

Szent Istvan University, Faculty of Veterinary Sciences
Department of Parasitology and Zoology

The seroprevalence of *Toxoplasma gondii* antibodies in cats from Hungary

By
Daniela Nieto

Supervisor:
Professor Róbert Farkas

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1. Introduction

Toxoplasma gondii is an ubiquitous parasite causing infection in a broad spectrum of domestic and wild animal species. It is a zoonotic protozoan which is especially dangerous for pregnant women as it may cause abortion or fetal developmental disorders and in immunocompromised individuals.

The felids are the definitive hosts and a wide range of animals serve as the intermediate hosts. Cats play an epidemiological role in the spread of the parasite in the environment because they can shed oocysts with their feces. Cats can obtain the infection by consumption of sporulated oocysts from the environment or by tissue cysts from infected intermediate hosts. The detection of infection is very difficult with finding of oocysts in the feces of cats because the shedding period is 1-3 weeks once in their lifetime. Even though cats can shed up to hundred thousands of oocysts of the parasite, the exact moment of shedding is challenging to determine. On the other hand, the infection caused by *T. gondii* cannot be diagnosed based on the morphology and size of the unsporulated oocysts, molecular biological studies are needed. The infection of cats can be determined easier by serological methods because antibodies against *T. gondii* circulate in the blood of the infected animals. These cats have most likely already shed oocysts in their feces and now just harbour the antibodies. The data about the seroprevalence of *T. gondii* infection in cats is an important parameter for Public Health, because it gives a suggestion about the contamination in the environment and therefore the risk it implies to humans (Gyorke et al., 2011).

The aim of my thesis is to study the occurrence of anti-*T. gondii* antibodies in the sera of 200 cats living in Budapest and the countryside with Immunofluorescence Antibody Test (IFAT). The other aim of this study is to evaluate whether there are relationships between the seroprevalence of the animals living in Budapest and the countryside, their age or gender.

2. Literature Review

2.1 History of *Toxoplasma gondii*

The parasite was discovered more than 100 years ago by Charles Nicolle and Louis Manceaux in 1908, while they worked at the Pasteur Institute in Tunis, Tunisia. They found the parasite in the tissues of a rodent having the species name *Ctenodactylus gundi*. Subsequently, they named the parasite *Toxoplasma gondii*. In Greek, 'toxos' means arc or bow and 'plasma' means anything that is shaped or molded. The name *gondii* comes from the name of the rodent in which it was discovered. The correct name should have been *Toxoplasma gundii*, but the scientists identified the rodent incorrectly as *Ctenodactylus gundi* (Dubey, 2010). *T. gondii* is regarded as one of the most prevalent parasites in the world (Sykes, 2013).

2.2 Morphology of *Toxoplasma gondii*

Toxoplasma gondii is a protozoan that belongs to the Phylum Apicomplexa. This species has 3 important infectious stages: oocysts, tachyzoites and bradyzoites (Dubey, 2010). The oocysts are approximately 11-13 micrometers in size; each has two sporocysts, each with 4 sporozoites (Bowman, 2013). The tachyzoites (from Greek: Tachos=speed; Zoon=animal) are the rapidly multiplying stage. They are crescent-shaped with a central nucleus (Dubey, 2010). Bradyzoites (Brados=slow; Zoon=animal) have a more posterior nucleus, they are more slender, contain glycogen and are resistant to digestion (Dubey, 2010; Bowman, 2013). Bradyzoites are the encysted stage found in the tissues of animals and humans. Bradyzoites are also called cystozoites. The size of tissue cysts varies; young cysts are small with only two bradyzoites and older cysts are larger with thousands of bradyzoites. Tissue cysts in the brain are around 70 micrometers and reaching up to 100 micrometers in the muscles (Dubey, 2010).

2.3 Life cycle of *Toxoplasma gondii*

The life cycle of *T. gondii* was discovered by Frenkel in 1970. This protozoan can infect all warm-blooded animals as intermediate hosts (including cats). The only definitive hosts are those species belonging to the family Felidae (wild and domestic cats). Only definitive hosts

can shed oocysts in their feces. The oocysts undergo sporulation in the environment within 1-5 days. Sporulated oocysts are the infective stage of the parasite. The definitive and intermediate host species become infected by ingesting sporulated oocysts. Once ingested, the wall of the oocysts is digested by the stomach. The sporocysts are released and undergo excystation and then the released sporozoites will penetrate the enterocytes. They develop into tachyzoites which spread to several tissues, the most predilection sites are the neural tissue (e.g. brain and eyes) and muscle tissues (e.g. skeletal and cardiac) and where tissue cysts are formed as early as 3 days post inoculation (Dubey, 2010). It was previously thought that tissue cyst formation was controlled by the host's immune system, but a later study from Dubey and Frenkel (1976) revealed that tissue cysts are an essential part of the life cycle of the parasite and independent from the immune system. These tissue cysts can remain viable and infective for the lifetime of the host (Dubey, 2008).

2.3.1 Life cycle in the definitive host

The definitive hosts may become infected after ingesting oocysts, tissue cysts or tachyzoites (Dubey, 2010). Cats also serve as intermediate hosts, because the tachyzoites and tissue cysts can form in the extraintestinal tissues (Bowman, 2013). The parasite undergoes an extraintestinal cycle in the host. After ingesting tissue cysts, these are digested in the stomach and small intestines. Bradyzoites are released and penetrate the enterocytes, developing into merozoites and replicate by several cycles of schizogony. Merozoites will transform into micro and macrogametes, which later fertilize and produce a zygote. The cat will shed unsporulated oocysts in their feces. Moreover, after ingesting sporulated oocysts, the sporozoites inside the oocyst will undergo enteroepithelial replication and develop into tachyzoites. Tachyzoites replicate quickly and reach visceral organs via the blood and lymph vessels (Sykes, 2013). Cats will shed oocysts after they ingest any of the three stages of the parasite; tachyzoites, bradyzoites or oocysts. The prepatent period and frequency of the shedding depends on which infectious stage the parasite is ingested (Dubey, 2010). The prepatent period varies between 3-10 days after ingesting tissue cysts and more than 18 days (19-48) after ingesting oocysts (Dubey, 2010; Bowman, 2013). Cats will shed *T. gondii* oocysts for 1-3 weeks. If reinfection occurs, the shedding is less and for a shorter period. Cats

that are co-infected with *Cystoisospora*, can trigger the shedding of *T. gondii* (Bowman, 2013). Cats can shed millions of oocysts, after ingesting only one bradyzoite and contrarily 100 oocysts may not infect cats (Dubey, 2008). Experimentally infected cats have shown to not shed oocysts again after they were reinfected with *T. gondii* (De Craeye et al., 2008). Cats that have undergone *Toxoplasma* infection and have shed oocysts are generally a minor source of infection. These cats are considered safer pets than those that have not contracted the infection yet (Bowman, 2013). *Toxoplasma gondii* is more efficiently transmitted by carnivorism in felines that ingest tissue cysts from intermediate hosts, whereas in intermediate hosts it is by the ingestion of sporulated oocysts (Dubey, 2010).

2.3.2 Life cycle in the intermediate hosts

The parasite can cause infection in animals and humans through the following ways:

1. Congenital: the tachyzoites are able to pass through the placenta and reach the fetus (Dubey, 2008).
2. Carnivorism: by the ingestion of tissue cysts in undercooked meat (Dubey, 2008).
3. Fecal-oral route: this explains the widespread infection of vegetarians and herbivores. The cats shed the oocysts in the feces and contaminate the environment (Dubey, 2008).
4. Infection by sexual transmission and transplacental infection has been documented in dogs (Sykes, 2013).
5. Dogs can mechanically transmit *T. gondii* oocysts after they have ingested feline feces (Sykes, 2013).

The parasite has an extraintestinal cycle in the intermediate hosts, when they ingest sporulated oocysts or tissue cysts. After the ingestion of *T. gondii* oocysts, the sporozoites are released in the intestinal lumen. They penetrate the enterocytes, undergo replication and develop into tachyzoites. The tachyzoites are the rapidly dividing stage and can spread to many tissues and further develop into bradyzoites. In case of immunodeficient or pregnant animals, the tachyzoites are what cause the most damage. Most animals can control the infection and the parasite remains enclosed as tissue cysts in the brain, muscles and visceral organs. Intermediate hosts may also become infected by ingesting *T. gondii* tissue cysts. The tissue

cysts are digested by the stomach and the resistant bradyzoites are released, these penetrate the enterocytes and develop into tachyzoites and these again into bradyzoites (Dubey, 2010; Bowman, 2013).

Some intermediate hosts such as pigs, mice and humans can become infected after ingesting only one oocyst. The ingestion of tissue cysts is less infective in mice than ingestion of oocysts (Dubey, 2008). When rodents are infected with *T. gondii*, the parasite causes a change in their behaviour, which makes rodents less afraid of cats. This increases the probability that the cat will become infected by eating the rodent and so that the parasite can replicate sexually and complete its life cycle in the definitive host (Sykes, 2013).

2.4 Clinical toxoplasmosis in cats

Cats that are immunocompetent, *T. gondii* usually causes a subclinical infection. On the contrary, the parasite may lead to chronic and clinical toxoplasmosis in immunosuppressed cats, caused either by corticosteroid treatment or viruses (D'Amore et al., 1997). Toxoplasma is an opportunistic infection in cats with virus infection (Svobodova et al., 1998). After a primary infection, if there is not a proper immune response; the tachyzoites are responsible for tissue damage. Clinical toxoplasmosis in cats is very rare, during the enteroepithelial stage of the parasite's cycle, only 10-20% of cats will show diarrhea for 1-2 weeks, which is generally self-limiting. Fatal extraintestinal toxoplasmosis has developed in cats that were co-infected with the Feline Leucosis Virus (FeLV), Feline Immunodeficiency Virus (FIV), Feline Infectious Peritonitis Virus (FIP) or received immunosuppressive treatment such as cyclosporine. The tissues most commonly affected are the central nervous system, liver, lungs and pancreas. The most severe signs are seen in kittens that are infected via the placenta or milk from their mothers. They usually die of lung or liver failure and ocular disease is commonly seen. The most common clinical signs of generalized toxoplasmosis in cats are lethargy, anorexia, respiratory signs and uveitis (Sykes, 2013).

2.5 Clinical toxoplasmosis in the intermediate hosts

Tachyzoites are the stage that cause tissue damage and the clinical signs depend on the number of tachyzoites in the host, the immune status of the host and the organs involved. Toxoplasmosis is usually a subclinical infection in immunocompetent animals. In immunosuppressed animals, the infection may cause acute systemic toxoplasmosis. Complications that may arise are interstitial pneumonia, myocarditis, hepatic necrosis, meningoencephalitis, chorioretinitis, lymphadenopathy and myositis. Common clinical signs are fever, diarrhea, coughing, dyspnea, icterus, seizures and death. *Toxoplasma* can cause abortion in small ruminants and pigs. Toxoplasmosis can cause liver disease in immunocompromised or young dogs (Khan and Line, 2010). Cattle and horses are resistant to clinical toxoplasmosis and clinical cases have not been reported (Dubey, 2008). Marine mammals have also been identified with clinical toxoplasmosis, indicating that the waters are also infected with *T. gondii* oocysts (Dubey, 2008). Even if animals are treated and recover from toxoplasmosis, they should be considered to be chronically infected with the parasite (Khan and Line, 2010).

Humans that have an intact immune system and are infected with *T. gondii*, can have mild clinical signs such as fever, muscle pain, lymphadenopathy, anorexia and sore throat. Toxoplasmosis is much worse in humans with immunodeficiency such as in fetuses, newborns and old adults. Transplacental transmission can result in abortion, malformations or mental retardation of the fetus. Pregnant women should avoid contact with cat feces and undercooked meat. The most important source of infection for humans is pork meat (Bowman, 2013).

The data about the seroprevalence of *T. gondii* infection in cats is an important parameter for Public Health, because it gives a suggestion about the contamination in the environment and therefore the risk it implies to humans (Gyorke et al., 2011).

2.6 Laboratory diagnosis of toxoplasmosis in cats

There are no pathognomonic signs for toxoplasmosis. For this reason specific laboratory methods must be used for the diagnosis (Sykes, 2013).

2.6.1 Laboratory methods

1. Fecal flotation method: *Toxoplasma gondii* oocysts are morphologically similar to the oocysts of *Hammondia*, *Besnoitia* and *Neospora* genus (Sykes, 2013; Deksne et al., 2013). Therefore their distinction is difficult. This method is not very indicative of clinical toxoplasmosis in cats, because most clinical cases occur after the shedding period. Cats shed the oocysts usually once in their lifetime for 1-3 weeks, so it is difficult to find oocysts at the precise moment of shedding (Sykes, 2013). In spite of cats excreting up to millions of oocysts, they are rarely found in fecal samples (Lopes et al., 2008).
2. Serology diagnosis in cats: this method is the most frequently used (Sykes, 2013). Serological tests cannot exactly determine when a cat got infected or has shed oocysts (Lappin, 1996). The antibodies produced against *T. gondii* are detected in the serum (Sykes, 2013).

Different techniques are available such as ELISA, Immunofluorescence Antibody Test (IFAT), Modified Agglutination Test (MAT), Latex Agglutination Assay (LA), Direct Agglutination (DA), Indirect Hemagglutination Assay (IHA) and Sabin-Feldman dye test. The agglutination assays can be used for cats and can detect all types of antibodies but they very poorly detect antibodies when only IgM type is present (Sykes, 2013).

The Direct Agglutination Assay is the main method used in humans and animals (Michalski et al., 2010), although there is no international standard when it comes to the detection of toxoplasmosis in cats, even when using the same method (Hornok et al., 2007). Various articles show different opinions about the serological methods used. According to Da Craeye et al. (2008), the Sabin Feldman dye test and IFAT have higher sensitivity and specificity than ELISA or Agglutination tests. Lopes et al. (2008) considered MAT the most sensitive and specific method. On the contrary, Salant and Spira (2004) used ELISA method as it was

considered to have higher specificity and sensitivity than the Latex Agglutination and Indirect Hemagglutination Assays.

Other serological assays can detect *T. gondii* immune complexes, but these are not available commercially (Sykes, 2013). Circulating Antigens: give more evidence about the presence of an infection and can be detected in the acute stage of an infection (Wang et al., 2012).

The detection of *T. gondii* DNA with PCR in biological samples is also a good diagnosis for toxoplasmosis, because of the high sensitivity and specificity (Wang et al., 2012).

2.6.2 Detection of IgM antibody in cats

IgM antibodies were detected within 2-4 weeks after infection of experimentally infected cats with ELISA, but these animals became negative within 16 weeks. IgM titers after 16 weeks have been detected in cats co-infected with FIV and with ocular toxoplasmosis. Some percentages of cats do not have detectable IgM titers. The IgM antibodies cannot be used to predict the exact moment when a cat is shedding oocysts. One study of cats with clinical toxoplasmosis, IgM titers were detected in 93% of cats and IgG in 60% of cats. Thus, IgM antibodies have a higher predictive value for clinical toxoplasmosis than IgG. In the case of cats with chronic toxoplasmosis, they are firstly IgM positive and later negative, but they can become positive again after reinfection due to immunosuppression by FIV or glucocorticoid treatment. In these cats with chronic toxoplasmosis, they do not show clinical signs of the infection, therefore the detection of IgM antibodies will not always indicate clinical toxoplasmosis (Sykes, 2013). The IgM antibodies are difficult to detect in feline sera and results using the Latex Agglutination and Indirect Hemagglutination methods have shown not to be accurate (Lappin and Powell, 1991).

2.6.3 Detection of IgG antibody in cats

With ELISA method, IgG antibodies can be detected in the serum of cats within 3-4 weeks after infection. Usually by the time these antibodies are detected, the shedding period of the oocysts has finished, thus IgG seropositive cats pose a lower threat to the Public Health (Sykes, 2013). In experimentally inoculated cats, the IgG can be detected for at least 6 years in the serum. The parasite and the antibodies produced against it can persist lifelong in the host (Sykes, 2013). Also confirmed by Salant and Spira (2004), IgG antibodies are known to remain high in cats for many years after infection. The low titers indicate a past infection and high titers indicate a more recent or current infection (Michalski et al., 2010), but detection of IgG antibodies do not give information when the infection occurred (Wang et al., 2012). It has been shown that healthy cats can have titers above 1:10,000, thus high titers of IgG do not accurately indicate a recent or active infection. Cats with low number of IgG titers can be considered seronegative based on the cut-off titer and serological method used (even though *T. gondii* persist in the tissues). Increasing IgG titers indicate a recent or active infection. The time from the first detectable antibody to the highest titer is seen in 2-3 weeks. Increasing IgG titers are seen in healthy and sick animals, so the presence of the antibody alone does not indicate clinical toxoplasmosis. In case of chronic toxoplasmosis, if reinfection occurs after immunosuppression, IgG titers seldom increase (Sykes, 2013).

2.7 Studies on *Toxoplasma gondii* infection in cats

Due to an estimation that 30-40% of cats are infected with *T. gondii* worldwide (Waap et al., 2012), the serology diagnosis makes it possible to determine the prevalence rates in the different countries. Cats that are seropositive are already likely to have shed *T. gondii* oocysts and the detection of antibodies in their serum is useful to indicate the degree of contamination of the parasite in the environment and the risk it poses for humans (Lopes et al., 2008). The prevalence of *T. gondii* antibodies in cats, gives an indirect indication of the presence of *T. gondii* contamination of the environment (Wang et al., 2012). The prevalence rates of *T. gondii* infection in cats, varies between countries, within a country, even within the same city (Sukhumavasi et al., 2012). One of the highest prevalence rates was detected in Majorca Spain

(84.7%), this indicates that feral cats have highly contaminated the environment and therefore humans are at risk of possible infection (Millan et al., 2009).

A study carried out in Romania used IFAT and ELISA, in which higher prevalence was found in older cats, with mixed diet, with outdoor access and from rural area. The sex and breed of the cats did not influence the prevalence rate (Gyorke et al., 2011). From Arad County, Romania, 42 feline fecal samples were collected, all showed negative results for *T. gondii* oocysts (Hotea et al., 2009).

In Czech Republic, sera samples from 390 cats were collected and screened for IgG and IgM antibodies using IFAT (cut off titer 1:10). The overall prevalence was 61.3%. Only one cat was positive for IgM antibody. The flotation method was used to detect *T. gondii* oocysts and were detected in one fecal sample (Svobodova et al., 1998).

In Poland, the sera samples from 135 domestic cats were sampled and IgG was detected using Indirect Agglutination Assay. Eighty-seven (64.4%) cats were seropositive (Michalski et al., 2010).

In Scandinavian and Baltic countries, the prevalence ranged from 42% in Sweden and up to 60.8% in Estonia (Uggla et al., 1990; Must et al., 2015). In Latvia, 80 feline fecal samples were examined for *T. gondii* oocysts. *Toxoplasma-like* oocysts were found in two samples (Deksne et al., 2013).

In Belgium the sera of 567 domestic cats were tested for IgG and IgM antibodies using IFAT. The cut off titer was 1:40. The seroprevalence was 25%. Only one cat was IgM positive and IgG negative, respectively. Older cats showed higher IgG titers (De Craeye et al., 2008).

In the Netherlands, the sera samples from 450 cats were collected. The overall seroprevalence was estimated to be 18.2% when the presence of IgG antibodies were detected with ELISA. The determined risk factors were: age, hunting behavior, former stray cat and feeding of raw meat (Opsteegh et al., 2012).

In Germany, a total of 18,259 cats' fecal samples were analyzed for the detection of *T. gondii* oocysts with PCR. In total, 68 (0.25%) samples were positive (Herrmann et al., 2010).

In Spain, the sera samples from 585 cats were tested for IgG antibodies using IFAT. The cut off titer was 1:80. The prevalence was 32.3%. Higher seroprevalence was detected in stray and farm cats (Miro et al., 2004).

In Turkey, the sera samples of 1121 stray cats were collected and tested for IgG antibodies using IFAT and ELISA. The cut off titer was 1:16. The overall prevalence was 33.5% with both assays (Can et al., 2014).

In Japan, a total of 1,447 cats were examined, *T. gondii* antibodies were detected using LA. Seroprevalence was 5.4% (78/1,447). Higher prevalence was seen in outdoor and older cats (Maruyama et al., 2003).

During a study in the United States, the sera samples were collected from 12,628 clinically sick cats for detecting IgG and IgM antibodies with ELISA. The overall seroprevalence was 31.6%. The age, gender and breed of cats influenced the seroprevalence (Vollaire et al., 2005).

In Mexico, 37 sera samples of 169 domestic cats had IgG antibodies. The seroprevalence was 21.8% determined with ELISA. Risk factors for infection rate were increasing age, female gender and feeding raw meat (Besne-Merida et al., 2008).

In Brazil, Bresciani et al. (2006) reported that 25% of 400 cats had *T. gondii* infection when they used IFAT (cut off titer 1:64).

2.8 Studies on *Toxoplasma gondii* infection in cats in Hungary

The first study to be carried on *T. gondii* in cats was in 1979. The unsporulated oocysts were searched in the fecal samples of 60 cats using the flotation method. No cats were found to be actively shedding oocysts at the time of examination (Hegedus, 1979).

A second survey was performed in 2004, the sera samples were collected from 264 cats and 112 dogs for studying *T. gondii* antibodies using the Complement Fixation Test. Seventy-three cats had antibodies and the seroprevalence was 28%. The study showed higher prevalence in outdoor and younger cats (between 1,5-5 years) from the countryside (47%), compared to those living in Budapest (17%). No correlation was found between the seroprevalence and the breed or gender of the cats (Chikan, 2004).

A few years ago Hornok et al. (2007) studied the presence of IgG antibodies developed against *T. gondii* in 330 cats using IFAT. The seroprevalence was 47.6% (157/330). The sera were diluted at 1:20 to 1:10,240. High prevalence 80.3% (126/157) was detected at high titers between 1:640 to 1:5120. The prevalence was 22.4% in urban cats, 50% in suburban cats and 61.3% in rural cats. Females (53.3%) had a higher prevalence than males (39.3%). This study determined that higher prevalence of *T. gondii* infection occurred in rural, female and older cats.

3. Materials and methods

3.1 Materials

Altogether sera samples were collected from 100 cats kept in Budapest and 100 cats from the countryside. There were 99 male and 101 female cats, some of them were castrated. The majority of cats (168) were non-purebred. Based on the age the cats belonged to the following groups: Group 1 (11 cats) < 1 year, Group 2 (53 cats) from 1 to 5 years of age, Group 3 (123 cats) between 6 and 15 years of age and Group 4 (13 cats) > 15 years.

The sera were stored at minus 20° C until examined at the Department of Parasitology and Zoology, Faculty of Veterinary Science, Budapest.

3.2 Serological Method

The IgG antibody of cats developed against *Toxoplasma gondii* were detected with an Indirect Immunofluorescence Antibody Test using ‘MegaFLUO TOXOPLASMA g.’ (Diagnostik Megacor Laboratory, Austria).

The test kit contained:

- 10 slides with 10 wells coated with toxoplasma tachyzoites
- 1 dropper bottle with 0,5 ml Positive control
- 1 dropper bottle with 0,5 ml Negative control
- 1 dropper bottle with 3,0 ml of anti cat-FITC Conjugate
- 1 dropper bottle with 3,0 Mounting fluid

Laboratory materials used:

- Phosphate buffered saline solution (PBS, pH 7,4)
- Micropipettes
- Mixing wells for sera and PBS dilutions
- Coverslips for the slides
- Humid chamber for incubation of slides at 37 degrees Celsius
- Fluorescent microscope (Carl Zeiss Jena, Germany) at 400x magnification

3.3 Methods

Firstly, the slides were removed from the foil pouch and placed on a plastic container. The slides contained *T. gondii* tachyzoites. Each slide contained 10 wells; the first and the second well of each slide were used for positive and negative control, respectively. The sera of cats were diluted in PBS at the standard titer of 1:50. This is the cut-off titer written in the manual. If the serum was positive it was furthered screened at every twofold serial dilutions from 1:50 until it was seronegative. After incubating the slides at 37°C for 30 minutes they were washed in PBS solution for removing the excess of unbound serum proteins. After this, the fluorescein isothiocyanate (FITC) labelled anti-cat IgG antibodies, as the conjugate, was placed in each well of the slides. The slides were incubated again for 30 minutes at 37°C and after that washed to remove the unreacted conjugate. Finally, the results were detected under the fluorescent microscope in a dark room. The positive reaction shows bright green fluorescent colour forming a complete ring around the membrane of the *T. gondii* tachyzoites (Figure 1). The negative reaction is red fluorescent colour on the membrane of the *T. gondii* tachyzoites due to Evan's Blue counterstain or just one pole of the membrane of the tachyzoite is stained.

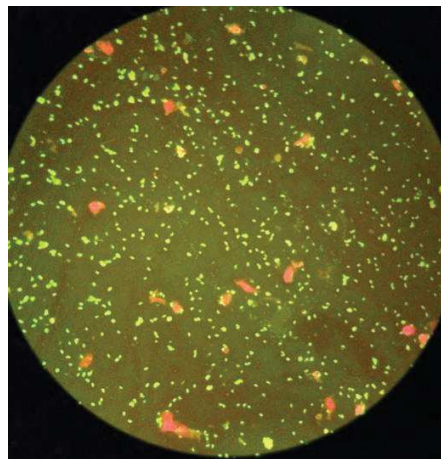


Figure 1: IgG antibody positive result
(The bright green fluorescent rings indicate positive IgG antibody reaction)

4. Results

The results are summarized in Table 1 and Figures 2-5.

Seropositivity of cats

From 200 cat sera samples 136 were found to be positive using the cut-off titer (1:50) (Table 1) and 64 were negative. The overall seroprevalence was 68.0% (Figure 2), with 69 positive cats from Budapest and 67 cats from the countryside (Table 1).

Table 1: Number of cats from Budapest and countryside having *T. gondii* IgG antibody

	Dilution of sera									
	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:25600
BP	69	53	44	36	18	12	7	3	1	0
C	67	50	42	37	27	20	7	3	2	0
Total	136	103	86	73	45	32	14	6	3	0

BP: Budapest

C: Countryside

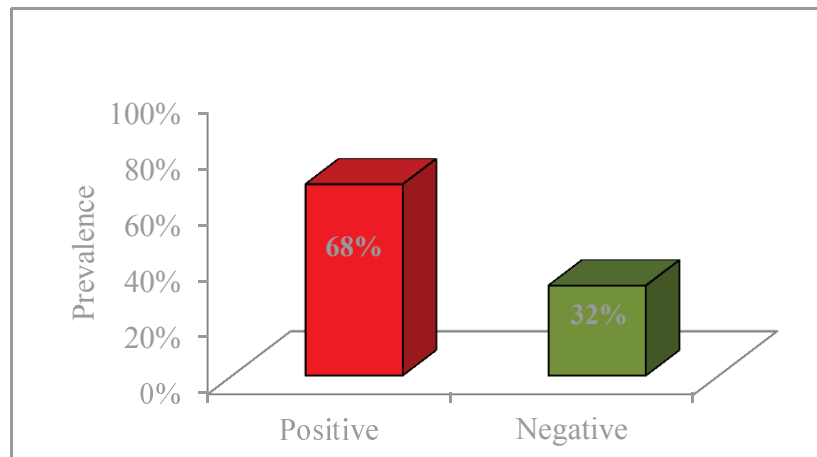


Figure 2: Prevalence of seropositive and seronegative cats

With increasing the dilution of the sera the number of positive samples decreased. The highest dilution of sera when more than half of the cats were still seropositive was 1:400 among the cats from Budapest as well as from countryside (Table 1, Figure 3).

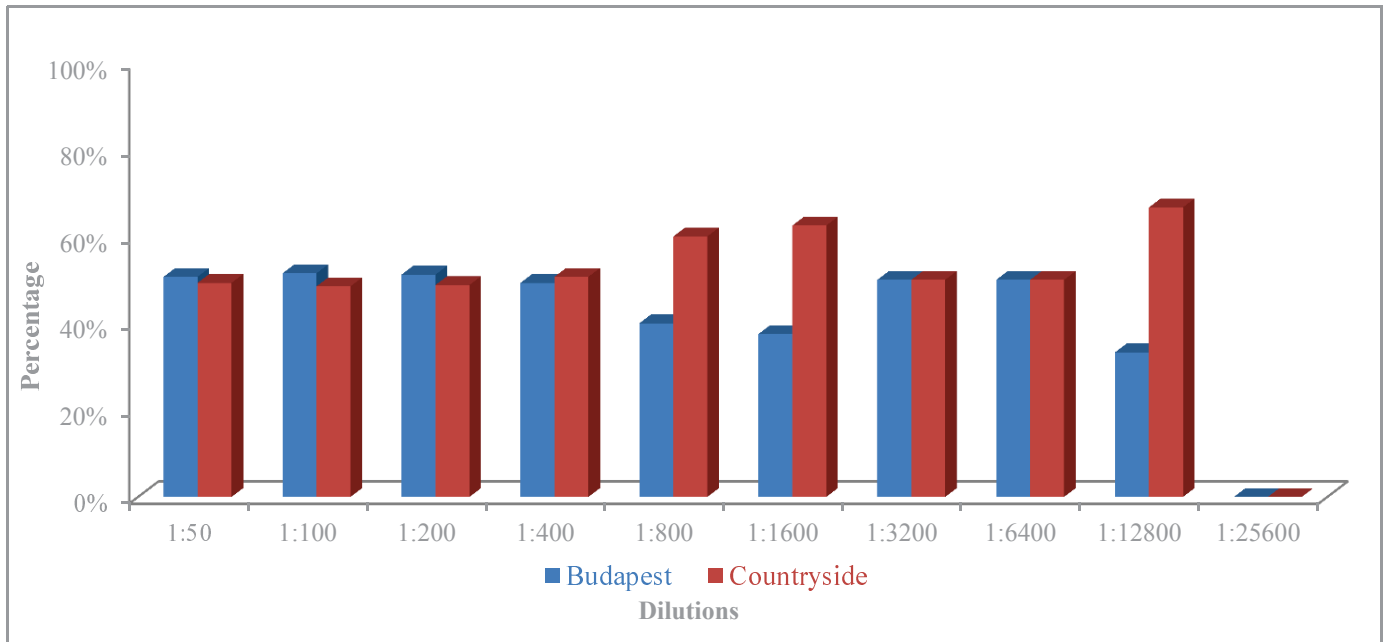


Figure 3: Seropositive samples at different dilutions

The seroprevalence of the tested cats from Budapest was almost the same as the seroprevalence of cats tested from the countryside in the first three dilutions (1:50, 1:100 and 1:200). In the next dilution (1:400) high percentage of cats from Budapest still remained positive comparing with the other group. In the next two dilutions (1:800 and 1:1600) the seroprevalence was higher among the cats from the countryside. From Budapest and countryside, equal positive values were seen at the dilutions of 1:3200 and 1:6400. The number of seropositive animals decreased with the dilution and no infected animals were found at the highest dilution (1:25600) (Table 1, Figure 3).

Seropositivity of male and female cats

Among the cats kept in Budapest, 33 out of 52 (63.5%) males had IgG antibody against *T. gondii*. The seroprevalence of females was higher (75%, 36/48) comparing with the results of males (Figure 4). Higher percentage of males (72.3%, 34/47) than females (62.3%, 33/53) were found to be seropositive which lived in the countryside (Figure 4).

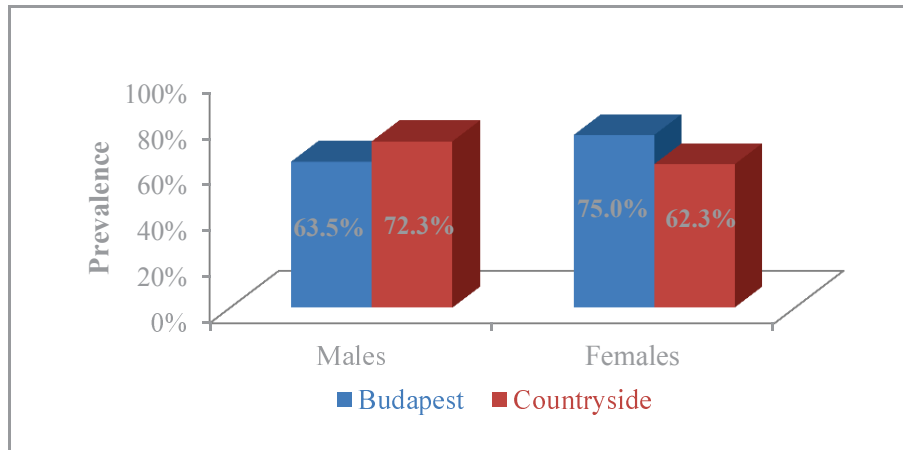


Figure 4: Seroprevalence according to gender

The seroprevalence of cats increased with age, in both groups, seen the highest in cats above 15 years of age (Figure 5). Among the cats which lived in the countryside, more than 60% of animals were seropositive above 1-year-old. This level of seropositivity was also found in cats kept in Budapest.

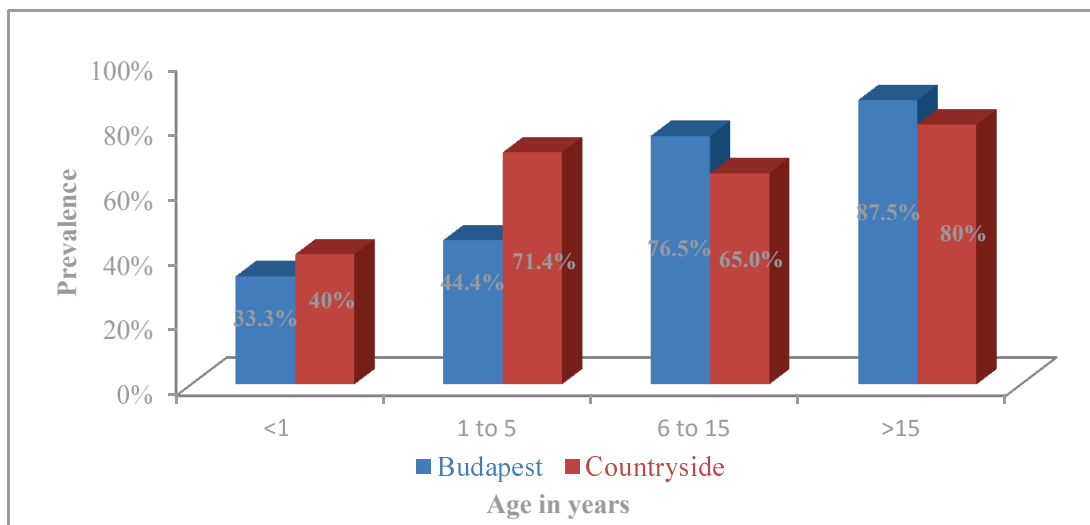


Figure 5: Seroprevalence of cats according to different age groups

5. Discussion

Toxoplasma gondii is a zoonotic parasitic species found worldwide, which has great importance in humans, especially pregnant women and immunocompromised patients (Dubey, 2010; Sykes, 2013; Bowman, 2013). It is also important in veterinary medicine because of causing abortion, mild or severe diseases in domestic animals (Khan and Line, 2010). Domestic and wild felids play a key role in the epidemiology of *T. gondii*, because they are the only definitive hosts of the parasite. The oocysts of *T. gondii* shed with the feces of the cats and contaminate the environment from where humans and all warm blooded animals as the intermediate hosts of the parasite can become infected (Dubey, 2010). Humans as well as carnivorous domestic animals can also become infected by eating the meat of infected animals, which contain tissue cysts (Dubey, 2008).

From epidemiological point of view it is important to know whether the domestic cats living around humans and domestic animals are infected with *T. gondii* or not. It is difficult to find the oocysts at the time of shedding because the infected cats shed oocysts for 1-3 weeks usually once in their lifetime (Sykes, 2013). According to the investigations done earlier, only a few carried out fecal examinations. In these studies, they found negative results (Hegedus, 1979; Hotea et al., 2009) or very few infected cats with shedding oocysts. In Germany, a large sample size (18,259 cats) was used and the rate of oocyst shedding was 0.25% (Herrmann et al., 2010). According to these reports, it is difficult to determine positive cats at the precise moment of shedding and it varies among the sample number examined. The likelihood of more positive cats should be found in larger sample numbers and in spite of cats excreting up to millions of oocysts, they are rarely found in fecal samples (Lopes et al., 2008).

Further difficulty arises with microscopical examination, since the oocysts of *T. gondii* cannot be distinguished from those of *Hammondia*, *Besnoitia* and *Neospora* spp. (Sykes et al., 2013; Deksne et al., 2013). Therefore, PCR detection of the *T. gondii* DNA has proven to be more accurate in detecting positive feline fecal samples (Wang et al., 2012). However, nowadays the most frequent method used for detecting *T. gondii* infection in cats is by serological methods (Sykes, 2013). The advantages of serological methods is that it gives an indirect

evaluation about the presence of *T. gondii* in the environment, thus providing the risks it poses for humans and warm blooded animals (Lopes et al., 2008; Gyorke et al., 2011; Wang et al., 2012).

With serological methods, the antibodies produced against *T. gondii* by the definitive hosts can be detected in their sera. Usually IgG and IgM antibody types are detected. The presence of IgG antibodies are found in a latent infection, as for IgM antibodies appear in more recent infections (Sykes, 2013). The importance of the different dilutions of the sera is that it may indicate an acute or chronic infection. As mentioned by Michalski et al. (2010), the low titers indicate a past infection and high titers indicate a more recent infection. There are some difficulties that arise when detecting IgM antibody type, because it is poorly detected with the Agglutination Tests (Lappin and Powell, 1991; Sykes, 2013) and some cats do not have detectable IgM titers (Sykes, 2013). For this reason, only IgG antibodies were detected in this study. It is important to mention that cats which are IgG seropositive, are considered safer pets and pose a lower risk to humans, because they most likely have shed *T. gondii* oocysts (Sykes, 2013; Bowman, 2013).

The estimated prevalence of infected cats with *T. gondii* is thought to be around 30-40% worldwide (Waap et al., 2012). However the number of *Toxoplasma* infected cats ranges from a few percentages up to 80-90%. It was reported from Mexico that the seroprevalence of the tested cats was 21.8% (Besne-Merida et al., 2008), 25% in Brazil (Bresciani et al., 2006) and 31.6% in the USA (Vollaire et al., 2005). In Far East Asia, the prevalence rates were much lower, only 5.4% in Japan (Maruyama et al., 2003). In Europe the percentage of the infected cats varied from 18.2% reported in the Netherlands (Opsteegh et al., 2012) up to 84.7% reported in Majorca, Spain (Millan et al., 2009). The results obtained in this study are similar to those reported from Czech Republic (61.3%) and Poland (64.4%) where the sera of 390 and 135 cats were studied using IFAT and Indirect Agglutination Assay, respectively (Svobodova et al., 1998; Michalski et al., 2010).

There are several different serological kits on the market. The disadvantage of this diagnostic technique is that all serological methods have shown different sensitivity and specificity as

discussed by many authors (Salant and Spira, 2004; Da Craeye et al., 2008; Lopes et al., 2008). For this reason and the sample size used, the seroprevalence rates vary regarding the data reported from other countries (Sukhumavasi et al., 2012). For instance, the scientists who worked with IFAT for detecting anti-*T. gondii* IgG used different cut-off titers. The cut-off titer was 1:10 in Czech Republic (Svobodova et al., 1998), 1:16 in Turkey (Can et al., 2014), 1:40 in Belgium (Da Craeye et al., 2008), 1:64 in Brazil (Bresciani et al., 2006) and 1:80 in Spain (Miro et al., 2004). Therefore it is almost impossible to compare the results with other published data. On the other hand, cats with low titers can be considered seronegative based on the cut-off titer used as discussed by Sykes (2013). As mentioned by several authors, there is no international standard for diagnosing *T. gondii* infection and results vary between countries, within a country and even within the same city (Hornok et al., 2007; Sukhumavasi et al., 2012).

The Spanish scientists concluded that the results also varied depending on the origin of the animals, whether they were stray or household cats (Miro et al., 2004). Opsteegh et al. (2012) confirmed the conclusion of Miro et al. (2004). Other scientists concluded that the prevalence was not influenced by the origin of cats (Deksne et al., 2013). Generally it can be stated that amongst stray cats, the seroprevalence of *T. gondii* infection may be higher because of outdoor access, therefore the cats may become infected by eating rodents and birds as the intermediate hosts of the parasite. Mostly, outdoor access was proven to be a risk factor for *T. gondii* infection in cats as reported by some authors (Maruyama et al., 2003; Gyorke et al., 2011). From Hungary, the author Chikan (2004) also concluded the same. Opsteegh et al. (2012) determined hunting behavior was also a risk factor.

In previous serological studies of cats carried out in Hungary, high seroprevalence rate was also found among rural cats (Chikan, 2004; Hornok et al., 2007). Forty-seven percent of rural cats out of 264 were found seropositive by Chikan (2004) who used the Complement Fixation Test. Three years later when Hornok et al. (2007) checked *Toxoplasma* infection in 330 cats with IFAT, they found that 61.3% of rural cats were seropositive. In our study the prevalence (67%) in rural cats was higher than those compared to the previous Hungarian studies. The difference in results may be due to the different sample size and serological method used. All

the cats collected in this study were domestic household pets. Concerning our findings, the seroprevalence of cats from Budapest and the countryside was 69% and 67%, respectively. This difference was not considered to be statistically significant. Based on these results it could be assumed that the infection was homogenously spread among the cat population in Hungary. However, this conclusion may not be true because only a limited number of cats were screened in this study, due to the difficulties of sampling and the financial situation.

According to the gender, this study showed higher seroprevalence (75%) in females than males (63.5%) amongst cats living in Budapest. On the contrary, male cats showed higher seroprevalence (72.3%) than females (62.3%) kept in the countryside. Some earlier studies reported that the rate of the seroprevalence did not differ between the female and male cats (Chikan, 2004; Gyorke et al., 2011), while other authors found a difference between the infection rate of females and males (Vollaire et al., 2005; Besne-Merida et al., 2008). Interestingly, females showed higher prevalence rates in two studies (Hornok et al., 2007; Besne-Merida et al., 2008). Due to these conflicting results, it is difficult to regard the gender as a strong risk factor for *T. gondii* infection in cats. Further investigation would be needed to figure out whether *T. gondii* infection is more prevalent in male or female cats and what is/are its reason(s).

Taking into account the age of the cats, this study showed that the seroprevalence of cats increased with advancing of age. The results showed the highest (> 80%) seropositivity above 15 year old cats, originating both from Budapest and the countryside. The majority of cats were infected with *T. gondii* above one year of age. This indicates that a high percentage of cats are infected with *T. gondii* from an early age in both Budapest and the countryside. The results are in connection with those from previous studies in Hungary. The seroprevalence was also found to increase with age reported by Hornok et al. (2007) and Chikan (2004). The relationship between seroprevalence rate of *T. gondii* infection and increasing age of cats, is in accordance with the majority of the results from other countries, such as in Belgium (De Craeye et al., 2008), Romania (Gyorke et al., 2011), Netherlands (Opsteegh et al., 2012), Japan (Maruyama et al., 2003), USA (Vollaire et al., 2005) and Mexico (Besne-Merida et al., 2008).

Finally, it can be concluded that the sample size used in this study was small, but the data confirmed that *T. gondii* infection is prevalent among local cats of Hungary, like reported all over the world. This accounts for the high amount of oocyst shedding in the environment and many animals and humans are at risk of infection. Further investigation would be needed on how to minimize the infection of *T. gondii* in cats and ultimately to the intermediate hosts and humans.

6. Abstract

The aim of this study was to detect the anti-*T. gondii* IgG antibodies in the sera collected from 100 cats living in Budapest and 100 kept in the countryside. The serological method used was a marketed Immunofluorescence Antibody Test (MegaFLUO TOXOPLASMA g). We also investigated the relationship between the seroprevalence rate of *T. gondii* infected cats with factors such as the origin of the cats, their gender and age.

A total of 136 (68%) cats were found to be seropositive, with 69 and 67 positive cats from Budapest and from the countryside, respectively. The cut off titer was 1:50. The number of seropositive cats decreased at higher dilutions, the highest dilution titer of positive sera was at 1:12800. The seroprevalence varied only slightly according to the gender of the cats from both Budapest and the countryside. More than 60% of the cats above 1 year old were seropositive, from both Budapest and countryside. Higher seroprevalence was found in older cats above 15 years of age.

Overall the results showed a high seroprevalence rate of *Toxoplasma gondii* infection among cats from Hungary and the most important associated factor determined was the increasing of age.

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