



## **Experimental Toxoplasmosis in Pigeons (*Columba livia*)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors AA, GE, DYG and AI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CB and AI managed the analyses of the study. Authors GE and DYG managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The purpose of the present study was to establish experimental model of toxoplasmosis in pigeons, to investigate pathogenesis and compare tissue lesions by clinical, histopathological, serological and bioassay techniques. Total of 60 unknown aged pigeons (*Columba livia*), 21 males and 39 females were used. They were divided into groups as oral (Group I and II) and parenteral (Group III, IV, V and VI) infection groups (Table 1). While some pigeons in Group IV showed acute infection signs such as anorexia, weight loss, pale cockscomb, bend of head and neck and partial paralyze; chronic infection signs such as anorexia, weakness, weight loss, diarrhea, difficulties in breathing,

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and conjunctivitis were seen in Group IV, V, and VI. In necropsy, the pigeons in Group IV had hyperemia and focal hemorrhages in the meninges and brain; the pigeons in Groups V and VI had yellowish color of the liver, the pigeons in Group V had the pale chest muscles, pericardial thickening and opaqueness. There were no macroscopic findings in pigeons in Group I and III. Histopathological examination revealed nonsuppurative meningoencephalitis and tachyzoites and bradyzoite cysts formation of *T. gondii* in brain tissue, lymphoid cell infiltration and necrotic focal hepatitis and nephritis in Group IV. While pigeons in Group V had nonsuppurative focal myositis, myocarditis, hepatitis, gastritis, enteritis, pneumonitis, and necrotic pancreatitis, one of them had toxoplasma bradyzoite cyst in the sinusoid in the liver. In group VI, nonsuppurative focal hepatitis, myocarditis, nephritis and necrotic pancreatitis were detected in pigeons. Bioassay tests were performed with tissue samples taken from seropositive pigeons and parasitic tachyzoites were isolated from the peritoneal fluid of the mice. Seropositivity in the oral and parenteral groups was determined by Sabin-Feldman Dye Test (SFDT) and Indirect Hemagglutination Assay (IHA). As a result; in similar studies that will be performed investigating pathogenesis of Toxoplasmosis and subclinical cases that may be overlooked, serologic tests and bioassay applications should be used together for the diagnosis of toxoplasmosis.

**Keywords:** Bioassay; histopathology; pigeon; serology; tachyzoites.

## 1. INTRODUCTION

Toxoplasmosis is an important zoonotic infection that caused by *Toxoplasma gondii* and the disease distributed worldwide and can affect mammals including human, domestic and wild animals and also wild and domestic avian species [1-6]. *T. gondii* infection is subclinical in many bird species [1,4,5,7-11]. In addition to natural toxoplasmosis cases in domestic birds, experimental studies have been conducted in many poultry species such as white quails, Japanese quails, chicken, broiler, pigeon, turkey and pheasants [4,5,7-15]. *T. gondii* infection has been reported in numerous domestic and wild avian species by serologic [16-19] and experimental studies [2,5,8,12,14,15] in Turkey. Pigeons have been shown to be susceptible to toxoplasmosis by natural studies [20-26] or experimental studies [2,3,27], and morbidity and mortality in pigeons have been reported to be higher than in other birds. *T. gondii* oocyte-contaminated soil, water and cats play an important role in the spread of toxoplasmosis [1,2,4,6,11,20]. Experimental studies have shown that the pigeons that are infected with *T. gondii* can spread the disease with their faeces and also pigeons living freely in rocky areas and cities have a risk of spreading the disease [2,20,21,23,25]. In addition, since pigeons are used in Chinese cuisine [19] and in the racing industry in Taiwan the disease has gained zoonotic importance. Seroprevalence studies for toxoplasmosis have been performed all over the world and it has been reported to be between 4-

5.9% [2,20-25,28,29]. Immunohistochemistry and histopathological examination are important to confirm the diagnosis of toxoplasmosis [22,30]. Immunohistochemistry is more appropriate in cases in which toxoplasmosis cannot be diagnosed in routine histopathological examination. For this reason, microscopic findings are very important in the diagnosis of toxoplasmosis [31]. The prevalence of *T. gondii* has been different in naturally [1,6,11,20-22,24-26,28-30,32-37] and experimentally [2,4,7-10,12,27] infected poultry. The serological diagnosis of toxoplasmosis is usually made by detecting specific antibodies by indirect fluorescent antibody test (IFAT) and Modified agglutination test (MAT) [26,27,37-40]. Additionally, bioassay tests are thought to be more useful if brain and heart are both used [1,11,14,41-44].

The objective of this study was to establish experimental model of toxoplasmosis in free-living pigeons in rocky areas and cities using oral and parenteral (intracerebral, intramuscular, intravenous) infection, and also to diagnose the disease using clinical, histopathological, bioassay, and serological methods and to obtain information about pathogenesis.

## 2. MATERIALS AND METHODS

**Animal material:** This study was conducted with unknown aged pigeons (*Columba livia*), 21 males and 39 females and all pigeons were obtained from a commercial farm in Kayseri/Turkey.

**Table 1. Experimental groups and inoculum doses that were used in the study**

Oral group ( $10^5$ tachyzoites)		Parenteral group ( $10^5$ tachyzoites)			
Control group (I. Grup)	Infection group (II. Grup)	Control group (III. Grup)	Intracerebral group (IV. Grup)	Intramuscular group (V. Grup)	Intravenous group (VI. Grup)

**Experimental plan:** *T. gondii* RH strain was isolated from human and was provided from the Ankara Refik Saydam Hifzisiha Research Centre. The required concentration of inoculums ( $10^5$  tachyzoites/0,5 ml) was obtained from tachyzoites. Before administrating *T. gondii*, bloods of five pigeons in each group were tested for sero-negativity. Sabin-Feldman Dye Test (SFDT) and Indirect Hemagglutination Assay (IHA) were used in serological detection of toxoplasmosis. Experimental groups and inoculum doses that were used in the study are shown in Table 1. While Group I was used as a control group for Group II, Group III was served as a control group (parallel concurrent) for Group IV, Group V and Group VI.

#### **Necropsy and histopathological examination:**

Following inoculation among the pigeons under the clinical observation, two of them died on the 5th and 9th days from the group II; two pigeons died on the 6th and 19th days in Group VI and one pigeon died on the fifth day in Group IV. Tissue samples (brain, heart, skeletal muscle, ovary, testis, gizzard, crop, liver, kidney, spleen, pancreas, and intestine) that were taken from pigeons died from acute toxoplasmosis in Group IV (n: 1) and non-specific reasons (n: 4) were fixed in neutral formalin solution. On the 13th day of post inoculation, six of the pigeons in Group IV that were showed neurological signs and all the remaining pigeons (n = 49) on the 45th day were anesthetized with 0.015 ml %2 xylazine [29] and then blood samples were collected through cardiac puncture and necropsies were performed. All the samples were fixed in 10% neutral formalin. Followed by routine procedure the samples embedded in paraffin and sectioned at 5  $\mu$ m. After staining with haematoxylin and eosin (HxE), sections were examined with light microscope. Also some tissue samples were collected from brain, heart, chest and leg muscles for bioassay.

**Serological examination:** Serums were obtained from blood samples during necropsy. Seropositivity was determined by SFDT and IHA. Fisher Exact test and SPSS 20.0 package program were used for statistical analysis of serological test results of SFDT and IHA.

**Bioassay:** After necropsy, tissues (brain, heart, and muscles of chest and legs) of the pigeons were bioassayed [1,11,23,26,33]. As reported previously, specific pathogen free (SPF) 3- to 6-wk-old Swiss Albino mice (20-25 g) were injected intraperitoneally with 0.5 ml suspension that was prepared from the brain, heart and skeletal muscles of each pigeon. Brain, heart, lung, skeletal muscle and other tissues were examined histopathologically for toxoplasmosis in mice which died between days of 5 and 11. Peritoneal liquid of each mouse was microscopically examined for the presence of tachyzoites [1,11,16,17,23,26].

### **3. RESULTS**

**Clinical findings:** While the pigeons that had neurological symptoms on day 13 in Group IV (n: 6) showed acute infection signs such as anorexia, weight loss, pale cockscomb, bend of head and neck and partial paralyze; chronic infection signs such as anorexia, weakness, weight loss, diarrhea, difficulties in breathing, and conjunctivitis were seen in Group IV (n: 2), Group V (n: 10) and Group VI (n: 7). No clinical signs were observed in pigeons of groups I and III.

**Serological findings:** Serum samples of pigeons were serologically examined with SFDT and IHA. Results are shown in Table 2 according to the experimental groups. The difference between SFDT and IHA was not statistically significant in all routes of administration ( $P>0.05$ ) (Table 3).

**Necropsy findings:** The pigeon died from acute toxoplasmosis in Group IV (n: 1) showed hyperemia and hemorrhages of meninges and brain. No pathological findings were observed during necropsy in pigeons which died due to housing conditions and nonspecific reasons (n: 4) in Group II and Group VI. On the 13th day of postinoculation, six of the pigeons in Group IV that had neurological symptoms were showed hyperemia and focal hemorrhages of meninges and brain. Necropsies were performed on the 45th day of postinoculation all the remaining pigeons (n: 49). The pigeons had yellowish colored liver in Groups V and VI. In group V, pigeons had pale chest muscles, and additionally,

**Table 2. Serological and bioassay results of oral and parenteral groups. \* Noninfectious death (housing conditions)**

Oral Group								Parenteral Group										
Serological Results				Bioassay Results				Serological Results (Dilutions)				Bioassay Results						
I. Group		II. Group		I. Group	II. Group	III. Group	IV. Group	V. Group		VI. Group		III. Group	IV. Group	V. Group	VI. Group			
SFDT	IHA	SFDT	IHA			SFDT	IHA	SFDT	IHA	SFDT	IHA	SFDT	IHA					
1	-	-	1/64	1/80	-	-	-	-	1/64	1/160	1/1024	1/160	1/64	1/160	-	+	+	+
2	-	-	1/16	1/80	-	-	-	-	1/256	-	1/16	-	-*	-*	-	+	+	-*
3	-	-	-*	-*	-	-*	-	-	1/16	1/80	1/256	1/160	1/256	1/160	-	+	+	+
4	-	-	1/64	-	-	-	-	-	-*	-*	1/1024	1/160	1/16	1/80	-	-*	+	+
5	-	-	1/64	1/80	-	-	-	-	1/64	1/160	1/64	1/80	1/16	-	-	+	+	+
6	-	-	-*	-*	-	-*	-	-	1/64	1/160	1/256	1/160	1/16	1/80	-	+	+	+
7	-	-	1/256	1/160	-	-	-	-	1/64	-	1/1024	1/160	1/64	1/160	-	+	+	+
8	-	-	1/64	1/80	-	-	-	-	1/256	1/160	1/64	1/80	1/16	-	-	+	+	+
9	-	-	1/16	-	-	-	-	-	1/16	-	1/64	1/80	-*	-*	-	+	+	-*
10	-	-	1/16	1/160	-	-	-	-	1/64	1/80	1/256	-	1/16	1/80	-	+	+	+

**Table 3. The difference between SFDT and IHA was not statistically significant in all routes of administration (P>0,05)**

Group names	Tests	Positive	Negative	P value
Group II (oral)	SFDT	8	0	P= 0,467
	IHA	6	2	
Group IV (intracerebral)	SFDT	9	0	P= 0,206
	IHA	6	3	
Group V (intramuscular)	SFDT	10	0	P= 0,211
	IHA	7	3	
Group VI (intravenous)	SFDT	8	0	P= 0,467
	IHA	6	2	

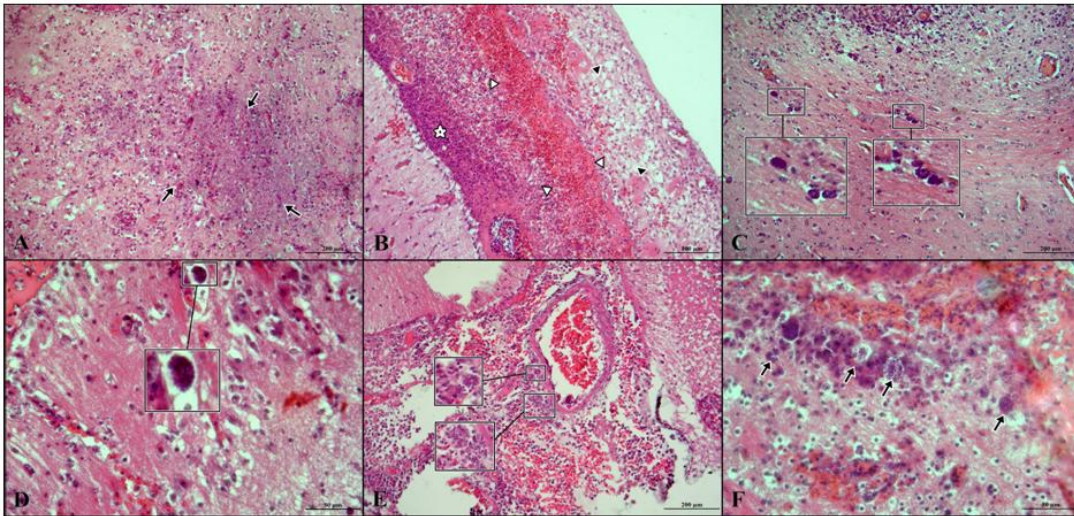
four pigeons had an opaque appearance and thickening of the pericardium in the heart. No pathologic findings were noted on macroscopic examination in Groups I, III and IV.

**Histopathological findings:** Nonspecific hyperemia and focal hemorrhages were detected in some of the tissue sections (liver, lung and intestine) that were prepared from the pigeons in Group I and III. However, no lesion associated with toxoplasmosis was found in these sections. There were no pathological findings of toxoplasmosis in pigeons in Group II. All pigeons in Group IV showed severe hyperemia and focal hemorrhages with necrosis in brain sections (Fig. 1A). Nuclear material consisting of fibrin and cell debris, lymphoid cell infiltrations and haemorrhages were observed in the meninges (Fig. 1B). A few strawberry-like bradyzoites containing crescent shaped tachyzoites in a macrophage cytoplasm were observed in the meninges. There was a large area of necrosis in the occipital and temporal lobes of the brain where there were disrupted nuclear chromatins, glia cells, macrophages, lymphocytes, and a mass of fibrins. A few strawberry-like bradyzoites containing crescent shaped tachyzoites in a macrophage cytoplasm were perivascularly observed in the peripheral necrotic areas in the meninges and substansia grisea (Fig. 1C, D). Additionally, free tachyzoites were also noted in paranchyma (Fig. 1E, F). There were also hyperemia and small, round and sharp-sided lipid vacuoles in the cytoplasm of hepatocytes in the liver of some pigeons in this group. In addition, focal necrotic areas and lymphoid cell infiltrations were observed. Whereas only one pigeon showed focal necrotic areas and lymphoid cell infiltrations in the kidney; some of the pigeons showed similar lesions in the liver.

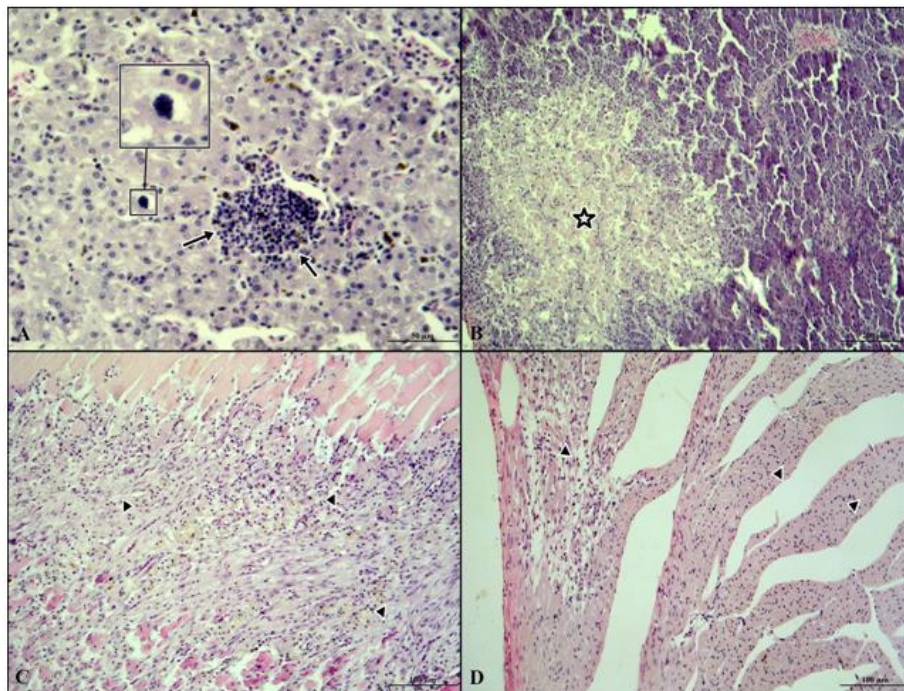
In group V, the focal lymphoid cell infiltration was seen in the brain of the one pigeon and in the

interglandular region of glandular stomach of another one. Furthermore, lymphoid cell infiltration was detected in intestine of one pigeon and lungs of another two pigeons. Almost all pigeons in group V had hyperemia, focal hemorrhages, necrosis and focal lymphoid cell infiltration foci in the kidneys. One of the pigeons had bradyzoite cyst in the sinusoid of the liver adjacent to the focal lymphoid cell infiltration area (Fig. 2A). Focal necrosis and lymphoid cell infiltrations was detected in the pancreas of two pigeons (Fig. 2B). Multifocal necrosis and lymphocyte-rich mononuclear cell infiltration in both centre and periphery of necrotic area were seen in skeletal muscles of all pigeons (Fig. 2C) and heart muscles of four pigeons (Fig. 2D). The appearance of the lesions in muscles was similar in Group VI. Hyperemia and focal hemorrhages were also observed in heart muscles of two pigeons and skeletal muscles of three pigeons in Group VI (Fig. 3A-B). There were also hyperemia and small, round and sharp-sided lipid vacuoles in the cytoplasm of hepatocytes in the liver of four pigeons in this group. In addition, focal necrotic areas and lymphoid cell infiltrations were observed in liver (Fig. 3C). Focal hemorrhages and focal lymphoid cell infiltration areas were observed in the kidneys. Focal necrosis and lymphoid cell infiltrations was detected in the pancreas of a pigeon in Group VI (Fig. 3D).

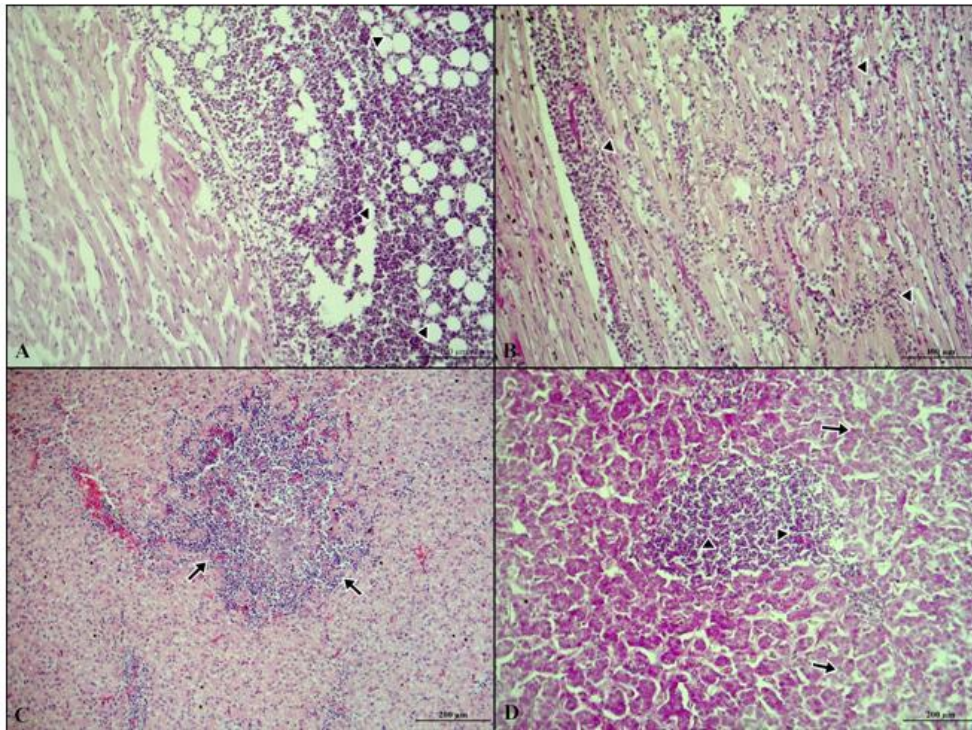
**Bioassay findings:** Bioassay was performed in mice from mixture suspension prepared from the tissues (brain, heart, chest and leg muscles) of pigeons. Results of the bioassay are given in Table 2. In addition, when mice died on 5th- 11th days, mice tissues (brain, heart, lung, liver etc.) were histopathologically tested for toxoplasmosis, and peritoneal fluid was tested for *T. gondii* tachyzoites. While no tachyzoites in histopathological examinations were detected in the organs of dead mice in sections, presence of tachyzoites were determined in the examination of peritoneal liquid.



**Fig. 1.** Large areas of necrosis (arrows) is characterized by nonsuppurative inflammation in the brain of pigeons (A); The appearance of nuclear material consisting of lymphoid cell infiltrations (white star), hemorrhage (white arrowheads), fibrin mass and cell debris (black arrowheads) (B); The appearance of strawberry shaped bradyzoite tissue cysts containing crescent shaped tachyzoites located perineuronally and perivascularly at the periphery of necrotic areas in substantia grisea in the brain of pigeons (C, D). The appearance of free tachyzoites (arrows) in substantia grisea, in the brain of pigeons (E, F). (Group IV), H&E stain



**Fig. 2.** One of the pigeons in Group V had a bradyzoite cyst structure within the sinusoid and the focal lymphoid cell infiltration (arrows) in the liver (A); The appearance of focal necrotic areas (star) in the pancreas of pigeons (B); In the skeletal (C) and heart (D) muscles of the pigeons; they had multifocal necrosis in muscle fibers and lymphocyte-rich mononuclear cell infiltration (arrowheads) in the necrotic areas. (Group V), H&E stain



**Fig. 3. In the heart (A) and skeletal (B) muscles of the pigeons; they had multifocal necrosis in muscle fibers and lymphocyte-rich mononuclear (arrowheads) cell infiltration in the necrotic areas; they also had focal necrotic areas and lymphoid cell infiltration (arrows) in liver (C); Focal necrosis (arrows) and (D)lymphoid cell infiltration (arrowheads) were observed in one pancreas of pigeons. (Group VI), H&E stain**

#### 4. DISCUSSION

In the literature, *T. gondii* oocysts were given orally in most of the studies [2,4,5,7-11,27,40, 44-46]. Furthermore, in another study [12], oocysts and bradyzoites were inoculated orally, and tachyzoites were inoculated intravenously. In addition, there are some studies in which the toxoplasma tachyzoites in quail, white turkeys and broilers are administered as oral, intramuscular, intravenous, intraperitoneal, cloacal and intracerebral [14,15,46]. There is also a study [3] in which pigeons were administered subcutaneously, intramuscularly and intravenously with *T. gondii* tachyzoites.

In the present study, in addition to oral and intravenous administration, *T. gondii* was given intramuscular and intracerebral routes similar with Atasever et al. [14,15]. In this study, it was investigated that if pigeons had infection despite some of the tachyzoites are destroyed because of the protective properties of gastric secretions.

Acute toxoplasmosis-related deaths in experimental infections and unexplained deaths

after inoculation have been reported in poultry [7-11]. In the present study similar results were observed. Deaths without infection (falling into the water and similar reasons) were observed in two pigeons in Group II, one pigeon in Group IV and three pigeons in Group VI. While six pigeons in Group IV had neurological symptoms on the 13th day of inoculation, necropsy examination revealed that hyperemia in the meninges and brain and focal hemorrhages were attributed to acute toxoplasmosis in this pigeons.

Rare clinical findings have been reported in experimental toxoplasmosis in poultry [6,7,12-15, 27,40,44,46-48]. In this study, neural symptoms characterized by torticollis, ataxia and tremor were detected in six pigeons that given *T. gondii* tachyzoites in Group IV. Weakness and weight loss were found in three pigeons in the same group and in all other groups except for those given orally. Although most of the *T. gondii* infections in poultry are subclinical, this study has shown that there are 6 pigeons with acute neurological findings unlike other researchers. Necrosis of the visceral organs, muscular

dystrophy, pericardial and myocardial thickening, gastric ulceration, lung hepatization, hepatosplenomegaly and enteritis have been reported as the prominent lesions for toxoplasmosis in poultry [1,6-15,40,46,47,49,50]. Similar macroscopic findings were observed in the present study in pigeons given tachyzoites through intramuscular, intracerebral and intravenous routes.

Unlike other researchers, large areas of necrosis in the pancreas were detected in two pigeons in Group V and one in Group VI. It was reported that histological features of experimental toxoplasmosis consist of necrotic hepatitis, splenitis, nephritis, pneumonia, non-suppurative encephalitis, pancreatitis, typhlitis, colitis, adrenalitis, oesophagitis, and gastric inflammation [7-13,40,45-47]. However, in the present study, necrotizing inflammation was observed only in the liver, brain, lungs, and spleen.

Natural [3,11,20,28,32,33,48,50] and experimental [2,4,5,7-10,12-15,27,40,45] toxoplasmosis have been serologically tested and found seropositive in many poultry species. Seroprevalence of toxoplasmosis has been determined in owls, pigeons, turkeys and ducks using MAT [11,21,22,28,32,33,48], in laying hens using MAT, IHA, IFAT, SFDT, ELISA and latex agglutination test (LAT) [36-39,46] and in pigeons using MAT, PCR, LAT, IHA, SFDT [21-26]. In addition, toxoplasmosis has been determined in pet birds using LAT and MAT [11,40,50]. Limited numbers of studies were conducted on toxoplasmosis in domestic and wild poultry in Turkey [16-19]. Seroprevalence of experimental toxoplasmosis in poultry has been detected using different serologic tests [2,7-10,14,15]. Researchers tested toxoplasmosis using MAT, LAT, IHA, and SFDT and concluded that MAT is more sensitive in detecting experimental toxoplasmosis in bobwhite quail, Japanese quails, turkey and pheasant [8,9,11,15]. In addition, MAT, ELISA, SFDT, LAT and IHA tests were used in experimental toxoplasmosis in turkeys and pheasants and MAT and ELISA were reported to be more sensitive compared with others [7,10,14]. Similarly, ELISA was used for serological detection of *T. gondii* infection in experimentally infected chickens and pigeons [2,34,37]. In the present study, SFDT and IHA were used in serological detection of toxoplasmosis. *T. gondii* specific antibody was detected in pigeons orally given  $10^5$  tachyzoites using the both tests (SFDT and IHA). All pigeons

in other groups were tested and found seropositive using the both methods (SFDT and IHA) and results were not statistically significant ( $P>0.05$ ).

In the diagnosis of toxoplasmosis in poultry as in other animal species, tachyzoites were obtained by bioassay method from mice. Natural [11,31,41,42,43,49,50] and experimental toxoplasmosis [2,5,7-10,12,15,27,44] cases have been confirmed using bioassay in many poultry species. In the present study, unlike other researchers [7-10,12,15,41-43,49,50] a single suspension has been prepared and bioassay were done in mice using samples (brain, heart, chest and skeletal muscles) from all pigeons that were seropositive. Tachyzoites of the parasite were detected in mouse peritoneal fluid and the bioassay results obtained in this study were in parallel with those reported in the literature. However, it has not been possible to comment on which of the tissues in the suspension given to the mice had the causative agent.

In the present study, all groups were seropositive except Group I and Group III. No pathological findings except lymphoid cell infiltration in kidney, pancreas, heart muscle and liver were observed in studies conducted in poultry with intravenous tachyzoites administration [12,14,16] was similar with the presented study which probably suggests that it may be related to the inactivation of the causative agent by circulating macrophage cells.

The absence of a positive result (histopathological and bioassay) other than serology in Group II is consistent with destruction of the agent by various enzymes released from the stomach and intestines.

As a result; nonsuppurative meningoencephalitis was detected microscopically and free tachyzoites and bradyzoites cyst formations related to toxoplasmosis were observed microscopically in brain tissues of pigeons in Group IV. Pigeons in this group also had microscopic findings of focal nonpurulent hepatitis and nephritis with focal foci of lymphoid cell infiltration and necrosis. One of the pigeons had bradyzoite cyst in the sinusoid of the liver, as well as nonsuppurative focal myositis, myocarditis, hepatitis, gastritis, enteritis, pneumonia in other pigeons. Nonsuppurative focal hepatitis, myocarditis, nephritis and necrotic pancreatitis lesions were observed microscopically in pigeons in group VI.



## 5. CONCLUSION

This study concludes that the intramuscular administration was the most effective route followed by intracerebral administration. For future seropositivity testing of subclinical cases the histopathological examination and bioassay applications should be performed together in order to determine *T. gondii* tachyzoites and bradizoites which will make differential diagnosis of dead and live animals.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

The experiments were carried out in accordance with the Guidelines for Animal Experimentation approved by Erciyes University, Experimental Animal Ethics Committee (permit no: 15/02/2008).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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