Pak. J. Agri. Sci., Vol. 60(3),455-461;2023
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/23.61
http://www.pakjas.com.pk

Seroprevalence of *Toxoplasma gondii* in dogs and cats in Khyber Pakhtunkhwa Pakistan

Arsalan Said[1], Rao Zahid Abbas[1,*], Irfan Khattak[2], Muhammad Kasib Khan[1], and Muhammad Kashif Saleemi[3]

[1]Department of Parasitology, University of Agriculture Faisalabad, Pakistan; [2]College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University, Mardan, Pakistan; [3]Department of Pathology, University of Agriculture Faisalabad, Pakistan

*Corresponding author's e-mail: raouaf@hotmail.com

Toxoplasma gondii is the most prevalent parasite that mostly affects warm-blooded animals, including humans. According to the Center for Disease Control (CDC), this parasite infects about one-third of the world's population of people. Lack of information regarding toxoplasmosis in the population of domestic and stray dogs and cats in Khyber Pakhtunkhwa was a major factor in the decision to conduct this study. Our aim was to use serological technique to establish the seroprevalence of *T. gondii* in dogs and cats. In this study, 405 dogs and 405 cats, blood samples were collected from various veterinary hospitals and privates pet clinics and an ELISA test was used to determine anti-*Toxoplasma* IgG antibodies. Information on four risk factors age, sex, deworming of dogs and cats and, area was collected from the owners by questionnaire. Total seroprevalence in dogs, and cats was 45.18% (183/405), and 51.60% (209/405), respectively. No significant difference was documented on basis of age and gender between dogs, and cats. Dogs and cats from urban areas showed lower seroprevalence than rural areas. Dogs that had received deworming showed lower seroprevalence. This research indicates that neglected parasite *Toxoplasma gondii* is prevalent in dogs and cats in Pakistan, which may have great public health importance.
Keywords: Toxoplasmosis, vertebrates, bradyzoites, tachyzoites, neglected.

INTRODUCTION

Toxoplasmosis, which affects both humans and animals, is caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*), which resides inside cells (Lazar *et al.,* 2021). It is the most prevalent parasite that mostly affects warm-blooded animals, including humans (more than 30 bird species and 300 mammal species) (Hill *et al.,* 2005). According to the Centers for Disease Control (CDC), this parasite infects about one-third of the world's population (Molan *et al.,* 2019). *T. gondii* is one of the top "Five Neglected Parasitic Infections" because of its chronic nature and frequent dissemination, which is most common in places with inadequate sanitation (Foroutan *et al.,* 2018).

The life cycle of toxoplasma is very complex. *Toxoplasma gondii* is a cyst-forming protozoan found in tissue. The life cycle of *T. gondii* is divided into two phases: asexual and sexual. The asexual phase occurs in the tissues of intermediate hosts (omnivorous or herbivorous), while the sexual phase occurs in the intestines of definitive hosts (felids). The three infectious phases of *T. gondii* life cycle are bradyzoites, tachyzoites enclosed in tissue cysts, and sporozoites contained in sporulated oocysts. The parasite can enter the intestines and then travel through the bloodstream to important organs like the brain and muscles. In most situations, infection does not manifest clinically, but it is possible to develop a life-threatening illness (Cenci-Goga *et al.,* 2011).

All warm-blooded vertebrates, including humans, may serve as intermediate hosts for *T. gondii* and possibly be infected by bradyzoites in meat, intrauterine tachyzoites, or sporulated oocysts, and felids are the definitive hosts (Dabritz and Conrad, 2010; Elmore *et al.,* 2010). *T. gondii* can be transmitted in a variety of ways; the sexual cycle occurs in a definitive host, and the asexual cycle is completed in omnivorous or herbivorous hosts by meat consumption and vertical transmission (Baneth *et al.,* 2016). All of these routes may work together to improve transmission, but they may also serve as a vehicle for selection, allowing strains in the environment to be separated (Lindsay and Dubey, 2011).

Said, A., R.Z. Abbas, I. Khattak, M.K. Khan and M.K. Saleemi. 2023. Seroprevalence of *Toxoplasma gondii* in dogs and cats in Khyber Pakhtunkhwa Pakistan. Pakistan Journal of Agricultural Sciences 60:455-461.
[Received 2 Apr 2023; Accepted 12 Jun 2023; Published 29 Sep 2023]

Humans contract toxoplasmosis by eating raw or undercooked meat, such as lamb or pork (Baneth *et al.,* 2016). The presence of toxoplasmosis infection in human communities that do not consume meat or raw or uncooked meat, however, shows that infection acquired via the environment, such as through oocysts in water, soil, or uncooked vegetables, may also play a role in transmission. According to certain studies, only a small percentage of affected people (0.1%) are infected congenitally (Lindsay and Dubey, 2011). Toxoplasmosis infection is diagnosed by isolating the parasite from blood or fecal sample, performing serological tests, or using polymerase chain reaction to detect particular DNA (PCR). Toxoplasmosis is routinely diagnosed using serological techniques, such as the enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), Sabine Feldman dye test, or a variety of agglutination tests, which are available for both animals and humans. In most clinical laboratories, ELISA is employed for regular screening of particular immunoglobulin (Ig) G and IgM, whereas other technologies are normally retained for reference laboratories (Rafique *et al.,* 2022). However, each of these tests has certain benefits as well as some drawbacks. The most dependable, useful, affordable, and commonly used test for determining whether animals have been exposed to *T. gondii* is the ELISA It is excellent for large-scale screening because only a small sample volume is needed, and the test can be semiautomated. Furthermore, ELISAs are beneficial in identifying the stage of infection since they can distinguish between immunoglobulin types. Sensitivity, specificity, and overall agreement (often represented by the Kappa statistic) with a reference test are all indicators of test performance. Commercial ELISA kits are now readily accessible for the detection of *T. gondii* antibodies in domesticated animals, making routine and extensive screening more feasible (Liyanage *et al.,* 2021).

Preventing oocyst shedding is critical for reducing *T. gondii* infection in humans (Tenter et al., 2000). Cats that hunt wildlife and live in the wild or on farms are at a higher risk of contracting *T. gondii* Parasites. Kittens produce more oocysts and are more susceptible to infection than adults. Efforts to produce a *T. gondii* vaccine for cats should be continued, resulting in improved human protection (Robert-Gangneux and Darde, 2012).

A dog can act as a mechanical vector for *T. gondii*, and due to its close contact with humans and other animals, it is a key risk factor for the transmission of this protozoan. For *Toxoplasma* oocytes assessment, both dogs and cats are sentinel creatures because most pet owners keep their dogs and cats in the same place. The owners, dogs, and cats are in close proximity to one another, which provides the perfect environment for toxoplasmosis to spread. Also, in outdoor environments, all three share the same atmosphere, which rises the spread of *Toxoplasma* (Hamidullah *et al.,* 2022). The prevalence of infection by *T. gondii*, which is widespread and occurs in different climates, is higher in felines than other animials and also in humans. The *T. gondii* infection has affected more than 6 million people worldwide. *Toxoplasma* seroprevalence, as determined by IgG antibodies, has variation worldwide. The prevalence of *T. gondii* was recorded as 47% in France, 46% in Tanzania, 30% in Europe, 23.9% in Nigeria, 12.3% in China, 10% in the United States, and 6.7% in Korea (Raza *et al.,* 2022). According to numerous studies conducted across the world on the prevalence of pet toxoplasmosis, the seroprevalence of the disease ranges from 8% to 47% in dogs and 12.5% to 51% in cats, respectively [Ahmad *et al.,* 2014; Zarra-Nezhad *et al.,* 2017; Huertas-López *et al.,* 2021; Hamidullah *et al.,* 2022; Konate *et al.,* 2023; Raza et al., 2023; Turlewicz-Podbielska *et al.,* 2023]. Recently, Hamidullah *et al.* (2022) from Pakistan, reported 22.5% and 21.8% seroprevalence of *T. gondii* in dogs with LAT and ELISA tests, respectively. Lack of information regarding toxoplasmosis in the population of domestic and stray dogs and cats in Khyber Pakhtunkhwa was a major factor in the decision to conduct this study. Our aim was to use serological technique (ELISA) to establish the seroprevalence of *T. gondii* in dogs and cats.

MATERIALS AND METHODS

***Study Site*:** Province: Khyber Pakhtunkhwa (KPK) is one of Pakistan's four administrative provinces. It is situated in the western part of the country and shares a border with Afghanistan. Geographically speaking, it is the smallest of the four provinces, but in terms of population and economic output, it ranks third in Pakistan. An equal number of dogs and cats were collected among the three study districts (Mardan, Swabi, and Nowshera) (Figure 1).

***Sample type and sample size*:** To determine sample size, the following formula was used (Daniel and Cross, 2018).

$$n = \frac{Z^2\, P(1-P)}{d^2}$$

Where n = sample size, Z = confidence level, P = expected prevalence and d = precision.

If no prevalence study is not conducted then Z=1.96 value for 95% confidence limits, the prevalence is assumed to be 50% and then P = Estimated prevalence (e.g., 0.5 for 50%) and Desired precision = d = (e.g., 0.05 for ± 5%) (Thursfield, 2018).

$$n = \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2} = 384$$

According to the formula, the sample size calculated was 384. In this study, a total of 810 samples from dogs and cats were collected between September 2019 and September 2020. Before the conduct of the study, permission obtained from the district director of livestock, who informed all veterinary doctors and veterinary assistants in the study area. To that end, the study included client-owned dogs who came to the Civil Veterinary Hospital in each district for vaccinations and

routine check-ups. Each dog and cat owner who provided a sample of their dog and cat was spoken to about the present research topic and asked to sign an informed consent form that included all of the written information about the study. A veterinary doctor or assistant, assisted in the collection of blood samples from dogs and cats. Stray dogs and cats were excluded from this study.

***Laboratory testing*:** A 3ml blood sample was collected from dogs and cats and transferred to a gel tube. Samples were carried to the laboratory, kept overnight at 4°C, and centrifuged at 2000 rpm for 4 min. The collected sera were stored at −20°C until testing. Serum was tested using an indirect commercial IgG ELISA kit (IDvet Innovative Diagnostics, ID Screen®, France) for detection of IgG antibodies against *T. gondii*. The ELISA was conducted according to the methods described by the manufacturer (Hamidullah *et al.*, 2022). Before the start of the test, the serum was placed outside the refrigerator to reach room temperature, which is 21°C (±5°C). All reagents were homogenized using vortexing. IgG diluent was used to dilute the test sera by a factor of 1+20. Added 190µl of dilution buffer 2 and 10 ml of the negative control to wells A1 and B1, 190µl of dilution buffer 2 and 10µl of the positive control to wells C1 and D1, and 190µl of dilution buffer 2 and 10µl of each test sample to the remaining wells. Plate was covered and left in an incubator for 45±5 minutes at 21°C (±5°C). Each well was washed three times with 300µl of the wash solution. Concentrated conjugate 10X is diluted to 1/10 in dilution buffer 3 to create conjugate 1X. The conjugate 1X (100 µl) were added to each well. Covered the dish and left it in an incubator for 31°C (±5°C). Each well was washed three times with 300µl of the wash solution. Each well received 100µl of the substrate solution. Covered the plate and left it in the dark for 15±2 minutes at 21°C (±5°C). To halt the reaction, 100µl of the stop solution were added to each well. The stop solution was added in the same order. At 450 nm, the O.D. was read and recorded.

***Statistical Analysis*:** The proportion of *T. gondii* seropositive dogs and cats and possible risk factors were evaluated by chi-square using Fisher's exact test. Statistical significance was defined as a p-value of 0.05. STATA version 17 was used for all data analysis (Stata Corporation, College Station, Texas, USA).

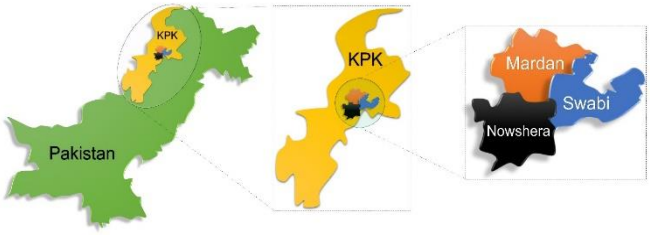

Figure 1. Map of Pakistan (green), indicating the location of the province of Khyber Pakhtunkhwa (KPK) (yellow) containing the three study districts: Nowshera (black), Swabi (blue), and Mardan (orange)

District wise Prevalence of *Toxoplasma gondii*: In dogs, the seroprevalence of *T. gondii* in districts Mardan, Swabi, and

Table 1. District wise Prevalence of *Toxoplasma gondii*

Districts	No. of samples	Dogs positive samples	Prevalence (%)	95% CI	P-value	Cats positive samples	Prevalence (%)	95% CI	P-value
Mardan	135	64	47.40	0.391-0.557		67	49.62	0.413-0.579	
Swabi	135	61	45.18	0.370-0.536	0.90	73	54.07	0.456-0.622	0.91
Nowshera	135	58	42.96	0.328-0.491		69	51.11	0.427-0.593	
Total	405	183	45.18	0.409-0.506		209	51.60	0.467-0.564	

Table 2. Risk Factors of *Toxoplasma gondii* in dogs.

Sr.	Questions	Total	Positive	Percentage (%)	95% CI	P-value (Chi-square)
1	Years					
	1-2	126	41	32.53	0.249-0.411	
	3-4	181	87	48.06	0.409-0.553	0.0700
	Greater than 4	98	55	56.12	0.462-0.655	
2	Gender					
	Male	215	108	50.23	0.436-0.436	0.1000
	Female	190	75	39.47	0.327-0.465	
3	Area				0.482-0.599	
	Rural	275	149	54.18	0.193-0.343	0.0007
	Urban	130	34	26.15		
4	Deworming					
	Yes	151	57	37.74	0.304-0.457	0.1400
	No	254	126	49.60	0.435-0.559	

Table 3. Risk Factors of *Toxoplasma gondii* in cats

Sr.	Questions	Total	Positive	Percentage (%)	95% CI	P-value (Chi-square)
1	Years					
	1-2	164	75	45.73	0.382-0.533	
	3-4	150	91	60.00	0.526-0.681	0.20000
	Greater than 4	91	43	47.25	0.373-0.574	
2	Gender					
	Male	189	83	43.91	0.370-0.510	0.10000
	Female	216	126	58.33	0.516-0.647	
3	Area					
	Rural	207	154	74.39	0.680-0.798	0.00001
	Urban	198	55	27.77	0.220-0.344	
4	Deworming					
	Yes	179	41	22.90	0.173-0.296	0.00001
	No	226	168	74.33	0.682-0.795	

Nowshera is 47.40% (64/135, 95% CI: 0.391%-0.557%), 45.18% (61/135, 95% CI: 0.370%-0.536%), and 42.96% (58/135, 95% CI: 0.328%-0.491%), and 49.62% (67/135, 95% CI: 0.413%-0.579%), 54.07% (73/135, 95% CI: 0.456%-0.622%), and 51.11% (69/135, 95% CI: 0.427%-0.593%) in dogs and cats, respectively. Overall prevalence of *T. gondii* in the three districts was 45.18% (183/405, 95% CI: 0.409%-0.506%) and 51.60% (209/405, 95% CI: 0.467%-0.564%) in dogs and cats. There was no significant difference in seroprevalence of *T. gondii* between the three districts (P> 0.05) (Table 1). Table 2 shows that the seroprevalence of *T. gondii* in dogs with risk factors. Comparing the total seropositive (183 subjects) with seronegative populations (222 subjects), the chi-square analysis confirmed that area (rural vs. urban) ($p < .05$) was a risk factor associated with *T. gondii* infection in our study. All other risk factors (age, gender, and deworming) were not statistically significant (P> .05) in this study. In cats, seropositive (183 subjects) and seronegative populations (222 subjects) were compared. The regression analysis confirmed that area (rural vs. urban) ($p < .05$) and deworming ($p < .05$) (yes vs. no) were risk factors associated with *T. gondii* infection in this study. All other risk factors, including age and gender, were not statistically significant (P> .05) in our study (Table 3).

DISCUSSION

There is always a requirement for *T. gondii* parasite prevalence in dogs and cats due to their role in oocyst adulteration of the environment and in human infection transmission (Miro *et al.,* 2004). Serodiagnosis is a good and reliable way to detect *T. gondii* infection in cats and dogs because clinical signs and symptoms cannot be used to diagnose the infection. Various tissues can be examined in order to diagnose *T. gondii* infection; however, doing so necessitates the death of the animals. As in the present work, we used more precise, contemporary, trustworthy, and sensitive procedures like indirect ELISA. In contrast to the current study, Ahmad *et al.* (2001) and Ahmad *et al.* (2014) showed that dogs and cats from the Faisalabad district and Khyber Pakhtunkhwa province had seroprevalence rates of 50% and 60%, and 28.43% and 26.43%, respectively. In the present study, the overall seroprevalence of *T. gondii* infection in cats was 51.60%, which was lower than that observed in KPK (74.6%), and Faisalabad (60%) [Ahmad *et al.*, 2001; Shahzad *et al.*, 2006], but higher than that observed in KPK (12.22%) and Sub-tropical Arid parts (26.43%) [Ahmad *et al.*, 2014; Raza *et al.*, 2022]. Recently, Majid *et al.* (2021) recorded 58.37% of cats seropositive against anti-*Toxoplasma* IgG antibodies, comparable to this study. Many research studies carried out in various parts of the world have found cat seroprevalence, including those conducted in Iran (Raeghi *et al.*, 2011), Korea (Lee *et al.*, 2010), China (Zhou *et al.*, 2009), the Czech Republic (Sedlak and Bartova, 2006), Brazil (Pena *et al.*, 2006), France (Afonso *et al.*, 2006), Spain (Miro *et al.*, 2004), and Japan (Maruyama *et al.*, 2003).

The total prevalence of *T. gondii* infection in dogs in this study was 45.18%, which was lower than that reported in Faisalabad, Pakistan (50%) and many other countries, such as Brazil (70.85%) and Senegal (67%) [Ahmad *et al.*, 2001; Rodrigues *et al.*, 2016], higher than that in Sub-tropical Arid parts (28.43%), and Lahore (39%), and Poland (28.92%) [Shahzad *et al.*, 2006; Ahmad *et al.*, 2014], but comparable with that detected in Lahore (46.88%) and 43% in Brazil (Jadoon *et al.*, 2009). Likewise, seroprevalence studies in dogs was also conducted in China (Wu *et al.*, 2011), Iran (Hosseininejad *et al.*, 2011), the Czech Republic (Sedlak and Bartova, 2006), Sri Lanka (Silva *et al.*, 1997), and Nigeria (Ogunrinade, 1978). It's possible that changes in the proportion of stray vs. domesticated animals, the persistence of oocysts in the environment, and the diagnostic procedures used account for differences in seroprevalence in cats and dogs across the globe (Dubey, 2004). The seroprevalence of *T. gondii* in cats is higher than in dogs in this study, as

supported by different studies conducted in various countries, i.e., Poland, Eastern China, Malaysia, and Thailand (Chandrawathani *et al.,* 2008; Liu *et al*., 2014; Huertas-López *et al.,* 2021; Turlewicz-Podbielska *et al.,* 2023). The variations in ecological and geographic parameters, the types of serologic tests employed, and the living circumstances of dogs and cats are likely to account for the differences in seroprevalence of *T. gondii* in dogs and cats (Liu *et al.,* 2014). However, seroprevalence tends to be higher in cat populations because they hunt prey for food. Regarding the cats hunting habits, 86% of the respondents had observed a cat hunt small animals (Byström, 2023). The cats were reported to hunt rats, snakes, lizards, birds, frogs, grasshoppers, and chameleons (Byström, 2023).

The higher likelihood of parasite interaction with age may explain the lower incidence seen in cats and dogs older than one year (Ahmand *et al.,* 2014). In a similar study, Wu *et al.* (2011) and Pena *et al.* (2012) found that elderly dogs and cats had increased seroprevalence. But in our study, the seroprevalence was higher in 3–4-year-old cats than in cats older than 4 years.

In our study, the difference was between male and female seropositivity, both in dogs and cats. In contrast to the current study, Ahmad *et al.* (2014) and DeFeo *et al.* (2002) showed that dogs and cats from Khyber Pakhtunkhwa province and Rhode Island had the same seroprevalence rates in gender groups, both in dogs and cats. Gender of dogs and cats was not associated with seroprevalence in our study, compared with the studies that correlate with the reports of Wanha *et al.* (2005), Dubey (1985), and Ahmed *et al.* (1983). Due to two factors, rural locations have higher environmental oocyst densities than metropolitan areas, which results in higher seroprevalence in cats and dogs from such places. In rural locations, domestic and wild felid populations are typically high. Second, metropolitan locations have less exposure to soil, which lowers the likelihood of coming into contact with oocysts, which are more frequently found in soil. Ahmad *et al.* (2014) reported considerably greater seroprevalence in rural dogs and cats, and this finding is consistent with the current investigation. Opsteegh *et al.* (2012) and Gyroke *et al.* (2011) also recorded greater seroprevalence in cats in rural areas.

In this study, a difference was found between the deworming group (yes vs. no) and seroprevelance both in dogs and cats. The animals that received deworming have a lower prevalence than those did not receive deworming. Similar studies were conducted, and the same result was found by Ahmad *et al.* (2014). In addition to demonstrating the prevalence of *T. gondii* infection in dogs and cats in Khyber Pakhtunkhwa, Pakistan, our study identified potential risk factors that may contribute to the spread of infection in these animals. This knowledge could be used as a guide when developing control measures to reduce the likelihood that humans would contract this significant zoonosis.

***Conclusion*:** Our findings generally indicated that infection is widespread in dogs and cats in the study locations and has contaminated the environment. Because of this, the human population is more susceptible to infection. In order to predict the future strategy for prevention and management of this zoonotic parasite in Pakistan, our work encourages further serological and molecular diagnosis of acute and chronic toxoplasmosis as well as genotyping of *T. gondii* strains from various hosts.

***Authors Contribution*:** Study Design Arsalan Said, Irfan Khattak, Rao Zahid Abbas, Muhammad Kasib Khan and Muhammad Kashif Saleemi

***Samples collection and interviewing*:** Arsalan Said

***Lab work*:** Arsalan Said and Irfan Khattak

***Data analysis and article write up*:** Arsalan Said, Irfan Khattak, Rao Zahid Abbas, Muhammad Kasib Khan and Muhammad Kashif Saleemi

***Conflict of interest*:** We have no conflict of interest to disclose regarding this manuscript. As a corresponding author, I confirm that the manuscript has been read and approved for submission by all the named authors.

***Ethical approval*:** Ethical approval was given by the Ethics committee of Agriculture University Faisalabad, Pakistan

REFERENCES

Afonso, E., P. Thulliez and E. Gilot-Fromont. 2006. Transmission of *Toxoplasma gondii* in an urban population of domestic cats (Felis catus). International Journal for Parasitology 36:1373-1382.

Ahmad, F., A. Maqbool, A. Mahfooz and S. Hayat. 2001. Serological survey of *Toxoplasma gondii* in dogs and cats. Pakistan Veterinary Journal 21:31-35.

Ahmad, N., H. Ahmed, S. Irum and M. Qayyum. 2014. Seroprevalence of IgG and IgM antibodies and associated risk factors for toxoplasmosis in cats and dogs from sub-tropical arid parts of Pakistan. Tropical Biomedicine 31:777-784.

Ahmed, B.A., S.M. Gaafar, W.E. Weirich and C.L. Kanitz. 1983. Relationship of *Toxoplasma* infections to other diseases in dogs. Veterinary Parasitology 12:199-203.

Baneth, G., S.M. Thamsborg, D. Otranto, J. Guillot, R. Blaga, P. Deplazes and L. Solano-Gallego. 2016. Major parasitic zoonoses associated with dogs and cats in Europe. Journal of Comparative Pathology 155:54-74.

Byström, R. 2023. The seropositivity of *Toxoplasma gondii* in free-roaming domesticated cats in Masai Mara, Kenya.

Cenci-Goga, B.T., P. V Rossitto, P. Sechi, C.M.E. McCrindle and J.S. Cullor. 2011. *Toxoplasma* in animals, food, and humans: an old parasite of new concern. Foodborne Pathogens and Disease 8:751-762.

Chandrawathani, P., R. Nurulaini, C. Zanin, B. Premaalatha, M. Adnan, O. Jamnah and T.C. Seah. 2008. Research Note Seroprevalence of *Toxoplasma gondii* antibodies in pigs, goats, cattle, dogs and cats in peninsular Malaysia. Tropical Biomedicine 25:257-258.

Dabritz, H.A. and P.A. Conrad. 2010. Cats and *Toxoplasma*: implications for public health. Zoonoses and Public Health 57:34-52.

Daniel, W.W. and C.L. Cross. 2018. Biostatistics: a foundation for analysis in the health sciences. Wiley. NY, USA.

DeFeo, M.L., J.P. Dubey, T.N. Mather and R.C. Rhodes III. 2002. Epidemiologic investigation of seroprevalence of antibodies to *Toxoplasma gondii* in cats and rodents. American Journal of Veterinary Research 63:1714-1717.

Dubey, J.P. 1985. Toxoplasmosis in dogs. Canine Practice (USA).

Dubey, J.P. 2004. Toxoplasmosis–a waterborne zoonosis. Veterinary Parasitology126:57-72.

Elmore, S.A., J.L. Jones, P.A. Conrad, S. Patton, D.S. Lindsay and J.P. Dubey. 2010. *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends Parasitology 26:190-196.

Foroutan, M., S. Dalvand, A. Daryani, E. Ahmadpour, H. Majidiani, S. Khademvatan and E. Abbasi. 2018. Rolling up the pieces of a puzzle: a systematic review and meta-analysis of the prevalence of toxoplasmosis in Iran. Alexandria Journal of Medicine 54:189-196.

Györke, A., M. Opsteegh, V. Mircean, A. Iovu and V. Cozma. 2011. *Toxoplasma gondii* in Romanian household cats: evaluation of serological tests, epidemiology and risk factors. Preventive Veterinary Medicine 102:321-28.

Hill, D.E., S. Chirukandoth and J.P. Dubey. 2005. Biology and epidemiology of Toxoplasma gondii in man and animals. Animal Health Research Reviews 6:41-61.

Hosseininejad, M., A. Malmasi, F. Hosseini, M. Selk-Ghaffari, N. Khorrami, M. Mohebali, S. Shojaee, A. Mirani, M. Azizzadeh and P. Mirshokraei. 2011. Seroprevalence of *Toxoplasma gondii* infection in dogs in Tehran, Iran. Iranian Journal of Parasitology 6:81-85.

Huertas-López, A., W. Sukhumavasi, G. Álvarez-García, S. Martínez-Subiela, D. Cano-Terriza, S. Almería, J.P. Dubey, I. García-Bocanegra, J.J. Cerón and C. Martínez-Carrasco. 2021. Seroprevalence of *Toxoplasma gondii* in outdoor dogs and cats in Bangkok, Thailand. Parasitology 148:843-849.

Jadoon, A., T. Akhtar, A. Maqbool, A.A. Anjum and A. Ajmal. 2009. Seroprevalence of *Toxoplasma gondii* in canines. The Journal of Animal and Plant Sciences 19:179-181.

Konate, Y., P. Goumou, L.W. soromou, K. Lancei, M. Keyra, M.F. Camara and Y. Sidime. 2023. Seroprevalence and Risk Factors of Toxoplasmosis in Cats and Women Undergoing Prenatal Consultation in Coyah, Guinea. Journal of Drug Delivery and Therapeutics 13:79-84.

Lazar, L.T.Y., M.S.J. Al-Ammash and K.S. Abass. 2021. *Toxoplasma gondii*: life cycle, pathogenesis, immune response: a review. Plant Archives 21:1057-1059.

Lee, S.E., J.Y. Kim, Y.A. Kim, S.H. Cho, H.J. Ahn, H.M. Woo, W.J. Lee and H.W. Nam. 2010. Prevalence of *Toxoplasma gondii* infection in stray and household cats in regions of Seoul, Korea. The Korean Journal of Parasitology 48:267-270.

Lindsay, D.S. and J.P. Dubey. 2011. *Toxoplasma gondii*: the changing paradigm of congenital toxoplasmosis. Parasitology 138:1829-1831.

Liu, Q.X., S. Wang, L.Q. Wang, J. Xing, W.J. Gao, G.F. Liu, B. Zhao, H.B. Zhang and L.H. Gao. 2014. Seroprevalence of *Toxoplasma gondii* infection in dogs and cats in Zhenjiang City, Eastern China. Asian Pacific Journal of Tropical Biomedicine 4:725-728.

Liyanage, K.L.D.T.D., A. Wiethoelter, J. Hufschmid and A. Jabbar. 2021. Descriptive comparison of ELISAs for the detection of *Toxoplasma gondii* antibodies in animals: A systematic review. Pathogens 10:605.

Majid, A., N. Ahmad, S. Haleem, S. Zareen, M. Taib, S. Khan and R. Hussain. 2021. Detection of toxoplasmosis in pets and stray cats through molecular and serological techniques in Khyber Pakhtunkhwa, Pakistan. BMC Veterinary Research 17:1-7.

Maruyama, S., H. Kabeya, R. Nakao, S. Tanaka, T. Sakai, X. Xuan, Y. Katsube and T. Mikami. 2003. Seroprevalence of *Bartonella henselae*, *Toxoplasma gondii*, FIV and FeLV infections in domestic cats in Japan. Microbiology and Immunology 47:147-153.

Miro, G., A. Montoya, S. Jiménez, C. Frisuelos, M. Mateo and I. Fuentes. 2004. Prevalence of antibodies to Toxoplasma gondii and intestinal parasites in stray, farm and household cats in Spain. Veterinary Parasitology 126:249-255.

Molan, A., K. Nosaka, M. Hunter and W. Wang. 2019. Global status of *Toxoplasma gondii* infection: systematic review and prevalence snapshots. Trop Biomed 36:898-925.

Ogunrinade, A.F. 1978. Studies on toxoplasmosis of domestic animals in Nigeria I: *Toxoplasma* antibodies in pet dogs in Ibadan area. Nigerian Medical Journal: Journal of the Nigeria Medical Association 8:446-448.

Opsteegh, M., R. Haveman, A.N. Swart, M.E. Mensink-Beerepoot, A. Hofhuis, M.F. Langelaar and J.W. Van der Giessen. 2012. Seroprevalence and risk factors for *Toxoplasma gondii* infection in domestic cats in The

Netherlands. Preventive Veterinary Medicine 104:317-26.

Pena, H.F. d J., R.M. Soares, M. Amaku, J.P. Dubey and S.M. Gennari. 2006. *Toxoplasma gondii* infection in cats from São Paulo state, Brazil: seroprevalence, oocyst shedding, isolation in mice, and biologic and molecular characterization. Research in Veterinary Science 81:58-67.

Raeghi, S., S. Sedeghi and S. Sedeghi. 2011. Prevalence of *Toxoplasma gondii* antibodies in cats in Urmia, northwest of Iran. Journal of Animal and Plant Sciences 21:132-134.

Rafique, A., S. Nasir, A. Ashraf, Z. Nawaz, F.M. Zahid, A. Abbas and S. Masood. 2022. Sero-Surveillance and Risk Factors Analysis of Caprine Toxoplasmosis in Faisalabad Punjab, Pakistan. Pakistan Veterinary Journal 42:102-106.

Raza, H., M.Y. Tipu, A. Ahmad, T. Umar, M. Suleman, S. Asfa, A. Sara and R, Ziaur. 2022. Screening of toxoplasmosis in cats through serology and PCR from selected districts of Khyber Pakhtunkhwa Pakistan. Pure and Applied Biology 12:78-86.

Robert-Gangneux, F. and M.L. Dardé. 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. Clinical Microbiology Reviews 25:264-296.

Hamidullah, M. Saqib, M.S. Sajid, S. Hussain, H.M. Rizwan, K. Ashfaq, S. Ghazanfer, A.A. Butt, M. Maqbool, S. Ahmad and O.A. Sparagano. 2022. Screening of Toxoplasmosis in Owned and Stray Dogs of District Faisalabad, Pakistan through Latex Agglutination and Indirect ELISA. Pathogens 11:1-9.

Sedlak, K. and E. Bartova. 2006. The prevalence of *Toxoplasma gondii* IgM and IgG antibodies in dogs and cats from the Czech Republic. Veterinary Medicine 51:555-558.

Silva, D.A.O., D.D. Cabral, B.L.D. Bernardina, M.A. Souza and J.R. Mineo. 1997. Detection of *Toxoplasma gondii*-specific antibodies in dogs. A comparative study of immunoenzymatic, immunofluorescent and haemagglutination titers. Memórias do Instituto Oswaldo Cruz 92:785-789.

Tenter, A.M., A.R. Heckeroth and L.M. Weiss. 2001. Erratum-*Toxoplasma gondii*: From animals to humans (International Journal for Parasitology (2000) 30 (1217-1258) PII: S0020751900001247). International Journal for Parasitology 31:217-220.

Thrusfield, M. 2018. Veterinary epidemiology. John Wiley & Sons. NY, USA.

Wanha, K., R. Edelhofer, C. Gabler-Eduardo and H. Prosl. 2005. Prevalence of antibodies against Neospora caninum and *Toxoplasma gondii* in dogs and foxes in Austria. Veterinary Parasitology 128:189-193.

Wu, S.-M., S.Y. Huang, B.Q. Fu, G.Y. Liu, J.X. Chen, M.X. Chen, Z.G. Yuan, D.H. Zhou, Y.B. Weng and X.Q. Zhu. 2011. Seroprevalence of *Toxoplasma gondii* infection in pet dogs in Lanzhou, Northwest China. Parasites & Vectors 4:1-5.

Zhou, P., H. Zhang, R.Q. Lin, D.L. Zhang, H.Q. Song, C. Su and X.Q. Zhu. 2009. Genetic characterization of *Toxoplasma gondii* isolates from China. Parasitology International 58:193-195.

Raza, H., M.Y. Tipu, A. Ahmad, T. Umar and M. Suleman. 2022. Asfa sakhawat, Sara Attique and Ziaur Rehman. Screening of toxoplasmosis in cats through serology and PCR from selected districts of Khyber Pakhtunkhwa Pakistan. Pure and Applied Biology 12:78-86.

Rodrigues, A.A., S.S. Reis, E. da Silva Moraes, E.M.A. do Nascimento Araújo, A. de Moura Zanine, T.V.C. Nascimento, J.L. Garcia and I.A.L. da Cunha. 2022. A systematic literature review and meta-analysis of Toxoplasma gondii seroprevalence in goats. Acta Tropica 106411.

Shahzad, A., M.S. Khan, K. Ashraf, M. Avais, K. Pervez and J.A. Khan. 2006. Sero-epidemiological and haematological studies on toxoplasmosis in cats, dogs and their owners in Lahore, Pakistan. Journal of Protozoology Research 16:60-73.

Turlewicz-Podbielska, H., J.J. Ruszkowski, M. Gogulski and M. Pomorska-Mól. 2023. Seroprevalence of Toxoplasma gondii in domestic cats, dogs and rabbits from Poland. Veterinary Research Communications:1-6.

Zarra-Nezhad, F., M.P. Borujeni, B. Mosallanejad and H. Hamidinejat. 2017. A seroepidemiological survey of Toxoplasma gondii infection in referred dogs to Veterinary Hospital of Ahvaz, Iran. International Journal of Veterinary Science and Medicine 5:148-151.