

## Investigation of Anti-*Toxoplasma gondii* Antibodies in Water Buffaloes (*Bubalus bubalis*) in Samsun and Afyon Provinces

Samsun ve Afyon yörelerindeki Mandalarda (*Bubalus bubalis*) Anti-*Toxoplasma gondii* Antikorlarının Araştırılması

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### ABSTRACT

**Objective:** The present study was conducted to investigate the seroprevalence of *Toxoplasma gondii* infection in water buffaloes.

**Methods:** The research was conducted on 131 water buffaloes from Samsun and Afyon provinces. Serum samples of buffaloes were tested for toxoplasmosis with the Sabin Feldman Dye Test (SFDT), using live tachyzoites and methylene-blue dye.

**Results:** 87.79% of buffaloes had anti-*Toxoplasma gondii* antibodies titrated 1:16 and more. No statistically significant difference was observed between the age and gender of buffaloes with infection ( $p>0.05$ ).

**Conclusion:** These results show that toxoplasmosis is quite common in water buffaloes in the regions of study.

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**Keywords:** Toxoplasmosis, SFDT, water buffaloes

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### ÖZET

**Amaç:** Bu çalışma mandalarda *Toxoplasma gondii* seroprevalansını belirlemek amacıyla yapılmıştır.

**Yöntemler:** Araştırma Samsun ve Afyon yörelerindeki 131 manda üzerinde yürütüldü. Mandalara ait serum örnekleri, canlı takizoitler ve metilen blue boyası kullanılarak Sabin Feldman Dye Test (SFDT) ile toksoplazmozis yönünden incelendi.

**Bulgular:** Mandaların %87,79'unda 1:16 ve üzeri titrede anti-*Toxoplasma gondii* antikorları tespit edilmiştir. Yaş ve cinsiyet ile enfeksiyon arasında istatistiksel olarak anlamlı bir fark bulunmamıştır ( $p>0,05$ ).

**Sonuç:** Sonuçlar bu bölgelerde mandalarda toksoplazmozisin oldukça yaygın olduğunu göstermiştir. (*Türkiye Parazitol Derg* 2014; 38: 220-2)

**Anahtar Sözcükler:** Toksoplazmozis, SFDT, manda

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## INTRODUCTION

Toxoplasmosis is an important zoonotic infection that affects both humans and animals, including water buffaloes. It is caused by *T. gondii*, which is an obligate intracellular parasite, and its worldwide distribution. Its definitive hosts are domestic cats and other Felidae, and it is able to develop in a wide range of intermediate hosts, including humans and domestic and wild animals as well as birds (1-3). The major routes of transmission of *T. gondii* to humans are; ingestion of oocysts in contaminated food and water and bradyzoites in undercooked/raw meat (3, 4).

Toxoplasmosis uncommonly causes clinically significant disease; it shows a few general symptoms such as fever, increase in body temperature, degeneration of retina and ataxi, although not specific for the disease (3, 5). Pyrexia, anorexia, conjunctivitis, and dyspnoea are observed in experimental toxoplasmosis in buffaloes (6).

Many seropositive animals harbor cysts in their tissues, particularly in their muscle. They would constitute infectious reservoirs for other animal species and humans. So, the determination of prevalence of *T. gondii* infection in water buffaloes may be of epidemiological importance (2, 3, 7).

*T. gondii* infection can be detected using serological and histological examinations. Sabin-Feldman Dye Test (SFDT), Indirect Hemagglutination (IHA), Indirect Fluorescent Antibody Test (IFAT), Enzyme-Linked Immunosorbent Assay (ELISA), Complement Fixation Test (CFT), and Latex Agglutination Test (LAT) are commonly used serological tests. SFDT is considered the most specific, sensitive, and gold standard test for the detection of antibodies to *T. gondii* (3, 8).

In Turkey, the seroprevalence of toxoplasmosis in water buffaloes has been investigated in only two studies. The first study was reported by Weiland and Dalchow (9) and the second by Cicek et al. (10). The researchers found the prevalence to be 83% and 31.13%, respectively, using SFDT. The purpose of this study was to determine the anti-*T. gondii* antibodies in water buffaloes in Samsun and Afyon provinces of Turkey.

## MATERIALS AND METHODS

This study was performed in Samsun and Afyon provinces of Turkey. Samsun is located in the Black Sea region of Turkey and between 41° N latitude and 36° E longitude, the other city, Afyon, is located in Central Anatolia and between 38° N latitude and 30° E longitude.

The age and sex of buffaloes were recorded. A total of 131 water buffaloes (54 males and 77 females) were examined. Of animals, 86 were from Samsun, and 45 were from Afyon. The age between 1 and 3 years was considered young, and up to 3 years was considered old (Table 1).

Serum samples were collected following centrifugation at 3000 rpm for 10 min of 5 ml of blood samples obtained from the jugular vein of water buffaloes. All sera were stored at -20°C and later assayed for *Toxoplasma* antibodies.

Testing for toxoplasmosis using SFDT

**Table 1.** Inspected water buffaloes age and gender

Age	Male (%)	Female (%)	Total (%)
≤3	48 (36.64)	34 (25.95)	82 (62.6)
>3	6 (4.58)	43 (32.82)	49 (37.4)
Total	54 (41.22)	77 (58.73)	131

**Table 2.** Sabin-Feldman Dye Test (SFDT) results with provinces and titers

Provinces	Titers				Negative	Total
	1:16	1:64	1:256	1:1024		
Samsun	36	29	7	2	11	86
Afyon	24	12	2	1	5	45
Total	60	41	9	3	16	131

Serum samples were tested for toxoplasmosis using SFDT with live tachyzoites and methylene-blue dye. Positive and negative controls and test sera were diluted with saline in a series of 4-fold serial dilutions (1/16; 1/64; 1/256; 1/1024). Each dilution, 25 µL, was transferred to a tube, and an equal volume of activator sera, which was seronegative for *T. gondii* and rich in C2, C3, C4, Mg2 and properdin, was added. For the antigen, 48-h passage of *T. gondii* Rh strain derived from periton fluid of 3-4-week aged white Swiss albino mice were used and per tube. The tubes were then incubated at 37°C for 50 min. Then, a 10-min incubation at 37°C was performed in the presence of 25 µL of alkaline methylene-blue (pH 11). After incubation, 20 µL of each sample was examined under the light microscope with a 400 objective. The result of SFDT was regarded as positive if more than 50% of tachyzoites remained unstained at titer of 1:16 or higher.

### Statistical analysis

The chi-square ( $\chi^2$ ) test was used to compare seroprevalence rates (SPR) relative to gender and age. Differences were considered statistically significant when P value was ≤0.05.

## RESULTS

Among 131 water buffaloes tested, 115 (87.79%) were found to be seropositive. From positive samples with SFDT, 60 samples (52.17%) were seropositive at 1:16 dilution, 41 samples (35.65%) at 1:64, 9 (7.83%) samples at 1:256, 3 (2.61%) samples at 1:1024 dilutions (Table 2). A total of 82 serum samples from young and 49 samples from old animals were examined. The seropositivity of *T. gondii* was found to be 86.59% and 85.71%, respectively. Relative to gender, the infection rate was found to be 85.19% in males and 87.01% in females.

No statistically significant difference was observed between the age and gender of buffaloes relative to infection using the chi-square test ( $p>0.05$ ).

## DISCUSSION

Toxoplasmosis is one of the most important zoonotic infections that affect both humans and animals. The frequency of infection is variable in the different regions of the world. Depending on factors such as age, education, sanitation, life and aliment style,

the seroprevalence of the disease ranges from 0% to 90% in humans (3). As well as all other the world toxoplasmosis is common in Turkey, and the seroprevalence has been reported in humans to be 23.1-57.6% (11).

Antibodies to *T. gondii* in water buffaloes have been reported in only two studies in Turkey. The prevalence was found to be 83% (9) and 31.13% (10) using SFDT. SFDT was also used in this study, and it is known to be the most reliable and sensitive method for the diagnosis of toxoplasmosis. Its main disadvantages are its high cost and the hazard of using live organisms in humans (3).

The overall prevalence recorded in water buffaloes in the present study was close to that reported in Weiland and Dalchow's (8) research (83%) but higher than that reported by Çiçek et al. (10) (31.13%). The reason for this may be geographical.

In other countries, other serological methods were mainly used to detect antibodies to *T. gondii* in buffaloes. The prevalence was found to be 27.2% and 3.85% in Brazil by LAT (12) and IFA (13); 8.8% and 14.33% in Iran by IFA (14) and by modified agglutination test (MAT) (15); 7.8% in Trinidad Island by LAT (16); 5.6% in Zimbabwe by MAT (17). In addition, antibodies to *T. gondii* were not found in any of the 75 buffaloes in Egypt by direct agglutination test (DAT) (18).

The results of the present study further confirm the high prevalence of *Toxoplasma* infections in water buffalo populations in Turkey. The reasons for this may be the presence of reservoir cats and farm animals; past infections; suitable temperature and humidity; potentiality of environment for sporulation of oocytes; traditional breeding systems; and suitable pastures.

## CONCLUSION

Toxoplasmosis in water buffaloes results economic losses because of abort and reduction of reproduction. Also, contaminated meats are risk for human toxoplasmosis. So, some precautions should be taken to decrease the prevalence of *T. gondii* in this region.

**Ethics Committe Approval:** Ethics Committee Approval was not received due to the retrospective nature of the study.

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**Hasta Onamı:** Çalışmamızın retrospektif tasarımından dolayı hasta onamı alınmamıştır.

## Hakem Değerlendirmesi: Dış Bağımsız

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