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TOXOPLASMA GONDII SEROPREVALENCE IN CATS IN ESTONIA

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<th>Description</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>BSR</td>
<td>bradyzoite-specific recombinant antigen</td>
</tr>
<tr>
<td><em>C. gundi</em></td>
<td><em>Ctenodactylus gundi</em></td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>e.g.</td>
<td><em>exempli gratia</em> (for example)</td>
</tr>
<tr>
<td>ECDC</td>
<td>European Centre for Disease Prevention and Control</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EMU</td>
<td>Eesti Maaülikool (Estonian University of Life Sciences)</td>
</tr>
<tr>
<td>et al.</td>
<td><em>et alii</em> (and others)</td>
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<tr>
<td>FeLV</td>
<td>feline leukaemia virus</td>
</tr>
<tr>
<td>FIV</td>
<td>feline immunodeficiency virus</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>i.e.</td>
<td><em>id est</em> (that is)</td>
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<tr>
<td>IgA</td>
<td>immunoglobulin A</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
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<tr>
<td>IgM</td>
<td>immunoglobulin M</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>SAG</td>
<td>surface antigen</td>
</tr>
<tr>
<td>spp.</td>
<td>species: plural</td>
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<tr>
<td>SRS</td>
<td>surface antigen related sequence</td>
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<tr>
<td><em>T. gondii</em></td>
<td><em>Toxoplasma gondii</em></td>
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<td>UV</td>
<td>ultraviolet</td>
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1 SUMMARY

TOXOPLASMA GONDII SEROPREVALENCE IN CATS IN ESTONIA

Toxoplasma gondii (T. gondii) is a cosmopolitan zoonotic parasite that can infect a wide variety of host species. The only definitive hosts of T. gondii are felids that are capable of shedding oocysts in their faeces. The parasite can cause a disease called toxoplasmosis, which can be potentially fatal to many hosts, including cats and humans. The cat is considered to be the most important host species from an epidemiological point of view. Recently in Estonia, studies on T. gondii seroprevalence in humans and in wild boars have been conducted, but epidemiological information on T. gondii infections in cats in Estonia has been lacking.

The present epidemiological cross-sectional study was conducted to obtain information on naturally-acquired T. gondii infections in cats in Estonia. The aims of the study were to estimate the prevalence of specific anti-T. gondii immunoglobulin G (IgG) antibodies in pet cats and shelter cats, and to determine and evaluate risk factors for T. gondii infections in cats in Estonia. No cat blood was drawn solely for this study; surplus blood samples originally taken for other diagnostic purposes were used. For risk factor analysis, owners and veterinarians were asked to fill questionnaires that included information about the cat’s signalment and for pet cats, their lifestyle.

The blood samples were collected in January–December 2013. The pet cat samples came from four different small animal clinics in Tartu, the shelter cat samples came from a shelter located in Tartu. Altogether 490 feline serum or plasma samples were obtained: 306 samples were from pet cats and 184 were from shelter cats. The samples were screened with a commercial direct agglutination test.

Specific anti-T. gondii IgG antibodies were found in 105 shelter cats and 193 pet cats, thus the seroprevalences in shelter cats and pet cats were 57.07% and 63.07%, respectively. The overall T. gondii seroprevalence in cats in Estonia was estimated to be 60.82%. Significant risk factors for T. gondii seropositivity included adult age, outdoor access, and hunting. Cats that lived in Tartu and that lived in a town were less often seropositive. Being fed raw meat was not a significant risk factor in this study. Domestic cats were more often seropositive than purebred cats, and significant differences between seroprevalences in different cat breeds were also found.

This study shows that T. gondii antibodies are highly prevalent in domestic cats in Estonia. Adult age, outdoor access and hunting being significant risk factors indicate that most T. gondii infections are acquired postnatally, and owners could prevent their cats from becoming infected
with *T. gondii*. Seropositive cats are believed to have already shed oocysts and caused environmental contamination with the parasite. Given that more than half of the cats in this study had outdoor access, the parasite is probably widely spread in the Estonian environment.

Key words: cats; cross-sectional study; epidemiology; seroprevalence; *Toxoplasma gondii*. 
2 KOKKUVÕTE

**TOXOPLASMA GONDII SEROPREVALENTSUS KASSIDEL EESTIS**


Käesolev epidemioloogiline läbilõikeuring käsitleb loomulikul teel omandatud *T. gondii* infektsioone kassidel Eestis. Uuringu eesmärkideks olid spetsiifiliste *T. gondii* vastaste immunoglobuliini G (IgG) antikehade levimuse hindamine kodu omavatel ja varjupaigas elavatel kassidel ning *T. gondii* infektsiooni riskitegurite kindlakstegemine ja hindamine kassidel Eestis. Spetsiiaalselt antud uuringu jaoks kassidelt verd ei võetud, vaid kasutati seerumit ja plasmat, mis jäid üle muudel eesmärkidel võetud vereproovidest. Riskifaktorite analüüsiks paluti omanikel ja loomaarstidel täita küsimustikke, mis sisaldasid informatsiooni kassi andmete ja kodu omavatel kassidel ka elustiili kohta.


Märksõnad: epidemioloogia; kassid; läbilõikeuuring; seroprevalentsus; *Toxoplasma gondii*.
3 INTRODUCTION

*Toxoplasma gondii* (T. gondii) is a microscopic intracellular coccidian parasite (Dubey, 2010). It can infect virtually all warm-blooded species, including domestic animals, wildlife and humans. The parasite can complete its lifecycle and reproduce sexually only in felids. *Toxoplasma gondii* is one of the most common and widespread parasites in the world and causes toxoplasmosis, a disease that is potentially fatal to many hosts, including cats and humans (Nissapatorn, 2009; Jokelainen, 2013).

*Toxoplasma gondii* is mainly a threat to the immunocompromised, but it is also capable of causing a serious disease or even death in immunocompetent humans and animals (Montoya and Liesenfeld, 2004; Dubey, 2010; Jokelainen, 2013). In women and female animals, *T. gondii* can cause reproductive disorders and birth of progeny suffering from toxoplasmosis.

Cats (*Felis catus*) are considered to play a key role in the parasite’s epidemiology. Environmental contamination with infectious forms of the parasite is mainly caused by domestic cats (Dabritz and Conrad, 2010). Infections in production animals and game are often due to ingestion of the parasites that have been shed into the environment. Humans most often become infected by eating tissues from these animals but also by directly consuming the infectious forms from the environment.

There is scarce information concerning the epidemiology of *T. gondii* infections in Estonia. Recent studies have estimated the seroprevalence to be 54.9% in humans living in Tartu (Birgisdottir *et al*., 2006), 46.2% among veterinarians, and 56.4% in the general human population (Janson *et al*., 2013). In 2013, the prevalence of anti-*T. gondii* immunoglobulin G (IgG) antibodies in wild boars in Estonia was estimated to be 24.0% (Velström *et al*., 2013). In Estonia, *T. gondii* prevalence in cats has not been previously studied. Given the high prevalences in other species, *T. gondii* infections in cats should be common.

The purpose of this study was to estimate the seroprevalence of *T. gondii* in cats in Estonia. Additionally, risk factors for *T. gondii* infection in cats were evaluated. By analysing risk factors, important information about the parasite’s epidemiology may be revealed. Moreover, preventive measures for *T. gondii* infections in cats can be suggested and applied to control the spread of this potentially dangerous parasite.
4 LITERATURE REVIEW

4.1 Toxoplasma gondii, a Zoonotic Parasite

4.1.1 Introduction of the Parasite

*Toxoplasma gondii* (*T. gondii*) is a ubiquitous intracellular coccidian parasite. The disease caused by *T. gondii* is called toxoplasmosis. Because of its veterinary and medical importance, *T. gondii* has been thoroughly studied, making it one of the best-known parasites. It is capable of infecting many different cells of a wide range of hosts (Dubey, 2010). Its definitive hosts are only felids, but the parasite is not host-specific, since it can infect virtually all warm-blooded animals. Humans can get the infection from animals, and animals from humans, thus the parasite is a zoonotic parasite.

*Toxoplasma gondii* is the only species in the genus. It was discovered in 1908 in a hamster-like rodent *Ctenodactylus gundi* (*C. gundi*) (Nicolle and Manceaux, 1908). In Greek, the word *toxo* means an arc or a bow, *plasma* means life. The parasite was named *T. gondii* because the authors had misspelt the host’s species name (*C. gondi* instead of *C. gundi*) (Dubey, 2010).

*Toxoplasma gondii* has three infectious stages: tachyzoite, bradyzoite, and sporozoite (oocyst). It has developed a wide range of potential routes of transmission. Transmission occurs mainly via a faecal-oral route, by carnivorism or transplacentally. *Toxoplasma gondii* is an obligatory intracellular parasite, but in the oocyst form, it can survive extracellularly for a long time (Dubey, 2010; Jokelainen, 2013).

4.1.2 Infectious Stages

4.1.2.1 Tachyzoites

Tachyzoites (*tachy* = fast in Greek) are approximately 2 × 6 μm and crescent-shaped (Figure 1). They dominate in acute toxoplasmosis. They have also been called endodyozoites, endozoites or previously trophozoites. By multiplying, they expand the population of the parasite in the host. Aggregates of tachyzoites are called clones, terminal colonies, or groups (Dubey *et al*., 1998).

The nucleus in tachyzoites is located toward the central area of the cell (Dubey *et al*., 1998). Despite having no visible means of locomotion, tachyzoites move by gliding, flexing, undulating, and rotating. Tachyzoites can penetrate a variety of cell types from a wide range of hosts and the penetration of the host cell’s plasmalemma takes 26 seconds (Dubey, 2010). After the penetration, the parasite is surrounded by a membrane derived from the host cell.
plasmalemma, which becomes the parasitophorous vacuole membrane. Tachyzoites modify the parasitophorous vacuole and its membrane with parasite proteins, forming a tubular membranous network within the vacuole. The parasitophorous vacuole membrane has pores in it and it allows charged molecules (up to 1,200 kDals, including proteins) to diffuse between the vacuole and host cell cytoplasm (Dubey, 2010). These changes create a parasite-friendly environment and allow the parasite to replicate within the host cell cytoplasm.

The replication of tachyzoites is asexual and it involves the formation of two progeny within the parent parasite, consuming it. This process is called repeated endodyogeny. When the host cell cannot support the growth of tachyzoites anymore, free tachyzoites are released, and they are capable of invading new cells. The rate of invasion and growth depends on the host cells and the strain of the parasite (Dubey, 2010).

Figure 1. The three forms of Toxoplasma gondii. A: tachyzoites in cell culture. B: an unsporulated oocyst. C: tissue cyst with bradyzoites from the brain of a mouse. Photos by Pikka Jokelainen, reproduced with her kind permission.
4.1.2.2 Bradyzoites

Bradyzoites (brady = slow in Greek) are the intracellular parasites encysted in tissue cysts in tissues (Dubey et al., 1998). This stage is also called cystozoite. Bradyzoites are 5–8.5 × 1–3 μm and crescent-shaped. The nucleus in bradyzoites is situated toward the posterior end of the parasite. Bradyzoites also divide by endodyogeny. Consequently, tissue cysts are formed in the parasitophorous vacuoles (Figure 1) and they can contain variable numbers of bradyzoites (Dubey, 2010). Smaller cysts are about 5 μm in diameter and contain two bradyzoites. Larger tissue cysts in the brain can reach a diameter of 70 μm, containing thousands of parasites. The cyst wall contains components from the host cell and parasite. Parasitophorous vacuoles with bradyzoites lack the tubular membranous network, which is present in vacuoles containing tachyzoites. Bradyzoites differ from tachyzoites biologically because they can survive the digestive process in the stomach, where tachyzoites are usually killed (Dubey, 2010).

The formation of tissue cysts is largely controlled by the host. Most frequently, tissue cysts develop in skeletal muscles, myocardium and brain, but they can also be found in lungs, eyes, liver, kidneys, and other organs (Dubey, 2010). They can persist for the life of the host. Unknown factors can lead to tissue cyst rupture, but it rarely happens. In immunocompromized hosts, the rupture can lead to multiplication of tachyzoites and active infection (Dubey, 2010).

4.1.2.3 Sporozoites

Sporozoites are the forms present in sporulated oocysts (Dubey et al., 1998). Oocysts are the products of sexual cycle of T. gondii. They are formed in the cat’s intestine. Unsporulated oocysts are spherical to subspherical and 10 × 12 μm in diameter (Figure 1). They have a wall that consists of two layers. Their sporulation occurs outside the cat, during which they become subspherical to ellipsoidal. The infectious sporulated oocysts are 11 × 13 μm in diameter and their wall consists of three layers (Dubey et al., 1998).

Sporulated oocysts contain two ellipsoidal sporocysts, 6 × 8 μm each. A sporocyst contains four sporozoites; thus there are eight sporozoites in one sporulated oocyst. Sporozoites are similar to tachyzoites, banana-shaped and 2 × 6–8 μm in diameter (Dubey, 2010). The location of their nucleus is subterminal. They can survive in the oocysts for many months. When sporulated oocysts are eaten by a host, the release of infectious sporozoites occurs in the intestinal tract, after which cells of intestinal mucosa are infected (Dubey, 2010).
4.1.3 Enteroepithelial Life Cycle

Presumably, practically all species of felids can be definitive hosts for *T. gondii*, which means they can shed the parasite’s oocysts. Cats become infected after ingesting tachyzoites, bradyzoites, or oocysts. Less than half of domestic cats shed oocysts after ingesting tachyzoites or oocysts, but nearly all of them shed oocysts after ingestion of tissue cysts (Dubey, 2010). The time between the ingestion and the shedding of oocysts is called the prepatent period. The length of the prepatent period does not depend on the dose of infectious forms, but varies according to the stage of the parasite ingested. Tissue cysts are associated with a short prepatent period of 3–10 days, whereas for oocysts, it is more than 18 days (Dubey, 2010). Prepatent periods after ingestion of tachyzoites are variable, but usually more than 13 days (Dubey, 2010).

Following tissue cyst ingestion, the cyst wall is dissolved by proteolytic enzymes in the cat’s digestive system, followed by the release of bradyzoites (Dubey, 2010). Some of the released bradyzoites initiate the development of *T. gondii* generations by penetrating the epithelial cells of small intestine, beginning the enteroepithelial cycle that will terminate in oocyst production (Dubey, 2010). Before gametogony, five morphologically distinct types of *T. gondii* (A to E) develop intracellularly in the intestinal epithelium (in enterocytes). Types C, D and E multiply by schizogony, which means that the nucleus divides two or more times without cytoplasmic division. The multiplying types, the schizonts, are surrounded by a parasitophorous vacuole. The daughter organism is called the merozoite. The schizont plasmalemma invaginates around each merozoite, forming the plasmalemma of the merozoite. The merozoites are thought to initiate gamete formation (Dubey, 2010).

The sexual cycle starts with the formation of gamonts in enterocytes of a definitive host 3–15 days after inoculation (Dubey, 2010). Gamonts (gametocytes) can be found throughout the small intestine. Female gamonts are subspherical and contain a centrally located nucleus. Male gamonts are ovoid to ellipsoidal and they divide to produce microgametes. Microgametes are elongated, biflagellate and consist mainly of nuclear material. They use the flagella to swim to macrogametes. After penetrating the mature macrogamete, fertilization occurs and a zygote is formed. A wall develops around the zygote and an oocyst is created. Oocysts are released into the intestinal lumen after the rupture of infected intestinal cells (Dubey, 2010).

Unsporulated oocysts are not infectious. Sporulation occurs in the environment depending on aeration, temperature, humidity, and takes 1–5 days (Dubey, 2010). During sporulation, the oocysts become infectious as sporocysts containing sporozoites are formed. The oocyst wall is susceptible to carbon dioxide (CO₂) and various enzymes that are present in the digestive tract.
The enzymes and CO₂ make the oocyst wall permeable for bile salts and trypsin, which stimulate the excystation of sporozoites from sporocysts. The described process naturally occurs in the digestive tract of a potential host. The sporocyst ruptures during excystation, and sporozoites are released (Dubey, 2010).

Cats that are infected by oocyst ingestion have not been shown to develop enteroepithelial stages of *T. gondii* (Dubey, 2010). It has been speculated that after ingestion of oocysts (if the cat gets infected), the released sporozoites convert to tachyzoites. The tachyzoites then become tissue cysts containing bradyzoites. The enteroepithelial cycle is initiated when a tissue cyst ruptures and bradyzoites travel back to the intestine to produce the enteroepithelial cycle that results in oocyst production (Dubey, 2010).

Tachyzoites are more sensitive to acid than bradyzoites, but some of them may survive acid-pepsin digestion in the stomach and initiate the enteroepithelial cycle (Dubey, 2010). Moreover, tachyzoites might penetrate pharyngeal-buccal mucosa before entering the oesophagus (Dubey, 2010).

### 4.1.4 Extraintestinal Life Cycle

The extraintestinal life cycle of *T. gondii* is the same for all hosts (Dubey, 2010). Extraintestinal development does not depend on whether tissue cysts or oocysts are ingested. The result of the extraintestinal life cycle is usually tissue cyst formation. Tissue cysts containing bradyzoites have been found in the brain, skeletal muscle, and liver of cats, and evidently occur also in mesenteric lymph nodes, lung, spleen and intestine (Dubey and Frenkel, 1974).

After ingestion of sporulated oocysts, excystation occurs, and sporozoites penetrate the cells of the intestinal epithelium (Dubey, 2010). Most of the sporozoites are found in the lamina propria where they multiply and become tachyzoites. Tachyzoites can multiply in almost any cell of the body; they are spread by lymph and blood circulation. In association with systemic immune response, by approximately the third week after infection, tachyzoites begin to disappear from visceral tissues and tissue cysts containing bradyzoites may be formed (Dubey, 2010).

The bradyzoite-induced cycle is similar to the oocyst-induced cycle. Also in cats, some bradyzoites (that do not initiate the enteroepithelial cycle) will penetrate into the intestinal lamina propria and begin development as tachyzoites (Dubey, 2010). The formation of tachyzoites takes more time when bradyzoites are ingested by non-felid hosts, but the tissue cysts develop with approximately the same speed (Dubey, 2010). After oocyst and bradyzoite ingestion, tissue cysts persist in several organs.
4.2 Transmission

*Toxoplasma gondii* is transmitted in diverse ways and it can exist without completing its life cycle (Jokelainen, 2013). The parasite can reproduce sexually as well as asexually, but it may also lie dormant in the host and survive in the environment. There are three main modes of transmission: acquired infection by ingesting tissue cysts, acquired infection by ingesting oocysts, and congenital infection (Figure 2). As the parasite has many different infection routes, its infections belong to the overlapping lists of foodborne, waterborne, soil-transmitted, milk-transmitted, cat litter box derived, iatrogenic, blood-borne, occupational, opportunistic, and zoonotic infections (Dubey, 2010; Jokelainen, 2013).

Figure 2. Simplified life cycle of *Toxoplasma gondii*: carnivorism, faecal-oral transmission from a cat to a rodent, and vertical transmission to a foetus. Illustration by Brian Lassen for Jokelainen, 2013. Reproduced by kind permission of Brian Lassen and Pikka Jokelainen.

Oocysts are shed by domestic cats and other felids (Figure 2). The environment can be contaminated with their faeces, and oocysts can be found in soil, water or feed. Most seropositive cats shed oocysts before seroconversion occurs (Dubey, 2010). The time the cat is shedding oocysts is called the patent period. The oocysts are shed for less than three weeks, usually only for one week (Dubey, 2010). Immunosuppression and hyperadrenocorticism may prolong the patent period (Dubey and Frenkel, 1974). Coprological surveys are unrewarding in estimating the prevalence of the parasite because it has been found that at any given time, less than 1% of cats are shedding oocysts (Jones and Dubey, 2010).
*Toxoplasma gondii* oocysts are resistant to various physical and chemical environmental influences (Dubey, 2010; Jokelainen, 2013). They can survive in the soil and cold water for years (Siński and Behnke, 2004; Dubey, 2010). The bilayered oocyst wall protects the infective sporozoites from common disinfectants such as chlorine-based products (Jones and Dubey, 2010). Oocysts are resistant to freezing, but temperatures higher than 60 °C can effectively inactivate them in a few minutes (Jones and Dubey, 2010). Because *T. gondii* oocysts are not destroyed by chemical and physical sewage water treatments, cat faeces should not be flushed down the toilet (Dabritz and Conrad, 2010; Jokelainen, 2013).

Land contamination with high levels of oocysts may cause the parasite’s spread to water, including surface and marine waters (Dubey, 2010). Molluscs are potential transport hosts for *T. gondii* oocysts. Oocysts in water may infect mammals such as sea otters, seals, and dolphins (Jones and Dubey, 2010). *Toxoplasma gondii* oocysts may pose a significant risk to recreational and drinking water worldwide, especially if the water is untreated or inadequately treated (unfiltered, not boiled). Ultraviolet (UV) irradiation has been shown to be effective against *T. gondii* oocysts and could potentially be used to disinfect drinking water and wastewater systems (Ware *et al*., 2010).

It is not known how immune status of the definitive host affects the shedding of oocysts. Whether or how commonly naturally infected cats shed oocysts more than once in their life is not clear either. Experimentally, cats have shed oocysts during primary infection and challenge, but during the secondary infection, less oocysts are shed (Dubey, 1995; Dubey, 2010). In a study by Dubey (1995), seronegative cats were infected with *T. gondii* bradyzoites and all of them excreted oocysts after the primary infection. Five of the cats were challenged 39 days after the first inoculation and none of them started re-shedding. The second challenge was done 77 months after the first inoculation, and four of nine cats started re-shedding. Thus, the cats were apparently immune to re-shedding 39 days after the first inoculation, but some of them had lost the immunity by 77 months post inoculation. Shedding and re-shedding of oocysts can be affected by several factors, including the age, nutritional and immune status of the cat, strain and stage of *T. gondii*, or number of tissue cysts eaten by the cat (Dubey, 1995).

Cats are considered to be clean animals and when they have the chance, they bury their faeces. Covered by soil, oocysts may have better chances to survive different environmental conditions. Oocysts can be further spread from the faeces by rain, snow and other climatic conditions, among other things (Dubey, 2010). They may be carried by flies and other insects, earthworms, or even on shoes contaminated with cat faeces, or dogs that have rolled in cat faeces. When a...
dog eats cat faeces containing *T. gondii* oocysts, the oocysts can pass unexcysted in dog faeces (Dubey *et al*., 2009). Humans are unlikely to be infected by touching a cat because oocysts have not been found in cat hair even at the time of peak oocyst shedding (Dubey, 1995).

The important stage in the life cycle of *T. gondii* is the stage in tissue cysts. They are destined to be eaten by carnivores and omnivores, including cats and humans. Tissue cysts are more labile than oocysts, they can be killed for example by heating (67 °C or higher), freezing (−20 °C for 54 hours or more), pasteurization, or salting (Dubey, 2010). However, when stored at temperatures favourable for the parasite, tissue cysts may survive for long periods (Work *et al*., 2000): rotting of tissues around tissue cysts from a corvid’s brain had no effect on infectivity.

The sources of infection for wild felids include live prey, eviscerated tissues from hunted game, and carcasses. Many species of felids, for example the cougar (*Felis concolor*), the lion (*Panthera leo*), the bobcat (*Lynx rufus*), and the pallas cat (*Felis manul*) have been proven to shed oocysts (Dubey, 2009). In addition, wild felids may get infected in zoos if given feed with infectious stages of *T. gondii* (Dubey, 2010).

Tachyzoites cannot survive outside of the host. When tissues containing tachyzoites are eaten, the parasites are usually destroyed by the acidic environment in the stomach (Dubey, 2010). Tachyzoites may spread via blood transfusion, organ transplantation, or transplacentally from a parasitemic mother to the foetus. Tachyzoites can also infect humans by entering the cornea, buccal mucosa, or through a stab wound, thus laboratory personnel and slaughterhouse workers must follow strict hygiene standards (Dubey, 2010).

Any type of raw milk from infected animals may contain infectious tachyzoites (Tenter *et al*., 2000). For example, kittens born to queens infected with *T. gondii* can become infected via suckling (Dubey *et al*., 2009). semen from infected males can contain infectious forms of *T. gondii* and cause sexual transmission of the parasite. Lopes and colleagues (2013) demonstrated that male sheep infected with *T. gondii* produced semen that caused natural infection in seronegative ewes. Moreover, transplacental transmission occurred in the infected ewes and caused infection in lambs born to them, proving that infectious semen can cause congenital toxoplasmosis.
4.3 Epidemiology

Toxoplasma gondii infection is widespread throughout the world. Environmental conditions affect the oocyst sporulation and survival. The prevalence of T. gondii is higher in warmer climates and low-lying areas compared with colder climates and mountain regions, and the spread of the parasite seems to be favoured also in humid areas (Dubey, 2010).

Cats get infected mainly by eating tissues from infected animals. Cats that have outdoor access or live in rural areas have more possibilities to catch and eat prey, thus they are more likely to be seropositive for T. gondii. Also, in cats that are fed raw meat by their owners, the prevalence of T. gondii infection is likely higher. In a study conducted by Jokelainen and colleagues (2012b), both outdoor access and providing the cat with raw meat in its diet were significant risk factors for seropositivity with odds ratios of 1.6 and 2.0, respectively.

As humans may get infected with oocysts on unwashed hands or vegetables, hygiene and other cultural habits play a role in the transmission of the parasite (Tenter et al., 2000). Health education could have an effect on the spread of T. gondii. Several outbreaks of toxoplasmosis in humans have been reported. The outbreaks are most commonly associated with consumption of contaminated drinking water or raw and undercooked meat, but have also resulted from handling or inhalation of soil or dust contaminated by cat faeces (Dubey, 2010).

In humans, eating and cooking habits also influence the spread of infection (Tenter et al., 2000). The prevalence of T. gondii in humans may be related to the prevalence of infection in food animals and consumption of raw or undercooked meat. In addition to meat of domestic animals, toxoplasmosis can be acquired from game meat. Privately consumed game is not subject to meat inspection in Estonia. Moreover, the current inspection does not attempt to detect T. gondii. Wild animals that carry T. gondii in their tissues may pose a risk for human infection when their carcasses are eviscerated or handled and when their insufficiently cooked meat is eaten (Jokelainen, 2013). Little is known about the prevalence of the infection in wild animals used for human consumption in Estonia. In a study conducted by Velström et al. (2013), 113 (24.0%) of 471 Estonian wild boars were defined as antibody positive for T. gondii. Seropositive animals are considered to harbour the infective stages of the parasite and can pose a risk for human infection.

Toxoplasma gondii has been shown to be common and endemic in Finland. In all host species examined in Finnish studies (domestic sheep (Ovis aries), moose (Alces alces), white-tailed deer (Odocoileus virginianus), roe deer (Capreolus capreolus), farmed wild boars (Sus scrofa),
European brown hare (*Lepus europaeus*), mountain hare (*Lepus timidus*), cats, Eurasian lynx (*Lynx lynx*), humans), antibodies against *T. gondii* were detected (Jokelainen, 2013). In Finnish moose, the latitude gradient observed in the prevalence of *T. gondii* was striking: seroprevalence in the south was over 15 times higher than in the north (Jokelainen *et al.*, 2010). A similar geographical north-south gradient in seroprevalence was detected in domestic sheep, farmed wild boars, and lynx (Jokelainen *et al.*, 2010; 2012a; 2013a).

The only wild cat living in the northern parts of Europe (Estonia, Finland, Sweden, and Norway) is the Eurasian lynx, which could potentially be a definitive host for *T. gondii*. In Finland, most of the wild lynx examined had serologic evidence of natural exposure to *T. gondii*, 86.1% of the 337 lynx hunted and examined were seropositive, but none of them was shedding oocysts at the time of sampling (Jokelainen *et al.*, 2013a). Heavier and older lynx were more often seropositive than lighter and younger lynx, and a north-south gradient in anti-*T. gondii* antibody prevalence was reported. However, no clinical or fatal toxoplasmosis was found in the lynx in the Finnish database. From Russia and Baltic countries, data on *T. gondii* infections in lynx are lacking (Jokelainen *et al.*, 2013a).

### 4.4 Host–parasite Relationships

#### 4.4.1 The Disease

The disease caused by *T. gondii* is called toxoplasmosis. Animals and humans mainly acquire toxoplasmosis naturally by consuming tissue cysts in infected meat, or food and water contaminated with oocysts from feline faeces. Bradyzoites from tissue cysts and sporozoites from oocysts multiply first in the intestinal epithelial cells (Dubey, 2010). The parasites spread to lymph nodes and other organs by lymph and blood circulation. Where tachyzoites multiply, organ damage is caused by focal areas of necrosis. Necrosis is often followed by inflammation. Clinical signs are associated with damage to various organs (Dubey, 2010; Dubey and Lappin, 2012).

Toxoplasmosis may be acute and cause severe clinical signs or even the host’s death (Jokelainen, 2013). More often the parasite does not cause any clinical signs, while the host mounts an effective immune response. Humoral antibodies against *T. gondii* are produced and tachyzoites begin to disappear from tissues. Simultaneously, tissue cysts containing bradyzoites are formed. Reactivation of a dormant infection can also occur, but when and why this may happen is often not known (Dubey, 2010).
Some host species (e.g. rats, cattle, Old World monkeys and horses) are more resistant to toxoplasmosis, while other species (especially Australian marsupials, European brown hares, mountain hares, Eurasian red squirrels (*Sciurus vulgaris*), and New World monkeys) are highly susceptible (Dubey, 2010; Jokelainen *et al*., 2011; Jokelainen and Nylund, 2012; Jokelainen, 2013). This variation might be associated with ecology, genetics and evolution. Moreover, susceptibility varies among individuals of one species, depending on age and other factors. The infection dose, infection route, and strain of the parasite may also cause differences in susceptibility (Dubey, 2010; Jokelainen, 2013).

### 4.4.2 Immunological Responses of the Host

*Toxoplasma gondii* is highly immunogenic and the host’s immunological mechanisms are complex (Dubey, 2010). *Toxoplasma gondii* is an intracellular parasite and cellular immunity is considered to be the most important response against it. However, humoral immunity also has a significant role in shaping the immune responses. Immunological responses are mainly mediated by lymphoid immune cells. The most important cytokine is interferon gamma. Humoral antibodies are effective against extracellular, but not intracellular parasites (Dubey, 2010).

Despite the host’s immunological responses to *T. gondii*, the infection usually becomes chronic and the host remains permanently infected. These responses are nevertheless needed for a balanced co-existence (Maubon *et al*., 2008). If the responses were highly efficient, the parasites would be killed and no co-existence would follow. If the response is insufficient, the parasites can proliferate uncontrollably and the host can die together with the parasites. Chronically infected hosts serve as an amplifying reservoir and, by migrating or travelling, they may transmit the parasite to new areas (Jokelainen, 2013). They harbour the parasite in their tissues for the rest of their lives (Dubey, 2010).

In immunocompetent mothers who have been previously, before conception, infected with *T. gondii*, immune mechanisms usually prevent transmission of the infection to foetuses (Elbez-Rubinstein *et al*., 2009). However, the immunity produced in response to *T. gondii* infection has been proven not to be fully protective. Congenital toxoplasmosis has been described in female animals and women who have been seropositive before conception. Reinfection of the seropositive mother may be caused by infection with another parasite strain (Elbez-Rubinstein *et al*., 2009), but it can also be due to reactivation of a chronic infection (Jokelainen, 2013). Women in Europe, for example, who are infected with a strain endemic to Europe, may be reinfected with an atypical strain from South-America and if that happens during pregnancy, the foetus may be congenitally infected (Elbez-Rubenstein *et al*., 2009).
4.5 Toxoplasmosis in Humans

4.5.1 Epidemiology

Seroprevalence of *T. gondii* is different in populations and it is thought to correlate with eating and hygiene habits, since a major source of infection is the oral route. Infections are found on all continents and up to a third of the world’s human population is estimated to have been exposed to the parasite (Tenter *et al*., 2000). In many countries, the prevalence of antibodies to *T. gondii* has decreased over the past decades (Jokelainen, 2013).

Incidence and prevalence of the infection in man varies with geographic regions and the population group. Seroprevalence is higher in warm climates, wet areas and low-lying areas than in cold climates, dry areas and mountainous regions (Dubey, 2010). Higher seroprevalence is associated with older age (Montoya and Liesenfeld, 2004). The infection is more common in abattoir workers, waste pickers, garbage handlers, and in humans who have experienced frequent contact with animals or soil (Dubey, 2010).

In Estonia, according to European Food Safety Authority EFSA, European Centre for Disease Prevention and Control ECDC (2013), the number of human cases of toxoplasmosis per year has varied. Data concerning human cases is available since 1997. The highest reported incidence rate has been 16 cases per year, in 2004. Terviseamet reports that in 2011, 2012, and 2013, no human cases of toxoplasmosis were reported. In March 2014, one human case was detected (Terviseamet, 2014).

In a study conducted by Birgisdottir *et al*., (2006), 1016 blood samples from people living in Tartu, Reykjavik, and Uppsala were tested for *T. gondii* immunoglobulin G (IgG) antibodies by an enzyme-linked immunosorbent assay (ELISA) method. The samples were collected in 1999–2001. The overall seroprevalence of *T. gondii* antibodies was 24.0%. In Tartu, the seroprevalence (54.9%) was higher than in Uppsala and Reykjavik (23.0 and 9.8%, respectively). Most likely, the difference can be explained by different environmental contamination with *T. gondii* oocysts, hygiene practices, and sanitary standards in these countries (Birgisdottir *et al*., 2006). Janson and colleagues (2013) compared zoonotic parasite infections in Estonian veterinarians and general population, suggesting veterinarians as a risk group due to frequent contact with potentially infected animals. The seroprevalence of *T. gondii*-specific IgG antibodies was 46.2% in veterinarians and 56.4% in general population, thus the seroprevalence in veterinarians was lower. Nevertheless, the general seroprevalence of *T. gondii* in Estonia was still high.
The reported incidence of congenital toxoplasmosis in humans varies between countries and regions. According to Carlier et al. (2012), lowest incidences have been detected in the United States, Austria, Sweden, and Norway (less than one case per 10,000 live births). In Denmark, Switzerland, and the United Kingdom, the incidence has been higher (1–3 cases per 10,000 live births). Brazil, Poland, France, Belgium, and Italy have had the highest incidence of congenital toxoplasmosis (3–10 cases per 10,000 live births). In some countries, routine surveillance for toxoplasmosis is implemented, but in others, for example Estonia, surveillance is designed to detect only symptomatic toxoplasmosis. In Estonia, during the past decades, only a few sporadic cases of congenital toxoplasmosis have been diagnosed (Masso, 2012). However, since pregnant women are not routinely screened for *T. gondii* infections and congenital toxoplasmosis can be challenging to diagnose, many cases may have been undetected.

### 4.5.2 Clinical Infections

*Toxoplasma gondii* rarely causes clinical illness in immune-competent persons, but the parasite can persist in tissues for a very long time and cause a life-long infection. Clinical disease in postnatally acquired infection begins with non-specific symptoms that are common in many diseases and thus toxoplasmosis is often unrecognized. Most common symptoms in humans are lymphadenopathy (cervical and occipital), ocular signs, listlessness, fatigue, headache, fever, maculopapular rash, muscle and joint pain (Montoya and Liesenfeld, 2004; Dubey, 2010). The signs may last for one to several weeks. Weakness, lymphadenopathy and malaise may persist for months. The infection may progress and virtually all organs can be involved (Montoya and Liesenfeld, 2004; Dubey, 2010). Myalgia may proceed to myositis, fatigue can precede myocarditis or pericarditis, pneumonia, nephritis, haemolytic anaemia, hepatitis, or polyneuritis. Retinochoroiditis can cause pain and tearing, photophobia, or even loss of vision. In pregnant women, toxoplasmosis can cause abortion.

In immunosuppressed individuals, toxoplasmosis can result in life-threatening complications. Toxoplasmosis is one of the most common diseases that caused death in people with acquired immunodeficiency syndrome (AIDS) before the currently used medical interventions (Nissapatorn, 2009; Dubey, 2010). Most commonly, toxoplasmosis in the immunocompromised occurs from reactivation of a latent *T. gondii* infection. The parasite damages the central nervous system, resulting in inflammation, haemorrhages and necrosis. Most common clinical signs are headaches, disorientation, drowsiness, paresis, reflex changes, convulsions, altered mental status and coma. *T. gondii* infection in human immunodeficiency virus (HIV)-infected people can also result in pneumonia, retinochoroiditis, or orchitis. Patients who receive immunosuppressive
therapy are also at risk of developing clinical toxoplasmosis (Montoya and Liesenfeld, 2004; Dubey, 2010). The infection can be fatal when a seronegative immunosuppressed person receives an infected transplant or has infected leukocytes transfused. Toxoplasmosis can also be reactivated by malignancies like lymphoma, myeloma and leukaemia (Dubey, 2010).

A pregnant woman may transmit *T. gondii* infection transplacentally to the foetus. Typically, this follows an acute asymptomatic infection in the mother. Congenital transmission in humans can occur when the woman is newly infected during pregnancy or when she was just infected before pregnancy (Elbez-Rubinstein *et al.*, 2009). Rarely, congenital transmission can occur when an infection that was acquired before pregnancy is reactivated because of the mother’s immunocompromised state or the mother is infected with another strain of the parasite (Elbez-Rubinstein *et al.*, 2009; Jokelainen, 2013). About 30–40% of babies born of infected mothers are infected. The risk of infection is lowest (10–15%) when the mother is infected in the first trimester and highest (60–90%) in the third trimester of pregnancy (Dubey, 2010). Even though fewer babies are infected early in pregnancy, they are more severely affected. More often, the baby has acquired *T. gondii* infection later in pregnancy and the clinical signs are less severe.

Transplacental infection can cause abortion and stillbirth. An infected infant may suffer from severe diseases like encephalomyelitis, epilepsy, microcephaly, thrombocytopenia, anaemia, retinitis or retinochoroiditis, and hydrocephalus (Montoya and Liesenfeld, 2004; Dubey, 2010). Children who are infected in utero can develop clinical signs several years after birth (later in childhood or even in adult life). The most common manifestation is ocular disease. Retinochoroiditis may result in microphthalmy, cataract, strabismus, nystagmus, and total blindness (Dubey, 2010).

### 4.6 Toxoplasmosis in Cats

#### 4.6.1 Epidemiology

In feline populations, *T. gondii* infection is widespread (Dubey, 2010). Cats get infected by eating *T. gondii* tissue cysts or rarely sporulated oocysts. Postnatally acquired infections are usually subclinical. Kittens can be infected congenitally and they are most likely to develop clinical signs of toxoplasmosis (Elmore *et al.*, 2010). Maternal antibodies in kittens can be detected until 12 weeks of age, after which they disappear (Dubey, 2010).

Most cats are infected with the parasite postnatally and seropositivity increases with the age of the cat. In Finland, *T. gondii* specific IgG antibodies were detected in 48.4% of cats (Jokelainen...
et al., 2012b). In the study, 445 purebred cats and 45 shelter cats were tested for seropositivity with a direct agglutination test. In Finnish cats, the odds of testing seropositive were about three times higher in adults than in those under one year of age. The odds of testing seropositive increased by 20% for every year increase in the animal’s age (Jokelainen et al., 2012b). In Latvia, 51.6% of cats were seropositive for *T. gondii*-specific antibodies and a positive correlation between the cat’s age and seroprevalence was also found (Deksne et al., 2013).

Seroprevalence of *T. gondii* varies in different feline populations, depending on the lifestyle and diet of the cats. Domestic cats usually have a lower seroprevalence than feral cats. Seroprevalence is higher in cats who are fed raw meat or who hunt for their food. In the aforementioned Finnish study (Jokelainen et al., 2012b), outdoor access and raw meat in the diet were important risk factors for seropositivity in domestic cats. Also, differences in seroprevalence between cat breeds were detected, but they could be due to different lifestyles (Jokelainen et al., 2012b). In the Latvian study, there were significant positive correlations between the seroprevalence and outdoor access with an odds ratio of 5.8 (Deksne et al., 2013). Possible associations with infections of other pathogens that induce immunosuppression in cats, for example feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) infections, have been suggested (Dubey 2010).

No official programmes have been developed for *T. gondii* monitoring in animals in Estonia. Cats are tested only when toxoplasmosis is suspected. In 2008–2010, no animal cases were detected in Estonia. In 2011, there were four reported cases in cats (European Food Safety Authority EFSA, European Centre for Disease Prevention and Control ECDC, 2013). According to Estonian Veterinary and Food Laboratory, no positive cases were reported in cats in 2012. In 2013, one seropositive cat was detected with a method based on agglutination reaction and one cat was reported to shed oocysts (Veterinaar- ja Toidulaboratorioorium, 2014).

### 4.6.2 Clinical Infections

In general, clinical signs of *T. gondii* infection in healthy adult cats are uncommon. However, any cat may develop clinical toxoplasmosis, with any organ involved, and even die of toxoplasmosis (Dubey and Lappin, 2012). In a Finnish study, the proportional mortality rate from toxoplasmosis in cats submitted for post-mortem examination was 3.1% (Jokelainen et al., 2012b).

The most common clinical signs are apathy, fever, anorexia, dyspnoea and tachypnoea, signs attributable to hepatitis and pancreatitis (jaundice, discomfort and pain on abdominal palpation,
abdominal effusion), diarrhoea and vomiting (Dubey, 2010; Dubey and Lappin, 2012, Jokelainen et al., 2012b). The enteroepithelial development of the parasite rarely causes diarrhoea (Dubey et al., 2009). Respiratory tract disease may cause coughing, rhinitis and diffuse harsh lung sounds. Neurologic signs can be mild (ear twitching, increased affectionate behaviour, partial blindness) or severe (seizures, torticollis, total blindness, circling, hypothermia, incoordination). Uveitis and chorioretinitis are common ocular manifestations caused by *T. gondii*. Uveitis may lead to glaucoma. Examples of ocular signs are aqueous flare, anisocoria, mydriasis, hyphaema, optic neuritis or optic nerve atrophy, and retinal haemorrhages. The parasite may cause dermal and subcutaneous nodules and ulceration or periarticular inflammation resulting in joint pain and lameness. Shifting leg lameness and stiffness of gait may occur (Dubey and Lappin, 2012).

Prenatal infection usually leads to more severe clinical signs because the kittens’ immune system is not mature yet and it cannot slow down the replication of tachyzoites effectively (Dubey et al., 2009). Kittens can be stillborn or die soon after birth. They often have ocular signs and death is usually caused by pulmonary or hepatic problems, after suffering from ascites, hepatomegaly and respiratory distress (Dubey et al., 2009; Dubey and Lappin, 2012).

Toxoplasmosis is a challenging clinical diagnosis, but should be one of the differential diagnoses, especially in young cats with acute interstitial pneumonia, acute necrotizing hepatitis, or non-suppurative meningoencephalitis (Jokelainen, 2013). When toxoplasmosis is suspected, a response to anti-toxoplasmic treatment (clindamycin, pyrimethamine, and sulfonamide) may aid in diagnosis (Lappin, 2010).

### 4.7 Diagnosis

#### 4.7.1 General Considerations

The diagnosis of toxoplasmosis is usually based on the clinical history, signs of illness, and the results of supportive laboratory tests. The signs of toxoplasmosis are not specific. For example, it has been stated that it is difficult to prove that *T. gondii* infection is responsible for a cat’s systemic illness (Javinsky, 2012).

Suitable specimens for detection or isolation of the parasite are body fluids, secretions, excretions, and tissue samples. Tissues may be sampled by biopsy or at necropsy. Aqueous humor and cerebrospinal fluid can be assessed in suspected toxoplasmic uveitis and encephalitis. *Toxoplasma gondii* infection is confirmed by directly detecting the organism in cells, body
fluids, secretions, excretions, or tissues. Also, inoculation of laboratory animals and tissue cultures can be used (Dubey, 2010).

4.7.2 Clinical Laboratory Findings

In acute systemic toxoplasmosis, routine blood parameters may be abnormal. In cats, for example, the most common haematologic findings are nonregenerative anaemia, neutrophilic leukocytosis, lymphocytosis, monocytosis, and eosinophilia (Dubey and Lappin, 2012). However, severely affected cats may be leukopenic. Biochemical abnormalities include hypoproteinaemia and hypoalbuminaemia. In chronic toxoplasmosis, hyperglobulinaemia may occur (Dubey and Lappin, 2012). Acute hepatic necrosis causes marked increases in serum bilirubin, alanine aminotransferase and aspartate aminotransferase activities. Acute muscle necrosis is differentiated by an increase in serum creatine kinase activity. Increases in serum amylase and lipase activities can be associated with pancreatitis, but these changes are inconsistent. Reduced serum total calcium (with normal serum albumin concentrations) may also occur in cats with pancreatitis. Urine analysis often shows bilirubinuria and proteinuria (Dubey and Lappin, 2012).

4.7.3 Diagnostic Imaging

Imaging studies are mainly used when cerebral or pulmonary toxoplasmosis is suspected. Myelography, magnetic resonance imaging, and computed tomography may detect multifocal or solitary lesions in the central nervous system (Walot et al., 1996). However, these findings are not pathognomonic for toxoplasmic encephalitis. A computed tomography scan can be used to demonstrate cerebral calcification in a foetus. When T. gondii infection in a foetus is suspected, repeated ultrasound examinations may reveal enlargement of cerebral ventricles, hepatic enlargement, and increased placental thickness (Montoya and Liesenfeld, 2004; Dubey, 2010).

In postnatally acquired infections, abdominal ultrasonography can be used to detect tissue or organ enlargement. Radiographic findings are unspecific, but in cats with acute toxoplasmosis, thoracic radiographs often reveal a diffuse interstitial to alveolar pattern with a “patchy” lobar distribution (Dubey, 2010). Alveolar coalescence may occur in severely affected animals, appearing on thoracic radiographs as diffuse, symmetric homogeneous increased density. Effusions can be detected both on thoracic and abdominal radiographs. Common abdominal radiographic findings are masses in the intestines and mesenteric lymph nodes (Dubey, 2010).
4.7.4 Serologic Procedures

4.7.4.1 Detection of Antibodies

Several serologic tests have been developed for the detection of immunoglobulin M (IgM), immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies formed against *T. gondii*. Immunoglobulins are best preserved at low temperatures (−20 °C or lower). None of the assays can alone confirm the diagnosis of toxoplasmosis definitively. After the first exposure to *T. gondii*, IgM levels increase and this is followed by increases in IgG levels. For example, in cats experimentally inoculated with *T. gondii*, 80% of them develop detectable IgM titers and 100% develop detectable IgG and IgA titers (Dubey and Lappin, 2012). The titer of antibodies can remain high for many years because tissue cysts stimulate a long-term humoral immune response. Recent infection can sometimes be verified by documentation of a positive IgM titer or an increasing IgG or IgA titer (fourfold) (Dubey and Lappin, 2012).

The reference serological test for human toxoplasmosis is the Sabin-Feldman dye test, which is based on a complement-mediated neutralizing type of antigen-antibody reaction (Dubey, 2010). The dye test uses live tachyzoites. It is highly sensitive and specific, but expensive and potentially unsafe. When the test serum does not contain antibodies to *T. gondii*, the organisms are stained uniformly with a dye (methylene blue). When specific antibodies are present in the test serum, cytolysis occurs, cytoplasm leaks out and tachyzoites remain unstained. The dye test is not very sensitive for diagnosing toxoplasmosis in cats (Dubey, 2010).

Agglutination tests are species independent and commercial kits are available (Dubey, 2010). For indirect haemagglutination test, erythrocytes are coated with a soluble antigen from tachyzoites. When the test serum contains antibodies, red blood cells are agglutinated. Primarily, the test measures IgG antibodies, thus it is usually negative during acute infection. Even though the test is simple and it does not require live antigen, it is not practical because of technical variables and its sensitivity is lower compared to other commonly used tests (Dubey, 2010).

The modified or direct agglutination test is a simple agglutination test. The test is commercially available (Toxo-Screen DA, bioMérieux, Charbonnières Beins, France) and it has been used extensively for the diagnosis of toxoplasmosis in animals and is highly sensitive (Dubey, 2010). As IgM antibodies are removed by adding 2-mercaptoethanol, the test detects only IgG antibodies. Serum, plasma and whole blood can be used for the test. Additionally, a specific test for IgM detection has been developed (Dubey, 2010).
In latex agglutination test, the test serum is added to soluble antigen coated on latex particles (Dubey, 2010). The test is commercially available, but it does not distinguish immunoglobulin classes. Complement fixation test is impractical because of complex procedures and lack of standardization of the test. The indirect fluorescent antibody test utilizes killed tachyzoites and fluorescent-labelled antispecies IgG. The results are viewed with a fluorescent microscope. An indirect fluorescent antibody test for IgM detection has also been developed (Dubey, 2010).

Enzyme-linked immunosorbent assay (ELISA) tests are commonly used to detect anti-*T. gondii* antibodies (Dubey, 2010). *Toxoplasma gondii* antigen is attached to a plastic surface, on which serum or plasma is added. If the specimen contains *T. gondii*-specific antibodies, they will bind to the antigen. The bound antibodies are detected by a second antibody that binds to the anti-*T. gondii* antibody. A colour change is used to identify the antibodies. Quantification of the colour that develops makes it possible to assess the reaction objectively. ELISAs specific for other types of antibodies than IgGs, for example IgMs, are also available (Dubey, 2010).

Immunoglobulin M immunoabsorbent agglutination assay test is IgM-ELISA combined with the agglutination test, where an enzyme conjugate is not needed (Dubey, 2010). Test sera are added to plates coated with antispecies IgM antibodies. After incubation and washing, whole tachyzoites are added. If the test serum contains IgM to *T. gondii*, it binds to the antispecies IgM and agglutinates the parasites. If the patient serum is negative for IgM, the parasites settle at the bottom of the well (Dubey, 2010).

In western blotting, a membrane transferred from a polyacrylamide gel is used and sera are reacted with *T. gondii* antigens on it (Remington *et al*., 2004). The reaction gives banding patterns of immunoglobulins that are compared to known molecular weight controls. Western blots of paired maternal and baby sera are useful for diagnosis of *T. gondii* infection in the foetus and newborn. The method should be used in combination with other serologic tests (for example IgM and IgA ELISA). When the newborn has acquired immunoglobulins through passive transfer from the mother before or at the time of parturition, the bands in the blots of mother and baby do not differ (other than in intensity). When the newborn is infected and produces its own IgG and IgM, the bands demonstrated in Western blots of serum from the infant are not present in blots of serum of the mother (Remington *et al*., 2004).

### 4.7.4.2 Avidity Tests

Avidity describes the strength of binding interactions between *T. gondii* antigen and specific antibody. During the acute stage of infection, IgG avidity values are low, but avidity becomes higher (stronger bonds) with the duration of infection. Tightness of the binding of the antibody to
the antigen is influenced by antigen-driven B-cell selection and it is established through chemical forces, for example hydrogen binding and electrostatic interactions (Remington et al., 2004).

In avidity tests, a protein-denaturing agent (for example urea) is used to break the antigen-antibody bond (Remington et al., 2004). The titer obtained reflects urea-resistant immunoglobulin and total immunoglobulin, and is determined using the ratios of urea-treated and urea-untreated samples (Remington et al., 2004).

The avidity test can be used to determine whether the infection with *T. gondii* is recent (within the prior four to five months) or older (Remington et al., 2004). It is most commonly used in pregnant women who have both IgG and IgM antibodies in their blood, and helps to determine whether the foetus is at high risk of *T. gondii* infection. Pregnancy and treatment of toxoplasmosis may delay the increase in avidity. Thus, a low-avidity result does not necessarily mean the patient acquired the infection recently. For proper interpretation, avidity test should be performed with a panel of other serologic tests (Remington et al., 2004).

### 4.7.5 Organism Detection

#### 4.7.5.1 Cytology

Tachyzoites can be detected in tissues and body fluids during acute toxoplasmosis. The selection of tissues to sample depends on the organs that are affected. In body fluids, inflammatory changes are usually present. The parasites can be found in blood, cerebrospinal fluid, fine-needle aspirates, and transtracheal or bronchoalveolar washings (Dubey, 2010). In cats with thoracic effusions or ascites, tachyzoites are commonly found in thoracic and peritoneal fluids.

#### 4.7.5.2 Faecal Examination

Cats may shed *T. gondii* oocysts, usually for one to two weeks after their first exposure to the parasite. While shedding, the cats are typically not clinically ill (Dubey, 2010).

Any of the standard faecal flotation techniques may be used for detection of *T. gondii* oocysts in feline faeces (Dubey, 2010). Oocysts are unsporulated and not infectious in fresh faeces. Oocysts of *T. gondii* and other similar coccidians (*Hammondia* and *Besnoitia* spp.) are morphologically indistinguishable. For definite differentiation by detection of deoxyribonucleic acid (DNA) of the parasite, faecal polymerase chain reaction (PCR) test should be used (Javinsky, 2012).
4.7.5.3 Bioassays and Inoculation of Cell Cultures

*T. gondii* can be cultivated in laboratory animals, chicken embryos, or cell cultures. The main hosts used for bioassays are mice, but hamsters, guinea pigs, and rabbits can also be used (Dubey, 2010).

Most studied strains of mice are susceptible to *T. gondii* infection. Mice may be inoculated via the subcutaneous, intraperitoneal, or oral routes with tachyzoites, bradyzoites, or oocysts (Dubey, 2010). After injection of sample material intraperitoneally, tachyzoites can be found in the peritoneal fluid and mesenteric lymph nodes. Tachyzoites may be isolated from mesenteric and intestinal lymph nodes, lungs, or brain obtained from mice that died or were killed while moribund. Films of body fluids or tissue imprints can be made and stained (Dubey, 2010).

Tachyzoites of virulent strains grow quickly and usually cause illness or even death in mice (Maubon et al., 2008). Avirulent strains replicate slowly, but the virulence of *T. gondii* may increase with frequent, rapid passages. Antibodies to *T. gondii* are developed 3 to 70 days after infection and can be found in the sera of inoculated mice (Dubey, 2010). Diagnosis should be confirmed by direct demonstration of the parasite. In survivors, tissue cysts should be sought 6–8 weeks after inoculation. After preparation, tissue cysts are easily seen in brain tissue at 100× magnification (Dubey, 2010). In cell cultures, tissue cyst yield is lower than in mice (Dubey, 2010).

Bioassays can also be conducted in cats (Dubey, 2010). Compared with mice, larger volumes of tissues can be fed to cats. For detection of *T. gondii* in samples with small numbers of tissue cysts (e.g. tissues from food animals), bioassays in cats have been used. After the multiplication of the parasite in the intestine of the cat, numerous oocysts are excreted in faeces (Dubey, 2010).

4.7.5.4 DNA Detection

Polymerase chain reaction (PCR) can be used for *T. gondii* DNA detection (Dubey, 2010). This method can be highly sensitive (a single tachyzoite can be detected), specific and fast. Many different protocols have been described. PCR can detect both acute and chronic subclinical infections. Real-time PCR can be used to quantify the DNA. Possible cross reactions between *T. gondii* and closely related parasites must be ruled out (Dubey, 2010).

4.7.5.5 Immunological Methods

For immunohistochemical staining, anti-*T. gondii* antibodies are used to detect the parasite in tissues (Jokelainen, 2013). With this method, tachyzoites can be differentiated from bradyzoites by using antibodies to stage-specific antigens (Dubey, 2010).
ELISAs for *T. gondii* antigen detection are also available, and can detect both free antigen and that bound in immune complexes. A positive result confirms *T. gondii* infection, but the circulation of antigen does not alone prove that the parasite is responsible for the clinical disease (Dubey and Lappin, 2012).

In *T. gondii*, several stage-specific surface antigens (SAGs) have been identified (Lyons *et al.*, 2002). For example, SAG1 and SAG-related sequences SRS1–SRS3 are present only on tachyzoites, but bradyzoite-specific recombinant antigen BSR4 is present only on bradyzoites. However, SAG3 is present on both stages. The development of stage-specific antibodies makes it possible to monitor the parasite’s stage conversion and differentiate acute infection from chronic infection (Lyons *et al.*, 2002).

### 4.7.6 Post-mortem Examination

Gross lesions observed in post-mortem examination of hosts with toxoplasmosis are usually unspecific (Jokelainen, 2013). Cats with systemic toxoplasmosis usually have thoracic and abdominal pathologies, for example pulmonary oedema, pneumonia, pleural effusion, hepatitis, hepatic necrosis, lymphadenomegaly, and splenomegaly (Jokelainen *et al.*, 2012b). They may also have icterus, pale mucous membranes, nasal discharge, and ocular discharge. Lesions in the brain and muscles are usually only microscopic (Jokelainen *et al.*, 2012b); e.g. non-suppurative myelitis and necrosis in the spinal cord may be seen in histopathological investigations.

### 4.8 Treatment

Drugs commonly used against *T. gondii* have beneficial action when there is active multiplication of the parasite, i.e. they suppress replication in the acute stage of the disease process. However, they are usually unable to eradicate infection and they probably have little effect on subclinical infection (Dubey, 2010).

In humans, combinations of sulfonamides (e.g. sulfadiazine) and pyrimethamine are used for therapy of toxoplasmosis (Montoya and Liesenfeld, 2004; Dubey, 2010). These drugs are synergists and exert their effect on two different steps in folic acid metabolism, inhibiting enzymes that are needed for folate biosynthetic pathways. Consequently, important biochemical processes are impaired, for example the synthesis of purines and pyrimidines is inhibited, which impairs the production of DNA. *Toxoplasma gondii* is more sensitive to the inhibition than mammalian cells: unlike its mammalian host, it cannot use preformed dietary folates (Aspinall *et al.*, 2002). Pyrimethamine should be accompanied by folinic acid or fresh brewers’ yeast.
Spiramycin is used in humans prophylactically during pregnancy to prevent transplacental transmission (Dubey, 2010).

The most effective drug for treating clinical toxoplasmosis in dogs and cats is clindamycin (Dubey and Lappin, 2012). Clindamycin binds to ribosomal subunits and inhibits protein synthesis (Beckers et al., 1995). The combination of pyrimethamine and rapid-acting sulfonamides, such as sulfadiazine, sulfamethazine or sulfamerazine, can also be used (Dubey and Lappin, 2012). In cats, the combination often results in toxicity because antifolate drugs can induce mental depression and bone marrow suppression (anaemia, leukopenia and thrombocytopenia). Side effects can often be prevented with folinic acid or brewer’s yeast, which is added to the animal’s diet (Dubey and Lappin, 2012).

Doxycycline and minocycline have also been proven to be effective in treating toxoplasmosis (Dubey and Lappin, 2012). Tetracyclines bind to ribosomal subunits and inhibit the protein synthesis of *T. gondii* in a concentration-dependent manner (Beckers et al., 1995). These drugs could be considered when side effects prevent the usage of clindamycin or antifolates or when co-infection with other pathogens sensitive to tetracyclines is present.

Several other drugs have been effective in the treatment of experimental toxoplasmosis, for example antifolates trimetrexate and piritrexim and a macrolide roxithromycin (Dubey and Lappin, 2012). For humans, macrolides azithromycin and clarithromycin have been used (Nissapatorn, 2009). Combinations, for example pyrimethamine and clindamycin or azithromycin and sulfonamides, have also been effective (Dubey and Lappin, 2012). Oocyst shedding period can be shortened by administration of clindamycin, ponazuril, toltrazuril, sulfonamides, or pyrimethamine in higher doses (Lappin, 2006; Javinsky, 2012).

In addition to *T. gondii*-specific drugs, supportive treatment should be provided as needed, despite this is rarely mentioned in literature discussing treatment options. The choice of treatment plan depends on the clinical signs and organ involvement.

### 4.9 Vaccination

Toxoplasmosis is a disease of great medical and veterinary importance, but the treatment of it is challenging. *T. gondii* vaccines might help to prevent and control the spread of the disease.

Thus far, inactivated, killed and crude antigen vaccines have not been efficacious enough to prevent toxoplasmosis in animal models (Liu et al., 2012). Attenuated live vaccines are thought to be protective, but the shelf-life of these vaccines is short, the vaccines are expensive, and the
attenuated organism could theoretically revert to a pathogenic strain. Subunit vaccines require adjuvants to enhance their immunogenicity (Liu et al., 2012). DNA vaccines can elicit cellular and humoral immune responses against toxoplasmosis, thus their usage can be a promising approach against both intracellular and extracellular parasites. Unfortunately, in higher primates and humans they generate a weak immune response (Liu et al., 2012).

Despite many attempts to find an effective vaccine that prevents toxoplasmosis, currently no vaccine is suitable for human use. Only one commercial vaccine has been licensed for use in ewes to avoid congenital infections and reduce the neonatal mortality in lambs. This vaccine uses an attenuated strain of *T. gondii* (Liu et al., 2012).

Feline *T. gondii* vaccines have been used experimentally and proven to be effective. A field trial was conducted by Mateus-Pinilla and colleagues (1999), where cats on commercial swine farms were vaccinated against *T. gondii* to reduce *T. gondii* seroprevalence in pigs. The trial lasted three years, and it was concluded that the feline *T. gondii* vaccine was effective in reducing the parasite prevalences on swine farms. Oocyst shedding in juvenile cats was decreased and the risk of *T. gondii* infection in finishing pigs was reduced. Thus, the risk of pork having infective forms of *T. gondii* was diminished.

### 4.10 Prevention and Control

Even though *T. gondii* is widespread, toxoplasmosis can be prevented. Humans should be careful when handling unprocessed meat. Infectious tissue stages of *T. gondii* are killed by water and soap, and therefore hands, utensils, appliances and all other materials should be cleaned with water and soap after contact with raw meat. Meat should be cooked thoroughly before eating and it should not be tasted during its preparation. *Toxoplasma gondii* tissue cysts are thought to be killed when the internal temperature of the meat is at least 66 °C, but to cover also other possible pathogens, a temperature of 72 °C should be reached. It appears also possible to kill *T. gondii* tissue cysts by freezing the meat (Dubey, 2010). *Toxoplasma gondii* oocysts in drinking water can be inactivated with UV irradiation, but the effectiveness of this method depends on the UV dose (Ware et al., 2010).

Gloves should be worn when gardening, changing cat litter, or handling potentially contaminated soil. Oocysts need 1–5 days for sporulation (Dubey, 2010), thus cat faeces should be disposed every day. All vegetables have to be washed before consumption. Especially people of the risk groups, pregnant women and immunocompromised persons, should not clean cat litter boxes, and contact with soil or raw meat should be avoided (Dubey, 2010).
In general, feline populations should be controlled with responsibility: domestic cats are not only predators but also have a role in spreading some infections. Pet cats should be kept indoors in order to keep them from ingesting prey infected with *T. gondii* and shedding oocysts in the environment (Jokelainen, 2013). Felids should not be fed raw meat or viscera. In zoos, felids should be housed separately from other animals (Dubey, 2010). All equipment that has been used to clean cat cages and litter boxes can be autoclaved or heated to 70 °C for at least 10 minutes to kill possible infectious parasites (Dubey, 2010). On farms, all dead animals, foetal membranes, and dead foetuses must be disposed of following local regulations, buried or incinerated (Dubey, 2010).
5 AIMS OF THE STUDY

The general aim of this study was to estimate the seroprevalence of *T. gondii* in cats in Estonia and to compare it with the results available from neighbouring countries. The specific aims were:

1) to estimate the prevalence of specific anti-*T. gondii* IgG antibodies in pet cats and shelter cats in Estonia;

2) to determine and evaluate risk factors for *T. gondii* infections in cats in Estonia.
6 MATERIALS AND METHODS

6.1 Study Design and Sampling

The study was a nationwide epidemiological cross-sectional study of naturally-acquired *T. gondii* infections in cats. Serology was used to reveal the proportion of cats that had previously encountered *T. gondii*. The null hypothesis was that the overall seroprevalence in cats in Estonia would not differ from the seroprevalences in cats reported from Finland and Latvia (Jokelainen *et al.*, 2012b; Deksne *et al.*, 2013).

The study area was Estonia. The sampling was carried out in 2013 (1 January–31 December). Two subgroups of the Estonian cat population (pet cats and shelter cats) were investigated.

The domestic cat population in Estonia comprises approximately 230 000–240 000 pet cats (Royal Canin representative, personal communication, 2012). Feral cat population is present, but there is no data about the population size.

The sample size for seroprevalence in cats was calculated beforehand by using OpenEpi software (Dean *et al.*, 2014). The calculation was based on expected seroprevalence of 50%. The target sample size required for the desired precision of the estimate of seroprevalence was 384 cats (95% confidence level).

The blood samples were leftover diagnostic samples, taken by veterinarians. No cat blood was drawn solely for this study. The plasma or serum samples from pet cats came from four small animal clinics located in Tartu. The samples were sent to Clinical Biochemistry and Haematology Laboratory of the Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences for haematological and biochemical analyses. The serum samples from shelter cats were taken from cats located in Shelter for Homeless Animals of Tartu. The sera and plasmas were separated and stored at −20°C until analysed.

At the beginning of sample collection, a popular science paper on *T. gondii* was published in the magazine Lemmik (Jokelainen *et al.*, 2013b). In the article, this study was mentioned. Veterinarians and cat owners were asked to participate in the study. The study was also advertised in online blogs, where the consent forms and questionnaires were available as well (http://parasitoloogiaestis.blogspot.com, http://parasitologyestonia.blogspot.com).
6.2 Questionnaires and Consent Forms

The questionnaires for risk factor evaluation were distributed to veterinarians working in the contributing clinics and the shelter. For pet cats and shelter cats, the questionnaires were different. The questionnaires for pet cat owners (Appendix I) included the cat’s signalment (date of birth, place of residence, sex, and breed) and lifestyle (diet, outdoor access, and hunting). For shelter cats, the questionnaires (Appendix II) included the cat’s sex, approximate age (kitten or adult), place where the cat was found, and breed. Cats younger than 12 months were regarded as kittens and cats older than one year were considered to be adults. For shelter cats, results of the test for FIV antibodies and FeLV antigen were also recorded (FASTest® FeLV-FIV, MEGACOR Diagnostik GmbH, Hörbranz, Austria).

The veterinarians asked the owners to fill a consent form along with the questionnaire. In addition, the EMÜ Small-animal Clinic has a general consent form that all clients are asked to fill in, thus a separate form for this study was not necessary there. At the other three clinics, the informed consent forms designed for this study were used. These also provided relevant information about this study to the owners.

The consent forms and all information obtained from cat owners in the questionnaires are stored securely and treated confidentially. The samples are stored coded.

6.3 Serology

The stored plasma and serum samples were analysed for anti-*T. gondii* antibodies. A commercial direct agglutination test (Toxo-Screen DA, bioMérieux, Marcy-L’Étoile, France), a screening test for specific anti-*T. gondii* IgG antibodies, was chosen as the serology method for this study. The principle of this method is the agglutination of formalin-treated *T. gondii* parasites if the sample contains specific IgG antibodies. Possible IgM antibodies are denatured by 2-mercaptoethanol.

The method was performed according to the manufacturer’s instructions. All plates included the negative and positive controls provided in the kit at two dilutions: 1:40 and 1:4000. The antigen control (all reagents but the serum) was carried out in two wells on each plate. The results were read after 18 hours and a metal box with a mirror inside was used to read the results from below the plate. Good lightning eliminated any problems caused by background colour in haemolysed samples.
All the samples were diluted to 1:40 and this dilution was the cut-off for seropositivity. We used a four-point scale to record the results (Figure 3):

0 – a button → negative

1 – a ring or mat covering less than half of the bottom of the well → negative

2 – a large mat covering at least half of the bottom of the well → positive

3 – an unshrunken mat covering the majority of the bottom of the well → positive.

Figure 3. Toxo-Screen DA test plate and the four-point scale for recording of results (0 and 1 – negative; 2 and 3 – positive).

These results were further interpreted as a dichotomous outcome: seropositive = 1 and seronegative = 0. Only clear positives were considered positive. All the rest, including the ones that showed borderline reactions, were considered negative.

6.4 Statistical Analysis

For evaluation of simple associations and for comparing binominal proportions, 2 × 2 tables and test statistics of open source software for epidemiological statistics were used (Dean et al., 2014). Confidence intervals were computed using mid-P exact (Lydersen et al., 2009). Two-tailed P-values ($P$) <0.05 were considered statistically significant. In risk factor analyses, cats for
which relevant information concerning a particular risk factor was not known (data not given in
the questionnaires) were excluded, thus the $2 \times 2$ tables contained different total numbers of cats.

The charts and tables were created using Microsoft Excel 2013. The same program was used for
descriptive statistics of the age categories.
7 RESULTS

7.1 Toxoplasma gondii Seroprevalence

In this study, 490 plasma or serum samples from cats were analysed: 306 (62.45%) of the samples were from pet cats and 184 (37.55%) were from shelter cats. Specific anti-\textit{T. gondii} IgG antibodies were detected in 298 of 490 cats at the sample dilution of 1:40. The seroprevalence was 60.82%.

Of the 184 shelter cats, 105 were seropositive for anti-\textit{T. gondii} IgG antibodies, thus the seroprevalence in shelter cats was 57.07%. Among the 306 pet cats, 193 were seropositive, and the seroprevalence of pet cats was thus 63.07%. The difference between the seroprevalences in pet cats and shelter cats was not statistically significant.

7.2 Risk Factor Analysis

7.2.1 Age

For most shelter cats, the exact age was not known and in the questionnaires, it was indicated whether the cat was a kitten or an adult. Nine shelter cats were known to be approximately one year of age, five were known to be two years old. Twelve cats from shelter were kittens. For ten shelter cats, the age-group (whether it was a kitten or an adult) was not known. For all pet cats, the age was given in years. Eighteen pet cats were kittens. Thus, 30 cats that were included in the study were less than one year of age. The mean age of cats included in the study was 7.14 years, the median age was 7 years and mode was 1 year. The youngest among the cats whose age was known were 3-month-old kittens. The oldest cats were 18 years old. The age-distribution is shown in Figure 4.

![Figure 4](Image)

Figure 4. A histogram of the age-distribution of the cats included in the study.
Five of the 30 kittens tested seropositive (16.67%). Of 450 adult cats, 286 tested seropositive (63.56%). Adult age was a significant risk factor when shelter cats and pet cats were analysed in one 2 × 2 table ($P < 0.001$) with an odds ratio of 8.68 (95% CI 3.43–25.94).

In shelter cats, seropositivity among kittens was 16.67% (95% CI 3.50–46.00%) and among adults, it was 59.26% (95% CI 51.56–66.53%). Adult shelter cats were significantly more often seropositive ($P < 0.01$) with an odds ratio of 7.20 (95% CI 1.69–49.65). In pet adult cats, the seroprevalence (65.32%, 95% CI 60.32–71.21%) was significantly higher ($P < 0.001$) than the seroprevalence in pet kittens (16.67%, 95% CI 5.01–40.05%) with an odds ratio of 9.62 (95% CI 2.93–42.48).

Seroprevalences for each age in years were calculated with 95% confidence intervals. The results are depicted in Figure 5.

Figure 5. *Toxoplasma gondii* seroprevalence (with 95% confidence intervals) by age in years, in cats in Estonia.
7.2.2 Location

Due to the location of the contributing small animal clinics and shelters in Tartu, 299 cats included in the study were from Tartu and altogether 365 cats were from Tartumaa. For some cats, place of residence or place where the cat was found was not given in the questionnaire.

The seroprevalence in cats that did not live in Tartu (66.42%) was significantly higher than in cats that lived in Tartu (54.52%; \( P < 0.05 \)). The lifestyle of these two groups was not significantly different (58.59% of cats that did not live in Tartu and 52.17% of cats that were from Tartu had outdoor access). The two groups differed significantly when their hunting was compared (59.38% of cats that were not from Tartu and 52.17% of cats from Tartu had been hunting, \( P < 0.05 \)). The seroprevalence of cats that lived in Tartumaa was lower but did not differ significantly from that of the cats from other counties.

When cats that lived in a town were compared with those that did not live in a town, their \( T. gondii \) seroprevalence was significantly lower (55.17%). In cats that lived or were found in other places than towns, the seroprevalence (71.43%) was significantly higher than the overall prevalence. Living outside of a town was a significant risk factor (\( P < 0.01 \)) with an odds ratio of 2.03 (95% CI 1.22–3.45). These two groups also differed significantly when their hunting was compared (63.79% of cats that did not live in a town and 47.92% of cats that lived in a town had been hunting; \( P < 0.05 \)).

7.2.3 Sex

The gender distribution in the sample was balanced: 224 of the cats were female and 251 were male. In shelter cats, the proportion of female cats was higher, whereas in pet cats, there were more males. For 15 cats, sex was unknown. The prevalence of anti-\( T. gondii \) antibodies was higher in male cats (61.75%) compared with females (58.93%), but the difference was not statistically significant. When the seroprevalences in sexes were analysed separately in shelter and pet cats, the differences were not significant either.

7.2.4 Lifestyle

The questionnaire for pet cats contained a question about the cat’s outdoor access. All cats who had outdoor access, whether supervised by the owner or not, were analysed as one group. For 32 of the pet cats, information on outdoor access was not available.

As 64 of the 128 cats with indoor lifestyle were seropositive, the prevalence of anti-\( T. gondii \) antibodies in them was 50.00%. The seroprevalence was significantly higher in cats that had
outdoor access \( (P < 0.001) \): 106 of 146 cats tested positive and the seroprevalence was 72.60%.
That is, outdoor access was a significant risk factor for \( T. gondii \) infection \( (P < 0.001) \) with an
odds ratio of 2.64 (95% CI 1.60–4.39).

All pet cats who had caught a prey at least once in their lifetime were regarded as hunters. In 41
questionnaires, information on hunting was not given. Cats that did not hunt had a significantly
lower seroprevalence (50.38%) compared with the cats that were reported to be hunters
(73.88%). Thus, hunting was also a significant risk factor for \( T. gondii \) seropositivity \( (P < 0.001) \)
with an odds ratio of 2.78 (95% CI 1.66–4.68).

7.2.5 Diet

Owners were asked whether they give their cats raw meat or not. Altogether 49 questionnaires
did not contain this information on the cat’s diet. The seroprevalence in cats receiving raw meat
was higher (68.18%) than the seroprevalence in cats that did not receive raw meat as part of their
diet (57.82%), but the difference was not significant.

7.2.6 Breed

Altogether 67 of the cats in the study were purebred cats of various breeds. Most of the cats, 390,
were domestic, and for 33 cats, breed was not known. Seroprevalence in domestic cats was
63.33%, which was significantly higher \( (P < 0.001) \) than seroprevalence in purebred cats
(37.31%; odds ratio 2.90, 95% CI 1.70–5.01). Among shelter cats, when analysed separately, the
difference between seroprevalences in purebred (57.14%) and domestic cats (56.29%) was not
significant. In pet cats, the seroprevalence in domestic cats (68.61%) was significantly higher
\( (P < 0.001) \) than in purebred cats (35.00%) with an odds ratio of 4.04 (95% CI 2.22–7.47).

In the study, 13 different cat breeds were represented. Cats of eight breeds were not statistically
analysed separately due to small numbers. The five most numerous breeds were the Persian, the
British Shorthair, the Siamese, the Scottish Fold, and the Sphynx. The seroprevalences in these
five breeds are shown in Table 1. The seroprevalences were compared with each other and with
the overall seroprevalence (the 33 cats with unknown breed status were excluded).

The breed with the largest number of representatives was the Persian. Six of them were
seropositive and 12 were seronegative, thus the seroprevalence in the Persian was 33.33%.
Among the British Shorthairs, three cats were seropositive and eight were seronegative
(seroprevalence 27.27%). In these two breeds, the seroprevalence was significantly lower than
the overall estimate \( (P < 0.05) \). The Siamese was represented by 11 individuals, eight of which
were seropositive, so the seroprevalence was 72.73%, which did not differ significantly from the general population. The lowest seroprevalence among these five breeds was in the Scottish Fold, as all seven cats tested seronegative (0.00%). *Toxoplasma gondii* seroprevalence in the Scottish Fold was significantly lower than the prevalence in the general cat population (*P* < 0.01). The highest seroprevalence among the five breeds was in the Sphynx – four out of five were seropositive (80.00%), but the difference when compared with the general estimate was not significant.

When seroprevalences between the breeds were compared, three differences were found to be significant. Seroprevalence in the Siamese was significantly higher than in the British Shorthair (*P* < 0.05) as well as than in the Scottish Fold (*P* < 0.01). Anti-*T. gondii* IgG seroprevalence was significantly higher in the Sphynx when compared with the Scottish Fold (*P* < 0.05). When each of the five most numerous breeds was compared to other purebred cats, the only significant difference was in the Siamese (*P* < 0.05), thus among purebred cats, the Siamese breed was a significant risk factor with an odds ratio of 5.94 (95% CI 1.44–30.64).

The lifestyle of purebred and domestic cats differed significantly. Of the cats with lifestyle information available, 28.08% of purebred cats had outdoor access, whereas 59.91% of domestic cats could go outside (*P* < 0.001). Of the 55 purebred cats with known hunting status, 12 (21.82%) had been hunting, whereas of the 210 domestic cats, 122 (58.10%) were hunters (*P* < 0.001).

### 7.2.7 FIV and FeLV Infections

The FeLV and FIV status was known for most of the shelter cats. Fourteen shelter cats had not been tested for FeLV and FIV infections. Seroprevalence in the 26 FIV-positive cats was higher (69.23%) than in FIV-negative cats (55.56%), but the difference was not significant (Table 1). Two of the cats tested for FeLV were FeLV-positive, and one of them was also seropositive for *T. gondii.*
Table 1. *Toxoplasma gondii* seroprevalence in cats in Estonia, by selected categories.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>N seropositive</th>
<th>Seroprevalence %</th>
<th>95% CI (mid-P exact)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet cat</td>
<td>306</td>
<td>193</td>
<td>63.07</td>
<td>57.53–68.29</td>
</tr>
<tr>
<td>Shelter cat</td>
<td>184</td>
<td>105</td>
<td>57.07</td>
<td>49.84–64.00</td>
</tr>
<tr>
<td>Kitten (&lt;1 year)</td>
<td>30</td>
<td>5</td>
<td>16.67</td>
<td>6.86–34.04</td>
</tr>
<tr>
<td>Adult (≥1 year)</td>
<td>450</td>
<td>286</td>
<td>63.56</td>
<td>59.01–67.87</td>
</tr>
<tr>
<td>Male</td>
<td>251</td>
<td>155</td>
<td>61.75</td>
<td>55.6–67.55</td>
</tr>
<tr>
<td>Female</td>
<td>224</td>
<td>132</td>
<td>58.93</td>
<td>52.39–65.17</td>
</tr>
<tr>
<td>Purebred cat</td>
<td>67</td>
<td>25</td>
<td>37.31</td>
<td>26.70–49.30</td>
</tr>
<tr>
<td>-Persian</td>
<td>18</td>
<td>6</td>
<td>33.33</td>
<td>16.1–56.43</td>
</tr>
<tr>
<td>-British Shorthair</td>
<td>11</td>
<td>3</td>
<td>27.27</td>
<td>9.205–57.1</td>
</tr>
<tr>
<td>-Siamese</td>
<td>11</td>
<td>8</td>
<td>72.72</td>
<td>42.9–90.79</td>
</tr>
<tr>
<td>-Scottish Fold</td>
<td>7</td>
<td>0</td>
<td>0.00</td>
<td>0.00–40.44</td>
</tr>
<tr>
<td>-Sphynx</td>
<td>5</td>
<td>4</td>
<td>80.00</td>
<td>35.97–97.97</td>
</tr>
<tr>
<td>Domestic cat</td>
<td>390</td>
<td>247</td>
<td>63.33</td>
<td>58.44–67.97</td>
</tr>
<tr>
<td>From Tartu</td>
<td>299</td>
<td>163</td>
<td>54.52</td>
<td>48.85–60.07</td>
</tr>
<tr>
<td>From place other than Tartu</td>
<td>134</td>
<td>89</td>
<td>66.42</td>
<td>58.05–73.87</td>
</tr>
<tr>
<td>From Tartumaa</td>
<td>365</td>
<td>210</td>
<td>57.53</td>
<td>52.41–62.50</td>
</tr>
<tr>
<td>From county other than Tartumaa</td>
<td>66</td>
<td>41</td>
<td>62.12</td>
<td>50.04–72.87</td>
</tr>
<tr>
<td>From a town</td>
<td>348</td>
<td>192</td>
<td>55.17</td>
<td>49.92–60.31</td>
</tr>
<tr>
<td>Not from a town</td>
<td>84</td>
<td>60</td>
<td>71.43</td>
<td>60.95–80.03</td>
</tr>
<tr>
<td>Indoors only</td>
<td>128</td>
<td>64</td>
<td>50.00</td>
<td>41.47–58.53</td>
</tr>
<tr>
<td>Outdoor access</td>
<td>146</td>
<td>106</td>
<td>72.60</td>
<td>64.84–79.21</td>
</tr>
<tr>
<td>Receives raw meat</td>
<td>110</td>
<td>75</td>
<td>68.18</td>
<td>58.97–76.17</td>
</tr>
<tr>
<td>No raw meat given</td>
<td>147</td>
<td>85</td>
<td>57.82</td>
<td>49.74–65.51</td>
</tr>
<tr>
<td>Has been hunting</td>
<td>134</td>
<td>99</td>
<td>73.88</td>
<td>65.82–80.61</td>
</tr>
<tr>
<td>Has never been hunting</td>
<td>131</td>
<td>66</td>
<td>50.38</td>
<td>41.93–58.81</td>
</tr>
<tr>
<td>FIV-positive</td>
<td>26</td>
<td>18</td>
<td>69.23</td>
<td>49.85–83.66</td>
</tr>
<tr>
<td>FIV-negative</td>
<td>144</td>
<td>80</td>
<td>55.56</td>
<td>47.4–63.42</td>
</tr>
<tr>
<td>FeLV-positive</td>
<td>2</td>
<td>1</td>
<td>50.00</td>
<td>4.454–90.55</td>
</tr>
<tr>
<td>FeLV-negative</td>
<td>168</td>
<td>97</td>
<td>57.74</td>
<td>50.18–64.95</td>
</tr>
<tr>
<td><strong>All</strong></td>
<td><strong>490</strong></td>
<td><strong>298</strong></td>
<td><strong>60.82</strong></td>
<td><strong>56.43–65.04</strong></td>
</tr>
</tbody>
</table>
8 DISCUSSION

8.1 Study Design

The cat is a definitive host of *T. gondii* and plays a key role in the parasite’s epidemiology. In cats in Estonia, *T. gondii* seroprevalence had not been comprehensively studied. As toxoplasmosis is a public health concern and as it can cause disease or even death in domestic as well as wild animals, information on *T. gondii* infections in cats is not only useful but necessary.

In this study, the sample size obtained exceeded the sample size calculated beforehand for seroprevalence estimation. This can be regarded as a success in sampling in co-operation with the clinics and shelter.

The obtained estimate of *T. gondii* seroprevalence (60.82%) indicates that cats of the studied population often encounter *T. gondii*. As the study was a cross-sectional study, risk factors and the presence of antibodies were measured at the same time. Whether the risky lifestyle, for example, preceded the seroconversion, is not known. Some investigated risk factors, such as breed and sex, were however time-invariant.

Even though oocyst shedding makes cats important hosts for *T. gondii*, serology gives more valuable information about the parasite than a study investigating faecal samples. Cats that are infected with *T. gondii* typically shed oocysts only once in their life and for a limited period of time. Faecal examination gives the proportion of cats shedding at the time of sampling, whereas serology provides data on the presence, prevalence, and spread of the infection (Jokelainen *et al.*, 2012b).

Most seropositive cats have already shed oocysts and caused environmental contamination with the parasite (Dubey, 2010). In this study, more than half of the cats (53.28%) with lifestyle information available had outdoor access. Majority of them were seropositive for *T. gondii*, which means that they have probably spread the oocysts into the environment at some point in their life. The high seroprevalence of *T. gondii* in cats in Estonia indicates that the parasite is widespread in the environment.

8.2 Methodology

The direct agglutination test has been widely used for screening for anti-*T. gondii* IgG antibodies in different animal species (Dubey, 2010; Jokelainen, 2013). The commercial test (Toxo-Screen DA) was chosen in order to obtain results comparable with other recently published studies. The
reading of the test results is fast and simple. In addition, the supervisors of this study were experienced with using the method.

According to the manufacturer, the sensitivity of the direct agglutination test is 96.20% (95% CI 94.55–97.39%) and the specificity is 98.80% (95% CI 96.46–99.60%) compared with the gold standard in human serology (the dye test). The specificity and sensitivity of this method for use in cats is unknown. In addition, the method has not been validated for haemolysed sera. Some of the serum and plasma samples in this study were haemolysed. However, Dubey (2010) has indicated and Jokelainen (2013) has confirmed that haemolysis does not affect the results of the direct agglutination test.

The direct agglutination test used in this study detects only IgG antibodies, which are long-lasting and thus relevant for epidemiological studies. Only one dilution was chosen for the samples, even though the manufacturer suggests using two dilutions (1:40 and 1:4000). The decision was made taking into consideration the limited sample quantities from many pet cats and the cost of the kits, as well as the limited benefit expected from using more than one dilution.

Since only the lower dilution (1:40) was used, there are expectedly some false-negative results due to the prozone phenomenon. The phenomenon occurs when the concentration of antibodies is excessively high compared to the antigen concentration. This inhibits the agglutination, as few or no antibodies bind more than one antigen particle. As a result, antibodies do not bridge between antigens and no agglutination occurs. Positivity of the sample is seen when the sample is sufficiently diluted (Jokelainen, 2013).

Statistical analysis was done using an open source software for epidemiological statistics (Dean et al., 2014), which calculates several test statistics. Mid-P Exact was selected for this work, as it has been recommended for biological studies like this one (Lydersen et al., 2009). Only simple associations were evaluated in this work, possible combined effect of several variables will be evaluated by building multivariable models later.

### 8.3 Sample Representativity and Risk Factor Analysis

#### 8.3.1 Location

The clinics and shelter that participated in the study were located in Tartu. Initially, clinics and shelters in counties other than Tartumaa were also contacted, but due to various reasons, blood samples from them were not received. Consequently, most of the cats included in the study were
from Tartumaa (84.69% of the cats with available information on living place or the place where the animal was found).

However, the seroprevalence in cats from Tartumaa did not differ significantly from the seroprevalence in cats from other counties. There were also no geographic differences in seroprevalence in wild boar (Velström et al., 2013), indicating the infection is endemic and the environmental T. gondii contamination is apparently evenly spread. Moreover, Estonia is a small country with total area of 45,227 km² (Kivilaid et al., 2014), and climatic conditions are similar in different parts of it (humid continental climate). Given that Tartu is not situated in any periphery of Estonia, the results of this study can be considered representative of the whole country.

The seroprevalence of T. gondii in cats that lived in Tartu (69.31% of the cats with available information on living or finding place) was significantly lower than the seroprevalence in cats from other places. This finding might be due to lifestyle differences, as more of the cats living outside of Tartu hunted, and hunting was identified as a significant risk factor for T. gondii infection. The same association seemed to be relevant when the seroprevalences of cats that live in a town and that do not live in a town were compared. T. gondii seroprevalence was significantly higher in cats living outside of towns, but these animals were also more likely to catch prey.

According to Jokelainen et al. (2012b), T. gondii seroprevalence in cats in Finland was 48.37%. Cats in Estonia had 1.66 times higher odds for testing seropositive for T. gondii (95% CI 1.29–2.14) and the difference in seroprevalences was significant (P < 0.001). In this study, the proportion of purebred cats was smaller, and compared with domestic cats, the purebred cats were significantly less often seropositive for T. gondii. The pet cat samples in this study were mostly taken from sick cats, whereas in the Finnish study, mostly healthy pet cats were sampled for research purposes. The health status of the cats might influence the results. Moreover, Estonia has a more southern location and higher temperatures. Toxoplasma gondii is considered to be more prevalent in southern areas and warmer climates (Dubey, 2010).

The results of this study were also compared with T. gondii seroprevalence in cats in Latvia, reported to be 51.6% (Deksne et al., 2013). Cats in Estonia had a significantly higher prevalence of anti-T. gondii antibodies (P < 0.05) with an odds ratio of 1.45 (95% CI 1.06–1.98). Similarly to this study, blood samples in the Latvian study were taken from cats at veterinary clinics for other diagnostic purposes and from shelter cats. The age of the cats included was also similar: the mean age of cats in both studies was 7.1 years. This comparison does not support the
generalization that *T. gondii* is more prevalent in warmer and more southern areas (Dubey, 2010), however the climate in the Baltic countries is quite similar. For currently unknown reasons, the prevalence of *T. gondii* in cats in Estonia is higher than in its neighbouring countries.

### 8.3.2 Age

In adult cats, *T. gondii* seropositivity was significantly higher than in young cats (<12 months old). Other studies also indicate that seropositivity increases with the age of the cat (Dubey, 2010; Jokelainen *et al*., 2012b). This indicates that the transmission of the parasite is mainly postnatal. Older cats have had longer time during which to encounter the parasite.

### 8.3.3 Breed

Altogether 67 (14.66%) out of 457 cats with known breed status were purebred cats. The cat population in Estonia is probably well represented, as most of the cats in Estonia are domestic. The seroprevalence in purebreds was significantly lower than in domestic cats. Even though there might be differences in susceptibility to *T. gondii* between purebred and domestic cats, the apparent difference might also be due to lifestyle differences. Outdoor access and hunting are both known risk factors for *T. gondii* infection, which was also confirmed in this study. The data of this study indicates that domestic cats are going outdoors and hunting more often than purebreds in Estonia.

Statistical analysis of the data in this study indicates that being of the Siamese breed is a significant risk factor among the purebred cats, and when compared with the British Shorthair and the Scottish Fold breeds. Also, being of the Sphynx breed was a significant risk factor in comparison with the Scottish Fold. The seroprevalence differences might be due to other factors than the breed differences, such as age, lifestyle or location of the living place of the cats. However, when outdoor access, hunting, and place of residence or place where the cat was found were compared, no differences that could explain these seroprevalence differences were detected.

The most numerous specific breed in the study was the Persian, followed by the Siamese, the British Shorthair, the Scottish Fold, and the Sphynx. Whether these are among the most common cat breeds in Estonia is not known because there are no statistics available on purebred cats in Estonia. When five of the most numerous breeds were compared, the one with the highest seroprevalence (the Sphynx) and the one with the lowest seroprevalence (the Scottish Fold) appeared to have similar data concerning other risk factors. All of the Scottish Folds and
Sphynxes with lifestyle information available were living indoors and had never been hunting. Most of them were living in towns, and the age distribution differences appeared minor. Thus, two different breeds with very similar lifestyles were found to have significantly different *T. gondii* seroprevalences.

However, the number of purebred cats included in this study was small. To draw any definite conclusions, more samples from purebred cats in Estonia should be analysed.

### 8.3.4 Pet and Shelter Cats

Almost 2/3 of the cats in the study were pet cats, above 1/3 were shelter cats. The proportions of these subgroups in the Estonian cat population are unknown. Since *T. gondii* seroprevalences in these two groups did not differ significantly, the overall seroprevalence estimate is likely not influenced by the sizes of the two groups included in the study, and the results can be considered representative of the whole cat population in Estonia.

Generally, the prevalence of *T. gondii* infection is higher in feral cats (Dubey, 2010). Most of the shelter cats have had outdoor access and a probably bigger proportion of them have caught prey when compared with pet cats, which should make seroprevalence in shelter cats higher, but this association was not found in this study. The proportion of kittens in shelter and pet groups did not differ significantly (7.41% of shelter cats and 6.25% of pet cats were less than one year of age). Unfortunately, for most adult shelter cats, the age was not known. It is possible the more detailed age distribution differs in the two groups, partly explaining the finding.

### 8.3.5 Lifestyle

The number of pet cats with indoor lifestyle (128 cats) was similar to the number of cats with outdoor access (146 cats). Hunting was even more equally divided, as 134 pet cats were reported to catch prey and 131 had reportedly never been hunting. Majority of the cats in Estonia are thus allowed access outdoors, and half of the cats are allowed to hunt prey.

Cats that have caught prey usually have outdoor access and the other way around, although there are exceptions. Cats that are always supervised by their owner might go outside, but never have the chance to hunt. Cats with indoor lifestyle might catch rodents living in the house. These two risk factors were analysed separately in this study and were both found to be significant. Cats with outdoor access have more opportunities to hunt, but they may also ingest oocysts, for example by eating grass contaminated with feline faeces or by drinking from water sources contaminated with feline faeces.
8.3.6 Diet

Inclusion of undercooked meat, viscera, or bones in the cat’s diet is considered an important risk factor for *T. gondii* infection (Dubey, 2010). Feeding raw meat to purebred cats was also a risk factor in a Finnish study conducted by Jokelainen *et al.* (2012b) with odds ratio of 2.0. In this study, the seroprevalences of pet cats that received raw meat in their diet and of those that did not were not significantly different, although the prevalence in cats that received raw meat was higher.

One of the reasons for the insignificance of an expectedly important risk factor in this study might be the inexactness of the diet question in the questionnaire. The word “meat” may stand for different types of animal products, for example beef, pork, and poultry, which indeed may carry the infective tissue cysts. However, eating raw fish is not considered to be a risk factor for *T. gondii* infection. Some fish (e.g. goldfish) may be infected with the parasite, but it has never been shown to multiply or persist in fish tissues (Dubey, 2010). For some of the cats included in the study, the raw meat that they received might be raw fish, if the owners understood the question differently than how it was meant.

8.3.7 Sex

The findings of this study did not show any significant difference in the seroprevalences of female and male cats. In a Finnish study (Jokelainen *et al.*, 2012b), a significantly higher seroprevalence (66.7%) of *T. gondii* was found in female cats with reproductive problems. In this study, clinical data was not analysed and such associations could not be made. The role of *T. gondii* in reproductive problems in cats as well as in other animals (e.g. sheep) in Estonia would be an interesting topic for future investigations.

8.4 Prevention of *Toxoplasma gondii* Infections

As *T. gondii* infections of cats are usually acquired during their lifetime, it is possible to prevent them. In this study, outdoor access and hunting were among the significant risk factors for *T. gondii* seropositivity. In order to prevent the infections, cats should not be allowed to hunt or roam outdoors freely. By limiting outdoor access, owners can protect their cat from becoming infected and developing possible clinical toxoplasmosis. Moreover, they protect other cats and other host species, including production animals and humans, from encountering infectious environmental forms of the parasite. Cats can spread *T. gondii* oocysts when they defaecate in
the environment. Outdoor access is risky for the cat, as well as to other hosts that share the environment with an oocyst-shedding cat.

Cats can become infected by ingesting raw meat (Dubey, 2010), and feeding the cat raw meat is a risk factor (Jokelainen, 2013). Even though raw meat in diet was not shown to be a risk factor in this study, owners should be suggested not to feed their cats raw meat. Sufficient cooking and assumedly also freezing kills the tissue-dwelling stages of *T. gondii*: cats can be provided with the nutrients they need without exposing them to infective *T. gondii* tissue cysts.

*Toxoplasma gondii* infection can be litter box derived, but the risk of acquiring the infection from cat faeces collected in litter boxes is negligible when protective hygiene measures are employed (Dabritz and Conrad, 2010). Cat faeces should be burned or collected and disposed of in garbage destined for landfills where waste material leaking into groundwater is prevented. Owners should be advised against flushing cat faeces down toilets because routinely used sewage treatments do not destroy *T. gondii* oocysts (Dabritz and Conrad, 2010; Jokelainen, 2013).

Cat owners have possibilities and a major role in preventing *T. gondii* infections. Cats are the most important host species of *T. gondii* epidemiologically, and cat owners could reduce the contamination of the environment with the parasite. Consequently, *T. gondii* infections in humans could also become less prevalent. Cats themselves also merit to be protected from the infection and the disease it can cause.

### 8.5 Possible Cases of Toxoplasmosis at EMÜ Small-animal Clinic

Toxoplasmosis is probably an underestimated disease due to its unspecific clinical signs and challenges in diagnosing the clinical disease. Veterinarians often do not include toxoplasmosis to the list of differential diagnoses. However, two interesting cases were noted by the author of this thesis at EMÜ Small-animal Clinic during the time period of January 2013–February 2014.

An 8-month-old female cat was presented with anorexia, dehydration, and lethargy that had lasted for four days. Reportedly, the cat was living indoors, had not received any raw meat in its diet, and had not been in contact with other animals. While being stabilized at the clinic, she vomited several times and was noted to have diarrhoea. Her faecal sample was sent to EMÜ Laboratory of Parasitology. Quantitative flotation was performed and the results showed 3.3 million *T. gondii*-like oocysts in one gram of faeces. After being treated for six days at the clinic, the cat was stable enough to go home. After receiving the results of faecal analysis, she was
started on a course of clindamycin (orally, 16 mg/kg twice a day). The cat’s condition improved without any drawbacks.

It must be noted that typically, cats that shed *T. gondii* oocysts are not clinically ill (Dubey, 2010) and the clinical signs of the cat presented above might have not been caused by clinical toxoplasmosis. No specific diagnostic tests were attempted to confirm or rule out toxoplasmosis as their cause. In addition, the oocysts shed were not confirmed to be *T. gondii* by, for example, faecal PCR.

For some cats included in the study, multiple serum or plasma samples taken on different times were available and analysed in 21 cats. For 20 cats, all samples had the same test results, but in one cat, the second and third result were different from the first one. The cat was an 18-year-old female that reportedly lived indoors, did not receive any raw meat in its diet, and had never caught prey. The cat was diagnosed to have pancreatitis and three blood samples were taken on different times for haematological evaluation. All of the samples were analysed in this study. The first blood sample tested negative with direct agglutination test, but the second sample, which was taken on day three, tested positive. The third sample was taken on day eight, and it also tested positive. Thus, the results indicate a seroconversion in an older cat with pancreatitis. Whether the pancreatitis was due to clinical toxoplasmosis, remained unknown.

### 8.6 Future Perspectives

The results of this study show that *T. gondii* is widely spread in cats in Estonia. Indirectly, it indicates the environment is also contaminated. It is thus likely the parasite is widely spread not only in cats, but also in other host species, including domestic animals and wildlife intended for human consumption. This is also supported by previous studies: about ¼ of wild boars in Estonia have encountered the parasite (Velström et al., 2013) and more than half of people have anti-*T. gondii* antibodies (Birgisdottir et al., 2006; Janson et al., 2013).

Few cases of *T. gondii* infections have been reported in cats in Estonia (European Food Safety Authority EFSA, European Centre for Disease Prevention and Control ECDC, 2013; Veterinaar-ja Toidulaboratorium, 2014). As the infections and toxoplasmosis are probably underdiagnosed, the awareness of this possibly fatal disease and zoonosis could be improved, both among cat owners and veterinarians. This could be done, for example, by arranging lectures and courses, publishing articles, and presenting in television and other channels such as social media. Cooperation with physicians is relevant: people should obtain similar, updated information from their family practitioners and their veterinarians.
The genotypes of *T. gondii* strains circulating in Estonia have not been determined. In Finland, genotype II is endemic. Genotype II has been considered to be of low virulence causing mainly chronic and subclinical infections, but it has been shown to kill cats as well as other host species in Europe (Spycher *et al.*, 2011; Jokelainen, 2013). When confirmed *T. gondii* oocyst shedding or clinical toxoplasmosis cases are detected in Estonia, it would be informative to determine the genotypes of the parasite strains. The same strains can infect also humans and other hosts, and as some strains can be more virulent, knowledge on local strains is relevant (Jokelainen, 2013).

In addition to the cat, there is possibly another definitive host for *T. gondii* in Estonia — the Eurasian lynx. To the author's knowledge, *T. gondii* infections in lynx in Estonia have never been studied. Two blood samples from lynx were sent to Clinical Biochemistry and Haematology Laboratory of the Institute of Veterinary Medicine and Animal Sciences during the study period and were included in the last plate of agglutination tests. The cut-off dilution for seropositivity was 1:40. Both lynx were found to be seropositive (seroprevalence 100%, 95% CI 22.36–100.00%). In Finland, the seroprevalence in lynx is 1.8 times as high as seroprevalence in cats (Jokelainen, 2013), thus a high seroprevalence is expected in lynx in Estonia. In addition, faecal samples of lynx would be interesting to analyse for *T. gondii*. Oocyst shedding in the Eurasian lynx has never been detected (Jokelainen, 2013), but as *T. gondii* seems to be widely spread in Estonia, such new findings could be yielded.
9 CONCLUSIONS

Toxoplasma gondii antibodies are very common in both pet cats and shelter cats in Estonia, and the seroprevalence is significantly higher than in cats in Finland and Latvia. This indicates that the zoonotic parasite is widely spread in its hosts as well as in the Estonian environment.

The risk factors for T. gondii seropositivity in cats in Estonia are older age, living outside of a town, hunting prey, having outdoor access, being a domestic cat, and among purebred cats, being of the Siamese breed. Seroprevalence increases by age, which indicates that feline T. gondii infections are usually postnatally acquired.
REFERENCES


http://www.vetlab.ee/?a=page&page=42f088c48f3e323aa1bbc (14.05.2014).


ACKNOWLEDGEMENTS

Thank you for your interest in this study.

Foremost, I would like to express my deep gratitude to Dr Pikka Jokelainen and Dr Brian Lassen, my supervisors, for their guidance, encouragement and immense knowledge. I have been extremely lucky to have supervisors who cared so deeply about this work. They have been patient with me and given me useful advice, answering all my questions promptly and thoroughly. I look forward to our future collaboration.

I acknowledge the contribution of the Clinical Biochemistry and Haematology Laboratory. Throughout the year, pet cat blood samples for this thesis were received, processed and collected there. I would especially like to thank the head of the laboratory, Mrs Külli Must, who helped me keep the sample registry up to date and reminded contributing veterinarians to fill the questionnaires. Her time and energy spent for this thesis are much appreciated. I am also grateful to Mrs Niina Sidorova for her contribution.

I also take this opportunity to express my profound gratitude to veterinarian Janne Orro-Taruste and the Shelter for Homeless Animals of Tartu (Tartu Koduta Loomade Varjupaik) for participating in this study. I am greatly indebted to Janne and the staff at Janne Orro Small Animal Clinic for collecting the shelter cat samples and filling the questionnaires for this thesis throughout the year 2013. I am also grateful for the pet cat samples from Janne Orro Small Animal Clinic.

I kindly thank the EMÜ Small-animal Clinic and Kristel Peetsalu, the head veterinarian of the Small-animal Clinic, for pet cat samples and filled questionnaires. I also thank small animal clinics Animal and Farmax for their participation in this study. I am grateful to all veterinarians, veterinary assistants and other staff who participated in the sample collection process and who allowed me to communicate with clients when they had questions about T. gondii or toxoplasmosis. The filled questionnaires from small animal clinic Farmax were especially amusing. Credit should be given to veterinarian Ave Kupper, who contributed to the study with the biggest number of patients: 48 cats!

I acknowledge the contribution of Pille Paats. I thank her for ensuring that all necessary equipment and reagents were available in the Laboratory of Parasitology. Working together with her has been enjoyable and refreshing. I would like to extend my thanks to Maarja Tagel, who kindly demonstrated the agglutination test to me and Pille.
The Society for the Advancement of Estonian Studies in Canada, Ellen and Eduard Kurvits Fund, is warmly thanked for partial financial support during this work.

I would like to acknowledge the continued support and encouragement provided by my family. I thank my parents for their unconditional love and care; I will always be grateful that they have made it possible for me and my brother to finish our university studies. I thank my brother for inspiring me and providing the much-needed entertainment during difficult times.

Finally, I wish to thank my cats for being there for me and forgiving me when I missed their feeding time because I was in the laboratory analysing samples of other cats. This thesis is dedicated to Kitu, Naatan and Murdik.
APPENDICES

Appendix I

The questionnaire about pet cats, in English and Estonian, used in the study on *Toxoplasma gondii* seroprevalence in cats in Estonia conducted in 1 January‒31 December 2013.

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**Questionnaire**

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<th>Breed:</th>
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<th>Has the cat ever gone hunting (catching small animals)?</th>
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<td>☐ no</td>
</tr>
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**Küsimustik**

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<td>☐ tõutu kodukass</td>
</tr>
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<td>☐ isane</td>
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Appendix II

The questionnaire about shelter cats, in English and Estonian, used in the study on *Toxoplasma gondii* seroprevalence in cats in Estonia conducted in 1 January–31 December 2013.

**Questionnaire**

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<td></td>
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<td>Breed:</td>
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<td></td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Found in countryside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>FeLV test:</td>
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<td></td>
</tr>
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**Küsimustik**

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