

Seroprevalence of Anti-*Toxoplasma gondii* Antibodies in the Rabbit Population of Lahore: Gender and Age-Based Analysis

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Abstract- Numerous mammalian and other warm-blooded animal species are susceptible to infection by *Toxoplasma gondii* (*T. gondii*). In these warm-blooded creatures, it mostly goes unnoticed but can occasionally result in neurological, ophthalmic, and reproductive issues, particularly in cases where a mammal contracts the infection while pregnant. Rabbits, like many other domesticated animals, can harbor *T. gondii* and spread the infection because of their widespread use as food and laboratory subjects. This research was done to find out anti-*T. gondii* antibodies in Lahore, Pakistan's rabbit (*Oryctolagus cuniculus*) population. Our research also aimed to determine if there was a difference in *T. gondii* infection rates based on gender and age. 273 Blood Samples were attained from 6 different areas of Lahore: Rehan Ishaq Rabbit Farm, Data Nagar, Walled City, Misri Shah, Karim Park, and Sanda Khurd and were analyzed using the commercial Indirect-Enzyme Linked Immunosorbent Assay (ELISA) kit. Chi-square and Pearson's Correlation tests were used for statistical investigations via GraphPad Prism software. Overall, 7.32 per cent (20/273) of rabbits were reported to have *T. gondii* antibodies. At the gender level, the findings showed a higher prevalence in males of 12.40% (16/129) than in females of 2.7% (4/144). The relationship between age and toxoplasmosis was found positive ($r = 0.098$). The observed seroprevalence of *T. gondii* is described as a health hazard to people who are in close contact

with rabbits. Therefore, the screening of animals is suggested before their use as food and for experimental work by humans.

Key words- ELISA, Rabbits, Risk factors, Seroprevalence, *Toxoplasma gondii*, Toxoplasmosis,

INTRODUCTION

Rabbits, pets, and humans are among the warm-blooded creatures susceptible to the zoonotic protozoan parasite *Toxoplasma gondii*. New vectors for host infection have been proposed and confirmed only lately. Since its discovery, the parasite has been observed in a wide range of hosts and environments across the globe and its DNA has been identified in mummies (Khairat *et al.*, 2013).

T. gondii seems to be extremely prevalent and pervasive about a third of the world's population has the parasite (Moncada and Montoya, 2012; Birgisdottir *et al.*, 2006; Flegr, 2013). *T. gondii* infections are frequently misdiagnosed, underreported, and underestimated in mammalian hosts. Evidence linking previously unrecognized chronic latent infections in humans to neurological or mental disorders like schizophrenia, for instance, has recently emerged (Webster *et al.*, 2012; Niebuhr *et al.*, 2008; Torrey *et al.*, 2007; Miman *et al.*, 2010; Pedersen *et al.*, 2011; Torrey *et al.*, 2012; Flegr, 2013).

There is a long history, especially in Asian countries, of raising rabbits (*Oryctolagus cuniculus*) specifically for human

consumption. Meat from *O. cuniculus* is much sought after in Europe and certain Asian countries as they are unique among food animals because of their high nutritional values and reproductive capacity (Rodriguez-Calleja *et al.*, 2006). Meat from rabbits is lean and healthy due to its small fat and cholesterol content and its plenty of healthy minerals and vitamins (Rodriguez-Calleja *et al.*, 2006). Proteins account for 80% of rabbit's total calorie value; its vitamin B12 level is high, ranking among the highest of the most common animal meat species; rabbit meat is low in salt (49.5 mg/100 g of meat, in the meat of the hind leg); and rabbit meat has low cholesterol (61.2 mg/100 g of meat) (Dalle-Zotte and Szendro, 2011). Further, the purine and uric acid levels in rabbit meat are quite low (Hernandez and Dalle-Zotte, 2010).

Toxoplasmosis, a zoonotic disease that influences meat production should be closely monitored for both public and animal health reasons. *T. gondii* infection in *O. cuniculus* is caused by swallowing oocysts that have been shed by cats into contaminated food or water. Parasite in humans and cattle occurs globally, thus there is a definite need for epidemiological inspection of this infection in animals that can be used as a source of food.

As a result of widespread *O. cuniculus* farming in Pakistan for both commercial and academic purposes, rabbits are now thought to be a potential human toxoplasmosis carrier. However, nothing is known regarding the origins of this *T. gondii* infection in rabbits in Lahore. This research work was therefore carried out to investigate the incidence of *T. gondii* contamination in rabbits as well as the parameters associated with seropositivity.

MATERIALS AND METHODS

This research was conducted in Lahore, Punjab, Pakistan. Located at 31.52° N, 74.36° E, 217 meters above sea level, and receiving an average of 628.8 millimeters of precipitation per year, Lahore is Punjab's most populated city. In order to choose 273 rabbits, a simple and convenience random sampling procedure was applied.

Collection of blood

The ear of the rabbit was rubbed for 1 minute before puncturing the vein, then the blood sample was drawn by using a 3cc syringe from the lateral marginal vein of the ear away from the base. Each rabbit had 2-3 mL of blood drawn, which was then put into a blood collection tube with gel and clot activator. All experiments were performed in accordance with relevant guidelines and regulations set by the ethical board of the University of The Punjab, Lahore. Our research work was reviewed and approved by the institutional ethics review board of University of The Punjab, Lahore which did not uncover any unethical issues.

Serum Separation

In accordance with the manufacturer's instructions, samples were centrifuged (3000 rpm for 5 minutes), and the resulting serum was stored at -20°C in cryovials (1.5 ml: Imec, China) until being put through an Indirect-ELISA test for *T. gondii*-specific IgG antibodies detection.

Sample Dilution

All serum samples were diluted (1:100) with sample diluent before starting the assay. For this purpose, 10ul serum sample and 1ml (1000ul) sample diluent were added in a tube to obtain 1:100 dilution and thoroughly mixed with a Vortex Mixer.

Washing solution Dilution

10 ml washing solution and 190 ml fresh and germ free re-distilled water was added to obtain 1:19 dilution.

Serodiagnosis

The sera were subjected to Indirect-ELISA for the detection of *T. gondii*-specific IgG antibodies in the sera. The commercial kit that was used for detection of ant-*T. gondii* antibodies was "Vet Line *Toxoplasma* ELISA (Nova Tec Immundiagnostica GMBH)" The animals were labelled as seropositive or seronegative based on the ELISA results. ELISA was performed according to the manufacturer instructions.

Statistical analysis

The number of positive samples in relation to different factors like sex and age were analyzed via GraphPad Prism (Version 9.5) software.

RESULTS

Analyze and understand all the provided review comments thoroughly. Overall, 7.32 per cent of rabbits were reported to have *T. gondii* antibodies (Table 1). At the gender level, the findings showed a higher prevalence in males of 12.12% (16/129) than in females of 2.7% (4/144) (Table 2). The relationship between age and toxoplasmosis was found positive (Pearson $r = 0.098$) (Table 3).

Table 1: Overall seroprevalence of *T. gondii* in rabbits.

Parameters	Frequency	Percentage (%)
Animals Negative	253	92.67
Animals Positive	20	07.32
Total	273	100%

Table 2: Relationship between toxoplasmosis and rabbit gender.

Samples	Male	Female	Total
Positive	16	4	20
Negative	113	140	253
Total	129	144	273

Chi-Square = 9.28, P-value = 0.0023, Statistically significant ($P < 0.05$)

Table 3: Relationship between toxoplasmosis and age in rabbits.

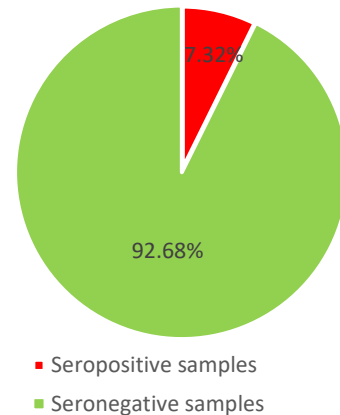
Age (Months)	Animals Examined	Animals Positive	Prevalence (%)
Group-I (1-4)	50	0	0
Group-II (5-8)	54	0	0
Group-III (9-12)	58	5	8.62
Group-IV (13-18)	70	7	10
Group-V (19-24)	41	8	19.51

Pearson $r = 0.098$

DISCUSSION

A conclusion section is not required. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

The present study showed an overall, 7.32% (20/273) seroprevalence of *T. gondii* in rabbits via Indirect-ELISA test (Fig. 1; Table 1). The same technique (Indirect-ELISA) was used to detect rates of seroprevalence of anti-*T. gondii* antibodies in different countries such as China (Zhou *et al.*, 2013), Mexico (Figueroa-Castillo *et al.*, 2006) and Algeria (Henneb *et al.*, 2019).



*Figure 1: Overall seroprevalence of *T. gondii* antibodies in rabbits*

The present results were in close agreement evaluated by Woźniak-Biel *et al.*, (2020) from Poland and by Elez *et al.*, (2017) from China. The present result was lower than the infection rate of 37.5% evaluated by Harfoush *et al.*, (2013) from China and 26.9% from Mexico by Castillo *et al.*, (2006). The various seroprevalence values could be attributed to variations in hygienic standards, climatic conditions, and the presence of diseased cats. Due to differential feeding for both genders and the sensitivity of the serological tests, toxoplasmosis rates have decreased (Miao *et al.*, 2013).

Even still, the detection of parasites in even a small number of animals raised the strong possibility that infective stages of the parasite were probably present in the local food and water supplies. Higher *T. gondii* prevalence rates were found in the current study based on rabbit origin, which may be related to variations in the cat population in the study area and partly because of poor sanitation and management conditions (Ogendi *et al.*, 2013). This finding demonstrates substantial variation between areas within the same country (Hejlek *et al.*, 1997).

Infection rates might vary from country to country because of factors like environmental contamination, the method utilized,

the climate, and the breeding system used. (Millar *et al.*, 2012).

Our study's secondary goal was to compare *T. gondii* seroprevalence rates among rabbits according to gender. (Table 2). 80% (16/20) male rabbits and 20% (4/20) female rabbits showed anti-*T. gondii* antibodies among the seropositive samples (Figure 2). Our study results were almost coincident with Luo *et al.* (2017) who reported similar results from the Hubei province of China. Despite the large gender gap in infection rates, sexual transmission of *T. gondii* in rabbits appears unlikely (Asgari *et al.*, 2015). Our research corroborated the idea that testosterone, which increases parasite susceptibility, was the reason that males were found to have more *T. gondii* infections than females (Morales-Montor *et al.*, 2004). When it comes to the transmission of parasites from one generation to the next, however, female animals can be held responsible. (Othman and Al-Azuheir, 2014).

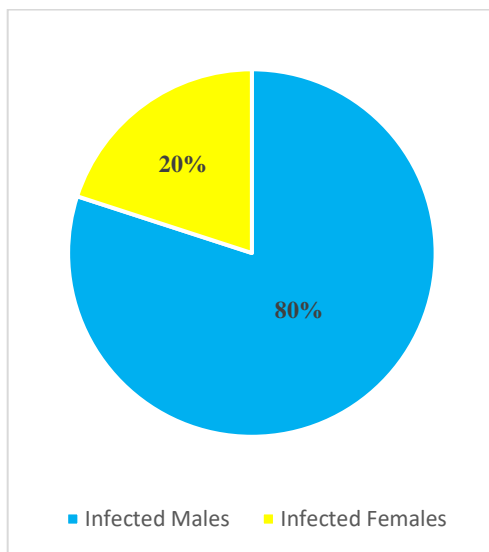


Figure 1: Seroprevalence of anti-*T. gondii* antibodies in male and female rabbits among seropositive samples

In this work, we also looked at the prevalence of *T. gondii* infection in various age groups of rabbits (Fig. 3; Table 3). Overall, the data showed a high positive correlation ($r=0.098$) between *T. gondii* infection and ageing animals, which was consistent with findings from multiple other researchers (Ntafis *et al.*, 2007; Tembue *et al.*, 2020; Wang *et al.*, 2011). The increased environmental contamination with oocysts that enter animals' digestive tracts and result in infection with time, according to research by Ottman and Al-Azuheir (2014), was

suggested by the positive correlation between *Toxoplasma* infection and age.

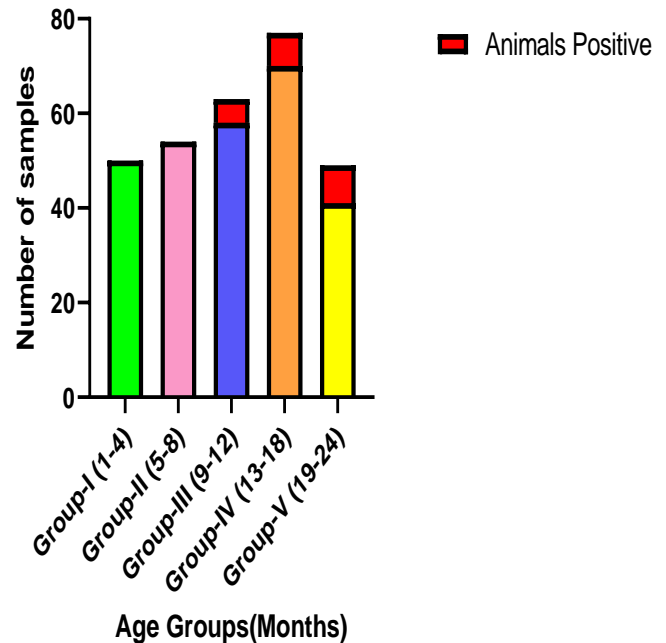


Figure 3: Comparison of seroprevalence of *Toxoplasmosis* in different age groups of rabbits

Congenital transmission in rabbits was also a possibility, according to Uhlková and Hübner (1973). Rabbits are likely to contract *T. gondii* by consuming food or water that has been tainted with the parasite's oocysts after coming into contact with felids. This suggests that rabbits play a part in the zoonosis and epidemiology of the parasite that has been found. Care should be exercised when interacting with animals and cleaning out cages because close contact between owners and their rabbits may increase exposure to *T. gondii*. However, the meat of rabbits should be thoroughly cooked and frozen to prevent toxoplasmosis in humans.

CONCLUSION AND RECOMMENDATIONS

The general seroprevalence of *T. gondii* antibodies in the rabbit population was 7.32%, in our study area. The occurrence of the *T. gondii* in males more than the female rabbits not supported the hypothesis of the sexual transmission of *T. gondii* from male to female rabbits. In light of the above discussion, it can be concluded that the higher infection rates in

the rabbits can prove to be a health hazard for the humans in the study area. Therefore, authorities should pay attention to this because rabbits might be the source of zoonotic transmission to humans. In the end, the authorities concerned are warranted to take serious measures to decrease this health menace in the current study area.

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DECLARATIONS

Ethical Approval

Using a syringe, only blood samples from the lateral marginal ear vein were taken. No physical harm was done to the rabbit while it was being handled to collect blood samples. Our research was reviewed and approved by the institutional ethics review board of Punjab University, which did not uncover any unethical issues.

Statements of consent

The publishing of the manuscript was approved by all authors. Agreement to take part the owners of the animals used in the study gave their informed consent.

Competing interests

The authors have no competing interests to disclose.

Authors contribution

The data was collected and examined by Muhammad Irfan and Hafsa Javed Butt under the supervision of Sadaf Aslam. The idea was originated from Muhammad Irfan and Abdul Majid Khan. 1st draft of the manuscript was written by Muhammad Irfan and Abdul Majid Khan. Final draft was organized by Muhammad Tahir and Laeeq Anwar and Anwaar Iftikhar. Each author made significant contributions and read and approved the final draft of the work.

Availability of data

All data are available upon request; to get the data

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