has two infectious forms: oocysts shed in the feces of felids (domestic and wild cats) and tissue cysts in chronically infected animals. Humans may become infected via the ingestion of food or water contaminated with oocysts, the consumption of tissue cysts in undercooked meats, or vertical transmission from the mother to the fetus. Previous studies have found that *T. gondii* strains from South America are genetically diverse and tend to be more virulent and causing of more severe diseases in infected hosts than strains from elsewhere and that higher anti-*T. gondii* IgG levels are correlated with severity of toxoplasmosis in humans. We hypothesize that more virulent strains can induce a stronger immune response to chronic infection than less virulent strains resulting in higher IgG antibody levels in the hosts. If true, we would expect higher IgG levels in the serum of animals and humans from South America than elsewhere. To test this hypothesis, we used the modified agglutination test (MAT) to measure anti-*T. gondii* IgG levels in serum samples from animal hosts (cats & dogs) from South America and elsewhere. In addition, human serum samples from USA and Brazil were compared. The IgG titers were then compared to determine if IgG level can be used as a marker to predict parasite virulence at the population level. Our results revealed that IgG titers were similar between cats from South American and other continents. Slightly differential IgG titers were observed in South American dogs compared to dogs from other continents, but this result needs to be confirmed using a higher sample size. There, however, was a large difference between IgG levels in humans from USA and Brazilian human serum samples, with the latter having much higher titers, suggesting that parasite genotypes may be associated with parasite virulence in humans.

INTRODUCTION

Toxoplasma gondii is a protozoan parasite that causes the disease toxoplasmosis in animals and humans. One third of the world's human population is chronically infected with this parasite, and it can infect all warm-blooded animals as an intermediate host (Tenter et al., 2000).

Toxoplasma gondii has two infectious forms: oocysts shed in the feces of felids (domestic and wild cats) and tissue cysts in chronically infected animals. Humans may become infected via ingestion of food or water contaminated with oocysts, the consumption of tissue cysts in undercooked meats, or vertical transmission from the mother to the fetus.

Previous studies have found that *T. gondii* strains from South America are genetically diverse from and tend to be more virulent and causing more severe diseases in humans than strains from elsewhere (Shwab et al., 2016). Also, another study found that higher anti-*Toxoplasma* IgG levels were correlated with severity of toxoplasmosis in humans (Bharti et al., 2016). We hypothesize that the suspected to be more virulent strains from South America can induce a stronger immune response to chronic infection than the less virulent strains from other continents, resulting in higher IgG antibody levels in the hosts. To test this hypothesis, we used the modified agglutination test (MAT) to measure anti-*Toxoplasma* IgG levels in serum samples from animals (cats & dogs) and humans from South America and elsewhere. The IgG titers were then compared to determine if IgG level can be used as a marker to predict parasite virulence at the population level.

MATERIALS AND METHODS

Serum Samples

obtained 477 (n=477) serum samples. Three hundred and twenty-five (325) of these samples were obtained from the parasitic diseases laboratory at the USDA. These samples include 119 from dogs and 206 from cats. One hundred and nine of the cat samples came from Egypt, 37 came from Brazil, 25 came from China and 35 came from Ethiopia. Twelve of the dog samples came from Sri Lanka, 27 came from Egypt, 24 came from Brazil, 29 came from Colombia, and 20 came from Vietnam. In addition, 82 human serum samples from Brazil and 70 cat samples from the University of Tennessee-Knoxville College of Veterinary Medicine were also included. All serum samples were stored at -20°C until use.

Serological Testing

All serum samples were tested for the presence of anti-*T. gondii* IgG using the MAT test (**Figure 1; Protocol in Appendix**). Whole-cell *T. gondii* antigen was used for IgG binding. Twenty-four human samples from Nashville, TN evaluated in a previous study (Mawlood et al., 2016) were also included to be used in comparison with the Brazilian human samples. The initial MAT test from 1:25 to 1:3,200 was performed as described by Desmonts & Remington (1980). Fifty microliters of each diluted serum sample, along with positive (a serum sample previously determined to have a titer of 1:200) and negative (uninfected fetal bovine serum) controls, was diluted with phosphate buffer solution (PBS) to 1:25 and added to the first row of a 96 well plate and 25 microliters were added to all other wells (**Figure 1**). From there 25 microliters of the diluted serum was diluted into the next well in the column, continuing this dilution serially until a dilution of 1:3200 was reached. Twenty-five microliters of an antigen mixture consisting of 2.5mL of an alkaline buffer, 35 μ L of 2-mercaptoethanol, 50 μ L of Evan's Blue Dye, and 150 μ L of whole-cell *T. gondii* antigen. The plate was then covered and incubated at 37°C in 5.0% CO₂ for 24 hours and the results were read. Wells with a blue pellet collected at the bottom were read as negative and those with a clear bottom or “mat” of diffuse cells were read as positive. Those serum samples that were positive to 1:3,200 were then further tested using this procedure, though starting at a higher dilution, to 1:409,600 or until a negative result was reached. Samples were considered seropositive at 1:25.

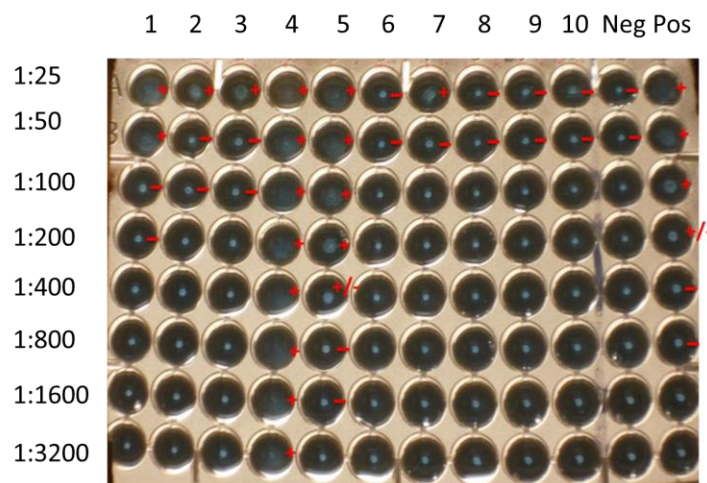


Figure 1. MAT test plate example. Wells with blue pellet were read as negative, while clear wells or those with a “mat” of cells were read as positive. A titer of $\geq 1:25$ was considered positive. Plates were incubated at 37°C in 5.0% CO₂ for 24 hours before being read.

Statistical analysis

Mann-Whitney U tests were performed to test the relationship between titer, country of origin, and species. Data was organized by sample and analyzed using JMP Pro version 14.0 to determine if the Anti-*Toxoplasma* IgG titer was associated with the country of origin of each sample for each species. Seronegative samples were not included in this analysis.

RESULTS

Cats

The most common, or mode of the, IgG titers among all the cat samples was found to fall between approximately 1:400 and 1:3,200 (**Figure 2; Table 1**). For positive samples, the titers range from 1:100 to 1:204,800. The mode of the IgG titers for the Egyptian cats was found to be 1:3200 (**Figure 5** in Appendix). For the Brazilian cats the mode was 1:400, 1:800, and 1:3200 (**Figure 6** in Appendix). For the Chinese cats it was 1:400 and 1:800 (**Figure 7** in Appendix). For the Ethiopian cats it was 1:200 and 1:600 (**Figure 8** in Appendix). For the Tennessean cats it was 1:1600 (**Figure 9** in Appendix). The Chinese and Brazilian were found to exhibit the highest IgG titers, at 1:409,600. The Ethiopian cats exhibited lower titers overall compared to the other cats. A direct comparison of the IgG titers of the South American cats (those from Brazil) against the cats from all other regions is shown in **Figure 17** (In Appendix). It can once again be seen that the mode of both sets of samples fell between approximately 1:400 and 1:3,200.

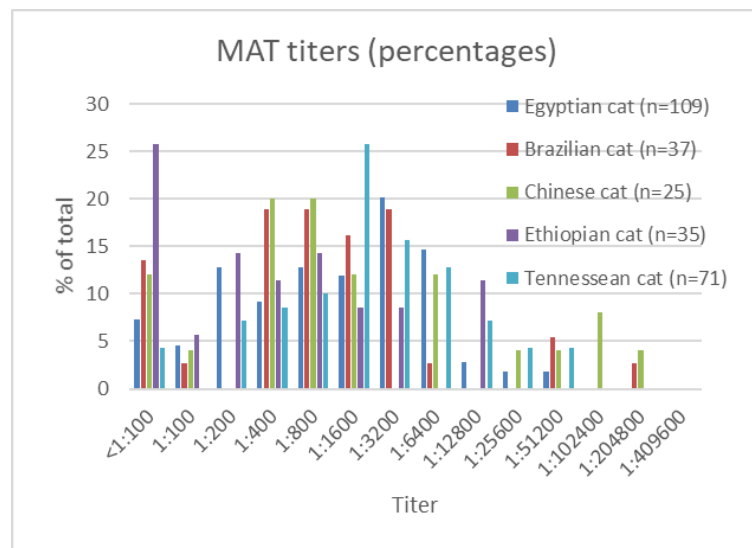


Figure 2. MAT titers of cat samples. The mode of the titers fell between approximately 1:400 and 1:3200 for cats from all locations including Brazil, Egypt, China, Ethiopia, and USA (Tennessee).

Table 1. MAT titers of cat samples. The number in parenthesis is the percentage of the cat samples of that titer versus the total number of samples from that country.

	Egyptian cat	Brazilian cat	Chinese cat	Ethiopian cat	Tennessean cat
<1:100	8 (7.34%)	5 (13.51%)	3 (12.00%)	9 (25.71%)	3 (4.23%)
1:100	5 (4.59%)	1 (2.70%)	1 (4.00%)	2 (5.71%)	0 (0.00%)
1:200	14 (12.84%)	0 (0.00%)	0 (0.00%)	5 (14.29%)	5 (7.04%)
1:400	10 (9.17%)	7 (18.92%)	4 (20.00%)	4 (11.43%)	6 (8.57%)
1:800	14 (12.84%)	7 (18.92%)	5 (20.00%)	5 (14.29%)	7 (9.86%)
1:1,600	13 (11.93%)	6 (16.22%)	3 (12.00%)	3 (8.57%)	18 (25.35%)
1:3,200	22 (20.18%)	7 (18.92%)	0 (0.00%)	3 (8.57%)	11 (15.49%)
1:6,400	16 (14.68%)	1 (2.70%)	3 (12.50%)	0 (0.00%)	9 (12.68%)
1:12,800	3 (2.75%)	0 (0.00%)	0 (0.00%)	4 (11.43%)	5 (7.04%)
1:25,600	2 (1.83%)	0 (0.00%)	1 (4.00%)	0 (0.00%)	3(4.22%)
1:51,200	2 (1.83%)	2 (5.41%)	1 (4.00%)	0 (0.00%)	3 (4.22%)
1:102,400	0 (0.00%)	0 (0.00%)	2 (8.00%)	0 (0.00%)	0 (0.00%)
1:204,800	0 (0.00%)	1 (2.70%)	1 (4.00%)	0 (0.00%)	0 (0.00%)
total	109	37	25	35	70

Dogs

MAT results for all dogs are summarized in **Figure 3**. For positive samples, the titers range from 1:50 to 1:800. The mode of the IgG titers for the Sri Lankan dogs was found to be 1:100 (**Figure 10** in Appendix). For Egyptian dogs the mode was 1:200 (**Figure 11** in Appendix). For Brazilian dogs the mode was 1:800 (**Figure 12** in Appendix). For Colombian dogs the mode was 1:50 (**Figure 13** in Appendix). For Vietnamese dogs the mode was 1:25 (**Figure 14** in Appendix). The Brazilian dogs were found to exhibit the highest IgG titers, at 1:3200 (**Figure 3; Table 2**). The Vietnamese dogs exhibited IgG titers that were overall lower than that of the other dogs. A direct comparison of the IgG titers of the South American dogs (those from Brazil or Colombia) against the dogs from all other regions is shown in **Figure 18** (In Appendix). Here it can be seen that the highest distribution of IgG titers fell between 1:100 and 1:200 for the dogs from countries not in South America, while for those countries in South America the highest distribution flanked those values and fell between 1:25 and 1:50 or 1:400 and 1:800.

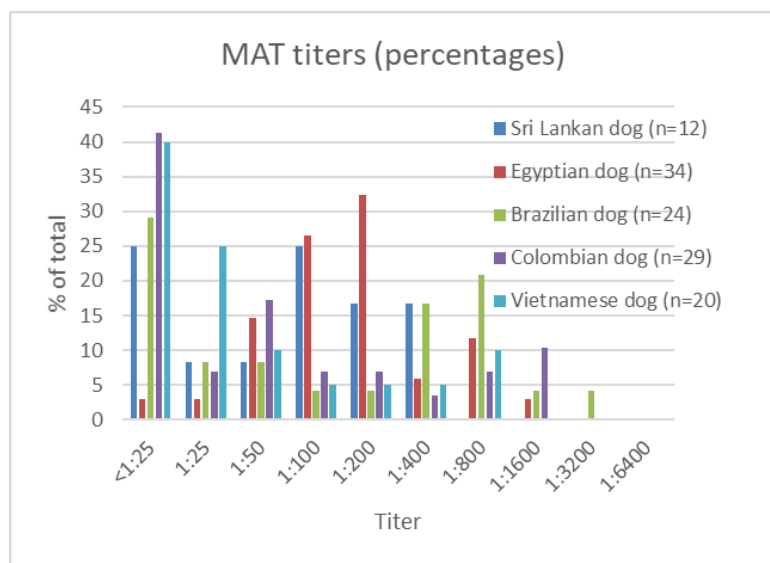


Figure 3. MAT titers of dog samples. The mode of the Brazil dog results fell between 1:400 and 1:800 and the Colombia dog between 1:25 and 1:100. The modes for the Sri Lanka dog, Egypt dog, and Vietnam dog fell a bit lower, between 1:100 and 1:200.

Table 2. MAT titers of dog samples. The number in parenthesis is the percentage of the dog samples of that titer versus the total number of samples from that country.

	Sri Lankan dog	Egyptian dog	Brazilian dog	Colombian dog	Vietnamese dog
<1:25	3 (25.00%)	1 (2.94%)	7 (29.17%)	12 (41.38%)	8 (40.00%)
1:25	1 (8.33%)	1 (2.94%)	2 (8.33%)	2 (6.90%)	5 (25.00%)
1:50	1 (8.33%)	5 (14.71%)	2 (8.33%)	5 (17.24%)	2 (10.00%)
1:100	3 (25.00%)	9 (26.47%)	1 (4.17%)	2 (6.90%)	1 (5.00%)
1:200	2 (16.67%)	11 (32.35%)	1 (4.17%)	2 (6.90%)	1 (5.00%)
1:400	2 (16.67%)	2 (5.88%)	4 (16.67%)	1 (3.45%)	1 (5.00%)
1:800	0 (0.00%)	4 (11.76%)	5 (20.83%)	2 (6.90%)	2 (10.00%)
1:1,600	0 (0.00%)	1 (2.94%)	1 (4.17%)	3 (10.34%)	0 (0.00%)
1:3,200	0 (0.00%)	0 (0.00%)	1 (4.17%)	0 (0.00%)	0 (0.00%)
1:6,400	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
total	12	34	24	29	20

Humans

MAT results for all humans are summarized in **Figure 4**. The mode of IgG titers of the Tennessean humans, as determined by Malwood et al. (2017), was found to be 1:400 (**Figure 15** in Appendix). For the Brazilian humans the mode was found to be 1:3200 and 1:6400 (**Figure 16**

in Appendix). The overall distribution of the IgG titers of the Tennessean humans was quite a bit lower than that of the Brazilian humans (**Figure 4; Table 3**).

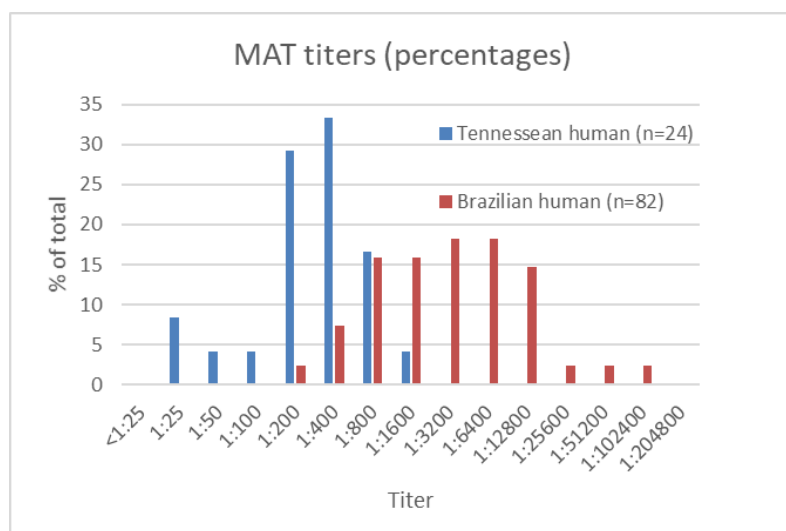


Figure 4. MAT titers of human samples from Brazil and USA (Mawlood et al., 2017). The mode for the samples from Tennessee humans fell between 1:200 and 1:400. The mode for the Brazilian human samples was considerably higher, around 1:3200 to 1:6400.

Table 3. MAT titers of human samples (Mawlood et al., 2017). The number in parenthesis is the percentage of the dog samples of that titer versus the total number of samples from that country.

	Tennessean human	Brazilian human
<1:25	0 (0.00%)	0 (0.00%)
1:25	2 (8.33%)	0 (0.00%)
1:50	1 (4.17%)	0 (0.00%)
1:100	1 (4.17%)	0 (0.00%)
1:200	7 (29.17%)	2 (2.44%)
1:400	8 (33.33%)	6 (7.32%)
1:800	4 (16.67%)	13 (15.84%)
1:1,600	1 (4.17%)	13 (15.84%)
1:3,200	0 (0.00%)	15 (18.29%)
1:6,400	0 (0.00%)	15 (18.29%)
1:12,800	0 (0.00%)	12 (14.63%)
1:25,600	0 (0.00%)	2 (2.44%)
1:51,200	0 (0.00%)	2 (2.44%)
1:102,400	0 (0.00%)	2 (2.44%)
1:204,800	0 (0.00%)	0 (0.00%)
total	24	82

DISCUSSION

We conclude that the serum samples from the more virulent South American strains of *T. gondii* did not exhibit differential IgG titers compared to cats from other continents. The cat samples tested tended to have fairly high titers overall, with the average seropositive titer being approximately 1:1600. In most sets of samples evaluated in this experiment, at least a few samples exhibited a negative seropositivity result. Due to the nature of this study, these results were not valuable but were included to increase the variety of titers shown. While the Ethiopian cat samples' mode and distribution was a bit lower than the general distribution and range of modes from all of the other cat samples, this difference was not deemed large enough to disrupt the overall pattern of distribution among the cat samples. The correlation between continent of origin and IgG titers for cats was not significant ($p=0.1228$, Mann-Whitney test).

The dog samples tested tended to exhibit somewhat low titers overall, with the common seropositive titer being approximately 1:200. Averaged together, the mode of the IgG titers of dogs from the two South American countries equals that of the overall mode of the non-South American dog samples. Although the mode of the titers of the Brazil dogs was slightly higher than that of the Colombia dogs, this difference needs to be confirmed using a larger sample size. While the overall IgG titers in cats were similar among samples collected from different locations, they were slightly higher than that in dogs. The correlation between continent of origin and IgG titers for dogs was not significant ($p=0.8484$, Mann-Whitney test).

However, we found a large difference between IgG levels from the USA (range: 1:25 to 1:1600) and Brazilian human serum samples (range: 1:200 to 1:102,400), with the latter having much higher titers, suggesting that parasite genotypes may be associated with parasite virulence in humans. However, as the actual genotype of the samples are unknown, this conclusion is based solely on the previously found data that *T. gondii* genotypes from South America are more virulent than their genotypes from other continents (Schwab et al., 2016). The correlation between continent of origin and IgG titers for humans was significant ($p<0.0001$, Mann-Whitney test).

Given the small sample size of the human samples from the USA and that these samples were tested by a different researcher than all of the other samples, additional studies with larger

sample sizes are necessary. Future studies should also be performed in order to determine if the results found are indicative of IgG host responses in other animals, such as sheep and cows, and in continents and countries not examined in this study. Also, further confirmation of the differential response of humans to more virulent *T. gondii* strains should be performed.

ROLE IN PROJECT

The student, Riley Byrd, solely performed all data collection, analysis, and interpretation. Advice on which project the student should conduct this thesis on was given by the PI, Dr. Chunlei Su. Some assistance and advice on the analysis and interpretation of the results was given by the PI and other members of the Su lab, but the student both conducted and finalized all analysis and interpretation.

REFERENCES

- Bharti, A. R., McCutchan, A., Deutsch, R., Smith, D. M., Ellis, R. J., Cherner, M., ... & Letendre, S. L. 2016. Latent *Toxoplasma* infection and higher *Toxoplasma gondii* immunoglobulin G levels are associated with worse neurocognitive functioning in HIV-infected adults. *Clinical Infectious Diseases* ciw655.
- Desmonts, G. E. O. R. G. E. S., & Remington, J. S. 1980. Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. *Journal of Clinical Microbiology* 11(6): 562-568.
- Dubey, J. P. 2004. Toxoplasmosis—a waterborne zoonosis. *Veterinary Parasitology* 126(1): 57-72.
- Mawlood, H. H., Mero, W. M., Su, C., & Isa, A. M. 2017. Comparison of MAT with ELISA and LAT tests in detecting *Toxoplasma gondii* antibodies in human sera. *Science Journal of University of Zakho* 5(1): 28-31.
- Shwab, E. K., Jiang, T., Pena, H. F., Gennari, S. M., Dubey, J. P., & Su, C. 2016. The ROP18 and ROP5 gene allele types are highly predictive of virulence in mice across globally distributed strains of *Toxoplasma gondii*. *International Journal for Parasitology* 46(2): 141-146.
- Tenter, A. M., Heckeroth, A. R., & Weiss, L. M. 2000. *Toxoplasma gondii*: from animals to humans. *International Journal for Parasitology* 30(12-13): 1217-1258.

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We would like to thank Dr. J. P. Dubey from the USDA for providing the animal serum samples, Dr. Solange Gennari at the University of Sao Paulo for providing the human serum samples, and Dr. John New at the University of Tennessee-Knoxville College of Veterinary Medicine for providing the Tennessean cat serum samples. We would also like to thank Brandon Buck, Alison Easter, and Matthew Scott for their contributions to this project as well as the rest of the Su Lab team.

APPENDIX

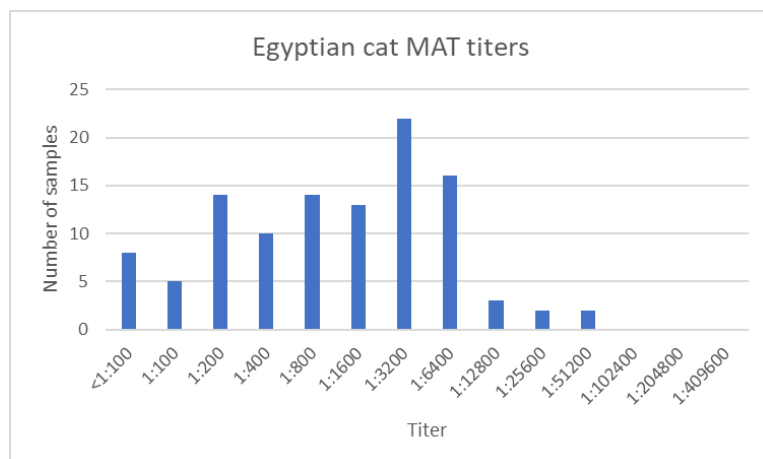


Figure 5. MAT titers of Egyptian cat samples.

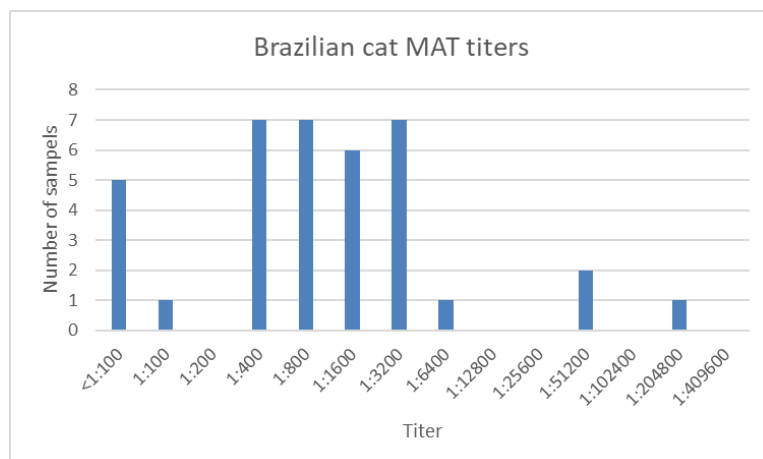


Figure 6. MAT titers of Brazilian cat samples.

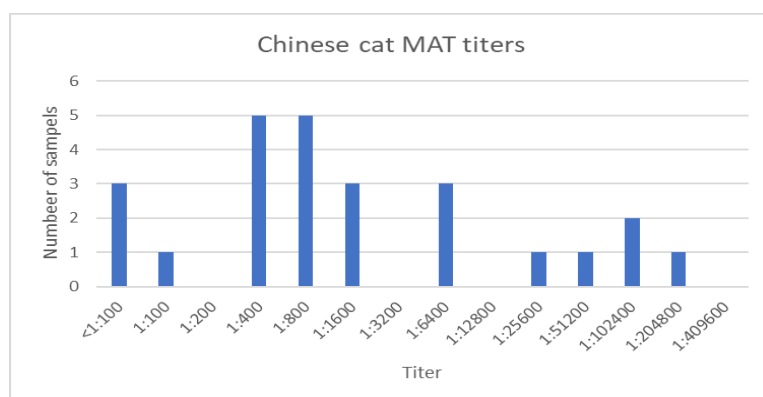


Figure 7. MAT titers of Chinese cat samples.

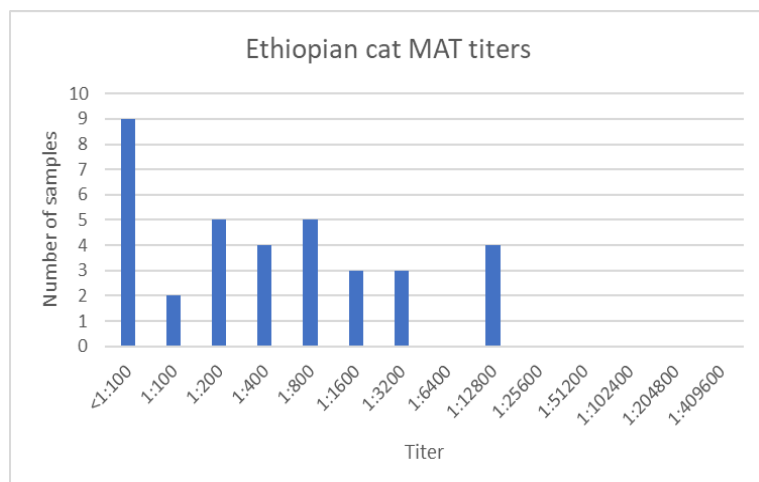


Figure 8. MAT titers of Ethiopian cat samples.

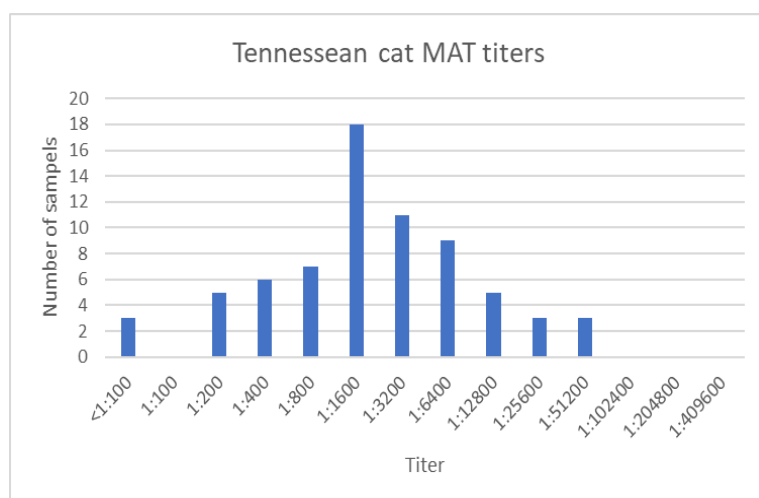


Figure 9. MAT titers of Tennessean cat samples.

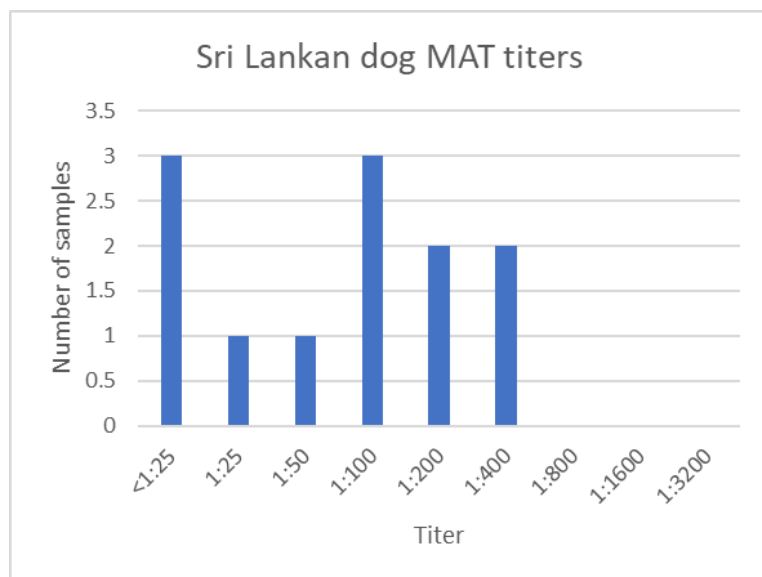


Figure 10. MAT titers of Sri Lankan dog samples.

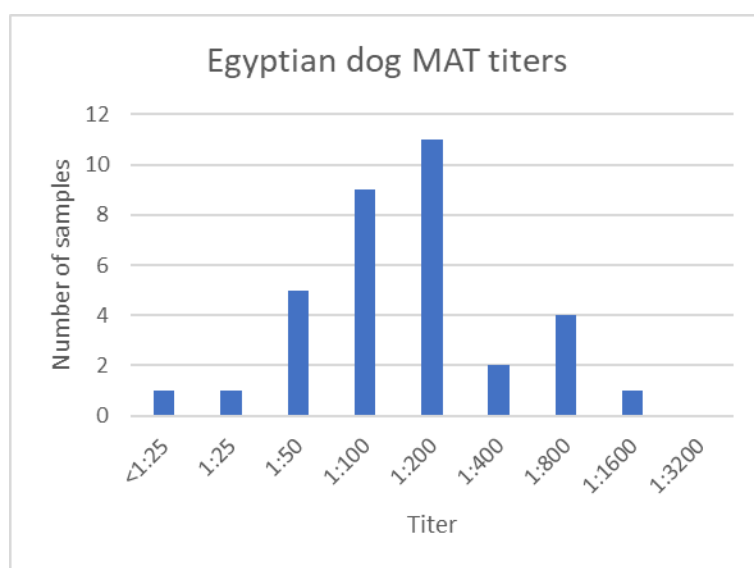


Figure 11. MAT titers of Egyptian dog samples.

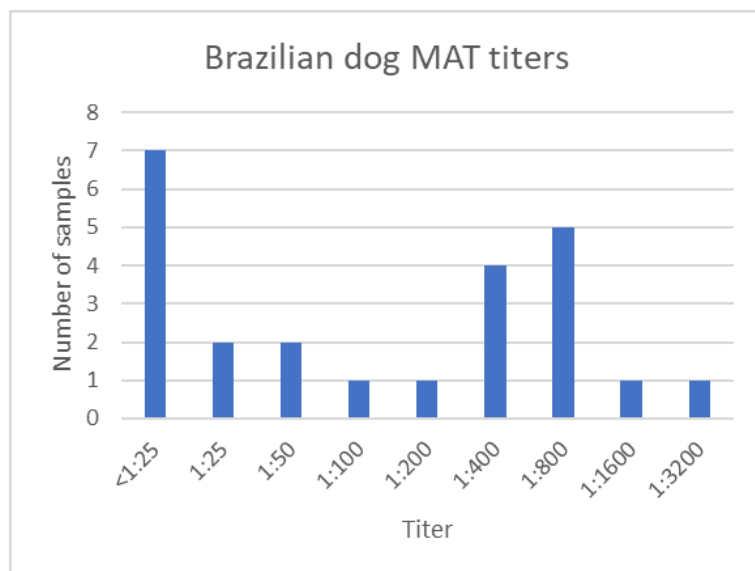


Figure 12. MAT titers of Brazilian dog samples.

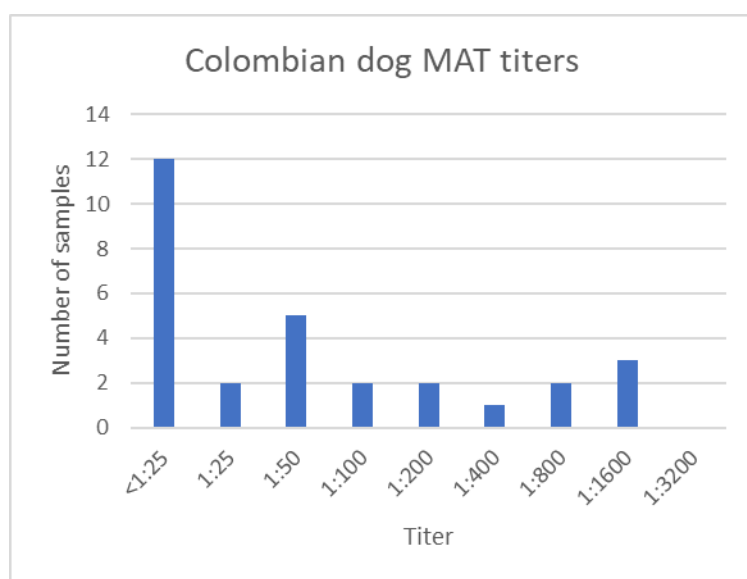


Figure 13. MAT titers of Colombian dog samples.

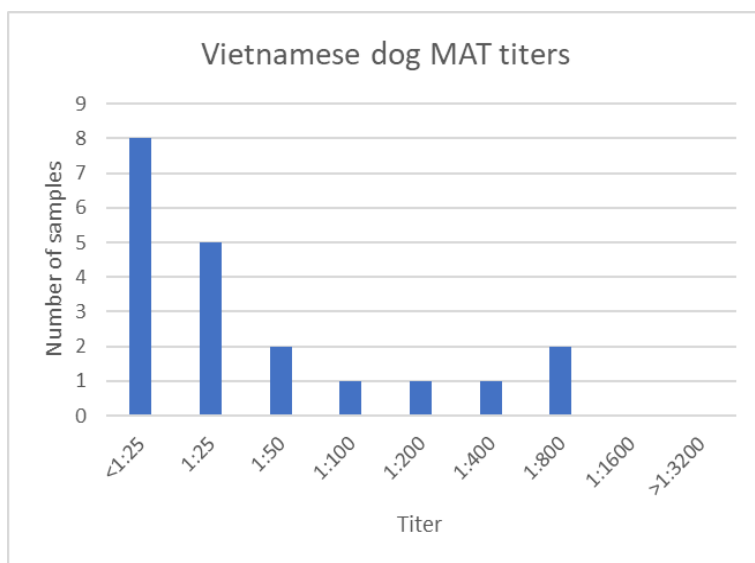


Figure 14. MAT titers of Vietnamese dog samples.

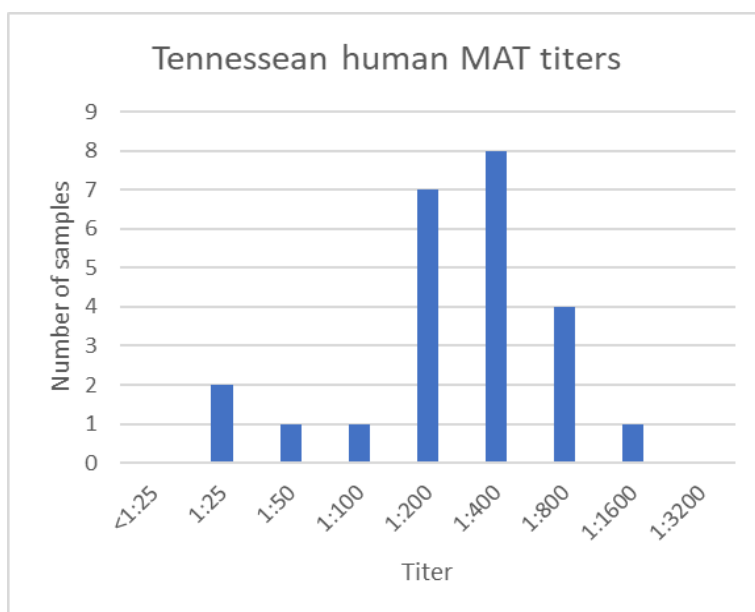


Figure 15. MAT titers of Tennessean human samples (Mawlood et al., 2017).

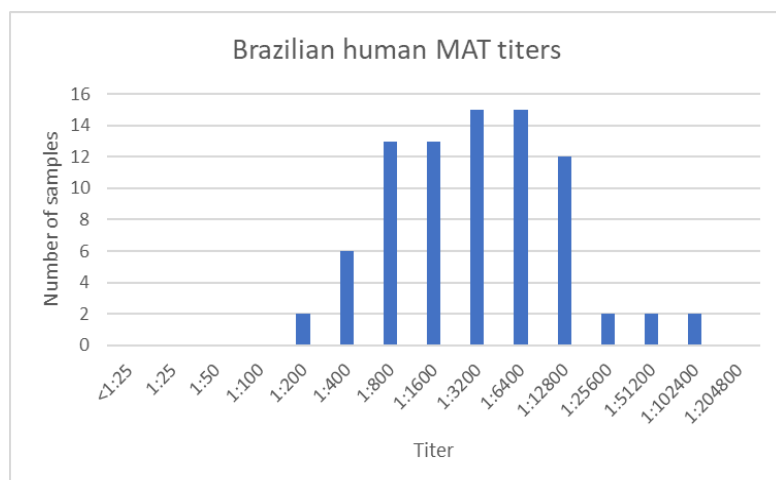


Figure 16. MAT titers of Brazilian human samples.

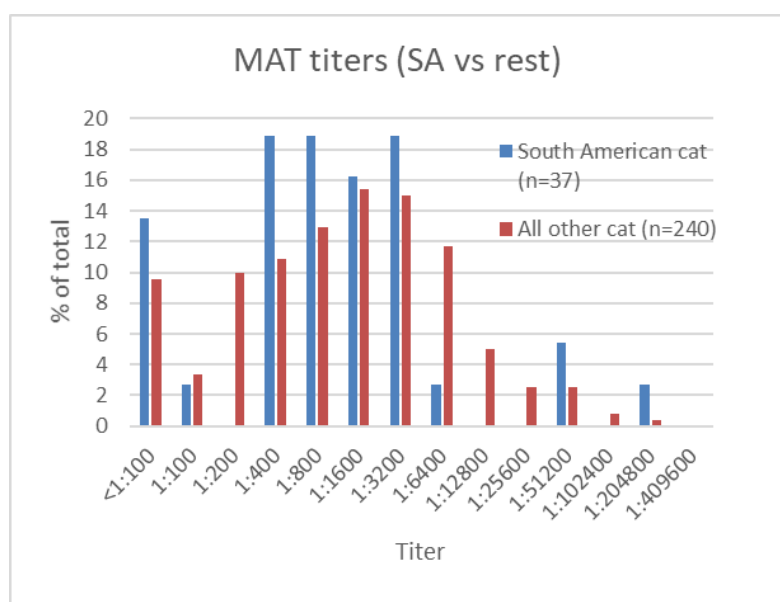


Figure 17. MAT titers of cat samples. Samples from South America are compared to non-South American regions. The modes of the titers from both regions are similar, approximately 1:400 to 1:3200.

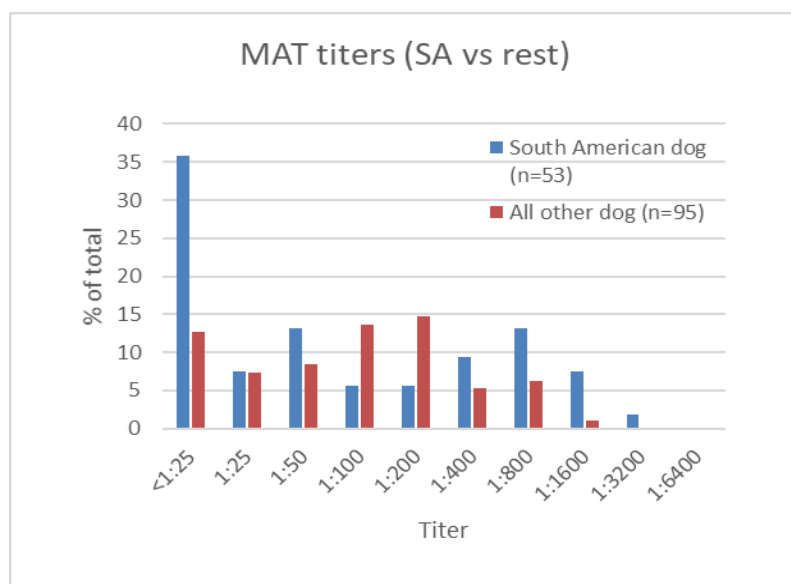


Figure 18. MAT titers of dog samples. Dog samples from South America are compared to non-South American regions. The modes of the titers from both regions are similar, approximately 1:100 to 1:200.

Supplementary Table 1. All relevant data from each serum sample.

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx351	1	TgCatEg23	Cat	Egypt	Africa	Yes	1:800
Tx351	2	TgCatEg24	Cat	Egypt	Africa	Yes	1:6400
Tx351	3	TgCatEg25	Cat	Egypt	Africa	Yes	1:400
Tx351	4	TgCatEg26	Cat	Egypt	Africa	Yes	1:100
Tx351	7	TgCatEg27	Cat	Egypt	Africa	Yes	1:3200
Tx351	8	TgCatEg28	Cat	Egypt	Africa	No	<1:25
Tx351	9	TgCatEg29	Cat	Egypt	Africa	Yes	1:800
Tx351	10	TgCatEg30	Cat	Egypt	Africa	Yes	1:1600
Tx351	11	TgCatEg31	Cat	Egypt	Africa	Yes	1:3200
Tx351	12	TgCatEg32	Cat	Egypt	Africa	Yes	1:1600
Tx351	13	TgCatEg33	Cat	Egypt	Africa	Yes	1:3200
Tx351	14	TgCatEg34	Cat	Egypt	Africa	Yes	1:3200
Tx351	15	TgCatEg35	Cat	Egypt	Africa	Yes	1:6400
Tx351	16	TgCatEg36	Cat	Egypt	Africa	Yes	1:12800
Tx351	17	TgCatEg37	Cat	Egypt	Africa	No	<1:25
Tx351	19	TgCatEg38	Cat	Egypt	Africa	Yes	1:3200
Tx351	20	TgCatEg39	Cat	Egypt	Africa	Yes	1:25
Tx351	21	TgCatEg40	Cat	Egypt	Africa	Yes	1:25
Tx351	22	TgCatEg41	Cat	Egypt	Africa	Yes	1:400
Tx351	23	TgCatEg42	Cat	Egypt	Africa	Yes	1:6400
Tx351	24	TgCatEg43	Cat	Egypt	Africa	Yes	1:200
Tx351	25	TgCatEg44	Cat	Egypt	Africa	Yes	1:12800
Tx351	26	TgCatEg45	Cat	Egypt	Africa	Yes	1:200
Tx351	27	TgCatEg46	Cat	Egypt	Africa	Yes	1:800
Tx351	28	TgCatEg47	Cat	Egypt	Africa	Yes	1:200
Tx345	1	TgCatEg1	Cat	Egypt	Africa	Yes	1:200
Tx345	13	TgCatEg2	Cat	Egypt	Africa	Yes	1:1600
Tx345	15	TgCatEg3	Cat	Egypt	Africa	Yes	1:200
Tx345	16	TgCatEg4	Cat	Egypt	Africa	Yes	1:1600
Tx345	18	TgCatEg5	Cat	Egypt	Africa	Yes	1:12800
Tx345	19	TgCatEg6	Cat	Egypt	Africa	Yes	1:51200
Tx345	20	TgCatEg7	Cat	Egypt	Africa	Yes	1:3200
Tx345	22	TgCatEg8	Cat	Egypt	Africa	Yes	1:6400

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx345	29	TgCatEg10	Cat	Egypt	Africa	Yes	1:1600
Tx345	30	TgCatEg11	Cat	Egypt	Africa	Yes	1:200
Tx345	31	TgCatEg12	Cat	Egypt	Africa	No	<1:25
Tx345	32	TgCatEg13	Cat	Egypt	Africa	Yes	1:1600
Tx345	33	TgCatEg14	Cat	Egypt	Africa	Yes	1:800
Tx345	34	TgCatEg15	Cat	Egypt	Africa	Yes	1:1600
Tx345	37	TgCatEg17	Cat	Egypt	Africa	Yes	1:800
Tx345	39	TgCatEg18	Cat	Egypt	Africa	Yes	1:100
Tx345	40	TgCatEg19	Cat	Egypt	Africa	Yes	1:50
Tx345	41	TgCatEg20	Cat	Egypt	Africa	Yes	1:3200
Tx345	42	TgCatEg21	Cat	Egypt	Africa	Yes	1:6400
Tx345	45	TgCatEg22	Cat	Egypt	Africa	Yes	1:25
Tx373	1	TgCatEg48	Cat	Egypt	Africa	Yes	1:800
Tx373	2	TgCatEg49	Cat	Egypt	Africa	Yes	1:3200
Tx373	3	TgCatEg50	Cat	Egypt	Africa	Yes	1:3200
Tx373	4	TgCatEg51	Cat	Egypt	Africa	Yes	1:3200
Tx373	5	TgCatEg52	Cat	Egypt	Africa	Yes	1:6400
Tx373	6	TgCatEg53	Cat	Egypt	Africa	Yes	1:800
Tx373	7	TgCatEg54	Cat	Egypt	Africa	Yes	1:3200
Tx373	8	TgCatEg55	Cat	Egypt	Africa	Yes	1:1600
Tx373	9	TgCatEg56	Cat	Egypt	Africa	Yes	1:3200
Tx373	11	TgCatEg58	Cat	Egypt	Africa	Yes	1:1600
Tx373	12	TgCatEg59	Cat	Egypt	Africa	Yes	1:3200
Tx373	14	TgCatEg60	Cat	Egypt	Africa	Yes	1:800
Tx373	15	TgCatEg61	Cat	Egypt	Africa	Yes	1:3200
Tx373	16	TgCatEg62	Cat	Egypt	Africa	Yes	1:800
Tx373	18	TgCatEg63	Cat	Egypt	Africa	Yes	1:25600
Tx373	19	TgCatEg64	Cat	Egypt	Africa	Yes	1:6400
Tx373	20	TgCatEg65	Cat	Egypt	Africa	Yes	1:3200
Tx373	21	TgCatEg66	Cat	Egypt	Africa	Yes	1:3200
Tx373	22	TgCatEg67	Cat	Egypt	Africa	Yes	1:400
Tx373	23	TgCatEg68	Cat	Egypt	Africa	Yes	1:6400
Tx373	25	TgCatEg69	Cat	Egypt	Africa	Yes	1:100

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx373	27	TgCatEg70	Cat	Egypt	Africa	Yes	1:200
Tx373	28	TgCatEg71	Cat	Egypt	Africa	Yes	1:200
Tx373	29	TgCatEg72	Cat	Egypt	Africa	Yes	1:200
Tx373	30	TgCatEg73	Cat	Egypt	Africa	Yes	1:100
Tx373	31	TgCatEg74	Cat	Egypt	Africa	Yes	1:400
Tx373	33	TgCatEg75	Cat	Egypt	Africa	Yes	1:400
Tx373	34	TgCatEg76	Cat	Egypt	Africa	Yes	1:6400
Tx373	36	TgCatEg77	Cat	Egypt	Africa	Yes	1:400
Tx373	37	TgCatEg78	Cat	Egypt	Africa	Yes	1:200
Tx373	38	TgCatEg79	Cat	Egypt	Africa	Yes	1:1600
Tx373	39	TgCatEg80	Cat	Egypt	Africa	Yes	1:3200
Tx373	40	TgCatEg81	Cat	Egypt	Africa	Yes	1:1600
Tx373	42	TgCatEg82	Cat	Egypt	Africa	Yes	1:800
Tx373	45	TgCatEg83	Cat	Egypt	Africa	Yes	1:400
Tx373	46	TgCatEg84	Cat	Egypt	Africa	No	<1:100
Tx373	49	TgCatEg85	Cat	Egypt	Africa	Yes	1:3200
Tx373	52	TgCatEg87	Cat	Egypt	Africa	Yes	1:1600
Tx373	54	TgCatEg89	Cat	Egypt	Africa	Yes	1:400
Tx373	55	TgCatEg90	Cat	Egypt	Africa	Yes	1:6400
Tx373	56	TgCatEg91	Cat	Egypt	Africa	Yes	1:800
Tx373	57	TgCatEg92	Cat	Egypt	Africa	Yes	1:6400
Tx373	58	TgCatEg93	Cat	Egypt	Africa	Yes	1:200
Tx373	59	TgCatEg94	Cat	Egypt	Africa	Yes	1:6400
Tx373	60	TgCatEg95	Cat	Egypt	Africa	Yes	1:400
Tx373	61	TgCatEg96	Cat	Egypt	Africa	Yes	1:6400
Tx373	62	TgCatEg97	Cat	Egypt	Africa	Yes	1:200
Tx373	63	TgCatEg98	Cat	Egypt	Africa	Yes	1:100
Tx373	64	TgCatEg99	Cat	Egypt	Africa	Yes	1:25600
Tx373	65	TgCatEg100	Cat	Egypt	Africa	Yes	1:6400
Tx373	66	TgCatEg101	Cat	Egypt	Africa	Yes	1:1600
Tx373	69	TgCatEg102	Cat	Egypt	Africa	Yes	1:200
Tx373	70	TgCatEg103	Cat	Egypt	Africa	Yes	1:400
Tx373	71	TgCatEg104	Cat	Egypt	Africa	Yes	1:800

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx373	72	TgCatEg105	Cat	Egypt	Africa	Yes	1:800
Tx373	73	TgCatEg106	Cat	Egypt	Africa	Yes	1:51200
Tx373	77	TgCatEg108	Cat	Egypt	Africa	Yes	1:200
Tx373	78	TgCatEg109	Cat	Egypt	Africa	Yes	1:800
Tx373	79	TgCatEg110	Cat	Egypt	Africa	Yes	1:3200
Tx373	80	TgCatEg111	Cat	Egypt	Africa	Yes	1:3200
Tx373	81	TgCatEg112	Cat	Egypt	Africa	Yes	1:3200
Tx373	82	TgCatEg113	Cat	Egypt	Africa	Yes	1:3200
Tx373	83	TgCatEg114	Cat	Egypt	Africa	Yes	1:3200
Tx373	84	TgCatEg115	Cat	Egypt	Africa	Yes	1:6400
Tx109	2	TgCatBr1	Cat	Brazil	South America	Yes	1:1600
Tx109	3	TgCatBr2	Cat	Brazil	South America	Yes	1:3200
Tx109	5	TgCatBr3	Cat	Brazil	South America	Yes	1:1600
Tx109	6	TgCatBr4	Cat	Brazil	South America	Yes	1:800
Tx109	8	TgCatBr5	Cat	Brazil	South America	Yes	1:3200
Tx109	9	TgCatBr6	Cat	Brazil	South America	Yes	1:400
Tx109	11	TgCatBr7	Cat	Brazil	South America	Yes	1:1600
Tx109	20	TgCatBr8	Cat	Brazil	South America	Yes	1:3200
Tx109	21	TgCatBr9	Cat	Brazil	South America	Yes	1:6400
Tx120	1	TgCatBr23	Cat	Brazil	South America	Yes	1:51200
Tx120	2	TgCatBr24	Cat	Brazil	South America	Yes	1:51200
Tx120	3	TgCatBr25	Cat	Brazil	South America	Yes	1:400
Tx120	4	TgCatBr26	Cat	Brazil	South America	Yes	1:800
Tx120	5	TgCatBr27	Cat	Brazil	South America	Yes	1:202800
Tx120	6	TgCatBr28	Cat	Brazil	South America	Yes	1:3200
Tx120	7	TgCatBr29	Cat	Brazil	South America	No	<1:100
Tx120	8	TgCatBr30	Cat	Brazil	South America	Yes	1:1600
Tx120	9	TgCatBr31	Cat	Brazil	South America	Yes	1:1600
Tx120	10	TgCatBr32	Cat	Brazil	South America	Yes	1:800
Tx120	11	TgCatBr33	Cat	Brazil	South America	Yes	1:800
Tx120	12	TgCatBr34	Cat	Brazil	South America	Yes	1:400
Tx120	13	TgCatBr35	Cat	Brazil	South America	Yes	1:100
Tx120	14	TgCatBr36	Cat	Brazil	South America	No	<1:100

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx120	15	TgCatBr37	Cat	Brazil	South America	Yes	1:800
Tx114	1	TgCatBr10	Cat	Brazil	South America	Yes	1:400
Tx114	7	TgCatBr11	Cat	Brazil	South America	Yes	1:800
Tx114	9	TgCatBr12	Cat	Brazil	South America	No	<1:100
Tx114	11	TgCatBr13	Cat	Brazil	South America	Yes	1:1600
Tx114	13	TgCatBr14	Cat	Brazil	South America	No	<1:100
Tx114	14	TgCatBr15	Cat	Brazil	South America	Yes	1:400
Tx114	18	TgCatBr16	Cat	Brazil	South America	Yes	1:3200
Tx114	20	TgCatBr17	Cat	Brazil	South America	Yes	1:400
Tx114	22	TgCatBr18	Cat	Brazil	South America	Yes	1:400
Tx114	23	TgCatBr19	Cat	Brazil	South America	No	<1:100
Tx114	24	TgCatBr20	Cat	Brazil	South America	Yes	1:3200
Tx114	25	TgCatBr21	Cat	Brazil	South America	Yes	1:3200
Tx114	26	TgCatBr22	Cat	Brazil	South America	Yes	1:800
Tx477	77	N/A	Cat	China	Asia	Yes	1:25600
Tx225	1	N/A	Cat	China	Asia	Yes	1:1600
Tx225	4	N/A	Cat	China	Asia	Yes	1:102400
Tx225	6	N/A	Cat	China	Asia	Yes	1:6400
Tx244	8	N/A	Cat	China	Asia	No	<1:100
Tx244	11	N/A	Cat	China	Asia	Yes	1:800
Tx251	20	N/A	Cat	China	Asia	Yes	1:800
Tx251	21	N/A	Cat	China	Asia	Yes	1:102400
Tx251	23	N/A	Cat	China	Asia	Yes	1:202800
Tx251	24	N/A	Cat	China	Asia	Yes	1:51200
Tx251	25	N/A	Cat	China	Asia	Yes	1:400
Tx251	26	N/A	Cat	China	Asia	Yes	1:400
Tx269	42	N/A	Cat	China	Asia	No	<1:100
Tx269	43	N/A	Cat	China	Asia	Yes	1:100
Tx269	44	N/A	Cat	China	Asia	No	<1:100
Tx269	45	N/A	Cat	China	Asia	Yes	1:400
Tx269	46	N/A	Cat	China	Asia	Yes	1:1600
Tx269	47	N/A	Cat	China	Asia	Yes	1:6400
Tx269	48	N/A	Cat	China	Asia	Yes	1:400

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx269	49	N/A	Cat	China	Asia	Yes	1:400
Tx601	3	N/A	Cat	China	Asia	Yes	1:800
Tx601	5	N/A	Cat	China	Asia	Yes	1:800
Tx601	9	N/A	Cat	China	Asia	Yes	1:1600
Tx601	10	N/A	Cat	China	Asia	Yes	1:6400
Tx601	11	N/A	Cat	China	Asia	Yes	1:800
Tx480	1	N/A	Cat	Ethiopia	Africa	Yes	1:800
Tx480	3	N/A	Cat	Ethiopia	Africa	Yes	1:800
Tx480	4	N/A	Cat	Ethiopia	Africa	Yes	1:200
Tx480	5	N/A	Cat	Ethiopia	Africa	No	<1:100
Tx480	6	N/A	Cat	Ethiopia	Africa	Yes	1:400
Tx480	8	N/A	Cat	Ethiopia	Africa	No	<1:100
Tx480	9	N/A	Cat	Ethiopia	Africa	Yes	1:200
Tx480	14	N/A	Cat	Ethiopia	Africa	Yes	1:3200
Tx480	15	N/A	Cat	Ethiopia	Africa	No	<1:100
Tx480	16	N/A	Cat	Ethiopia	Africa	Yes	>1:12800
Tx480	17	N/A	Cat	Ethiopia	Africa	No	<1:100
Tx480	18	N/A	Cat	Ethiopia	Africa	Yes	1:800
Tx480	19	N/A	Cat	Ethiopia	Africa	No	<1:100
Tx480	20	N/A	Cat	Ethiopia	Africa	Yes	1:3200
Tx480	21	N/A	Cat	Ethiopia	Africa	Yes	1:400
Tx480	22	N/A	Cat	Ethiopia	Africa	Yes	1:100
Tx480	25	N/A	Cat	Ethiopia	Africa	Yes	1:200
Tx480	26	N/A	Cat	Ethiopia	Africa	Yes	1:800
Tx480	27	N/A	Cat	Ethiopia	Africa	Yes	1:200
Tx480	28	N/A	Cat	Ethiopia	Africa	Yes	1:1600
Tx480	29	N/A	Cat	Ethiopia	Africa	Yes	1:800
Tx480	31	N/A	Cat	Ethiopia	Africa	Yes	1:400
Tx480	32	N/A	Cat	Ethiopia	Africa	No	<1:100
Tx480	33	N/A	Cat	Ethiopia	Africa	Yes	1:3200
Tx480	34	N/A	Cat	Ethiopia	Africa	No	<1:100
Tx480	35	N/A	Cat	Ethiopia	Africa	No	<1:100
Tx480	36	N/A	Cat	Ethiopia	Africa	No	<1:100

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx480	38	N/A	Cat	Ethiopia	Africa	Yes	1:1600
Tx480	40	N/A	Cat	Ethiopia	Africa	Yes	1:1600
Tx480	43	N/A	Cat	Ethiopia	Africa	Yes	>1:12800
Tx480	44	N/A	Cat	Ethiopia	Africa	Yes	1:400
Tx480	45	N/A	Cat	Ethiopia	Africa	Yes	1:100
Tx480	46	N/A	Cat	Ethiopia	Africa	Yes	1:200
Tx480	48	N/A	Cat	Ethiopia	Africa	Yes	>1:12800
Tx480	49	N/A	Cat	Ethiopia	Africa	Yes	>1:12800
N/A	116-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:400
N/A	117-7	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	117-5	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	117-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	114-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	114-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	109-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:800
N/A	108-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	107-6	N/A	Cat	USA (Tennessee)	North America	Yes	1:800
N/A	104-14	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	104-13	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	104-6	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	103-5	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	103-4	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	103-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	103-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	103-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	102-2*	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	102-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:800
N/A	423-6	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	419-4	N/A	Cat	USA (Tennessee)	North America	No	<1:200
N/A	395-11	N/A	Cat	USA (Tennessee)	North America	Yes	1:800
N/A	394-2	N/A	Cat	USA (Tennessee)	North America	No	<1:200
N/A	424-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:25600
N/A	419-7	N/A	Cat	USA (Tennessee)	North America	Yes	1:200

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
N/A	421-8	N/A	Cat	USA (Tennessee)	North America	Yes	1:200
N/A	398-6	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	398-7	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	399-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	399-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:400
N/A	396-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	420-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	398-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	439-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	395-12	N/A	Cat	USA (Tennessee)	North America	Yes	1:200
N/A	438-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:12800
N/A	438-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:400
N/A	439-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	439-5	N/A	Cat	USA (Tennessee)	North America	Yes	1:12800
N/A	439-7	N/A	Cat	USA (Tennessee)	North America	No	<1:200
N/A	442-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	443-1	N/A	Cat	USA (Tennessee)	North America	No	<1:200
N/A	526-4	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	447-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:12800
N/A	443-5	N/A	Cat	USA (Tennessee)	North America	Yes	1:200
N/A	475-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	526-6	N/A	Cat	USA (Tennessee)	North America	Yes	1:200
N/A	476-22	N/A	Cat	USA (Tennessee)	North America	Yes	1:400
N/A	443-6	N/A	Cat	USA (Tennessee)	North America	Yes	1:400
N/A	526-4	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	472-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:800
N/A	474-4	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	442-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:12800
N/A	443-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:400
N/A	104-6	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	114-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	114-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	103-5	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
N/A	103-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:1600
N/A	104-13	N/A	Cat	USA (Tennessee)	North America	Yes	1:800
N/A	106-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	362-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:12800
N/A	356-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	519-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:51200
N/A	504-4	N/A	Cat	USA (Tennessee)	North America	Yes	1:51200
N/A	513-1	N/A	Cat	USA (Tennessee)	North America	Yes	1:51200
N/A	513-6	N/A	Cat	USA (Tennessee)	North America	Yes	1:3200
N/A	445-3	N/A	Cat	USA (Tennessee)	North America	Yes	1:6400
N/A	225-4	N/A	Cat	USA (Tennessee)	North America	Yes	1:25600
N/A	225-2	N/A	Cat	USA (Tennessee)	North America	Yes	1:25600
Tx256	1	N/A	Dog	Sri Lanka	Asia	No	<1:25
Tx256	2	N/A	Dog	Sri Lanka	Asia	Yes	1:100
Tx256	11	N/A	Dog	Sri Lanka	Asia	Yes	1:200
Tx256	15	N/A	Dog	Sri Lanka	Asia	Yes	1:200
Tx256	16	N/A	Dog	Sri Lanka	Asia	Yes	1:100
Tx256	22	N/A	Dog	Sri Lanka	Asia	Yes	1:400
Tx256	23	N/A	Dog	Sri Lanka	Asia	Yes	1:25
Tx256	25	N/A	Dog	Sri Lanka	Asia	No	<1:25
Tx256	48	N/A	Dog	Sri Lanka	Asia	No	<1:25
Tx256	51	N/A	Dog	Sri Lanka	Asia	Yes	1:400
Tx256	52	N/A	Dog	Sri Lanka	Asia	Yes	1:50
Tx256	54	N/A	Dog	Sri Lanka	Asia	Yes	1:100
Tx496	5	TgDogEg1	Dog	Egypt	Africa	Yes	1:200
Tx496	6	TgDogEg2	Dog	Egypt	Africa	Yes	1:400
Tx496	7	N/A	Dog	Egypt	Africa	Yes	1:25
Tx496	9	TgDogEg3	Dog	Egypt	Africa	Yes	1:1600
Tx496	10	N/A	Dog	Egypt	Africa	Yes	1:50
Tx496	11	TgDogEg4	Dog	Egypt	Africa	Yes	1:800
Tx496	12	TgDogEg5	Dog	Egypt	Africa	Yes	1:50
Tx496	13	TgDogEg6	Dog	Egypt	Africa	Yes	1:800
Tx496	14	TgDogEg7	Dog	Egypt	Africa	No	<1:25

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx496	15	N/A	Dog	Egypt	Africa	Yes	1:100
Tx496	17	TgDogEg8	Dog	Egypt	Africa	Yes	1:50
Tx496	18	TgDogEg9	Dog	Egypt	Africa	Yes	1:100
Tx496	21	N/A	Dog	Egypt	Africa	Yes	1:200
Tx496	23	TgDogEg10	Dog	Egypt	Africa	Yes	1:100
Tx496	25	TgDogEg11	Dog	Egypt	Africa	Yes	1:200
Tx496	26	TgDogEg12	Dog	Egypt	Africa	Yes	1:100
Tx496	27	TgDogEg13	Dog	Egypt	Africa	Yes	1:50
Tx496	28	N/A	Dog	Egypt	Africa	Yes	1:100
Tx496	29	TgDogEg14	Dog	Egypt	Africa	Yes	1:100
Tx496	30	N/A	Dog	Egypt	Africa	Yes	1:200
Tx496	31	N/A	Dog	Egypt	Africa	Yes	1:200
Tx496	32	TgDogEg15	Dog	Egypt	Africa	Yes	1:200
Tx496	33	TgDogEg16	Dog	Egypt	Africa	Yes	1:200
Tx496	34	N/A	Dog	Egypt	Africa	Yes	1:200
Tx496	37	TgDogEg17	Dog	Egypt	Africa	Yes	1:200
Tx496	38	TgDogEg18	Dog	Egypt	Africa	Yes	1:200
Tx496	40	N/A	Dog	Egypt	Africa	Yes	1:800
Tx496	43	TgDogEg19	Dog	Egypt	Africa	Yes	1:400
Tx496	45	N/A	Dog	Egypt	Africa	Yes	1:800
Tx496	46	TgDogEg20	Dog	Egypt	Africa	Yes	1:100
Tx496	47	N/A	Dog	Egypt	Africa	Yes	1:50
Tx496	48	TgDogEg21	Dog	Egypt	Africa	Yes	1:100
Tx496	49	TgDogEg22	Dog	Egypt	Africa	Yes	1:200
Tx496	50	N/A	Dog	Egypt	Africa	Yes	1:100
Tx215	3	N/A	Dog	Brazil	South America	Yes	1:50
Tx215	4	N/A	Dog	Brazil	South America	Yes	1:400
Tx215	12	N/A	Dog	Brazil	South America	No	<1:25
Tx215	13	N/A	Dog	Brazil	South America	Yes	1:50
Tx215	14	N/A	Dog	Brazil	South America	No	<1:25
Tx215	23	N/A	Dog	Brazil	South America	Yes	1:3200
Tx215	24	N/A	Dog	Brazil	South America	No	<1:25
Tx220	5	N/A	Dog	Brazil	South America	Yes	1:800

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx220	13	N/A	Dog	Brazil	South America	Yes	1:800
Tx220	14	N/A	Dog	Brazil	South America	No	<1:25
Tx220	18	N/A	Dog	Brazil	South America	No	<1:25
Tx220	19	N/A	Dog	Brazil	South America	Yes	1:200
Tx224	9	N/A	Dog	Brazil	South America	Yes	1:100
Tx224	18	N/A	Dog	Brazil	South America	No	<1:25
Tx224	20	N/A	Dog	Brazil	South America	Yes	1:25
Tx243	8	N/A	Dog	Brazil	South America	Yes	1:1600
Tx243	9	N/A	Dog	Brazil	South America	Yes	1:25
Tx248	1	N/A	Dog	Brazil	South America	Yes	1:400
Tx248	3	N/A	Dog	Brazil	South America	Yes	1:400
Tx248	8	N/A	Dog	Brazil	South America	Yes	1:400
Tx248	10	N/A	Dog	Brazil	South America	Yes	1:800
Tx248	17	N/A	Dog	Brazil	South America	No	<1:25
Tx248	18	N/A	Dog	Brazil	South America	Yes	1:800
Tx248	21	N/A	Dog	Brazil	South America	Yes	1:800
Tx235	16	N/A	Dog	Colombia	South America	No	<1:25
Tx235	19	N/A	Dog	Colombia	South America	No	<1:25
Tx235	39	N/A	Dog	Colombia	South America	Yes	1:25
Tx235	44	N/A	Dog	Colombia	South America	Yes	1:50
Tx238	51	N/A	Dog	Colombia	South America	No	<1:25
Tx238	1	N/A	Dog	Colombia	South America	No	<1:25
Tx238	3	N/A	Dog	Colombia	South America	No	<1:25
Tx238	6	N/A	Dog	Colombia	South America	Yes	1:50
Tx238	9	N/A	Dog	Colombia	South America	Yes	1:50
Tx238	14	N/A	Dog	Colombia	South America	Yes	1:50
Tx238	15	N/A	Dog	Colombia	South America	No	<1:25
Tx238	16	N/A	Dog	Colombia	South America	Yes	1:100
Tx238	26	N/A	Dog	Colombia	South America	Yes	1:200
Tx238	37	N/A	Dog	Colombia	South America	Yes	1:1600
Tx247	15	N/A	Dog	Colombia	South America	Yes	1:800
Tx247	28	N/A	Dog	Colombia	South America	Yes	1:1600
Tx247	46	N/A	Dog	Colombia	South America	Yes	1:200

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
Tx249	10	N/A	Dog	Colombia	South America	Yes	1:800
Tx250	15	N/A	Dog	Colombia	South America	No	<1:25
Tx250	33	N/A	Dog	Colombia	South America	Yes	1:400
Tx250	37	N/A	Dog	Colombia	South America	Yes	1:100
Tx250	38	N/A	Dog	Colombia	South America	No	<1:25
Tx250	39	N/A	Dog	Colombia	South America	No	<1:25
Tx250	41	N/A	Dog	Colombia	South America	Yes	1:1600
Tx250	43	N/A	Dog	Colombia	South America	Yes	1:25
Tx250	48	N/A	Dog	Colombia	South America	No	<1:25
Tx250	49	N/A	Dog	Colombia	South America	No	<1:25
Tx250	47	N/A	Dog	Colombia	South America	No	<1:25
Tx254	5	N/A	Dog	Colombia	South America	Yes	1:50
Tx241	1	N/A	Dog	Vietnam	Asia	No	<1:25
Tx241	2	N/A	Dog	Vietnam	Asia	Yes	1:50
Tx241	3	N/A	Dog	Vietnam	Asia	Yes	1:200
Tx241	5	N/A	Dog	Vietnam	Asia	Yes	1:400
Tx241	6	N/A	Dog	Vietnam	Asia	Yes	1:25
Tx241	7	N/A	Dog	Vietnam	Asia	No	<1:25
Tx241	8	N/A	Dog	Vietnam	Asia	Yes	1:25
Tx241	9	N/A	Dog	Vietnam	Asia	No	<1:25
Tx241	10	N/A	Dog	Vietnam	Asia	No	<1:25
Tx241	11	N/A	Dog	Vietnam	Asia	No	<1:25
Tx241	12	N/A	Dog	Vietnam	Asia	Yes	1:800
Tx241	13	N/A	Dog	Vietnam	Asia	No	<1:25
Tx241	14	N/A	Dog	Vietnam	Asia	No	<1:25
Tx241	15	N/A	Dog	Vietnam	Asia	Yes	1:100
Tx241	16	N/A	Dog	Vietnam	Asia	Yes	1:800
Tx241	17	N/A	Dog	Vietnam	Asia	Yes	1:25
Tx241	18	N/A	Dog	Vietnam	Asia	Yes	1:25
Tx241	19	N/A	Dog	Vietnam	Asia	No	<1:25
Tx241	20	N/A	Dog	Vietnam	Asia	Yes	1:50
Tx241	2	N/A	Dog	Vietnam	Asia	Yes	1:25
N/A	1	N/A	Human	Brazil	South America	Yes	1:3200

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
N/A	3	N/A	Human	Brazil	South America	Yes	1:6400
N/A	4	N/A	Human	Brazil	South America	Yes	1:12,800
N/A	5	N/A	Human	Brazil	South America	Yes	1:800
N/A	9	N/A	Human	Brazil	South America	Yes	1:1600
N/A	10	N/A	Human	Brazil	South America	Yes	1:6400
N/A	11	N/A	Human	Brazil	South America	Yes	1:800
N/A	12	N/A	Human	Brazil	South America	Yes	1:6400
N/A	13	N/A	Human	Brazil	South America	Yes	1:1600
N/A	14	N/A	Human	Brazil	South America	Yes	1:6400
N/A	15	N/A	Human	Brazil	South America	Yes	1:3200
N/A	16	N/A	Human	Brazil	South America	Yes	1:6400
N/A	21	N/A	Human	Brazil	South America	Yes	1:3200
N/A	25	N/A	Human	Brazil	South America	Yes	1:6400
N/A	26	N/A	Human	Brazil	South America	Yes	1:1600
N/A	27	N/A	Human	Brazil	South America	Yes	1:800
N/A	29	N/A	Human	Brazil	South America	Yes	1:800
N/A	35	N/A	Human	Brazil	South America	Yes	1:6400
N/A	36	N/A	Human	Brazil	South America	Yes	1:1600
N/A	37	N/A	Human	Brazil	South America	Yes	1:12800
N/A	38	N/A	Human	Brazil	South America	Yes	1:12800
N/A	43	N/A	Human	Brazil	South America	Yes	1:6400
N/A	48	N/A	Human	Brazil	South America	Yes	1:200
N/A	49	N/A	Human	Brazil	South America	No	<1:200
N/A	58	N/A	Human	Brazil	South America	Yes	1:800
N/A	64	N/A	Human	Brazil	South America	Yes	1:400
N/A	65	N/A	Human	Brazil	South America	Yes	1:12800
N/A	67	N/A	Human	Brazil	South America	Yes	1:12800
N/A	69	N/A	Human	Brazil	South America	Yes	1:25600
N/A	70	N/A	Human	Brazil	South America	Yes	1:3200
N/A	75	N/A	Human	Brazil	South America	Yes	1:3200
N/A	174	N/A	Human	Brazil	South America	Yes	1:3200
N/A	175	N/A	Human	Brazil	South America	Yes	1:12800
N/A	185	N/A	Human	Brazil	South America	Yes	1:400

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
N/A	199	N/A	Human	Brazil	South America	Yes	1:51200
N/A	206	N/A	Human	Brazil	South America	Yes	1:12800
N/A	209	N/A	Human	Brazil	South America	Yes	1:6400
N/A	234	N/A	Human	Brazil	South America	Yes	1:51200
N/A	243	N/A	Human	Brazil	South America	Yes	1:6400
N/A	246	N/A	Human	Brazil	South America	Yes	1:800
N/A	247	N/A	Human	Brazil	South America	Yes	1:1600
N/A	250	N/A	Human	Brazil	South America	Yes	1:12800
N/A	257	N/A	Human	Brazil	South America	Yes	1:12800
N/A	349	N/A	Human	Brazil	South America	Yes	1:800
N/A	357	N/A	Human	Brazil	South America	Yes	1:1600
N/A	364	N/A	Human	Brazil	South America	Yes	1:6400
N/A	365	N/A	Human	Brazil	South America	Yes	1:3200
N/A	367	N/A	Human	Brazil	South America	Yes	1:12800
N/A	364	N/A	Human	Brazil	South America	Yes	1:400
N/A	365	N/A	Human	Brazil	South America	Yes	1:1600
N/A	367	N/A	Human	Brazil	South America	Yes	1:3200
N/A	368	N/A	Human	Brazil	South America	Yes	1:400
N/A	370	N/A	Human	Brazil	South America	Yes	1:800
N/A	373	N/A	Human	Brazil	South America	Yes	1:1600
N/A	374	N/A	Human	Brazil	South America	Yes	1:1600
N/A	376	N/A	Human	Brazil	South America	Yes	1:400
N/A	383	N/A	Human	Brazil	South America	Yes	1:400
N/A	386	N/A	Human	Brazil	South America	Yes	1:800
N/A	391	N/A	Human	Brazil	South America	Yes	1:3200
N/A	398	N/A	Human	Brazil	South America	Yes	1:1600
N/A	400	N/A	Human	Brazil	South America	Yes	1:1600
N/A	409	N/A	Human	Brazil	South America	Yes	1:12800
N/A	422	N/A	Human	Brazil	South America	Yes	1:800
N/A	424	N/A	Human	Brazil	South America	Yes	1:6400
N/A	155	N/A	Human	Brazil	South America	Yes	1:102400
N/A	292	N/A	Human	Brazil	South America	Yes	1:3200
N/A	298	N/A	Human	Brazil	South America	Yes	1:6400

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
N/A	299	N/A	Human	Brazil	South America	Yes	1:25600
N/A	308	N/A	Human	Brazil	South America	Yes	1:6400
N/A	310	N/A	Human	Brazil	South America	Yes	1:3200
N/A	311	N/A	Human	Brazil	South America	Yes	1:12800
N/A	321	N/A	Human	Brazil	South America	Yes	1:3200
N/A	332	N/A	Human	Brazil	South America	Yes	1:6400
N/A	336	N/A	Human	Brazil	South America	Yes	1:12800
N/A	452	N/A	Human	Brazil	South America	Yes	1:102400
N/A	466	N/A	Human	Brazil	South America	Yes	1:1600
N/A	467	N/A	Human	Brazil	South America	Yes	1:800
N/A	468	N/A	Human	Brazil	South America	Yes	1:3200
N/A	469	N/A	Human	Brazil	South America	Yes	1:3200
N/A	471	N/A	Human	Brazil	South America	Yes	1:1600
N/A	482	N/A	Human	Brazil	South America	Yes	1:800
N/A	484	N/A	Human	Brazil	South America	Yes	1:800
N/A	25	N/A	Human	USA (Tennessee)	North America	Yes	1:25
N/A	20	N/A	Human	USA (Tennessee)	North America	Yes	1:25
N/A	27	N/A	Human	USA (Tennessee)	North America	Yes	1:50
N/A	3	N/A	Human	USA (Tennessee)	North America	Yes	1:100
N/A	84	N/A	Human	USA (Tennessee)	North America	Yes	1:200
N/A	86	N/A	Human	USA (Tennessee)	North America	Yes	1:200
N/A	6	N/A	Human	USA (Tennessee)	North America	Yes	1:200
N/A	50	N/A	Human	USA (Tennessee)	North America	Yes	1:200
N/A	49	N/A	Human	USA (Tennessee)	North America	Yes	1:400
N/A	51	N/A	Human	USA (Tennessee)	North America	Yes	1:400
N/A	94	N/A	Human	USA (Tennessee)	North America	Yes	1:400
N/A	44	N/A	Human	USA (Tennessee)	North America	Yes	1:400
N/A	10	N/A	Human	USA (Tennessee)	North America	Yes	1:200
N/A	52	N/A	Human	USA (Tennessee)	North America	Yes	1:200
N/A	35	N/A	Human	USA (Tennessee)	North America	Yes	1:200
N/A	57	N/A	Human	USA (Tennessee)	North America	Yes	1:400
N/A	36	N/A	Human	USA (Tennessee)	North America	Yes	1:400
N/A	34	N/A	Human	USA (Tennessee)	North America	Yes	1:800

Toxo designation	Sample ID	T. gondii Strain ID	Species	Country of Origin	Continent of Origin	Seropositivity	Final Titer
N/A	85	N/A	Human	USA (Tennessee)	North America	Yes	1:400
N/A	91	N/A	Human	USA (Tennessee)	North America	Yes	1:800
N/A	56	N/A	Human	USA (Tennessee)	North America	Yes	1:800
N/A	12	N/A	Human	USA (Tennessee)	North America	Yes	1:400
N/A	30	N/A	Human	USA (Tennessee)	North America	Yes	1:800
N/A	92	N/A	Human	USA (Tennessee)	North America	Yes	1:1600

***Toxoplasma* Modified Agglutination Test (MAT)**

Materials:

- a). **TgMAT antigens, 2×10^8 /ml.** *Toxoplasma* whole-cell antigen, formalin-fixed tachyzoites.
- b). **Alkaline Buffer.** (Dissolve 7.01g NaCl, 3.09g boric acid (H_3BO_3), and 1g sodium azide in 950 ml distilled water, add 20 ml of 1N NaOH and adjust pH to 8.95. Add 4g of bovine plasma albumin, bring volume to 1 liter using distilled water.)
- c). **Phosphate Buffered Saline (PBS).** (dissolve 7.20g NaCl, 1.48g Na_2HPO_4 (anhydrous), 0.43g KH_2PO_4 (anhydrous) in 1 liter of distilled water).
- d). **Positive control serum**
- e). **Negative control serum**
- f). **2% Evans blue dye** (2g in 100 ml distilled H_2O)
- g). **2-mercaptoethanol.**
- h). **96-well U-bottom microtiter plates**
- i). **Serum samples.** Note: The minimum amount of serum for a sample is 3 μl .
Note: All reagents should be stored at 4°C , except serum at -20°C .

Procedure:

1. Make 1:25 dilution of serum samples and the controls in PBS.

PBS	72.0 μl	
Serum sample or control	3.0 μl	
Total volume	75.0 μl	Mix well.
2. To a 96-well U-bottom microtiter plate, transfer 50 μl of diluted samples to the first and fifth rows from column 1 to column 10 of the plate. Transfer 50 μl of diluted negative and positive controls to the first wells of columns 11 and 12, respectively (20 samples, 1 negative and 1 positive control).
See **Template 1** for sample layout.
3. Add 25 μl of PBS to the rest of the wells.
4. Using a multichannel pipette, take 25 μl of diluted samples (10 samples) from row 1, make serial dilution to row 4, remove 25 μl from the last dilution and discard. Repeat the procedure for the 10 samples in row 5. For negative and positive controls, make serial dilution to 1:3,200 and remove 25 μl from the last dilutions.
5. Prepare antigen mixture (each 96-well plate):

Alkaline Buffer	2.5 ml
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2-mercaptoethanol	35 µl
Evans blue dye (2 mg/ml in H ₂ O)	50 µl
<u>TgMAT antigen</u>	<u>150 µl</u>
Total	2.735 ml

6. Mix antigen well by pipetting, immediately transfer 25 µl antigen mixture to each well using multichannel pipette. To prevent carryover of serum, the pipette tips should not touch the bottom of wells. Tap the plate lightly to bring the liquid to the bottom of the wells.

Note: Each well has 3×10^5 tachyzoites.

7. Cover the plate with sealing tape and incubate at 37°C for 16-24 hours. A pellet at the bottom of the well means negative. Samples without pellets are positive.

8. For positive samples with titers $\geq 1:200$, further test can be performed to determine titers. Serial dilutions include 1:25, 50, 100, 200, 400, 800, 1600, and 3200. See **Template 2** for sample layout.

Reference:

Desmonts G., Remington JS. Direct agglutination test for diagnosis of *Toxoplasma* infection: Method for increasing sensitivity and specificity. J. Clin. Microbiol. 1980. 11:562-568.

Dubey JP, Desmonts G. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 1987. 19:337-339.

Note:

One milliliter TgMAT antigens can test six and half of 96-well plates. Each plate can screen 20 serum samples. Therefore, a total of 130 samples can be screened.

Template 1. Serial dilution of serum samples for MAT screening (20 samples/plate)

Row 1	1	2	3	4	5	6	7	8	9	10	Negative control	Positive control
Row 5	11	12	13	14	15	16	17	18	19	20		
1:25	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>
1:50	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:100	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:200	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:25	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:50	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:100	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:200	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS

Diagram illustrating the serial dilution process for MAT screening. The table shows the volume of serum (50 ul) and PBS (25 ul) added to each well. Blue arrows indicate the transfer of 25 ul from one well to the next. Yellow boxes labeled "Discard 25 ul" indicate the volume discarded from wells 12, 13, 14, 15, 16, 17, 18, 19, and 20. The final dilution is 1:3200.

Template 2. Serial dilution of serum samples for titration (10 samples per plate)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Negative control	Positive control
1:25	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>	50 <u>ul</u>
1:50	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:100	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:200	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:400	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:800	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:1600	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS
1:3200	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS	25 <u>ul</u> PBS



The diagram illustrates the serial dilution process. Blue arrows on the right side of the table indicate the transfer of 25 ul of sample from one row to the next. The arrows start from the 'Sample 10' column and point to the 'Sample 1' column of the next row. This process is repeated for all rows from 1:25 down to 1:3200. A final arrow points from the bottom row (1:3200) to a yellow box labeled 'Discard 25 ul'.

Example of MAT test results (8 titers for each sample):

