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Determination of the Prevalance of Toxoplasmosis in Cats with Immunochoromatographic Rapid Tests Kits in Kırıkkale University Veterinary Faculty Animal Hospital

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ABSTRACT

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* that can cause disease in all warm-blooded animals. In the transmission cycle of the disease, cats serve as the primary/definitive hosts, and transmission occurs through direct and indirect oral ingestion of oocysts spread by the definitive hosts. To diagnose the disease, a variety of methods are employed, including fluorescent diagnostic techniques, indirect hemagglutination tests, modified agglutination tests, ELISA, polymerase chain reaction (PCR), Sabin-Feldman dye tests, and immunochromatographic rapid diagnostic test kits. In clinical settings, rapid diagnostic test kits are the preferred option due to their ease of access, cost-effectiveness, and rapid results. The objective of this study is to ascertain the prevalence of toxoplasmosis in cats in the Kirikkale region and to highlight the efficacy of rapid diagnostic kits in this regard. The study material consisted of 50 cats brought to the Kırıkkale University Veterinary Faculty Animal Hospital for diagnosis and treatment of various disease presentations. Toxoplasma was detected using rapid diagnostic kits. The diagnostic tests performed on the blood samples taken from the 50 cats for the purposes of diagnosis and treatment revealed that three of them were positive. The screening revealed a prevalence of toxoplasmosis in the sample population of 6%. It has been determined that cats can harbor this disease despite exhibiting symptoms compatible with toxoplasma. The use of rapid diagnostic kits for screening cats is a viable and practical solution. The study objective was to contribute to the development of control policies for cats in the context of public health and disease control policies. The results of this study will serve as a source of information for future studies.

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INTRODUCTION

Toxoplasmosis is a disease caused by the *T. gondii* and has a global distribution. It has a zoonotic potential and has been observed in all haematophagous animals, including birds and humans (Dubey 2010). This infection represents a significant public health concern due to its epidemiology and pathogenesis. It is commonly reported in humans and animals due to its easily transmissible nature (Dumanlı & Aktaş, 2010; Marquert et al., 2000). Despite the prevalence of the disease, the number of clinically diagnosed cases remains low (Dumanlı et al., 2013). Of particular concern is the congenital transmission potential of *T. gondii*, particularly in the asymptomatic form. This underscores the importance of addressing the disease for the benefit of future generations (Jones et al., 2003).

Toxoplasma has a complex life cycle that includes 3 stages as trophozoits, bradyzoits and sporozoits (Mevelec et al, 2020). Trophozoites are responsible for acute infections in tissues and can spread to almost all organs quickly. Most of the pathologies are composed by trophozoites (Bernal and Gennari, 2019). Bradyzoites are found in cysts formed by trophozites. They cause a life-long chronic inflamation and procure the hosts immunity stability (Di Cristina et al, 2008). Sporozoits include sporants and oocycts in the intestinum, and has the ability to actively infect (Dubey and Frenkel, 1972; Tenter et al, 2000; Dumanlı et al, 2013).

Toxoplasmosis has two hosts in its transmission cycle. Felidae serve as the definitive hosts, while other warm-blooded animals act as intermediate hosts. Additionally, toxoplasmosis can result in asexual proliferation in felidae, with this family serving as an intermediate host (Bernal and Gennari, 2019). The disease is transmitted via direct oral ingestion of oocysts, which are spread in definitive hosts by water or food contaminated with oocysts. In addition, the disease can be transmitted by ingestion of tissues containing trophozoites or bradyzoites (Karakavuk et al., 2021). Following ingestion of oocysts and cysts by intermediate hosts, sporozoites and trophozoites are released into the intestinal lumen. Once the oocysts have passed the intestinal epithelial barrier, they undergo endodyogeny in the parasitophorous vacuoles of various cells. Consequently, the trophozoites develop and spread throughout the organism. It is also possible for trophozoites to infect the fetus, which can result in disruption of the placental barrier. (Courret et al., 2006; Persson et al., 2009). The acute clinical picture is caused by trophozoites, while the latent, chronic, lifelong presentation is caused by encysted bradyzoites. (Chen et al., 2022).

Toxoplasmosis can be identified by detecting *T. gondii* antibodies, which has remained a relevant diagnostic method over the past decade. Numerous factors that influence the prevalence of the disease, with a particularly high prevalence observed in feral cats (Dubey et al., 2020). It should be noted that the prevalence of the agent varies from region to region within the same country. In studies employing indirect and immunofluorescent antibody tests, the antibody seroprevalence of the agent has been reported to range from 15% to 82% in Brazil (Munhoz et al., 2017; Neto et al., 2018; Cardia et al., 2013). In China, the rate was found to be between 11% and 63% using indirect agglutination methods (Qiu et al., 2020; Hou et al., 2018; Cong et al., 2018). In Egypt, the rate is approximately 95% using a modified agglutination test (Al-Kappany et al., 2011). A study conducted with 1,490 animals in Thailand revealed a prevalence rate of 4.8% (Jittapalapong et al., 2010). In Turkey, the prevalence rate has been reported to range from 34% to 66% using the Sabine Feldman Dye method (Yücesan et al., 2019; Ercan & Kırmızıgül, 2019).

There is still no clarity regarding the diagnosis of toxoplasmosis (Dubey, 1995). Toxoplasmosis can be identified through several tests, including flourescent tests, indirect hemagglutination tests, modified agglutination tests, ELISA, PCR, Sabin-Feldman dye, and immunochromatographic rapid tests

(Lappin et al. 1989; Liesenfeld et al. 1996). The materials used in rapid tests for detecting *T. gondii* antigens or antibodies vary, but the underlying working principle is consistent across all tests. The objective of this study is to identify the prevalence of *T. gondii* with lateral flow immunochromatographic rapid tests (RIDXTM Toxoplasma Ab Test, Korea), which include surface antigen (SAG 1: p.30) + dense granule protein (GRA 1: p.24). These rapid tests are designed to detect antibodies to *T. gondii* in blood samples. The objective was to evaluate the prevalence of toxoplasmosis in Kırıkkale and assess the threat to human and animal health.

MATERIAL and METHOD

This study was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (Approval no: 22.07.2024-E.265544).

Study Material

The animal material of the study was comprised of 50 cats from Kırıkkale University Veterinary Faculty Animal Hospital. The study population was consisted of domestic and shelter cats. All cats' ages are >1 year and the presence of any clinical symptoms compatible with toxoplasmosis was not sought for incorporation in the animals to be included in the study

Sample Collection and Implement Rapid Tests

Blood samples were collected from animals' vena cephalica antebrachiums to collecting tubes as 2 milliliters. These samples were centrifugated at 3000 rpm for 10 minutes to obtain serum. Rapid diagnostic tests were performed in the same day for detecting toxoplasmosis antibodies. 10 microliters of the obtained serum samples were added to the sample well on the rapid diagnostic test kits (Asan Easy Test® Seoul, Korea) and then 15 microliters of toxoplasma reagent was applied to the same well. 10 minutes later results were recorded. Seeing the control line on the result area was searched all rapid test kits for determine the activation of test kits. If the test section line was seen, the result was evaluated as positive.

Statistical Analyses

In this study the prevalance of toxoplasmosis was calculated with descriptive statistical methods. Positive results were calculated as a percantage

RESULTS

The efficacy of immunochromatographic rapid tests was evaluated in a cohort of 50 cats. The test results are presented in Table. Three cats had positive results with toxoplasmosis (%6) and they were older than 3 years old and youger than 4 years old. In the study, nine cats' blood samples were collected for the purpose of detecting whole blood results prior to the administration of routine vaccinations. No positive results for toxoplasmosis were identified in these cats. The positive cats had different clinical symptoms consistent with various diseases. Stranguria was seen on the first positive cat. The second cat showed diarrhea, and the third cat had ocular lesions.

Table. The ratio and numbers of antibody positive cats

	Animals	**Rapid Test Result Positive**	**Rapid Test Result Negative**
n	50	3	47
% (ratio)	100	6	94

DISCUSSION

Cats are considered the definitive host for toxoplasmosis. This disease is the most common protozoal pathogen in humans and is found worldwide in both humans and warm-blooded animals. (Jones et al 2018). There are many studies on the seroprevalence of toxoplasmosis. According to these studies, *T. gondii* antibody ratio was %62.3 in Albania with IFAT (Silaghi et al 2014). In Algeria seroprevalence of toxoplasmosis was found to be %50 (Yekkour et al 2017). In Iran, using different serologic diagnostic methods, the seroprevalence was between %2.7 and %82.2 (Derakhshan ve Mousevi 2014, Hamidinejat et al 2011, Asgari et al 2018). It was reported that the percentage of toxoplasmosis antibodies in Iraq was between %30.4 and %45.5 (Al-Rahmani et al 2010, Switzer et al 2013). In Türkiye, the presence of antibodies ranging from %34.2 to %66.6 was detected in studies conducted using IFAT, ELISA and dye test methods (Yücesan et al 2019, Can et al 2014, Ercan and Kırmızıgül 2019, Erkılıç et al 2016). It is reported that this situation reaches up to %81 in Europe (Dubey et al 2020). In another study, the seropositivity of toxoplasmosis was found to be 48% using the Sabin Feldman test in previous years in Türkiye (Yasa Duru et al, 2017). In this study, the ratio of toxoplasma positive animals was found to be %6. We thought that this proportional difference between the two studies was related to the diagnostic method. In a recent study conducted with a rapid diagnostic kit in another region of our country, 5.5% of cats were found seropositive (Aktemur, 2021). It seems that the results of this study are compatible with studies performed with the same method. Studies show that toxoplasmosis is still active in our region. It is reported to be found at very high rates in neighboring regions, as well as in humans and animals in European countries with similar climates (Dubey et al. 2020, Molan et al. 2019).

It was reported that toxoplasmosis was seen in cats living in urban areas more than those living alone (Abbas et al 2021). The agent continues to exist in central areas in cities over cats living in periurban regions. According to studies, it has been seen that cats can carry the agent even if they live alone at home (Sroka et al 2018). For cats that are definite hosts, regardless of what was the enviromental living conditions, the factor can persist to continue living. Enviromental conditions of Türkiye are suitable for the toxoplasmosis life cycle. The stray cat's population is not known exactly except that domestic cats number is detected as more and less because there is no system to find real population of domestic cats. As a definitive host for toxoplasmosis, Türkiye’s cat's living standards allow the disease to become a public health problem.

Toxoplasmosis occurs in cats of all ages, regardless of gender and breed (Dubey 2020). Pneumonia is the most common clinical symptom (Dubey 2010). Icterus, anorexia, vomiting, paresis and dermatitis take place in toxoplasmosis clinical table (Dubey 2020). Besides that oculer lesions are identified in infected cats. It was reported that retinochoroiditis, chorioretinitis, optic neuritis and anterior uveitis were detected (Ali et al, 2021). In the diagnosis of the disease, the presence of Ig G and Ig M is usually releaved serologically (Remington et al. 1995). Disease pathogenesis blocks the identification of agents. Antibodies can be found animals that haven’t any clinical symptoms (Dubey et al 2020, Ali et al 2021). In line with the aforementioned information, in this study, the presence of

antibodies to the agent was investigated regardless of clinical complaints. In the findings obtained, clinical complaints were detected in animals with positive disease, while antibodies could not be detected in healthy animals.

The diagnosis includes the serological presence of IgG and IgM antibodies in toxoplasmosis. There are several ways to detect antibodies serologically, including MAT, ELISA, PCR, IMX, Sabin-Feldman, and dye tests. It's also included in immunochromatographic-based diagnostic test kits (Luo et al., 2018). Onosakponome et al. (2020) found that the rapid tests had a specificity of 46.7% and a sensitivity of 81.7%. Hassaneina and Shehata (2018) also found that the rapid tests had a specificity of 54.4% and a sensitivity of 100% in humans. A study on using immunochromatographic rapid diagnostic test kits to diagnose toxoplasmosis in cats found that they had a specificity and sensitivity of 98.63% and 100%, respectively (Villanueva-Saz et al., 2023). Both human and veterinary studies show that rapid tests are useful diagnostic tools when laboratory techniques aren't available. In clinical settings, rapid tests can be a great way to diagnose toxoplasmosis.

CONCLUSION

In conclusion, we determined the prevalence of toxoplasmosis in cats in Kırıkkale. Determining the prevalence of toxoplasmosis, which has zoonotic potential for the region, is important for the existence of the disease, as assumes a role for cats in human cases as a source, and for establishing the control methods of toxoplasmosis. We recommended that larger studies be conducted on this subject using advanced molecular and serological diagnostic methods.

Ethical Statement

This research article has not been published anywhere before.

Ethics Committee Approval

This study was approved by the Kırıkkale University Animal Experiments Local Ethics Comuttee (Approval no: 22.07.2024-E.265544)

Author Contributions

Research Design (CRediT 1) Author 1 (%40) – Author 2 (%30) – Author 3 (%30)
Data Collection (CRediT 2) Author 1 (%40) – Author 2 (%30) – Author 3 (%30)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%40) – Author 2 (%30) – Author 3 (%30)
Writing the Article (CRediT 12-13) Author 1 (%40) – Author 2 (%30) – Author 3 (%30)
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Conflict of Interest

The authors have no relevant interests

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