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DETECTION OF *TOXOPLASMA GONDII* IN PET CATS AND PET OWNERS IN LAHORE, PAKISTAN

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Abstract

Toxoplasmosis is a zoonotic disease caused in humans by a parasite named as *Toxoplasma gondii*, through fecal-oral route. Cats are the primary hosts of *Toxoplasma gondii* oocysts and excrete these oocysts into their fecal matter. Recently, in Pakistan the trend of keeping pets has increased. The maximum number of pets in Lahore is cats, which may pose a great threat of causing this parasitic disease in their owners. The aim of this research project was to detect the presence of *Toxoplasma gondii* in pet cats and their owners. The study included 155 cats and 45 pet owners. Fecal samples were collected and were subjected to microscopy. Microscopic analysis confirmed that 9 cat samples were suspected highly positive for Toxoplasma gondii related oocysts. Molecular identification confirmed the presence of *Toxoplasma gondii* in 1 sample. No pet owners were found to be infected with the parasite. It was discovered that the infected cat was an outdoor Persian female cat, which was not given treatments for mild flu this year. It was also observed through further investigation that the cat often used to mate with stray cats. The owner was not infected with the parasite as they used to clean the litter after 8 hours, so the chances of contact with the oocyst were minimal. All these finding reveal that stray cats might be a potential carrier of *Toxoplasma gondii*, whereas the pet cats kept at home are being provided with essential treatments suggested by the vets. It also reveals that maintenance of hygienic conditions by owners can help minimize the chance of *Toxoplasmosis*.

Keywords

Toxoplasmosis, Toxoplasma Gondii, Hydrocephaly, Retinochondritus, Pet Cats Diseases, Parasitic Diseases

1. Introduction

Toxoplasma gondii is a known zoonotic parasite which infects warm blooded animals worldwide (Slifko *et al.*, 2000). Toxoplasmosis is the disease caused by Toxoplasma gondii, which is transmitted

into animals and humans through fecal-oral route (Dubey, 2004). T.gondii is a small and arc shaped parasite which uses body flexions for motility and uses endodygeny process for reproduction. Discovery of whole life cycle of T.gondii in cats in early 1970s led to confirmation that the cats are primitive host of this parasite and cause primary infection in the warm blooded animals as cats shed a lot of oocysts of T.gondii in environment via fecal route (Hutchison *et al.,* 1971). There are mainly 3 infective stages of T.gondii:

- Sporozoites (Oocysts)
- Bradyzoites (Tissue cysts)
- Tachyzoites

Two types of phases are seen in the life cycle of T.gondii where in definitive hosts (cats) the sexual reproduction occurs in the intestine and asexual reproduction occurs in the infected warm blooded animals (Tenter et *al.,* 2000). *Toxoplasma gundi* has high prevalence in warm-blooded animals mainly mammals (Lopes *et al.,* 2011) *Toxoplasma gondii* infecting marsupials, flying foxes, and marine dolphins has proven to be highly problematic and fatal (Gonzales-Viera *et al.,* 2013). Marine mammals are most susceptible to this parasite as its oocysts can survive up to 4 years in salty water. It mainly infects dolphin, whales, sea otters and different species of seals (Gibson *et al.,* 2011). Presence of T.gondii cysts in marine mammals can show the communication rate of T.gondii in water. This parasite can be transmitted in humans through vertical and horizontal transmission In vertical transmission the parasite is directly passed to the fetus in infected mother's womb through placental route. The bradyzoites already present in the mother already affected from the parasitic infection or the fresh tachyzoites are the infectious parasite that can travel through the placental route into the fetus (Petersen *et al.,* 2010).

If the fetus in its early stage is infected by the parasite, miscarriage occurs. But if the fetus survives the infection and is born live, there are chances that it suffers from hydrocephaly and retinochoroiditis.

Hydrocephaly is the condition of brain in which the pressure inside it is increased due to accumulation and leakage of cerebrospinal fluid. This accumulation cause high liquid pressure in the brain of the new born babies and hence the brain looks bigger in size than the normal one (Asgari *et al.,* 2013). It is reported that high mortality rate of infants due to toxoplasmosis is because of hydrocephaly (Hutson *et al.,* 2015).The other condition is Retinochondritus in which the infection occurs in the posterior segment of the eye and becomes inflammated. Babies suffering from toxoplasmosis are seen to have developed congenital retinochondritus

(Dodds *et al.,* 2004).Children which are infected with toxoplasmosis have difficulty in understanding, seeing and learning processes (Diebler *et al.,* 1985).

Horizontal transmission includes ingestion of sporulated oocyst by humans through fecal-oral route, blood transfusions, and organ transplants, soil contamination and uptake of infected meat (Dubey *et al.,* 2005). This type of transmission occurs when they body comes in direct contact with the oocyst of the parasite by any of the latter means.

Toxoplasmosis does not show fatal symptoms when transmitted horizontally, where as in vertical transmission the symptoms are life threatening. In adults no obvious symptoms of toxoplasmosis are except for the flu, muscle ache, fever and swollen lymph nodes for few weeks. Moreover in vertical transmission the patients either die or develop certain syndromes for life time (Mutlaq *et al.,* 2017). Toxoplasmosis has been a real threat to humans in past decades due to the high consumption of meat and poultry products but due to awareness the threat has been decreased but the recent trend of keeping pets at home has given rise to increased threat of toxoplasmosis. According to studies 1/3rd of the human population is at risk of being infected by the T.gondii (Al Hamdani *et al.,* 1997).

Pakistan is one of those countries where pet lovers are in a considerable number and about 30% of people in Pakistan are in direct contact with the cats. And among them almost half of them have Persian cats and the others treat stray cats as their own and feed them daily. So it concludes that people in Pakistan are also at potential risk of developing toxoplasmosis with this increasing trend. Adult which are immuno compromised are also at risk of disease and can transmit it to the next generations.

Women who get pregnant in first time are more at risk for development of this disease in her child. Increased pet ownership has cause potential threat of toxoplasmosis in the pregnant females and their children. Cats are the distinctive host of the parasites that is why the infected cats may excrete a lot of Oocysts in their feces and pet owners are more likely to get in contact with the litter and may develop toxoplasmosis. According to an estimate around 40%-60% of the domestic and stray cats worldwide are reported to have infection (Meireles *et al.,* 2004; De Craeye *et al.,* 2008).This research project focuses to find the prevalence of *Toxoplasma gondii* in pet cats and pets owners in Lahore, Pakistan. This project

also predicted the percentage of population of Lahore which is at risk due to this parasite.

2. Methodology

The study comprised of 200 samples (155 pet cats and 45 owners).The owners were told the purpose of study and threats caused by *Toxoplasma gondii* in humans. Before collection of samples, history of the subjects was recorded which included the number of pet cats of an owner, their age, gender, food and kind of treatments they had previously taken (Table 1)

Table 1: Primer Used In This Study

Forward Primer	5'-CCTGGTGTCTCTTCAAGCGT-3'
Reverse Primer	5'-AAAGGAGAATGAGCGCACGA-3'

2.1 Sample Preparation

3g fecal sample was taken in a beaker and 20ml distilled water was added to it. The mixture was stirred and gently strained in the beaker (Figure 1). The filtrate was now ready to be processed for detection of Toxoplasma gondii.

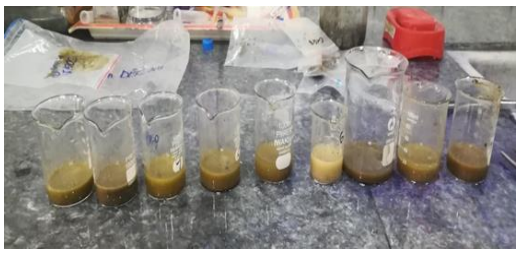

Figure 1: Strained and Filtered Fecal Samples For Flotation Method

2.2 Experimental Design

Experimental design includes microscopic analysis followed by molecular analysis which includes PCR testing. These two processes will confirm the presence of the parasite in the samples.

For microscopic analysis the samples were subjected to flotation method with zinc sulphate solution. The floating eggs were then pippeted out into vial containing double distilled water. Single drops from the vials were taken on glass slides along with 2µL of Lugol's stain. The slides were thoroughly observed under microscope for the presence of oocysts/eggs

The samples were subjected to DNA extraction with standard phenol-chloroform method to confirm the presence of DNA in the samples. The purified DNA were then subjected to PCR with H. hammondi-specific primer pair (Forward and Reverse) at following conditions (Table 1).The further processed includes the commercial sequencing and bioinformatics analysis of the positive samples.

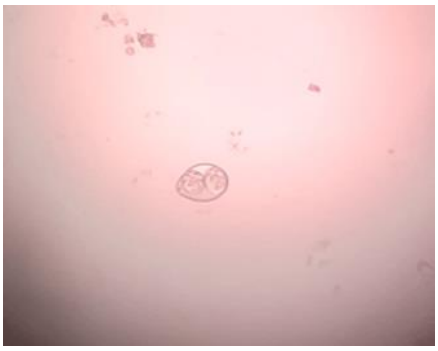

Figure 2: *Toxoplasma Gondii* Oocyst Under 40X Objective Of Microscope

3. Results and Discussion

The results of microscopic analysis show that 15 fecal samples were positive for the presence of

oocysts/eggs. 9 samples were found highly positive for *Toxoplasma gondii* oocysts (Figure 2) and the other 6 showed nematode eggs (Figure 3). All the samples were subjected to DNA extraction in order to eliminate the risk of leaving behind any sample containing the oocysts, which were left in the microscopic analysis. All the purified samples were run on 1% agarose gel. The results show bands in 15 samples which were the same samples detected for presence of oocysts and eggs in microscopy.

The suspected samples were subjected to polymerase chain reaction with forward and reverse primer set (Nabi *et al.*, 2018).

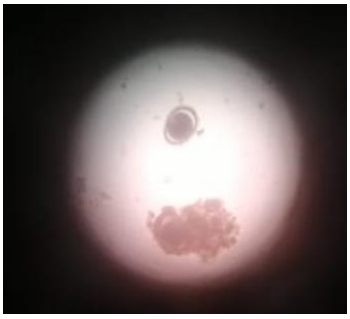

Figure 3: Nematode (Ascaridoedia Egg) Observed Under 10X Objective Of Microscope

In molecular analysis, 1 sample was found to be positive for *Toxoplasma gondii* presence (Figure 4).

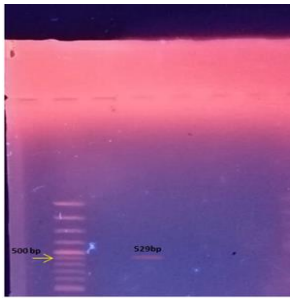

Figure 4: Agarose Gel Showing Amplification Of Gene

Table 2: PCR Conditions For 30 Gene Amplification

Steps	Conditions
Step 1	Initial Temp: 94°C 4 min Denaturing: 94°C 1 min
Step 2 **(30 cycles)**	Annealing : 59°C 30 sec Extension: 72° C 2 Min
Step 3	Final Extension: 72° C 10 min

The positive sample was commercially sequences and the following sequence was obtained.

(CCTGGTGTCTCTTCAAGCGTGAGACCGC
GGAGCCGAAGTGCGTTTTCTTTTTTTGAT
TTTTTTTTGTTTTTTCACAGGCGAGCTCG
CCTGTGCTTGGAGCCACAGAAGGGACAG
AAGTCGAAGGGGACTACAGACGCGATGC
CGCTCCTCCAGCCGTCTTGGAGGAGAGA
TATCAGGACTGTAGATGAAGGCGASGGT
GAGGATGAGGGGGTGGCGTGGTTGGGAA
GCGACGAGAGTCGGAGAGGGAGAAGAT
GTTTCCGGCTTGGCTGCTTTTCCTGGAGG
GTGGAAAAGAGACACCGGAATGCGATCT
AGACGAGACGACGCTTTCCTCGTGGTGA
TGGCGGAGAGAATTGAAGAGTGGAGAA
GAGGGCGAGGGAGACAGAGTCGGAGGC
TTGGACGAAGGGAGGAGGAGGCGTAGG
AGAGG)

The obtained sequences was run on BLAST and found to be 99.7% similar to that of *Toxoplasma gondii* in NCBI BLAST.

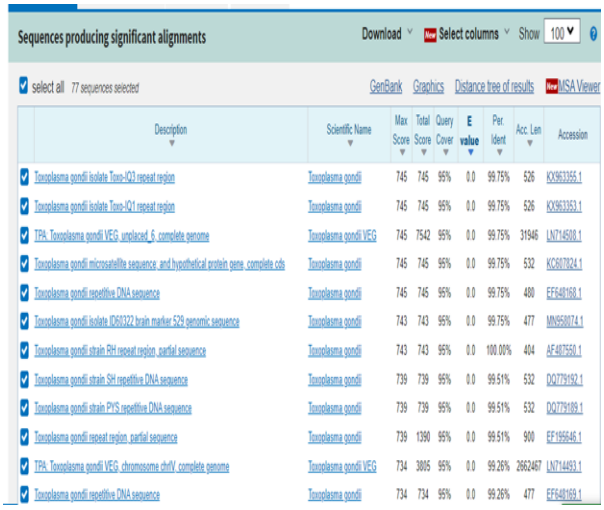

Figure: 5 BLAST Results Showing the Similarity

According to the data collected, it was observed that 91.11% of total pet owners were female, whereas only 8.89% were males (Figure 6) and the average number of cats kept by owners were 3 (Figure 7). 84.44% of the pet cats used to share their litter boxes with other cats and the rest 15.56% do not share the litter boxes. The same percentage was calculated for the indoor and outdoor cats respectively (Figure 8). Only 2.2% cats were not treated for mild flu this year and was infected with *Toxoplasma gondii* oocysts (Figure 9). 71.11% cats involved in the study were persian cats (Figure 10).

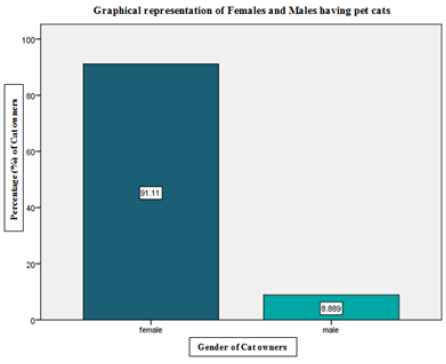

Figure 6: Gender of Pet Owners

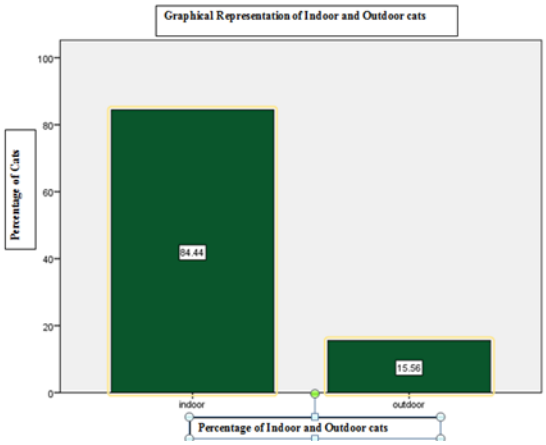

Figure 7: Average Number of Cats Kept By Owners

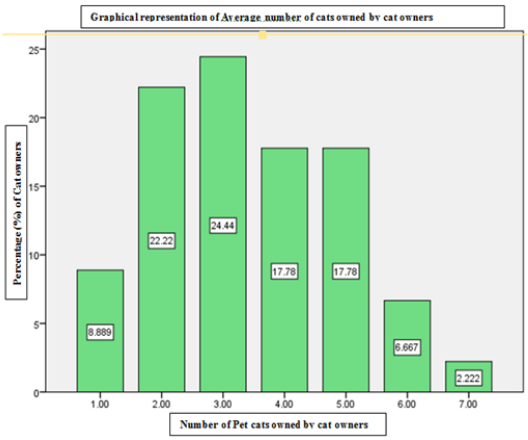

Figure 8: Estimation of Indoor and Outdoor Cats

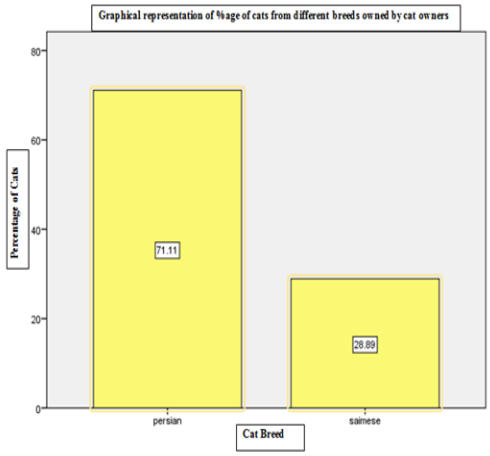

Figure 9: Medical History of the Cats Involved In Study

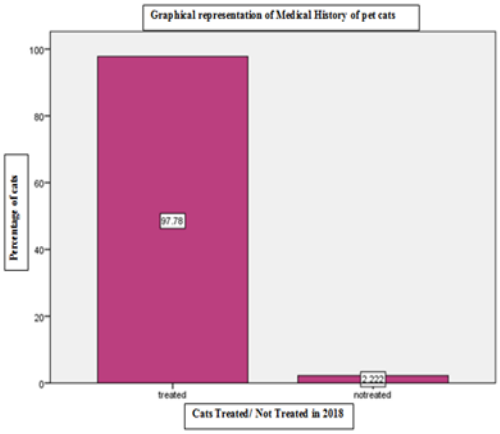

Figure 10: Breed of Cats Involved In Study

It was observed that the infected cat was Persian female cat which often used to mate with stray cats. This can be one of the vital reasons for the cat to be infected by the parasite. This, cat was also not given treatment for mild flu or any other vaccination the previous year due the unknown reasons. Owner of the cat told the hygiene conditions were maintained and the litter box was cleaned after every 8 hours. This can be the main reason why the owner was not infected with the parasite.

Moreover it was found that among cat owners , 91% were females, which is an alarming sign, because if the parasitic contamination is present in the city then the new generations will be at risk of the parasitic diseases. A study shows that toxoplasmosis is becoming a threat in the world as it infects around 50% population of world. The symptoms shown by the disease are however asymptomatic but may cause serious human pathologies by weakening the immune system (Hill & Dubey., 2002).

Similarly in a study, for detection of *Toxoplasma gondii* B1 gene two methods were compared. Microscopy of the tissue samples was done with the formalin fixation method whereas molecular analysis was done with the help of polymerase chain reaction. The results from both techniques were different. Microscopic analysis confirmed presence of one infected mouse but on the other hand PCR results were not positive. This was due to sensitivity and rapidity of PCR process (Veronesi *et al.,* 2017).

Toxoplasma gondii mainly infects the nervous tissue and have devastating effects on the eyes of the unborn. A study in Germany shows that every year 6339 pregnant women are being detected positive for the seroprevelence of *Toxoplasma gondii* presence (Wilking *et al.,* 2016). In another study, done on prevalence of *Toxoplasma gondii* oocysts in 470 fecal samples of cats brought at pet center. Results of copro-pcr showed that 2.3% (11/470) cats were found positive for presence of *Toxoplasma gondii* oocysts (Nabi *et al*., 2018).

A study was conducted in Mooro Bay area in order to estimate the environmental oocyst burden. 326 fecal samples from cats were collected and were tested with flotation process using zinc sulphate solution, 3 /326 samples i.e. 0.9% samples were found positive for *Toxoplasma gondii* oocyst. It was concluded that every 4thcat out of 434 cats was infected (Leutenegger *et al.,* 2007).

So it can be concluded on the basis of the results that a very low risk of *Toxoplasma gondii* contamination is present in the pet cats owners but yet we cannot say that is safe to keep cats

without maintaining proper conditions and taking them for medical checkups regularly. Otherwise the whole nation will be at risk of developing this silent enemy which will affect our future generations.

4. Conclusion

This study confirms that the cat owners are certainly at potential risk of being infected by the *Toxoplasma gondii* oocysts. Although no owner was found to be infected with parasite but one female Persian cat was infected with Toxoplasma gondii. The infected cat was an outdoor cat and was not treated since one year. Details also revealed that the cat used to mate with stray cats, which can be a strong reason for its infection with the parasitic oocysts. It was also observed that the pet owner of the infected cat took all possible safety measures to avoid direct contact with the cat feces. This concludes that it is important for pet owners to maintain hygiene conditions and get their cats treated in order to avoid Toxoplasmosis and certain other parasitic diseases. Awareness about keeping and handling pets must be spread among the pet owners and local people of the city before they adopt pets, in order to avoid chances of occurrence of such diseases in country.

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