PREVALENCE OF TOXOPLASMOSIS IN FELINES

T H E S I S

Submitted

in partial fulfillment of requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

BY

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I hereby declare that the experimental research work and interpretation of thesis entitled **"PREVALENCE OF TOXOPLASMOSIS IN FELINES"** or part thereof has not been submitted for any of the other degree or diploma of any university or scientific organization. The source of material used and all assistance received during the course of investigation have been duly acknowledged.

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We also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other university.

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Place- Shirwal

Date -

(Vidhate Pritesh Shivaji)

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%	Percent
ºC	Degree Celsius
CFT	Complement fixation test
µg	Microgram
CBC	Complete Blood Count
PC	Positive control
dl	Decilitre
g	Gram
µl	Microliter
et al.	And others
Fig	Figure
Hb	Haemoglobin
i.e	That is
M	Molar
mg	Milligram
ml	Millilitre
e.g.	Example
No.	Number
pH	Power of hydrogen
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
TEC	Total Erythrocyte Count
TLC	Total Leukocyte Count
WBC	White Blood Cell
ELISA	Enzyme linked immunosorbent assay
EDTA	Ethylene Diamine Tetra Acetic acid
DLC	Differential Leukocyte Count

DNA	Deoxyribose Nucleic Acid
MAT	Microscopic agglutination test
bp	Base pair
CI	Confidence interval
RNA	Ribonucleic acid
spp.	Species
pmol	Picomol
UV	Ultraviolet
SE	Standard Error Mean
rpm	Revolutions per minute

INTRODUCTION

CHAPTER I

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite with worldwide distribution. As definitive hosts of this parasite, cats play a very important role within the life cycle of *T. gondii*, facilitating the genetic recombination between strains, also as environmental contamination and felines are the sole animals that pass oocysts in their faeces (Elmore *et al*., 2010). They acquire the infection post-ingestion of undercooked meat containing tissue cysts and ingestion of vegetables or water contaminated with sporulated oocysts (Tenter *et al*., 2000).

The parasite was first found in laboratory animals (Dubey, 2007). Its medical importance remained unknown until 1939 when *Toxoplasma gondii* was identified conclusively in tissues of a congenitally-infected infant in New York City, USA (Wolf *et al*., 1939), and its veterinary importance became known when it had been found to cause abortion storms in sheep in 1957 in Australia (Hartley and Marshall, 1957). It belongs to Phylum Apicomplexa, Class Sporozoasida, Subclass Coccidiasina, Order Eimeriorina, and Family *Toxoplasmatidae* (Hill, 2005). There's just one species of genus Toxoplasma which is *Toxoplasma gondii*.

In a national study of humans for seroprevalence of toxoplasmosis in India showed 22.40% (328/1464) prevalence by IgG-ELISA. (Singh *et al*., 2014). The sero-prevalence of 35.10% by IgG-ELISA was seen in humans of Maharashtra (Singh *et al*. 1994). A similar study of chickens in Maharashtra seroprevalence was found 17.94% (133/741) by MAT which might support the hypothesis of spreading of toxoplasmosis by meat feeding (Sreekumar *et al*., 2003).

Copro-prevalence of *Toxoplasma gondii* in cats of Banglore region was observed 6.25% by 529 bp repetitive gene. (Gautham, 2014) Serological survey in India detected 33.7% of positive cases of cats using indirect heamagglutination (Chhabra *et al*., 1985)

Overall global pooled seroprevalence of *Toxoplasma gondii* in domestic cats reported from 1967 to 2017 was 35% which ranged between zero to 97 percent. In Australia 52% seroprevalence was seen whereas in Africa 51% was observed which was higher than other countries. In Asia, seroprevalence was lowest (27%). Highest pooled seroprevalence was noted in male domestic cats of Australia, Europe and Africa 62%, 46% and 43% respectively. (Montazeri *et.al.,* 2020)

Owing to its zoonotic importance, after birth, humans are usually infected with *Toxoplasma gondii* by ingestion of oocysts. Symptoms are fever, malaise and lymphadenopathy (Montoya and Remington 2006) also pulmonary and multivisceral involvement is observed. (Carme *et al*., 2002). *Toxoplasma gondii* develop ocular disease usually retinochoroiditis (Holland, 2003). Congenital infection can result in a miscarriage, still-birth, a live infant with classic signs of congenital toxoplasmosis like hydrocephalus or microcephalus, cerebral calcifications and retinochoroiditis (McAuley *et al*., 1994).

In felines, pneumonia is the most vital clinical manifestation noticed in toxoplasmosis. Common clinical manifestations are hepatitis, pancreatic necrosis, myositis, myocarditis, uveitis, dermatitis and encephalitis. Clinical signs in acute systemic infection include lethargy, anorexia, pyrexia or hypothermia. Dyspnoea occurs along with cardiac involvement. Jaundice, abdominal distention and nervous signs are seen (Barrs, 2013). Clinical toxoplasmosis is most severe in congenitally infected kittens. The enteroepithelial phase of infection is asymptomatic or there's transient small-bowel diarrhoea. The extraintestinal phase of infection is clinically silent most of the times. Risk factors for clinical disease include FIV, FeLV or FIP, neoplasia and chronic administration of immunosuppressive drugs such

as steroids and cyclosporin. Clinical cases of toxoplasmosis are way more frequent in cats than in dogs (Dubey and Raton, 2010).

The detection of *Toxoplasma gondii* in faecal and tissue samples is performed by microscopic examination using Giemsa, Haematoxylin and Eosin (HE) stain. The isolation of *Toxoplasma gondii* by bioassay using laboratory animals is taken into account because it's the gold standard method. Serological tests, like dye test (DT), modified agglutination test (MAT), enzyme-linked immunosorbent assays (ELISA), immunosorbent agglutination assay (ISAGA), indirect fluorescent antibody test (IFAT) and indirect haemagglutination assays (IHA), are developed to detect different antibody classes or antigens. (Quan *et al*., 2015).

Molecular diagnostics of toxoplasmosis were generally done by detection of specific DNA sequences. Some use B1 gene that has 35 copies within the genome, but others use DNA R529 bp fragment that has 200-300 copies in genome, internal tran-scribed spacer - 1 that consists 110 gen copies, or 18s rRNA gene sequence. (Jones *et al*., 2014). Repetitive 529 bp sequence and Tox4 and Tox5 primers have utilized to detect *Toxoplasma gondii* within pig tissue and compared it with mouse bioassay and histopathology (Singh, 1997). The advance of molecular-based copro-diagnostic method is hoped to be utilized in detecting *T. gondii*.

As *toxoplasma gondii* is one of the important pathogens affecting felines with significant zoonotic potential. As well as, there are very few studies indicating its prevalence from adjacent area. Also, there are no studies which confirm *toxoplasma gondii* by molecular methods like PCR. The present study is designed with following objectives.

Objectives:

1. To study the prevalence of toxoplasmosis in cat.
2. To study clinical observations in affected cat.
3. Confirmation of toxoplasmosis by Polymerase chain reaction.
4. Hematobiochemical alterations in cat infected with toxoplasmosis.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The literature pertaining to the prevalence, isolation of *Toxoplasma gondii* oocysts from fecal samples, Toxo IgG rapid test kit, polymerase chain reaction, clinical observations of cats associated with Toxoplasmosis and hematobiochemical alterations in cats was reviewed and it is presented below.

2.1 Prevalence of toxoplasmosis and gastrointestinal parasites

Kulkarni (1972) examined 40 cats out of which 72.5% of cats were infected with parasites. It was noted that age group of 3-12 months were highly infected compared to others and males (85.71%) suffered higher infection. Prevalence in Mumbai region was; Tococara cati 27.5%, Toxocara canis 5%, Strongyloides 5% and Ancylostoma caninum 15%.

Dubey *et al.* (2004) worked to determine the prevalence of *Toxoplasma gondii* in 58 domestic cats from 51 homes from Santa Isabel do Ivai, Parana´ State, Brazil where a water-associated outbreak of acute toxoplasmosis had occurred in humans. Antibodies to *Toxoplasma gondii* were found with the modified agglutination test in 49 of 58 (84.4%) cats at a serum dilution of 1:20. Tissues (brain, heart, and skeletal muscle) of 54 of these cats were bioassayed in *T. gondii*–free, laboratory-reared cats; *Toxoplasma gondii* oocysts were excreted by 33 cats that were fed feline tissues.

Dubey and Karhemere (2004) studied prevalence of *Toxoplasma gondii* in chickens from Democratic Republic of Congo, Mali, Burkina Faso, and Kenya. The prevalence of *Toxoplasma gondii* antibodies in sera of 50 free-range chickens from Congo was 50% based on the modified agglutination test (MAT). antibody titers were 1:5 in 7, 1:10 in 7, 1:20 in 6, 1:40 in 1, and 1:160 or more in 4 chickens. Hearts, pectoral muscles, and brains of 11 chickens with titers of 1:20 or more were bioassayed individually in mice; *Toxoplasma gondii* was isolated from 9, from the hearts of 9,

brains of 3, and muscles of 3 chickens. Tissues of each of the 14 chickens with titers of 1:5 or 1:10 were pooled and bioassayed in mice; *Toxoplasma gondii* was isolated from 1 chicken with a titer of 1:10. Tissues from the remaining 25 seronegative chickens were pooled and fed to 1 *T. gondii*–free cat. Feces of the cat were examined for oocysts, but none was seen.

Sroka *et al.* (2004) assessed the prevalence of *Toxoplasma gondii* infection in cats from southwestern Poland using serology, coproscopy and PCR methods.
The positive results in IFAT for anti-*Toxoplasma gondii* IgG and IgM antibodies were found in 143 of 208 tested cats (68.8%). In 23.1% of cat sera anti- *Toxoplasma gondii* IgM antibodies were found. The prevalence of anti-*Toxoplasma* antibodies was significantly greater in older cats (>1 year) (83.5%) than in younger cats (48.3%) ($P<0.05$), in females (74.1%) than in males (58.8%) ($P<0.05$), and in cats kept outdoors than indoors (69.7% vs. 16.7%) ($P<0.01$).

Vollaire *et al*. (2005) determined regional seroprevalence estimates of Toxoplasma gondi-specific IgM and IgG in clinically ill cats throughout the United States. Serum from 12,628 clinically ill, client-owned cats was taken. *Toxoplasma gondii*-specific IgM and IgG antibodies were detected by use of ELISAs. Overall, 31.6% of the cats were seropositive for T. gondii-specific IgM, IgG, or both. Percentage of cats seropositive for T. gondii antibodies ranged from 16.1% (southwestern United States) to 43.5% (northeastern United States). Males were more likely than females to be seropositive for T. gondii antibodies.. Seroprevalence increases as cat's age and is higher in male and domestic shorthair cats, compared with females and other breeds.

Jones and Dubey, (2010) stated that determination of antibodies is a better measure of the prevalence of T. gondii in cats than fecal examination for oocysts because only a few (1%) cats actually shed oocysts at any given time.

Javadi *et al.* (2010) detected antibodies of *Toxoplasma gondii* 50 cats using an enzyme-linked immunosorbent assay. Immunoglobulin M (IgM) and

immunoglobulin G (IgG) were measured. Fifteen (30%) of the cats were found to be seropositive. No statistically significant difference was found amongst different age groups, but the male cats showed significantly higher antibody titres than female cats. A significant positive correlation was found between IgM and IgG of the cats.

Mircean *et al.* (2010) conducted a study in the Transylvania region. Total 414 faecal Concurrent infections with two or more parasites were recorded in 17.6% cats.The detected parasites were Toxocara cati (20.3%), Ancylostoma spp. (10.1%), Isospora rivolta (8.9%), Isospora felis (5.3%), Aelurostrongylus abstrusus (5.6%), Strongyloides spp. (3.4%), Capillaria aerophila (3.1%), Taenia taeniaeformis (2.7%), T. gondii/H. hammondi (1.2%), Sarcocystis spp. (1%), Giardia duodenalis (0.7%) and Dyphillidium caninum (0.2%). Risk factors for infection with parasites in cats were identified to be age and medium (rural or urban area).Thus, I. felis, I. rivolta and T. cati were more common in cats less than/equal to 1-year old,and Ancylostoma spp. and A. abstrusus were more prevalent in cats older than 1-year of age. 72.4% of the owners applied anthelmintic treatments to their cats, more commonly in urban areas (87.3%) (4 treatments/year) than in rural areas (12.7%) (1treatment/ year). 66.3% of the owners knew about the zoonotic potential of some parasites from cats, and the main source of information was veterinarians (65.4%).

Kulasena *et al.* (2011) studied seroprevalence of toxoplasmosis in Colombo and Shreelanka. Seropositivity increased with age and was higher in stray cats versus pet cats and higher in females. Percentage positivity of males were 25.8 and females 32.7. Prevalence by age group was less than one year-11.1%, one to two years 16.7%, two to four years 50%, greater than 16 years 44.44%. Stray cats had 39.3% seroprevalence and household cats had 31.4%.

Jokelainen *et al*. (2012) investigated different aspects of feline toxoplasmosis. Blood samples of 445 purebred pet cats and 45 shelter cats were screened for *Toxoplasma gondii*–specific immunoglobulin G antibodies with a direct agglutination test. The

overall seroprevalence was 48.4%; older cats and cats receiving raw meat in their diet were more often seropositive. Fecal samples were obtained from 131 shelters cats; 2 of the cats were found shedding *T. gondii*–like oocysts, and the oocysts shed by 1 of the 2 were confirmed as *Toxoplasma gondii* with polymerase chain reaction.

Gautham (2014) studied prevalence of Toxoplasmosis by fecal flotation and copro-PCR in Banglore. *Toxoplasma gondii* like oocyst was seen in two(1.38%) fecal sample by flotation out of total 144 samples and both samples were from animal shelters/stray cats(2.44% prevalence). Total 80 fecal samples were subjected to DNA isolation followed by 529 bp repetitive PCR, out of them five (6.25%) cats were positive for Toxoplasmosis. Prevalence of gastrointestinal parasites in cats was Toxocara catti 25(17.36%), Ancylostoma ova 15(10.42%), Isospora oocysts 5(3.47%), *Toxocara cati plus* Ancylostoma ova 18 (12.5 %) and total 53 (37.86%).

Must *et al.* (2017) carried out the study to evaluate *Toxoplasma gondii* seroprevalence in Birman, British Shorthair, Burmese, Korat, Norwegian Forest Cat, Ocicat, Persian, and Siamese. Plasma samples were analysed for the presence of immunoglobulin G antibodies against *Toxoplasma gondii* with a commercial direct agglutination test at dilution 1:40. Overall, 41.12% of the 1121 cats tested seropositive, and the seroprevalence increased with age. The Burmese had the lowest seroprevalence (18.82%) and the Persian had the highest (60.00%).

Njuguna *et al.* (2017) determined prevalence of GIT parasites in cats kept by households in Thika region, Kenya. Fecal samples were collected randomly from 103 cats and analyzed. In descending order, the prevalence of the detected protozoa parasites was *Isospora* spp. 43.7% (95% CI: 40.4–47%), *Cryptosporidium* spp. 40.8% (95% CI: 37.5–44.1%), *Toxoplasma gondii*7.8% (95% CI: 4.5–11.1%), and *Entamoeba* spp. 2.9% (95% CI: 1.6–6.2%).The prevalence of the observed helminths was *Strongyloides stercoralis* 43.7% (95% CI: 40.4–47%), *Toxocara cati* 23.3% (95% CI: 20–26.6%), *Ancylostoma* spp. 9.7% (95% CI: 6.4–13%), *Dipylidium*

caninum 8.7% (95% CI: 5.4–12.0%), and *Acanthocephala* spp. 1.9% (95% CI: 1–4.2%). The percentage of cats excreting at least one species of parasite was 73.2% (95% CI = 69.9–76.5%).

Wang *et al.* (2017) investigated the seroprevalence of *Toxoplasma gondii* infection in domestic cats in central China, 843 serum samples were collected in Henan province between March 2015 and May 2016 and tested for IgG antibodies against *Toxoplasma gondii* using the enzyme-linked immunosorbent assay (ELISA). The overall seroprevalence of *Toxoplasma gondii* was 21% (178/843). No significant difference was observed based on the sex of cats ($p > 0.05$). Significantly higher seroprevalence ($p < 0.05$) was observed in mixed-breed cats (24%) compared to purebred cats (17%). Seroprevalence in rural cats (29%) was significantly higher ($p < 0.01$) than in urban cats (16%), and increased significantly ($p < 0.01$) with age.

Hanafiah *et al.* (2018) determined the copro-prevalence of *Toxoplasma gondii* using polymerase chain reaction (PCR) with repetitive 529 bp gene and to construct the phylogenetic tree of *Toxoplasma* oocyst from pet cats in Yogyakarta. 9 of 132 pet cat samples which serologically positive for *Toxoplasma* were used in this research. To determine the copro-prevalence of *Toxoplasma gondii* in pet cat, 10 g of feces samples taken from practitioners and household cats in Yogyakarta were used in the PCR method utilizing repetitive 529 bp gene sequences. The result shows that copro-prevalence by PCR using repetitive 529 bp gene was 33.3% (3/9).

Latha *et al.* (2018) did fecal microscopy and seroprevalence in Thrissur, Kerala.The T. gondii oocyst was detected in 4.47% of 313 feline faecal samples. On molecular detection by PCR, the B1 gene of Toxoplasma sp. was detected in six out of 61 soil samples. None of the 61 water samples showed presence of the parasite. Overall seroprevalence of 52.46% was recorded in the 161 human serum samples. Practice of rearing of more than three cats and those humans involved in gardening and

agricultural activities had higher prevalence of T. gondii antibodies. Seroprevalence of the parasite in older individuals and in women with history of gynaecological disease conditions and on non-vegetarian diet was higher.

Sah *et al.* (2018) determined the seroprevalence of *Toxoplasma gondii* in cats and human in Inaruwa and surrounding areas of Sunsari district, Nepal. 44 samples from cats and 150 from human were collected and tested immediately using lateral flow chromatographic immunoassay (Toxo IgG/IgM Combo Rapid test®). Seroprevalence of toxoplasmosis was detected 36.36% (95%CI: 22.41- 52.23%) in cats and 12.67% (95% CI: 7.80- 19.07%) in human. Toxoplasmosis was found highly significantly associated with abortion (58.33%, OR= 15.4, P=0.0001) in humans. Regarding occupation, 20.83% butchers were seropositive followed by farmers (15.52%), housewives (10.0%) and diagnostic lab technicians (8.0%). Female and higher age group showed high prevalence of toxoplasmosis in all studied species.

Awobode *et al.* (2020) studied the shedding proportion of T. gondii-like oocysts in cats and soil contamination levels were investigated in three communities in Ibadan, Nigeria. Soil (n = 204) and feral cat faecal samples (n = 14) were examined for the presence of oocysts using a modified sucrose flotation technique. Cat sera (n = 15) were also analysed for IgG antibodies to T. gondii by ELISA. T. gondii-like oocysts were identified in 21.4% (95% CI: 4.6–50.8) of the total cat faecal samples. The prevalence was 50% (95% CI: 6.7–93.3), 0% and 10% (95% CI: 0.3–44.5) in Akinyele, Laniba and Ajibode communities respectively. T. gondii IgG antibody was present in 86.7% of the screened cat sera (including the copropositive cats). The seroprevalence in cats was 75% in Akinyele, 0% Laniba and 90.9% for Ajibode community (P >0.05). Oocysts were recovered from 1.5% (95% CI: 0.50–4.23) of the soil samples screened and were identified from 3.8% (95% CI: 0.13–10.58) of the soil collected in Akinyele community. Akinyele also recorded the highest number of infected cats.

Montazeri *et al.* (2020) estimated seroprevalence of *T. gondii* ranged between 0–97% in domestic cats The global pooled seroprevalence of *T. gondii* reported from 1967 to 2017 was 35% (95% CI: 32–38%) in domestic cats. The seroprevalence was higher in Australia and Africa where the *T. gondii* seropositivity in domestic cats was 52% (95% CI: 15–89%) and 51% (95% CI: 20–81%), respectively. The lowest seroprevalence estimated was in Asia (27%, 95% CI: 24–30%). Only one study was performed in Antarctica (Kerguelen Archipelago) and reported a seroprevalence of 51% (95% CI: 45–57%) in 276 feral cats .Significant geographical differences were observed in pooled *T. gondii* seropositivity rates among domestic cats. In the subgroup analysis, the global pooled seroprevalence of toxoplasmosis was equal (33%, 95% CI: 29–37%) in male and female domestic cats. The highest pooled seroprevalence in male domestic cats was observed in Australia (62%, 95% CI: 54–70%) followed by Europe (46%, 95% CI: 38–53%) and Africa (43%, 95% CI: 12–75%) (Table 3). Similarly, the highest pooled *T. gondii* seroprevalence in female domestic cats was observed in Australia (68%, 95% CI: 61–75%) followed by Europe (47%, 95% CI: 38–56%) and Africa (45%, 95% CI: 8–82%). The estimates of the global seroprevalence of *T. gondii* in male and female wild felids were 61% (95% CI:27–95%) and 57% (95% CI: 19–96%), respectively.

2.2 Isolation of *Toxoplasma gondii* oocysts from faecal sample of cats

Dubey (1976) observed a longer prepatent period of 19 to 48 days in cats fed with mice infected for one or two days. A short prepatent period of 3 to 10 days was seen in cats fed mice infected for ten or longer days. Parental inoculation of tachyzoites, bradyzoites, sporozoites and oocyst ingestion followed a longer pre patent period.

Dubey *et al.* (1980) fed cats with caprine and equine tissues and observed the prepatent period of five days and two to ten days respectively.

Tiemann (1981) carried out an experiment in which a total of 1697 small rodents were trapped and their muscles were examined for sarcocysts and cystozoites before being fed to 27 dye-test negative cats. The cat faeces were then examined for oocysts and sporocysts. Only two cats shed oocysts of *T. gondii*. Ten cats were also fed diaphragm from 1278 slaughtered pigs to test the reliability of the cat-feeding test and all shed *T. gondii* oocysts 8–28 days after starting the feeding. Nine of the ten cats had positive dye-test titres four weeks after first excreting Toxoplasma oocysts.

Dubey and Johnstone (1982) observed death in three of seven littermate kittens attributed to neonatal toxoplasmosis. Histological studies were carried out and there was evidence of pneumonitis, hepatitis, myocarditis, encephalitis, and retinitis.

Frenkel and Smith (1982) observed that the most effective immunization procedures suppressing *T. gondii* oocyst shedding in 90 per cent of cats after homologous bradyzoites challenge with bradyzoites infections orally and tachyzoite injected subcutaneously. Almost all kittens in which primary infection had resulted in oocyst production were immune. Only 60 per cent of cats developed immunity after subcutaneous infection with sporozoites. Little immunity developed after infection with bradyzoites and tachyzoites from non-oocyst producing lines with sporulated oocysts given orally or from use of killed vaccines. Kittens that developed antibody but had not shed oocysts were rarely immune. Immunity to heterologous challenge was found in 90 per cent of cats that had previously shed oocysts. In those that had developed antibody only the proportion of immune, animals after first challenge (25%) increased to 71 per cent after the 2nd challenge.

Frenkel and Smith (1982a) conducted an experiment with monensin in the food at 0.02 per cent to prevent shedding of Toxoplasma oocysts in all of nine experimentally infected kittens. The medicated food was well tolerated and accepted.

Nakao *et al.* (1982) carried out latex agglutination test and showed that none of the12 cats less than a year were positive for antibody titers and 12 (19.3%) of 116 older cats were positive with antibody titers of 1:64 or higher.

Varga (1982) conducted a microscopic examination of faecal samples taken from 200 cats of various ages which revealed small numbers of *Isospora bigemina* like oocysts (10 X 12 μm diameter) in two kittens aged four and six months. The identity of the oocysts as *T. gondii*, or *H. hammondi*, was confirmed by oral infection of mice and further passage of cysts found in the brain of the mice after one month. By the same technique *T. gondii* cysts were detected in the diaphragm muscle from three of 50 randomly selected pig carcasses at a Budapest-abattoir in 1978-79.

Kuhlhorn (1983) suggested that T. gondii oocysts are probably distributed by flies, in the same way as has been observed with coccidial oocysts. The faeces of cats were particularly attractive to flies of 166 species belonging to 27 families of Diptera which were attracted to the faeces.

Chhabra *et al.* (1985) conducted indirect haemagglutination test on 3761 serum samples from nine species of animals and positive results were found (titres between 1:4 and 1:1024) in 25 per cent of 1227 sheep, 30 per cent of 961 goats, 12 per cent 0f 603 horses, 19 per cent of 243 cattle, 16 per cent of 108 buffaloes, 32 per cent of 178 pigs, 31 per cent of 175 dogs, 34 per cent of 80 cats and 10 per cent of 186 bandicoots.

Svoboda and Svobodova (1987) studied the occurrence of T.gondii antibodies in 620 cats and concluded that antibodies were less frequent in animals kept exclusively in flats, and more frequent in cats in the habit of catching rodents or other mammals and birds. It was observed that antibodies were less frequent in cats fed exclusively heat-treated food but more frequent in cats fed raw pork, beef and poultry meat.

Knaus and Fehler (1989) carried out the faecal examination of 264 stray cats by the floatation technique and did not detect the presence of T.gondii oocysts. They concluded that faecal examination did not reflect the true infection rate based on serological results. The proportion of cats disseminating the oocysts in this area was 40 per cent and most of them were aged less than one year.

Guevara *et al*. (1990) examined 200 cat faecal samples collected from different parts of Mexico City by the floatation technique. The result of faecal sample examination showed that no T.gondii oocysts were found in the faecal sample, eight contained Isospora felis oocysts, 26 contained Toxocara cati and Toxascaris leonina eggs. The previous survey in 1977 indicated that 7 of 200 cats in Mexico City excreted T.gondii oocysts.

Shastri and Ratnaparkhl (1992) examined fresh faecal samples of 9 cats from different parts of Parbhani and recovered coccidian oocysts of T. gondii, I. rivolta and I.felis. They infected three experimental kittens free from coccidial infection with the tissues of infected kittens and T.gondii oocysts were found in the faecal samples seven to eight days later.

Anaya *et al*. (1997) examined 362 faecal samples from cats belonging to different ages, breeds and sexes. The faecal sample were screened by Faust technique and 145 (40.0%) were positive for coccidian oocysts which were identified according to longitudinal diameter. Seventy nine cats (54% of those infected) had I. felis, 15 (10.9%) I. rivolta, one cat (0.7%) Besnoitia besnoiti, two (2.1%) H. hammondi, one (0.7%) Sarcocystis and five (3.1%) had T. gondii oocysts. Mixed infection was found in 39 cats (26.9%).

Ragozo *et al*. (2002) examined faecal samples of 138 stray cats in Brazil for the presence of gastrointestinal parasitic infection. Eighty (57.97%) of the faecal samples were positive. The most frequent protozoan to be detected was I. felis (26.09%) followed by I. rivolta (26.64%), C. parvum (1.45%) and Sarcocystis spp. (0.72%). Of the helminths, T. cati showed highest occurrence (31.16%), followed by Ancylostoma ova (8.70%). They observed mixed infection in 25 cats (18.12%), with T. cati, Isospora oocysts and T. cati and Ancylostoma ova.

Borkovcova (2003) conducted a study to determine the incidence of T. gondii and other intestinal parasites in the rural areas of Czech Republic. They examined 358 cat faecal samples and found that 3.4 per cent of them excreted oocysts measuring 10-13 µm diameter which were either Toxoplasma/Hammondia oocysts.

Dubey (2005) reported that the prepatent period varied with the stage of T. gondii ingested by the cat. The prepatent period after ingesting bradyzoites was short (three to ten days) while it was long (18 days or longer) after ingesting oocysts or tachyzoites, irrespective of the dose.

Dabritz *et al*. (2007) examined 326 faecal samples of cats by zinc sulfate faecal floatation of which only three (0.9%) faecal samples contained T. gondii like oocysts.

Haydee *et al*. (2007) examined 326 fecal samples collected from cat shelters, veterinary clinics, cat owning households and outdoor location by zinc sulfate solution floatation technique. Only three (0.9%) faecal samples of collected in Marro Bar area of California contained T. gondii like oocysts.

Hooshyar *et al*. (2007) conducted research by examination of 50 faecal, blood and tissue samples of stray cats in Kashan, Central Iran. IgG specific antibody to T. gondii was assessed using indirect fluorescent antibody test of which 86 per cent (43 of 50 cats) were positive. In the faecal sample examined no oocysts were detected by both direct and indirect methods.

Adams *et al*. (2008) conducted an investigation on gastrointestinal parasites present in feral cats in Christmas Island with particular reference to the T. gondii. They examined 28 faecal samples and none were positive for T. gondii. The gastrointestinal parasites detected were T. cati (present in 15 of 28 faecal samples), Strongyloides sp. (13/28), Aelurostrongylus abstrusus, (7/28), an unidentified capillarid (6/28) and Ancylostoma ova (4/28).Further serum samples of 25 cats were collected and serological examination was carried out. Based on serology T. gondii was the most common parasite detected with antibodies in 24 serum samples by IFA

and 23 serum samples by LAT. High seroprevalence of T. gondii in these cats indicated a high level of exposure to the parasite in that environment.

Schares *et al*. (2008) examined 24,106 faecal samples of cats from Germany and other European countries microscopically to know the prevalence of cats shedding T. gondii or H. hammondi oocysts. Oocysts of 9-15 µm size with morphology similar to that of T. gondii and H. hammondi were found in 74 samples (0.31%). A total of 54 samples were further characterized to achieve a specific diagnosis and to determine the genotype of T. gondii isolates by PCR and PCR-RFLP. T. gondii and and H. hammondi were found in 26 (0.11%) and 22 (0.09%) of the samples respectively.

Elmore *et al.* (2010) stated that within a month of initial infection by the parasite, maximum oocyst shedding occurs and is completed within 21 days of infection most of the times.

Amany *et al*. (2012) examined 100 samples of cat faeces for presence of T. gondii oocysts using Sheather's sugar flotation and reported that the prevalence of T. gondii oocysts was two per cent at Sharkia Province in Egypt.

Veronesi (2016) detected intestinal parasites microscopically in 26 cat faeces (33.33%), e.g. Cystoispora felis and Toxocara cati in nine cats (11.54%), respectively, Giardia duodenalis in eight cats (10.26%), mixed infections were recovered between C. felis and G. duodenalis (n = 1, 1.28%) and between T. cati and C. felis (n = 1, 1.28%).

Montazeri *et al.* (2020) The global pooled seroprevalence of toxoplasmosis was equal (33%, 95% CI: 29–37%) in male and female domestic cats and the difference between them was not significant. examined the faecal samples collected from 160 cats, aged two months to 16 years old. They reported that no oocyst of T. gondii was found in faeces but when indirect immunofluorescence test was carried out on the same cats, 37 per cent were positive for IgG and none for IgM detection.

Nasiru Wana(2020) Using copro-microscopy, 200 cat faeces were screened for the presence of T. gondii-like oocysts, out of which 7/200 (3.5%) were detected as positive. More T. gondii-like oocysts were found in Free roaming cats (5/100, 5.0%) compared to Petcats (2/100, 2.0%).

2.3 Toxo IgG Rapid test kit- Lateral immune-chromatography

Dubey and Frenkel, (1972) stated that in experimental infections, cats usually stop shedding T. gondii oocysts by the time they seroconvert. 30 percent of cats in the present study were seropositive, they had probably shed oocysts and contaminated the environment.

Chhabra (1985) did a serological survey of latent Toxoplasma prevalence on 3761 animals in northern India by the microtitre indirect haemagglutination test, 23.7 per cent were found to have antibody titres ranging from 1:4 to 1:1024. Seropositivity was recorded in 25.3 per cent of 1227 sheep, 30.3 per cent of 961 goats, 11.8 per cent of 603 horses, 19.3 per cent of 243 cattle, 15.7 per cent of 108 water buffaloes, 31.5 per cent of 178 pigs, 30.9 per cent of 175 dogs, 33.7 per cent of 80 cats and in 9.7 per cent of 186 bandicoot rats.

Javadi *et al.* (2010) detected antibodies of *Toxoplasma gondii* 50 cats using an enzyme-linked immunosorbent assay. Immunoglobulin M (IgM) and immunoglobulin G (IgG) were measured. Fifteen (30%) of the cats were found to be seropositive.

Lilly and Wortham (2013) stated that while serological testing may be applicable for determining parasite exposure, it is likely to vastly overestimate human health risk from cats.

Sah *et al.* (2018) carried out study to determine the seroprevalence of *Toxoplasma gondii* in sheep, cattle, cats and human in Inaruwa and surrounding areas of Sunsari

district, Nepal. Altogether 336 blood samples, of which 50 from sheep, 92 from cattle, 44 from cats and 150 from human were collected and tested immediately using lateral flow chromatographic immunoassay (Toxo IgG/IgM Combo Rapid test®). Seroprevalence of toxoplasmosis was detected 12.00% (95% CI: 4.53- 24.31%) in sheep, 8.70% (95% CI: 3.83- 16.42%) in cattle, 36.36% (95%CI: 22.41- 52.23%) in cats and 12.67% (95% CI: 7.80- 19.07%) in human.

2.4 Polymerase chain reaction (PCR)

Lappin *et al.* (1996) carried out PCR in the aqueous humour of cats by targeting *T. gondii* B1 gene. They detected *T.gondii* in aqueous humour by PCR from two (8.7%) of 23 healthy cats and eight (18.6%) of 43 cats with uveitis. They suggested that PCR is a novel method for detection of *T.gondii* in clinical samples.

Samad *et al*. (1996) conducted research to improve the DNA purification methods from *T. gondii* tachyzoites and tissue specimens for amplification. They observed no difference in the results of electrophoresis of the extracted DNA samples with or without RNAse in tachyzoites samples. They established a PCR which was sensitive enough to detect 100pg of *T. gondii* DNA.

Homan *et al*. (2000) used PCR technique based on amplification of non-coding 529 bp DNA fragment in *T.gondii* genome and described it as a very sensitive method for the diagnosis of toxoplasmosis in clinical samples. The assay was reported to be more sensitive than B1 PCR and allowed a fair estimation of brain cyst numbers.

Sroka *et al.* (2018) Faecal samples of 41 cats were examined for the presence of oocysts/DNA *Toxoplasma gondii* by microscopy and Real-time/nested PCR. After flotation (with $NaNO_3$), pellets from faecal samples were disrupted by 10 cycles of freezing (liquid nitrogen) and warming. Among the 41 faecal samples examined, the presence of structures resembling *Toxoplasma gondii* oocysts was found in 2 samples

(4.9%), and for one of these samples (2.4% of the total) the result was also confirmed by PCR.

Switaj *et al*. (2005) reviewed the recent molecular diagnostic methods for *T. gondii* infections and suggested that multicopy sequence specific for *T. gondii*, e.g., the B1 gene or the 529-bp sequence, are especially useful in molecular tests.

Belli *et al.* (2006) suggested that in the external environment, sporozoites are protected from desiccation and chemical disinfection by the oocyst wall. This unique structure guarantees successful disease transmission and is as vital to the coccidian parasite as the exoskeleton is to insects without which they would die. They reviewed the early work and combined it with newer molecular data to describe the present understanding of the coccidian oocyst wall.

Salant *et al.* (2007) conducted a PCR method to detect *T. gondii* oocysts DNA in faecal samples. They extracted the DNA from oocysts by successfully disrupting them by freeze thawing coupled with mechanical means. The test based on amplifying a 529 bp repeated sequence proved sensitive for detecting one to two oocysts in 200 µg of stool sample. CoproPCR of 122 faecal samples from cats of Jerusalem revealed that 11 were positive for Toxoplasma oocysts which were negative by microscopy.

Kelly *et al*. (2009) studied in detail about the oocyst wall formation and composition in coccidian parasites. The study revealed that the oocyst wall had more than 90 per cent protein of which few of the proteins were studied. The oocyst wall was resistant to a variety of environmental and chemical insults. This resilience allowed oocysts to survive for long periods, facilitating transmission from host to host. The bilayer wall formed by the sequential release of the contents of two specialized organelles - wall forming body one and wall forming body two was found in the microgametocyte stage of coccidia. The oocyst wall had high levels of dityrosine bonds. These

dityrosine crosslinked proteins provided a structural matrix for assembly of the oocyst wall and contributed to its resilience.

Herrmann *et al*. (2010) collected a total of 18,259 feline faecal samples from cats in Germany collected and analyzed for the presence of *T. gondii* oocysts between June 2007 and December 2008. Forty-six *T. gondii* positive samples were genetically characterised using nine PCR-restriction fragment length polymorphism (RFLP) markers which included newSAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico. DNA from oocysts was extracted as subjected to thaw freeze (-20ºc, 10 min)/thaw (room temperature, two min) cycles. The DNA was extracted with 500 µl phenol/chloroform/isoamylalcohol (in a 25:24:1 ratio) until the supernatant was without visible contaminants

Mancianti *et al.* (2010) studied the seroprevalence, oocyst shedding rate and presence of *T. gondii* DNA in faeces collected from an urban population of colony cats in Florence (Tuscany). Fifty European shorthair feral cats were examined for anti-*T gondii* specific antibodies by a modified agglutination test (MAT), and for oocysts by microscopic examination and for faecal protozoal DNA, by means of a nested polymerase chain reaction (n-PCR) protocol. Twenty-two out of 50 serum samples (44%) were MAT positive. *Toxoplasma gondii* oocysts were not detected in any of the faecal samples examined. Eight out of 50 faecal specimens (16%) were n-PCR positive and sequencing of the bands was specific for *T. gondii*. Detection by combination of the two methods was higher than single technique and enhanced the detection of *T. gondii* up to 48 per cent.

Salant *et al.* (2010) compared three coprological diagnostic methods for detection of *T. gondii* in cats. The three methods included microscopy, bioassay and copro PCR. They reported that detection by microscopy was positive occasionally while copro-PCR and bioassay positive results were obtained continuously from two to 24 days post infection. Copro-PCR was considered as a new gold standard for determining

potential cat infectivity. Its technological advantage over bioassay makes it superior for large-scale screening.

Berger *et al*. (2011) undertook a study to determine the prevalence of oocyst shedding by cats and to assess the level of infection with *T. gondii* in meat-producing animals in Switzerland via detection of genomic DNA (gDNA) in muscle samples. A total of 252 cats (44 stray cats, 171 pet cats, 37 cats with gastrointestinal disorders) from January 2007 and August 2008 were analyzed coproscopically (flotation method with 44 per cent zinc chloride solution (w/v) with a specific gravity of 1.3), and subsequently species-specific identification of *T. gondii* oocysts was achieved by polymerase chain reaction (PCR). gDNA was subsequently isolated from purified oocysts with the DNeasy Kit according to the manufacturer's instructions (Qiagen, Switzerland). They reported that only one of the cats shed *T. gondii* oocysts, corresponding to a *T. gondii* prevalence of 0.4 per cent.

Kumar *et al.* (2011) obtained a positive signal of PCR assay on a 200-300 fold repetitive 529 bp for the efficacy in amplifying the DNA of theoretically lowest number of tachyzoites of *T. gondii*, RH strain. They reported that PCR assay was found sensitive in detecting as low as 9.75 pg of DNA equivalent of 78 tachyzoites of *T. gondii* and suggested that the assay may be tried on clinical samples.

Weifeng *et al*. (2011) determined the seropositivity, distribution of genotypes and mouse virulence of *T. gondii* from stray cats in Beijing, China. A total of 64 serum samples, 23 faeces and tissue samples were collected from stray cats in Beijing. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT). 57.8 per cent (37/64) of these stray cats had titers of 1:20 or higher and were considered positive with infection. *Toxoplasma gondii* oocysts were not found in faeces of the 23 cats (microscopy after Sheather's sugar flotation). Tissues of 23 cats were bioassayed in mice and 11 *T. gondii* isolates were obtained.

Emily and Caroline (2013) studied the prevalence of Toxoplasma in cat populations by PCR detection in cat faeces to more accurately estimate the numbers of cats. DNA

sequencing was used to confirm the identity of the PCR products. Of the 49 cats tested, nine yielded PCR products of the expected size. Six out of nine were determined by sequence analysis to be false positive while three products were true positives. Overall, six per cent of cats examined were found to be actively shedding oocysts. They detected false positives at a higher rate than true positives, emphasizing the need for confirmatory analysis.

Fuller and Robert (2013) reviewed about the Toxoplasma as a public health problem. The author suggested that waterborne outbreaks of *T. gondii* have focused attention on the importance of oocysts shed in the faeces of infected cats. Cat faeces deposited annually into the environment in the United States was approximately 1.2 million metric tons. The annual oocyst burden measured in community surveys was three to 434 oocysts per square foot and was greater in areas where cats selectively defecated. Because a single oocyst can possibly cause infection, this oocyst burden represented a major potential public health problem. The proper disposal of cat litter, keeping cats indoors, reducing the feral cat population, and protecting the play areas of children was suggested to reduce the oocyst burden.

2.5 Clinical observations of cats associated with Toxoplasmosis

Dubey and Frenkel (1972) infected felines with Toxoplasma oocysts by oral and subcutaneous route and noted that newborn kittens were highly susceptible to acute infection and none of the adult cats showed clinical signs. There was less infectivity, pathogenicity and lesions in kittens fed orally with cysts than by the subcutaneous route. Ante-mortem diagnosis of clinical toxoplasmosis in cats is rare. Cats usually shed T. gondii oocysts for 1 to 2 wk, and they rarely have clinical signs of disease during the oocyst shedding period. Cats usually develop T. gondii antibodies after the cessation of oocyst shedding.

Svoboda and Svobodova (1985) carried out an experiment on stray cats out of which a pregnant cat showed symptoms of bristled hair, slight conjunctivitis, occasional sneezing and moaned frequently. On the fifth day it delivered apparently healthy kittens. All were sacrificed and examined for toxoplasmosis. Sabin- Feldman, Complement fixation and microprecipitation tests were positive in all cats. Oocysts were found in the faeces of the mother and two kittens. Toxoplasmosis developed in mice inoculated with mixed samples of brain, liver and spleen from all 4 cats.

Lappin *et al.* (1996) noted behavioural changes in 3/11 cases (27.27%) and anorexia in 4/11 cats (36.36%). Icterus was seen in 2/11 cats(18.18%) and respiratory distress in 5/11 cases(45.45%).

Dubey *et al.* (2009) observed that clinical signs reflect inflammation of the liver, lungs, and CNS. Affected kittens may have an enlarged abdomen because of enlarged liver and ascites. Encephalitic kittens may sleep most of the time or cry continuously. Anorexia, lethargy, and dyspnea due to pneumonia have been commonly recognized features of postnatal toxoplasmosis. Other clinical signs include persistent or intermittent fever, anorexia, weight loss, icterus due to hepatitis or cholangiohepatitis, vomiting, diarrhea, abdominal effusion, hyperesthesia on muscle palpation, stiffness of gait, shifting leg lameness, dermatitis, loss of vision, and neurologic deficits. In 100 cats with histologically confirmed toxoplasmosis, clinical syndromes were

diverse but infection of pulmonary (97.7%), CNS (96.4%), hepatic (93.3%), pancreatic (84.4%), cardiac (86.4%), and ocular (81.5%) tissues were most common. Clinical signs may be sudden or may have a slow onset. The disease may be rapidly fatal in some cats with severe respiratory or CNS signs. Anterior or posterior uveitis involving one or both eyes is common. Iritis, iridocyclitis, or chorioretinitis can occur alone or concomitantly. Aqueous flare, keratic precipitate, lens luxation, glaucoma, and retinal detachment are common manifestations of uveitis. Chorioretinitis may occur in both tapetal and nontapetal areas. Ocular toxoplasmosis occurs in some cats without polysystemic clinical signs of disease.

Lappin (2010) noted that when *toxoplasma gondii* cyst is fed in experimental infections, self-limiting small intestinal diarrhoea is seen for one to two weeks after initial feeding. This phenomenon is observed in only 10% to 20% of cats.

Barrs (2013) observed affected cats for the clinical symptoms and followed up on treatment. Clinical disease occurs in a small proportion of cats during primary infection and in reactivated latent infections where immunosuppression induces cyst rupture, tachyzoite replication and tissue damage. Risk factors are FIV, FeLV or FIP, neoplasia and chronic administration of cyclosporin. Disease can be acute or chronic and focal or systemic. Hepatic, pulmonary, CNS and pancreatic involvement is present. Clinical signs in acute systemic infection can include lethargy, anorexia, pyrexia or hypothermia, dyspnoea (pulmonary, pleural space or cardiac involvement), jaundice, abdominal distension (peritoneal effusion) and diffuse or multifocal CNS signs. Acute infections are often fatal, especially in kittens born to queens infected during pregnancy. Focal infections include uveitis, chorioretinitis and focal CNS infection. Using ELISA tests, positive IgM titres develop in 80% of cats 1 to 4 weeks post infection and are usually negative by 16 weeks after infection. Positive IgG titres develop 3 to 4 weeks post infection and peak 2 to 4 weeks after initial detection. In recrudescent infections, there may be no rising IgG titre and IgM titres may remain negative.

Dubey and Prowell (2013) observed A 6-mo-old domestic male cat was hospitalized because of lethargy, anorexia, fever, and diarrhoea. Numerous (6 million in 1 sample) *T. gondii* oocysts were found in feces of the cat and antibodies to *T. gondii* (titer 1:800) were found in its serum by the modified agglutination test. The cat was medicated orally with Clindamycin for 10 days; it became asymptomatic after 10 days and was discharged from the hospital.

Evans *et al.* (2017) described acute respiratory distress syndrome (ARDS) and septic shock in a cat with disseminated toxoplasmosis. A 2-year-old neutered male domestic shorthair cat was presented for acute respiratory distress which was receiving cyclosporine for treatment of eosinophilic dermatitis. Thoracic radiographs revealed severe mixed nodular interstitial and alveolar patterns. An endotracheal wash was performed, which confirmed a diagnosis of pulmonary toxoplasmosis. Despite initial treatment with oxygen supplementation and intravenous clindamycin, the cat developed refractory hypoxemia and hypotension requiring mechanical ventilation and vasopressor support within 24 hours of hospital admission. Cardiac arrest occurred 56 hours after admission. Necropsy was performed and histopathology revealed protozoal organisms disseminated throughout the heart, lungs, liver, and brain. The clinical and necropsy findings presented here are consistent with ARDS secondary to disseminated toxoplasmosis in a cat.

Desmettre (2020) noted that all the elements increasing the risk of predation by the definitive host are then favourable to the parasite. Numerous experimental animal model studies have shown that T. gondii infection is associated with predatory risk behaviours such as an attraction of infected mice to cat urine. Infection with the parasite is associated with a demethylation of the promoters of certain genes in the cerebral amygdala of the intermediate hosts, modifying dopaminergic circuits associated with fear. Similarly, T. gondii has been linked to behavioural changes in humans. Toxoplasma infection is classically associated with the frequency of schizophrenia, suicide attempts or "road rage.

2.6 Hematobiochemical alterations

Advincula *et al.* (2010): tested 60 cats anti-*Toxoplasma gondii* antibodies using the latex agglutination test in Sta Rosa and San Pedro, Laguna, Philippines. Results revealed that 28 (46.67%) of the 60 cats were seropositive. There were more household cats (28.33%) which showed seropositivity compared to stray cats (18.33%), however the difference was statistically insignificant ($p>0.05$) . Hematologic tests through complete blood count showed significantly ($p<0.05$) higher number of seropositive cats with abnormalities on hemoglobin level, red blood cell count, segmenter (neutrophil) and monocyte counts compared to the control. Other parameters such as percent packed cell volume, white blood cell count, eosinophil and lymphocyte counts showed insignificant ($p>0.05$) results across seropositive cats and the control. Blood chemistry analysis showed significantly higher ($p<0.05$) potassium level irregularities in seropositive cats relative to the seronegative cats. Other parameters such as amylase, blood sugar, blood uric acid, creatinine and blood urea nitrogen were statistically insignificant ($p>0.05$).

Javadi *et al.* (2010) detected antibodies of *Toxoplasma gondii* 50 cats using an enzyme-linked immunosorbent assay. Comparison of haematological data between two groups of the cats (IgM <1/64, n=35; IgM ≥1/64, n=15) showed that packed cell volume (PCV), red blood cell (RBC) and monocyte values in cats with higher IgM titres were significantly higher than cats with lower IgM. It is suggested that in cases with higher values of PCV, RBC and monocyte in routine complete blood count profile of apparently normal cats, toxoplasmosis should be considered.

Jokelainen *et al*. (2012) concluded that marked elevations in the liver enzyme alanine aminotransferase (ALT) i.e. 1000 U/L is a consistent finding in cats with generalized toxoplasmosis Among 193 cats submitted for necropsy during a 3.5-year period, 6 (3.1%) had been diagnosed with generalized toxoplasmosis and were retrospectively further investigated. The main pathological lesions included acute

interstitial pneumonia, acute necrotizing hepatitis, and nonsuppurative meningoencephalitis with glial granulomas.

Cucos *et al*. (2015) observed the common changes in routine hematology include anemia, typically increased neutrophils, lymphocytes and eosinophils; while the changes in serum biochemistry screening in some cases showed liver dysfunctions, hyperglobulinemia. Routine haematology and serum biochemistry tests were not specific and the results depend on the extent of systemic involvement. Ophthalmological signs were observed in 19 of the 22 cases examined (86 %), representing the most frequent clinical manifestation. In three out of 22 cases, the ocular signs were associated with neurological signs, such as seizures, behavioural and personality changes. The common changes in routine hematology include anemia, typically increased neutrophils, lymphocytes and eosinophils; while the changes in serum biochemistry screening in some cases showed liver dysfunctions, hyperglobulinemia.

Cohen *et al.* (2016) examined a 22-month-old indoor/outdoor neutered male domestic short-haired cat had a history of progressive lethargy, vomiting, and decreased appetite. Abdominal ultrasound revealed an irregular hyperechoic mass in the mid-abdomen. He was unresponsive to symptomatic medical management and was euthanized after 3 days of hospitalization. A diagnosis of disseminated extraintestinal toxoplasmosis was made based on the finding of intracytoplasmic protozoan parasites on histopathological examination of mesenteric lymph nodes, hepatic and intestinal samples, and on immunohistochemistry. Nonregenerative anemia, neutrophilic leukocytosis, lymphocytosis, monocytosis, and eosinophilia, in addition to a hypoproteinemia and hypoalbuminemia was found.

Veronesi (2016) tested 78 cats , Sixteen faeces (20.52%) tested positive for T. gondii DNA; 12 samples were positive only at B1-PCR, two at 529-bp RE-PCR and two at both genetic targets (overall agreement = 82.11%).Two stool samples were microscopically positive for T. gondii-like oocysts and also tested positive by both

B1 and 529-bp RE-PCRs and 4 (5.13%) with the 529-bp RE amplification. Thirty-three (42.3%) sera tested positive for antibodies; of which, seven were found to have T. gondii DNA-positive results using the B1 genetic target (overall agreement = 57.77%).

Bastan and Bulent(2018) studied serologically positive cats by ELISA and observed Anaemia (decreased Hb, RBC, PCV) in 8 cats (57.1%), monocytosis in 6 cats (42.8%), neutrophilia in 5 cats (35.7%), hypoalbuminemia in 5 cats (35.7%) and increased AST and ALT levels in 3 cats (21.4%) were detected.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

The present study was conducted to study the prevalence of toxoplasmosis in cats. Investigations comprised of screening of 106 cats irrespective of clinical signs and symptoms by fecal microscopy. Serology and confirmation of cases by Polymerase chain reaction was performed in selected 51 samples. Hematobiochemical alterations were assessed in positive cases.

3.2 Place of research work

Present research work was carried out in the Department of Veterinary Epidemiology and Preventive Medicine and Department of Veterinary Pathology Krantisinh Nana Patil College of Veterinary Science, Shirwal, District-Satara in addition to Disease Investigation Section, Pune, Government of Maharashtra.

3.3 General material

3.3.1 Glassware and plastic ware

All the glassware and plastic ware used during present study were obtained from Borosil Ltd, Mumbai (India), HiMedia Pvt Ltd. (India). All the glassware and plastic were properly cleaned, dried and sterilized prior to use for the research work.

3.3.2 Chemicals, Buffers and Reagents

All the chemicals, buffers used during course of study were standard and of molecular grade which were obtain from HiMedia Pvt Ltd. (India).

3.3.3 Equipments

All required scientific instruments and equipments like Micropipettes, Incubator, thermocycler, Gel electrophoresis, Weighing balance and Water bath, were available in Department of Veterinary Epidemiology and Preventive Medicine, Department of Veterinary Pathology of Krantisinh Nana Patil College of Veterinary Science, Shirwal and Disease Investigation Section, Pune, Government of Maharashtra were used for carrying out the present research study.

3.4 Methods

3.4.1 Collection of fecal, blood and serum samples

The fecal samples of cats were collected in Shirwal, Mumbai and Pune area from Teaching veterinary clinical complex, Shirwal, Veterinary clinics, Animal Rescue Trusts, owned cats and stray cats. Samples were collected within twelve hours after defecation. Massive shedding of oocyst occurs irrespective of clinical signs hence samples were collected even without presence of any clinical symptoms or age. (Plate3.1)

Table 3.1: Source wise details of sample collected

Place of collection	No. of samples collected
Animal shelters (Stray Cats)	61
House hold cats (Pet)	45
Total	106

Collected fecal samples were subjected to fecal floatation method and results were noted. Blood samples were collected from the cephalic vein of cats in K3-EDTA vials and transported to the laboratory under chilling conditions. These

blood samples were subjected for haematobiochemical studies and rapid test kits. Fecal samples were stored at -20^{0}C till further use. Data related to age, sex, location were collected on sampling day. (Plate 3.2)

3.4.2 Collection of fecal sample

- Sterile 30 ml containers
- Pair of gloves
- Collection sticks

3.4.2. Fecal flotation with the use of Sheather's solution

3.4.2. (a) Material

- Double distilled water
- Centrifuge tube
- Clean grease free slide and coverslip
- Glass rod
- Sheather's solution
- 3.4.2. (b) Method

    1. Three grams of fecal sample was taken and thoroughly mixed with 15 ml of double distilled water to dissolve particles in centrifuge tube.
    2. Centrifuge tubes were spun @1500rpm for 10 minutes in fixed angle centrifuge.
    3. Supernatant was discarded and sediment was resuspended in Sheather's solution(106gm sucrose, 1000ml ddw, 0.8ml phenol with specific gravity 1.26)

4. Tube was spun @2000 rpm for 10 minutes and supernatant was separated for microscopic examination and D.N.A. isolation.
5. Sample was taken on glass slide and covered with coverslip and visualized under 100X and 400X.(Dubey JP and Beattie CP.,1988)

3.5 Toxoplasma IgG Antibody detection by rapid Test kit (PetX Toxo Ab)

The Toxoplasma IgG antibody rapid test (PetX Toxo Ab) is a test cassette to diagnose the presence of anti-Toxoplasma IgG in animal's blood specimen Assay Time: 5-10 minutes

- Specimen: Serum, plasma
- PRINCIPLE-The Toxoplasma IgG Antibody Test is based on sandwich lateral flow immunochromatographic assay.
- Reagents and materials-Test devices, Disposable capillary droppers, Assay buffer
- Test procedure-

1. All materials, including specimen and test device were allowed to recover to 15-25^{0}C before running the assay. Test device was placed horizontally.
2. Using the capillary dropper 1 drop of the prepared specimen was placed into the sample hole "S of the test device
3. Then 2 drops (approx. 80 µL) of the assay buffer were poured into the sample hole immediately.
4. Test results were interpreted in 5-10 minutes. Result after 10 minutes is considered as invalid.

Interpretation of results

1. IgG Positive (+): The presence of "C" line and "IgG" line, which indicates that the animal has been contracted to *Toxoplasma gondii*.
2. Invalid: No coloured line appears in C zone. No matter if IgG line appears.

3.6 Diagnosis by molecular methods

3.6.1 Genomic DNA extraction

3.6.1 (a) Materials:

- Microcentrifuge tubes (HiMedia)
- Micropipettes
- Micro tips
- Microcentrifuge
- Vortex Mixer (genei)
- Water bath
- Liquid nitrogen
- Glass beads

The genomic DNA was extracted from the supernatant which was separated after floatation of fecal sample from cats using the MB544 HiPurA® Stool DNA Purification Kit from HiMedia Laboratories Pvt. Ltd., Mumbai as per their recommended standard protocol with slight modifications.

3.6.1(b) List of Materials provided in kit

Table 3.2: MB544 HiPurA® Stool DNA Purification Kit component

Reagent provided	Quantity
• TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0)	75ml
• Lysis Solution (AL)	30ml
• Stool Lysis Buffer (SL1)	15ml
• Inhibitor Removal Solution (IRSH)	20ml
• Wash Solution Concentrate (WSP)	30ml
• Binding Solution (SB)	15ml
• Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	15ml
• Proteinase K	25mg
• RNase A Solution (20 mg/ml)	1.25ml
• HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	50
• Collection Tube(Uncapped), Polypropylene (2.0 ml)	50
• Collection Tube, Polypropylene (2.0 ml)	100

3.6.1(c) Procedure for extraction of DNA

1. The supernatant collected in the step of fecal flotation by Sheather's solution was taken; 200µl supernatant was mixed with 900 µl of T.E. buffer and spun at 2500×g for 15 minutes to remove sugar solution.
2. Supernatant was discarded and pellet was used for mechanical disruption of oocyst.
3. The 500 µl lysis solution provided in the kit was mixed with the pellet and equal volumes of glass beads were added.
4. Vortexing of sample was performed for 30 minutes with intermittent freeze-thawing every 10 minutes.(Salant *et al.*,2007).
5. 1 minute dip in liquid nitrogen for freezing and 1 minute dip at 60ºC for thawing was performed.
6. 200µl supernatant was collected in new 2 ml capped tube.

7. **Lysis**
   To 200 µl of resuspended solution 20 µl of the Proteinase K Solution (20 mg/ml) was added and mixed by vortexing and incubated for 30 minutes at 55°C.
8. To obtain RNA-free genomic DNA, 25 µl of RNase A solution (DS0003) added, mixed, and incubated for 5 minutes at room temperature (15-25°C).
9. Lysis - 200 µl of Stool Lysis Buffer (SL1) (DS0085) added and vortexed thoroughly (about 15 seconds) and incubated at 70°C for 10 minutes.
10. Inhibitor removal - 250 µl of Inhibitor Removal Solution (IRSH) (DS0066) added vortexed for few seconds and incubated at 4°C for 5 minutes.
11. Centrifuged the tube for 1 minute at 10,000 x g (12,000 rpm) at room temperature.
12. Binding- Transferred the supernatant to a clean collection tube (2.0 ml), added 200 µl of Binding Solution (SB) (DS0067) and vortexed for few seconds.
13. Load onto HiElute Miniprep Spin Column (Capped) [DBCA03] - Loaded the lysate on the HiElute Miniprep Spin Column (capped) and centrifuged for 1minute at 10,000 x g (12,000 rpm) at room temperature. Discarded the flow-through.
14. Added 500 µl of diluted Wash Solution (WSP) (DS0019) and centrifuged at 10,000 x g (12,000 rpm) at room temperature for 1 minute. Discarded the flow-through. Repeated the wash step one more time.
15. Flow-through was discarded and centrifuged the HiElute Miniprep Spin column (Capped) at 10,000 x g (12,000 rpm) at room temperature for an additional 1 minute to remove any residual ethanol.
16. DNA Elution- Transferred the column to a fresh uncapped collection tube 2.0 ml and added 200 µl of Elution Buffer (ET) (DS0040) directly onto the centre of the column membrane. Centrifuged the tube for 1 minute at 10,000 x g (12,000 rpm) at room temperature.
17. Transferred the eluate to a fresh capped 2ml collection tube for longer DNA storage and kept at -20ºC.

3.7 Molecular method

3.7.1 Polymerase Chain Reaction (PCR)

For confirmatory diagnosis of toxoplasmosis, total 51 samples were processed. Polymerase Chain Reaction (PCR) assay was used on samples positive by fecal microscopy, rapid test kit and clinically suspected cases. This technique is too expensive to be used widely; it is more and appropriate for differential diagnosis. All the chemicals and reagents required for Polymerase Chain Reaction (PCR) were supplied by HiMedia Pvt Ltd. (India).

3.7.2 Details of the primers used in PCR

The Tox4, Tox5 primers (Burg *et al.1989*) were used for molecular detection of DNA R529 bp fragment that has 200-300 copies in genome, internal transcribed spacer – 1. For the present study; the primers were procured from BioResource Biotech Pvt. Ltd.

Table 3.3: Synthetic oligonucleotide design of the primers:

Primer name	**Sequence 5'-3'**	**Target Length bp**	**Reference**
TOX4	(50-CGCTGCAGGG AGGAAGACG AAAGTTG-30)	529 bp	Burg *et.al.* (1989)
TOX5	(50-CGCTGCAGAC CAGTGCATCTGGATT-30)		

3.7.3 DNA amplification

The isolated DNA samples were subjected to 529 bp fragment that is repeated 200- to 300-fold in the genome of *Toxoplasma gondii* (Homan 2000). Details of primers are given in Table 3. The PCR reaction was carried out in 25µl volume mixture including of 12.5µl 2x PCR-Master-Mix (0.05 units/µl Taq DNA Polymerase in reaction buffer, 4mM MgCl2, 0.4mM dATP, 0.4mM dCTP, 0.4 mM dGTP and 0.4 mM dTTP (HiMedia). To formulate a final concentration of 1X, 1 µl of forward and reverse primers (10pmol/µl), 5 µl of DNA template, and nuclease free water was added to make 25 µl final volumes. The DNA amplification reaction was performed in a Prima-Duo Thermal Cycler (HiMedia) with a preheated lid. (Plate 3.3)

Table 3.4: Steps and conditions of thermal cycling

Primers (Forward and Reverse)	Cycling conditions **(Hanafiah *et al.* 2018)**				
	Initial denaturation	Denaturation	Annealing	Extension	Final Extension
Tox4 (Forward) Tox5 (Reverse)	94^{o}C 7 minutes	95^{o}C 1 minute	60^{o}C 1 minute	72^{o}C 1 minute	72^{o}C 10 minutes
		Repeated for 35 cycles			

3.7.4 Electrophoresis of PCR products

After completion of PCR, the PCR products were analyzed and confirmed by Horizontal gel electrophoresis (HiMedia Laboratories Pvt. Ltd.)(Plate 3.4) The gel casting tray was placed on a leveled surface and gel comb was then placed across the gel casting tray, so that the teeth of the comb remains 1 mm above the base of the tray. The agarose gel was prepared and electrophoresis was performed as below:

1. Agarose gel was prepared by dissolving 550 mg Agarose (1.1%) in 50 ml of 1x Tris Acetate- EDTA (TAE) in a glass flask and mixed it carefully in a microwave and then again repeated until clear solution was formed.
2. After cooling to about 50 °C, 2.7 µl of Ethidium bromide (HiMedia, India) was then added at the base of the flask and mixed thoroughly. It was allowed to cool before solidification and was poured on a plastic tray fixed on a gel caster containing a plastic comb for the wells formation.
3. The molten agarose was then poured onto the gel casting tray and was kept undisturbed for about an hour to solidify. After the solidification of gel the comb was removed.
4. The solidified gel with the gel casting tray was then submerged in the electrophoresis tank with the wells at the cathode end of the tank with sufficient quantity (about 1 mm)of electrophoresis buffer (TAE, 1 X) above the surface of the gel.
5. About 10 µl of PCR product was mixed with 2 µl of Bromophenol blue gel loading dye (6X) and was loaded in to the wells.
6. Simultaneously, 100 bp DNA ladder (HiMedia India Pvt. Ltd) was loaded in one of the wells as a molecular weight marker.
7. Electrophoresis assembly was run at 140 volts and the progress of mobility was monitored by the forward migration of dye. (36 minutes).

3.7.5 Detection and identification of PCR product

Gel was visualized under a UV transilluminator and photographed by HiMedia UV transilluminator cover system using camera. Visible band of appropriate size of 529 bp for *Toxoplasma gondii* were considered positive.

3.8 Hematobiochemical studies

Hematological estimations were carried in animals found positive for toxoplasmosis by fecal flotation and PCR and antibody detection kit. The blood samples were collected from cephalic vein of the cat in the K3-EDTA vials for haematology and sterile vials for serological studies. They were analysed for hematological parameters such as hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) using automated hematology analyzer HORIBA ABX Micros ESV 60. Differential leukocyte count (DLC) performed as per the methods described by Benjamin (2001). Biochemical parameters were evaluated such as B.U.N., Creatinine, Total Bilirubin, SGPT, ALP and Total proteins using reagents manufactured by ERBA Scientifics Pvt. Ltd., Germany. Trivitron healthcare biochemical analyser was used.

3.8.1 Differential Leucocyte Count (DLC)

3.8.1. (a) Preparation of Blood smear

1. Drop of blood was placed on clean, grease free slide.
2. Drop was spread using edge of another slide at an angle of $45^{0.}$
3. Slide was allowed to dry and labelled.

3.8.1. (b) Staining of blood smear (Leishman's staining)

1. Blood smeared glass was kept on flat surface and 5-10 drops of Leishman's stain was poured and kept for 1-2 minutes
2. Double quantity of distilled water was added and mixed by blowing of air.
3. Diluted stain was kept for 10 minutes.
4. Slide was washed under running water to remove excess stain.
5. Slide was examined under oil immersion (100X) objective.

3.8.1. (c) Examination of slide

1. Slide was observed low power as well as oil immersion lens with cedar wood oil.

2. Counting was done for at least 100 leucocytes by battlement method i.e. count 4 fields horizontally, then 4 fields to the inside of slide, 4 fields again horizontally, 4 fields to the outside of slide and then repeat.

3.9 Statistical analysis

The data collected for various parameters were statistically analyzed by using standard methods described by Snedecor and Cochran (1994). All values in the text are expressed as percentage (%) and mean ± S.E. SPSS 16.0 software was used for statistical analysis.

Plate 3.1: Study animals in animal shelters

Plate 3.2: Fecal sample collection

Plate 3.3: Thermal cycler

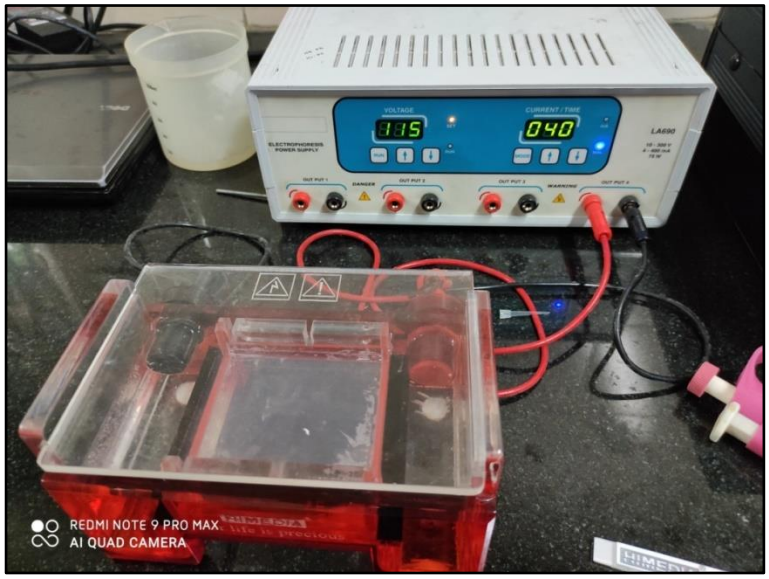

Plate 3.4: Gel electrophoresis used during the study

b

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The prevalence of *Toxoplasma gondii* oocysts in cats in Pune city, Mumbai and Shirwal area was studied and the results of the study are presented below.

4.1 Prevalence by copro microscopy

4.1.1 Prevalence of *Toxoplasma gondii* oocysts in cat faecal sample

Out of 106 faecal samples examined, 3(2.83%) samples were found positive for *T.gondii* like oocysts by Sheather's floatation technique. One sporulated oocyst (Plate 4.1) and two unsporulated oocysts (Plate 4.2) were found. An oocyst which resembled morphological structure of *Toxoplasma gondii* were found in 4.91% of faecal samples collected from the animal shelters (Table 4.1, Figure 4.1). No oocysts were detected in faecal samples collected from household cats by copromicroscopy. This was probably because of feeding commercial diet and absence of hunting activities which exposes to zoonotic diseases via raw meat consumption. Also, numbers of fecal samples collected from shelters were more than household cats.

Table 4.1: Prevalence based on housing of cats

Sr.No	Housing	No. of samples	No. of positive samples	Percent positivity (%)
1.	Animal shelters (Stray Cats)	61	3	4.91
2.	House hold cats (Pet)	45	0	0
3.	Total	106	3	2.83

Results were in accordance with Latha *et al.* (2018) in which fecal microscopy and seroprevalence studies were done in Thrissur, Kerala. The *T. gondii* oocyst was detected in 4.47% of 313 feline faecal samples. Mostly because of matching environmental conditions similar results were observed in our studies. Similarly, Gautham, N. (2014) studied prevalence of Toxoplasmosis by fecal flotation and copro-PCR in Banglore. *Toxoplasma gondii* like oocyst was seen in two(1.38%) fecal sample by flotation out of total 144 samples and both samples were from animal shelters/stray cats(2.44% prevalence).

This results were comparable with NasiruWana (2020) who used copro-microscopy to screen for the presence of *T. gondii*-like oocysts in 200 cat faeces, out of which 7/200 (3.5%) were detected as positive. More *T. gondii*-like oocysts were found in Free roaming cats (5/100, 5.0%) compared to pet cats (2/100, 2.0%).Similarly, low prevalence of cats shedding *T. gondii* or *H. hammondi* oocysts were seen by Schares *et al.* (2008) who examined 24,106 faecal samples of cats from Germany and other European countries microscopically. Oocysts of 9-15 µm size with morphology similar to that of *T. gondii* and *H. hammondi* were found in 74 samples (0.31%). In accordance to this, Amany *et al.* (2012) reported that the prevalence of *T. gondii* oocysts was two per cent at Sharkia Province in Egypt by examination of 100 samples of cat faeces for presence of *T. gondii* oocysts using Sheather's sugar flotation method. Also, Berger *et al.* (2011) reported that only one of the cats shed *T. gondii* oocysts, corresponding to a *T. gondii* prevalence of 0.4 per cent.

In contrast to this, Awobode *et al.* (2020) identified *T. gondii*-like oocysts were in 21.4% (95% CI: 4.6–50.8) of the total cat faecal samples. The prevalence was 50% (95% CI: 6.7–93.3) in Akinyele community which is higher than current findings.

4.1.2 Prevalence of different helminth eggs with mixed parasitic infections.

Fecal samples were examined for presence of different helminth eggs along with *Toxoplasma gondii* to check prevalence of different gastrointestinal parasites in cats as well to increase awareness in owners. (Table 4.2, Figure 4.2).

Table 4.2: Prevalence of different helminth eggs with mixed parasitic infections.

Sr.No	Parasite	Animal shelters (Stray Cats)	House hold cats (Domestic Cats)	Total (106)	Percent positivity (%)
1.	*Toxoplasma gondii*	3	0	3	2.83
2.	*Ancylostoma spp*	2	2	4	3.77
3.	*Isospora spp spp*	5	3	8	7.54
4.	*Strongyloides*	3	3	6	5.66
5.	*Toxocara cati*	19	4	23	21.69
6.	*Ancylostoma spp and Toxocara cati*	2	0	2	1.88
7.	*Toxocara cati and Strongyloides*	5	0	5	4.71
		39	12	51	48.11

Total 106 fecal samples from various locations were examined by Sheather's sugar flotation method for the presence of gastrointestinal parasites in cats. An overall prevalence was found to be 48.11% (n=51) out of which 39(36.79%) cats were from shelters and 12(11.32%) household/ pet cats. Lesser prevalence was observed in pet/ household cats compared to stray cats; this was because of regular deworming and prompts veterinary care.

Prevalence percentage of various parasites was *Ancylostoma* spp 3.77 (n=4) (Plate 4.3), *Isospora* spp 7.54(n=8) (Plate 4.4), *Strongyloides* 5.66 (n=6) (Plate 4.5), *Toxocara cati* 21.69 (n=23) (Plate 4.6 and 4.7). Highest prevalence of *Toxocara cati* was found in cats. Mixed prevalence of *Ancylostoma* spp and *Toxocara cati* 1.88

(n=2) (Plate 4.8) as well as *Toxocara cati* and *Strongyloides* 4.71 (n=5) (Plate 4.9) was observed in cats.

The overall prevalence of gastrointestinal parasites was lower than findings of Kulkarni (1972) in Mumbai region who examined 40 cats. Out of which 72.5% of cats were infected with parasites. This can be related to difference in sample size as well as variation in geographical area as in our research, Pune and Shirwal region were more focused but prevalence *Toxocara cati* 27.5% and *Strongyloides* 5% was similar to current findings. Prevalence of *Ancylostoma caninum* (15%) was higher than current findings.

These results were in accordance with findings of Gautham (2014). Prevalence of gastrointestinal parasites in cats from banglore area was; *Toxocara cati* 25(17.36%), *Ancylostoma* spp ova 15(10.42%) which was lower than our study and *Isospora* spp oocysts 5(3.47%). Mixed infection of *Toxocara cati plus Ancylostoma* spp ova 18 (12.5 %) was seen compared with 1.88%. Total 51(48.11%) cats were infected by various parasites compared with 53 (37.86%) cats.

4.2 Prevalence of *Toxoplasma gondii* by Antibody detection kit (PetX Toxo Ab)

Total 51 Blood samples were collected for the detection of antibodies of *Toxoplasma gondii* in cats which were showing clinical signs such as diarrhoea, icterus, anemia, ocular problems, lethargy and behavioural changes. Asymptomatic cases were also screened by antibody detection kit. Serum sample was used for sandwich ELISA based rapid test kit according to manufacturer's instructions. Appearance of IgG line was considered as positive.

4.2.1 Prevalence of *Toxoplasma gondii* by Antibody detection kit based on housing

Out of 51 samples, 16 were found to be positive for the presence of IgG antibodies (Plate 4.10). Samples from animal shelter/ stray cats were 27, out of them 9(33.33%) were positive and 4(16.66%) out of 24 samples were positive that were

collected from household/pet cats. Prevalence study indicated that seropositivity was lower in pet cats that were fed with commercial diets compared to stray cats which were admitted in shelters for various treatments (Plate 4.11) (Table 4.3, Figure 4.3)

Table 4.3: Prevalence of *Toxoplasma gondii* by Antibody detection kit

Sr.No	Housing	No. of samples	No. of positive samples	Percent positivity (%)
1.	Animal shelters (Stray Cats)	27	9	33.33
2.	House hold cats (Pet)	24	4	16.66
3.	Total	51	16	31.37

The results of present study were in accordance with Sah *et al.* (2018) who detected antibodies for *Toxoplasma gondii* using lateral flow chromatographic immunoassay (Toxo IgG/IgM Combo Rapid test®) in Nepal region. 16 out of 44 (36.36%) cats were positive for it. Similarly, Javadi *et al.* (2010) detected antibodies of *Toxoplasma gondii* 50 cats using an enzyme-linked immunosorbent assay for IgG and IgM. Seropositivity in cats was 30% (n=15). Also, Chhabra *et al.* (1985) did a serological survey of latent Toxoplasma prevalence study on 80 cats in northern India by the microtitre indirect haemagglutination test. Seropositivity was recorded in 33.7 per cent of 80 cats which was found to be similar to results of our study.

The results of seropositivity according to area were comparable with studies of Wang *et al.* (2017) who tested 843 serum samples collected in Henan province for IgG antibodies and concluded that seroprevalence in rural cats (29%) was significantly higher ($p < 0.01$) than in urban cats (16%), and increased significantly ($p < 0.01$) with age.

In contrast to this, Sroka *et al.* (2018) assessed the prevalence of *Toxoplasma gondii infection* in cats. Serology, coproscopy and PCR assay was performed. In

total, 208 cats (139 females and 68 males), aged 0.5–12 years (mean=2.6) from 25 localities in southwestern Poland were examined by indirect immunofluorescence assay (IFAT) to estimate the *Toxoplasma gondii serological* status. The positive results in IFAT for anti-*Toxoplasma gondii IgG* and IgM antibodies were found in 143 of 208 tested cats (68.8%) which are higher than our findings.

Absence of oocyst in fecal samples of cats which were positive for the presence of antibodies by rapid test kit proved hypothesis of Dubey and Frenkel, (1972) which depicted that, in experimental infections, cats usually stop shedding *T. gondii* oocysts by the time they seroconvert. Seropositivity in 30% cats suggests that they must have already contaminated environment by oocyst shedding.

4.2.2 Age wise sero-prevalence

Age wise seroprevalence ranged from 17.64 to 50. In kittens, 0-6 month's age range-three (17.64) kittens were seropositive out of 17. Four (28.57%) were seropositive for the age range of 6 months to 1 year out of 14 cats. In 1-6 years and 6< years seroprevalence was 41.66% and 50% respectively i.e. five out of 12 and four out of eight respectively.

Table 4.4: Age wise sero-prevalence

Sr. No.	Age group	No. of samples	No. of positive samples	Percent positivity (%)
1.	0-6 months	17	3	17.64
2.	6 months to 1 year	14	4	28.57
3.	1-6 years	12	5	41.66
4.	6 and above	8	4	50
	Total	51	16	31.27

It was noted that seroprevalence was lower in young age group compared to older age group. Higher age group had more chances of exposure to *T. gondii* throughout the lifespan. (Table 4.4, Figure 4.4)

These results were in accordance with the findings of Must *et al.* (2017) who stated that seroprevalence increased with age. Overall, 41.12% of the 1121 cats tested seropositive, and the The Burmese had the lowest seroprevalence (18.82%) and the Persian had the highest (60.00%). Also Sroka *et al.* (2018) assessed that the prevalence of anti-Toxoplasma antibodies was significantly greater in older cats (>1 year) (83.5%) than in younger cats (48.3%) ($P<0.05$). As well as Vollaire *et al.* (2005) detected that seroprevalence increases with the age of cats.

4.2.3 Sex wise seroprevalence

Table 4.5: Sex wise seroprevalence

Total 24 samples from males and 27 samples from female were subjected to the sandwich ELISA test kit, out of them 16 were positive. Seropositivity in male samples were 25.92%, seven out of 24 and in female samples, nine out of 27 i.e. 33.33% sero-prevalence. The seropositivity was higher in females than in males. (Table 4.5, Figure 4.5)

Sr. No.	SEX	No. of samples	No. of positive samples	Percent positivity (%)
1.	Male	24	7	25.92
2.	Female	27	9	33.33
	Total	51	16	31.27

The above results were similar to the findings of Sroka *et al.* (2018) which stated seropositivity in females were higher (74.1%) than in males (58.8%). Also, Montazeri *et.al*. (2020) found out the global pooled seroprevalence of toxoplasmosis was equal (33%, 95% CI: 29–37%) in male and female domestic cats and the difference between them was not significant. Increased seroprevalence in females can be related to sampling size and population as well as it might be related to increase in hunting activity along with roaming to feed kittens.

In contrast to this, Javadi *et al.* (2010) detected antibodies of *Toxoplasma gondii* 50 cats using an enzyme-linked immunosorbent assay. Fifteen (30%) of the cats were found to be seropositive and male cats showed significantly higher antibody titres than female cats. Also Bastan and Bulent (2018) observed higher prevalence in male (57.1%) cats than females (42.8 %). Also, Jokelainen *et al.* (2012) investigated different aspects of feline toxoplasmosis and noted that older cats were often found to be positive along with higher seroprevalence in cats which were fed with raw meat and two cats were found shedding oocyst which resembled *Toxoplasma gondii*. Similarly, Vollaire *et al.* (2005) noted that males had more seroprevalence than females.

4.3 Molecular detection

Nowadays, PCR based assay in infectious diseases are transforming clinical practice thoroughly. It is possible to give timely and accurate diagnosis in critical cases which helps to devise early therapeutic control of diseases, especially in the zoonotic ones. Copro-PCR is emerging tool compared to fecal microscopy.

4.3.1 Fecal DNA isolation

The fecal DNA isolation of *Toxoplasma gondii* was difficult because of unavailability of standardised methods or Toxoplasma specific commercial kits. Disruptions of oocysts were done with mechanical method. Repeated freeze thawing combined with use of glass beads facilitated disruption of oocysts by vortexing it for significant time. DNA was extracted using MB544 HiPurA® Stool DNA Purification Kit from HiMedia Laboratories Pvt. Ltd., Mumbai as per their recommended standard protocol with slight modifications suggested by Salant *et al.*, (2010).

4.3.2 *Toxoplasma gondii* specific PCR

Fecal samples were subjected to DNA isolation and subsequently PCR which were positive by fecal microscopy and or rapid antibody detection kit as well as presence of clinical signs. TOX 4 and TOX 5 primers based on 529bp repetitive gene

which are *T. gondii* specific were used for copro-PCR. Optimum annealing temperature was found to be 61º in the process of standardisation of PCR.

4.3.3 DNA confirmation by agarose gel electrophoresis

PCR products were run on the 1.1% agarose gel electrophoresis along with the positive and negative control as standard. Expected amplicon size i.e. 529 bp were obtained by amplification of fragment of *T. gondii* DNA. (Plate 4.12). On completion of electrophoresis, the gel (1.1%) was visualized using HiMedia UV max transilluminator). Discrete bands were noticed under luminance of UV light showing presence of DNA fragments. Three samples (5.88%) which were positive by fecal microscopy out of 51 suspected cases were confirmed by PCR assay.

It indicates that fecal microscopy along with PCR is better technique to confirm a presence of oocyst as *toxoplasma gondii* thus helping to devise accurate therapeutics and control of zoonosis in general population. (Table 4.6, Figure 4.6). Microscopically, *Besnoitia* and *Hammondia* oocysts are morphologically similar to *toxoplasma gondii* in cats. (Salant *et al.*, 2010)

Table 4.6 Method wise distribution of *toxoplasma gondii* positive cases

Sr. No.	Total number of animals	No. of cases positive for presence of oocyst	No. of cases positive by PCR	Cases positive for presence of antibodies
1.	106	2.83% (3/106)	5.88% (3/51)	31.27% (16/51)

The results of current study were in agreement with Gautham (2014) who performed DNA extraction from 80 fecal samples followed by 529 bp repetitive PCR, out of them five (6.25%) cats were positive for Toxoplasmosis. Likewise, Veronesi *et al.* (2016) tested 78 faeces of cats out of which two (2.56%) samples were positive by 529bp PCR, 12 samples with B1-PCR and two with both protocols. Two samples were positive by microscopy as well as 529 bp PCR and two samples were positive

by PCR but not microscopy. Similarly Sroka *et al.* (2018) examined 41 faecal samples and two samples (4.9%) were positive by fecal flotation technique out of which one was confirmed by PCR indicating 2.4% copro-prevalence. Likewise, Homan (2000) performed PCR by same sequence of primers with 529bp amplicon repeated 200- to 300-fold in the genome of *Toxoplasma gondii*

In contrast to this Hanafiah *et al.* (2018) performed copro prevalence in Yogyakarta area and repetitive 529 bp gene was used. The result indicated 33.3% (3/9) prevalence. It is also noted that in contrast to this study copro-prevalence was performed on the cats which were serologically positive which explains higher copro-prevalence than this study.

Result in the current study was in accordance with Salant *et al.* (2010) who compared microscopy, bioassay and copro PCR for detection of *Toxoplasma gondii* in cats in which it is stated that even if the microscopy renders the sample as positive occasionally, Copro-PCR gives consistent and quick results compared to microscopy and bioassay. In contrast to this Jones and Dubey, (2010) suggested that Toxoplasma specific antibody detection is better measure of prevalence than oocyst detection mainly because of very less(1%) percentage of active oocyst shedding in cats at given time.

Within a month of initial infection by the parasite, maximum oocyst shedding occurs and is completed within 21 days of infection most of the times. (Elmore *et al.* 2010) Serological survey of latent Toxoplasma prevalence on 80 cats in northern India by the microtitre indirect haemagglutination test recorded Seropositivity in 33.7 per cent of 80 cats. (Chhabra 1985) Thus, while serological testing may be applicable for determining parasite exposure, it is likely to vastly overestimate human health risk from cats (Lilly and Wortham 2013). Sensitivity of serological test was not taken into account in current study and antibody titre might differ from case to case. Therefore serology might not be reliable method in cases which requires confirmatory diagnosis as well as to measure zoonotic potential.

Common method to measure oocyst shedding in feces of cats is fecal flotation followed by microscopic examination which is cumbersome and requires trained person. PCR have better accuracy to check, confirm and differentiate between oocysts of similar morphological structure and size.

Hence, PCR is reliable and accurate method than copro-microscopy. It may be considered as alternative method for the screening of toxoplasmosis.

4.4 Clinical signs and symptoms

Cats affected with toxoplasmosis show very less clinical signs or no detectable signs most of the times at given time. Also recording of all clinical parameter can be difficult in cats because of less compliant nature of cats especially in case of stray cats. (Table 4.7, Figure 4.7)

Table 4.7: Clinical signs and symptoms

Sr. No.	Observed signs and symptoms	Cases positive for toxoplasma	Percentage %
1.	Diarrhoea	5	26.31
2.	Ocular problems	2	10.52
3.	Skin disorders	2	10.52
4.	Lethargy and dullness	8	42.10
5.	Anorexia	5	26.31
6.	Icterus	3	15.78
7.	Respiratory distress	1	5.26
8.	Pale mucus membrane	4	21.05
9.	Asymptomatic	10	52.63
10.	Behavioural changes	3	15.78
	Total	19	

Cats were observed for clinical signs and symptoms with the special attention to symptoms which were specific to toxoplasmosis as well as general wellbeing. Most of the cats were asymptomatic in nature without any obvious signs and symptoms 10(52.63%) cats were asymptomatic out of 19.

Lethargy and dullness (Plate4.13) were seen in eight (42.10%) cases and anorexia was noted in five (26.31%) cats. Icterus was evident in three (15.78%) cases and was confirmed with biochemical reports with elevated total bilirubin cases. (Plate 4.14, 4.15) Diarrhoea was confirmed with loose consistency of stools as well as increased frequency of fecal samples in five (26.31%) cases.

Ocular problems were noted in two (10.52%) cats such as uveitis (Plate 4.16) and conjunctivitis (Plate 4.17) as well as pale mucous membrane was noticed in four (21.05%) cases which were later confirmed with haematological report. Total two (10.52%) cases were found to be positive for toxoplasmosis with dermatological problems. (Plate 4.18) Out of 19 cats, one (5.26%) was noted with respiratory distress. Behavioural changes were noted in 3(15.78%) cats.

In accordance to this, Bastan and Bulent (2018) observed 14 *Toxoplasma gondii* IgG seropositive cats. Of 14 cats, neural symptoms such as behavioural changes, seizures, ataxia and nystagmus were detected in 11(78.57%) cats, uveitis in 5(35.71%) cats and diarrhoea in 4(28.57%) cats. Also studies of Barrs (2013) suggested presence of uveitis, chorioretinitis and focal CNS infection in cats positive for toxoplasmosis. Similarly, Svoboda and Svobodova (1985) observed conjunctivitis in positive cats which were also seen in our studies.

Present study was in accordance with Dubey and Prowell (2013) who observed lethargy, anorexia and diarrhoea in cases of toxoplasmosis. As well as Lappin M. R. (2010) noted that when *toxoplasma gondii* cyst is fed in experimental infections, self-limiting small intestinal diarrhoea is seen for one to two weeks after initial feeding. This phenomenon is observed in only 10% to 20% of cats.

Also, this study was in accordance with Barrs (2013) who suggested hepatic, pulmonary, CNS and pancreatic involvement in positive cases along with lethargy, anorexia and dyspnoea. Similarly**,** Dubey *et al.* (2009) noted that inflammation of liver is common finding which was confirmed by alterations in liver function test in this study and anorexia, lethargy, and dyspnoea was also common. Icterus due to hepatitis was observed in 84.4% cases and ocular problems in 81.4% cases. Unilateral or bilateral anterior uveitis was detected that ocular toxoplasmosis is seen without apparent signs from other systems.

Present study was in agreement with findings of Dubey and Beattie (1988). In which it was suggested that nonspecific clinical signs are seen in cases of toxoplasmosis and they don't suffice for definite diagnosis as it mimics several other infections clinically. Highest prevalence was noted in asymptomatic cases.

Accordingly, Lappin *et al*. (1996) noted behavioural changes in 3/11 cases (27.27%) and anorexia in 4/11 cats (36.36%). Icterus was seen in 2/11 cats (18.18%) and respiratory distress in 5/11 cases (45.45%). Infection with *toxoplasma gondii* is linked to demethylation of the promoters of certain genes in the cerebral amygdala of the intermediate hosts and modifies dopaminergic circuits associated with fear (Desmettre, 2020). Similarly, Cucos *et al*. (2015) noted alterations in behaviour with personality changes.

In contrast to current study, Lappin *et al.* (1989) observed higher percentage of ocular problems i.e. in 60% cats and respiratory distress was seen in one (6.66%) of cats. It was also suggested that anterior uveal changes were present because of selective immune complex deposition/immunological response outside of eye and organism replication. Specific antigen can react with immune competent lymphocyte after migration to eye might stimulate severe uveal reaction. Also, Cucos *et al*. (2015) detected ophthalmic signs in 86 % of cases.

Higher no of asymptomatic cases of toxoplasmosis are related to method of detection as 16 out of 19 cases were serologically positive and three of them were

confirmed for shedding of oocysts. Clinically ill cats have not confirmed to be shedding an oocyst and it is seen that healthy looking cats can shed oocysts without any clinical signs and infection persists as subclinical infection (Dubey, 2010). Also, Jokelainen et al. (2012) observed that 529 bp copro-PCR positive cats didn't develop any clinical signs until 10 days post shedding.

Toxoplasma gondii specific antibodies are detectable after two to four weeks of post-infection, this can attributed to absence of notable symptoms in seropositive population. Lethargy and dullness is common manifestation of disease. Jaundice is seen in this study as it can occur due to various hepatic disorders along with toxoplasmosis as well as migration of tachyzoites into circulation thereby reaching liver. Elevation in the serum level of ALT was also noted in cases of jaundice indicating hepatic disorders. Rapid intracellular replication of tachyzoites occurs, causing necrosis of infected tissues and can be distributed in body initiating inflammation and damage. Correspondingly, Cucos *et al*. (2015) detected hepatic disorders in seropositive cats.

4.5 Haematological alterations

Samples positive for toxoplasmosis by serology and presence of oocyst in fecal samples were subjected to hematobiochemical studies. (Table 4.8, Figure 4.8)

Parameters like Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte count (TEC), Total Leukocyte count (TCL) and Differential leukocyte count using automated haematology cell analyser ABX Micros ESV 60 (HORIBA) were estimated.

4.5.1 Haemoglobin (Hb)

The mean ± S.E. value of haemoglobin (gm/dl) of the cat's positive for toxoplasma were 12.38 ± 0.80. Total 3 (15.78%) cases of cats were seen with severe anaemia and 1 (5.26%) was seen with haemoglobin just below normal average range.

Total 4 (21.05%) cats were anaemic. Total 15 (78.4%) cat were having normal haemoglobin range.

Table 4.8: Haematological values of cats found positive for toxoplasmosis.

Sample no	Name	Hb (g/dL)	PCV (%)	TEC ($\times 10^{6}$/µL)	TLC ($\times 10^{3}$/µL)
1	Falguni	11.1	37.5	7.6	15.8
2	Cocco	11.6	34.8	7.2	6.5
3	Bunti	12	35.8	8.44	11.9
4	Chai	12.3	36.8	7.88	9
5	Chuha	14.8	42.3	9.25	26.9
6	Danis	14.9	44.7	9.99	14.4
7	Fiona	12.2	36.6	8.78	19.3
8	Garfield	10	30.4	6.08	13.7
9	Gypsi	13.7	41.1	9.26	9.6
10	Good boy	5.5	15.8	3.72	11.6
11	Hornuz	15.2	45.2	9.52	14.3
12	Leo	13.9	41.3	9.13	4.6
13	Metro	15.1	43	9.41	10.4
14	Mewtwo	14.5	43.5	9.73	15.6
15	Mothi	12.1	36.6	8.38	17
16	Nikku	18.4	54.2	11.75	14.1
17	Paul	6	18.8	4.69	17.5
18	Pillu	5.9	18.8	5.54	29.8
19	Zoya	16.1	48.1	10.89	8
	Mean ± SE	**12.38 ± 0.80**	**37.12±2.32**	**8.27± 0.47**	**14.21±1.44**
	Reference Range Vaden *et al*.(2009)	10.5–14.9	31–46	6.8–10.0	4.5–15.7

In contrast to this, low levels of haemoglobin were seen by Advincula *et al.* (2010). Also Bastan and Bulent (2018) detected decreased Hb level in 8 cats (57.1%). As well as, Cucos *et al*. (2015) detected anaemia in sero-positive cases.

4.5.2 Packed Cell Volume (PCV)

The mean ± S.E. value of PCV (%) in the present study was 37.12±2.32 in cats positive for toxoplasmosis. Total 4 (21.05%) cats were showing PCV level below normal range and 15 (78.4%) cases had normal PCV range.

Results were in contrast with Bastan and Bulent (2018) who detected decreased PCV level in 8 cats (57.1%). Also, Lappin *et al.* (1996) observed 28.177±7.04 mean ± S.E. value of PCV.

4.5.3 Total Erythrocyte Count (TEC)

The mean ± S.E of Total Erythrocyte Count (TEC) in cats affected were 8.27± 0.47, which indicated normal reference range. Total 4 (21.05%) cases had low T.E.C. count compared to others.

Present study was in accordance with Lappin *et al.* (1996) observed 8.38±1.96 mean ± S.E. value of Total Erythrocyte Count.

In contrast to our findings Advincula *et al.* (2010) reported decreased Total Erythrocyte Count (TEC) ranging 3.3x106-5.7x106/mm3. Also Bastan and Bulent (2018) observed decreased (TEC) level in 8 cats (57.1%).

4.5.4 Total Leucocyte Count (TLC)

The mean ± S.E. value of total leucocyte count (TLC) of affected cats were 14.21±1.44. Values were near the upper range of reference range.

These results were in contrast with studies of Advincula *et al.* (2010) who observed elevated total leucocyte count.

4.5.5 Differential Leucocyte Count (DLC)

Differential Leucocyte Count (DLC) is measure of percentage leucocytes, also known as white blood cells present in the blood. (Table 4.9, Figure 4.8)

Table 4.9: Differential Leucocyte count of cats found positive for toxoplasmosis

Sr. No.	Name	Neutrophils %	Eosinophils %	Lympho-cites%	Mono-cytes%	Baso-phils %
1	Falguni	74	3	22	1	0
2	Cocco	88	0	11	1	0
3	Bunti	67	2	30	0	0
4	Chai	49	4	46	1	0
5	Chuha	76	2	21	1	0
6	Danis	56	4	39	1	0
7	Fiona	80	4	15	1	0
8	Garfield	78	6	15	1	0
9	Gypsi	80	5	14	1	0
10	Good boy	49	13	37	1	0
11	Hornuz	70	2	2	1	0
12	Leo	62	2	35	1	0
13	Metro	52	7	40	1	0
14	Mewtwo	43	2	54	1	0
15	Mothi	18	11	70	1	0
16	Nikku	77	4	18	1	0
17	Paul	52	4	43	1	0
18	Pillu	69	2	28	1	0
19	Zoya	59	2	38	1	0
	Mean ± SE	**63.10±3.87**	**4.15±0.74**	**30.42±3.84**	**0.94±0.05**	**0±0**
	Reference Range Fielder (2015)	**45-64**	**0-4**	**27-36**	**0-5**	**0-1**

4.5.5. (a) Neutrophils

The Mean ± S.E. value of neutrophils (%) was 63.10±3.87 which was near upper range of normal values. Total 10 (52.63%) animals were having neutrophilia and 2 (10.52%) showed neutropenia.

These results were similar with studies of Advincula *et al.* (2010) who observed increase in neutrophils with a value range of 79-96% neutrophils. Also, Bastan and Bulent (2018) observed neutrophilia in 5 cats (35.7%) positive for toxoplasmosis.

4.5.5. (b) Eosinophils

The mean ± S.E. value of Eosinophils (%) of affected cats was 4.15±0.74 which was slightly higher than normal range. Total 5 (26.31%) cats had eosinophilia out of 19.

These results were in accordance with the findings of Lappin *et al.* (1989) who observed eosinophilia in 13.33% of cats. As well as Cucos *et al*. (2015) detected eosinophilia in sero-positive cats.

4.5.5. (c) Lymphocyte

The mean ± S.E. value of lymphocyte (%) of affected cats in the present study was 30.42±3.84. Total 8(42.10%) cats had lymphocytosis and 8 cats had lymphopenia. Lymphocytosis could be associated with proliferation of B-lymphocytes which can result in increased antibody synthesis by plasma cells either due to a concurrent infection or induced immune response to past infections. This is explained in our experiment with lymphocytosis being seen in cases with increased serological titre of antibodies. Lymphopenia could be seen in early infection where adequate immune response is not available.

Similar results were seen by Javadi *et al.* (2010) who observed increased number of lymphocytes in cases having higher serological titre of antibodies. Also,

Lappin *et al.* (1989) observed lymphocytosis in 6.66% of cats. As well as Cucos *et al*. (2015) detected lymphocytosis in sero-positive cats.

4.5.5. (d) Monocytes

The mean ± S.E. value of monocytes (%) was 0.94±0.05 which indicated normal range of felines.

These results were in accordance with Advincula *et al.* (2010) who observed normal range of monocytes in affected cats.

This result was in contrast with Javadi *et al.* (2010) who observed monocytosis in seropositive cases of toxoplasmosis mostly because author has documented cases of chronic toxoplasmosis and our studies asymptomatic cases were seen frequently. Also, Bastan and Bulent (2018) observed monocytosis in clinically positive cats. As well as Lappin *et al.* (1989) noted monocytosis in 6.66% of cats.

4.5.5. (e) Basophils

No basophils were detected in Differential Leucocyte Count of any positive cats.

4.10 Biochemical parameters

Results of biochemical parameters are given in Table 4.10, Figure 4.9.

4.6.1 Blood urea nitrogen (BUN)

The mean ± S.E. value of Blood urea nitrogen (BUN) in cases positive for toxoplasma was 46.13±9.36. It shows increased BUN in cases of toxoplasma positive cats. Increase can be attributed to various conditions apart from toxoplasmosis. It can be also related to common conditions occurring in cats such as dehydration which is seen in cats due to lower water intake.

In similar study by Advincula *et al.* (2010) it was observed that blood urea nitrogen was statistically insignificant ($p>0.05$). Also, Lappin *et al.* (1996) observed

81.36±136.48 mean ± S.E. value of Blood urea nitrogen (BUN). These results were in contrast with studies of Bastan and Bulent (2018) who observed normal range of Blood urea nitrogen in toxoplasmosis affected cats.

Table 4.10 Biochemical parameters

Sr. No	Name	**B.U.N.** (mg/dl)	**Creatinine** (mg/dl)	**Total Bilirubin** (mg/dl)	**SGPT** (IU/L)	**ALP** (IU/L)	**Total Protein** (g/dl)
1	Falguni	17.1	1	0.2	74	151	7.7
2	Cocco	175	10.2	0.2	31	81	8.2
3	Bunti	32	1.4	0.4	57	207	6.5
4	Chai	30.1	1.2	0.3	79	223	6.3
5	Chuha	32.1	1.9	0.6	43	693	8.1
6	Danis	21.1	1.4	0.6	91	307	6.1
7	Fiona	19.1	1.2		58	98	6.8
8	Garfield	76.1	2.8	0.5	204	91	8.7
9	Gypsi	26.1	1.2	0.5	61	207	7.7
10	Good boy	86.1	4.1	0.4	54	31	9.2
11	Hornuz	19.1	1.3	0.3	31	188	7.4
12	Leo	28.1	1.6	0.2	89	196	6.1
13	Metro	28.1	1.3	0.6	51	68	8.1
14	Mewtwo	27	1.9	0.5	35	51	6.9
15	Mothi	22.1	1.5	0.4	86	415	6.9
16	Nikku	22.1	1.7	0.2	91	117	7.4
17	Paul	98.1	4.5	0.2	146	93	8.7
18	Pillu	88.1	2.4	0.2	74	48	7.2
19	Zoya	29.1	1.4	0.5	55	225	7.4
	Mean ± SE	46.13 ±9.36	2.28 ±0.49	0.37 ±0.03	74.21 ±9.55	183.68 ±36.09	7.44 ±0.20
	Reference Range Vaden *et al.*(2009)	15-32	0.9-2.1	0.1-0.3	29-145	10.0-72	6-8.4

4.6.2 Creatinine

The mean ± S.E. value of Creatinine in affected cats was 2.28±0.49 which is slightly higher than normal reference range. Slightly higher value was observed because of history of chronic kidney disease in one cat.

Results were in accordance with Advincula *et al.* (2010) who observed creatinine was statistically insignificant ($p>0.05$). Also Bastan and Bulent (2018) observed normal range of total bilirubin in toxoplasmosis affected cats. As well as Lappin *et al.* (1996) observed 2.17±3.86 mean ± S.E. value of creatinine.

4.6.3 Total Bilirubin

The mean ± S.E. value of Total Bilirubin of positive cats was 0.37±0.03 which is slightly elevated in cats.

These results were similar with studies of Bastan and Bulent (2018) who observed normal range of total bilirubin in toxoplasmosis affected cats.

4.6.4 SGPT (Serum glutamic pyruvic transaminase)/ALT

The mean ± S.E. value of ALT of affected cats was 74.21±9.55 which indicates normal range. Total two (10.52%) out of 19 cases were seen with increased serum levels of ALT.

Similar results were seen by Bastan and Bulent (2018) in three (21.4%) out of 14 cats which were seen with elevated levels of ALT.

In contrast to this Jokelainen *et al.* (2012) observed a distinct elevation in the liver enzyme alanine aminotransferase (ALT) i.e. 1000 U/L was a consistent outcome in cats with generalized toxoplasmosis. Since cases detected in our studies were serologically positive, elevated ALT was not apparent in most of the cases but two of them which showed oocyst shedding stage thus revealed increased serum values. Also Lappin *et al.* (1989) noted increased serum level of ALT in 20% of cats and Lappin *et al.* (1996) observed 40.55±44.22 mean ± S.E. value of ALT.

4.6.5 ALP (Alkaline phosphatase)

The mean ± S.E. value of ALP (Alkaline phosphatase) of cats positive for toxoplasmosis was 183.68±36.09. It indicates that higher ALP is seen in cats positive with toxoplasmosis. This was a steady finding in most of the cases.

Findings of this study was in agreement to Dubay (2005) who stated that, though increased serum alkaline phosphatase (ALP) is seen in positive cats with hepatic necrosis, it less frequently seen.

In contrast to current study, Lappin *et al.* (1989) observed increased ALP in just one (6.66%) case out of 15 cases.

4.6.6 Total Proteins

The mean ± S.E. value of Total Proteins was 7.44±0.20. Out of 19, three (15.78%) cats showed minor elevation in the total proteins of affected cats.

In accordance to current study, Dubey (2005) suggested increase in serum total protein level.

In contrast to current study, Barrs (2013) stated that there can be slight reduction in total serum protein level in acute illness due to proteinuria.

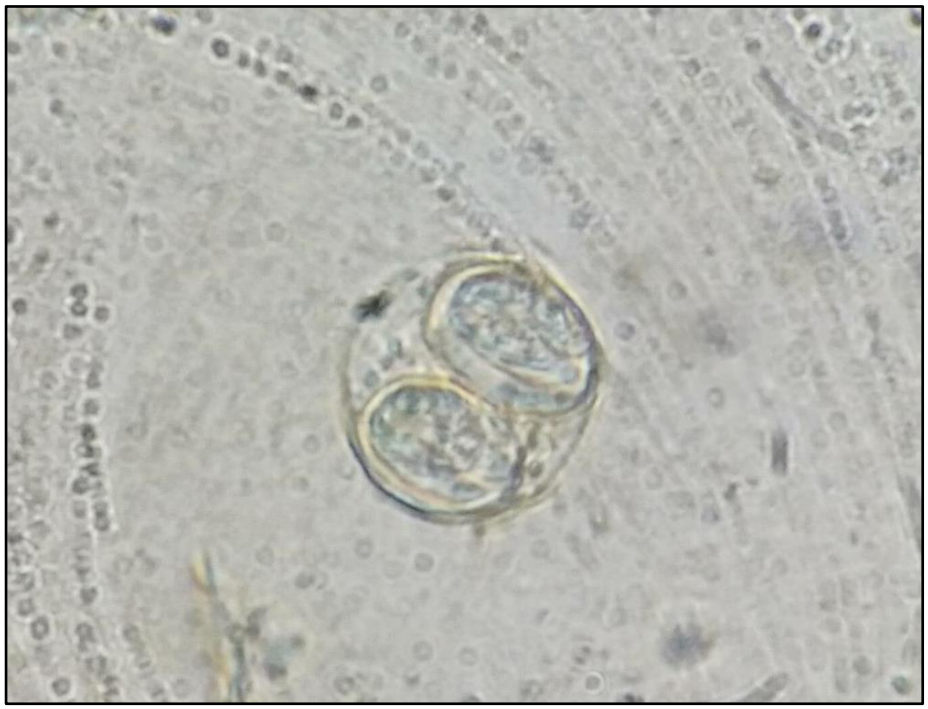

Plate 4.1: Toxoplasma gondii sporulated oocyst

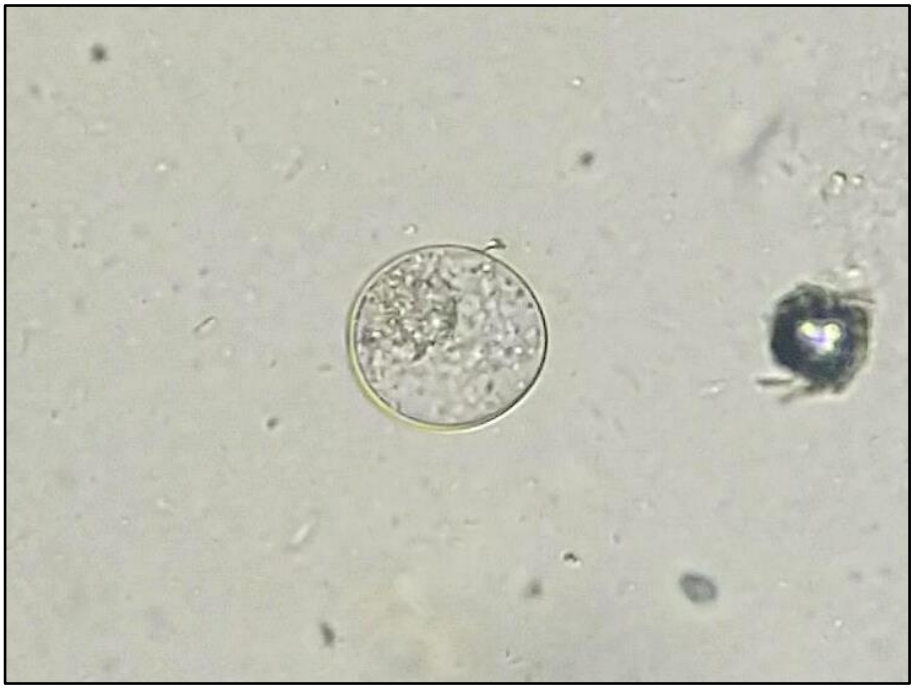

Plate 4.2: Toxoplasma gondii unsporulated oocyst

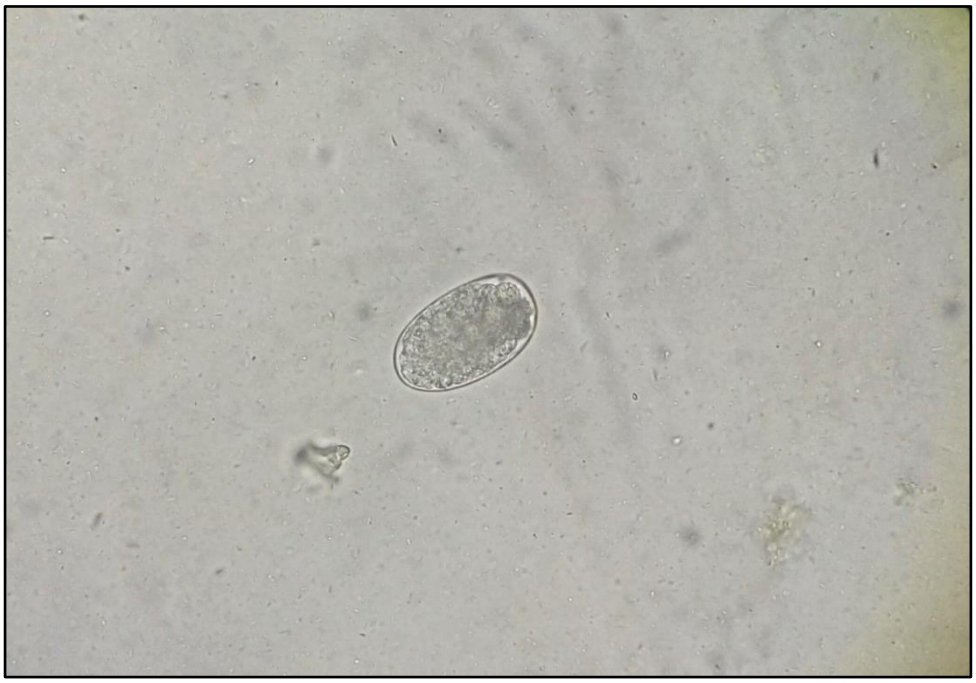

Plate 4.3: ***Ancylostoma spp***

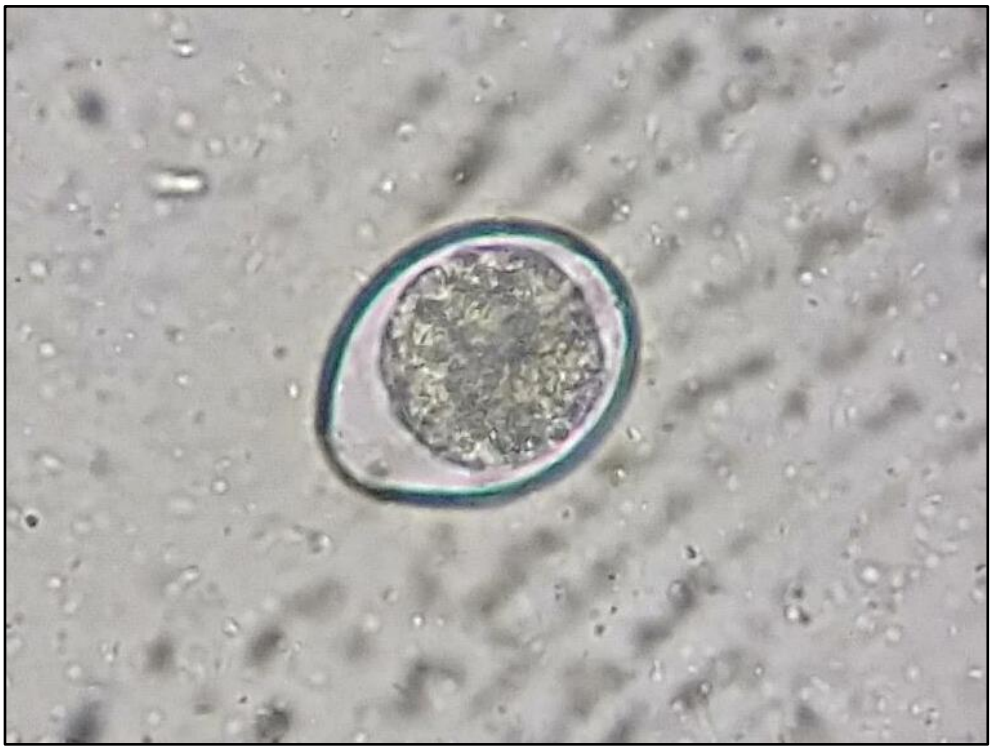

Plate 4.4: Isospora spp

b

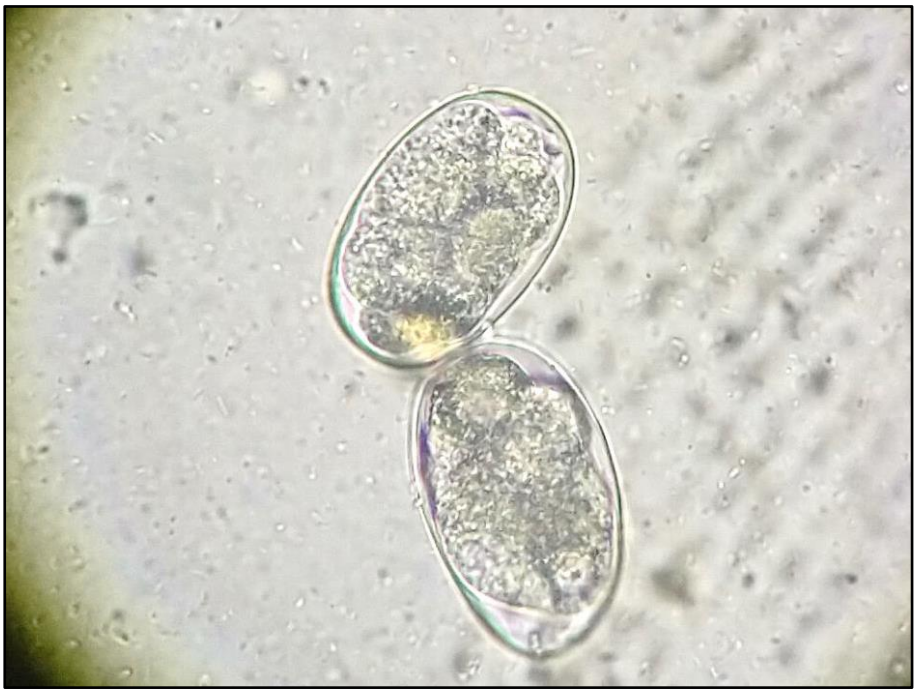

Plate 4.5: Strongyle

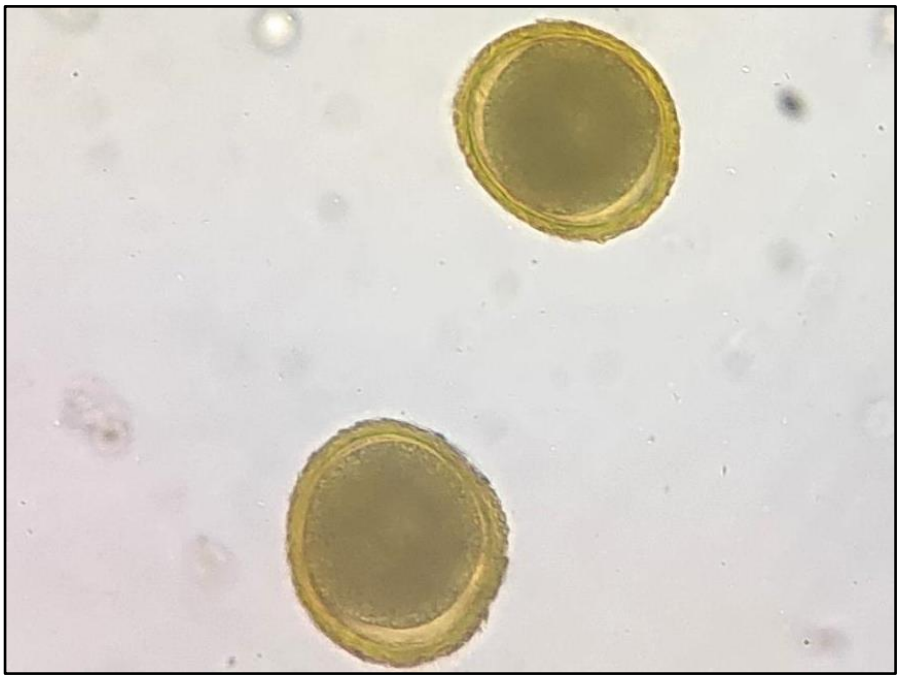

Plate 4.6: Toxocara cati

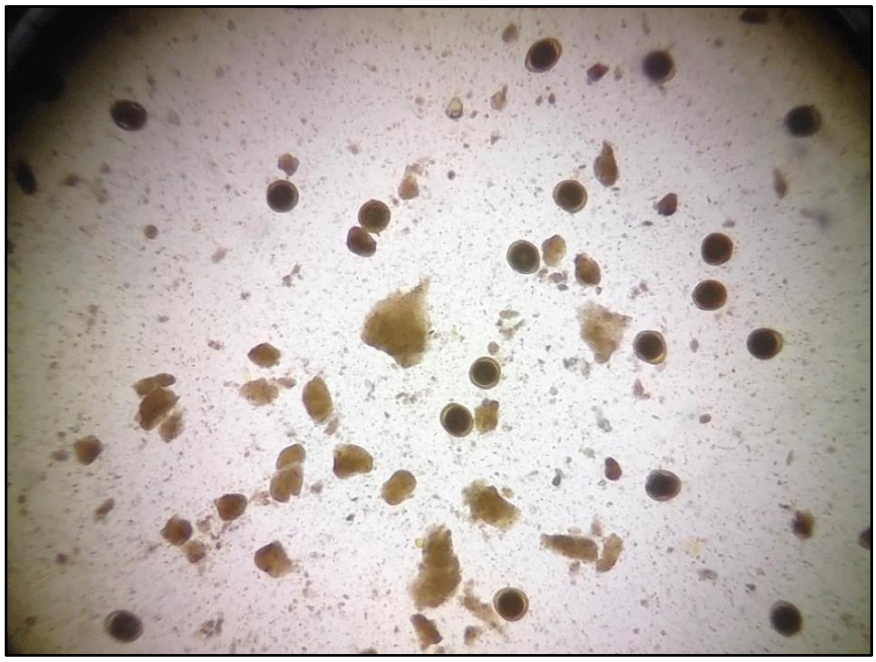

Plate 4.7: Toxocara cati severe infection

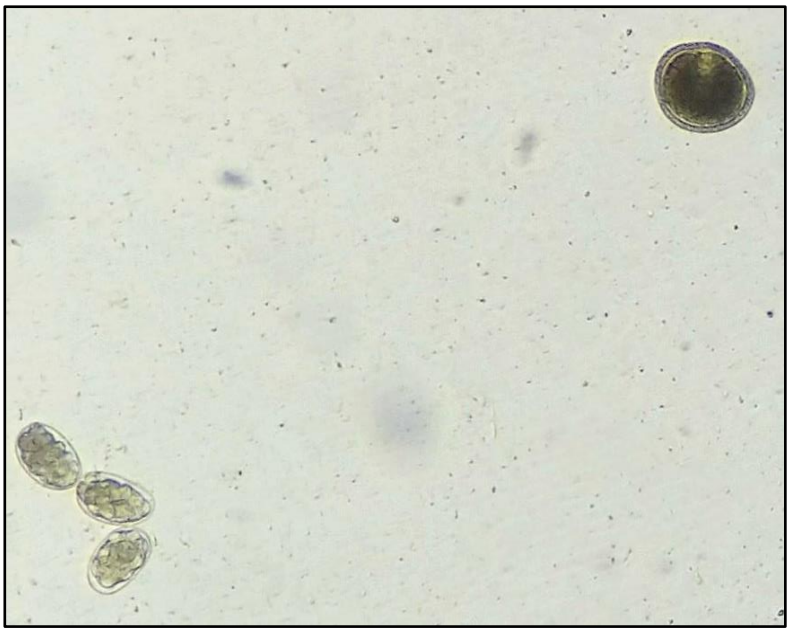

Plate 4.8: Mixed infection Ancylostoma and Toxocara

d

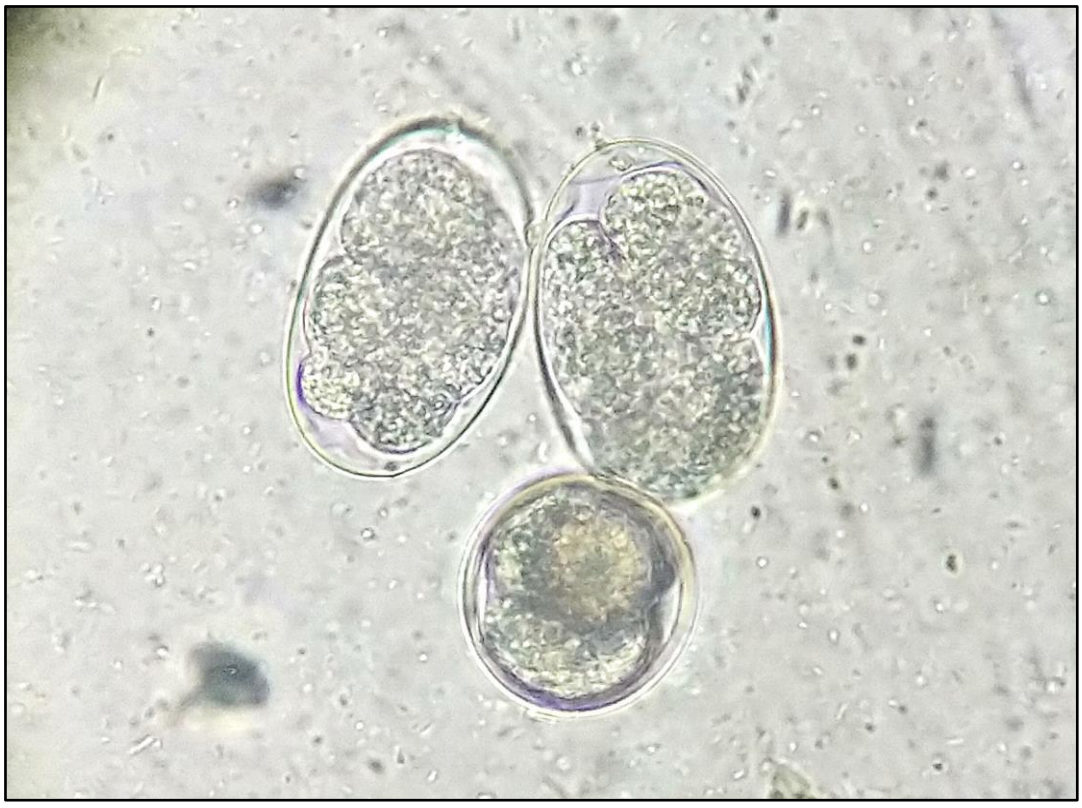

Plate 4.9: Mixed infection *Toxocara cati and Strongyloides*

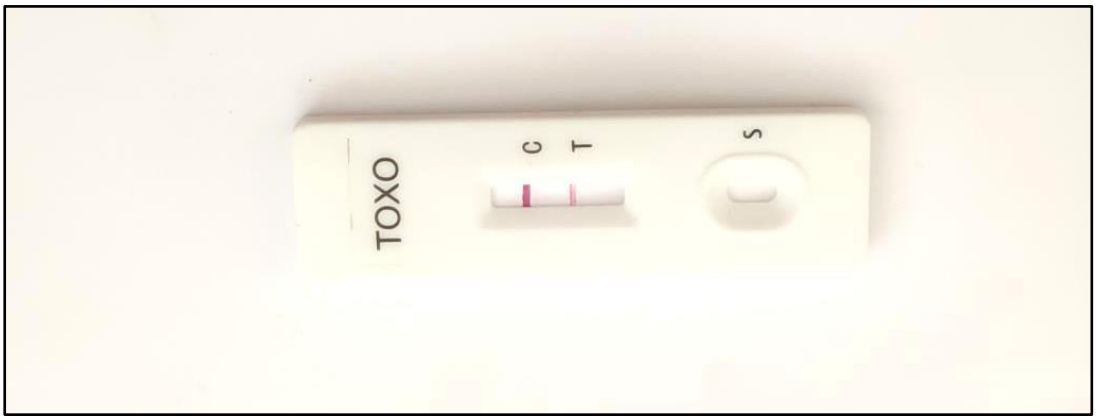

Plate 4 .10: Rapid antibody test kit(PetX Ab) showing control and test line

Plate 4.11: Stray cat feeding on raw meat

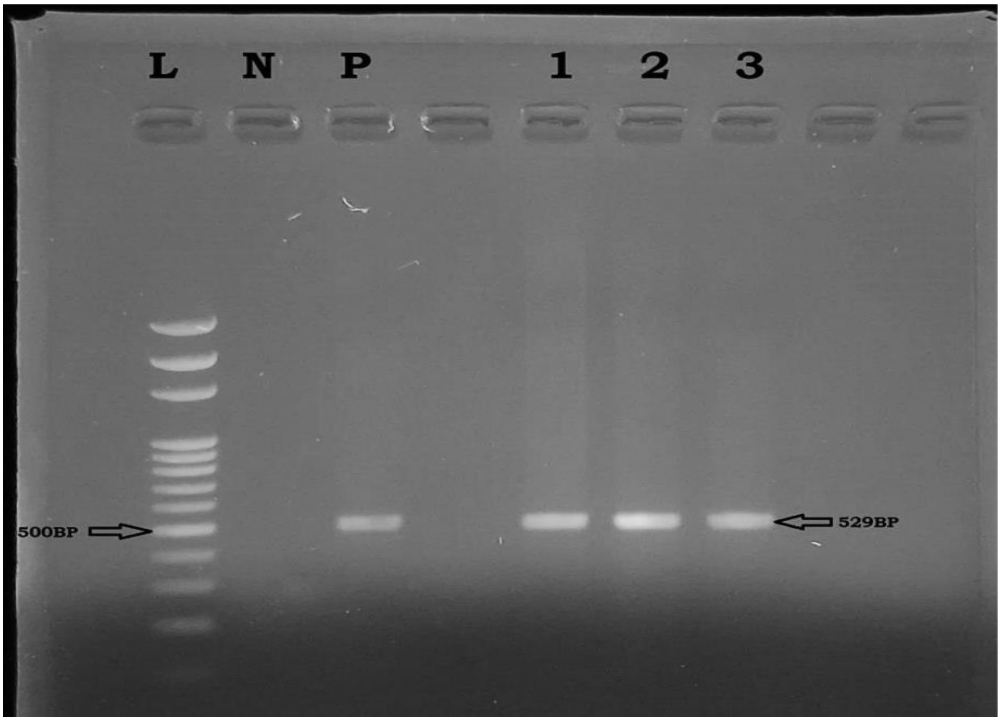

Plate 4.12: Positive results obtained at 529bp in PCR

Lane L: DNA ladder 100bp, Lane N: Negative control,

Lane P: Positive control, Lane 1-3: DNA isolates

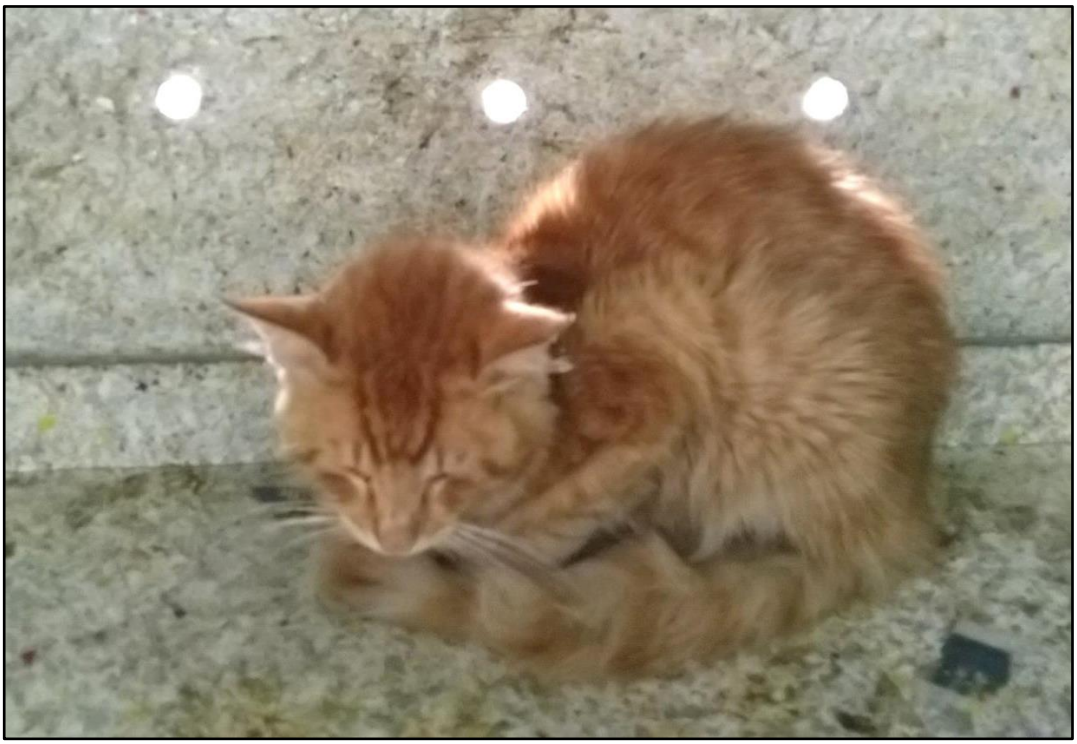

Plate 4.13: Lethargy and dullness

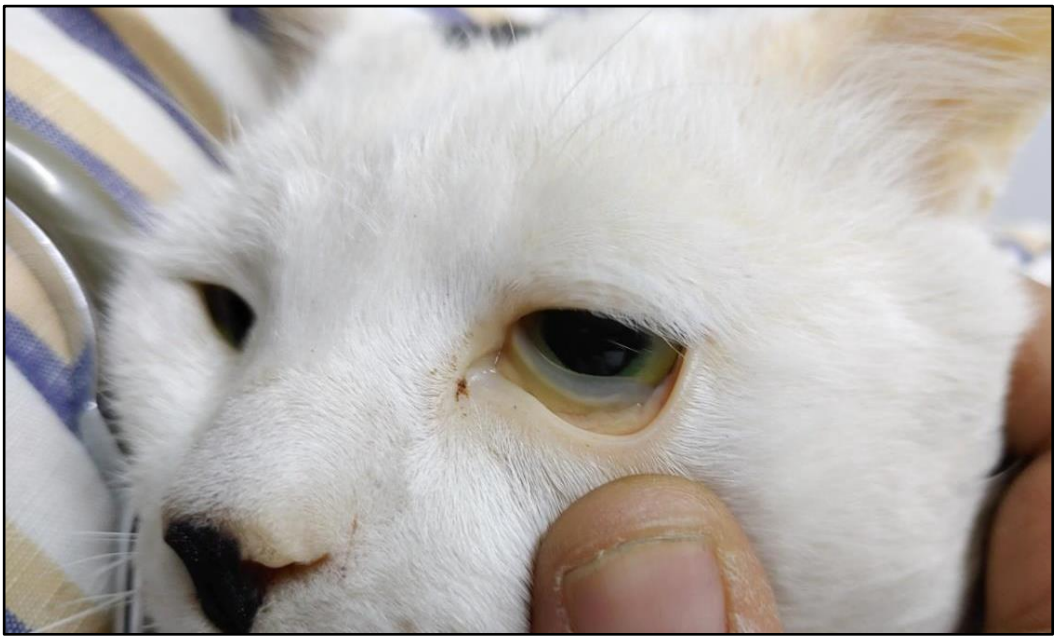

Plate 4.14: Icteric conjuctival mucous membrane

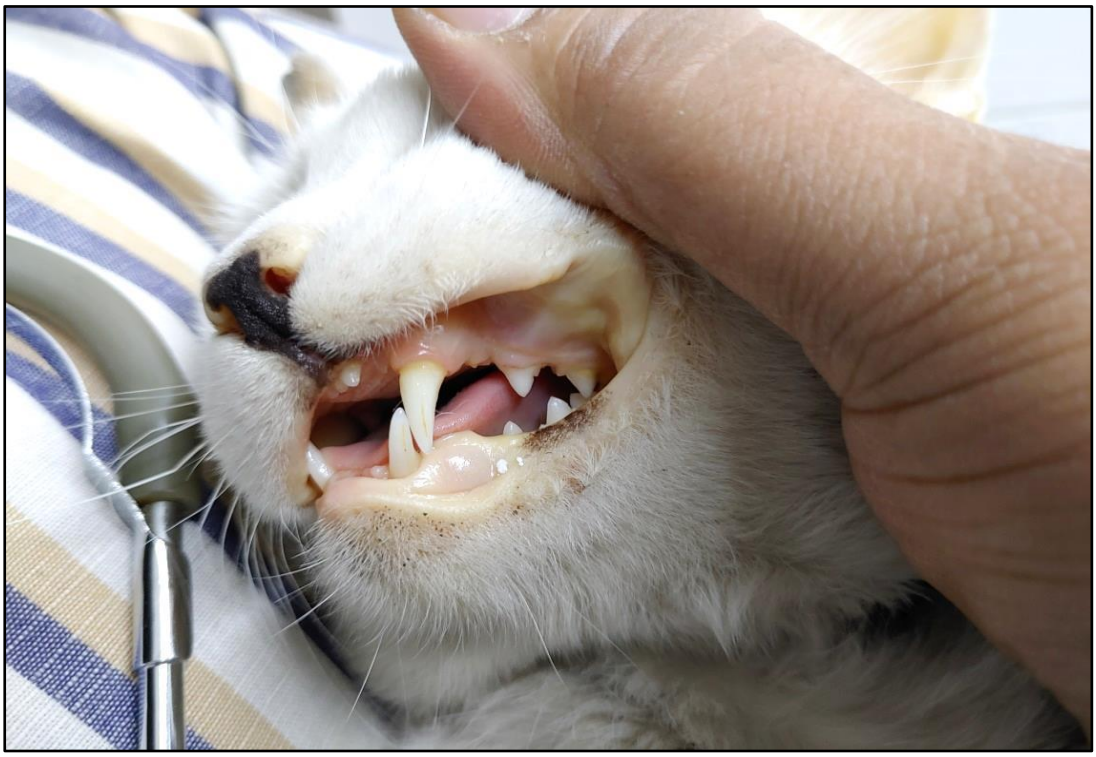

Plate 4.15: Icteric buccal mucous membrane

Plate 4.16: Anterior uveitis

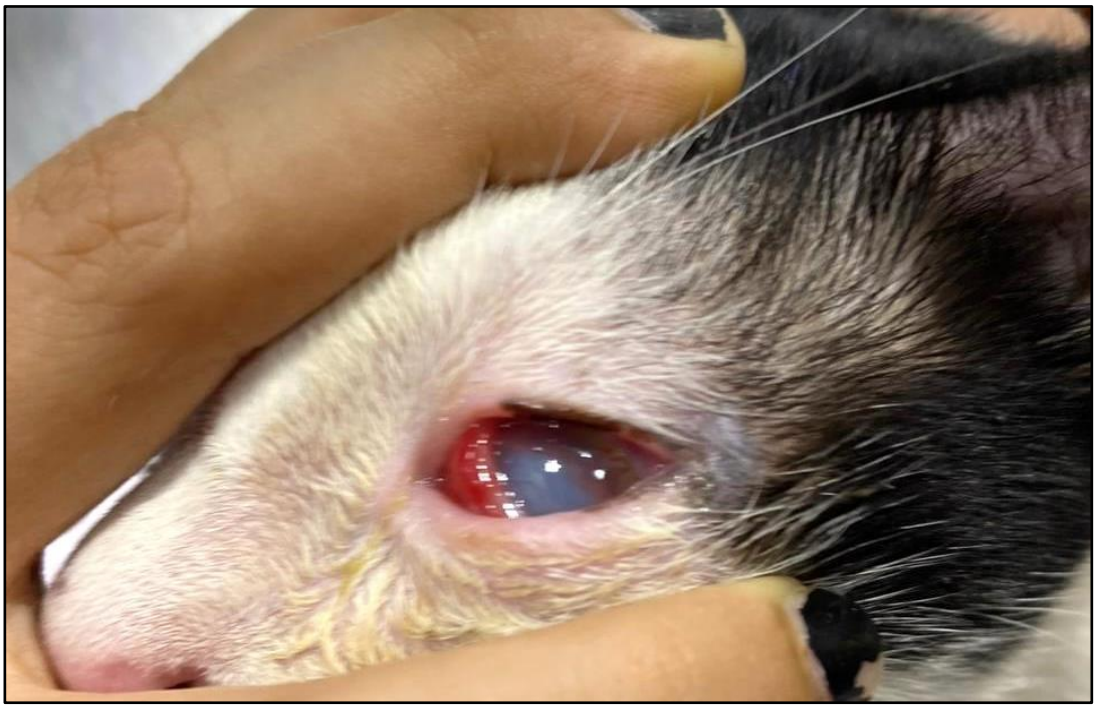

Plate 4.17: Conjunctivitis

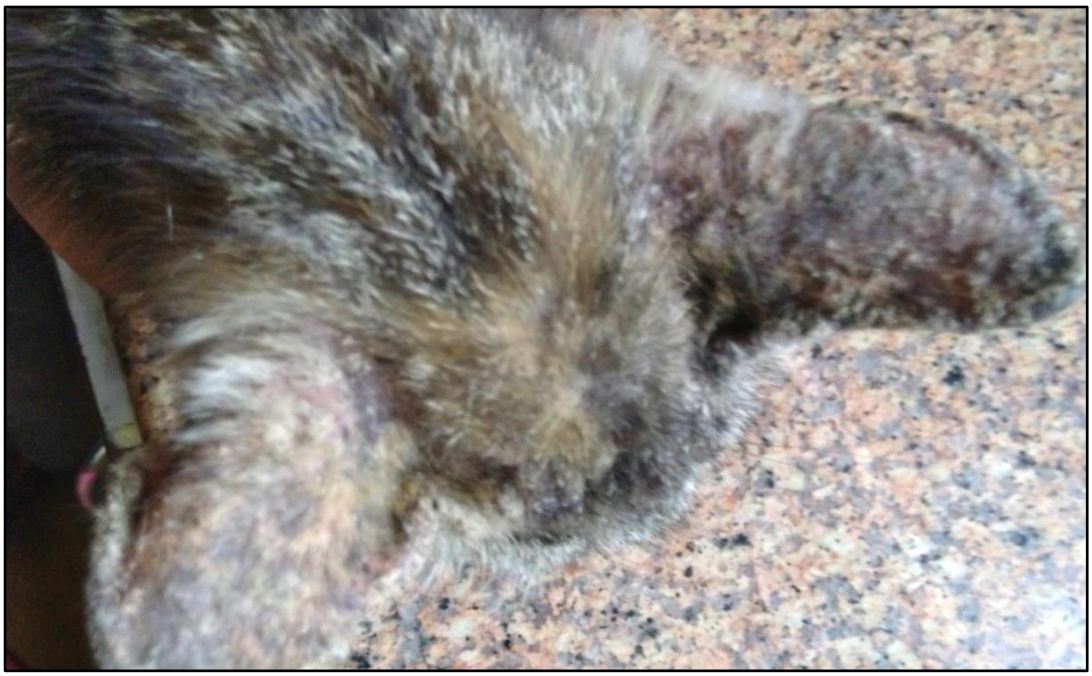

Plate 4.18: Dermatological affections

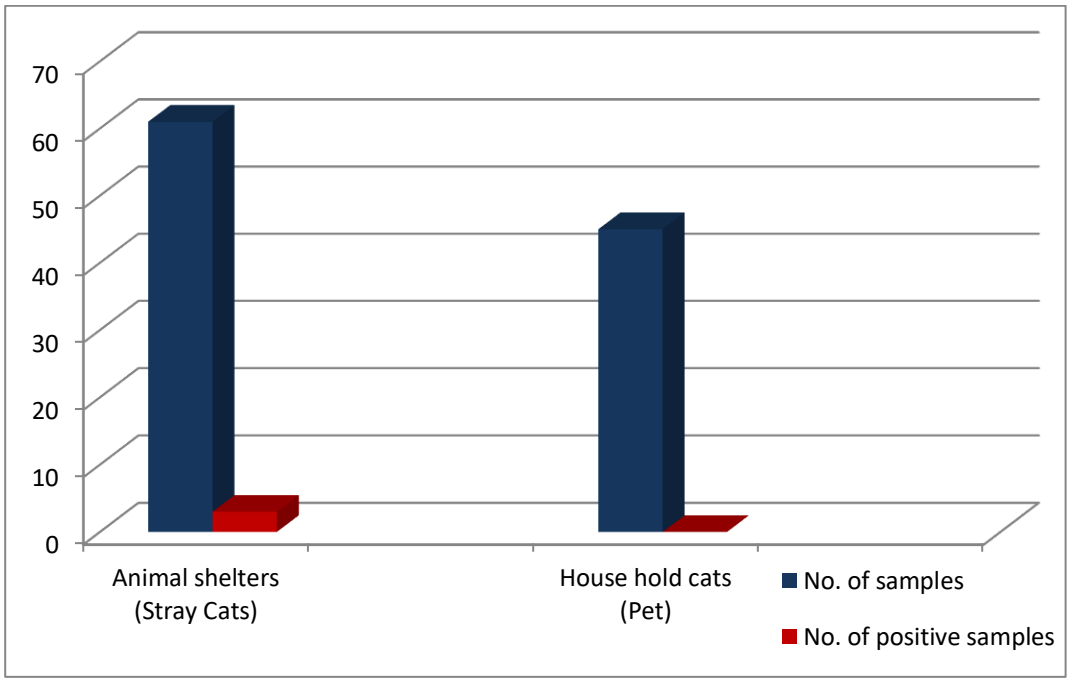

Figure 4.1: Prevalence of *Toxoplasma gondii* oocysts in cat's faecal sample

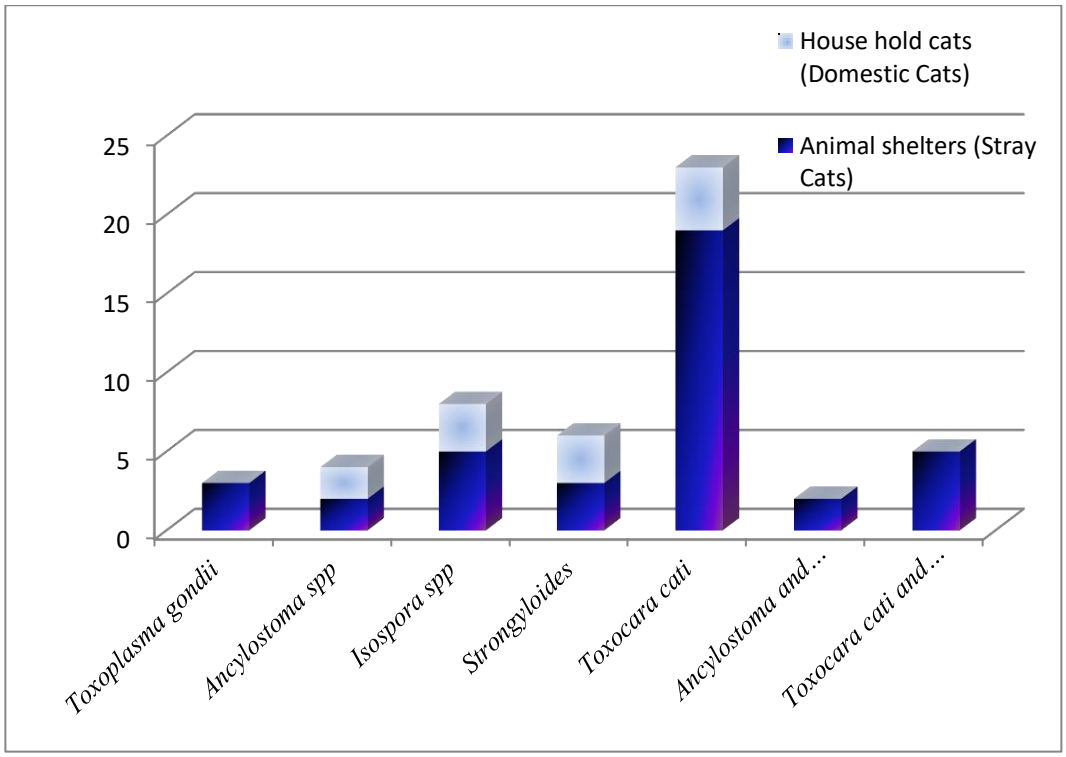

Figure 4.2: Prevalence of different helminth eggs with mixed parasitic infections.

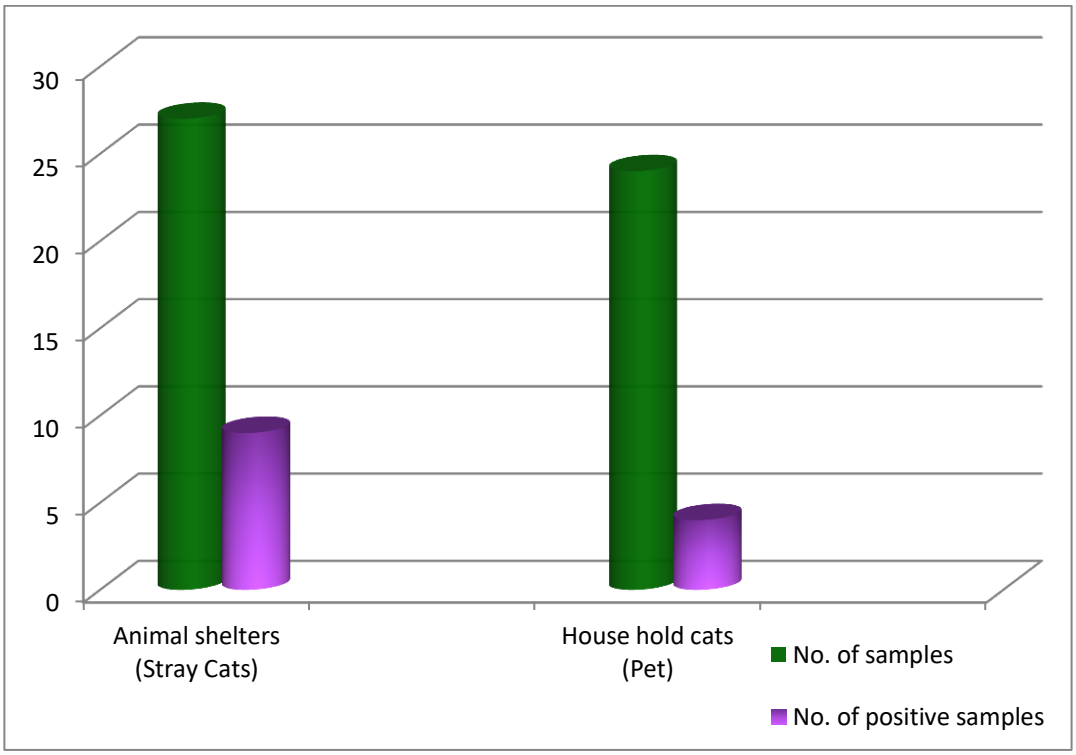

Figure 4.3: Prevalence of *Toxoplasma gondii* by Antibody detection kit (PetX Ab)

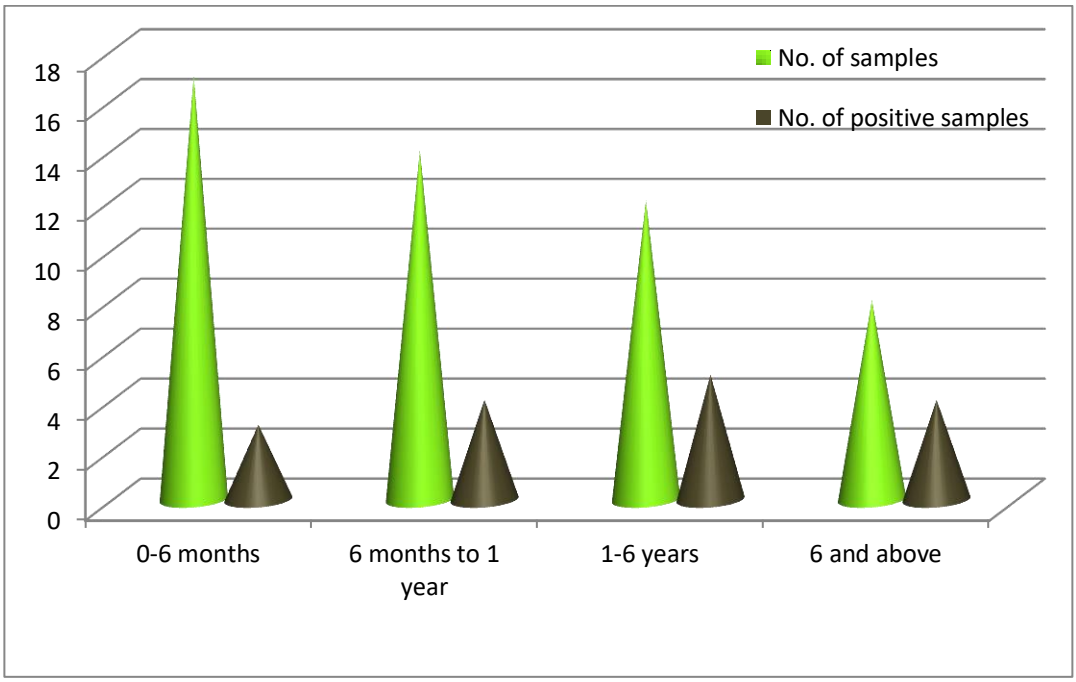

Figure 4.4: Age wise sero-prevalence

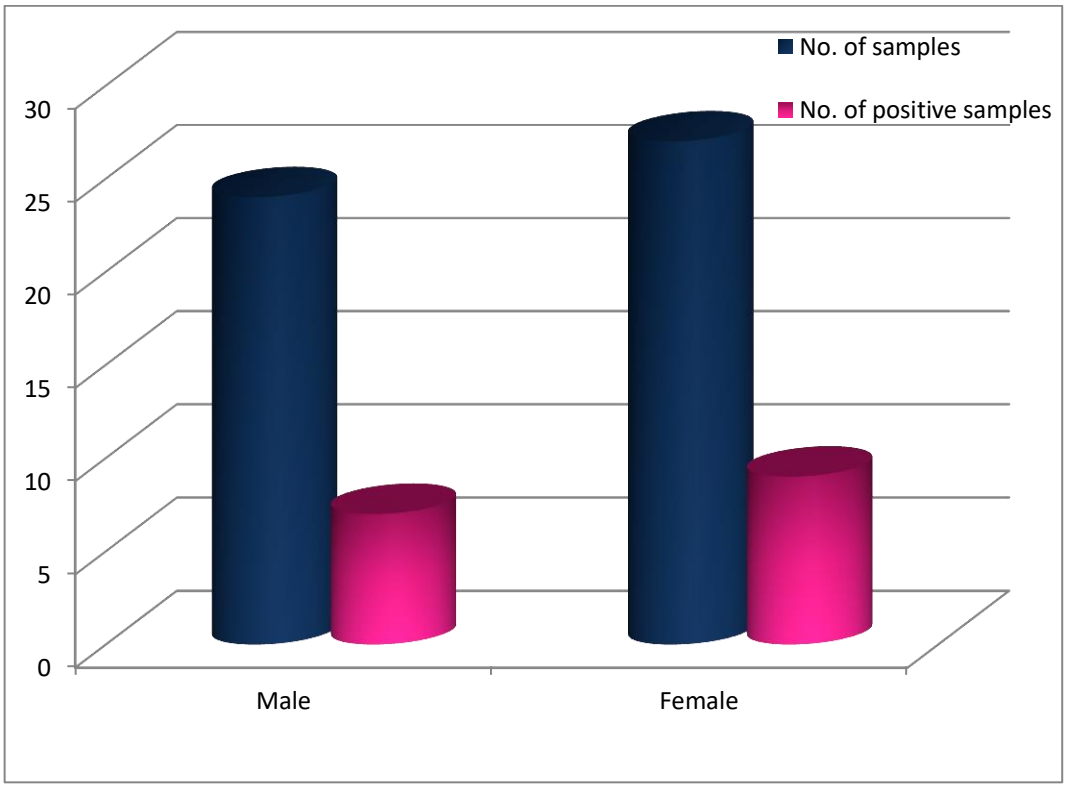

Figure 4.5: Sex wise seroprevalence

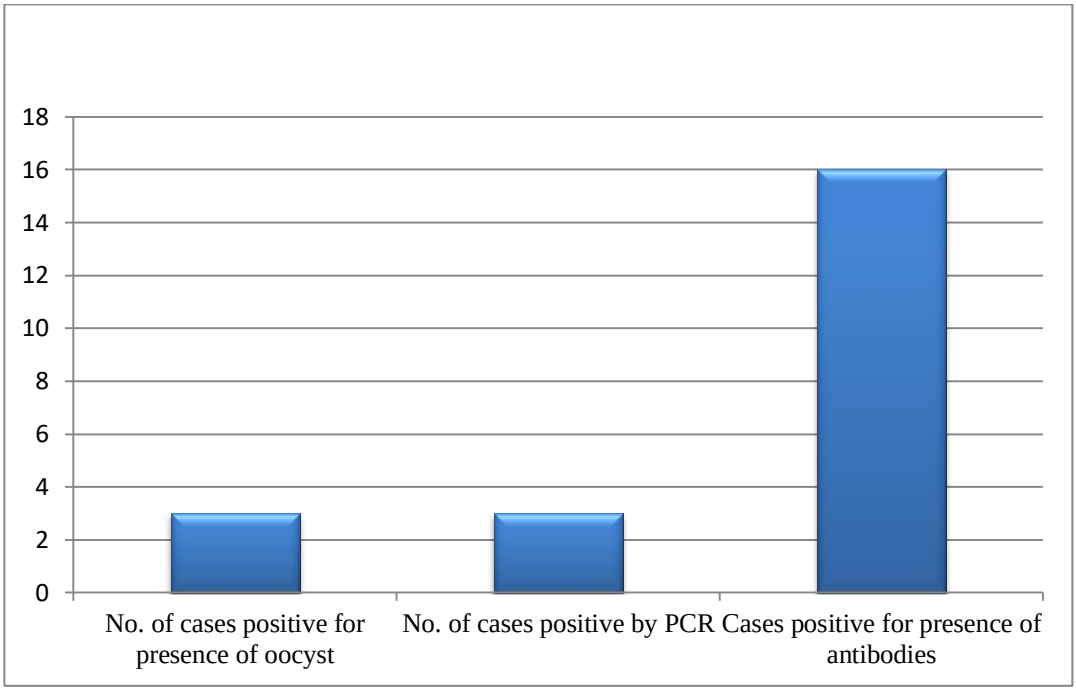

Figure 4.6: Method wise distribution of *toxoplasma gondii* positive cases

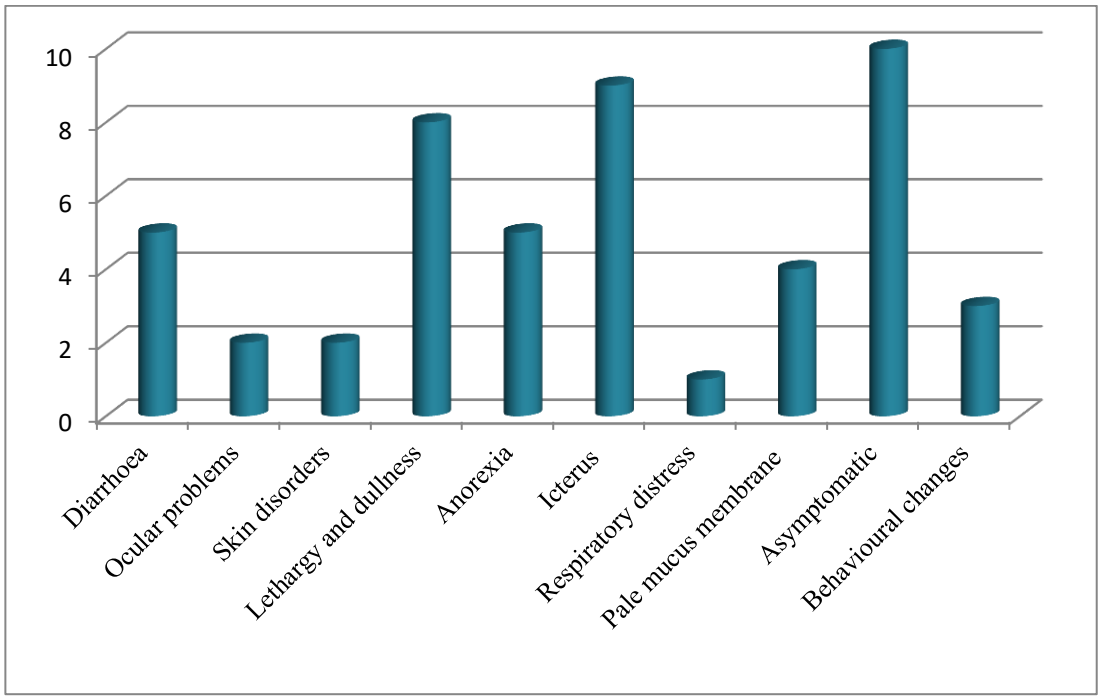

Figure 4.7: Clinical signs

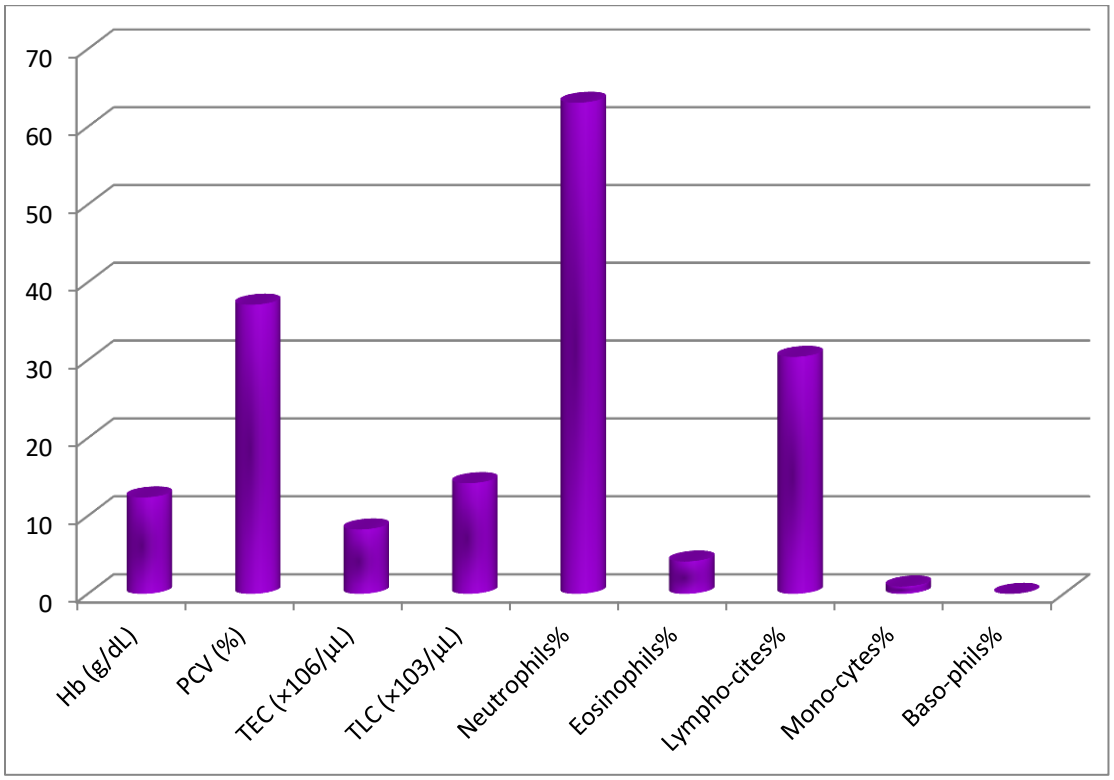

Figure 4.8 Haematological parameters (Mean ± SE)

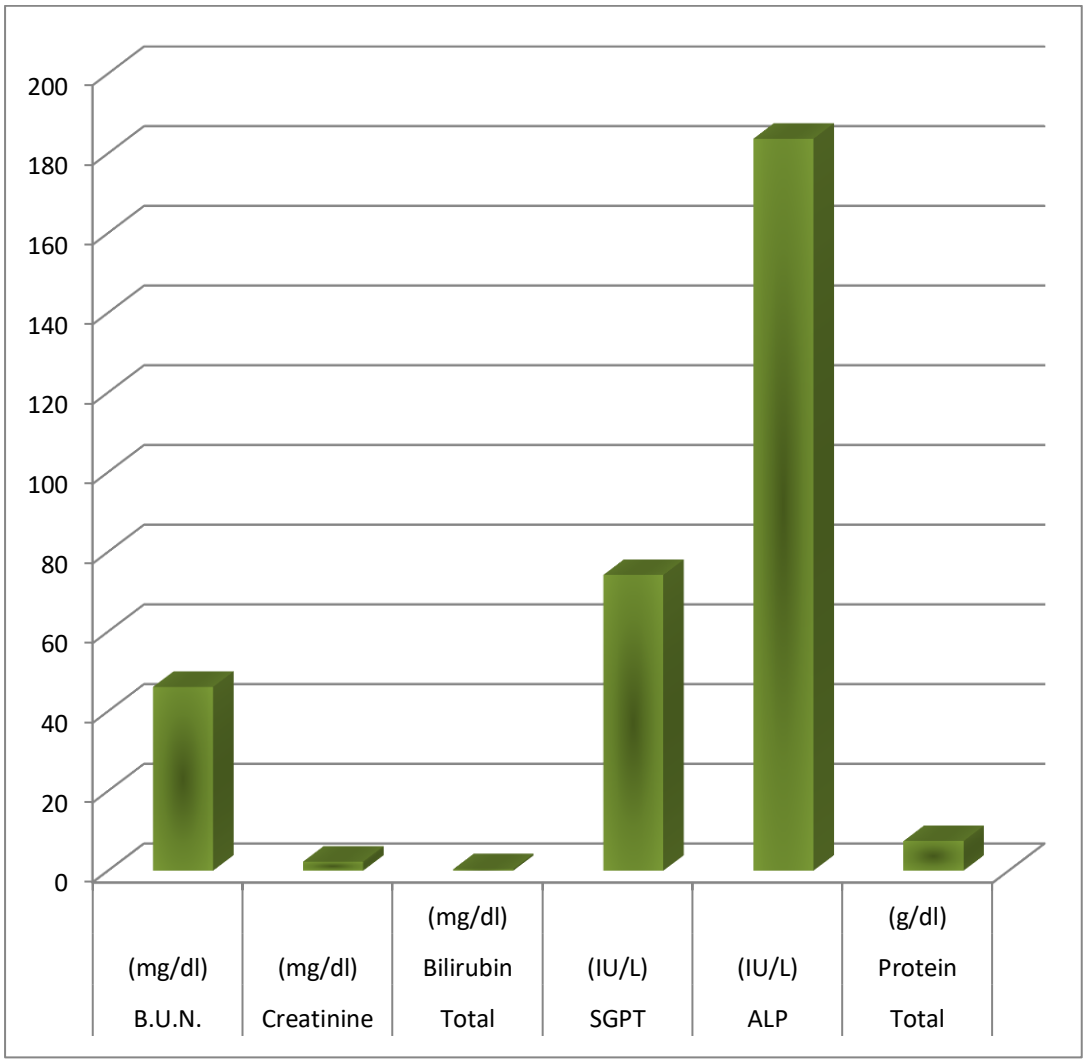

Figure 4.9 Biochemical parameters (Mean ± SE)

SUMMARY AND CONCLUSIONS

CHAPTER V

SUMMARY AND CONCLUSIONS

Toxoplasmosis is the major zoonotic disease involving all warm blooded animals. Oocyst shedding is mainly seen in cats being definitive host. The present study was aimed to find out prevalence of toxoplasmosis in Shirwal, Pune and Mumbai region which will help veterinarians in differential diagnosis.

Total 106 fecal samples from clinically suspected cases and apparently healthy cats were screened by using fecal flotation technique for presence of oocysts. . The overall copro-prevalence of *toxoplasma gondii* in cats was found to be 2.83% (3/106). The prevalence based on housing and ownership was 4.91% (3/61) in animal shelters (Stray Cats) and 0% (0/45) in House hold cats (Pets).

At the same time prevalence of different helminth eggs with copro-microscopy was 48.11% (51/106). Higher prevalence of gastrointestinal parasites was noted in shelter (stray) cats than household (Pet) cats.

The overall sero-prevalence of toxoplasmosis by rapid test (PetX Toxo Ab) was 31.37% (16/51) with increased seropositivity in 33.33% (9/27) stray cats than pet cats 16.66% (4/24).

Age wise seroprevalence ranged from 17.64% to 50%. In kittens of 0-6 month's age range, seropositivity was observed in 17.64% (3/17). Seropositivity for the age range of 6 months to 1 year was 28.57% (4/14). In 1-6 years and greater than 6 years of age, seroprevalence was 41.66% and 50% i.e. 5/12 and 4/8 respectively. It was noted that seroprevalence was lower in young age group compared to older age group. Higher age group had more chances of exposure to *T. gondii* throughout the lifespan.

In sex wise seroprevalence studies, 25.92% (7/24) males and 33.33% (9/27) females were found to be positive for the presence of *toxoplasma gondii* specific

antibodies by rapid antibody detection kit. The seropositivity was higher in females than in males. Increased seroprevalence in females can be related to sampling size and population as well as it might be related to increase in hunting activity along with roaming to feed kittens.

Total 51 animals were subjected to PCR assay which were suspected for toxoplasmosis by rapid antibody detection kit, copro-microscopy and presence of clinical signs. For copro-PCR, 529bp repetitive gene was targeted. Samples positive by copro-microscopy were confirmed by PCR and prevalence based on PCR from fecal samples was 5.88% (3/51).

Most of the cats were asymptomatic in nature without any obvious signs and symptoms 52.63% (10/19). In cases positive for Toxoplasmosis, lethargy and dullness were observed in 42.10% (8/19), icterus in 15.78% (3/19), diarrhoea in 26.31% (5/19), ocular problems in 10.52% (2/19), pale mucous membrane in 21.05% (4/19), dermatological problems 10.52% (2/19) and respiratory distress in 5.26% (1/19). Anorexia was seen in 26.31% (5/19) and behavioural changes in 15.78% (3/19).

The study of hematobiochemical alternations can aid to suspect cases of toxoplasmosis in differential diagnosis. The mean ± S.E. value of Hemoglobin (gm/dl) of the cat's positive for toxoplasma were 12.38 ± 0.80. Total 4(21.05%) cats were anaemic. The mean ± S.E. value of PCV (%) in the present study was 37.12±2.32 in cats positive for toxoplasmosis. Total 4(21.05%) cats were showing PCV level below normal range and 15(78.4%) cases had normal PCV range. The mean ± S.E of TEC ($\times 10^6$ /µl) in cats affected were 8.27± 0.47, which indicated normal reference range. Total 4 (21.05%) cases had low TEC count.

The mean ± S.E. value of TLC ($\times 10^3$ /µl) of affected cats were 14.21±1.44. Values were near the upper range of reference range. The Mean ± S.E. value of neutrophils (%) was 63.10±3.87. Total 10 (52.63%) animals were having neutrophilia and 2(10.52%) showed neutropenia. The mean ± S.E. value of Eosinophils (%) of

affected cats was 4.15±0.74 which was slightly higher than normal range. Total 5 (26.31%) cats had eosinophilia out of 19.The mean ± S.E. value of Lymphocyte (%) of affected cats in the present study was 30.42±3.84. Total 8(42.10%) cats had lymphocytosis and 8 cats had lymphopenia. The mean ± S.E. value of monocytes (%) was 0.94±0.05 which indicated normal range of felines.

The mean ± S.E. value of BUN (mg/dl) in cases positive for toxoplasma was 46.13±9.36 which showed increased BUN. The mean ± S.E. value of Creatinine (mg/dl) in affected cats was 2.28±0.49 which is slightly higher than normal reference range. The mean ± S.E. value of total Bilirubin (mg/dl) of positive cats was 0.37±0.03 which is slightly higher. The mean ± S.E. value of ALT (IU/L) of affected cats was 74.21±9.55 which indicates normal range. The mean ± S.E. value of ALP (IU/L) of cats positive for toxoplasmosis was 183.68±36.09. It indicates that higher ALP is seen in cats positive with toxoplasmosis. The mean ± S.E. value of total Proteins (g/dl) was 7.44±0.20.

Thus, it may be concluded from the above study that,

- The overall copro-prevalence was detected in 2.83% of cats on the basis of detection of oocyst and sero-prevalence was 31.37% by rapid antibody detection kit.
- Seropositivity was higher in (33.33%) stray cats than household cats (16.66%) and higher seroprevalence was noted as age increases (50.00%) as well as the seropositivity was higher in females (33.33%) than in males (25.92%).
- Samples positive by copro-microscopy for presence of oocysts were confirmed by PCR assay and copro-PCR is important tool in identification of active oocyst shedding cases.

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VITA

VITA

Dr. Pritesh Shivaji Vidhate was born on 28th of December, 1995 in Ratnagiri. The author grew up understanding animals under the guidance of his father who belongs to the veterinary field. He developed a liking for the veterinary field while seeing his father curing ailing animals and their trips for bird watching.

The author's father had a transferable job, and hence the author had to change four schools before reaching his Matriculation. These transfers introduced the author to the rural landscapes and the issues related to the livestock. The author moved to Pune city to complete his higher secondary studies from Modern College, Shivajinagar, Pune. He secured first class with distinction and second class during S.S.C and H.S.C examinations. The author pursued his passion for veterinary field from B.V.Sc. & A.H. from Nagpur Veterinary college and KNP College of Veterinary Science, Shirwal from 2013 to 2018.

During his Masters doctor Pritesh has participated in the total 6 workshops and conferences including, national and state level as well as World Small Animal Veterinary Association conference.

In order to gain practical expertise he has been involved into veterinary practice from last 2 years. He has worked in polyclinics, and volunteered at animal rescue trust.

The author is a multifaceted person with an affinity for long rides, nature treks along with wildlife and travel photography in his leisure time. He is an avid reader and lover of sarcasm.

THESIS ABSTRACT

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a)	Title of the thesis	:	PREVALENCE OF TOXOPLASMOSIS IN FELINES
b)	Full name of student	:	Vidhate Pritesh Shivaji
c)	Name and address of Major Advisor	:	Dr. Milind D. Meshram Professor, Department of Veterinary Epidemiology and Preventive Medicine, Krantisinh Nana Patil College of Veterinary Science Shirwal, Dist. Satara
d)	Degree to be awarded	:	M.V.Sc.
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f)	Major subject	:	Veterinary Epidemiology and Preventive Medicine
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i)	Signature of Student	:	
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ABSTRACT

Toxoplasmosis is the major zoonotic disease involving all warm blooded animals and oocyst shedding is mainly seen in cats being definitive host. The present study entitled, "Prevalence of toxoplasmosis in felines" was undertaken to detect the prevalence of *toxoplasma gondii* in Shirwal, Pune and Mumbai region. The overall copro-prevalence of *toxoplasma gondii* in cats was 2.83%. Prevalence of different gastrointestinal parasites eggs was 48.11%. Out of total parasitic eggs, *Ancylostoma* spp, *Isospora* spp, *Strongyloides* and *Toxocara cati* was observed in 3.77%, 7.54%, 5.66% and 21.69% of cats respectively. Higher prevalence of gastrointestinal parasites was noted in stray cats. The sero-prevalence by rapid test (PetX Toxo Ab) was 31.37% with increased seropositivity in 33.33% stray cats

than pet cats 16.66%.Most of the seropositive cases were from the age group of greater than 6 years of age. Higher seroprevalence in 33.33% females was observed than 25.92% males. Samples positive by copro-microscopy were confirmed by 529bp repetitive gene copro-PCR and prevalence based on copro-PCR was 5.88%.

Highest no. of seropositive cases were asymptomatic (52.63%).Normal ranges of hematological parameters were observed with increased serum level of BUN, ALP and total bilirubin.

The cat can be suspected for Toxoplasmosis despite non-specific alterations in hematobiochemical parameters, on the basis of clinical signs, presence of *toxoplasma gondii* specific antibodies in serum, and confirmatory diagnosis such as PCR assay is required.

प्रबंध सारांश

अ)	प्रबंधाचे शीर्षक	:	टोक्सोप्लासमोसीस या आजाराचे मांजरामधील प्रमाण.
ब)	विद्यार्थ्यांचे पूर्ण नाव	:	प्रितेश शिवाजी विधाटे
क)	मार्गदर्शकाचे नाव व पत्ता	:	डॉ. एम. डी. मेश्राम प्राध्यापक पशुवैद्यकीय साथरोग व प्रतिबंधात्मक औषध शास्त्र विभाग क्रां. ना. पा. पशुवैद्यकीय महाविद्यालय शिरवळ जि. सातारा
ड)	पदवीचे नाव	:	एम.व्ही. एस्सी
इ)	पदवीचे वर्ष	:	२०२१
फ)	विषय	:	पशुवैद्यकीय साथरोग व प्रतिबंधात्मक औषध शास्त्र
ग)	प्रबंधातील एकूण पाने	:	६५
ह)	प्रबंध सारांशामधील एकूण शब्द	:	१९७
म)	विद्यार्थ्यांची स्वाक्षरी	:	
भ)	सादरकर्ता अधिकाऱ्याची स्वाक्षरी नाव व पत्ता	:	डॉ. एम. डी. मेश्राम प्राध्यापक पशुवैद्यकीय साथरोग व प्रतिबंधात्मक औषध शास्त्र विभाग क्रां. ना. पा. पशुवैद्यकीय महाविद्यालय शिरवळ जि. सातारा

सारांश

टोक्सोप्लासमोसीस हा सर्व उष्ण रक्तीय प्राण्यांना होणारा आजार असून तो एका प्रजाती पासून दुसऱ्या प्राण्यांच्या प्रजाती मध्ये पसरतो.

टोक्सोप्लासमोसीस या आजाराचे प्रमाण ओळखण्यासाठी "टोक्सोप्लासमोसीस या आजाराचे मांजरामधील प्रमाण" हा अभ्यास शिरवळ, पुणे व मुंबई या भागात केला गेला. एकूण 2.83% मांजरीच्या विष्ठेमध्ये टॉक्सोप्लाजमा गोंडी ची ऊसिस्ट सापडली. विविध आंत्रकृमीचे प्रमाण 48.11% आढळले. अंकायलोस्टोमा, आयसोस्पोरा, स्ट्रॉंगायडल आणि टोक्सोकॅरा कॅटीचे प्रमाण अनुक्रमे 3.77%, 7.54%, 5.66% आणि 21.69% मांजरी मध्ये आढळले. भटक्या मांजरी मध्ये कृमींचे प्रमाण जास्त आढळले. जलद प्रतिपिंडे चाचणी (पेट एक्स ए.बी.) ने सापडलेले सेरो प्रमाण 31.37% इतके होते व भटक्या मांजरी मधील (33.37%) प्रमाण, पाळीव (16.66%) मांजरीपेक्षा जास्त आढळले. सगळ्यात जास्त सेरो प्रमाण 6 वर्षापेक्षा अधिक वयाच्या मांजरीमध्ये आढळले. माद्यां मधले सेरो प्रमाण (33.33%) हे नरांपेक्षा (25.92%) अधिक आढळले.

विष्ठा सूक्ष्मदर्शी चाचणीमध्ये सकारात्मक आढळलेल्या नमुन्यांची ५२९ बेस् पेअर रिपीटेटीव जीन पीसीआर ने पुष्टी करण्यात आली. सगळ्यात जास्त मांजरी (52.63%) या लक्षण विरहित आढळल्या. रक्त तपासणी सामान्य तर सेरम तपासणी मध्ये बी.यु.एन , ए.एल .पी व टोटल बिलिरुबिन वाढलेले आढळले.

आजाराची लक्षणे व टॉक्सोप्लास्मा गोंडी आजाराची प्रतिपिंडे आढळल्यास आढळल्यास आणि रक्त व सेरम चाचणीत विशिष्ट बदल नसले, तरीही टोक्सोप्लासमोसीस या आजाराचे निदान केले जाऊ शकते. मात्र पुष्टी विष्ठा पीसीआर करणे गरजेचे आहे.