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Diploma thesis

Toxoplasma gondii – Prevalence and Risk Factors for Small Felidae at the Zoos in the Czech Republic

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Statement of authorship

I hereby declare that I have written my diploma thesis named *Toxoplasma gondii* – prevalence and risk factors for small felidae at the zoos in the Czech Republic by my own. All literature sources used in this thesis are cited according to citation norm ČSN ISO 690 and requirements of the Faculty of Tropical AgriSciences, CULS Prague and are listed in chapter References.

In Prague, date..... .....

signature

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Abstract

Toxoplasma gondii is an obligate intracellular parasite which was discovered in 1908 by Charles Nicolle and Louis Manceaux. This parasite attack almost all species of warm-blooded animals, including humans. The most important role in its life cycle play felids which serve as a definitive host. The main aim of this thesis was the verification of presence of *T. gondii* oocysts in faeces of small felidae at the zoos in the Czech Republic. Sample collection was done from July 2013 to January 2014 in zoos Jihlava, Olomouc and Ostrava. In total, 700 samples from nine species of small felidae were investigated. Oocyst prevalence was determined by flotation method, using sucrose solution and subsequently by microscope examination of samples. Overall, it was found 1.43% positive and 1.00% dubious findings. The results showed that in the incidence of positive findings, there is no statistically significant difference between individual zoos, nor between sexes of observed animals. Possible sources of infection for felids bred in zoological gardens are discussed.

Key words: toxoplasmosis, feline, *Toxoplasma gondii*, risk factors, zoological garden

Abstrakt

Toxoplasma gondii je obligátní intracelulární parazit, který byl objeven roku 1908 Charlesem Nicollem a Louisem Manceauxem. Tento parazit napadá téměř všechny druhy teplokrevných živočichů, včetně člověka. Nejdůležitější roli ve vývojovém cyklu hrají kočkovité šelmy, které slouží jako definitivní hostitel. Cílem této práce bylo ověření výskytu oocyst *T. gondii* v trusu malých kočkovitých šelem v zoologických zahradách v ČR. Sběr vzorků probíhal v období od července 2013 do ledna 2014 v zoologických zahradách Jihlava, Olomouc a Ostrava. Celkem bylo vyšetřeno 700 vzorků trusu od devíti druhů malých kočkovitých šelem. Prevalence byla stanovena pomocí flotační metody za použití cukerného roztoku a následným mikroskopickým vyšetřením vzorku. Celkově bylo zjištěno 1,43% pozitivních a 1,00% dubiózních nálezů. Výsledky prokázaly, že ve výskytu pozitivních nálezů není žádný statisticky významný rozdíl mezi jednotlivými zoologickými zahradami, ani mezi pohlavími sledovaných zvířat. Možné zdroje infekce jsou diskutovány.

Klíčová slova: toxoplazmóza, kočkovité šelmy, *Toxoplasma gondii*, rizikové faktory, zoologická zahrada

Content

List of Abbreviations

AC/HS test: Acetone [AC]-fixed vs. formalin [HS]-fixed tachyzoites test

AIDS: Acquired immunodeficiency syndrome

CNS: Central nervous system

CVS: Chorionic villus sampling

DALY: Disability-adjusted life years

DAT: Direct agglutination test

DNA: Deoxyribonucleic acid

ELISA: Enzyme Linked Immuno-Sorbent Assay

FIV: Feline immunodeficiency virus

FeLV: Feline leukemia virus

HPP: High pressure processing

HSCT: Haematopoietic stem cell transplantation

IFA: Indirect Fluorescent Antibody test

IgG, IgM, IgA or IgE: Immunoglobulin G, M, A, E

ISAGA: Immunosorbent agglutination assay

MAT: Modified Agglutination Test

MLE: Multilocus enzyme electrophoresis

MS: Microsatellite

OT: Ocular toxoplasmosis

PCR: Polymerase chain reaction

RFLP: Restriction fragment length polymorphism

RNA: Ribonucleic acid

ROP: Rhoptry protein

SFDT: Sabin-Feldman dye test

T. gondii: *Toxoplasma gondii*

UV: Ultraviolet

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite which has very high medical and veterinary importance with a major public health impact. It belongs to phylum Apicomplexa which contains also other important protozoan pathogens such as *Plasmodium* spp. (malaria), *Cryptosporidium* spp. (cryptosporidiosis), or *Eimeria* spp. (coccidiosis in animals and humans). The parasite was discovered by Nicolle and Manceaux in 1908 on the rodent gundi (*Ctenodactylus gundi*) which was used in Tunisian laboratory for leishmaniasis research. At the same time, it was also discovered by Splendore in Brazil on the rabbit but it was misdiagnosed as *Leishmania*. Name *Toxoplasma* comes from Greek *tóxikon* (poisoned arrow) and it was named after its arc-like shape.

Toxoplasma gondii has three stages – tachyzoites (in groups), slow-growing bradyzoites (encysted in tissues) and oocysts, which include two sporocysts, each containing four sporozoites. It is closely related to *Neospora caninum* because of similar morpholgy.

This parasite cause disease toxoplasmosis and has a wide range of intermediate hosts throughout the world, affecting humans and majority of warm-blooded animals, including domestic and wild animals, birds and even marine mammals. The world prevalence varies greatly, depending on diagnostic test used, food handling practices, eating habits, level of immunity and many other factors. It is estimated that 30 to 50% of the world´s human population is infected. In animals, it may represents a serious problem of significant economic importance due to losses in animal productivity, abortions, or neonatal complications. Toxoplasmosis is considered as one of the most common parasitic disease which can be found worldwide from Alaska to Australia.

T. gondii has a complex life cycle consisting of sexual and asexual phase. Sexual multiplication occurs only in the definitive hosts – domestic and wild felids, after ingestion of tissue cysts. After parasite replication, cats excrete millions of the resistant oocysts into the environment. Released oocysts sporulate and become infective to other animals and humans. Sporulated eggs are ingested by intermediate hosts, in which the parasite reproduce asexually and invade their tissues and organs. Infected animals are consumed by felids and the life cycle resumes.

After ingestion, the parasite penetrates intestinal wall and invade various organs of the body, including mostly CNS, muscles and eyes and there persists for the whole life of intermediate host. The most susceptible are fetuses, newborns, pregnant women, patients who are immunosuppressed by AIDS or cancer and transplant recipients. Although most of the cases

are asymptomatic, several features such as lymphadenopathy, malaise, myalgia, encephalitis, mental retardation, blindness or ocular problems may occur. It can be also associated with the occurence of brain tumors and can cause schizophrenia and alterations in behaviour.

The parasite may be transmitted by several ways and alternate between definitive and intermediate host, and *vice versa* and also between different definite or intermediate hosts. The main modes of transmission are vertical (congenital) and horizontal (ingestion of tissue cysts or oocysts) infection. Other, less common routes of infection are organ or bone marrow transplantation and blood transfusion from infected donor, inhalation of oocysts and transmission via sperm.

There are several methods for *T. gondii* detection. Specific diagnostic tools for toxoplasmosis are serologic tests, including IgG, IgM, IgA and IgE antibodies by ELISA, SFDT, IFA, MAT or ISAGA; amplification of specific nucleic acid sequences (PCR), histologic demonstration of the parasite and its antigens and last but not least, isolation of oocysts by centrifugation using various solutions and isolation of the parasite in laboratory animals by usage of bioassay.

Treatment, especially in ocular toxoplasmosis, is sometimes very difficult and there are no drugs which can be used against latent form of the disease. However, combination of pyrimethamine and sulfadiazine, which is widely used, seems to be helpful in 60-70% of acute cases. At present, no human or animal vaccine is available for commercial use but there is still considerable progress towards its development.

Small wild cats live worldwide and can be found in semideserts, open plains but even in rocky mountains (Hellyer and Aspinall, 2005). The role of *T. gondii* as a cause of morbidity or mortality in these felines is little known (Silva et al., 2001a) but as well as domestic cats, also wild felids may be infected and may disseminate oocysts in the environment because rodents and birds make up the main component of their diet. Wild felids which are bred in zoos play an important role in dissemination of *T. gondii* within the zoo environment and they can be possible source of infection for other zoo animals and also for humans involved with them. For zoo animals which do not have developed resistance to *T. gondii*, including marsupials, koalas or New World monkeys, the disease is often fatal. Moreover, the majority of wild feline species kept in zoos are threatened and toxoplasmosis may cause the failure of their breeding success. Therefore, research in this field is essential and monitoring of *T. gondii* prevalence in these animals is necessary.

2. Literature Review

2.1 General Overview and Taxonomy

Toxoplasma gondii is an ubiquitous apicomplexan parasite which cause protozoan disease toxoplasmosis (Boughattas et al., 2010; Oliveira Braga et al., 2012; Zulpo et al., 2012). It belongs to the family of the *Sarcocystidae* in the class of the coccidia (Omata et al., 1990; Hill et al., 2005; Lee et al., 2010) and it is the only species of the genus *Toxoplasma.* In 1969 Siim et al. showed that there are two sporocysts, each containing four sporozoites (see Appendix 1) within the oocyst (Dubey et al., 1998a). This study strengthened the hypothesis that *T. gondii* is related to the genus *Isospora.* Later, schizogonic and gametogonic processes identical with those of coccidians had been described in *T. gondii* and the parasite were classified under the order *Eimeriorina* (Hutchison et al., 1970).

This intracellular parasite has high medical and veterinary importance (Hill et al., 2008; Dabritz and Conrad, 2010; Elmore et al., 2010) with a major public health impact (Zhu et al., 2012). Public health organizations, such as the World Health Organization or European Food Safety Authority, have repeatedly advised the collection of accurate epidemiological data on this parasite (EFSA, 2007; Hamidinejat et al., 2011) but only a few countries regularly monitor toxoplasmosis in humans and even much less monitor it in animals (Tenter et al., 2000).

Word *Toxoplasma* comes from the Greek *tóxikon* (poisoned arrow, from *tóxon* [bow]) and *plásma* (something molded). This genus of intracellular parasitic protozoa was named for its arc-like shape (Black and Boothroyd, 2000; Ajioka and Morrissette, 2009; Muñoz-Zanzi et al., 2010).

Taxonomic classification (Dubey, 2010a):
Phylum: Apicomplexa; Levine 1970
Class: Sporozoasida; Leukart 1879
Subclass: Coccidiasina; Leukart 1879
Order: Eimeriorina; Leger, 1911
Family: Sarcocystidae; Poche 1913
Genus: *Toxoplasma*; Nicolle and Manceaux 1908
Species: *Toxoplasma gondii*; Nicolle and Manceaux 1909

2.2 History

The earliest observations of coccidia were done by Hake in 1839 (Woodcock, 1911; Dobell, 1922), but discovery of parasite *Toxoplasma gondii* was done by Charles Nicolle and Louis Manceaux in 1908. It was observed in the blood, spleen, and liver of a North African rodent called gundi - *Ctenodactylus gundi* (see Figure 1) (Ajioka and Morrissette, 2009; Muñoz-Zanzi et al., 2010; Flegr, 2013). This rodent was being used in the laboratory of Charles Nicolle at the Pasteur Institute in Tunis for leishmaniasis research (Dubey, 2009c; Dubey, 2010a). Name for the parasite should have been *Toxoplasma gundii* but Nicolle and Manceaux had incorrectly identified the host as *Ctenodactylus gondii* (Dubey, 2008; Dubey, 2010a). The species *T. gondii* was formally designated at that time and the definitive description of the organism was published in 1909 (Ajioka and Morrissette, 2009; Muñoz-Zanzi et al., 2010). Also Alfonso Splendore (1908) discovered the same parasite (Black and Boothroyd, 2000) in a rabbit but he wrongly identified it as *Leishmania* and he did not named it (Dubey, 2008). *Toxoplasma gondii* is closely related to *Neospora caninum* because of similar morphology and on the basis of their similarity, these two parasites were confused in the past (Sedlák and Bártová, 2006; Hamidinejat et al., 2011). Brief history related to *Toxoplasma gondii* is described in table (see Appendix 2).

Figure 1: African rodent *Ctenodactylus gundi* (photograph by Z. Chalupa)

2.3 Epidemiology

Toxoplasma gondii has a wide range of intermediate hosts, including humans and several animal species, particularly mammals and birds (FAO, 1998) throughout the world (Meireles et al., 2004; da Silva et al., 2006; Pas and Dubey, 2008). Today, up to 200 different animals are known as intermediate host for *T. gondii* (Hooshyar et al., 2009), including cattle (Spencer et al., 2003; Ghazaei, 2006), pigs (Dorny and Fransen, 1989; Dubey et al., 2005, Dubey, 2009b), horses (FAO, 1998; Spencer et al., 2003), goats (Dubey, 2004; Bártová and Sedlák, 2012), sheep (Nesbakken and Skjerve, 1996; Dubey, 2004; Dubey, 2009a), chickens (Dubey et al., 2002a; Hill and Dubey, 2013) and rabbits (Hill and Dubey, 2002; Aghwan et al., 2010; Alvarado-Esquivel et al., 2013) but it is also common in wild carnivores (Hill et al., 2005) such as bears (Hill and Dubey, 2002; Dubey and Jones, 2008), foxes and coyotes (Dubey et al., 2004), racoons (Hancock et al., 2005) and skunks (Hill et al., 2005). The parasite can occur in wild cervids (Spencer et al., 2003; Dubey et al., 2004), marsupials, ungulates, monkeys (Spencer et al., 2003; Hill et al., 2005), seals (Honnold et al., 2005), sea otters (Boothroyd, 2009; Hill and Dubey, 2013) and bats (Choi et al., 1987) too.

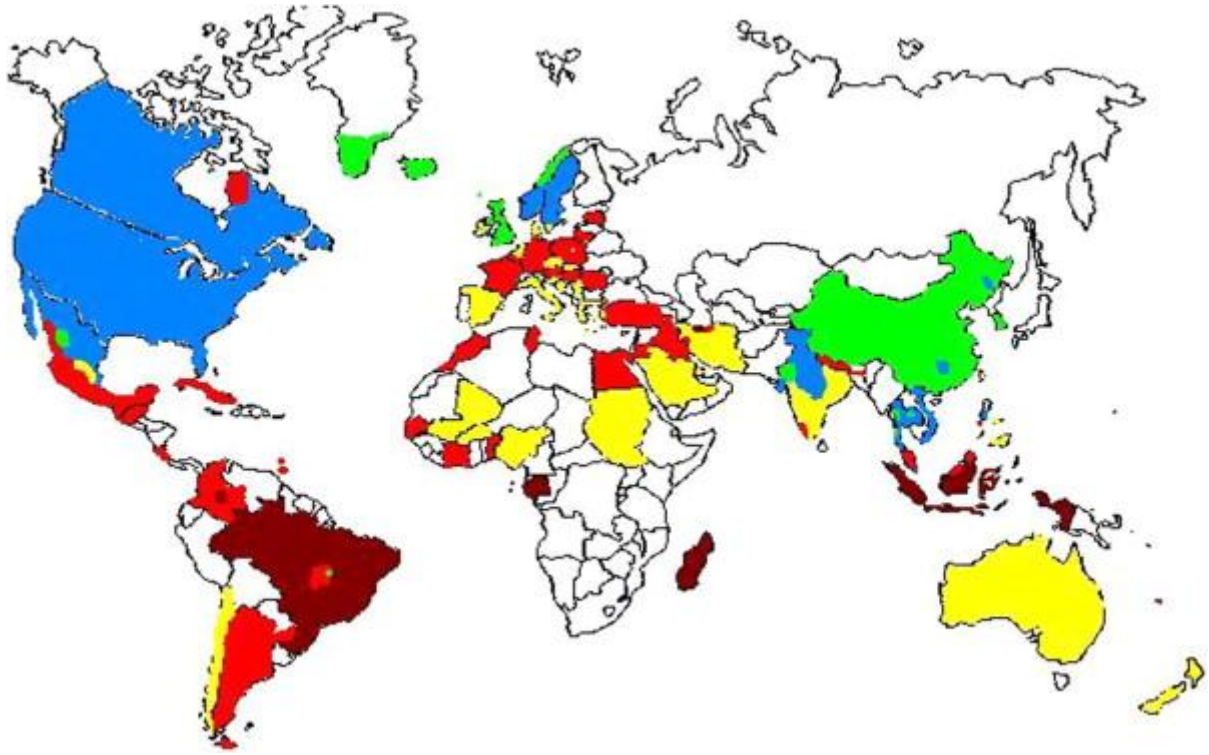
2.4 Prevalence

Toxoplasmosis is considered as one of the most common parasitic diseases in humans and probably in all warm-blooded animals (Suzuki, 2002; Kiliç et al., 2008; Dubey, 2010a). The world prevalence rates differ between individuals reports due to factors including diagnostic test used, personal hygiene and food handling practices, eating habits, level of the host immunity, rate of environment contamination by oocysts and the presence of cats in outdoor or indoor setting (Swai and Schoonman, 2009; Wu et al., 2011).

2.4.1 Prevalence of Toxoplasmosis in Humans

This parasite infects 30% to 50% of the world´s human population (Navarro et al., 1998; Kim et al., 2008). In continental Europe, it is estimated that 50 to 80% of the population had contact with parasite (Pasquali, 2004; Michalski et al., 2010) but according to the literature data (Canfield et al., 1990; Dickman, 1996; Dabritz et al., 2007a; Hooshyar et al., 2007; Hill et al., 2008; Boughattas et al., 2010; Kulasena et al., 2011), toxoplasmosis does not occur only in Europe but it is also widely prevalent in other continents (Michalski et al., 2010). Infection with this parasite has been found worldwide from Alaska to Australia (see Figure 2) (Hill et al., 2005). In developed nations, *Toxoplasma gondii* is the most common protozoan parasite (Tenter et al., 2000; Flegr, 2007). Its prevalence in humans varies from region to region

depending on ecological, socioeconomic and cultural factors (Pappas et al., 2009; Boughattas et al., 2010; Muñoz-Zanzi et al., 2010) and ethnic groups or geographical boundaries (Dubey and Frenkel, 1974). For example in France the prevalence is very high because of very frequent consumption of raw or undercooked meat and in contrast, the high prevalence in Central and South America is due to high contamination of environment by *T. gondii* oocysts (Hill and Dubey, 2002). In fact, the prevalence of toxoplasmosis not correlated to clinical cases. Particular risk factors are characterized by pregnancy and immunosuppression (Pasquali, 2004).



Dark red – prevalence above 60%
Light red – prevalence 40 to 60%
Yellow – prevalence 20 to 40%
Blue – prevalence 10 to 20%
Green – prevalence less than 10%
White – absence of data

Figure 2: World prevalence of *T. gondii* in humans (Pappas et al., 2009)

2.4.2 Prevalence of Toxoplasmosis in Animals

Among animals reared for food, *T. gondii* is mostly isolated in pigs, sheep, goats and rabbits (Lukešová and Literák, 1998) in comparison with other animals (Dubey and Frenkel, 1974; Flegr and Hrdý, 1994; Dubey, 1996), such as cattle, horses and water buffaloes, where the infection is less prevalent (see Figure 3). Among wild game, *T. gondii* infection is most

prevalent in black bears and in white-tailed deers (Hill and Dubey, 2002). It is due to different organotrhophism of the parasite and the number of tissue cysts produced in certain organs. Therefore, not all infected animals are of the same public health significance (Tenter, 2009).

Swines

Pigs are the most important animal source of toxoplasmosis throughout the world (Dubey and Frenkel, 1974). They are very important in epidemiology because of common consumption of pork meat (Sroka et al., 2007). Very high prevalence of infection in reared swines is indicated due to serological and parasitological surveys (Dubey and Frenkel, 1974). The parasite in these animals occurs in the form of tissue cysts (Sroka et al., 2007).

Cattle

Viable *T. gondii* is rarely found in beef meat (Dubey and Frenkel, 1974; Dubey, 1996; Hill and Dubey, 2013) but if cattle carry infectious tissue cysts in their meat they may be an important source of human infection because beef meat is often consumed raw or undercooked in many countries (Opsteegh et al., 2011). Cattle are resistant to infection and are not important hosts for Toxoplasma (Dubey, 2010a), therefore the ingestion of beef meat is not considered important in the epidemiology of this parasite (Silva et al., 2001b; Hill and Dubey, 2013). Animals may be serologically negative 2 years after inoculation (Dubey, 1996) and they seem to be able to eliminate the parasite (Lüder and Gross, 1998). Tissue cysts are also less frequent in buffaloes (Tenter et al., 2000).

Horses

The low prevalence of *T. gondii* recorded in horses indicates that they are more resistant than other animal species and they are less sensitive to the pathogenic effect of the parasite. Nevertheless, tissue cysts were isolated from horses. This indicates that consumption of horsemeat is a potential source of infection (Jakubek et al., 2006) but the risk of acquiring infection is not great (Dubey et al., 1999). In horses is also wide variation of *T. gondii* prevalence, like in other animal species (De Craeye, 2012).

Sheep and goats

Toxoplasma gondii is a very common infectious agent among sheep and goats (Dubey, 1996) due to the continuous contamination of pastures and therefore among these animals the prevalence of this parasite is very high (Flegr and Hrdý, 1994). Since sheep and goats are

important hosts of *T. gondii*, they represent a major risk for human beings (Bártová and Sedlák, 2012; Hill and Dubey, 2013).

Rabbits

Although rabbits are highly susceptible to *T. gondii* infection, attention to toxoplasmosis in rabbits is less than to toxoplasmosis in other farm animal species (Hejlíček and Literák, 1994). There are some differences in degree of infection, depending on husbandry conditions, individual susceptibility and type of feeding. Infected rabbits are also considerable source of infection for cats and humans (Alvarado-Esquivel, 2013). Toxoplasmosis in rabbits deserves epizootiological even hygienic attention (Hejlíček and Literák, 1994).

Chickens

Althought their naturally resistance to clinical toxoplasmosis, chickens considered one of the most important host in the epidemiology of *T. gondii* infection in many countries because they act as an efficient prey for domestic and wild cats (Hill and Dubey, 2013). In organic and free range poultry, the prevalence can be very high (up to 100%) but in spite of this, in chickens reared in intensive breeding it can be almost zero (Dubey, 2010b).

Because of difficult direct detection of *T. gondii* oocysts from soil, chickens (especially free range chickens) are one of the best indicators for soil contamination. They are fed from the ground and are directly exposed to infection by oocysts. Therefore chickens have been used for detection of soil contamination (Dubey, 2010b; Hill and Dubey, 2013).

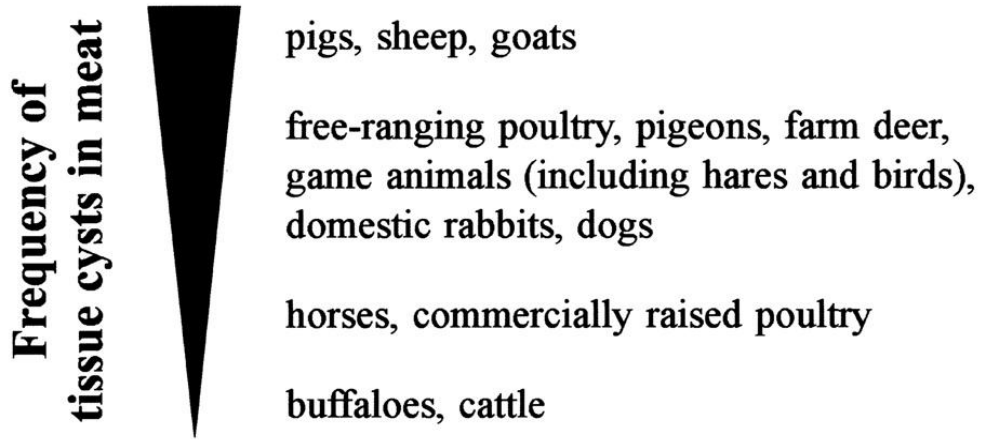


Figure 3: Importance of animals reared for meat and milk in the transmission of *T. gondii* (Tenter et al., 2000)

2.4.3 *Toxoplasma gondii* and Cats

The majority of domestic and wild cats (Kikuchi et al., 2004; Dubey, 2007) are important for *T. gondii* because they are definitive host for this parasite (Jackson and Hutchison, 1989; Galván Ramírez et al., 1999; Rosa et al., 2010). Cats play also an important role in the spreading of toxoplasmosis (Omata et al., 1990; Karatepe et al., 2008; Lee et al., 2010). After ingesting of only one bradyzoite or one tissue cyst (Dubey et al., 2002b; Hill and Dubey, 2002, Dubey, 2009c), cats may excrete millions of environmentally resistant oocysts in their faeces (Omata et al., 1990; Haddadzadeh et al., 2006; Zulpo et al., 2012). Contamination of the environment by oocysts is widespread because estimated population of domestic and feral cats is around 140 millions of individuals (Hill and Dubey, 2013) and 20g of their stool contain approximately 20 millions of oocysts. After faecal decomposition, the soil contamination can be as high as 100,000 oocysts per gram. Excreted oocysts after sporulation remain infectious for more than 1 year (Webster, 2001) and represent one of the main infection sources for intermediate hosts (Galván Ramírez et al., 1999; Dubey, 2004) in which the tissue cysts developed after ingestion of these oocysts (Moura et al., 2007). Although cats shed oocysts for only a short period during their lives (Jones et al., 2001; Dubey et al., 2006; Hooshyar et al., 2009) – usually for only two weeks (Bowie et al., 1997; Shuhaiber et al., 2003), the quantity of oocysts produced after primary infection may be up to 810 million oocysts (Tenter, 2009; Dabritz and Conrad, 2010; Cork, 2011). Oocyst production in cats may be related to dose, strain and cat age (Dubey, 2005; Dabritz et al., 2007a). After secondary infection the oocysts reshedding is rare (Tenter et al., 2000), thus there is a lower risk of oocyst shedding in older cats than in younger (Lüder and Gross, 1998). Cats immunosuppressed with FIV or FeLV do not shed oocysts for any longer time or in any greater numbers, or oocyst reshedding does not appear at all (Lappin et al., 1996). The prevalence of *T. gondii* infection in indoor cats is lower than in feral outdoor cats because of access to outdoor environment (Lucas et al., 1999; Dubey et al., 2002b). At present, the prevention of infection by *T. gondii* is the best strategy (Kijlstra et al., 2008; Meerburg and Borgsteede, 2011).

2.5 Economic Importance

Toxoplasma gondii represents serious problems for most of domestic animals. In intensive even in extensive breeding toxoplasmosis can become an infection of significant economic importance with regard to animal production. Socioeconomic impact of *T. gondii* infection in animals is mainly considered as a public health problem but the infection may cause also

notable economic losses in animal productivity by reproductive disorders (Sroka et al., 2007). Clinical toxoplasmosis also brings economic losses linked to abortions and neonatal complications and mortality (Buxton et al., 2007; Raeghi et al., 2011; Stormoen et al., 2012).

2.6 Life Cycle

The causative agent *Toxoplasma gondii* is a facultatively heteroxenous protozoan organism (Tenter et al., 2000; Pasquali, 2004; Haddadzadeh et al., 2006), which is found worldwide reflecting the distribution of felids (Tenter et al., 2000; Hill et al., 2008).

The parasite´s life cycle (see Figure 6) has been well categorized (Lindsay et al., 2001; Hill et al., 2007). It is a complex, has asexual and sexual part (Dubey, 2004; Sedlák and Bártová, 2006). In 1970´s, several investigators found that the sexual cycle occurs only in felines (Boothroyd, 2009). Integral parts of the life cycle of *T. gondii* are tachyzoites, bradyzoites and oocysts. Among all three infectious stages, oocysts are more virulent than bradyzoites or tachyzoites. Cystless or oocystless strains do not exist in the nature (Dubey et al., 2002a). The main aim of the parasite during its life cycle is to be transmitted from an intermediate hosts to the definitive host. Due to this, *Toxoplasma* can manipulate the host behavior and thereby specifically increase the probability that the host will be captured by a predator. It is based on changes of chemical messages in the CNS (Webster, 2001; Flegr, 2013). This is a considerable selective advantage to the parasite (Boothroyd, 2009).

The life cycle begins by sexual multiplication of the parasite which occurs only in the definitive host including domestic and wild cats and all members of the family Felidae (Flegr and Hrdý, 1994; Dubey and Jones, 2008). Cats may be infected by any of the three infectious stages of *T. gondii*: tachyzoites in groups, bradyzoites in tissue cyst, and sporozoites in oocysts (Dubey, 2002; Pasquali, 2004) but the most common is infection by tissue cysts (Dubey and Frenkel, 1974). After ingestion, tissue cysts are digested by proteolytic enzymes in the small intestine, and bradyzoites are released (Dubey, 1997; Blader and Saeij, 2009). During the second week, bradyzoites replicate asexually (extra-intestinal phase) and then possibly return to the small intestine via parasitemia (Dubey, 2002). They penetrate into intestinal epithelial cells and there the sexually mature stage of the parasite is completed (enteroepithelial phase) (Dubey, 1998a; Kikuchi et al., 2004; Karatepe et al., 2008) and bradyzoites transform into oocysts (Hill and Dubey, 2002). This results in the dissemination of millions of the environmentally resistant, unsporulated oocysts (see Figure 4) in the cat faeces (Silva et al., 2001b; Dabritz et al., 2007a; Oliveira Braga et al., 2012). The prepatent period (pp; days to shedding of oocysts) is different after ingesting bradyzoites versus

tachyzoites or oocysts (Dubey, 2002). Shedding of oocysts by cats starts 2 to 10 days after ingesting of cyst and lasts approximately 20 days (Lüder and Gross, 1998; Lucas et al., 1999) In contrast, when cats are infected by oocysts, shedding starts after 19 to 36 days and lasts for only 2 to 5 days (Lüder and Gross, 1998; Dubey, 2002). Usually oocyst shedding peaked 1–3 days after the cat started with oocyst excretion (Dubey, 2002). Shedding of oocysts lasts in a limited period and released oocysts are long-lived (Mead et al., 1999; Holland, 2003; Elmore et al., 2010). Due to release of oocysts in the environment, the presence of cats is an important risk factor for *T. gondii* infection of other animals or humans (Tavassoli et al., 2013). They are essential for the maintenance of the disease (Dubey, 1998a; Dubey et al., 2002b; Deksne, 2012) and represent the starting point of the whole cycle (Opsteegh et al., 2012).

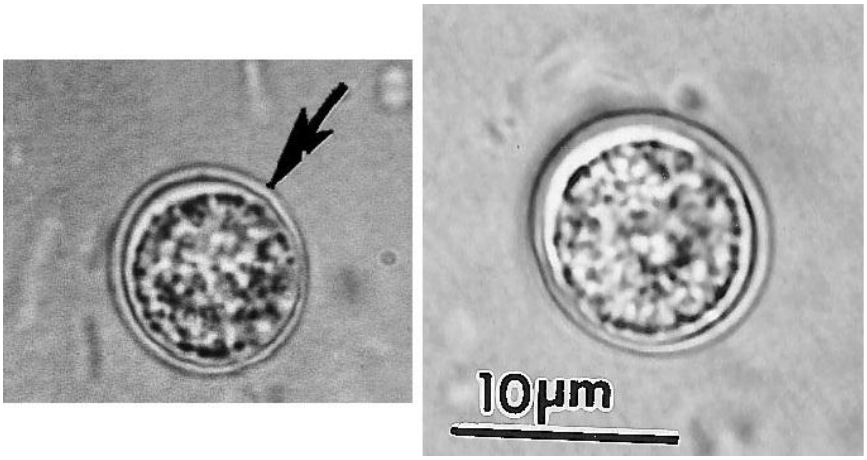


Figure 4: Unsporulated oocysts (Dubey et al., 1998a; Hill and Dubey, 2002)

After 1 to 5 days (Bowie et al., 1997; Lucas et al., 1999; Shuhaiber et al., 2003), released oocysts undergo the sporogony (see Appendix 3) in the environment and become infective to other animals and humans (Dubey et al. 1998a; Zulpo et al., 2012). Sporulated oocysts (see Figure 5) can survive in the environment (in moist shaded soil or sand) for several months due to their resistance to environmental conditions (Webster, 2001; Karatepe et al., 2008). Then the excreted sporulated eggs are consumed by farm or wild animals (Meerburg and Borgsteede, 2011).

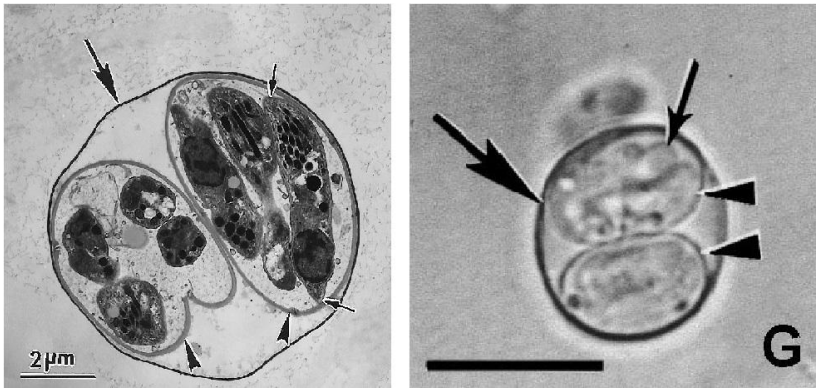
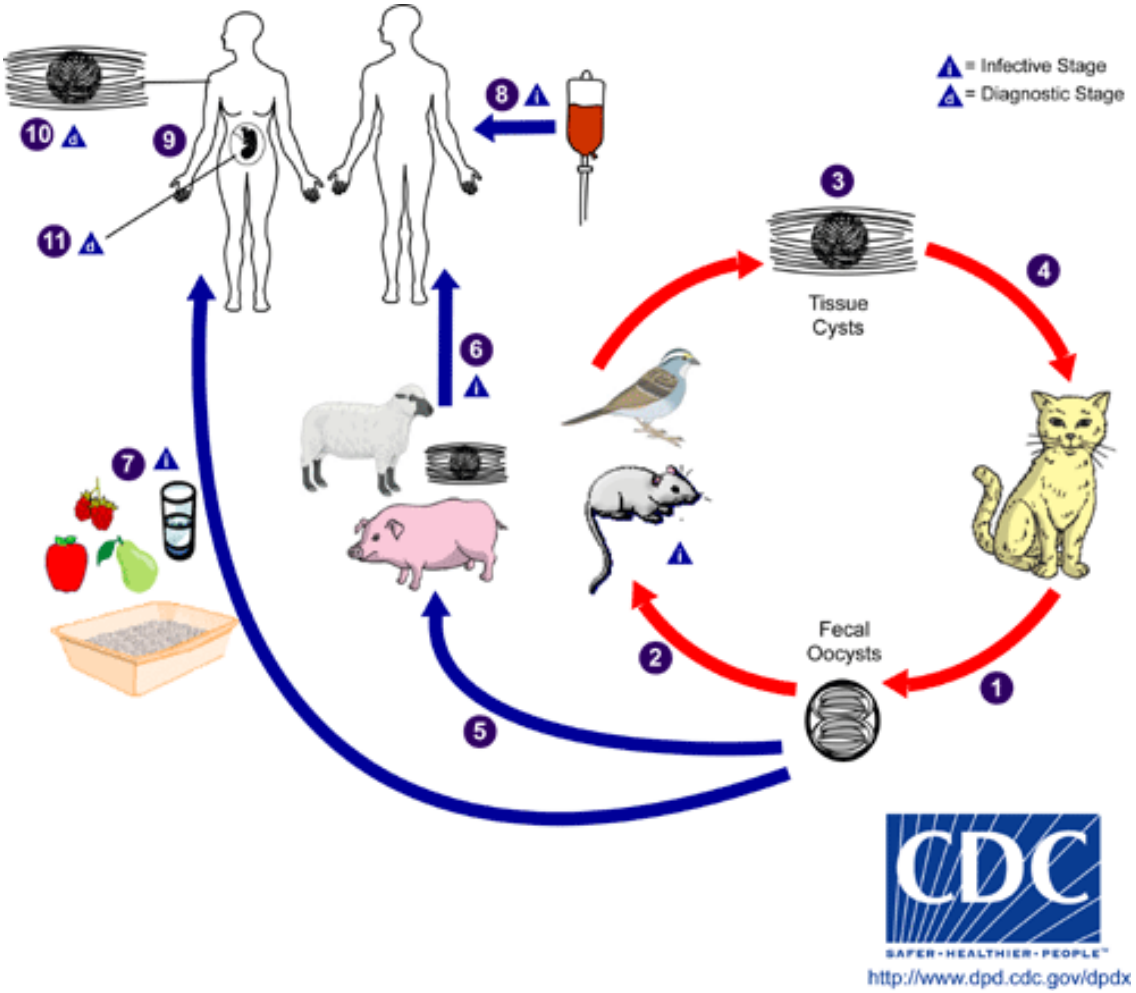


Figure 5: Sporulated oocyst (Dubey et al., 1998a; Hill and Dubey, 2002)

After ingestion of sporulated oocysts by intermediate hosts, icluding farm animals, wild game species, rodents, birds and many others warm-blooded animals which can form the reservoir for *T. gondii* (Dubey, 2004; Dubey and Jones, 2008; Hill et al., 2008), the parasite reproduce asexually by endodyogeny (see Appendix 4) in almost all nucleated cells (Tenter, 2009; Melo et al., 2011) and tissue cysts may develop in their meat (Tenter et al., 2000; Meerburg and Borgsteede, 2011). Host cell invasion is an active process which is essential for survival and replication of the parasite. During the invasion of host cells, *T. gondii* discharges proteins from its secretory organelles, including micronemes, rhoptries, and dense granules (Thirugnanam et al., 2013).

In intermediate hosts, *Toxoplasma* exists in two interconvertable stages – slow-growing, encysted bradyzoites and the lytic, invasive and active tachyzoites (Dzierszinski et al., 2004; Elmore et al., 2010; Webster and McConkey, 2010). Sporulated oocysts transform into fast replicating tachyzoites and infect different organs in the host body, including the CNS (Bohne et al., 1999; Weiss and Kim, 2000) and male and female reproductive organs (Dalimi and Abdoli, 2013). Some of tachyzoites differentiate into bradyzoites (Bohne et al., 1999; Weiss and Kim, 2000) and remain viable over the animal lifespan (Dubey, 2004; Hill et al., 2008). If they are released from tissue cysts, they will again undergo transformation back to tachyzoites (Mead et al., 1999; Holland, 2003).

In addition to the extraintestinal cycle observed in all intermediate hosts of *T. gondii*, there were observed 5 asexual types (types A–E) before the development of the sexual cycle in the cat´s intestine (Dubey, 2002; Dubey, 2008). Prey animals harbour the parasite and are ready to infect the predators – felids and migrating animals are introducing the parasite to new areas (Deksne, 2012). Cysts in intermediate hosts are consumed by felids, and the life cycle resumes (Cork, 2011).



1. Unsporulated oocysts are shed in the cat´s faeces
2. Oocysts take 1-5 days to sporulate in the environment and become infective. Intermediate hosts in nature become infected after ingesting soil, warer or plant material contaminated with oocysts
3. Oocysts transform into tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites
4. Cats become infected after consuming intermediate hosts harbouring tissue cysts
5. Cats may also become infected directly by ingestion of sporulated oocysts. Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment.
6. Humans can become infected by any of several routes: eating of undercooked meat of animals harbouring tissue cysts

7. Consuming food or water contaminated with cat faeces or by contaminated environmental samples (such as faecal-contaminated soil or changing the litter box of a pet cat)
8. Blood transfusion or organ transplantation
9. Transplacentally from mother to fetus
10. In the human host, the parasites from tissue cysts, most commonly in skeletal muscle, myocardium, brain, and eyes; these cysts may remain throughout the life of the host. Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained biopsy specimens
11. Diagnosis of congenital infections can be achieved by detecting *T. gondii* DNA in amniotic fluid using molecular methods such as PCR

Figure 6: Life cycle of *T. gondii* (Centers for Disease Control and Prevention)

2.7 Morphology

The basic biology as well as ultrastructure of development stages was well established in 1983 (Boothroyd, 2009). Morphology of tachyzoites and bradyzoites is quite similar (see Figure 7).

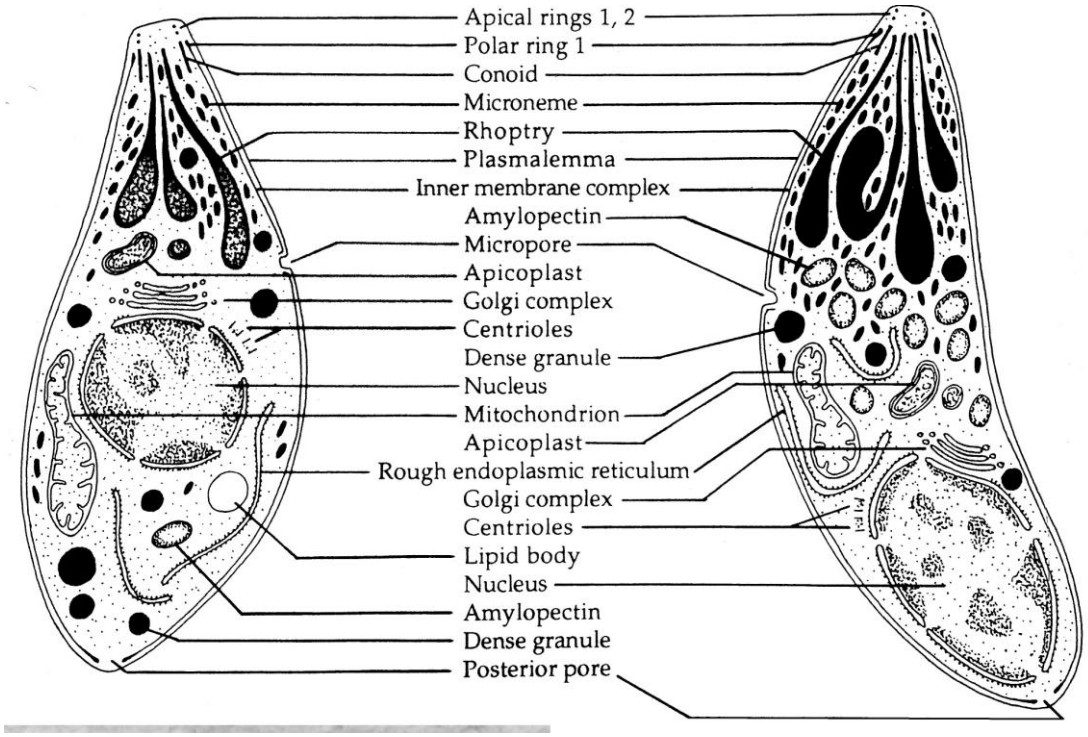


Figure 7: Morphology of tachyzoite (left) and bradyzoite (right) (Dubey et al., 1998a)

Tachyzoites

The term "tachyzoite" comes from Greek word *tachos* (speed) and replaces the previously used terms trophozoite (from Greek *trophicos*), endodyozoite or endozoite. This final term was proposed by Frenkel (1973) and describes the rapidly multiplying stage of the parasite in different tissues of intermediate hosts and in non-intestinal epithelial cells of definitive hosts (Dubey, 2010a). Tachyzoites (see Figure 8) have often crescent shape with pointed anterior end and a rounded posterior end and they are approximately 2x6 μm in size. They consist of various organelles (e.g. nucleus, apical and polar rings, rhoptries, micronemes, ribosomes and many others) (Gangneux and Dardé, 2012). Rhoptries and micronemes participate in preliminary attachment of the parasite into the host cells (Wellington et al., 2009). Although tachyzoites can move, they have no visible means for locomotion. In locomotion, there are no differences between tachyzoites, bradyzoites, and sporozoites (Dubey, 2010a). This form of the parasite was found by Nicolle and Manceaux (1908) in the gundi and it is directly connected with acute toxoplasmosis (Dubey, 2008).

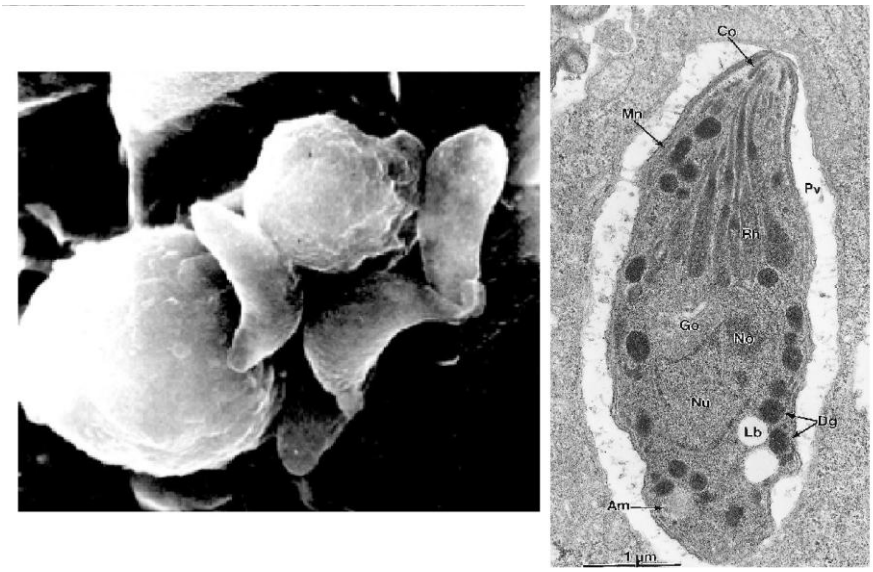


Figure 8: Tachyzoites (Dubey et al., 1998a; Lass, 2010)

Bradyzoites and tissue cysts

The term "bradyzoite" comes from Greek word *brady* (slow) and was also proposed by Frenkel (1973). It describes the encysted form of the parasite in tissues. Another used term for bradyzoites is cystozoites. Dubey and Beattie (1988) proposed the term tissue cyst to avoid confusion between the terms pseudocysts (cysts in tissues) and oocysts (in faeces) (Dubey, 2008; Dubey, 2010a). Tissue cysts grow and remain intracellular as the bradyzoites.

Structurally, bradyzoites differ only slightly from tachyzoites. The nucleus is situated toward the posterior end, whereas in tachyzoites, the nucleus is centrally located. The content of rhoptries is usually electron dense (in tachyzoites is labyrinthine) and vary with the age of the tissue cyst because some bradyzoites degenerate with advanced age of tissue cysts. Bradyzoites are more slender and less susceptible to destruction by proteolytic enzymes (Weiss and Kim, 2000; Dubey, 2010a). The bradyzoite cyst wall is thick, elastic and is built from chitin and glycoproteins secreted by the parasite (Boothroyd, et al., 1997; Dzierszinski et al., 2004).

Tissue cysts (see Figure 9) have different sizes. Young ones may be as small as 5 µm and contain only two bradyzoites and older ones may contain hundreds of bradyzoites (Weiss and Kim, 2000; Sullivan and Jeffers, 2012). The size varies also between brain cysts (rarely reach 70 µm) and intramuscular cysts (up to 100 µm). Although tissue cysts may attack almost all cells, they are the most prevalent in neural and muscular tissues (e.g. brain, eyes or skeletal and cardiac muscles) (Dubey, 2010a; Sullivan and Jeffers, 2012). The tissue cyst wall is elastic and thin and encloses hundreds of bradyzoites (see Figure 9). Both, bradyzoites and tissue cysts are associated with the chronic phase of toxoplasmosis (Dubey, 2010a).

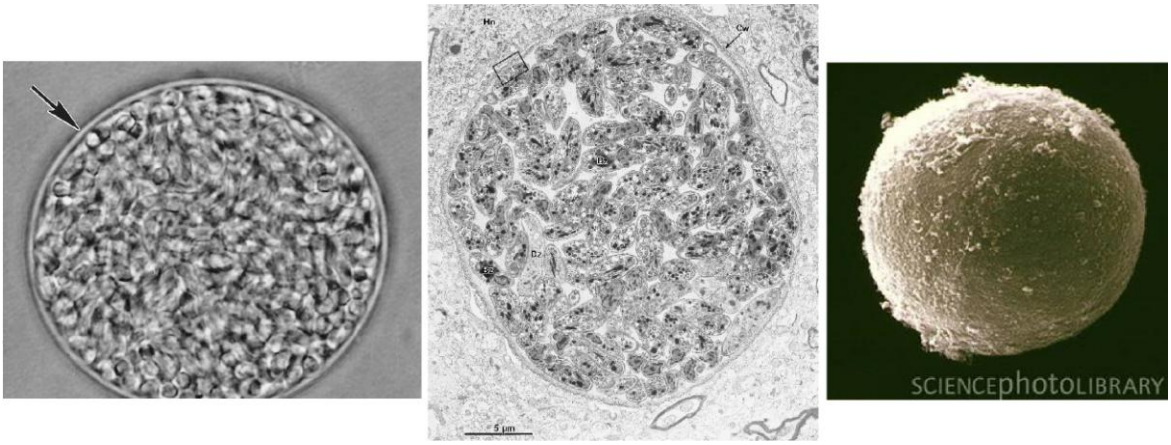


Figure 9: Tissue cysts with bradyzoites (Dubey et al., 1998a; Science Photo Library)

2.8 Genetic Aspects

All stages of *T. gondii* have a haploid nucleus, except the unsporulated oocyst. The genome of T. gondii has 14 chromosomes and represents a total length of 65 mega base pairs (De Craeye, 2012).

Several methods for classification of *T. gondii* have been developed over the years. One of the first methods for strain typing was multilocus enzyme electrophoresis (MLE). By using of

several polymorphic enzymes, this method places the majority of isolates into three zymodymes Z1, Z2 and Z3 (Dardé et al., 1992). Because of high number of purified parasites is needed for MLE, some new methods were therefore developed. These methods are PCR-RFLP and length polymorphism of microsatellites (MS) and are based on the parasite´s genomic DNA and PCR (De Craeye, 2012). Classification of genotype is made by polymorphous loci analyzation and up to 50 markers was described. Analyses based on RFLP shows that *T. gondii* consists of only three clonal lineages. Designated types I, II and III can occur in animals even in humans (Howe et al., 1997; Ajzenberg et al., 2002a; Montoya et al., 2008) and appear predominantly in Europe and North America (Sanecka and Frickel, 2012; Su et al., 2012). In Europe is the predominant type II (Dardé et al., 1992). Type I strains was found mostly in humans and types II and III predominated in animals (Howe et al., 1997; Literák et al., 1998). These clonal lineages developed after a single genetic cross (Su et al., 2003). From the point of view of infectivity, type I strains are responsible for acute infection and the most commonly isolated strain from clinical outbreaks is type II (Sullivan and Jeffers, 2012). The majority of isolates from South America and Africa are genetically different (Su et al., 2012). These "atypical" strains are not insignificant anomalies in the parasite population but they are important members of the gene pool, providing better representation of the host range utilized by *T. gondii* (Wendte et al., 2011).

By clonning the ROP2 gene into expression plasmid pcDNA3 and expression it by eukaryotic cells, there is a potential opportunity to produce a diagnostic kit for evaluation of immune response against toxoplasmosis and usage this expressed ROP2 gene as a candidate for recombinant vaccines (Khosroshahi et al., 2008).

2.9 Pathogenicity

After ingestion of sporulated cyst or tissue cyst of *T. gondii*, the parasite penetrates intestinal epithelial cells and disseminates through the body into the blood and lymph. Then, the tissue phase of infection develops in intermediate hosts (Luft et al., 1993). Viable organisms are released and invades various organs of the body including central nervous system (CNS) (Bohne et al., 1999; Weiss and Kim, 2000), muscles (Pfaff et al., 2014), and epithelial cells of the small intestine (Dubey, 1998a; Webster, 2001; Karatepe et al., 2008) where are encysted (Dubey and Jones, 2008; Miller et al., 2009; Khademvatan et al., 2013). Infection may also cause some adverse effects on reproductive function of the host (Martínez-García et al., 1996; Dalimi and Abdoli, 2013). It is important that parasite forms cysts in neurons, glial cells and astrocytes in the brain (Sabah and Mahfoth, 2009; Khademvatan et al., 2013) and have ability

to change the behavior of the host to increase the probability of transmission (Khademvatan et al., 2013). In AIDS patients any organ in the body may be infected, including, dermis, testis and spinal cord, but the most frequent is infection of the brain (Hill and Dubey, 2002). The main aim of the organism is efficiently infects but not kill an intermediate host (Sanecka and Frickel, 2012). It persists in different tissues of the body for the whole life of the host (Navarro et al., 1998; Hill and Dubey, 2002). Many factors such as individual genetic predisposition, the state of the immune system, parasite doses, virulence of the infecting strain, the timing of infection (e.g. prenatal and postnatal infection or infection in first trimester of pregnancy) and affected part of the brain, may influence the effect of *T. gondii* infection and therefore clinical signs and course of the disease may differ among humans and animals (Wellington et al., 2009).

Invading the small intestine in ruminants produce less pathogenic effects because they have very long intestine and thus provident a large number of host cells (Tamasaukas et al., 2010).

2.10 Clinical Signs in Humans

It was shown that *Toxoplasma gondii* is the third most frequent causative agent of foodborne illnesses which cause death (Mead et al., 1999; Meerburg and Borgsteede, 2011) or when hospitalization of infected patients is needed (Mead et al., 1999). On the other hand, toxoplasmosis is an infection that is typically manifested as asymptomatic (Montoya and Liesenfeld, 2004; Fernandes et al., 2012). The most susceptible individuals are fetuses, newborns, pregnant women and immunosuppressed patients (Remington et al., 2004; Fernandes et al., 2012). The public health relevance of toxoplasmosis relates to congenital and postnatal infection (Holland, 2003; Flegr, 2007; Muñoz-Zanzi et al., 2010) and related neonatal mortality, occurence of eye lesions and heavy brain alterations (Navarro et al., 1998). *T. gondii* nfection could be also associated with the occurence of brain tumors (Thirugnanam et al., 2013) and it may also cause schizophrenia in patients with genetic even non-genetic predispositions (Flegr, 2013). When the tachyzoites proliferate, they can cause tissue destructive inflammatory disease contrary to tissue cysts, which do not stimulate inflammation (Mead et al., 1999; Holland, 2003). An overstimulation of the immune system can lead to hyperinflammation with equally fatal consequences to the host and thus, *T. gondii* needs to strike balance between inducing and evading the immune response to reach the quiescence phase of infection (Blader and Saeij, 2009). Over all, only a small percentage (< 1%) of exposed adults develops clinical toxoplasmosis (Howe et al., 1997; Literák et al.,

1998). Some cases were reported in humans who had eaten naturally infected pork (Dubey et al., 1995a; Choi et al., 1997; Dubey et al., 2005).

2.10.1 In Healthy Persons

Most infections (90%) in immunocompetent humans (persons with normal immune function) are asymptomatic (Kasper and Buzoni-Gatel, 1998; Kim et al., 2008; Abhilash et al., 2013) but in the reamining 10% sometimes devasting disease can occur (Hill and Dubey, 2002; Abhilash et al., 2013). The clinical picture varies widely with a large proportion of the human population (Ajzenberg et al., 2002b; Cork, 2011). Possible mild symptoms and clinical signs may be cervical lymphadenopathy, fever or flu-like illness, malaise (Jones et al., 2001; Pasquali, 2004; Abhilash et al., 2013), myalgia, maculopapular rash (Alvarado-Esquivel et al., 2009; Hooshyar et al., 2009), encephalitis, mental retardation, blindness or ocular problems (Stanford et al., 2003; Alvarado-Esquivel et al., 2009; Meerburg and Borgsteede, 2011). Occasionally, permanent neurological damage, with or without hydrocephalus, myocarditis, polymyositis, hepatitis, or chrioretinitis can occur (Omata et al., 1990; Lee et al., 2010; Zulpo et al., 2012). It can leads to lifelong persistence or slow replicating of the parasite (Lüder and Gross, 1998).

2.10.2 In Immunocompromissed Persons

Toxoplasmosis can be life-threating to these individuals because they represent the group at highest risk of developing symptomatic toxoplasmosis (Pasquali, 2004; Hill et al., 2008). If the course of disease is not recognized and treated early it can be frequently fatal (Luft et al., 1993; Pasquali, 2004). Although high frequency of non-viable symptoms this zoonosis can cause major systemic diseases in patients with AIDS or cancer or in transplant recipients on immunosuppressive drugs (Navarro et al., 1998; Jones et al., 1999). Clinical toxoplasmosis can occur by reactivation of the latent infection (Kasper and Buzoni-Gatel, 1998; Jones et al., 1999; Kim et al., 2008), when the immune system is not able to control parasite replication (Fuentes et al., 2001; Fernandes et al., 2012). Also cerebral toxoplasmosis is often seen in these patients (Van de Venter, 2000). Infection in immunocompromised individuals can cause pneumonitis, myocarditis, meningoencephalitis, hepatitis, chorioretinitis (Van de Venter, 2000; Blader and Saeij, 2009), encephalitis which is one of the major causes of death in AIDS patients (Luft et al., 1993; Dubey et al., 1995a; Dubey, 1996), blindness (Dubey et al., 1995a), damages on the brain, eyes or other organs (Hill and Dubey, 2002) or combinations of these. Development of encephalitis can occur in up to 40% of HIV infected patients (Dubey, 1997;

Pasquali, 2004). When the AIDS epidemy was erupting, it became quickly clear that *T. gondii* is an important causative agent of CNS infection in AIDS patients (Boothroyd, 2009). Also serious pathological changes can be seen postmortem (Sroka et al., 2007). Special attention should be given to immunosuppressed patients because *T. gondii* bradyzoites can be activated when immunodeficiency develops and this is a major cause of mortality in AIDS patients (Luft et al., 1993; Pfaff et al., 2014).

2.10.3 Congenital Toxoplasmosis

The major clinical problem in human is congenital infection of fetus resulted from primary infection during pregnancy (Lüder and Gross, 1998; Hooshyar et al., 2007; Jones and Dubey, 2010). As a result of reactivation, vertical transmission was also described in immunocompromised women (Bachmeyer et al., 2006) and more rarely in immunocompetent women (Kodjikian et al., 2004). When pregnant women previously exposed to *T. gondii* are infected during pregnancy, significant prenatal damages of the brain, eyes or other organs can occur in their fetuses (Ajzenberg et al., 2002b; Rorman et al., 2006; Cork, 2011) and moreover, if acute infection occurs early in pregnancy, the transplacental infection can result in foetal death (Alford et al., 1974; Dubey et al., 1995a; Van de Venter, 2000), stillbirth (Pappas et al., 2009) or woman can give birth prematurely (FAO, 1998). Many parameters influence the gravity of symptoms but the period of gestation, when infection occurs, is the most important. If infection appears in the third trimester, it is usually asymptomatic (Pasquali, 2004), but probability of infection of the fetus is 50–60%. In the case of infection in the first trimester, the infection is transmitted to the fetus only in about 10% of cases (Flegr, 2013). As represented by disability-adjusted life years (DALY), the disease burden of congenital toxoplasmosis is the highest among all food-borne pathogens (Havelaar et al., 2007). The cases where toxoplasmosis was acquired congenitally are more severe than the postnatally ones (Singh, 2003).

2.10.4 Neonatal and Postnatal Toxoplasmosis

Toxoplasma gondii is an important causative agent of neonatal mortality (Navarro et al., 1998). The organism crosses the placenta and can cause a range of birth defects (Alford et al., 1974; Dubey et al., 1995a; Ghazaei, 2006). The amount of damage done to the mother and the fetus/baby depends on the stage of pregnancy at the time of infection (Ghazaei, 2006). Although most new-borns are asymptomatic or with subclinical presentation of the disease, there are numerous manifestation of congenital toxoplasmosis, including intracranial

calcifications, convulsion, psychomotor retardation, strabismus, chorioretinitis, microcephaly, and hydrocephaly (Sáfadi et al., 2003; Hooshyar et al., 2009; Jones and Dubey, 2010), blindness, epilepsy (Dubey, 1996; Jones et al., 1999), disorientation, drowsiness, reflex changes and may become comatose (Hill and Dubey, 2002). In acute infection, the non-specific symptoms such as convulsion, splenomegaly, hepatomegaly, anemia, and jaundice can occur (Alford et al., 1974; Pasquali, 2004). Infants often develop CNS disorders and ocular disease (FAO, 1998).

2.10.5 Ocular Toxoplasmosis

This form of the disease can occur in newborns who were exposed to *T. gondii in utero* and usually results in congenital infection (Havelaar et al., 2007; Hooshyar et al., 2009; Jones and Dubey, 2010). It is possible that ocular toxoplasmosis develop a long time after an acquired *T. gondii* infection (Holland, 2003). Whereas the parasite itself is responsible for the development of scars (see Figure 10), immunological mechanisms might trigger pathological alterations of the retina. Also hormones might be involved in the infection because deterioration of ocular toxoplasmosis can be observed during pregnancy (Lüder and Gross, 1998). Some ocular and neurological deficits can be observed (Pappas et al., 2009), including visual impairment or glaucoma (Wellington et al., 2009). Patients with ocular toxoplasmosis have a life-long risk of recurrences (Holland, 2003).

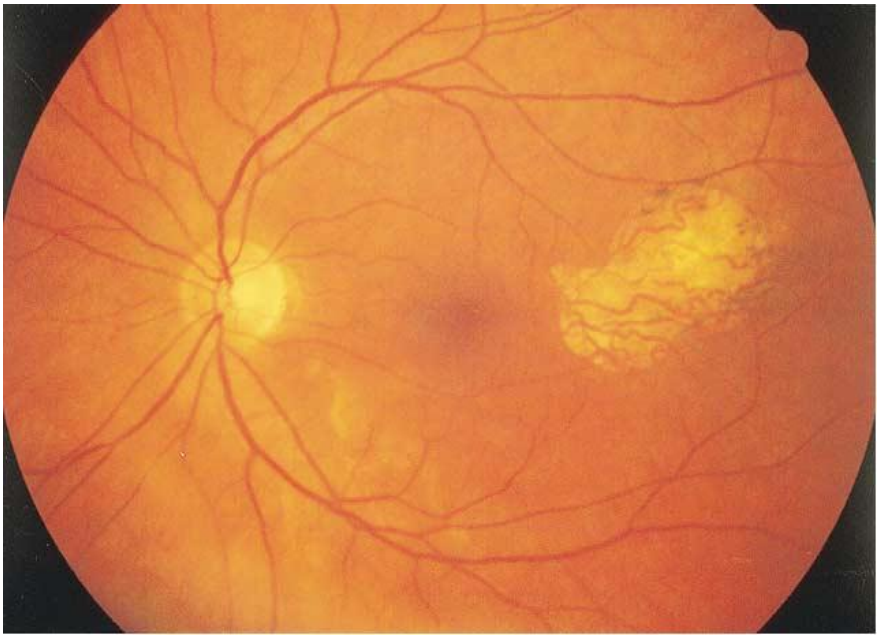


Figure 10: Scars on eye from toxoplasmic retinochoroidits (Holland, 2003)

2.10.6 Latent (chronic) and Acute toxoplasmosis

Usually, a latent form of the disease develops (Hill and Dubey, 2002; Sedlák and Bártová, 2006; Weiss and Dubey, 2009). The infection is turned into latent phase after a short period of acute toxoplasmosis (Dubey and Jones, 2008; Miller et al., 2009; Khademvatan et al., 2013). The dormant form of the parasite is predominantly found in the CNS and muscular tissue for the whole life of infective person (Tenter et al., 2000; Flegr, 2007) and tissue cysts of *T. gondii* persist for lifetime of the host without provoking any immune attack (Dubey et al., 1998a; Deksne, 2012; Fernandes et al., 2012). In this stage of disease, reproduction of bradyzoites is very slowly (Flegr, 2013). This is very beneficial for the parasite because while the host is unaware, the parasite stays dormant waiting for the host to be eaten by another host (Deksne, 2012). In a primary acute infection or in reactivation of a previously acquired latent form, tachyzoites can be often seen (Abhilash et al., 2013). Latent infection may be reactivated especially in patients with AIDS, where reactivation causes encephalitis (Porter and Sande, 1992). Acute form of toxoplasmosis is characterized by the rapid reproduction of tachyzoites in cells and is usually a self-limited. Typical symptoms in this phase of the disease are malaise, fever, fatigue, headache, and cervical lymphadenopathy. The form resulting from the infection by oocysts has usually worse course than form acquired from tissue cysts (Flegr, 2013).

2.11 Clinical Signs in Animals

Evidence of infection in animals is widespread and clinical or subclinical form usually develops. The disease is the most life-threating particularly for animals that have not developed resistance to *T. gondii*, such as marsupials, koalas and New World monkeys (family *Callitrichidae, Cebidae, Aotidae, Pitheciidae, Atelidae*). For these animal groups the infection is often severe or fatal (Epiphanio et al., 2000; Spencer et al., 2003; Hill et al., 2008). Clinical and subclinical toxoplasmosis was found also in wild cervids, ungulates, and marine mammals (Hill et al., 2005). Symptoms in native fauna include poor coordination, blindness, lethargy, respiratory and enteric distress, and often sudden death (Canfield et al., 1990; Dickman, 1996).

In farm animals, especially sheep and goats demonstrating high levels of infection and are associated clinical disease. Also sheep and goat milk which contain *T. gondii* tachyzoites can result in clinical infection in offsprings (Stormoen et al., 2012; Hill and Dubey, 2013). The most frequent symptoms in clinical form are abortions, stillbirths, and neonatal deaths of lambs (FAO, 1998; Stormoen et al., 2012).

In cattle and buffaloes, natural infection by *T. gondii* does not appear to cause clinical disease nor abortions (Opsteegh et al., 2011), probably due to their resistance against infection (Dubey, 2010a) and ability of parasite elimination (Lüder and Gross, 1998). Also in horses, there is no definitive evidence of T. gondii causing clinical disease (Jakubek et al., 2006).

In pigs is clinical toxoplasmosis accompanying mainly by anorexia, fever, dyspnea, and limb weakness (Dubey, 2009b). In affected sows were reported also neurological signs, vomiting, depression, recumbency, prostrations, and abortions (Kim et al., 2009). On necropsy, evidence of encephalitis, pneumonitis, and lymph node necrosis may occur (Kumagai et al., 1988; Dubey, 2009b).

Infection of rabbits with T. *gondii* is generally not manifested by clinical signs of disease. However in some cases clinical manifestations of the disease were described. It was represented by injury to the CNS, paralysis of hind limbs, abortion, and congenital hydrocephalus, together with more general symptoms such as loss of apetite, emaciation, rhinitis and enteritis (Hejlíček and Literák, 1994). Also ocular and lower respiratory tract disease (Alvarado-Esquivel et al., 2013), fever, anorexia, lethargia, nasolacrimal discharge, tremors, uncoordinated gait, and diarrhoea were observed. The pathological lesions of toxoplasmosis in the liver and spleen may occur (Haziroğlu et al., 2003).

Rarely, toxoplasmosis can cause clinical disease in chickens (Dubey, 2010b). Chickens can harbour mouse-virulent T. gondii without showing any clinical signs (Kaneto et al., 1997; Dubey et al., 2002a). However, anorexia, emaciation, diarrhoea, blindness, jerking of neck and head, paralysis and loss of eye sight, torticollis, ability to stand and lateral recumbency may be seen (Dubey, 2010b). Occasionally, hyperthermia (Kaneto et al., 1997) and affecting of retina may occur (Dubey, 2010b).

In cats, the infection is mainly asymptomatic (Tenter et al., 2000; Hamidinejat et al., 2011**)** but it can occasionally cause fetus abnormalities, fever, anorexia, abdominal and joint pain, ocular and neurological disease leading to blindness, seizures and loss of control over urination and defecation, or infection in the liver (see Appendix 5). The most common findings are pulmonary lesions leading to rapidly evolving pneumonia (De Craeye, 2012). Infection of cats usually results in a single acute phase when oocysts are excreted before developing of immunity (Dickman, 1996). This infection is important mainly clinically in cats (Hamidinejat et al., 2011).

2.12 Antigens and Immunity

The whole immune system plays an important role in the course of the infection and in the evolution of toxoplasmosis (Wellington et al., 2009). The presence of *T. gondii* cysts in body tissues induces local inflammation, and bradyzoites release various antigens and other substances (e.g. dopamine) into surrounding tissues (Flegr, 2013). *T. gondii* antigens are unique. Different tachyzoite and bradyzoite antigens cover the surface of the parasite (Arabpour et al., 2011). They can be of membranous or cytoplasmic origin and may reflect a common biochemical thread which goes through complicated two-host life cycle of this parasite. Antigens can be identified by several immunologic assays (Kasper, 1989).

The immune system of intermediate hosts presents challenges but also opportunities for *T. gondii* (Sanecka and Frickel, 2012). Immunocompetent patients are usually able to to control *T. gondii* infection (Lüder and Gross, 1998). Once infected, the host acquired lifelong immunity which is induced by the persistence of the parasite in an encysted form (Azevedo et al., 2010) but after natural or artificial immunosuppression (AIDS, immunosuppression in oncological or transplant patients), the latent form of the disease quickly transit into a new acute phase (Flegr, 2013). The chronically infected patients and animals usually develop lifelong immune protection against reinfection (Khosroshahi et al., 2008) because with a persistent infection, the prevalence of antibodies accumulates with age (Dubey, 2010a; Opsteegh et al., 2012). In pregnant women, the maternal immunity appears to protect the fetus against the infection and the woman is not at risk for a congenitally infected fetus when anti-*T. gondii* IgG antibody is confirmed before pregnancy (Azevedo et al., 2010). The most of patients with disseminated toxoplasmosis have specific IgG antibodies but in contrast, IgM antibodies were not detected in these patients (Lüder and Gross, 1998).

All seropositive cats probably shed oocysts (Silva et al., 2001a; Dubey et al., 2002b; García-Márquez et al., 2007) and as a consequence they develop a long-lasting humoral immune response against tachyzoites and bradyzoites (Maksimov et al., 2013). Cats usually acquire immune-based protection within approximately 2 week after primery infection (Dubey and Frenkel, 1974; Dubey, 2004), but the protection is not life-long (Tenter et al., 2000; Pasquali, 2004) and cats can reshed oocysts (Dubey, 1996). There is an evidence which indicate that immunity may loss with advanced age (Opsteegh et al., 2012). Seroprevalence of *T. gondii* in cats varies depending on the type of cats (feral vs. domestic), age of cats, method of serologic testing, and geographic location (Dubey et al., 1995b). Individual cats have different abilities to produce anti-*T. gondii* antibodies (Afonso et al., 2006). Kittens can develop detectable

antibody titres at a very young age and can also obtain high IgG antibody titres via colostrum (Opsteegh et al., 2012).

Cattle, in contrast to other animals, do not show high titers against T. gondii. The risk of human infection from seropositive cattle is low (Opsteegh et al., 2011).

2.13 Detection Methods

There are many tests for detection of *T. gondii* infection in humans and animals (Hill et al., 2006a). The main tools for diagnosis are serologic tests, amplification of specific nucleic acid sequences (e.g. PCR), histologic demonstration of the parasite or antigens (e.g. immunoperoxidase stain), isolation of the organism, sequencing of parasite DNA/RNA, or bioassay in laboratory animals (Hill and Dubey, 2002). However, the confirmatory methods using bioassay are expensive, require the killing of experimental animals, and may not always indicate true infection (Dabritz et al., 2007b). Also toxoplasma skin test, demonstration of antigemia and antigen in serum and body fluids, or antigen-specific lymphocyte transformation can be used but these methods are more likely rare (Dalgıç, 2008). Generally, *T. gondii* infection can be diagnosed indirectly (e.g. IgG and IgM antibodies) or directly, e.g. by PCR, histology and by detection of bradyzoites in tissue sections (Abhilash et al., 2013).

Detection of antibodies

T. gondii specific IgG, IgM, IgA or IgE antibodies can be detected by different serological methods and it is usually the primary diagnostic method in the parasite infection determining (Karatepe et al., 2008; Wellington et al., 2009). MAT and ELISA are usually widely used within the serologic tests (Zhu et al., 2012).

In detection of IgG antibodies, serodiagnosis includes titration of specific immunoglobulin G, showing past exposure of the organism to *T. gondii* parasite (Suzuki et al., 2001) because IgG antibodies usually appear within 1-2 weeks after acquiring of infection and persist for whole life of the host. The most common tests used for IgG detection are SFDT, ELISA, IFA, IgG avidity test and different agglutination tests (Montoya and Liesenfeld, 2004). During pregnancy, it is important to collect serum samples in 3 weeks intervals for determining the change in antibody titers for the evaluation of infection (Rorman et al., 2006) because IgG antibodies can cross the placenta (Dubey, 2008). In differentiation of probable acute or chronic infection during pregnancy was proven to be helpful the usage of the differential agglutination (AC/HS) test (Dannemann et al., 1990; Montoya, 2002).

At suspicion to recent exposure of the organism to *T. gondii* or to ongoing acute infection, screening for specific IgM antibodies is usually done (Suzuki et al., 2001). Although these titers may be negative within a few months (Liesenfeld et al., 1997), sometimes can be detected even 12 years after the acute infection (Dalgıç, 2008). In opposite, in some patients positive *T. gondii*-specific titers can be found also during the chronic phase of infection (Liesenfeld et al., 1997) and it is also useful for detection of IgM antibodies in cord blood for the diagnosis of congenital toxoplasmosis because IgM antibodies do not cross the placenta (Dubey, 2008). However, positive IgM test results can be interpreted as a true-positive result of an infection acquired in the past (Montoya, 2002), or as a false-positive result (Liesenfeld et al., 1997; Wilson et al., 1997). For the measurement of IgM antibodies are widely used tests including capture IgM-ELISA, IFA test and IgM immunosorbent agglutination assay (ISAGA) (Dubey, 2008).

Tests for IgA antibodies detection are predominantly used in fetuses and newborns because they are more sensitive than those used for detection of IgM antibodies. ELISA and ISAGA can be used in detection of IgA antibodies (Montoya, 2002).

IgE seropositivity lasts less than that with IgM and IgA and therefore it is suitable for detection infection in patients with recently acquired toxoplasmosis and also for congenitally infected children and those with congenital toxoplasmic chorioretinitis but on the other hand, unlike with IgA, it does not seem to be helpful for diagnosis in the foetuses and newborns. IgE antibodies are detected by ELISA test (Montoya, 2002).

PCR

Toxoplasmosis can be also detected by another serologic method – polymerase chain reaction (PCR), which is commonly used in testing the amniotic fluid to determine whether the pregnant women and fetuses are infected (Jones et al., 2003). Specificity and predictive value of PCR on samples from amniotic fluid is close to 100% (Hohlfeld et al., 1994; Romand et al., 2001). The sensitivity of PCR from amniotic fluid may be influenced by the stage of pregnancy in which maternal infection occurs (Romand et al., 2001) but even by antitoxoplasma drugs used for treatment of the disease. This method is also successful in detection of *T. gondii* DNA in body fluids and tissues in cases of congenital, ocular, and cerebral and disseminated toxoplasmosis (Dalgıç, 2008). PCR was a revolution in the diagnosis of infectious agents but on the other hand, it is quite expensive and technically difficult and therefore it has limited use in diagnosing of toxoplasmosis. Nevertheless, this method is very useful in parasite detecting in the environment (Boothroyd, 2009).

Sabin-Feldman Dye Test

This test was developed by Albert Sabin and Harry Feldman in 1948 (Dubey, 2008), in addition, it was the first test developed for the laboratory diagnosis of *T. gondii* (Rorman et al., 2006) and probably the greatest advancement in the field of toxoplasmosis (Dubey, 2008). This test detects the presence of anti-*T. gondii* specific antibodies (total Ig) (Rorman et al., 2006) and is highly sensitive and specific with no false results (Dubey, 2008). Nowadays, it is still considered as the "gold standard" (Rorman et al., 2006).

Direct agglutination test (DAT)

The development of a simple DAT helped greatly in the serological diagnosis of toxoplasmosis in humans and animals. It had been developed by Fulton (1956), and then improved many times and in 1987 it was called modified agglutination test by Dubey and Desmonts. This MAT was widely used for the diagnosis of toxoplasmosis in animals. The great advantage is that there is no special need of equipment in this test (Dubey, 2008).

Histologic diagnosis

This method is used for demonstration of presence of *T. gondii* tachyzoites and to etablish the diagnosis of the acute infection (Montoya, 2002) but for example in conventionally stained tissue sections, it is sometimes difficult to demonstrate tachyzoite presence. Tachyzoites can be detected in different tissues or in smears of various body fluids, including cerebrospinal or amniotic fluid (Dalgıç, 2008), saliva, sputum, urine, tears, semen or milk of intermediate hosts (Tenter, 2009). In some cases, evaluated tissues can be infected at levels too low to detect the parasite, or postharvest processing of meat (chilling and heating) may render *T. gondii* tachyzoites nonviable (Dubey et al., 2005) and therefore detection of specific antiboides in serum is better way in these cases (Sroka et al., 2007). Especially definitive diagnosis of cerebral toxoplasmosis requires demonstration of tachyzoites in brain (Mesquita et al., 2010).

Detection of *T. gondii* DNA

The first reported detection was done by Burg (1989) from tachyzoite using the B1 gene in a PCR. Genrally, this method was proven very useful in the diagnosis of clinical toxoplasmosis and some another subsequent PCR test have been developed using different gene targets (Dubey, 2008).

Isolation of oocysts from faecal samples

Because *T. gondii* oocysts are only 10 μm in size and the period of shedding is limited, the chance of detecting is not so big (Marchiondo et al., 1976; Lucas et al., 1999; Karatepe et al., 2008). Oocysts are best demonstrated by centrifugation using various types of solutions (e.g. Sheather´s sugar solution, Zinc Sulphate solution) (Dubey and Lappin, 2006; Karatepe et al., 2008). Since *T. gondii* oocysts are morphologically similar to oocysts of *Hammondia hammondi*, *Besnoitia orcytofelisi* and *B. darlingi* (Dubey and Lappin, 2006) it is necessary to observe the criteria for *T. gondii* oocysts diagnosis, including size, shape and presence of characteristics elements (e.g. polar cap, colour, aspect of oocyst wall etc.) (Chartier and Paraud, 2012). *T. gondii* oocysts are usually detected in < 1% of cat´s faecal samples (Dubey et al., 1995b) but the seroprevalence can be much higher (Opsteegh et al., 2012). Therefore, it is suitable to complete the oocyst detection with serologic surveys (Dubey, 2004; Karatepe et al., 2008; Dubey et al., 2009). A modified CsCl method that easily purifies *T. gondii* oocysts from cat faeces was also described (Staggs et al., 2009). Because the mother licks the kittens' anus and sometimes eats their faeces, oocysts excreted by kittens can be detected by examining the mother´s excreta (Dubey, 2002).

For *T. gondii* oocyst detection in environmental samples see Appendix 6.

Toxoplasmosis detection in pregnancy

When toxoplasmosis infection is suspected in women during or before pregnancy, the diagnosis should be done as soon as possible because delay in diagnosis may lead to abortion (Arabpour et al., 2011). The diagnosis is established usually on the basis of antibody detection using serological investigation. Within the acute infection, IgG and IgM antibody level generally rise during the first or second week of infection (Jenum et al., 1997). Although elevated levels of *T. gondii*-specific IgG antibodies do not differentiate recent infection from an infection acquired in the past, the time of infection can be determined by detection of *T. gondii*-specific IgM antibodies. Negative IgM result with a positive IgG result indicates that infection was acquired at least six months ago (Wilson et al., 1997). It is suitable to complete this test with SFDT, ELISA, or differential agglutination (Lüder and Gross, 1998; Jones et al., 2003).

Prenatal diagnosis

When the acute infection of the mother occurs, it should be determined whether the foetus is infected or not. There are several methods for diagnosis of the infection. For example

chorionic villus sampling (CVS) is possible to use but it is not suitable so much because it can only show placental but not fetal infection. The widely used method is cordocentesis for the determination of fetal IgM status. Other possibility is evaluation of haematological and liver function but still fetal blood sampling may not bring a reliable conclusion because fetal IgM or IgA antibodies may not be produced before 22 weeks of gestation (Dalgıç, 2008). It was proven that serologic tests on fetal blood have low sensitivity (Hezard et al., 1997). In addition, other problem in fetal blood sampling can be false-positive results. The most sensitive detection method seems to be PCR assay of amniotic fluid because it is rapid and accurate. However, if the concentration of the parasite in the amniotic fluid is low, DNA amplification may be the only positive result (Dalgıç, 2008). In opposite, using PCR test on the amniotic fluid in HIV-infected women is not convenient because there is a risk of transmission the HIV virus to the fetus during amniocentesis procedure (Montoya, 2002).

Diagnosis in newborns

Detection of toxoplasmosis in neonates involves a combination of parasite isolation, serologic tests, and nonspecific findings (Montoya and Liesenfeld, 2004). Serologic follow-up of the infants is recommended for the first year of their life in case, when the infection is suspected. Maternal IgG antibodies present in newborns may reflect infection of the mother acquired recently or in the past. Although shelf life of maternal IgG antibodies transferred to the baby is approximately 1 month, it can be still detected in newborns for several months but disappear completely within one year (Rorman et al., 2006). During anti-parasitic therapy, the antibody production may be delayed and occsionally may be completely stopped Dalgıç, 2008; Wellington et al., 2009). The highly sensitive for the diagnosis of congenital toxoplasmosis is detection of IgM and IgA antibodies (Wellington et al., 2009) but IgA antibodies are sensitive much more (Dalgıç, 2008). *T. gondii*-specific IgA antibodies may be present even though there are no IgM antibodies and also *vice versa* (Montoya, 2002). Serum samples from the umbilical cord may be contaminated with maternal blood (Dalgıç, 2008) and therefore, when IgA antibodies are detected in newborn, tests should be repeated at 10 days after birth to determine, whether the measured samples are not contaminated by mother (Montoya, 2002). Serum samples from peripheral blood are rather preferred. Other successfully used methods for detection of toxoplasmosis involve isolation of the parasite (e.g. by mice inoculation, inoculation in tissue cultures of urine, placental tissue, or peripheral blood) or amplification of *T. gondii*-specific DNA (e.g. PCR) (Dalgıç, 2008). Infants with suspicion to congenital toxoplasmosis should be always monitored through ophthalmologic

examination, tomography or ultrasound of the brain if there is no presence of hydrocephalus or calcification (Montoya, 2002; Wellington et al., 2009).

2.14 Transmission and Risk Factors

Toxoplasmosis is one of the most common parasitic zoonoses worldwide (Dubey, 2004; Rosa et al., 2010; Raeghi et al., 2011). The parasite transmission may alternate between definitive and intermediate hosts, and *vice versa*, and also between different definitive or intermediate hosts (Tenter, 2009). However, in some countries there are data suggesting that the transmission pathways can be more complex than previously thought (Cork, 2011). The parasite can be transmitted within and between different hosts in several ways (Tenter et al., 2000; Shuhaiber et al., 2003; Haddadzadeh et al., 2006). The three main modes of transmission are congenital (vertical) infection and ingestion of tissue cysts or oocysts - horizontal infection (see Figure 11) (Shahmoradi et al., 1993). Although horizontal transmission via tachyzoites is biologically possible, it is not important epidemiologically (Dubey and Frenkel, 1974). Less important ways are then blood transfusion and organ or bone marrow transplantation (Shahmoradi et al., 1993). Infections which are induced by oocyst ingestion are generally more severe than tissue cyst-induced ones (Choi et al., 1997).

Cats play a key role in spreading of the disease, nevertheless *T. gondii* infection is not transmitted by bites or scratches from and infected cat (Lindsay and Dubey, 2007). Not cat itself but oocysts shed in cat faeces to the environment represent the more significant infection risk (Lüder and Gross, 1998; Oliveira Braga et al., 2012) and although cats shed oocysts in their faeces, oocysts cannot be found on their coat (Dubey, 1995). Research on transmission of *T. gondii* oocysts in the faeces of cats was initiated and proven by Hutchison in 1965 (Hutchison et al., 1970; Frenkel, 1973). It was showed that veterinarian workers and people handling with cats are not more likely to be infected than other people who are not in contact with cats (Shuhaiber et al., 2003).

The frequency of transmission is very variable, owing to temperature and humidity variation (Montoya and Liesenfeld, 2004). Oocysts may persist in the environment for long periods, especially in warm and humid zones (Dubey, 2004; Wu et al., 2011); therefore infection is more common at lower altitudes with warmer climate than in mountainous regions (Ghazaei, 2006).

For the disease spreading and surviving of the parasite are more important animal hosts because they clearly outnumber human hosts living on this planet (Deksne, 2012).

2.14.1 Transmission to Humans

In humans, the disease is acquired mainly by eating of raw or undercooked meat, organs and tissue contaminated with *T. gondii* bradyzoites (Dubey et al., 2002a; Meireles et al., 2004; Kijlstra and Jongert, 2008), including pork **(**Weigel et al., 1999; Dubey et al., 2005), mutton (Kapperud et al., 1996; Cork, 2011), lamb (Baril *et al.*, 1999; Cook et al., 2000), venison or game (Cook et al., 2000), mincemeat products (Kapperud et al., 1996), even meat of kangaroos (Dubey et al., 1988) or reindeers (Oksanen et al., 1997; Dubey et al., 2002c) and rarely beef meat (Cook et al., 2000). Eating of raw shellfish is also considered as a potential source of infection (Jones et al., 2009; Muñoz-Zanzi et al., 2010). Professions such as slaughterhouse workers, butchers and hunters are more susceptible to infection because of frequent handling with meat and evisceration of carcasses (Dubey et al., 2004; Tenter, 2009). However, in the past, the risk for *T. gondii* infection was much higher because freezing of meat was less common. Also improving of livestock rearing has led to decline of toxoplasmosis (Jones et al., 2001). Very important are dietary preferences, habits and some cultural rituals which can increase risk of transmission (Van de Venter, 2000).

Some studies focused on investigation of infection outbreaks have suggested that *Toxoplasma gondii* could be also waterborne **(**Bowie et al., 1997; Isaac-Renton et al., 1998; Shuhaiber et al., 2003**)** and drinking water may be contaminated by sporulated oocysts (Heukelbach et al., 2007; Huong and Dubey, 2007; Kijlstra and Jongert, 2008). Runoff water which contains *T. gondii* oocysts (Bahia-Oliveira et al., 2003) can enter world´s oceans and thus, marine enivronment may be also contaminated (Hill and Dubey, 2013).

Other very common route of transmission is via ingestion of sporulated oocysts during contact with oocyst-contaminated soil (Choi et al., 1997; Dubey et al., 2005; Hooshyar et al., 2007), especially by gardening (Weigel et al., 1997; Jones et al., 2001) and contact with contaminated cat faeces (while handling cat litter box) due to poor hygiene practices (Kapperud et al., 1996; Nesbakken and Skjerve, 1996; Dabritz et al., 2007a). There is also high risk for children playing in sandboxes because they can be in contact or they can ingest sand which is contaminated by cat faeces (Kiliç et al., 2008). Also infection by inhalation of sporulated oocysts is possible, but very rare (Tenter, 2000; Dubey, 2008; Torrey and Yolken, 2013). This way of transmission can occur indirectly through eating of food contaminated by sporulated oocysts (inadequately washed fruit and vegetables) **(**Jackson and Hutchison, 1989; Huong and Dubey, 2007; de Camps et al., 2008).

Although pasteurization kill *T. gondii* in milk, unpasteurized, raw milk and products made from this milk are still sold in many countries. Therefore, goat cheeses (Dubey, 2010a) and

raw milk of goats (Sacks et al., 1982; Hill and Dubey, 2013; Tavassoli et al.¸2013) and sheep (Fusco et al., 2007; Tavassoli et al., 2013) infected by tachyzoites, could be a source of infection. Among the food, shelled eggs are not considered as a source of infection for humans and animals because they have not been found to be infected with *Toxoplasma gondii* (Hill and Dubey, 2013).

For unborn fetuses is very dangerous infection by tachyzoites which is transmitted vertically through the mother´s placenta (Jones et al., 2001; Thiangtum et al., 2006; Kim et al., 2008). Other routes of human infection, including transplantation of an organs or bone marrow (Hill and Dubey, 2002) and blood transfusion (Nunura et al., 2010; Strabelli et al., 2012) from infected donor, are less common.

Other considered risk factors which play a key role in the spreading of the disease, are owning cats (Baril et al., 1999; Wu et al., 2011), washing of kitchen knives and other cooking equipment infrequently (Kapperud et al., 1996), having poor hand hygiene (Baril et al., 1999; Swai and Schoonman, 2009) and last but not least, globalization. Hundreds of millions people cross borders and together with rapid movement of food of plant and animal origin, they can spread disease rapidly to new and distant areas and environments (Van de Venter, 2000).

2.14.2 Transmission to Animals

Among animals, the disease is usually spread also by ingestion of infected tissue cyst or sporulated oocysts. Access of animals to the outdoor environment and hunting of prey (Silva et al., 2007; De Craeye et al., 2008; Oliveira Braga et al., 2012) is one of the strongest risk factors identified (Opsteegh et al., 2012) because there is a permanent contact with parasite in the living environment (Michalski et al., 2010). As the main source of *T. gondii* infection for these animals are considered to be birds and small rodents (Dubey et al., 1995a) but also invertebrates. Also ingestion of carcasses up to several days after death may present a significant risk for infection (Dickman, 1996). Comparison of domestic and stray cats pointed out, that both indoor and wild cats may become infected by ingesting of tissue cyst in meat. Domestic cats are often fed by raw meat, whereas wild cats feed predominantly on garbage that may contain infected meat (Karatepe et al., 2008). Moreover feral cats become infected after weaning, when they start with hunting (Lucas et al., 1999). Licking of kitten´s anus by cat is also potential risk factor for disease transmission (Dubey, 2002).

A significant problem in livestock breeding is infection of animals on pastures which are infested by *T. gondii* sporulated oocysts (Flegr and Hrdý, 1994; Hooshyar et al., 2007; Tavassoli et al., 2013) and through contaminated water (Ghazaei, 2006). Animals under

organic farming or free ranging are much more susceptible to infection. Defined standards for these types of breeding are less stringently and outdoor access is considered a requirement labeling (Dubey et al., 2012). Therefore, animals are in higher risk of exposion to organic material potentially contaminated with cat faeces and oocysts (Dubey et al., 2002a; Dubey et al., 2012; Hill and Dubey, 2013). Other infection sources represent feeds (e.g. hay, grains and forage) stored in barns with open access for cats (Dubey et al., 1995a; FAO, 1998**)**, or bedding contaminated with the cat faeces (FAO, 1998). *T. gondii* oocysts can be also transported mechanically through the keeper´s clothing, boots and cleaning equipment (de Camps et al., 2008), by cockroaches, dung beetles, and earthworms (Hill and Dubey, 2002; Thiangtum et al., 2006), or by wind and rain (Tenter, 2009). In these cases, infection depends greatly on the density of cat population in given area (Navarro et al., 1998).

Recent studies show that *T. gondii* can be transmitted by semen to female animals (Arantes et al., 2009; de Moraes et al., 2010; Dass et al., 2011).

The data suggests that urbanisation may heighten interaction between wild and domestic animals, providing opportunities for T. gondii to expand its host range (Hill et al., 2008).

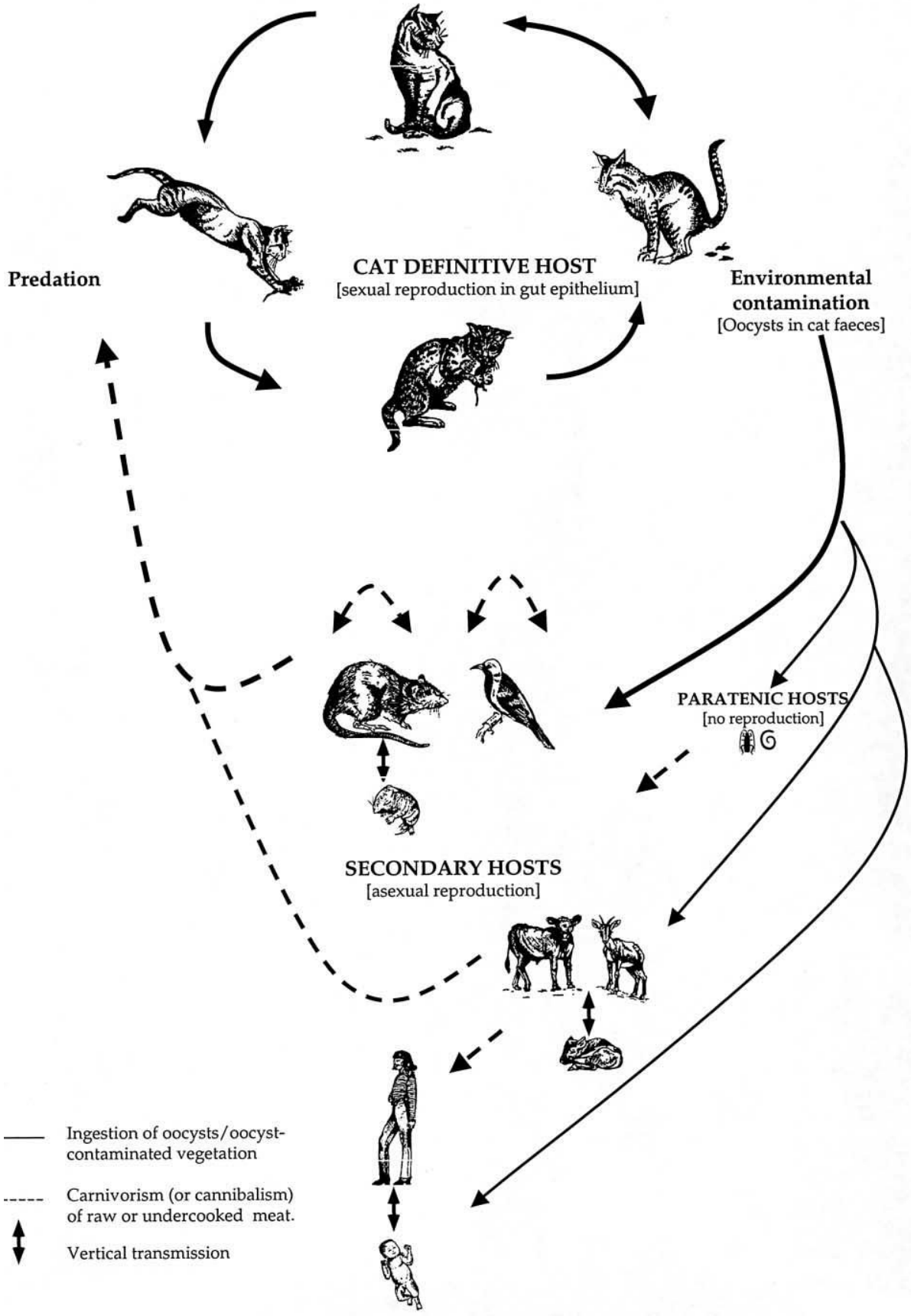


Figure 11: Ways of transmission of *T. gondii* (Webster, 2001)

2.15 Prevention

Control of the prevalence of *T. gondii* and effective prevention of toxoplasmosis in animals and humans require good diagnostic or testing methods that are appropriate for the specific species (Hill et al., 2006a). The data suggest that it is possible to significantly reduce the risk of *Toxoplasma gondii* infection (Ghazaei, 2006). The best approach to minimize or prevent the risk of transmission and protective factors are following:

- avoid direct contact with cat faeces (Pasquali, 2004)
- wear gloves during handling with manure or soil (Hill and Dubey, 2002; Gangneux and Dardé, 2012)
- cover the children´s sandpit when not in use (Torrey and Yolken, 2013)
- cooking all meat thoroughly to a minimal temperature of 67 °C (Ghazaei, 2006; Tenter, 2009) or adhere meat-free diet (Roghmann et al., 1999)
- use only pasteurized milk or milk products (Pasquali, 2004)
- washing hands well with soap and warm water after handling with raw meat, gardening, cat litter box or cats (Lopez et al., 2000; Ghazaei, 2006; Muñoz-Zanzi et al., 2010**)**
- freezing of meat before sale/consumption (Nesbakken and Skjerve, 1996; Tenter, 2009; Muñoz-Zanzi et al., 2010)
- washing or peeling of fruit and vegetable before consumption (Muñoz-Zanzi et al., 2010; Gangneux and Dardé, 2012)
- living at a high altitude or in arid climate (Jones et al., 2001)
- the elimination of cats, rodents (Kijlstra et al., 2008; Hill and Dubey, 2013) and insect from the farm environment (FAO, 1998; Tenter, 2009), and avoid being of healthy animals in proximity to seropositive animals in farming areas (Weigel et al., 1997; Zhu et al., 2012)
- feeding animals on sterilised food and controlling access of cats to feed stores (Hill and Dubey, 2002; Tenter, 2009)
- keeping a high standard of hygiene in the kitchen – washing any cutting boards, surfaces and knives (Nesbakken and Skjerve, 1996; Dubey, 1998b; Ghazaei, 2006)
- pregnant women and immunosuppressed patients should not handle with raw meat and cat litter box (Hill and Dubey, 2002; Ghazaei, 2006)

- removing of faeces from felid yards before nightfall may restrict opportunities for ingestion of oocysts by wildlife animals, and avert the sporulation of oocysts (Hill et al., 2008)
- usage of anticoccidial drugs, adequate nutrition (Rehman et al., 2011; Chartier and Paraud, 2012; Andrews, 2013)
- serological monitoring of farm animals (Sroka et al., 2007).

In immunosuppressed patients may be used trimethoprim-sulfamethoxazole as a preventive measure. Sometimes Spiramycin is used in Europe as a prophylactic during pregnancy but it is not proven in the US. All these drugs act on tachyzoites, rather than on bradyzoites in tissue cysts and they may control active infection but can not eliminate chronic infection (Dubey, 2010a). Healthcare workers should educate women of childbearing age on information about *T.gondii* transmission (Wellington et al., 2009).

Preventive measures may be also useful for restriction of parasite transmission between free-ranging and breeding animals (Hill et al., 2008). The prevention of cat infection is likely to be highly effective in reducing of oocyst shedding (Opsteegh et al., 2012) and it would be a fundamental prophylactic measure because cats are responsible for the dissemination of toxoplasmosis (Zulpo et al., 2012). It is recommended to place feline drinking bowls at the high to avoid faecal contamination (Rehman et al., 2011; Chartier and Paraud, 2012; Andrews, 2013) because water may act as a reservoir of oocysts and they may be spread between animals in the group (Mitchell et al., 2012). Vaccination of animals is also possible way how to prevent spreading of the disease but it is not currently feasible with the use of a single vaccine (Dubey, 1996).

After herd outbreak of infection, it is necessary to protect healthy, non-infected animals. Several preventive measures should be done, including:

1. Identification and isolation of all affected animals and treating them
2. Reduction of stocking rate of the animals in affected pens
3. Ensuring that all feedstuffs are fed in troughs (avoiding of faecal contamination)
4. Providing of extra bedding (reducing of oocyst concentration) (Radostits and Stockdale, 1980)
5. Vaccination of animals (reduction of fetal damage, reducing the number of *T. gondii* tissue cysts, and preventing the formation of oocysts in cats) (Dubey, 1996)

2.16 Treatment

After detection of *T. gondii*, treatment should be done as soon as possible (Taylor et al., 2003; Chartier and Paraud, 2012). It can be done by different products but which agent should be used depends on the stage of infestation (Andrews, 2013). The drugs which are routinely used have beneficial effect on toxoplasmosis in the acute stage because of their affecting on actively multiplying tachyzoites (Wellington et al., 2009). On the other hand, they are ineffective in eradicating the encysted form of the parasite (Luft et al., 1993; Guerina et al., 1994**)**. The most common in treatment of immunocompetent individuals is pyrimethamine in combination with sulfadiazine **(**Dubey and Frenkel, 1974; Guerina et al., 1994; Dubey, 2010a). The use was firstly demonstrated in 1953 by Eyles and Coleman and till now it remains the gold standard for anti-*T. gondii* therapy (Weiss and Dubey, 2009). These drugs were found helpful in 60–70% of cases (Stanford et al., 2003; Meerburg and Borgsteede, 2011). The treatment can be supplemented with folinic acid and yeasts (Alford et al., 1974). Also antipsychotic medications (e.g. chlorpromazine) or mood stabilizers have an inhibitory effect on *T. gondii* but their therapeutic significance remains to be elucidated (Jones-Brando et al., 2003). The dosage is usually expressed in mg/kg as the individual daily dose and it is calculated according to body weight (Taylor and Bartram, 2012). Although all current available drugs can treat *T. gondii* infection, they may have some side effects and in some patients are poorly tolerated (Blader and Saeij, 2009). In addition, resistance to some of these drugs was noted (Aspinall et al., 2002). As a supplement during treatment, there is possibilty to use herbal drug called Ropadiar (based on *Oreganum* extracts) which reduces parasite multiplication and enhances the host immunity development (CABI, 2010; Ropapharm).

Women infected during pregnancy should be treated no for themselves but to prevent the spreading of the disease to their children (Stanford et al., 2003; Meerburg and Borgsteede, 2011). Accurate treatment can decrease the risk of fetus infection up to 50% (Arabpour et al., 2011). To prevent the transplacental transmission of the parasite, spiramycin found to be effective but it has no effect on foetal lesions. When infection *in uteri* is documented, the mother should be treated by pyrimethamine with combination of sulphadiazine and supplemented with folinic acid (Lüder and Gross, 1998). Infected children should be treated after birth up to one year of age with the same drugs. Follow up with treatment is important up to adolescence (Wellington et al., 2009). Because of possible side effects of pyrimethamine and sulfonamides during early pregnancy, the development of new drugs for pregnant women with primary infection is needed (Lüder and Gross, 1998).

If the infection occurs in immunocompromised patients, they should be treated immediately (Stanford et al., 2003; Meerburg and Borgsteede, 2011). Even in these patients, the standard combination of pyrimethamine and sulfadiazine is preferred. Because of frequent serious side effects, treatment by alternative drugs is possible (Prášil, 2009). An alternative drug to sulfonamides is clindamycin supplemented with pyrimethamine, or atovaquone which can kill tachyzoites and can reduce the number of cysts. Atovaquone is therefore suitable also as prevention against toxoplasmic encephalitis (Lüder and Gross, 1998; Hill and Dubey, 2002). As a prophylaxis, the highly effective antibiotics are trimethoprim or sulfamethoxazole (Montoya and Liesenfeld, 2004). In patients undergoing immunosuppression due to stem cell transplantation, co-trimoxazole is used as a prophylaxis of cerebral toxoplasmosis. Nevertheless, another therapeutic measure should be used due to an increased risk of myeloxicity (Prášil, 2009). In patients after allogeneic haematopoietic stem cell transplantation (HSCT), the most promising results were reported after use of clindamycin, folic acid and high-dose pyrimethamine (Wellington et al., 2009).

Especially in ocular toxoplasmosis, the treatment is sometimes very difficult (Stanford et al., 2003; Meerburg and Borgsteede, 2011). Treatment with pyrimethamine and sulfadiazine began in the early 1950´s. It was proven that this treatment resulted in resolution of chorioretinitis in adults. Since there is no effect on latent form of this parasite, drug therapy is usually administrated only if there is reactivation of the infection (Weiss and Dubey, 2009). Sulfadiazine may be substituted by clindamycin (Prášil, 2009), which can be injected directly in the eye. For reduction of inflammation, combination of drugs supplemented with corticosteroides may be used (Stanford et al., 2003; Meerburg and Borgsteede, 2011).

Nowadays, no human vaccine against chronic infection is available (Dubey, 2008; Gangneux and Dardé, 2012), but in the last few years, the vaccine which is based on the S48 strain was developed for veterinary use (Dubey, 2009a; Kur et al., 2009). Nevertheless, this vaccine is expensive, has short shelf life and has several side effects and therefore it is not suitable for human nor animal use (Kur et al., 2009). On the other hand, in the last few years there was considerable progress towards the development of new vaccines for humans and animals (Wellington et al., 2009).

2.17 Resistance and Killing of *T. gondii*

Appropriate conditions may contribute to the survival of the parasite (Meerburg and Kijlstra, 2009). Generally, all forms of *T. gondii* parasite are sensitive to heat and freeze and they can be killed by proper cooking, pasteurization, or chilling (FAO, 1998; Hill et al., 2006b).

2.17.1 Tachyzoites

This form of the parasite is generally not considered as important source of oral transmission (Powell et al., 2001) but plays the major role in vertical transmission (Tenter, 2009). Tachyzoites are not very resistant and they are able to survive in only limited conditions (Dubey and Frenkel, 1974). Outside the host, they are usually killed rapidly (Tenter, 2009). Due to their sensitivity to gastric juice and proteolytic enzymes, they can be destroyed immediately after ingestion (Powell et al., 2001; Tavassoli et al., 2013). However, children have a lower concentration of proteolytic enzymes in their gastrointestinal tract and therefore, they are more susceptible to infection than aduls do. In addition, tachyzoites may be present also in adult humans because some meals may rise up the stomach pH up to five for several hours so tachyzoites can penetrate into the small intestine. Occasionally, they can survive in acid pepsin solution up to 2 hours (Tenter, 2009). In contaminated milk, tachyzoites can be killed by proper pasteurization (FAO, 1998; Dubey, 2010a; Hill and Dubey, 2013).

2.17.2 Bradyzoites and Tissue Cysts

In contrast to tachyzoites, bradyzoites are more resistant to digestive enzymes (pepsin, trypsin) (Tenter, 2009) and they can survive up to 3 hours in acid pepsin solution (Dubey, 1998b). But at room temperature, they do not survive more than a few hours. Fortunately, tissue cysts are not environmentally resistant (Dubey, 1996). Humans usually become infected by improperly cooked meat (Meerburg and Borgsteede, 2011), therefore cooking of meat to temperature 67 °C or higher is safe way to kill tissue cysts (Frenkel and Dubey, 1972; Silva et al., 2001b). Under household conditions, it is necessary to cook meat for a prolonged period of time to reach the temperature that kills all tissue cysts in all parts of the meat (Lundén and Uggla, 1992). In laboratory trials, tissue cysts remained viable at 60°C for 4 minutes and for 10 minutes at 50°C. However survival of tissue cysts at lower temperatures depends on the duration of cooking (Tenter, 2009). During microwave cooking the meat is heated unevenly, therefore not all tissue cysts are killed and some of them still remain infectious (Lundén and Uggla, 1992).

Also other postharvest processing, such as freezing, kills tissue cysts in meat (Choi et al., 1997; Dubey et al., 2005). Freezing of meat at -12°C for two or three days render tissue cysts nonviable (Dubey, 1996; Silva et al., 2001b**;** Opsteegh et al., 2012) but although most of tissue cysts present in meat are killed at this temperature, occasionally some of them may survive and infect the intermediate host. In meat refrigerated at 1-4°C, tissue cysts remain still infectious for up to three weeks (Tenter, 2009).

Some studies have suggested that tissue cysts can be killed by gamma irradiation at a dose of 0.4 kGy and higher (Dubey and Thayer, 1994; Tenter, 2009**).** Recently was shown that also high pressure processing at 300 MPa or higher can inactivate tissue cysts under laboratory conditions (Lindsay et al. 2005). Another method for killing *T. gondii* is usage of commercial procedurs, such as curing with salt, sucrose or low temperature smoking (Lundén and Uggla, 1992; Dubey et al., 2005). Nevertheless, the concentration of the salt solution and the temperature of storage affect the survival time of tissue cysts and according to this, survival time varies greatly. Under laboratory conditions, tissue cysts were killed in 6% NaCl solution at temperatures from 4 to 20°C but in aqueous solutions with lower concentration of salt, they survived for several weeks (Tenter, 2009).

2.17.3 Oocysts

Unsporulated oocysts lose their ability to sporulate after freezing at -21°C for one day or at -6°C for 7 days and after heating at 50°C for 10 minutes (Dubey et al., 1970) but after sporulation, they can survive even in unfavorable environmental condition for several months or years (see Appendix 7) (Lucas et al., 1999**)**. Fluctuating temperatures do not affect their survival time neither in soil nor in water and they can remain infective even severe winters (Dumètre and Dardé, 2003).

Oocysts may survive short periods of cold and dehydration and in shaded and moist soil or sand, they can remain infective for up to 334 days (Marchiondo et al., 1976) and even up to 18 months under various temperatures in experimentally infected soil **(**Dumètre and Dardé, 2003; Opsteegh et al., 2012). In water and seawater, infectivity of sporulated oocysts lasts for at least 54 months at 4°C (Dubey, 1998c; Lindsay and Dubey, 2009), but there is possibility to remove them from water by oysters (Lindsay et al., 2001).

T. gondii oocysts may be also present on food (Dubey et al., 1998b). Especially cool and moist fruits or vegetables may provide optimal survival conditions for oocysts. It was suggested that oocysts can survive up to 8 weeks on raspberries stored at 4°C (Kniel et al., 2002) because refrigerator conditions are not sufficient to prevent development of oocyst infectivity (Lindsay et al., 2002).

Although increasing temperatures decrease survival time of oocysts, freezing is not always reliable method and oocysts can survive up to 28 days at -21°C (Dubey et al., 1970). Direct exposition to sunlight or drying of uncovered suspension may be moderately deleterious to sporulated oocysts (Dumètre and Dardé, 2003).

Due to their high impermeability, oocysts are very resistant to various types of disinfectants (Dubey et al., 1970; Lucas et al., 1999; Tenter, 2009). For example in an aqueous 2% sulfuric acid or 2.5% potassium dichromate, oocysts may remain viable for several years (Dumètre and Dardé, 2003). Nevertheless, there is a possibility to kill them by sulfuric acid, ethanol, ammonium, and iodine (Dubey, 2004; Jones and Dubey, 2010).

Within physical methods, HPP technology seems to be effective in removal or killing of *T. gondii* oocysts. Study of Lindsay et al. (2005) showed that oocysts which were exposed to pressure of 340 to 550 MPa for 1 minute were rendered noninfectious. Same as in tissue cysts, also in oocysts may be used gamma irradiation for their inactivation at minimum dose of 0.5 kGy (Dubey et al., 1998b). Oocysts may be also rendered nonviable by UV radiation at a minimum dose of 1,000 mJ/cm^2 (Wainwright et al., 2007). Compared to UV radiation, *T. gondii* are highly resistant to an ozone exposure (Dumètre et al., 2008). According to high resistance of oocysts, it is still necessary to develop new procedures for their inactivation or elimination in contaminated surfaces (Dubey et al., 1998b).

3. Aim and Hypothesis

The main aim of the thesis is verification of presence of *Toxoplasma gondii* in small felidae at the zoos in the Czech Republic.

H1: Presence of *Toxoplasma gondii* oocysts in faeces of small felidae bred in zoos in the Czech Republic will be lower than 1%.

H2: There will be no significant difference in the prevalence of *Toxoplasma gondii* between individual zoos.

H3: There will be no significant difference in the prevalence of *Toxoplasma gondii* between males and females.

4. Materials and Methods

Finding of scientific publications in databases WoS, Scopus, CAB Abstracts, PubMed Central, SpringerLink, etc. for literature review. Key words for searching were toxoplasmosis, feline, *Toxoplasma gondii*, risk factors, zoological garden.

In total, 700 samples were collected. In Olomouc, Ostrava and Jihlava zoos (see Appendix 8) were faeces samples collected fresh from the floor (de Camps et al., 2008) by staff of individual zoos (see Appendix 9) during routine cleaning of cages. Samples were collected from July 2013, three times a week till the end of October 2013 and from November 2013 were collected weekly. Interval for collecting was 25 weeks (July 2013 to January 2014). The samples of faeces were collected from five small Felidae species in each zoological garden. In Olomouc Zoo from Leopard cat (*Prionailurus bengalensis*) (see Appendix 10), Fishing cat (*Prionailurus viverrinus*), Amur Leopard cat (*Prionailurus bengalensis euptilura*), Arabian wildcat (*Felis silvestris gordoni*), European wildcat (*Felis silvestris silvestris*). In Ostrava Zoo European wildcat (*Felis silvestris silvestris*) (see Appendix 11), Geoffroy´s cat (*Leopardus geoffroyi*) (see Appendix 12), Sri Lankan Rusty-spotted cat (*Prionailurus rubiginosus phillipsi*) (see Appendix 13), Fishing cat (*Prionailurus viverrinus*) (see Appendix 14) and Serval (*Leptailurus serval*) (see Appendix 15). In Jihlava Zoo Arabian wildcat (*Felis silvestris gordoni*) (see Appendix 16), Jungle cat (*Felis chaus*) (see Appendix 17), European wildcat (*Felis silvestris silvestris*), Amur Leopard cat (*Prionailurus bengalensis euptilura*) (see Appendix 18) and Geoffroy´s cat (*Oncifelis geoffroyi*).

In each zoological garden have been Felidae kept separately even in pairs or groups of mother and offsprings. Every place for breeding of cats consists of cage with open yard covered by sand; roofed section and resting or sleeping box (see Appendix 19). Cats are able to climb up a trees and therefore exhibits are closed on the top. All cages have been cleaned every day by the staff of zoological garden. Placing of examined animals showed in Table (see Appendix 20).

Feed rations for cats in Olomouc zoo consists of chicks – 0.3 kg for Arabian wildcat and Leopard cat; 0.4 kg for European wildcat and Amur Leopard cat and 0.5 kg for Fishing cat (1st day), hamsters or mice (2^{nd} day), chicken (4^{th} day), beef meat (5^{th} day) and chicks (6^{th} day). In the 3^{rd} and 7^{th} day, cats are on hunger strike. In Ostrava zoo feed rations consist of 70 dag of meat for Serval and Fishing cat, 30 dag of meat for European wildcat, 8 mice, 4 chicks and 2 rats for Sri Lankan Rusty-spotted cat and 20 to 30 dag of meat (or similar feed ration as in Sri Lankan Rusty-spotted cat) for Geoffroy´s cat. Also rabbits and guinea pigs are used.

Feed rations for all cats are modified according to body condition, season and need (e.g. fattening before winter). Cats in Jihlava zoo are fed by freshwater fish (see Appendix 21) (e.g. Crucian carp, Perch) on Monday, chickens on Tuesday, rabbits on Wednesday, rats and mice on Thursday, guinea-pigs on Friday and by rabbits on Saturday. On Sunday are cats on hunger strike. For feeding are also used hamsters, quails and beef meat. Rats, mice and guinea-pigs came from own breeding of zoological garden, the beef meat is bought in abattoirs or from private persons and rabbits, bunnies (2 to 3 days old) and fish came from factory farming. Chickens are bought live and then freshly killed in zoological garden.

Generally, beef and pork meat, rabbits, fish and chickens came from large-scale breeding centres or abattoirs, bunnies and sometimes meat were bought from small-scale breeder and guinea pigs, rats and mice were from own breeding, or from specialized breeding centres for laboratory animals. In Jihlava zoo, 5 to 6 day-old live chicks were bought and feshly killed directly in the zoo. All cats in each zoological garden were given fresh water that had been from freely accessible stores in the zoos.

As a preventive measure against parasites worming is used. In Olomouc zoo were all cats wormed by Cestal Cat Flavour® (Ceva Sante Animale) on the 25th of November. In Ostrava zoo are all cats regularly wormed in January/February before vaccination and otherwise on the basis of laboratory analysis of samples which are done every two months. During collecting interval was warming done only once in European wildcat in October by preparation Advocate Small Cats® (Bayer Animal Health). Worming in Jihlava zoo is done regularly each three months. Cats were wormed on the 14th of August and on the 27th of November by preparation Vitaminthe® (Virbac Animal Health).

The faeces samples were collected into reclosable plastic sample containers (see Figure 12) and placed into refrigerator at 4-8 °C (Dubey et al., 2005). Date of collection and species of cat were noted on plastic sample containers. After transportation of samples into laboratory were faeces mixed with water (see Appendix 22), crushed (see Appendix 23), filtered through gauze and put into test-glasses (all test-glasses were marked by number) (see Appendix 24). Then, all samples were centrifugated again at 2,500 rpm for 3 minutes. The supernatant was removed and the sediment was mixed with sucrose solution (800 ml of water + 1 kg of sugar) of specific density 1.15 (Dubey et al., 1970; Dubey et al., 2006; Pena et al., 2006; de Camps et al., 2008; Raeghi et al., 2011) and centrifugated again for 3 minutes at 2,500 rpm. With usage of microbiological wire loop, the surface membrane was collected and put on a glass slide under the microscope. The oocysts were identified under the microscope at 500 x magnification.

Collected samples were evaluated according following scale:

- negative

± dubious

+ a few oocysts in the sample

+ + light infection; oocysts found regularly

+ + + strong infection; oocysts in almost every microscoped field

The data were analyzed by STATISTICA 12 CZ programme. All data were qualitative and for analysis were used Frequency and Contingency tables. All tests were performed at a significance level of 0.05 and results were rounded off to two decimal numbers. To test the effect of sex on oocyst excretion, animals were divided into groups of males, females and males + females.

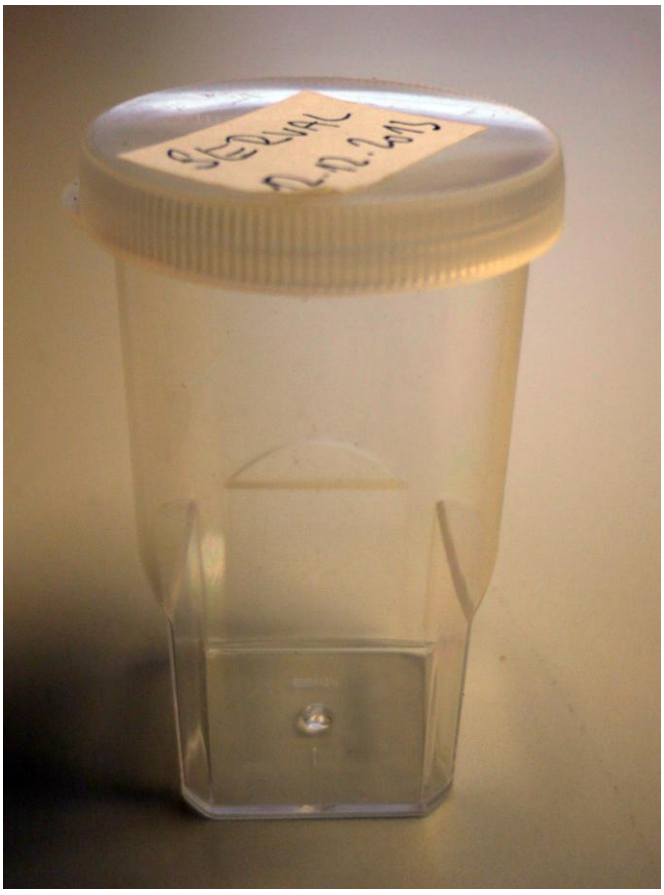
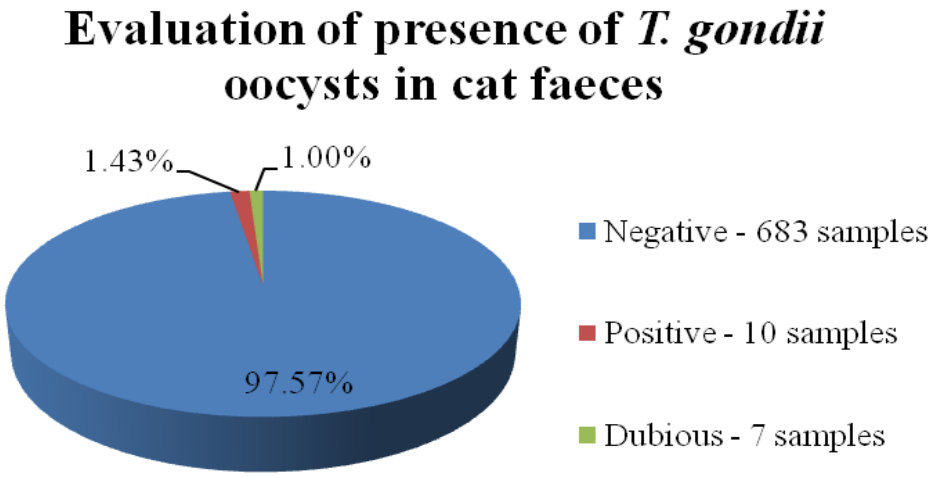


Figure 12: Reclosable plastic sample container (Kudrnáčová, 2014)

5. Results

H1: Presence of *Toxoplasma gondii* oocysts in faeces of small felidae bred in zoos in the Czech Republic will be lower than 1%.



Graph 1: Presence of *T. gondii* oocysts in cat faeces

Table 1: Evaluation of samples in Jihlava zoo

Species	*T. gondii* N	*T. gondii* P	Total
Arabian wildcat	30	0	30
Jungle cat	72	0	72
European wildcat	58	0	58
Amur leopard cat	56	1	57
Geoffroy´s cat	31	0	31
Total	247	1	248

Table 2: Evaluation of samples in Olomouc zoo

Species	*T. gondii* N	*T. gondii* P	*T. gondii* D	Total
Arabian wildcat	36	0	2	38
Leopard cat	38	0	0	38
European wildcat	37	1	0	38
Amur leopard cat	33	4	1	38
Fishing cat	37	0	1	38
Total	181	5	4	190

Table 3: Evaluation of samples in Ostrava zoo

Species	*T. gondii* N	*T. gondii* P	*T. gondii* D	Total
Sri Lankan rusty-spotted cat	52	0	0	52
European wildcat	50	0	1	51
Fishing cat	49	2	0	53
Geoffroy´s cat	53	0	0	53
Serval	51	2	0	53
Total	255	4	3	262

N – negative P – positive D – dubious

Number of *T. gondii* positive samples was 1.43% and this result rejects H1 that the incidence of *T. gondii* oocysts in cat faeces is lower than 1%.

Samples were examined by direct microscopy and data were evaluated by statistical programme using Frequency table. Results were put into the pie graph (see Graph 1).

Jihlava 248 samples/1 positive = 0.4%
Olomouc 190 samples/5 positive = 2.63%
Ostrava 262 samples/4 positive = 1.53%

In detail, tables with results of *T. gondii* oocysts occurence in collected samples are above. Table with all results placed in Appendix 25.

H2: There will be no significant difference in the prevalence of *Toxoplasma gondii* between individual zoos.

H2 was confirmed because there is no statistically significant difference between individual zoos in number of samples positive to *T. gondii* oocysts (Pearson´s chi-squared test: $\chi^2 = 8.83$; df = 4; p = 0.07).

Table 4: Evaluation of samples in all zoos

ZOO	*T. gondii* N	*T. gondii* P	*T. gondii* D	Total
Jihlava	247	1	0	248
Olomouc	181	5	4	190
Ostrava	255	4	3	262
Total	683	10	7	700

N – negative P – positive D - dubious

H3: There will be no significant difference in the prevalence of *Toxoplasma gondii* between males and females.

H3 was confirmed because there is no statistically significant difference in the oocyst excretion between individual sexes (Pearson´s chi-square test: $\chi^2 = 4.69$; df = 4; p = 0.32).

Table 5: Evaluation of samples between individual sexes of cats

T. gondii	Sex F	Sex M	Sex B	Total
N	191	243	249	683
P	1	6	3	10
D	2	4	1	7
Total	194	253	253	700

N – negative P – positive D – dubious

F – female M – male B – both

There was found statistically significant difference in oocyst excretion between individual animal species (Pearson´s chi-square test: $\chi^2 = 28.17$; df = 16; p = 0.03).

From all 9 animal species tested, 4 of them were positive to *T. gondii*; mostly Amur leopard cat which was kept in Jihlava and Olomouc zoos and in both cases was found to be positive. For detail informations see Table 6.

Table 6: Evaluation of samples in all felid species

Species	*T. gondii* N	*T. gondii* P	*T. gondii* D	Total
Arabian wildcat	66	0	2	68
Jungle cat	72	0	0	72
European wildcat	145	1	1	147
Amur leopard cat	89	5	1	95
Geoffroy´s cat	84	0	0	84
Leopard cat	38	0	0	38
Fishing cat	86	2	3	91
Sri Lankan rusty-spotted cat	52	0	0	52
Serval	51	2	0	53
Total	683	10	7	700

European wildcat was only species kept in all three zoos. There was found no statistically significant difference in positive samples of European wildcat between individual zoos (Pearson´s chi-square test: $\chi^2 = 4.77$; df = 4; p = 0.31).

Table 7: Evaluation of samples in European wildcat

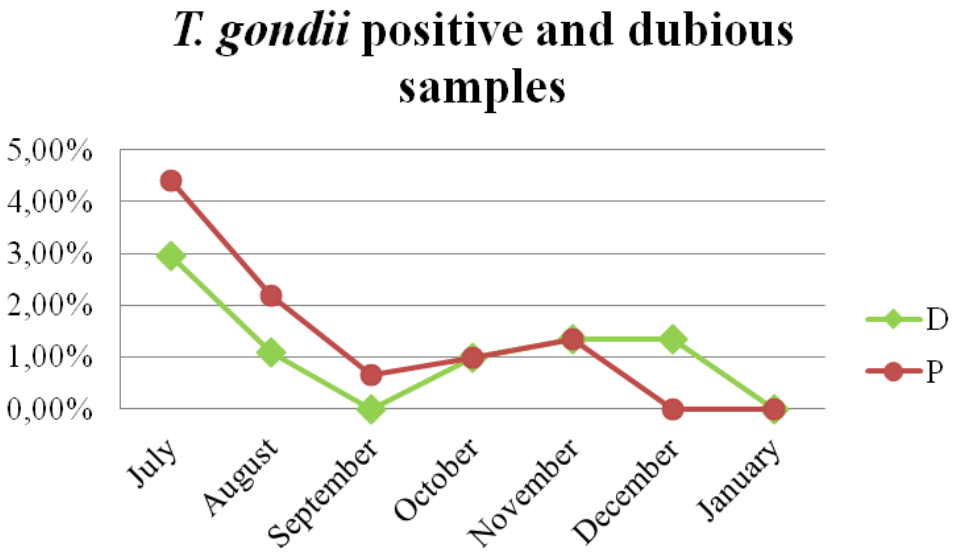
ZOO	*T. gondii* N	*T. gondii* P	*T. gondii* D	Total
Jihlava	58	0	0	58
Olomouc	37	1	0	38
Ostrava	50	0	1	51
Total	145	1	1	147

During months testing, there was found no statistically significant difference in oocyst shedding ($\chi^2 = 12.42$; df = 12; p = 0.41).

Only 697 samples were tested because in 3 samples the date of faeces collection was not clearly marked on the plastic sample containers.

Table 8: Evaluation of samples in individual months

Month	*T. gondii* N	*T. gondii* P	*T. gondii* D	Total
July	63	3	2	68
August	176	4	2	182
September	152	1	0	153
October	100	1	1	102
November	72	1	1	74
December	73	0	1	74
January	44	0	0	44
Total	680	10	7	697



Graph 2: *T. gondii* positive and dubious samples within the months

Occurence of positive and dubious samples among individual months

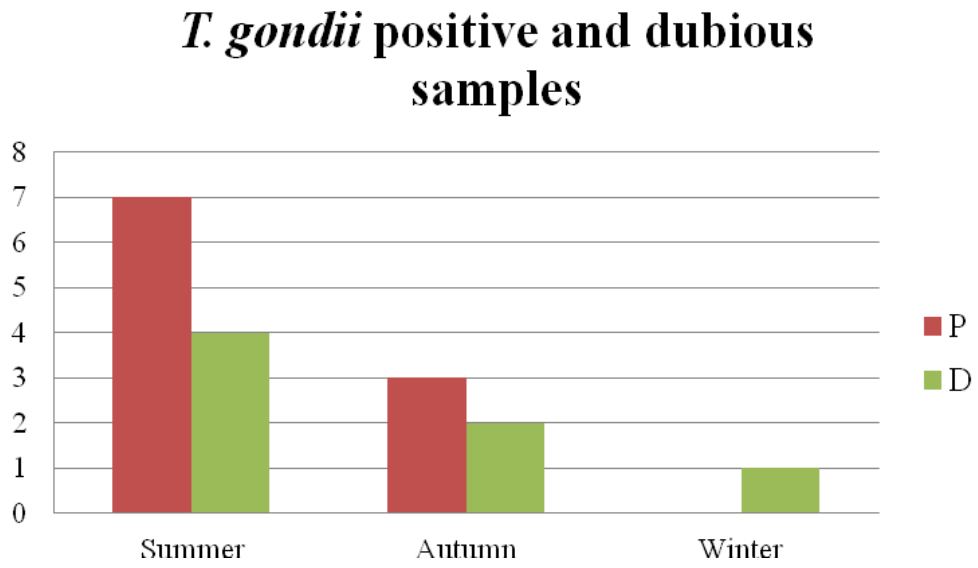
Also between tested seasons, there is no statistically significant difference in oocyst shedding (Pearson´s chi-square test: $\chi^2 = 7.15$; df = 4; p = 0.13).

Again only 697 samples were tested because of unclear collection date marked.

Table 9: Evaluation of samples in different seasons

Season	*T. gondii* N	*T. gondii* P	*T. gondii* D	Total
Summer	239	7	4	250
Autumn	324	3	2	329
Winter	117	0	1	118
Total	680	10	7	697

The highest prevalence of *T. gondii* oocysts was in summer. Then the tendency is decreasing.



Graph 3: *T. gondii* positive and dubious samples within the seasons

The highest prevalence of *T. gondii* oocysts in faecal samples was in summer. In winter, no positive sample was found and only 1 dubious was detected.

In 139 out of 700 samples were identfied 150 positive findings (19.86%) of other intestinal parasites. Concrete species, number of positive findings and occurence of parasites in % are mentioned below in Table 10.

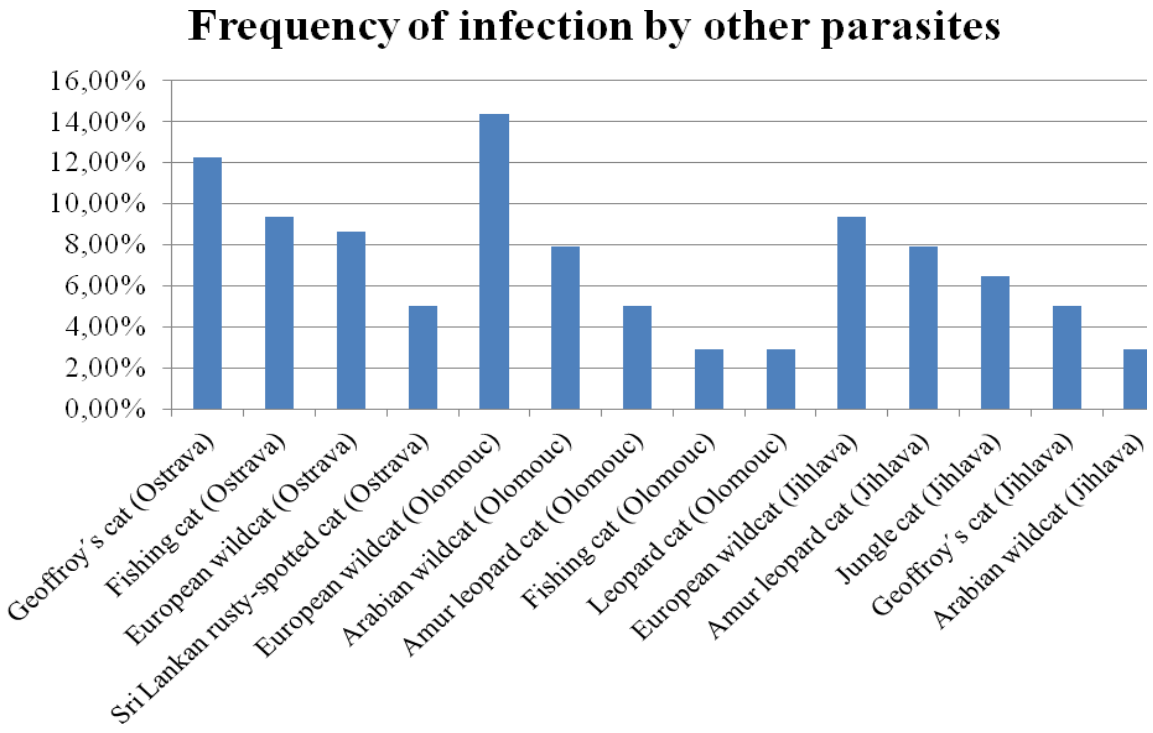
Table 10: Occurence of other intestinal parasites

Species	Positive samples	Occurence [%]
Cryptosporidium	1	0.14%
Giardia	60	8.57%
Isospora	8	1.14%
Toxascaris	29	4.14%
Toxocara	52	7.43%

Infestation of individual cat species by other intestinal parasites shown in Table 11 and in Graph 4.

Table 11: Frequency of infection in cats by other parasites

ZOO	Species	Positive samples/species (from 139 total positive)	%
Ostrava	Geoffroy´s cat	17	12.23%
Ostrava	Fishing cat	13	9.35%
Ostrava	European wildcat	12	8.63%
Ostrava	Sri Lankan rusty-spotted cat	7	5.04%
Olomouc	European wildcat	20	14.39%
Olomouc	Arabian wildcat	11	7.91%
Olomouc	Amur leopard cat	7	5.04%
Olomouc	Fishing cat	4	2.88%
Olomouc	Leopard cat	4	2.88%
Jihlava	European wildcat	13	9.35%
Jihlava	Amur leopard cat	11	7.91%
Jihlava	Jungle cat	9	6.47%
Jihlava	Geoffroy´s cat	7	5.04%
Jihlava	Arabian wildcat	4	2.88%



Graph 4: Frequency of infection in cats by other parasites

Influence of *T. gondii* to occurence of other parasites found in collected samples was also evaluated, using Contingency table.

Table 12: *T. gondii* influence on the presence of other parasites

	T. gondii	Other parasites N	Other parasites P	Other parasites D	Line sums
Frequency	N	549	109	25	683
Columnar freq.		98.04%	96.46%	92.59%	
Line freq.		80.38%	15.96%	3.66%	
Total freq.		78.43%	15.57%	3.57%	97.57%
Frequency	P	8	2	0	10
Columnar freq.		1.43%	1.77%	0.00%	
Line freq.		80.00%	20.00%	0.00%	
Total freq.		1.14%	0.29%	0.00%	1.43%
Frequency	D	3	2	2	7
Columnar freq.		0.54%	1.77%	7.41%	
Line freq.		42.86%	28.57%	28.57%	
Total freq.		0.43%	0.29%	0.29%	1.00%
Frequency	All groups	560	113	27	700
Total freq.		80.00%	16.14%	3.86%	

6. Discussion

Only species of Felidae family can produce environmentally resistant oocysts of *T. gondii* in their faeces. When sporulated oocysts are ingested by humans or animals, they may develop a *T. gondii* infection called toxoplasmosis. The majority of these infections are asymptomatic. Tissue cysts of *T. gondii* are formed in different body tissues of infected intermediate hosts and when these infected tissues are ingested by felids, toxoplasmosis may also develop in them. Transmission to non-carnivorous animals, such as rabbits and rodents, requires ingestion of sporulated oocysts. *T. gondii* is involved in the natural food chain and small animals become vectors of infection for predators, including domestic and feral cats, felids kept in zoos and for other carnivorous species as well (Marchiondo et al., 1976).

Due to the mild climate, Czech Republic may be relatively hostile and unfavorable for *T. gondii* and this can lead to a lower prevalence of oocysts in felines living in zoos in the Czech Republic than those living in the more humid and warmer areas.

Even though there are many studies on the occurence of *T. gondii* oocysts in the faeces of felines in other countries, within the Czech Republic we found only results of study Lukešová and Literák (1998) from zoos in the CR. This study shows 6.63% prevalence of oocysts in faeces of 6 Felidae species.

Although oocysts are excreted by cats only for a short period (Raeghi et al., 2011) after primary infection, as well as in studies conducted throughout the world (Dubey et al., 1970; Dubey and Frenkel, 1974; Dubey et al., 2004; Dubey, 2005), *T. gondii* oocysts were found in the faeces of cats also in this case. The total prevalence in this study was 1.43%, representing 10 positive samples out of 700. This result is moreless comparable with results of studies Dubey et al. (1995) showing that out of 274 samples were 5 (1.82%) determined as positive; Dabritz et al. (2007a) where was 0.92% (3) positive from 326 samples; Pena et al. (2006), who found oocysts in 1.27% (3) from total amount of 237 samples; Herrmann et al. (2010), who reported 0.25% (46) positive samples out of 18,259, and Berger-Schoch et al. (2011), who detected 1 positive sample (0.40%) from 252. Also Schares et al. (2008) give in their´s article a similar prevalence of *T. gondii* oocysts in faecal samples of 24,106 cats from various European countries, which was 0.31%.

In the study of Raeghi et al. (2011), 130 faecal samples of felines were investigated. From those, 3 (2.31%) were positive for the presence of oocysts.

The possible presence of *T. gondii* oocysts in faecal samples was investigated in eight feral cats and two cats kept in zoological gardens in research of Marchiondo et al. (1976). The results showed that of 10 samples examined, there were 9 positive.

Omata et al. (1990) indicate in their article that all cats which were inoculated (=fed) artificially with *T. gondii* cysts, start with oocyst shedding in the faeces between the 4^{th} and 11^{th} day. After re-inoculation with cysts, other excretion of oocysts was not found. After ingestion of oocysts, cats started with oocyst shedding between the 19^{th} and the 27^{th} day and the total number of oocysts was more than 10^8, in contrast to present study, where the presence of oocysts in samples was rather smaller. Also results of study Dubey et al. (2005) indicate that in faecal samples of cats fed by pork meat, the number of subsequently excreted oocysts was low.

Surprising result was found by Meloni et al. (1993), who reported that in their study was 18.2% of samples positive for the presence of *T. gondii* oocysts.

Lukešová and Literák (1998) present that in their research were 4 (out of 39 samples) identified as positive for *T. gondii* in Geoffroy´s cat in Jihlava zoo, in contrast to present work, where no positive or dubious sample was found in Geoffroy´s cat. Only one sample out of 248 collected in Jihlava was positive, namely in Amur leopard cat.

In Ostrava zoo was excretion of *T. gondii* oocysts proven in Fishing cat and Serval (both have 2 positive samples). In Olomouc zoo were found 4 positive and 1 dubious sample in Amur leopard cat and 1 in European wildcat. Of all faecal samples collected from Arabian wildcat and Fishing cat were 3 identified as dubious.

In our study, we decided to compare the incidence of *T. gondii* in various zoos and between various sexes of the animals. The results did not show any statistically significant difference between zoos nor animal genders. The results correspond with tests on *T. gondii* seropositivity in felids, conducted by Kim et al. (2008) in different regions of Korea. In their´s article, they state that there is no statistically significant difference between the sexes of the animals, nor among the surveyed regions.

During testing of different felines, there was found no statistically significant difference between species. Of all 9 species tested were 4 of them positive for the presence of oocysts; mostly then Amur leopard cat, which is kept in Jihlava and Olomouc zoos and in both cases was found to be positive. Also in the study of Lukešová and Literák (1998) was the excretion of *T. gondii* oocysts demonstrated in Amur leopard cat.

The only species bred in all three zoos was European wildcat. There was no statistically significant difference between various positive samples of these cats in individual zoos.

Contrary to study of Lukešová and Literák (1998), where were found at least 4 positive samples in breeding pair and other 4 in female of European wildcat, in present study was found 1 dubious sample in male of European wildcat kept in Ostrava zoo and in pair of European wildcats in Olomouc zoo was proven 1 positive sample.

The results showed that the highest incidence of positive samples was found in the first month tested (July). During the following months, the incidence of positive samples continuously decreased. Also from the point of view of seasons, the highest incidence was recorded in the summer, which supports the claim of Marchiondo et al. (1976) and Meerburg and Kijlstra (2009) that the prevalence of *T. gondii* is greater at higher ambient temperatures.

This result also confirm the study of Herrmann et al. (2010), in which was found the highest prevalence of *T. gondii* oocysts in the period from July to September 0.37% (21/3,734), which correspond with the results of our study. The second highest incidence was found in the period October – December 0.27% (19/7,023). This result is in contradiction with the statement of Marchiondo et al. (1976) and Meerburg and Kijlstra (2009). The incidence in other periods was January – March 0.16% (4/2,549) and April – June 0.03% (1/2,953).

Other intestinal parasites (a total of 150 positive findings) were detected in 139 (19.86%) samples out of 700. At 7.91% positive samples (11/139 positive) occured always two different kinds of other intestinal parasites at the same time. The overall prevalence of other intestinal parasites was *Cryptosporidium* 0.14% (1/700), *Giardia* 8.57% (60/700), *Isospora* 1.14% (8/700), *Toxascaris* spp. 4.14% (29/700) and *Toxocara* spp. 7.43% (52/700). The most affected species by other intestinal parasites is European wildcat kept in Olomouc zoo 14.39% (20 positive samples from total amount of 139 positive), following by Geoffroy´s cat 12.23% (17/139) in Ostrava zoo. Other occurences in infected cats are below 10%.

In the study carried out by Miró et al. (2004) in Spain, intestinal parasites were identified in 28.76% (107/382) of faecal samples from feral and domestic cats and cats living on farms. Among parasites which were detected belonged *Toxocara* 18.32% (70/382), *Toxascaris* 1.31% (5/382), further *Ancylostoma*, *Capillaria*, *Aelurostrongylus*, *Taenia*, *Dipylidium* a *Cystoisospora*. The results indicate that the occurence of *Toxascaris* in the research of Miró et al. (2004) was comparable with our results but the incidence of *Toxocara* was much higher. Other intestinal parasites mentioned above were not detected in present study in investigated samples. In contrast to the presence of mixed samples in present study wich was 7.91%, Miró et al. (2004) further mention in their´s article that during their research was found 30.8% (33/107) of mixed samples.

The same types of intestinal parsites that are presented in our study were found by Al-khushali (2007) during research in Iraq. The occurence of parasites was *Isospora* 23.81% (30/126), *Cryptosporidium* 7.94% (10/126), *Giardia* 3.97% (5/126), *Toxocara* 46.03% (58/126) and *Toxascaris* 32.54% (41/126). Except *Giardia* where the incidence was lower, prevalence of other parasites was many times higher than in our case.

Report of Ferreira et al. (2011) showed that in Portugal was found 5% (1/20) positive samples to *Giardia*, 5% (1/20) to *Isospora* and 10% (2/20) to *Toxocara* in domestic cats and 50% (1/2) in shelter cats. One of the two samples was identified as mixed. However, only a small number of samples were investigated.

In carrying out the study in feral cats in Turkey by Karatepe et al. (2008) was found an occurence of *Isospora* oocysts in 12.50% (9/72) samples – 8.11% ♀ (3 positive out of 37 tested) and 17.14% ♂ (6 positive out of 35 tested). *Toxocara* oocysts were present in 15.28% (11/72) samples – 8.11% ♀ (3/37) and 22.86% ♂ (8/35). Other parasite detected was *Toxascaris*, present in 20.83% (15/72) samples – 18.92% ♀ (7/37) and 22.86% ♂ (8/35).

Vanparijs et al. (1991) reported that of 30 samples of faeces from felines collected in Belgium which were investigated by flotation method, 83.33% were positive for parasites. The most frequently occuring species was *Toxocara* (60%). Other parasites found icluded *Ancylostoma*, *Taenia* and *Coccidia*.

In comparison with the results of Meloni et al. (1993) who indicate the prevalence of *Isospora* 15.1%, in study of Collins et al. (1983) of 71 investigated faecal samples of cats, only 4.23% (3/71) was positive to *Isospora*, which is comparable with the results of our research.

Significantly higher prevalence of *Giardia* 37% (37/100) and *Cryptosporidium* 11% (11/100) in observed cats was found by Dabritz et al. (2007b) in study made in California. These values are vastly different from our results where the prevalence of *Giardia* was 8.57% and *Cryptosporidium* 0.14%. Higher incidence of parasites detected by Dabritz et al. (2007b) could be affected by the warmer climate of California.

Although the prevalence of *T. gondii* oocysts in our study is low (1.43% of positive samples), in many studies conducted worldwide (Collins et al., 1983; Vanparijs et al., 1991; Miró et al., 2004; Dubey et al., 2006; Dabritz et al., 2007b; Hooshyar et al., 2007; Karatepe et al., 2008) no oocysts were detected in the faeces of cats. Nevertheless, Dubey et al. (2006) mentioned that this result is surprising and unexpected.

On the contrary, research made by Dubey et al. (2004) showed that cats that were fed by hearts of bears, shed oocysts in their faeces although the hearts were negative for bioassay in mice.

Differences in the prevalence of *T. gondii* in cats observed in this and other studies may be due to different ecological, geographical and living conditions and welfare of cats but also by food habits and preferences. These factors are mentioned also by Wu et al. (2011).

It is difficult to compare different studies with each other due to differences in using of various diagnostic methods, origin and quantity of samples and the overall time of conducted research. In present study was used flotation method using the sucrose solution which is used by many researchers (Dubey et al., 1970; Dubey, 2005; Dubey et al., 2006; Pena et al., 2006; de Camps et al., 2008; Schares et al., 2008; Raeghi et al., 2011). This method is often used because the number of *T. gondii* oocyst in the faeces samples may be too small to be detected by direct smear. However, it is appropriate to supplement the flotation method with serological survey which is good indicator of *T. gondii* infection in cats (Dubey et al., 1995b; Hill and Dubey, 2002; Kim et al., 2008). In many studies (Dorny and Fransen, 1989; Dubey et al., 2006; Hooshyar et al., 2007; de Camps et al., 2008; Raeghi et al., 2011) the samples were collected at once and therefore it is possible that some samples were not positive, although cats could be infected at the time of research.

Probably the most important source of infection for cats observed in this work could be pork and rabbit meat, which cats are fed especially in Ostrava and Jihlava zoos. Beef and chicken meat and fish are not considered as an important source of infection because the presence of cysts in tissues of these animals is very small null. This risk factor is mentioned also in articles of Lukešová and Literák (1998), Dabritz et al. (1997a), or Lopes et al. (2008). Feral cats roaming in zoos may also present a significant source of toxoplasmosis transmission to exotic wild felids kept in zoos. This was also demonstrated by Lukešová and Literák (1998) and Jones and Dubey (2010). However, cats kept in zoos can also play an important role in the spreading of infection, both among themselves and among other animals. This theory supports also statement of Lukešová and Literák (1998) and de Camps et al. (2008). In present study had examined cats free access to outdoor runs and probably had the opportunity to hunt small animals, such as rodents. These preys can be another source of infection for cats. All runs for cats were always cleaned by one zoo worker. It is therefore possible that oocysts excreted by cats in one paddock could be transferred through keeper´s clothes and boots or by cleaning equipment to other paddocks and thus other cats could be infected.

7. Conclusion

This master thesis was focused on the verification of presence of *T. gondii* in felids and determination of prevalence of *T. gondii* oocysts in cat faeces with special attention to small Felidae species. For analysis were used samples collected in Jihlava, Olomouc and Ostrava zoos. The overall prevalence of *T. gondii* oocysts in cat faeces was 1.43%. Occurence of oocysts positively correlates with seasons but probably also with sex of animals. The highest prevalence was detected in summer and it seems that males are more susceptible to infection. However, the prevalence of *T. gondii* infection depends on many factors, including age, type of environment, geographical region, breeding and hygienic conditions, diet, and many others. The low prevalence of *T. gondii* oocysts in cat faeces in the present and previous studies may lead to underestimating of the health risks connected with this parasite but it does not diminish the importance of oocysts in maintaining of *T. gondii* in the environment.

Excreted and sporulated oocysts may be an important source of infection not only to bred felids and other animals in zoos but even for zoo keepers and visitors. In addition, in some animal species kept in zoos it can cause high percentage of mortality or failure in their breeding.

Because of versatility of *T. gondii* it is difficult to determine strategies for control and prevention of the disease which would be effective worldwide but it is recommended to restrict the oocyst transmission and reduce contamination of the surrounding environment because it takes as little as 24 hours for oocysts to sporulate and become infective.

Cages and paddocks should be cleaned daily and equipment used for faeces removing should be cleaned as well. Proper removing of faeces and keeping of hygienic standarts may prevent transmission of the disease within the zoo environment. Also feeding of previously frozen meat is likely to reduce *T. gondii* infection in cats and other animals in the zoos.

For further research, I would recommend also blood samples collection and analyzing these samples by some laboratory test, and also bioassay on mice for obtaining more specific results. Serological survey is very suitable because positive results indicate that the cat has been already infected whereas negative results suggest that the cat has not been yet infected and is still very susceptible to infection.

This study has an important implication because contamination of zoo animals and environment by *T. gondii* should be monitored and prevented. We consider these results to be useful because they can be used as a basis of further research which may monitor epidemiology of *T. gondii* in felines in zoological gardens more detailed.

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Figure Resources

Figure 1: African rodent *Ctenodactylus gundi.* Available at http://www.biolib.cz/en/image/id138629/: Accessed 2014-04-09.

Figure 2: Pappas G, Roussos N, Falagas ME. 2009. Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. International Journal for Parasitology 39 (12): 1385-1394.

Figure 3: Tenter AM, Heckeroth AR, Weiss LM. 2000. *Toxoplasma gondii*: from animals to humans. International Journal for Parasitology 30 (2-3): 1217-1258.

Figure 4: Dubey JP, Lindsay DS, Speer CA. 1998a. Structures of *Toxoplasma gondii* Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. Clinical Microbiology Reviews 11 (2): 267-299.

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Figure 5: Dubey JP, Lindsay DS, Speer CA. 1998a. Structures of *Toxoplasma gondii* Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. Clinical Microbiology Reviews 11 (2): 267-299.

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Figure 6: Life cycle of *T. gondii*. Available at http://www.cdc.gov/parasites/toxoplasmosis/biology.html: Accessed 2014-04-09.

Figure 7: Dubey JP, Lindsay DS, Speer CA. 1998a. Structures of *Toxoplasma gondii* Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. Clinical Microbiology Reviews 11 (2): 267-299.

Figure 8: Dubey JP, Lindsay DS, Speer CA. 1998a. Structures of *Toxoplasma gondii* Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. Clinical Microbiology Reviews 11 (2): 267-299.

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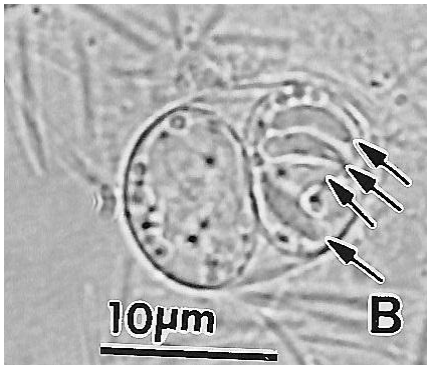
Figure 10: Holland GN. 2003. Ocular toxoplasmosis: a global reassessment: Part I: epidemiology and course of disease. American Journal of Ophthalmology 136 (6): 973-988.

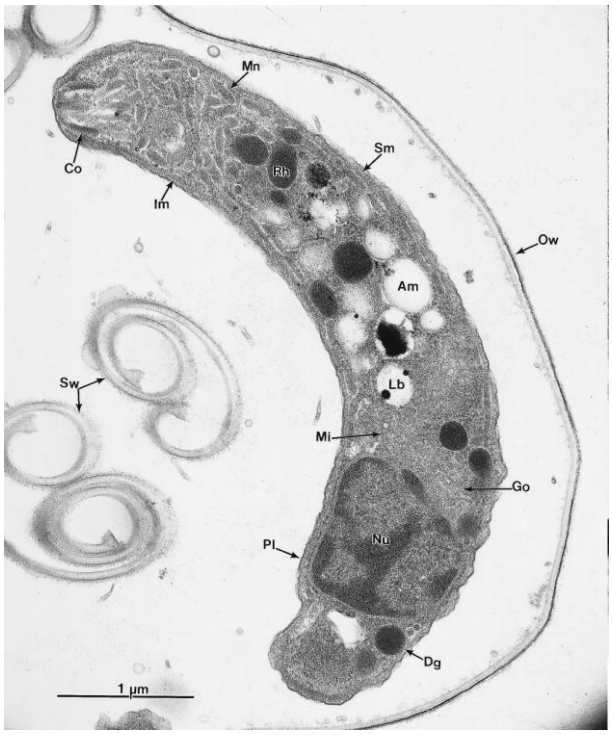
Figure 11: Webster JP. 2001. Rats, cats, people and parasites: the impact of latent toxoplasmosis on behaviour. Microbes and Infection 3 (12): 1037-1045.

9. List of Figures, Graphs and Tables

10. List of Appendices

Appendix 1: Sporocyst and sporozoite





Excysted sporozoite, still within an oocyst (Dubey et al., 1998a)

Appendix 2: History of *Toxoplasma gondii*

Finding	
Etiologic agent	
Parasite found in the rodent *Ctenodactylus gundii*, Tunisia	Nicolle and Manceaux, 1908
Parasite found in a rabbit, Brazil	Splendore, 1908
Parasite named *Toxoplasma gondii*	Nicolle and Manceaux, 1909
First viable *T. gondii* isolated from an animal	Sabin and Olitsky, 1937
First isolation of *T. gondii* from human	Wolf, Cowen and Paige, 1939
Human and animal *T. gondii* proven identical	Sabin, 1941
Pathogenesis of toxoplasmosis	Frenkel and Friedlander, 1951
Morphology and life cycle	
Term tachyzoite proposed	Frenkel, 1973
Endodyogeny of tachyzoite described	Goldman, Carver and Sulzer, 1958
Ultrastructure of tachyzoite described	Gustafson, Agar and Cramer, 1954; Sheffield and Melton, 1968
Tissue cyst recognized	Levaditi, Schoen and Sanchis Bayarri, 1928
Tissue cyst described cytologically	Frenkel and Friedlander, 1951; Frenkel, 1956
Ultrastructure of tissue cyst described	Wanko, Jacobs and Gavin, 1962; Ferguson and

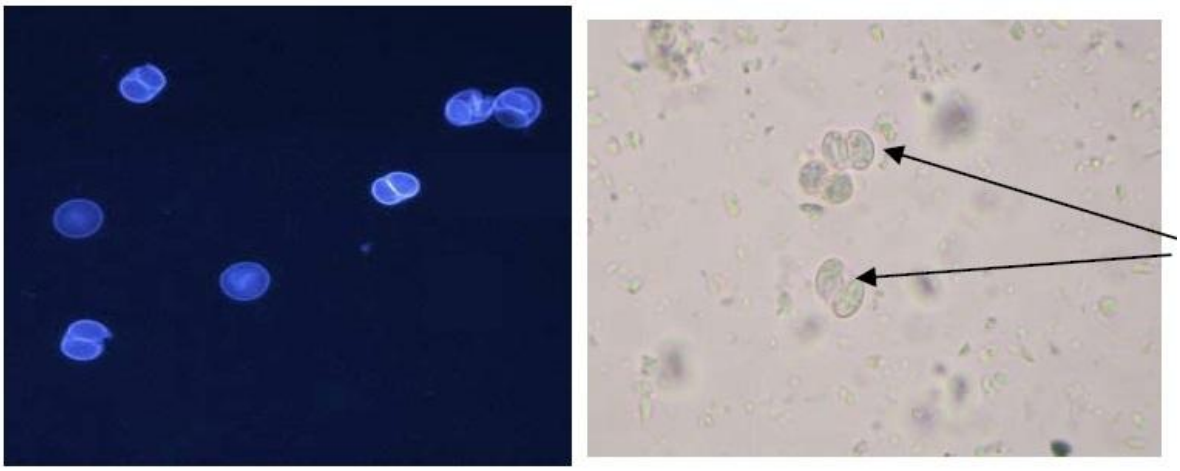
	Hutchison, 1987
Term bradyzoite proposed	Frenkel, 1973
Term tissue cyst proposed	Dubey and Beattie, 1988
Recognition of bradyzoite resistance to digestive enzymes	Jacobs, Remington and Melton, 1960
Development of tissue cysts and bradyzoites described	Dubey and Frenkel, 1976
Complete review of bradyzoite and tissue cysts biology	Dubey, Lindsay and Speer, 1998
Coccidian phases described	Frenkel, Dubey and Miller, 1970; Hutchison et al., 1970; Dubey and Frenkel, 1972; Sheffield and Melton, 1970
Oocyst morphology described	Dubey, Miller and Frenkel, 1970b
Five asexual *T. gondii* types (A - E) described	Dubey and Frenkel, 1972
Ultrastructure of coccidian stages described	Sheffield, 1970; Piekarski, Pelster and Witte, 1971; Ferguson, Hutchison, Dunachie and Siim, 1974
Transmission	
Congenital transmission in humans	Wolf, Cowen and Paige, 1939
Carnivorous transmission	Weinman and Chandler, 1954
Transmission by meat found in humans	Desmonts, Couvreur, Alison, Baudelot and Gerbeaux, 1965
Faecal-oral transmission	Hutchison, 1965
Definition of definitive and intermediate hosts, shedding of oocysts only by felids	Frenkel, Dubey and Miller, 1970; Miller, Frenkel and Dubey, 1972
Description of toxoplasmosis outbreak by oocyst inhalation by human	Teutsch, Juranek, Sulzer, Dubey and Sikes, 1979
Genetics and different *T. gondii* genetic strains	
Recombination and genetic crosses	Pfefferkorn and Pfefferkorn, 1980
RFLP used to group *T. gondii* strains into 3 types (I, II, III)	Sibley, Leblanc, Pfefferkorn and Boothroyd, 1992
Immunity and protection	
T. gondii neutralizing antibody recognized	Sabin and Suchman, 1942
Antibodies found to kill extracellular *T. gondii*	Sabin and Feldman, 1948
Protection transferred by immune lymphoid cells	Frenkel, 1967
Interferon γ found to be main cytokine for protection	Suzuki, Orellana, Schreiber and Remington, 1988
Toxoplasmosis in humans	
First proven case of congenital toxoplasmosis	Wolf, Cowen and Paige, 1939
Clinical signs (hydro or microcephalus, chorioretinitis, intracerebral calcification) described	Sabin, 1942
First case of acquired toxoplasmosis in child	Sabin, 1941
Fatal toxoplasmosis in adults	Pinkerton and Weinman, 1940

Recognition of lymphadenopathy as the most frequent symptom	Siim, 1956
Susceptibility to toxoplasmosis in AIDS patients recognized	Luft, Conley, Remington, Laverdine, Wagner, Levine, Craven, Strandberg, File, Rice and Meunier-Carpenter, 1983
Chronic infection	Plaut, 1946
Toxoplasmosis in animals	
Toxoplasmosis found in domestic animal (dog)	Mello, 1910
Epidemic toxoplasmosis abortions in sheep recognized	Hartley and Marshall, 1957
Toxoplasmosis in animals reviewed critically	Dubey and Beattie, 1988
Diagnosis	
Novel Sabin-Feldman dye test described	Sabin and Feldman, 1948
Toxoplasma skin test as a survey tool	Frenkel, 1948
Tests developed to detect IgM antibodies in cord blood	Remington, Miller and Brownlee, 1968
Simple direct agglutination test developed (DAT, MAT)	Desmonts and Remington, 1980
PCR test developed to detect *T. gondii* DNA using B1 gene	Burg et al., 1989
Treatment	
Sulfonamides found effective against *T. gondii*	Sabin and Warren, 1942
Pyrimethamine found synergestic with sulfonamides against tachyzoites	Eyles and Coleman, 1953
Folic acid and yeast improves activity of sulfadiazine and pyrimethamine	Frenkel and Hitchings, 1957
Spiramycin found to have anti-toxoplasmic activity	Garin and Eyles, 1958
Clindamycin found to be anti-toxoplasmic	McMaster, Powers, Finerty and Lunde, 1973; Araujo and Remington, 1974
Prevention and control	
Prophylactic treatment and screening of pregnant women	Thalhammer, 1973, 1978
Hygienic measures advocated to prevent human exposure to oocysts	Frenkel and Dubey, 1972
Thermal curves to kill *T. gondii* in meat by cooking, freezing, and irradiation constructed	Dubey, Brake, Murrell and Fayer, 1986
Animal production practices developed to reduce *T. gondii* infection	Dubey, Weigel, Siegel Thulliez, Kitron, Mitchell, Mannelli, Mateus-Pinilla, Shen, Kwok and Todd, 1995b
Vaccination	
A vaccine to reduce fetal losses in sheep commercialized	Wilkins and O´Connell, 1983

Ts-4 vaccine for intermediate host	Waldeland and Frenkel, 1983
T-263 vaccine to prevent oocyst shedding by cats	Frenkel, Pfefferkorn, Smith and Fishback, 1991

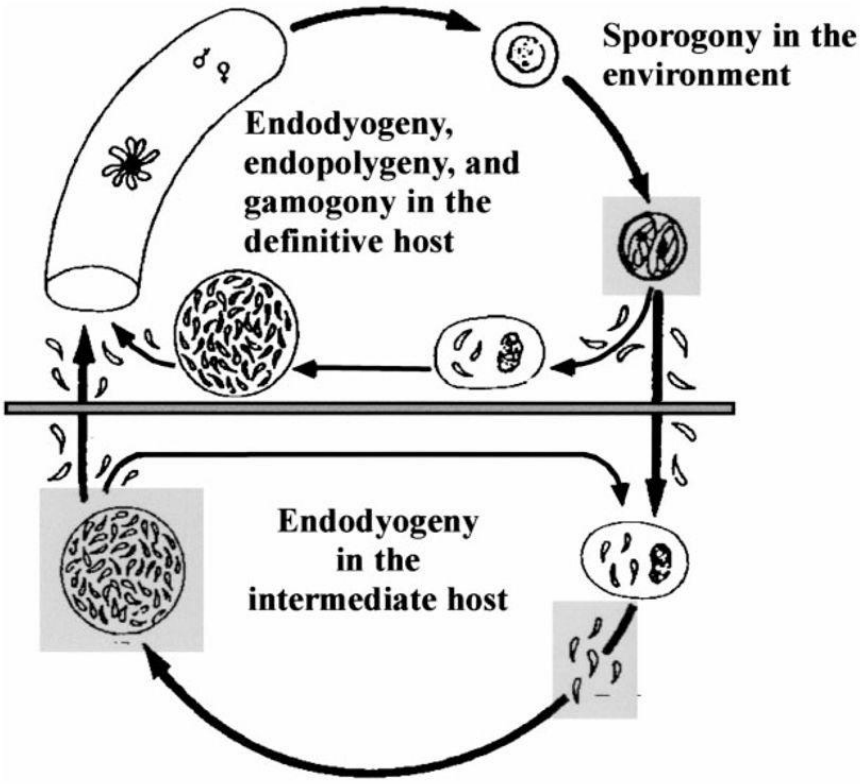
(According to Dubey, 2008)

Appendix 3: Oocyst sporogony



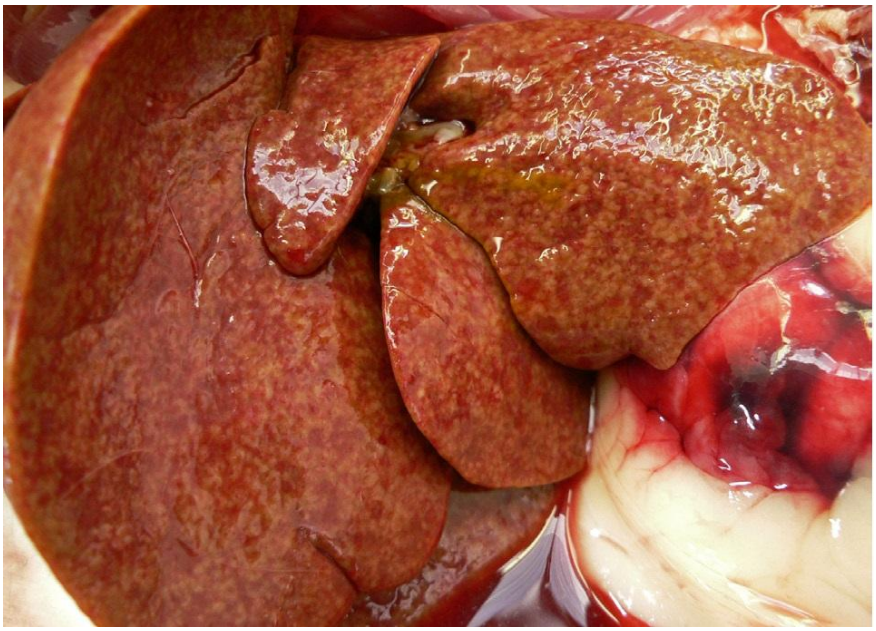
(Lass, 2010)

Appendix 4: Endodyogeny



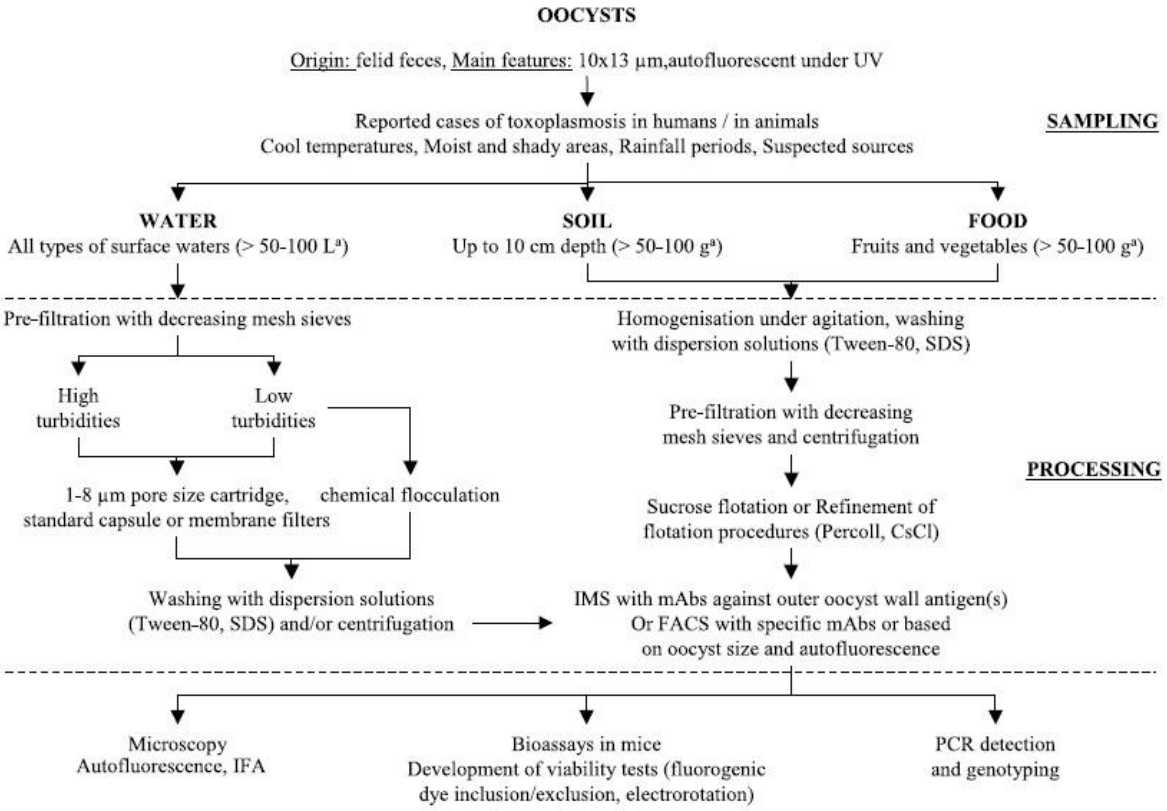
(Tenter et al., 2000)

Appendix 5: Liver infection in cat



(Nagel et al., 2013)

Appendix 6: Oocyst detection in environmental samples



(Dumètre and Dardé, 2003)

Appendix 7: Duration of infectivity of *T. gondii* oocysts under environmental conditions

Temperature (°C)	Conditions	Survival time[a]
	Indoors	
-20	water	14-28 days
-10/-5	water	106 days
0	water	13 months
+4	water	54 months
	faecal suspension	183-410 days[b]
	faecal deposits	214-410 days[b]
	on berries	56 days
+10/+25	water	200 days
+22,5	water	306-410 days[b]
	faecal suspension	153-410 days[b]
	faecal deposits	107-306 days[b]
+23 to +29	moist potted soil	117 days
+30	water	107 days
+35	water	32 days
+37	water	91-306 days[b]
	faecal suspension	46-199 days[b]
	faecal deposits	30-153 days[b]
+40	water	9 days
+45	water	1 day
+50	water	30-60 minutes
+55/+58	water	< 15 minutes
+60/+70	water	< 1 minute
	Outdoors	
-20 to +35	faecal deposits in soil	18 months
-6 to +39	water	122-306 days/153-410 days[c]
	faecal suspension	76-306 days/91-306 days[c]
	faecal deposits	46-183 days/76-334 days[c]
+15 to +30	faecal deposits in soil	56-357 days
+20 to +27	moist soil	106 days

a Overall infectivity of the oocyst suspension determined by bioassays in mice

[b] Duration of infective oocysts (first number) in uncovered suspension and in covered suspension (second number)

[c] Uncovered (first number) and covered (second number) suspension exposed to direct sunlight/or placed in a shady situation

(According to Dumètre and Dardé, 2003)

Appendix 8: Map indicating locations of involved zoos

(http://commons.wikimedia.org/wiki/File:2000px-Czech_Republic_-_zoos_map.png)

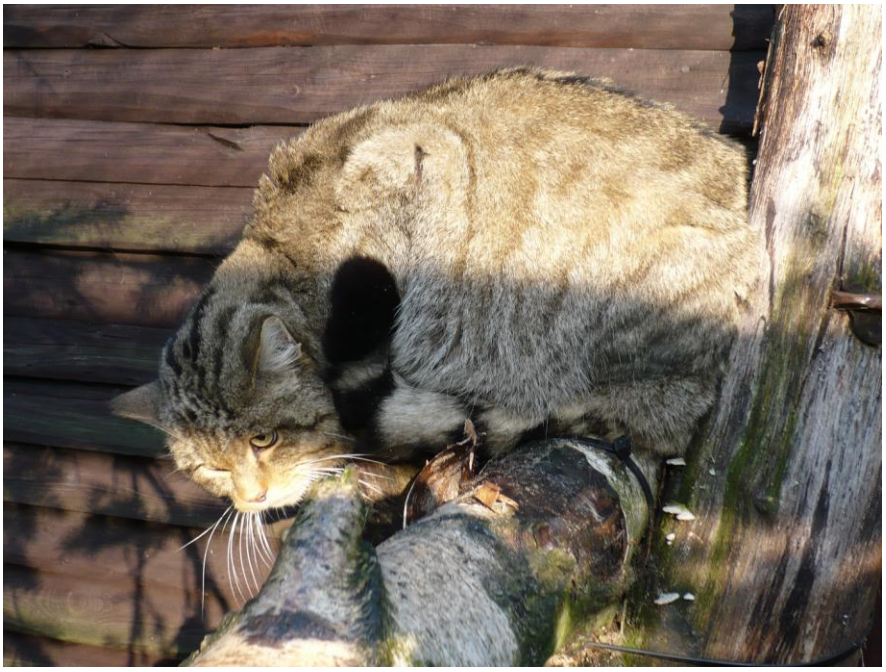
Appendix 9: Sample collecting

(Kudrnáčová, 2014)

Appendix 10: Leopard cat

(http://www.biolib.cz/cz/image/dir360/id28452/?viewall=1&termflt=4947)

Appendix 11: European wildcat



(Kudrnáčová, 2014)

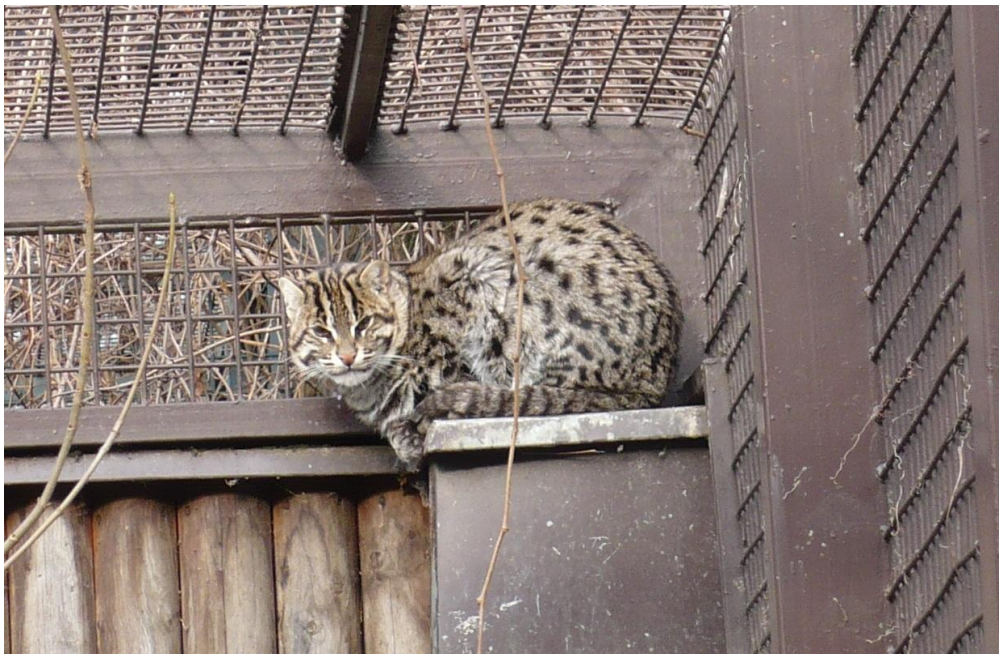
Appendix 12: Geoffroy´s cats

(Kudrnáčová, 2014)

Appendix 13: Sri Lankan rusty-spotted cat

(http://www.zoo-ostrava.cz/cz/novinky/794-novym-druhem-v-zoo-ostrava-je-vzacna-kocka-cejlonska/)

Appendix 14: Fishing cat



(Kudrnáčová, 2014)

Appendix 15: Serval

(Kudrnáčová, 2014)

Appendix 16: Arabian wildcat

(Kudrnáčová, 2014)

Appendix 17: Jungle cat

(http://kisspanda.rajce.idnes.cz/ZOO_Jihlava_17.3.2011?order=create&src=0#353_Kocka_bazinna.jpg)

Appendix 18: Amur leopard cat

(Kudrnáčová, 2014)

Appendix 19: Place for breeding of cats

(Kudrnáčová, 2014)

Appendix 20: Placing of animals

Zoo	Felidae species	Number and sex
Olomouc	Leopard cat	1 ♀
	Fishing cat	1 ♂ + 1 ♀
	Amur Leopard cat	on the beginning - 1 adult ♀ + offsprings 1 ♂, 1 ♀ August/September - ♂ bite and kill the mother and sister
	Arabian wildcat	2 ♀
	European wildcat	1 ♂ + 1 ♀
Ostrava	European wildcat	1 ♂
	Geoffroy´s cat	1 ♂
	Sri Lankan Rusty-spotted cat	1 ♂ + 1 ♀
	Fishing cat	1 ♂
	Serval	1 ♂ + 1 ♀
Jihlava	Arabian wildcat (Felis silvestris gordoni)	1 ♀ (died in September)
	Jungle cat	1 ♂ + 1 ♀
	European wildcat	1 ♂
	Amur Leopard cat	1 ♀
	Geoffroy´s cat	1 ♀ (leave in September)

(Kudrnáčová, 2013)

Appendix 21: Fish as feedstuffs

(Kudrnáčová, 2014)

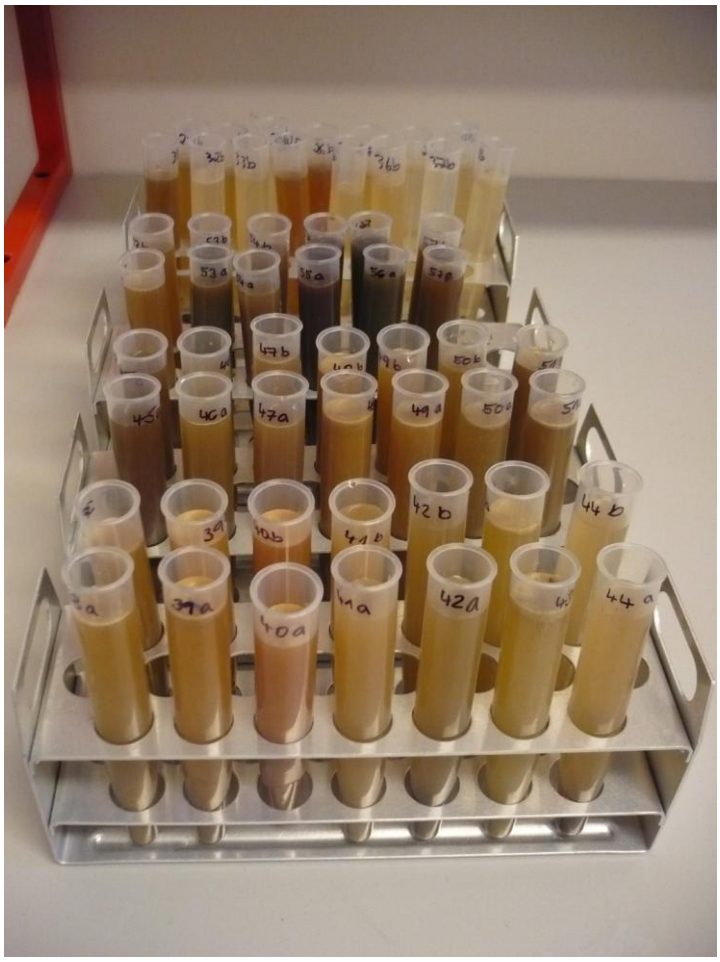
Appendix 22: Soaking of faeces

(Kudrnáčová, 2013)

Appendix 23: Crushing of faeces

(Kudrnáčová, 2013)

Appendix 24: Test-glasses with samples



(Kudrnáčová, 2013)

Appendix 25: Results of positive samples

ZOO	Species	Month	Season	Sex	*T. gondii*	Other parasites	Type of parasite
Jihlava	Arabian wildcat	August	Summer	F	N	P	*Giardia*
Jihlava	Arabian wildcat	July	Summer	F	N	P	*Giardia*
Jihlava	Arabian wildcat	July	Summer	F	N	P	*Giardia*
Jihlava	Arabian wildcat	August	Summer	F	N	P	*Giardia*
Jihlava	Jungle cat	December	Winter	B	N	P	*Giardia*
Jihlava	Jungle cat	December	Winter	B	N	P	*Giardia*
Jihlava	Jungle cat	August	Summer	B	N	P	*Giardia*
Jihlava	Jungle cat	August	Summer	B	N	P	*Toxascaris leonina*
Jihlava	Jungle cat	November	Autumn	B	N	P	*Toxocara cati*, *Toxascaris leonina*
Jihlava	Jungle cat	November	Autumn	B	N	D	*Toxascaris leonina*
Jihlava	Jungle cat	December	Winter	B	N	P	*Giardia*
Jihlava	Jungle cat	October	Autumn	B	N	P	*Giardia*
Jihlava	Jungle cat	July	Summer	B	N	P	*Toxocara cati*
Jihlava	European wildcat	January	Winter	M	N	P	*Giardia*

Jihlava	European wildcat	November	Autumn	M	N	P	*Toxocara cati*
Jihlava	European wildcat	November	Autumn	M	N	D	*Toxocara cati*
Jihlava	European wildcat	September	Autumn	M	N	D	*Isospora*
Jihlava	European wildcat	December	Winter	M	N	P	*Toxocara*
Jihlava	European wildcat	August	Summer	M	N	P	*Toxascaris, Toxocara*
Jihlava	European wildcat	September	Autumn	M	N	P	*Giardia*
Jihlava	European wildcat	October	Autumn	M	N	P	*Giardia*
Jihlava	European wildcat	November	Autumn	M	N	P	*Toxocara cati*
Jihlava	European wildcat	August	Summer	M	N	P	*Giardia*
Jihlava	European wildcat	January	Winter	M	N	P	*Toxocara cati*
Jihlava	European wildcat	September	Autumn	M	N	P	*Toxocara cati*
Jihlava	European wildcat	October	Autumn	M	N	P	*Toxocara*
Jihlava	Amur leopard cat	September	Autumn	F	N	P	*Giardia*
Jihlava	Amur leopard cat	October	Autumn	F	P	P	*Isospora, Toxocara*
Jihlava	Amur leopard cat	December	Winter	F	N	P	*Toxocara, Isospora*
Jihlava	Amur leopard cat	December	Winter	F	N	P	*Toxocara*
Jihlava	Amur leopard cat	September	Autumn	F	N	P	*Giardia*
Jihlava	Amur leopard cat	August	Summer	F	N	P	*Toxocara*
Jihlava	Amur leopard cat	October	Autumn	F	N	P	*Toxocara cati*
Jihlava	Amur leopard cat	October	Autumn	F	N	P	*Toxocara, Isospora*
Jihlava	Amur leopard cat	December	Winter	F	N	P	*Toxocara cati*
Jihlava	Amur leopard cat	August	Summer	F	N	P	*Toxocara*
Jihlava	Amur leopard cat	November	Autumn	F	N	D	*Isospora*
Jihlava	Geoffroy´s cat	October	Autumn	F	N	P	*Giardia*
Jihlava	Geoffroy´s cat	July	Summer	F	N	P	*Isospora*
Jihlava	Geoffroy´s cat	September	Autumn	F	N	P	*Giardia*
Jihlava	Geoffroy´s cat	September	Autumn	F	N	P	*Toxocara cati, Toxascaris leonina*
Jihlava	Geoffroy´s cat	October	Autumn	F	N	D	*Toxascaris leonina*
Jihlava	Geoffroy´s cat	July	Summer	F	N	P	*Giardia*
Jihlava	Geoffroy´s cat	August	Summer	F	N	P	*Giardia*
Olomouc	Arabian wildcat	July	Summer	F	N	P	*Toxocara cati*
Olomouc	Arabian wildcat	July	Summer	F	N	P	*Toxocara*

Olomouc	Arabian wildcat	July	Summer	F	D	P	*Giardia*
Olomouc	Arabian wildcat	July	Summer	F	N	P	*Giardia*
Olomouc	Arabian wildcat	August	Summer	F	N	P	*Giardia*
Olomouc	Arabian wildcat	August	Summer	F	D	D	*Giardia*
Olomouc	Arabian wildcat	August	Summer	F	N	P	*Toxocara*
Olomouc	Arabian wildcat	September	Autumn	F	N	P	*Toxascaris*
Olomouc	Arabian wildcat	September	Autumn	F	N	P	*Toxocara, Giardia*
Olomouc	Arabian wildcat	November	Autumn	F	N	P	*Toxocara cati*
Olomouc	Arabian wildcat	December	Winter	F	N	P	*Giardia*
Olomouc	Leopard cat	July	Summer	F	N	P	*Toxascaris*
Olomouc	Leopard cat	August	Summer	F	N	P	*Toxascaris*
Olomouc	Leopard cat	September	Autumn	F	N	P	*Giardia*
Olomouc	Leopard cat	December	Winter	F	N	P	*Giardia*
Olomouc	European wildcat	July	Summer	B	N	P	*Giardia*
Olomouc	European wildcat	July	Summer	B	N	P	*Toxocara, Giardia*
Olomouc	European wildcat	July	Summer	B	N	P	*Toxascaris, Giardia*
Olomouc	European wildcat	July	Summer	B	N	P	*Toxascaris leonina*
Olomouc	European wildcat	August	Summer	B	N	P	*Toxascaris*
Olomouc	European wildcat	August	Summer	B	N	D	*Toxascaris*
Olomouc	European wildcat	August	Summer	B	N	P	*Toxascaris*
Olomouc	European wildcat	August	Summer	B	N	P	*Toxascaris*
Olomouc	European wildcat	August	Summer	B	N	D	*Toxascaris*
Olomouc	European wildcat	August	Summer	B	N	P	*Toxascaris*
Olomouc	European wildcat	August	Summer	B	N	P	*Giardia*
Olomouc	European wildcat	August	Summer	B	N	D	*Toxascaris*
Olomouc	European wildcat	August	Summer	B	N	P	*Toxascaris*
Olomouc	European wildcat	August	Summer	B	N	P	vajíčka roztočů
Olomouc	European wildcat	September	Autumn	B	P	P	*Isospora*
Olomouc	European wildcat	September	Autumn	B	N	P	*Toxascaris*
Olomouc	European wildcat	September	Autumn	B	N	P	*Toxascaris*
Olomouc	European wildcat	September	Autumn	B	N	P	*Toxascaris*
Olomouc	European wildcat	November	Autumn	B	N	P	*Giardia*
Olomouc	European wildcat	November	Autumn	B	N	P	*Toxocara cati*
Olomouc	European wildcat	January	Winter	B	N	P	*Giardia*

Olomouc	Amur leopard cat	July	Summer	M	N	P	*Giardia*
Olomouc	Amur leopard cat	July	Summer	M	N	P	*Toxascaris leonina*
Olomouc	Amur leopard cat	July	Summer	M	P	N	-
Olomouc	Amur leopard cat	July	Summer	M	N	D	*Giardia*
Olomouc	Amur leopard cat	August	Summer	M	N	P	*Giardia*
Olomouc	Amur leopard cat	August	Summer	M	D	N	-
Olomouc	Amur leopard cat	August	Summer	M	P	N	-
Olomouc	Amur leopard cat	August	Summer	M	P	N	-
Olomouc	Amur leopard cat	August	Summer	M	P	N	-
Olomouc	Amur leopard cat	September	Autumn	M	N	P	*Toxascaris, Toxocara*
Olomouc	Amur leopard cat	September	Autumn	M	N	P	*Toxascaris*
Olomouc	Amur leopard cat	November	Autumn	M	N	P	*Cryptosporidium*
Olomouc	Fishing cat	July	Summer	B	D	P	*Toxascaris*
Olomouc	Fishing cat	September	Autumn	B	N	P	*Giardia*
Olomouc	Fishing cat	November	Autumn	B	N	P	*Giardia*
Olomouc	Fishing cat	January	Winter	B	N	P	*Giardia*
Ostrava	Sri Lankan rusty-spotted cat	September	Autumn	B	N	D	*Toxocara cati*
Ostrava	Sri Lankan rusty-spotted cat	September	Autumn	B	N	P	*Giardia*
Ostrava	Sri Lankan rusty-spotted cat	September	Autumn	B	N	P	*Giardia*
Ostrava	Sri Lankan rusty-spotted cat	September	Autumn	B	N	P	*Toxocara cati*
Ostrava	Sri Lankan rusty-spotted cat	October	Autumn	B	N	P	*Toxocara cati*
Ostrava	Sri Lankan rusty-spotted cat	November	Autumn	B	N	D	*Toxascaris leonina*
Ostrava	Sri Lankan rusty-spotted cat	November	Autumn	B	N	P	*Giardia*
Ostrava	European wildcat	July	Summer	M	N	P	*Toxocara cati*
Ostrava	European wildcat	August	Summer	M	N	P	*Toxocara cati, Giardia*
Ostrava	European wildcat	August	Summer	M	N	P	*Giardia*
Ostrava	European wildcat	August	Summer	M	N	P	*Toxocara cati*
Ostrava	European wildcat	September	Autumn	M	N	P	*Giardia*
Ostrava	European wildcat	September	Autumn	M	N	P	*Giardia*

Ostrava	European wildcat	September	Autumn	M	N	D	*Giardia*
Ostrava	European wildcat	September	Autumn	M	N	D	*Giardia*
Ostrava	European wildcat	October	Autumn	M	N	P	*Giardia*
Ostrava	European wildcat	November	Autumn	M	N	P	*Toxocara cati*
Ostrava	European wildcat	December	Winter	M	D	D	*Isospora*
Ostrava	European wildcat	January	Winter	M	N	P	*Giardia*
Ostrava	Fishing cat	July	Summer	M	N	P	*Toxocara cati*
Ostrava	Fishing cat	August	Summer	M	P	N	-
Ostrava	Fishing cat	August	Summer	M	N	D	*Toxocara cati*
Ostrava	Fishing cat	August	Summer	M	N	P	*Giardia*
Ostrava	Fishing cat	August	Summer	M	N	P	*Giardia*
Ostrava	Fishing cat	September	Autumn	M	N	D	*Toxascaris leonina*
Ostrava	Fishing cat	September	Autumn	M	N	D	*Giardia*
Ostrava	Fishing cat	September	Autumn	M	N	P	*Giardia*
Ostrava	Fishing cat	September	Autumn	M	N	D	*Giardia*
Ostrava	Fishing cat	October	Autumn	M	N	D	*Giardia*
Ostrava	Fishing cat	October	Autumn	M	D	N	-
Ostrava	Fishing cat	October	Autumn	M	N	P	*Giardia*
Ostrava	Fishing cat	October	Autumn	M	N	P	*Giardia*
Ostrava	Fishing cat	November	Autumn	M	N	D	*Toxascaris leonina*
Ostrava	Fishing cat	November	Autumn	M	D	N	-
Ostrava	Fishing cat	November	Autumn	M	P	N	-
Ostrava	Fishing cat	December	Winter	M	N	P	*Giardia*
Ostrava	Geoffroy´s cat	August	Summer	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	August	Summer	M	N	P	*Giardia*
Ostrava	Geoffroy´s cat	August	Summer	M	N	D	*Toxocara*
Ostrava	Geoffroy´s cat	August	Summer	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	September	Autumn	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	September	Autumn	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	September	Autumn	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	September	Autumn	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	September	Autumn	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	September	Autumn	M	N	D	*Toxocara cati*
Ostrava	Geoffroy´s cat	October	Autumn	M	N	D	*Toxocara cati*
Ostrava	Geoffroy´s cat	October	Autumn	M	N	D	*Toxocara cati*
Ostrava	Geoffroy´s cat	October	Autumn	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	October	Autumn	M	N	D	*Toxocara cati*
Ostrava	Geoffroy´s cat	November	Autumn	M	N	P	*Toxocara cati*
Ostrava	Geoffroy´s cat	November	Autumn	M	N	D	*Toxocara cati*
Ostrava	Geoffroy´s cat	December	Winter	M	N	P	*Toxocara cati*
Ostrava	Serval	July	Summer	B	P	N	-
Ostrava	Serval	July	Summer	B	P	N	-

Sex: M – male B – both *T. gondii*, other parasites: P – positive D - dubious

F – female N – negative