

RESEARCH ARTICLE

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Toxoplasmosis under coproscopic diagnosis in cats

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*Corresponding author; E-mail: bbizhga@ubt.edu.al & bejobizhga@yahoo.com**Abstract**

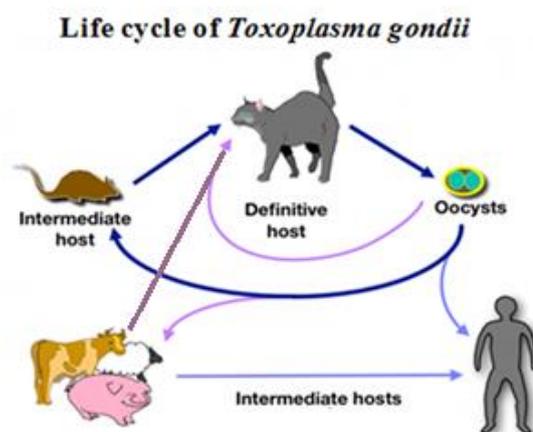
Toxoplasmosis is a disease caused from *Toxoplasma gondii*, an protozoan parasitic organism. Toxoplasmosis is common in any warm blooded animals. Intermediate hosts can be infected with the parasite, but need cats (definitive host) to complete the life cycle of *T. gondii*. Toxoplasmosis resulted present in 1.8% of heads of cats examined in this study where 442 cats household or in contact with the human, were examined under coproscopic examination for 10 years period (2006-2016) in Tirana area. According to age, 6/168 (3.22%) were young (up to 1 year old), 2/153 (1.3%) adults (1 to 8 years old) and 0/121 (0%) were old (>9 years old). Positive cats were free to have an almost linear stretch across age 0-1 years old. Above adult categorie in old cats is no cat was diagnosed positive during coproscopic examinations (over 9 years). Although no statistical differences were found between negative and positive cats regarding gender ($p > 0.05$). Statistical differences were found between negative and positive cats regarding age ($p < 0.05$). Parasitic loads resulting in variations, but with higher values and a danger for the infestation potential capacities of the environment, food and water. The large number of oocysts need checks and monitoring of the feline population and their treatment of faecal is the main way of knowing the epidemiological situation and the risk minimization by toxoplasmosis. While monitoring of contact with cats and preserving environmental quality, food and water is the main road to avoid infestation of intermediate hosts including man. Hight parasitic loud in cats positive for toxoplasmosis increase more potential capacities for free cats that move in large environments required for food causing the infested premises. The most important element is the treatment of faecal. They collected and not processed or burned. By throwing waste in the premises of rain or winds through trasport they become a source of occurrence of the infection.

Keywords: *Toxoplasma gondii*; infection; host; oocyst; coproscopy.

1. Introduction

Toxoplasmosis is a worldwide infectious disease caused by *Toxoplasma gondii* which is an intracellular obligatory protozoan capable of infecting animals and humans [3, 5, 8]. This parasite can be occasionally transmitted by ingestion of oocysts excreted on feces of felids, reportedly known as definitive hosts, or more commonly by the ingestion of cysts that are located into the musculature of intermediate hosts [3, 7, 9]. The life cycle of *Toxoplasma gondii* is complex where feline and specially cats (*Felis catus*) are the definitive host and produce oocysts arising in the environment with faeces and contaminate water and food [13]. The life cycle of *Toxoplasma gondii* is complex and involves two types of host, definitive and intermediate [10, 11, 12, 14]. Wild and domestic cats are the only definitive hosts for *Toxoplasma gondii*. *Toxoplasma gondii*

parasite can only produce oocysts (eggs) when infecting a cat. When a cat ingests an infected prey (or other infected raw meat) the parasite is released into the cat's digestive tract [9, 14]. The organisms then multiply in the wall of the small intestine and produce oocysts during the intrainestinal infection cycle. These oocysts are then excreted in great numbers in



the cat's feces. Cats previously unexposed to *T. gondii* will usually begin shedding oocysts between three and 10 days after ingestion of infected tissue, and continue shedding for around 10 to 14 days, during which time many millions of oocysts may be produced [3, 4, 10]. Oocysts are very resistant and may survive in the environment for well over a year [4]. During the intrainestinal infection cycle in the cat, some *T. gondii* organisms released from the ingested cysts penetrate more deeply into the wall of the intestine and multiply as tachyzoite forms. These forms then spread out from the intestine to other parts of the cat's body, starting the extraintestinal infection cycle. Other animals, including humans, are intermediate hosts of *T. gondii*. Oocysts passed in a cat's feces are not immediately infectious to other animals [1, 18]. They must first go through a process called sporulation, which takes one to five days depending on environmental conditions. Once sporulated, oocysts are infectious to cats, people, and other intermediate hosts. Intermediate hosts become infected through ingestion of sporulated oocysts, and this infection results in formation of tissue cysts in various tissues of the body. Tissue cysts remain in the intermediate host for life and are infectious to cats, people and other intermediate hosts if the cyst-containing tissue is eaten [19].

2. Material and Methods

Using routine techniques of staining with color is vital for finding and differentiation of Mc Master for oocysts and oocysts count in one gram of faeces. Qualitative methods of modifying known as Kato-Katz test [2, 6, 16, 19]. This method was modified with vital dye and unless differentiation of oocysts was used for the evaluation and quantification of oocysts. To determine the level of infestation of cats was asked to assess the oocysts in cat faeces [3, 4, 7]. Animals represented in most human cases cat under the auspices of which was collected until 5 grams of faeces. Stools are examined in veterinary hospitals and parasitology lab at FVM with a combinations of the two coproscopic methods. Laboratory work

consisted in using of the modified model KKK using Gimsae and blue methilen as working solutions [2, 4, 7, 14, 16, 19]. To count of oocysts in faeces Mc Master technique was used with saline solution. The salt was ZnCl₂ solution saturated in NaCl [3, 17].

2.1. Coproscopic and stain technique

Coproscopic technique is performed in 5 g faeces mixed with 45 ml saline solution (density 1.4). For simple quantitative examinations in the faeces of the beaker after centrifugation set for examination entrances and material moved by sliding. Oocysts microscopically evaluated navigation, cellular structures 10 µm thick walls and finding matured sporozoites. Faeces are processed with Gimsae and the blumetilen blue to do while counting the oocysts are applied dilutions of the order 100-1000 x due to the large number of oocysts per gram/faeces [3, 7, 14]. Faecal samples were placed inside a gauze that functioned as filter. From this solution was taken for the preparation of samples and filling lames Mc Master. Smears were told the air hot and painted (Amato Neto et al., 1996). In an other method the number of oocysts was determined by using a quantitative method of counting oocysts and calculated in each 40x optical field and level of dilution. For each sample were observed 2 microscopic smears which in the case of quantitative accounting of the 0.04 g faeces [3, 7, 14].

3. Results and Discussion

When a cat is infested (not only through the meat) the parasite in the intestine produces oocysts during what is known as the cycle of infection intrainestinal. These oocysts come in large numbers in the stools of the cat. Cats begin to produce oocysts 10 days after receiving infested tissue, a process that continues for 10-14 days [3, 4, 7, 10, 14]. Oocysts spreads by contaminated water into the environment and food. These oocysts are spread in environmental way and distributed through rain and surface water, causing pollution. Infested intermediate host when oocysts of sporular take food or water. The hosts

intermediate according to their type and depending on the mode of feeding, can be infested with various stages of the route with the oocysts mature sporozoites with tachisoid the pseudocyst or bradisoid of cysts. These forms of the parasite, arrive at the hosts digestive swallowing broker, cross the intestinal walls with SRE mediator cells where within which become tachisoids and spread throughout the body [9, 15, 17]. This is a diffusion stage of tachisoids plurivisceral that multiply rapidly through endodiogeny. They arrive at those outlets with hematogenous or lymphatic routes. Replication continues to rupture the host cell. The process usually continues until the development of specific immunity, or more rarely to death hosts. Toxoplasmosis is a protozoal disease which affected millions of people worldwide [1, 3, 8, 12, 15]. In general, this disease develops without clinical signs in individuals imunocopetents, but causes abortions, complications of multiple births, mental retardation, severe problems ocular and acoustical children infected in the street congenital and clinical manifestations with fatal outcome on individuals defects of the immune system [8, 13]. It causes miscarriages, neonatal death in animals (ruminants) and considerable economic losses for farmers. Tirana is the capital of Albania, with about 800,000 inhabitants. Tirana surface is approximately 31 km², while the entire district area is 1288 km² and includes four sites: Tirana, Vora, Krraba, Kamza and 150 villages. Number of cats in this environment is estimated (5500-6000 ± 500). Most of them are under human care. Even those who live free (a number around 2500-3000 ± 250), however, are somehow in

contact with humans for their food and some times were they are illnis [3]. Of these cat population we collected and examined samples taken from 82 samples, representing about 20% of the total screen samples. Results of the study belong to a period of 10 years from 2006 to 2016 [3, 4]. Sampling of cats was conducted individually. Most cases were brought samples of animal owners. This was done in this way because the time required for sporulation occurs the oocysts (2-5 days) when they have achieved or be sporular phase during sporulation occurs [18]. This means that the stools should not be totally fresh, because the process of sporulation occurs happens in the environment. Samples were collected by the owner or the use of approximately for direct sampling of cats relates to the characteristics of these animals as aggressiveness and small measure. In addition, in cases of sampling in the rectum (hospitals and pet clinics) samples generally were attempted to be kept under conditions that enable sporulation occurs. *T. gondii* oocysts were recovered and are calculated after dyeing easy, because oocysts stained with distinct and clearly identified [3, 6, 16]. They appear as color allowing their identification by several other representatives of the family Toxoplasmatidae and quantified. According to age cats were divided in three categories young, adults and olds. In young category are included cats up to 1 year old), in adults are included cats 1 to 8 years old and in old category included cats >9 years old [3, 7, 14]. According to sex cats were divided in two categori males and females [3, 4].

Table 1. Results of coproscopic examinations at cats.

Age	FVM			Petlife Hospital			Other Hospitals			Pet Clinics			Total		
	Head	Positive		Head	Positive		Head	Positive		Head	Positive		Head	Positive	
		no	%		no	%		no	%		no	%		no	%
Young	36	2	5.55	43	1	2.32	26	1	3.85	63	2	3.17	168	6	3.22
Adult	29	0	0	38	1	2.63	28	0	0	58	1	1.72	153	2	1.3
Old	27	0	0	35	0	0	24	0	0	35	0	0	121	0	0
Total	92	2	2.17	116	2	1.72	78	1	1.18	156	3	1.92	442	8	1.8

Of 442 controlled samples only 8 (1.8%) were positive for *T. gondii* oocysts. The population studied

comprised 239 females and 203 males; 4 females and 4 males were positive. Among the positive samples

the oocysts for gram faeces over 1 000 000 was the most frequently seen (4/8; 50%), and only one young cat had a oocysts number 3 800 000 and diagnosed with acute infection. The most common symptoms of toxoplasmosis include fever, loss of appetite, and lethargy [8]. Other symptoms may occur depending on whether the infection is acute or chronic, and where the parasite is found in the body. In the lungs, *T. gondii* infection can lead to pneumonia, which will cause respiratory distress of gradually increasing severity [12]. Toxoplasmosis can also affect the eyes and central nervous system, producing inflammation of the retina or anterior ocular chamber, abnormal pupil size and responsiveness to light, blindness, incoordination, heightened sensitivity to touch, personality changes, circling, head pressing, twitching of the ears, difficulty in chewing and swallowing food, seizures, and loss of control over urination and defecation [13]. According to age, 6/168 (3.22%) were young (up to 1 year old), 2/153 (1.3%) adults (1 to 8 years old) and 0/121 (0%) were old (>9 years old) [3, 7, 14]. During the examination in the parasitology lab, FMV during practical work (2006-2016) of 92 samples only 2 cats (17.2%) resulted positive for *Toxoplasma gondii* and average parasitic loads was 480,000 oocysts/g/faeces. From 116 examined samples in Petlife Hospital (2010-2014), only 2 (1.7%) fecal samples tested positive and average parasitic loads 800,000 oocysts /g/ faeces. From cats in other veterinary hospitals (2010-2016) only 1 (1.28%) of 78 samples resulted positive for *Toxoplasma gondii* and the parasitic load resulted 2.2 million oocysts/g faeces. From cats examinations in veterinary pet clinics (2006-2016) 3 samples (1.92%) of 156 resulted positive for *Toxoplasma gondii* and the average parasitic load resulted 1.6 million oocysts/g/faeces [3,4]. From a total of 442 cat fecal samples examined 8 (1.8%) resulted positive and averaged parasitic load was 1.2 million oocysts/g/faeces. Almost cats are cat owners and it clearly identifies with the risk that people of toxoplasmosis [14]. This is why the famous proverb cat or child who circulates and is well known by

people who raise animals, especially cats. The first mother in pregnancy although it may be animal or human (female) in the acute form of the disease suffers from severe phenomena which are attributed toxoplasmatic cyst formation in his tissues, especially nervous tissue [8]. Most descendants die in life stem, some of them die in the first month of life, while the tiny portion that survives suffers serious consequences unrecoverable for life, they have a depressed immune system and are destined to be affected rather rare and die and infestations of light and banal [12, 15]. Positivity represents approximate value with field researchers who report about 1% of the value of prevalence in the cat during coproscopic examinations [18]. Although no statistical differences were found between negative and positive cats regarding gender ($p > 0.05$). Statistical differences were found between negative and positive cats regarding age ($p < 0.05$). Older cats tended to have low rates of toxoplasmosis copropositivity [3, 5]. Prevalence may result small but in terms of the biology of the parasite offset by higher load values and the elimination of parasite oocysts in faeces. In one of the positive cats number of oocysts per 1 gram faeces resulted in approximately 3.8 million oocysts/g/faeces [3, 18]. Toxoplasmosis resulted in the prevalence of relatively low value, but parasitic loads positive cases resulted in very high. Outdoor human activities involving soil contact such as gardening and playing in sandboxes may increase people's exposure to oocysts and infection [7, 14]. For this reason, exposed human populations need to be made become aware of potential environmental contamination with *T. gondii* oocysts by cats. Large numbers of oocysts in the faeces in positive cats clearly show the infestation great risk of having a sick animal. This increase the risk of both innovation. First, the fact that a portion of the cats may result negative because it may have passed or may be in a transitional stage, not the elimination of oocysts. And secondly the fact that the level of infestation probably expected to be slightly higher in free-living cats because are lack of veterinary services [3, 9]. Significant differences were noted between parasitic

loud, but these differences are explainable by known biology and characteristics of the parasite and parasitism in feline. The disadvantage of the work had to do with manipulating the samples as painting or spin increasing the risk of contamination. The study pointed out that the definition of oocysts in the final host can serve as a tool in routine laboratory diagnosis. In addition determination of the number of oocysts shows the high risk of portraying cats for animal owners, veterinarians and for the entire range of intermediate hosts. There was observed that the prevalence of parasitic load or affected by sex. Almost the same infestation values were noted between the two different sex cats. Positive cats were free to have an almost linear stretch across years of age 0-1 years old. Above adult category in old cats no cat was diagnosed positive during coproscopic examinations (over 9 years). Despite the prevalence of infestation can result small (1.8% in our study) will suffice and only a dirty cat in a high level environment. This increase more potential capacities for free cats that move in large environments required for food causing the infested premises [17]. The most important element is the treatment of faecal. They collected and not processed or burned. By throwing waste in the premises of rain or winds through transportation they become a source of occurrence of the infection. This refers to the large number of oocysts that cats dissipating into the environment (thousands of oocysts) and the degree of faeces in the environment. The cats underwent routine clinical examination, and a questionnaire was applied to owners to collect information on animal age, gender, neutering status, domiciled or not, eating and hygiene habits and city area. Information on risk factors was also collected in the questionnaire and included type of housing (farmhouse, townhouse or apartment), kitchen access, meat consumption, raw or cooked meat consumption, milk and filtered water consumption, prey access, sanitary habits (litter box or yard) and outdoor access. The prevalence of toxoplasmosis infection found was not statistically different from a previous study conducted in Tirana city [3]. No differences were

found among the risk factors studied, probably due to the fact that cat feeding was not based on pork, pre-frozen meat and pasteurized milk. Despite the low prevalence of toxoplasmosis infection found in the present study, veterinarians of Tirana and surrounding areas should continuously educate pet owners on the important role cats play in the spread of toxoplasmosis and the importance of daily cleaning litter boxes and keeping their cats indoors. Tirana showed a relatively high feline toxoplasmosis coprovalence, which may be due to the sample studied of owned cats with adequate eating habits and restricted outdoor access, but also may be associated to low local environmental exposure. Sometimes the oocysts can be found in the feces, but this is not a reliable method of diagnosis because they look similar to some other parasites. Also, cats shed the oocysts for only a short period of time and often are not shedding the oocysts when they are showing signs of disease. The domestic cat (*Felis catus*) is infested by parasites in the intestines and intractintestinal cycle develops [9]. Oocysts are very resistant and can survive in the environment for more than a year [14]. These oocysts sporulated environmental and distributed through rain and surface water, contaminating food of animals and humans [8, 10]. Felid other cats and more frequently infested by eating rodents that have cysts in the musculature and brain [5, 7, 11]. But they can be infested with mature oocysts that is contaminated with soil, water, vegetation, and in one way or another of them swallowed [11]. Outbreaks of acute toxoplasmosis worldwide in coming intermediate host being added and has an interest in knowing the epidemiological [10, 12, 14]. Research on the prevalence of *Toxoplasma* oocysts in water and food are still rare and difficult [13]. Oocysts can be detected by examination with coproscopic method and high-density solutions and stains [2, 3, 7, 11, 16, 19]. In most invasions of toxoplasmosis in animals as well as people spend without clinical signs and only in few cases where the parasite reveals a high degree of pathogenicity causing the death of host [5, 8, 15].

4. Conclusions

The *Toxoplasma gondii* oocysts were found in the stools of the cats using dye to differentiate oocysts and Mc Master with salt solution to evaluate the parasitic load in positive samples. The percentage of *T. gondii* infection is similar to what is reported for cats by the authors in the field (1.8% of controlled cats). The tendency for growing in our conditions is related to the expansion of the past two decades and the lack of regular veterinary service. The painting techniques can not only discover the faeces of cats and oocysts but can be converted easily and successfully in a quantitative method. Results of the study indicate the need for care in several directions. For those who keep cats at home, every day should launder their stools, because oocysts want at least 24 hours to become infective oocysts. Before this time oocysts are safe. After cleaning should be thoroughly washed hands. Councils become more vulnerable to the veterinarian handling the animals and controlling products and meat by-products, but also employees in markets that have contact with these products. Rules become naturally stronger for all those who have contact with the street cats. For anyone who manages or is in contact with cats while it becomes expectant mother must undergo serological examination before being pregnant. Identification of anti-*Toxoplasma gondii* antibodies in the blood in high levels confirms that the infestation has been taken in the past without causing any problems and the host has acquired immunity against the parasite. This immunity will be transmitted to the offspring who will be out of any danger of infestation by *Toxoplasma gondii*. This rule is to ensure a normal pregnancy, is valid for any mother giving birth to a healthy offspring from *Toxoplasma gondii* (including humans). Being the main street of cat and human infestation is the living meat consumption (crude) or undercooked, then it is advisable avoiding such food. It's good that pregnant women not to manipulate the raw flesh, but when it is then necessary either to use plastic gloves or after any manipulation should clean your hands, tables, knives

and everything that has been in contact with crude meat. Likewise, when you touched crude flesh should avoid contact of hands with mucous membranes of the mouth, nose and eye. After all the work of kindergarten if they are not using gloves, hands should be clean and should not be consumed crude vegetable fruit without thoroughly cleaned, because it may be contaminated by the feces of cats. Feeding the cats kept under human care with cooked foods or custom would be a measure to prevent their infestation, but it will not be applied to cats (soft or wild) circulating in nature that can easily be infested by toxoplasmosis. Thus care should be taken for the decline in touch with those cats. We are in a time when the country needs a coordinated national program (several services simultaneously) for serological evaluation of toxoplasmosis throughout the intermediate host. This program value except the recognition that the situation will provide and how to prevent and control the toxoplasmic infestation. This program should aim at controlling all intermediate host (including humans) excluding cats when their serological check for the presence of *Toxoplasma* has no value.

6. References

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