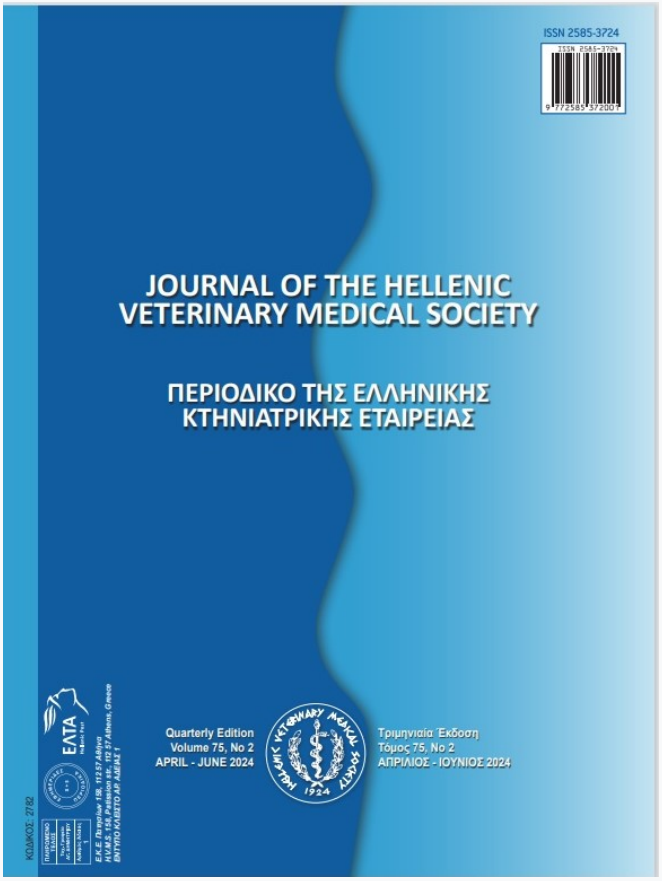
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 2 (2024)



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doi: 10.12681/jhvms.34354

To cite this article:

Yucesan, B., Kilic, S., Alçıgır, M., Babur, C., & Özkan, Ö. (2024). Histopathological, Immunohistochemical and Molecular Detection of Toxoplasma gondii in Organs and Tissues of Experimentally Infected Mice . *Journal of the Hellenic Veterinary Medical Society*, *75*(2), 7339–7350. https://doi.org/10.12681/jhvms.34354

J HELLENIC VET MED SOC 2024, 75 (2): 7339-7350
ΠΕΚΕ 2024, 75 (2): 7339-7350

Research article
Ερευνητικό άρθρο

Histopathological, Immunohistochemical and Molecular Detection of *Toxoplasma gondii* in Organs and Tissues of Experimentally Infected Mice

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ABSTRACT: With *Toxoplasma gondii* mouse experiments, it is aimed to determine its distribution to organs and tissues histopathologically, immunohistochemically, and molecularly. In our study, *T. gondii*_TR01 tachyzoites were injected intraperitoneally into the mice. The parasite was determined in daily concentrations in blood, peritoneal fluid, liver, kidney, heart, lung, intestine, and central nervous system sections.
Mild necrosis and degeneration of hepatocytes and degeneration of cortical and corticomedullary tubular epithelium in construction were observed as a result of histopathological examinations. Mild degeneration was observed in the heart. Regional hyperemic capillaries were found in the lung. Degeneration was observed in intestinal epithelial cells, and no necrosis was observed in villi and glandular epithelium. There was degeneration and necrosis of cerebral cortical neurons in the CNS. In immunohistochemical staining, reactions were mostly found in the liver, kidney, and intestine, while relatively low levels were found in the lung, heart, and brain. RT-PCR targeting the *T. gondii* B1 gene region molecularly was used. On the first and last days, it is mostly found in the liver, lung, and peritoneal fluid. At least it spread to the brain and heart. Tissue cysts were not found in all tissues. The spread to the heart is low. The liver, kidneys, and peritoneal fluid are most affected, and the brain is the least affected. Necrosis was detected in all tissues except the intestine. Organ and tissue spreads should be determined for drug and vaccine studies *of T gondii.* This study will assist in meeting this demand.

Keywords: Histopathology; Immunohistochemical; PCR; Tissues; *Toxoplasma gondii*

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Date of initial submission:
Date of acceptance:

INTRODUCTION

Toxoplasmosis is a multisystem disease caused by *Toxoplasma gondii* (*T. gondii*), an obligate intracellular protozoan, and can affect all vertebral organisms due to its characteristics. Studies have revealed that 25%-30% of the world's population is infected with *T. gondii* (Montoya and Liesenfeld, 2004). Economic and sociocultural habits, dietary patterns, meat cooking methods, meat and vegetable consumption, vegetable, fruit, and hand washing habits, quality of drinking and utility water, and sanitation processes may affect T gondii seroprevalence (Jones et al., 2007; Jones et al., 2003). This parasitic disease caused by T. gondii can affect all vital organs in the human body, especially in the acute period. It can be found in all fluids, such as blood, cerebrospinal fluid (CSF), semen, tears, saliva, and urine, and causes permanent fetal destruction and miscarriage through transplacental transmission. It is also known that T. gondii can survive for 50 days at 4°C and can be transmitted by whole blood and leukocyte transfusion (Karimi et al., 2014; Robert-Gangneux and Dardé, 2012).

After the primary infection, the oocysts that cats scatter into nature with their feces play a very important role in the transmission of the disease. Tachyzoites, the parasitic form of *T. gondii* in the acute phase, are the rapidly multiplying and spreading forms. Some tachyzoites transform into bradyzoites in tissue cysts, which are the life forms of the latent period, after about 3 days of division. Bradizoids are the only stage in the feline gut that leads to the enteroepithelial developmental period. When cats become infected by eating the tissue cyst, the bradyzoites that are released while passing through the stomach enter the enterocytes and form the stage that ends with the production of oocysts. Others invade the lamina propria, transform into tachyzoites, and reach the liver and mesenteric lymph nodes in about 8 hours, initiating the chronic stage (Dubey, 2016).

Diagnosis of toxoplasmosis: It can be diagnosed by serological detection of anti-Toxoplasma antibodies, by mouse or cell culture inoculation experiments, by histological evaluation from tissue sections, or by searching for tachyzoites in smears prepared from body fluids (Liu et al., 2015). The detection of *T. gondii* DNA by molecular methods has opened an important era, especially for the detection of congenital toxoplasmosis (Weiss and Dubey, 2009).

Today, the spread of this disease, which is known to affect all organs and has serious consequences, and the issue of which organs it settles in and in which periods arouse curiosity. The aim of this study is to define the distribution of *T. gondii* in all body tissues and fluids histopathologically, immunohistochemically, and molecularly with mouse experiments. With these methods, it is aimed at detecting the changes that may occur in human tissues with the distribution in mice with the profile closest to human tissues. The days on which the parasite reached these tissues, its location, and the infective changes it made were opened in two infected animals every day, and the changes in their organs were screened. In this way, the organ spread graph of the parasite was also obtained.

MATERIALS AND METHODS

In this study, 3-4-week-old and 17-18-gram healthy white male Swiss-Albino (Mus musculus albino) mice were obtained from the General Directorate of Public Health Experimental Animal Production Laboratory. The mice used in the study were "particularly pathogen-free (SPF)." Staff members with certificates for the care and use of experimental animals looked after mice and carried out studies in the laboratory setting. During the study, temperature, humidity, and light controls were made in the place where the animals stayed. The animals were fed calves' fattening meal and sterile water. This study was performed with 15 male Mus musculus albino mice with *T. gondii* seronegative.

The ethics committee approval of this study was obtained by the Veterinary Control Center Research Institute Local Ethics Committee/Ankara within the framework of the Permit to Work with Experimental Animals, with the decision numbered 2021/09 on June 21, 2022.

Samples and tissue collection

The study was performed in the Etlik Veterinary Control Central Research Institute Laboratories Experimental Animals Unit. The tachyzoites of the *T. gondii* TR 01 (Yucesan et al., 2021) strain used as antigen came from the peritoneal fluid of Swiss Albino mice that were kept in the National Parasitology Reference Laboratory of the General Directorate of Public Health and were infected intraperitoneally 50 hours ago. This strain is used in the Sabin Feldman Dye Test (SFDT), which is accepted as the gold standard in the world, in the same laboratory. Peritoneal exudate from mice was diluted with sterile normal saline to 2.5 x 105/mL. As a result, these diluted tachyzoites were injected intraperitoneally in 15

mice at a dose of 200μL (5x104) per mouse (Ekmen and Altıntaş, 1973; Ozkan et al., 2008). Thus, these mice were infected with the *T. gondii* TR_01 strain. Then, two experimental animals were euthanized by the cervical luxation method every day until all the mice died. These mice were opened, and their organs, blood, and peritoneal fluid were taken. The time taken for all animals to die after inoculation was 5 days. Therefore, the study took six days. All organs (heart, brain, liver, lung, large intestine, small intestine, and kidneys) were separated for histopathological and molecular analysis after removal. Organs are separated in a sterile environment with disposable materials.

Necropsy

A species-specific necropsy procedure was followed in the experiment after euthanasia. The abdominal and thoracal cavities, as well as the skull, were opened after skinning and cleaning muscles. After examining the changes in all tissues and organs in these cavities, the head area was removed, and the calvarium was removed. Liver, kidney, heart, lung, and intestine sections, in addition to the central nervous system, including the cerebrum, cerebellum, pons, and medulla oblongata, were taken out. The color, consistency, and other morphological changes were examined carefully and photographed. Tissues from all organs were sampled and placed in a 10% buffered formalin solution in plastic jars.

Histopathological Examination

Histopathological conditions in the organs were performed at Kırıkkale University, Faculty of Veterinary Medicine, Department of Pathology, Kirikkale, Turkiye. First, studies were conducted to confirm the infection and show how the infection damages and affects the microarchitecture of tissues. After formalin fixation for 48 h, all tissue samples from experimental animals were sliced and placed into plastic containers. They were processed routinely at graded alcohol and xylol series in an automatic tissue processor (Leica, TP1020, Germany). Tissues were embedded in paraffin wax (Thermo Shandon, EG 1150, Germany). Tissue sections were sectioned at a rotary microtome (Shandon, AS325). Five micron-thick sections were cut from paraffin blocks and stained with hematoxylin and eosin staining (H&E) according to the method described by Luna (1968). Histological findings in all organs were evaluated under digital light microscopy (Olympus BX51, Japan) and photographed in camera attachment (Olympus DP25 camera, Japan).

Immunohistochemical examination

Formalin-fixed, paraffin-embedded (FFPE) tissues were used. The tissue sections were sectioned at the same thickness. Deparafinization in xylene and degraded ethanols and rehydration in phosphate-buffered saline (PBS, Sigma Aldrich tablet) solution were completed for sections at the first step. The immunohistochemical method was carried out according to the manual of the Steptavidin-Biotin Complex Peroxidase (streptABC-P) kit (Novocastra, Leica, RE7120-K). Antigen retrieval solution was performed using citrate buffer solution (10x diluted in PBS; pH = 6.0) for 25 minutes in an 800-watt microwave oven. A 3% H_2O_2-methanol solution for 20 minutes relieved the removal of peroxidase activity in tissues. After that, the protein-blocking serum was dripped into tissue sections. Thereafter, primary antibodies (T. gondii-specific serum (1/250 at dilution, cat number: LS-C312239, Lot-181329/1 ml, Life Span)) were dripped into sections and incubated overnight at +4°C. Following this, biotinylated and HRP-conjugated antibodies were added after incubation with primary antibodies and incubated for 30 minutes at 37°C. The sections were kept under control with diaminobenzidine (DAB) chromogen for 5 minutes. Washing procedures were run with a 10x diluted PBS solution twice for 5 minutes, excluding after blocking serum. For counterstaining, the sections were kept in Gill's haematoxylin for 3 minutes. They were then passed through alcohol and xylene and mounted using a non-aqueous mounting medium, Entellan (Merck®). As a positive control, tissues that were previously diagnosed as *T. gondii*-positive were selected. A specific antibody against *T. gondii* was applied to these tissues under the same conditions. As a negative control, the sections were treated with phosphate-buffered saline (PBS) instead of primary antibodies. Moreover, mouse tissues in the experiment were incubated with antibodies against *Neospora caninum*, and the same method was applied to sample organ sections. The above-mentioned antibody dilutions were determined by trying out optimal reactions for each antibody in accordance with the datasheet of the product company.

Scoring and Statistical analysis

For scoring histopathological findings, each tissue section, including several organs, was obtained by counting 10 fields at 400x magnification (10 HPFs). Lesions were scored semiquantitatively as (0): no lesion, (1): a few lesion, (2): mild, and (3): strong.

These lesions were calculated according to the mean score after lesion types at each organ of the animal were performed.

Degeneration, necrosis, inflammation, and vascular changes were evaluated histopathologically. Toxoplasma-infected, immunopositive cells in each organ were counted. Their averages were calculated in a Microsoft Excel spreadsheet on Windows 10. The post-hoc Tukey test using the total number of animals as a basis confirmed the one-way analysis of variance (one-way ANOVA) method's results for immunopositivities. For histopathological lesions (degeneration, necrosis, inflammation, and vasculatory changes), one-way ANOVA was preferred and applied to the results for each animal. Thus, the difference between the tissues was evaluated according to statistical results. The statistical difference was found meaningful as $p < 0.05$ and graphed using GraphPad Prism Software version 8.4.2 (GraphPad Software, San Diego, California, USA).

Molecular identification

This study was carried out in the laboratories of the University of Health Sciences. A molecular study was carried out in organs, blood, and peritoneal fluid.

DNA ısolation and RT-PCR

According to the manufacturer's instructions, *T. gondii* DNA extraction from organs (brain, lung, kidney, intestine, heart, and liver), peritoneal fluid, and a blood sample was performed by the Nucleic Acid Extraction Kit (Bioeksen R&D Technologies, Cat No. B200-20). The concentration of DNA in each sample was measured by a spectrophotometer (Thermoscientific Nanodrop-ND 2000) for qualitative and quantitative analyses. The daily changes in the organs, blood, and peritoneal fluid of the mice were determined by calculating the daily density changes of the B1 gene (target gene) until all mice died completely by RT-PCR.

Real-Time PCR: The detection of the *T. gondii* B1 gene was performed by real-time quantitative PCR according to the method described by Sroka et al. (2018) (Sroka et al., 2018). For real-time PCR amplification, forward primers (*T. gondii*, F: 5'-CCCTCTGCTGGCGAAAAG-3'), reverse primers (*T. gondii*, R: 5'-AGCGTTCGTGGTCAACTATCG-3'), and TaqMan probes (6FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA) were used to specifically amplify the T. gondii B1 gene. The PCR mixture consisted of 10 µl of Premix Ex Taq master mix (Takara Bio Inc.), 5 µL of oligo mix containing 500 nmol forward, 500 nmol reverse, and 250 nmol TaqMan probe targeting the *T. gondii* B1 gene, and 5 µl of DNA template. PCR amplification was performed on the CFX-96 (Bio-Rad, California, USA) thermal cycler. The cycling conditions were as follows: initial denaturation at 95°C for 30 sec, followed by 40 cycles of denaturation at 95°C for 5 sec, followed by annealing and extension at 55°C for 5 sec. Negative and positive controls (the latter consisting of 10-fold dilutions of the *T. gondii* TR 01 strain) were amplified in parallel.

RESULTS

Histopathological findings

General lesions

Liver: In all cases, hepatocytes locating at the perihpey of the central vein included clear vacuoles in the cytoplasm. Nuclei had exantric localization in the cytosol. Some nuclei were lost or karyopyknotic in appearance. In some fields, necrotic cells were observed with shrunken and dark-pink cytoplasm. Among them, necrotic cells had totally lost their nuclei and cytoplasmic borders. There was no inflammatory cell infiltration or vasculatory changes, including severe hyperemia, edema, or hemorrhagia.

Kidney: In many cases, tubule epitheliums were degenerated in general. Cortical tubules were severely affected from both degeneration and necrotic changes. The nuclei and cytoplasm in terms of degeneration and necrosis had similar in appearence. There were again no inflammatory cell infiltration and vasculatory changes apart from milder hyperemia in glomerules.

Heart: In all cases, myocardiocytes were generally affected by paranchyme degeneration. The cells include shrunken and dark pink to red cytoplasm. Nuclei were generally pyknotic in appearance. Necroses were not as dense as in the liver or kidney. There were no other changes as in previous organs..

Lung: In all cases, there was desquamation in alveolar epitheliums. Some of the cells contained karyopyknosis. Necrosis was seen in only one case. Leukostasis was present. But neutrophil infiltration was not observed. Other vasculatory lesions, including edema and eritrodiapedesis, were encountered only in a couple fields.

Intestine: Villus and gland epitheliums were des-

quamated. Some nuclei of them were pyknotic in appearance, in contrast to some that had karyolytic and shrunken cytoplasm. In some fields, necrotic cells were encountered in both epitheliums lying on the lamina epithelialis and glands. There were trivial inflammatory lesions and vasculatory lesions in a few fields.

Central nervous system: Neurons in the cerebrum lost chromatin, and cellular borders were not conspicuous. The degenerations in cortical neurons were more severe than necrotic changes. Necrosis was seen moderately in one case, in contrast to other cases that showed milder necrotic changes in neurons. Cerebellum lesions were similar to those in cerebral neurons. In the cerebellum, Purkinje cells were more affected by degeneration. Some of the cytoplasms were encountered in shrunken and dark pink appearances. The medulla oblongata and pons did not contain any lesions.

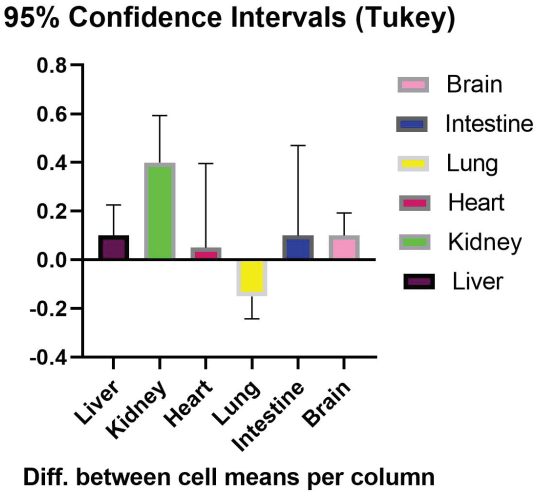
Lesions on the basis of degeneration, necrosis, inflammation, and vasculatory changes were scored meanly in Table 1 and evaluated statistically in Table 2. The comparative scores according to organs were illustrated in Graph 1. The findings belonging to these organs were illustrated in Figure 1.

Table 1: Mean score of lesion distribution in the organs

Cases	Liver	Kidney	Heart	Lung	Intestine	CNS
Degeneration	2	1.6	1.2	1	1	2
Necrosis	1.6	0.8	0.8	0.4	0	1.2
Inflammation	0	0	0	0	0	0
Vasculatory changes	0	0	0	0	0	0

Table 2: Statistical histological results obtained from Two-way ANOVA

Source of Variation	% of total variation	P value	P value summary	Significant?	Geisser-Greenhouse's epsilon
Sample x Lesion	7,006	0,0656	Ns	No	
Sample	1,244	0,9911	Ns	No	
Lesion	5,325	0,0033	**	Yes	0,6581
Subject	70,38	<0,0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Sample x Lesion	5,350	20	0,2675	F (20, 75) = 1,638	P=0,0656
Sample	0,9500	4	0,2375	F (4, 15) = 0,06628	P=0,9911
Lesion	4,067	5	0,8133	F (3,291, 49,36) = 4,980	P=0,0033
Subject	53,75	15	3,583	F (15, 75) = 21,94	P<0,0001
Residual	12,25	75	0,1633		



Graph-1. Lesional distribution comparatively according to organs

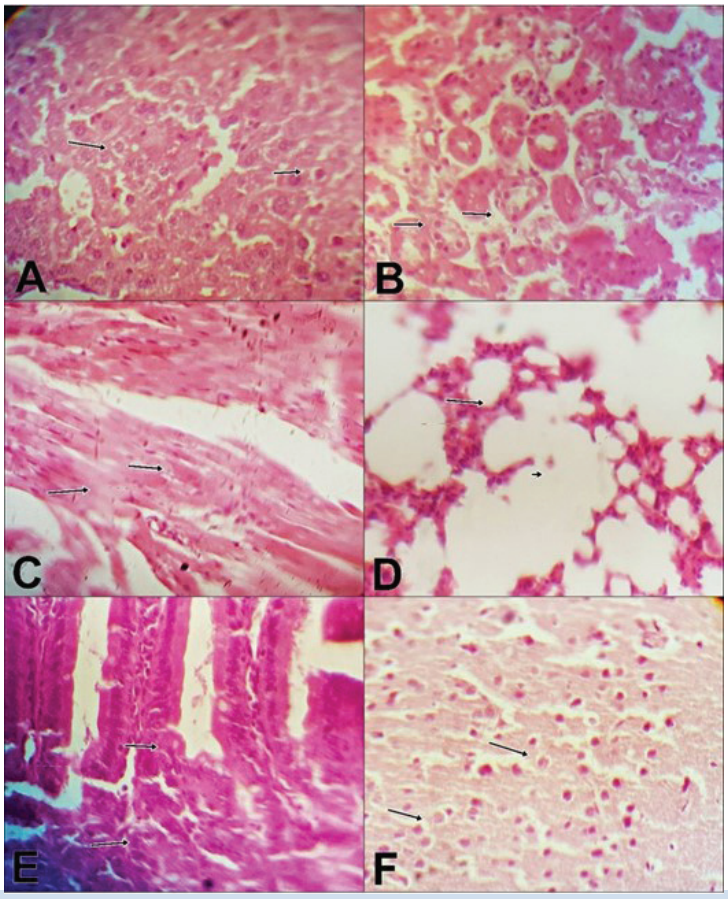


Figure 1. Degeneration in cells (arrows), (A) Hepatocyte (B) Renal tubule epithelium (C) Cardiomyocyte (D) Alveoler epithelium (E) Intestinal epithelium and glandl epithelium (F) Neuron of brain cortex, H&E staining, x400 magnification.

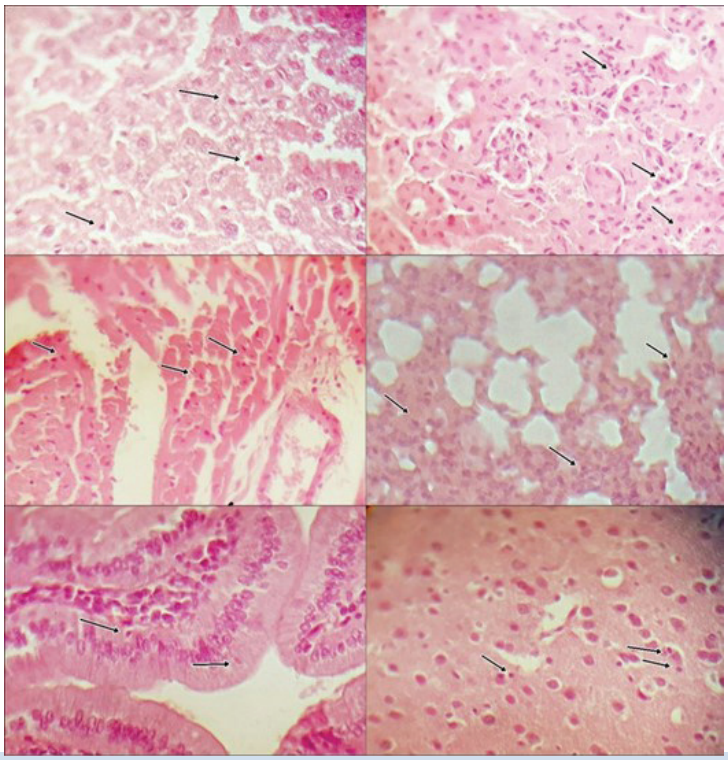


Figure 2. Tachyzoid in cells (arrows), (A) Hepatocyte (B) Renal tubule epithelium (C) Cardiomyocyte (D) Alveoler epithelium (E) Intestinal epithelium and glandl epithelium (F) Neuron of brain cortex, H&E staining, x400 magnification.

Distribution of lesions according to days of the experiment

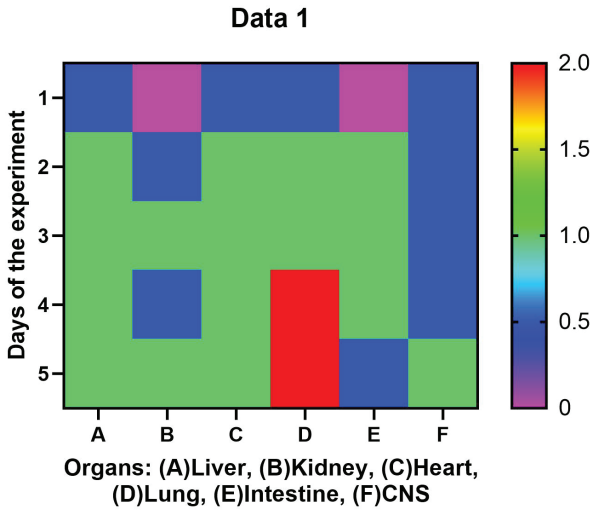
1st day of experiment: In the liver, necrosis in hepatocytes was milder than degeneration. Degeneration in hepatocytes was widespread, beginning from the central vein to the periphery of the hepatic lobules. Kupffer cells were activated in the central and midzonal areas. Tachyzoites were more common in central areas. In the kidney, tubule epitheliums locating at the cortical and corticomedullary levels were degenerated. However, medullary renal tubules were not affected by either degeneration or necrosis. Glomerules were hyperemic and hypercellularized in appearance. Podocytes in glomeruli were activated in many glomeruli. Tachyzoites were often found in the cortical and corticomedullary tubule epithelium. In the muscles of the heart, cardiomyocytes were mildly degenerated. Necrosis in cardiomyocytes was also mildly seen in some areas. Tachyzoites were found sparsely in cardiomyocytes. In the lung, hyperemic capillaries were found in some areas. In alveolar lumina, there were edema and desquamated epitheliums. Additionally, inflammatory cell infiltration was encountered at the periphery of hyperemic vessels and the interstitium. Tachyzoites were found haphazardly in pneomocytes. In the intestine, parasite-infected epithelium was generally degenerated. There was no necrosis in such villus and gland epitheliums. Tachyzoites were found in villus epitheliums in general. Inflammatory cell infiltration was not encountered in the propria mucosa. In the CNS, cerebral cortical neurons were affected by degeneration and necrosis. Gliosis and satellitosis were evaded in the paranchyme.

2nd day of experiment: In the liver, degeneration and necrosis in hepatocytes were more moderate than on the previous day of the experiment. In kidney tubules, epithelium was degenerated and necrotic in appearance, as it had been the previous day. However, corticomedullar and medullar tubules were also more affected by degeneration. In the lung, hyperemia, infiltramatory cell infiltration, and vasculatory changes were again seen mildly in terms of lesional distribution. In the intestine, necrosis as well as degeneration were present mildly in some villus epithelium. Inflammatory cells were mildly infiltrated in the propria mucosa, dissimilarly from the previous day of the experiment. In these organs, tachyzoites were encountered as many times as on the first day of the experiment.

3rd day of experiment: The lesions and parasitic distribution were the same as on the second day of the experiment. However, degeneration and necrosis were found in kidney tubules. Moreover, the lungs were more hyperemic in appearance. Degeneration and necrosis were more common in cortical neurons in the brain.

4th day of experiment: The lesions were found to be the same on the 2nd and 3rd days of the experiment. However, renal lesions, including degeneration and necrosis, were dense in corticomedullary tubules. Additionally, infiltratory cell infiltration and vasculatory lesions in lung lesions were moderately common in many areas. Lesions and parasitic distribution were likely to be at the remaining organs.

5th day of experiment: The results in organs were similar to the fourth day of the experiment. But renal lesions were less common in a few areas. Parasitic distribution was found according to degeneration and necrosis in tubules.



Graph 2. Total lesion score in organs according to days of experiment

Immunohistochemical detection of *Toxoplasma gondii* antigen in organs

Immunopositivities were reacted as red-brown to dark-brown according to the density of *T.gondii* antigen. The reactions were mostly obtained from the liver, kidney, and intestine, respectively. Immunopositivities were located in the cytoplasm of hepatocytes, and Kupffer cells were located at the periphery of central areas of the hepatic lobes. In the kidney, it was found positive in the cytoplasm of tubule epitheliums, in particular cortical tubules. The cytoplasm of cardiac myocytes reacted positively in the heart. In the lung, alveolar epitheliums and bronchiolar epitheli-

ums were evidently positive. In the CNS, mostly neuronal cytoplasm in the brain cortex and Purkinje cell cytoplasm were found positively, as well as glial cells in the substantia alba. Other parts of the CNS were not conspicuously reactive.

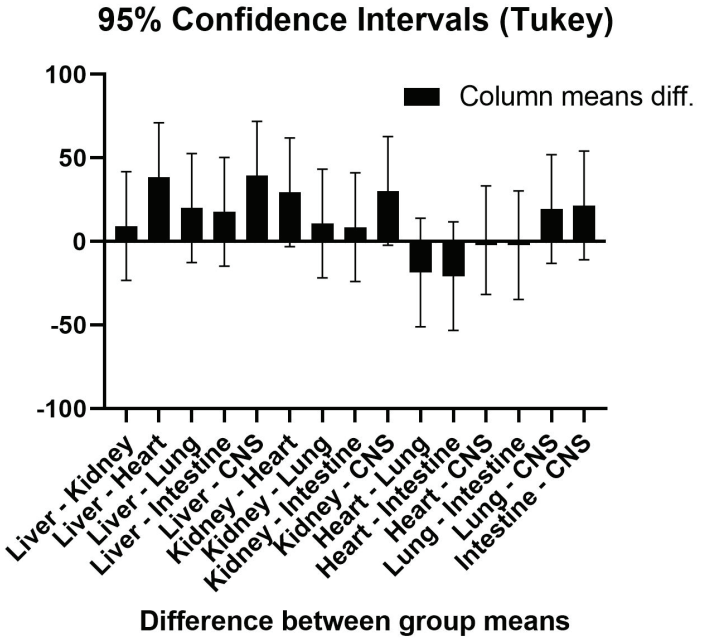
The mentioned reactions in the lung, heart, and brain were found to be relatively low when compared to other organs, including the liver and kidney. In particular, liver reactions were statistically meaningful in these organs. The immunopositivities were summarized in Tables 3 and 4. Comparative reactions were illustrated in Graph 3. The findings are shown in Figure 2.

Table 3: Mean immunoposivities of *T.gondii* in the organs

Cases	Liver	Kidney	Heart	Lung	Intestine	CNS
T.gondii Ag	63±8.92	53.8±6.42	24.4±6.77	43±8.58	45.2±8.81	23.6±3.69

Table 4. Statistical immunohistochemical results obtained from One-way ANOVA

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Liver vs. Kidney	9,200	-23,30 to 41,70	No	ns	0,9488
Liver vs. Heart	38,60	6,100 to 71,10	Yes	*	0,0135
Liver vs. Lung	20,00	-12,50 to 52,50	No	ns	0,4246
Liver vs. Intestine	17,80	-14,70 to 50,30	No	ns	0,5488
Liver vs. CNS	39,40	6,900 to 71,90	Yes	*	0,0113
Kidney vs. Heart	29,40	-3,100 to 61,90	No	ns	0,0925
Kidney vs. Lung	10,80	-21,70 to 43,30	No	ns	0,9040
Kidney vs. Intestine	8,600	-23,90 to 41,10	No	ns	0,9613
Kidney vs. CNS	30,20	-2,300 to 62,70	No	ns	0,0792
Heart vs. Lung	-18,60	-51,10 to 13,90	No	ns	0,5026
Heart vs. Intestine	-20,80	-53,30 to 11,70	No	ns	0,3826
Heart vs. CNS	0,8000	-31,70 to 33,30	No	ns	>0,9999
Lung vs. Intestine	-2,200	-34,70 to 30,30	No	ns	>0,9999
Lung vs. CNS	19,40	-13,10 to 51,90	No	ns	0,4574
Intestine vs. CNS	21,60	-10,90 to 54,10	No	ns	0,3428



Graph 3. Column bar graph showing comparatively of immunopositivities in organs.

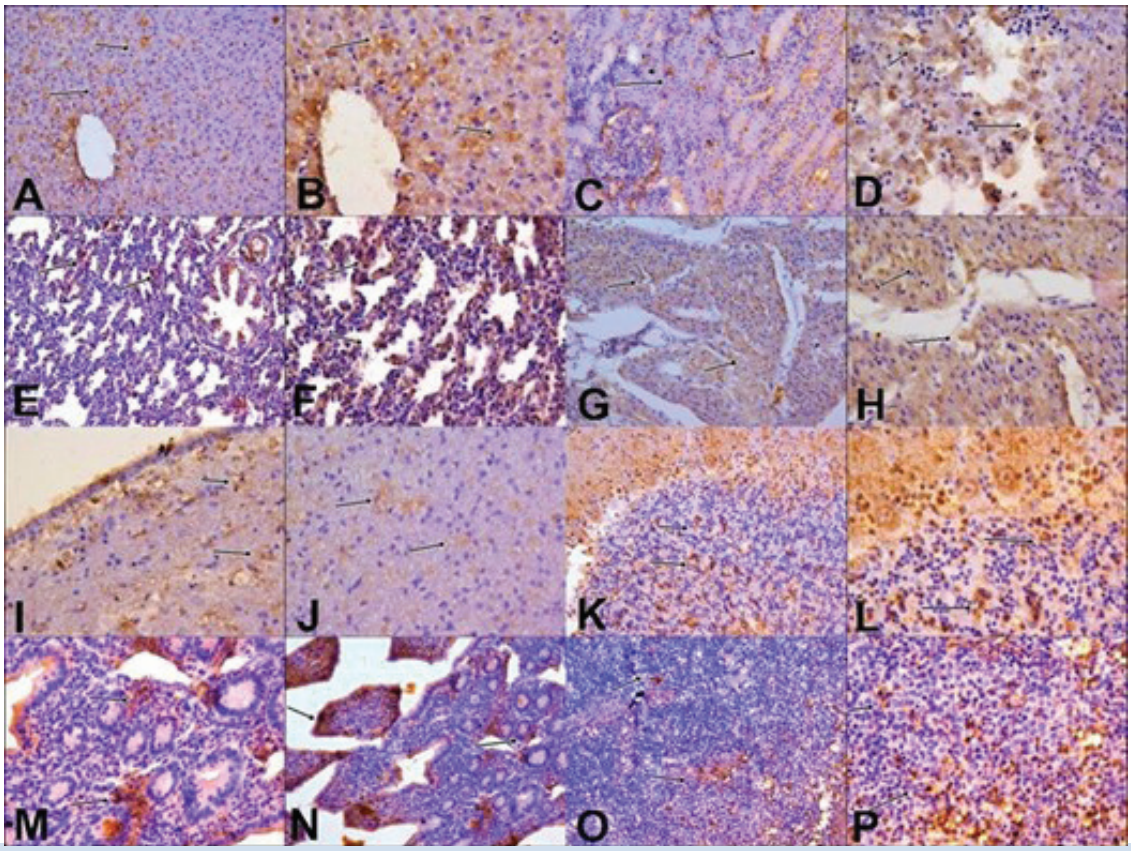


Figure 3. Immunopositive cells (arrows) by *T. Gondii* antibody. (A-B) Hepatocytes, D-D) Tubules epithelium of renal cortex (E-F) Alveoler epithelium and bronchioler epitheliums (G-H) cardiomyocytes (I-J) Neurons of brain cortwx (K-L) Purkinje cells and granular layer calls in cerebellum (M-N) Intertinal epithelium and glad epitheliums, (O-P) Lymyphoid follicles, (A-C-E-G-I-K-M-O, x 100 magnification) and (B-D-I-J-L-N-P, x250 magnification), strept ABC-P immunostaining, DAB chromogen

PCR detection of *Toxoplasma gondii* in samples

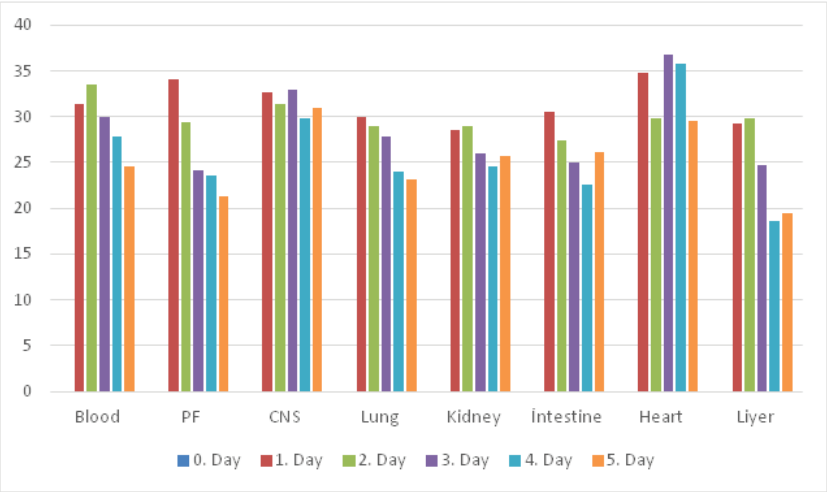
Molecular analyses are compatible with immunohistochemical analyses. As a result of PCR tests, all tests were positive except the first day. As a result of PCR analysis, it is seen that the positivity rates in all organs are increasing within days. Daily density changes were calculated as a result of RT-PCR analysis. CT values are given in Graph 4 as a result of RT-PCR. With the CT value, the amount of cycle that the system can detect is determined. In other words, fewer cycles are required as it can be detected more quickly in cases where it is more intense. That is, the low lines in Graph 4 show the areas with the highest density.

The highest spread in the first day is in the kidney, liver, and lung, respectively, and the least spread is in the heart and peritoneal fluid. On the last day, it is mostly distributed in the liver and peritoneal fluid. It is seen at least in the brain and peritoneal fluid. As a result, we can say that it is eliminated more quickly in the peritoneal fluid and is always present in high concentrations in the liver and lungs. It is found at least in the brain and heart. At least, it spread to the brain and heart.

The density of the genomic material obtained by RT-PCR is given in Table 5 and Graph 4.

Table 5. The density of the genomic material obtained by RT-PCR

	Blood	**PF**	**CNS**	**Lung**	**Kidney**	**Intestine**	**Heart**	**Liyer**
0. day	0	0	0	0	0	0	0	0
1. day	31,38	34,07	32,73	29,905	28,525	30,58	34,85	29,3
2. day	33,525	29,44	31,385	28,985	28,93	27,45	29,85	29,86
3. day	30,005	24,15	32,99	27,87	25,925	25,025	36,755	24,665
4. day	27,8	23,585	29,775	24,015	24,61	22,63	35,73	18,56
5. day	24,515	21,33	31,02	23,16	25,71	26,165	29,585	19,45



Graph 4. The density of the genomic material obtained by RT-PCR

DISCUSSION

Toxoplasma gondii is an intracellular protozoan that can spread to all warm-blooded mammals and their organs and body fluids. With the invasion of tachyzoites, it can envelop all tissues and form necrotic tissues in the following periods.

The virulence of the *T. gondii* strain is influenced by the type of host cell, infection rate, susceptibility, route of administration, and host and tissue selection of the parasite.

It is reported that the strain of the agent used in the infection has individual virulence characteristics in injected animals. Although some strains can be fatal in a short time after intraperitoneal injection, others cause chronic infection and cyst formation (Eissa et al., 1990; Zenner et al., 1998; Zenner et al., 1999). In this context, knowing which animal was infected with which strain may be useful in predicting the results of organ involvement.

Sabin Feildman Dye test is performed with antigen from mice infected with T. gondii TR 01, and this antigen is killed within 4-6 days when administered intraperitoneally to mice. After the injection, it was observed that the mice's hair became erect, they lost weight, their water and food intake decreased, and they showed signs of disease. The study lasted 6 days in total, and degeneration, necrosis, inflammatory cells, and vascular-based changes (edema, bleeding, and hyperemia) in tissues were evaluated histopathologically in this way semi-quantitatively. These results were prepared by counting the 10 sites of each tissue on each necropsy day. While scoring, we averaged the numerical expressions obtained in each tissue sample taken for each necropsy day. In the histopathological analysis, scoring was done as 0, 1, 2, and 3 semi-quantitatively.

A comparison of differences such as degeneration, necrosis, and vascular changes is mentioned in the lesions. The subject is the variety of organs. In our table, the difference between both the lesion diversity and the tissue or organ in question was found to be statistically significant. In other words, it has been determined that toxoplasmosis causes different lesions in different tissues. Degeneration was mostly seen in the liver and CNS. Necrosis was detected in all tissues except the intestine. This situation informs us that the parasite invasion in the liver is intense and shows parallels with other studies.

In the immunohistochemistry method, the most data obtained by counting 10 fields from the organs on each necropsy day is presented as an average. In other words, the averages given by T. gondii for all days are presented according to the organs.

When the numerical values in the organs are evaluated statistically, the liver, and central nervous system, liver and heart are seen to be significantly different according to the degree of closeness of the organs.

Polymerase chain reaction (PCR) is widely used for the detection of T. gondii in amniotic fluid, blood, and tissues in situations that are difficult to interpret serologically (Chabbert et al., 2004). Among the PCR targets used to detect T. gondii from blood, fluids, and tissue samples, the B1 gene is the most widely used (Tsutsui et al., 2007). Although laborious, expensive, time-consuming, and dangerous for laboratory personnel, it is the standard for detecting infection in tissues in mouse experiments (Homan et al., 2000).

Garcia et al. (2006) detected T. gondii in muscle and brain tissue in their study, and tissue cysts could not be detected histopathologically (Garcia et al., 2006). Samico-Fernandes et al. (2017) biologically tested tissue samples from seropositive pigs in mice. Tissue samples were subjected to histopathology, immunohistochemistry, PCR, and sequencing, and T. gondii DNA was positive (Samico-Fernandes et al., 2017). In another study, brain, muscle, and liver tissues of wild and domestic rodents were analyzed by GRA6 PCR. Mice were sacrificed 50 days after infection, and the brains of all vaccinated mice were examined by direct microscopy for the presence of T. gondii cysts (Saki and Khademvatan, 2014). In our study, it was aimed at detecting T. gondii in all tissues, peritoneal fluid, and blood. With PCR, the spread was detected mostly in the liver and peritoneal fluid. Histopathologically, no cyst form was observed in this study.

Chronic infections of T gondii cause a buildup of cysts that cause tissue damage in the brain and muscles. Cysts in the motor areas of the brain also affect some kinematic movement parameters (Galván-Ramírez et al., 2019). In the study of Samico et al. (2017), mice were sacrificed after being infected, and the brain, heart, skeletal muscle, lung, liver, and kidney were obtained. For histopathology, it was stained with hematoxylin and eosin, as in our study (Samico et al., 2017).

Tissue cysts in mice were formed 2 or 3 days after parenteral inoculation of tachyzoites and tissue cysts persist throughout the life of the host (Galván-Ramírez et al., 2019). However, tissue cysts were not detected in our study.

Histopathological lesions in the brain also depend on the type of T. gondii strain. Histopathological appearances in the brains of mice infected with T. gondii strain ME49 have been reported as endothelial hypertrophy, gliosis, and peripheral inflammatory cell migration to the brain (Fuentes-Castro et al., 2017). In our study, cerebral cortical neurons were affected by degeneration and necrosis.

Toxoplasma gondii infections are asymptomatic (95%) in the acute and chronic phases. The chronic phase continues throughout the life of the host. However, in immunocompromised patients, tachyzoites formed as a result of rapid replication of T. gondii and reactivation of bradyzoites can potentially cause serious symptoms such as encephalitis, which can be fatal (Blume and Seeber, 2018). The CNS has the highest frequency of parasites for T gondii in chronic infections (Zhao and Ewald, 2020). However, since all mice died after infection in this cellular study, no tissue cysts associated with infection were observed in the chronic form. Therefore, since tissue cysts are a life-threatening form, it is better that they were not detected histopathologically and immunohistochemically in our study, and that they were the tissue least affected by T. gondii in the PCR test. As in the brain, no tissue cysts were found in other liver, heart, kidney, lung, or intestinal tissues.

The death of mice within 6 days in our study suggests that the T. gondii TR 01 strain is a highly aggressive agent for mice.

Intraperitoneal inoculation of the Prugniaud strain resulted in its spread to the brain in mice and rats, among other organs. Histopathological changes, consisting of tissue degeneration, necrosis, and infiltration of inflammatory cells in some organs, have been reported in mice infected with the Prugniaud strain (Barakat et al., 2022; Zenner et al., 1999). It caused a more serious disease presentation than the Beverly strain, and its spread in the CNS was found to be high. In another study with mice, necrosis and spread in the liver, spleen, and pancreas and the absence of pseudocysts were mentioned (Sukthana et al., 2003). In our study, spread to the CNS was minimal in histopathological, immunologic, and PCR tests. It is thought that the reason for this may be that the CSF barrier has little penetration of the infective agent. T. gondii infects the liver first in all three studies and remains in high concentration until death. The low spread to the heart is also similar to other studies (Eissa et al., 1990; Sukthana et al., 2003; Waree et al., 2007; Zenner et al., 1998).

Galván-Ramírez et al. (2019) reported mild to moderate chronic inflammation in both peribronchiolar and parenchymal tissue in the lungs (Galván-Ramírez et al., 2019). In our study, desquamation and leukocytosis were present in the lung alveoli. However, there was no neutrophil infiltration.

CONCLUSIONS

In conclusion, the *T. gondii* TR 01 strain appears to have a lethal effect on mice. All mice are lost after an acute infection. Following inoculation, the parasite was found to invade all tissues and organs tested. The change in lesions is related to the duration of exposure. *T. gondii* parasite is a protozoon that has the potential to develop changes in tissues with this

spread, and it has the potential to be widely seen on the earth's surface. It is important to know the distribution of the parasite in the tissues and the changes it makes in today's world, where drug and vaccine studies are increasing rapidly. In this context, the parasite is still a parasite that needs to be investigated, and diagnosis and treatment are mandatory.

ETHİCS STATEMENT

Ethical approval of this study was obtained from the Local Ethics Committee of the Veterinary Control Central Research Institute with permission to work with experimental animals, dated June 21, 2021, and numbered 2021/09.

AUTHORS' CONTRIBUTIONS

Conceptualization: BY, SK, MEA, CA, OO; Data curation: BY, MEA; Formal analysis: BY, MEA; Funding acquisition: BY, MEA; Investigation; BY, SK, MEA, CB, OO; Methodology: BY, SK, MEA, OO; Project administration: BY, SK, OO; Resources; BY, MEA; Supervision; Validation; BY, SK, MEA, OO; Roles/Writing - original draft: BY, SK, MEA, OO; Writing - review & editing: BY, SK, MEA, OO.

DECLERATION OF CONFLICTING INTERESTS

The authors declare that they have no conflicts of interest.

FINANCIAL SUPPORT

There is no financial support in this study.

ACKNOWLEDGEMENT

Non

REFERENCES

Barakat AM, El Fadaly HAM, Selem RF, Madboli AE-NA, Abd El-Razik KA, Hassan EA, Alghamdi AL. Elmahallawy EK (2022) Tamoxifen Increased Parasite Burden and Induced a Series of Histopathological and Immunohistochemical Changes During Chronic Toxoplasmosis in Experimentally Infected Mice. Frontiers in microbiology. 13.

Blume M, Seeber F (2018) Metabolic interactions between Toxoplasma gondii and its host. F1000Res, 7. doi: 10.12688/f1000research.16021.1.

Chabbert E, Lachaud L, Crobu L, Bastien P (2004) Comparison of two widely used PCR primer systems for detection of Toxoplasma in amniotic fluid, blood, and tissues. Journal of clinical microbiology. 42(4): 1719-1722.

Dubey JP (2016) Toxoplasmosis of animals and humans: CRC press, USA

Eissa MH, Antonious S, Salama M, Fikry A, Morsy T (1990) Histopathological studies of acute, chronic and congenital infections of toxoplasmosis in mice. Journal of the Egyptian Society of Parasitology. 20(2): 805-816.

Ekmen H, Altinas K (1973) Bir köpekte Toxoplasma gondii izolmanı. Türk Hijiyen ve Tecrubi Biyologi Dergisi. 33; 17-20.

Fuentes-Castro BE, Reyes-García JG, Valenzuela-Vargas MT, Martínez-Gómez F (2017) Histopathology of murine toxoplasmosis under treatment with dialyzable leukocyte extract. Memórias do Instituto Oswaldo Cruz. 112: 741-747.

Galván-Ramírez ML, Salas-Lais AG, Dueñas-Jiménez SH, Mendizabal-Ruiz G, Franco Topete R, Berumen-Solís SC, Perez LRR, Franco Topete K (2019) Kinematic Locomotion Changes in C57BL/6 Mice Infected with Toxoplasma Strain ME49. Microorganisms, 7(11). doi:10.3390/microorganisms7110573

Garcia JL, Gennari SM, Machado RZ, Navarro IT (2006) Toxoplasma gondii: detection by mouse bioassay, histopathology, and polymerase chain reaction in tissues from experimentally infected pigs. Experimental parasitology. 113(4): 267-271.

Homan W, Vercammen M, De Braekeleer J, Verschueren H (2000) Identification of a 200-to 300-fold repetitive 529 bp DNA fragment in Toxoplasma gondii, and its use for diagnostic and quantitative PCR. Int J Parasitol. 30(1): 69-75.

Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M (2007) Toxoplasma gondii infection in the United States, 1999 2004, decline from the prior decade. Am J Trop Med Hyg. 77(3): 405-410.

Jones JL, Kruszon-Moran D, Wilson M (2003) Toxoplasma gondii infection in the United States, 1999-2000. Emerging infectious diseases 9(11): 1371.

Karimi G, Mardani A, Zadsar M (2014) Toxoplasma and blood transfusion. Iranian Journal of Parasitology. 9(4): 597.

Liu Q, Wang Z-D, Huang S-Y, Zhu X-Q (2015) Diagnosis of toxoplasmosis and typing of Toxoplasma gondii. Parasit Vectors. 8(1): 1-14.

Montoya J, Liesenfeld O (2004) Toxoplasmosis. Lancet. 363: 1965-1976. doi:https://doi.org/10.1016/S0140-6736(04)16412-X

Özkan AT, Çelebi B, Babür C, Lucio-Forster A, Bowman DD Lindsay DS (2008) Investigation of anti-Toxoplasma gondii antibodies in cats of the Ankara region of Turkey Using the Sabin-Feldman dye test and an indirect fluorescent antibody test. Journal of Parasitology. 94(4): 817-820.

Robert-Gangneux F, Dardé ML (2012) Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 25(2): 264-296.

Saki J, Khademvatan S (2014) Detection of Toxoplasma gondii by PCR and mouse bioassay in rodents of Ahvaz District, Southwestern Iran. Biomed Res Int, 2014:383859.

Samico-Fernandes EFT, Samico-Fernandes MFT, de Albuquerque PPF, de Almeida JC, de Souza Santos A, da Rocha Mota A, Neto OLS, Mota RA (2017) Toxoplasma gondii in backyard pigs: seroepidemiology and mouse bioassay. Acta Parasitologica. 62(2): 466-470.

Sroka J, Karamon J, Dutkiewicz J, Wójcik-Fatla A, Cencek T (2018) Optimization of flotation, DNA extraction and PCR methods for detection of Toxoplasma gondii oocysts in cat faeces" Annals of Agricultural and Environmental Medicine. Vol. 25, No. 4, doi: 10.26444/aaem/97402.

Sukthana Y, Waree P, Pongponratn E, Chaisri U, Riganti M (2003) Pathologic study of acute toxoplasmosis in experimental animals. Southeast Asian journal of tropical medicine and public health. 34(1): 16-21.

Tsutsui V, Freire R, Garcia J, Gennari S, Vieira D, Marana E, Purudencio LB, Navarrol IT (2007) Detection of Toxoplasma gondii by PCR and mouse bioassay in commercial cuts of pork from experimentally infected pigs. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 59: 30-34.

Waree P, Ferguson DJ, Pongponratn E, Chaisri U, Sukthana Y (2007) Immunohistochemical study of acute and chronic toxoplasmosis in experimentally infected mice. Southeast Asian journal of tropical medicine and public health. 38(2): 223.

Weiss LM, Dubey JP (2009) Toxoplasmosis: a history of clinical observations. Int J Parasitol. 39(8): 895-901.

Yucesan B, Guldemir D, Babur C, Kilic S, Cakmak A (2021) Whole-genome sequencing of a Toxoplasma gondii strain from a Turkish isolate using next-generation sequencing technology. Acta Trop. 218: 105907. doi:10.1016/j.actatropica.2021.105907

Zenner L, Darcy F, Capron A, Cesbron-Delauw MF (1998) Toxoplasma gondii: kinetics of the dissemination in the host tissues during the acute phase of infection of mice and rats. Experimental parasitology. 90(1): 86-94.

Zenner L, Foulet A, Caudrelier Y, Darcy F, Gosselin B, Capron A, Cesbron-Delauw MF (1999) Infection with Toxoplasma gondii RH and Prugniaud strains in mice, rats and nude rats: kinetics of infection in blood and tissues related to pathology in acute and chronic infection. Pathology-Research and Practice. 195(7): 475-485.

Zhao X-Y, Ewald SE (2020) The molecular biology and immune control of chronic Toxoplasma gondii infection. J Clin Invest. 130(7): 3370-3380.