The seropositivity of *Toxoplasma gondii* in free-roaming domesticated cats in Masai Mara, Kenya

- a zoonosis perspective

Ronja Byström

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Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme

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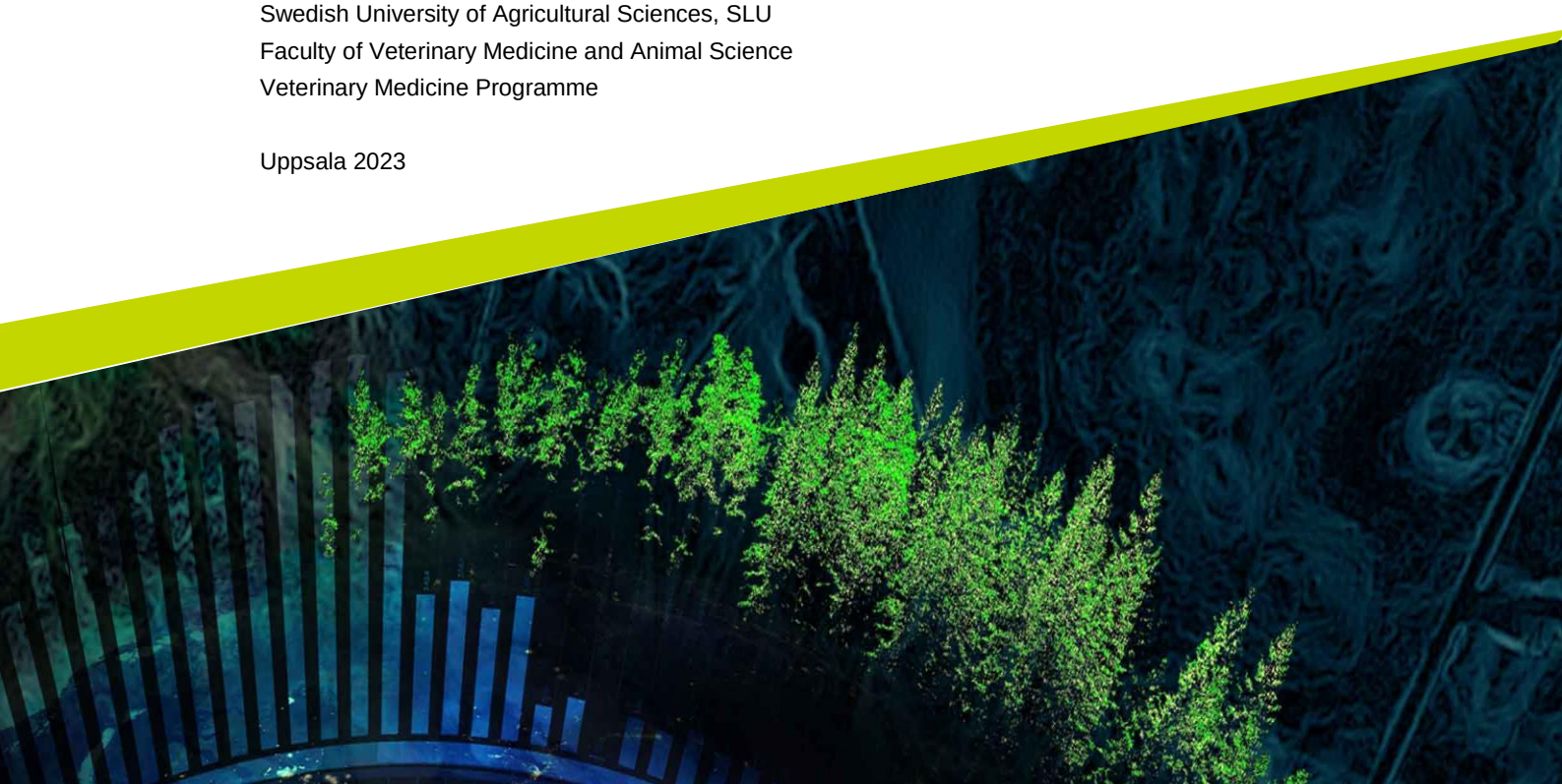

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- a zoonosis perspective

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Supervisor:	**Jane Morrell, Swedish University of Agricultural Sciences, Department of Clinical Sciences**
Assistant supervisor:	Therese Hård, Borås Djurpark/Boras Zoo, Sweden
Examiner:	Eva Axner, Swedish University of Agricultural Sciences, Department of Clinical Sciences

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Swedish University of Agricultural Sciences
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Abstract

Toxoplasma gondii is an intracellular parasite with felids as the definitive host. It can infect almost every animal, including humans, as its intermediate host. It is an important zoonosis, especially for immune-deprived people, such as patients with human immunodeficiency virus (HIV), and pregnant women. In patients with acquired immunodeficiency syndrome (AIDS) it can cause encephalitis and if a woman becomes infected during pregnancy, it can cause brain damage to the foetus or result in miscarriage. The parasite is spread throughout the world, but little research about the prevalence in cats has been carried out in African countries.

This thesis was performed in Masai Mara North Conservancy in Kenya and aimed to investigate the seroprevalence of toxoplasma IgG antibodies in domestic cats. In order to gain information regarding the local people's interactions with these cats a questionnaire was used to interview 100 households in the area. The questionnaire was performed with help from an interpreter because the majority of people did not speak or understand English.

In total 47 cats were sampled, of which 20 were males and 27 were females. Of the cats, 89.4% had IgG antibodies against toxoplasma, i.e. 90% of the males and 88.9% of the females. No statistically significant difference between the genders could be found. With sample size calculation, the seroprevalence in the area can be estimated to be 89.4 +/- 10% CI80.

The result of the interviews revealed that interactions with cats are more abundant in this community than hypothesized. Of 100 households, 69% answered that they owned one or more cats and 86.2% of non-cat households invited other peoples' cats into their homes. The majority of the households reported feeding the cats, with milk and meat being the most common food. The interviews also revealed that women have the most contact with cats.

Only 26% (26/100) believed that cats can transmit disease to humans and of them, seven households could name a disease they thought could be transmitted from cats to humans. Two of them believed that cats become venomous when ingesting a snake and that the cat then can transmit the venom to humans. Other diseases mentioned were rabies, tetanus, tuberculosis, and diarrhoea. No one mentioned toxoplasmosis and when told about the disease no one could recall hearing about it before.

The result of this report shows a high seropositivity for antibodies to *Toxoplasma gondii* in domestic cats in this area. It also showed that people, especially women, have close contact with cats and highlights the importance of education and information regarding zoonotic diseases. In addition, there was an interest from the community to know more about the topic, which would facilitate information dissemination.

Keywords: Toxoplasma, Toxoplasmosis, Domestic cat, Kenya, Masai Mara, Zoonosis, Public Health, Seropositivity, Seroprevalence

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
ECM	Extracellular matrix
HIV	Human immunodeficiency virus
MNC	Mara North Conservancy
OOPG	Oocyst per gram of feces
PVM	Parasitophorous vacuole membrane
SLU	Swedish University of Agricultural Sciences

1. Introduction

The domesticated cat (*Felis catus*) is an important and beloved pet in many households around the world, as they serve both as a companion animal and rodent control. The majority of cats have owners and are to some extent dependent on humans. But the cats' ability to adapt to various environments has made it possible for the species to survive even without human interaction (Spotte 2014), creating populations of feral and free-roaming cats on nearly every continent (Lepczyk *et al.* 2015)

A review by Lepczyk *et al.* (2015) found that feral and free-roaming domesticated cats are present in both urban and rural environments and that their interaction with wildlife, domesticated cats, and direct or indirect contact with humans are a possible disease transmission hazard for several diseases, including zoonoses.

One zoonosis where cats play an important role in transmission is toxoplasmosis (Taylor *et al.* 2007;Kochanowsky & Koshy 2018). Toxoplasmosis is caused by an intracellular protozoan parasite, *Toxoplasma gondii*, and cats are the definitive hosts for the parasite (Taylor *et al.* 2007;Kochanowsky & Koshy 2018). Infection in humans is usually subclinical, but it can cause severe symptoms in immuno-suppressed patients or pregnant women (Kochanowsky & Koshy 2018).

Toxoplasma gondii needs interaction with the cat gut-epithelium for sexual reproduction and the formation of oocysts, which are then disseminated via the feces into the environment (Kochanowsky & Koshy 2018). Transmission of toxoplasma to humans is either from consuming vegetables or water contaminated by oocysts or by ingestion of tissue cysts in farmed animals. Feral and free-roaming cats have been identified as a major transmission hazard of toxoplasmosis to humans (Nyambura Njuguna *et al.* 2017; Nisbet *et al.* 2018)

This study was conducted on feral and free-roaming domesticated cats in Masai Mara North conservancy (MNC) Kenya. MNC is a private national reserve with its main objective to improve ecosystem management (Mara North Conservancy 2017). To achieve this aim, the conservancy practices eco-tourism and has close relationships with the local Maasai population. The conservancy leases land from the Maasai people in exchange for wildlife conservation. To minimize wildlife-

human conflicts the MNC utilizes different projects to help the Maasai to protect their livestock, which in turn enables high numbers of predators, such as lions. Furthermore, MNC supports projects within the conservancy to improve wildlife and human health.

This study aims to investigate if the domesticated cat population in the area has antibodies against *Toxoplasma gondii,* and therefore could act as a source of infection. It also aims to study the interactions between humans and cats in an attempt to recognize possible transmission hazards. This is the first investigation of *Toxoplasma gondii* in this group of domesticated cats; it is a part of a larger project to increase the social status of the cat and appraise diseases important for conservancy and human health. The result of this study and other studies concerning the domesticated cat will serve as a science-based foundation for recommendations regarding human health, vaccination and/or neutering programs for the cats in MNC area.

2. Literature Review

2.1 Toxoplasma gondii

Toxoplasma gondii is a protozoan belonging to the phylum Apicomplexa, which is characterized by occurring intracellularly and using both asexual and sexual reproduction (Taylor *et al.* 2007). It goes under the family Sarcocystiidae in which the protozoans have a final and an intermediate host. Sexual reproduction occurs in the final hosts and asexual reproduction in the intermediate. Several Sarcocystiidae can infect a range of animals and are not host-specific. Toxoplasma shows a complete lack of species-specificity for intermediate hosts but can only replicate sexually in felids, which makes felids the final host.

2.1.1 The lifecycle of *Toxoplasma gondii*

The parasite uses the epithelium of the cat gut in order to differentiate into male and female gametocytes, which allows sexual reproduction (Frenkel *et al.* 1970; Kochanowsky & Koshy 2018). As a result, cats can shed millions of oocysts in their feces which then contaminate the soils and water nearby (Kochanowsky & Koshy 2018). The peak of oocyst shedding occurs approximately one week after infection, with between hundred thousand to a million oocyst per gram of feces (OOPG) (Zulpo *et al.* 2018). The oocysts are then ingested by an intermediate host (Kochanowsky & Koshy 2018). The full lifecycle is shown in Figure 1.

The intermediate host can be any animal, including humans or birds (Taylor *et al.* 2007; Centers for Disease Control and Prevention 2020). When an intermediate host gets infected, *Toxoplasma gondii* penetrates the intestinal wall and disseminates through the body by the hematogenous route (Taylor *et al.* 2007; Kochanowsky & Koshy 2018). This state of the parasite is called tachyzoite. The tachyzoites then enter a cell and replicate asexually (Taylor *et al.* 2007). When the cell contains 8-16 tachyzoites it ruptures, and new cells are infected. This is the acute phase of infection and is commonly suppressed by the host's immune system which forms antibodies against the tachyzoites. In response, the parasite forms cysts containing thousands of organisms called bradyzoites. In the cysts, the replication rate of the bradyzoites is slow and the cysts are kept intact by the host's acquired immunity; this is the latent state of the infection. If the host's immunity is compromised the cyst can rupture, releasing bradyzoites, and the parasite becomes re-activated.

The major organ for encystment in humans and rodents is the brain, but *Toxoplasma gondii* can also encyst in cardiac and skeletal muscle (Kochanowsky & Koshy

2018). The intermediate host remains infected throughout its life, as the bradyzoites lie latent in its cysts. The lifecycle of *Toxoplasma gondii* is completed when a felid ingests an intermediate host, but felids can also be infected directly by ingestion of sporulated oocysts in the environment (Dubey 1995; Centers for Disease Control and Prevention 2020). For infection route and importance in humans see the section "2.3 Toxoplasmosis in humans".

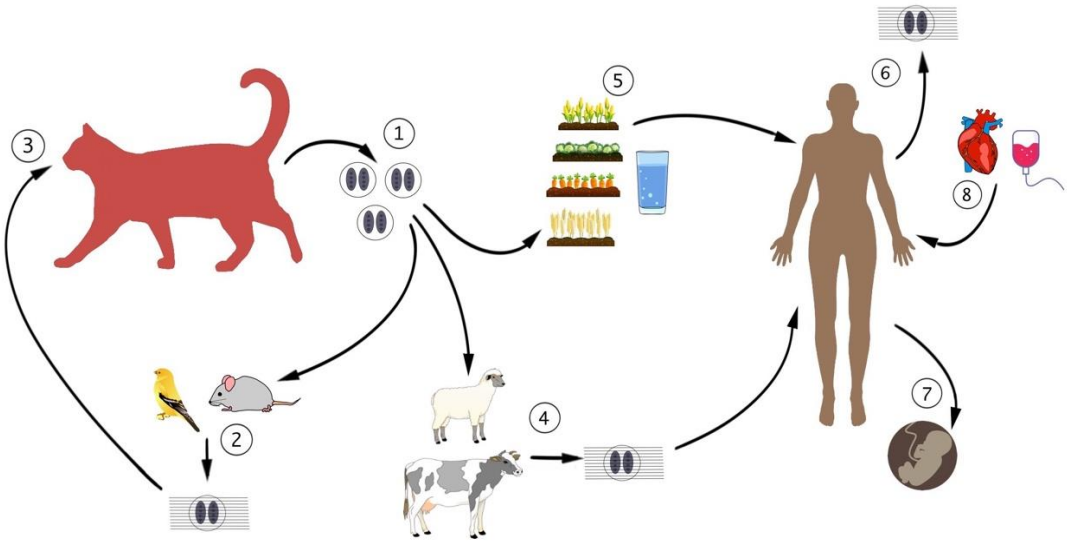

Figure 1. Lifecycle of Toxoplasma gondii. This Figure is composed by pictures from pixabay.com.

1. Oocysts shed in cat feces. 2. Oocysts ingested by prey and formation of tissue cysts. 3. Prey consumed by cat. 4. Oocysts ingested by farmed animals and formation of tissue cyst. 5. Oocysts contaminating soil and water. 6. Oocyst consumed by human either in vegetables or meat, forming tissue cysts. 7. Transplacental infection to fetus. 8. Infected transplantation organs.

2.1.2 The structure of tachyzoites and cell invasion

Tachyzoites are crescent shaped with a pointed anterior end and a rounded posterior end (Dubey 2022 pp. 7-13). They are approximately 2x6 μm and the body is covered with subpellicular microtubules arranged in a spiral. This structure is used by the protozoan for transport in the extracellular matrix (ECM) and to enter a host cell. It can move by gliding, flexing, undulating, and rotating.

The tachyzoite glides on the surface of a host cell with a twisting motion (Suss-Toby *et al.* 1996). The parasite then rearranges itself so that the anterior end attaches against the host cell membrane and the gliding stops prior to entering. The protozoan enters the cell by active penetration and endocytosis and becomes covered in a membrane called a parasitophorous vacuole membrane (PVM) (Dubey 2022 pp. 12-13) The PVM covers the parasite during the whole duration of the asexual replication phase, until the parasites become too numerous for the host cell

to support their growth. The tachyzoites then initiate their exit from the host cell by producing a certain protein (TgPLP1) (Dubey 2022 pp 15), and new cells are infected (Taylor *et al.* 2007).

2.1.3 Bradyzoites and tissue-cysts

Bradyzoites are, as described earlier, the latent phase of the infection and are found in tissue-cysts of the intermediate host (Taylor *et al.* 2007;Watts *et al.* 2015).

Watts *et al.* (2015) discovered that the tissue-cysts containing bradyzoites are more active than previously thought. The study was made *in vivo* in mouse brains and investigated the cyst wall and the density of bradyzoites within a tissue cyst. They showed that the tissue wall is more defined earlier in the latent phase and that the density decreased with increasing time of infection. In addition, the study confirmed previous work that showed an increase in cyst diameter with time. But in contrast to other reports, the mean bradyzoite count in tissue cysts was not linear to the cyst diameter. The study found that the most active period was between week 3 to 4 after infection, with an increase of 1.8-fold. The replication rate then diminishes, between weeks 5 to 8, but the diameter of the cyst still increases during the same time period. This illustrates the dynamic qualities of the bradyzoites.

2.1.4 Oocysts

The oocysts contain two sporocysts, which in turn contain four sporozoites (Frenkel *et al.* 1970;Speer *et al.* 1998) and it develops to the infectious stage, i.e. it sporulates, outside of the cats' body (Frenkel *et al.* 1970). The oocyst is covered in a three-layered wall (Speer *et al.* 1998) enabling the oocysts to survive in extreme condi-tions (Dumètre *et al.* 2013).

The oocysts can persist at 10-25°C for 200 days without losing infectivity and 13 months at 0°C in laboratory settings (Dubey 1998). At higher temperatures, the infectivity decreases depending on time and temperature. To become noninfective the oocysts require storage at 35°C for 62 days, 40°C for 20 days, 45°C for 2 days, 50°C for 120min, 55°C for 10 min and 60°C for 1 minute.

In outdoor conditions, with fluctuating temperatures between 6-35°C, the oocysts can be infective for approximately 50-400 days depending on the humidity (Yilmaz & Hopkins 1972). If the oocysts are kept moist and in shade, the parasite is infective for the longer time interval.

2.2 *Toxoplasma gondii* in cats.

Cats, like other animals, can be infected by oocysts from the environment as well as from tissue cysts in meat (Dubey 2006). However, the parasite appears to be more biologically adapted to transmission by carnivorism in cats, as a higher number of cats shed oocysts if infected by bradyzoites compared to oocysts.

2.2.1 Immunity to *Toxoplasma gondii* in cats.

The majority of adult cats do not show clinical signs of toxoplasmosis, even if they have ingested large numbers of Toxoplasma gondii (Dubey 1995). Instead, immunity develops after oocyst shedding. The duration of this immunity is not yet fully understood (Dubey 1995), and can vary between 36-77 months (Dubey 1995; Zulpo *et al.* 2018).

The immunity seems to decrease with time (Dubey 1995; Zulpo *et al.* 2018) and factors such as the nutritional status of the cat, age at primary infection, and secondary infections can impact the immunity (Dubey 1995). In addition, the strain of *Toxoplasma gondii* appears to affect immunity (Zulpo *et al.* 2018). If a cat is infected a second time with a heterologous strain the immunity is less effective (Zulpo *et al.* 2018), compared to when infected with a homologous strain, where the majority of cats do not shed oocysts (Davis & Dubey 1995; Dubey 1995; Zulpo *et al.* 2018).

Davis & Dubey (1995) reported that the acquired immunity does not prevent the early development stages of *Toxoplasma gondii* in the intestines of felids but prevents oocyst formation and oocyst shedding. The authors speculate that the antigens presented by the early stages of the parasite are not recognized by the hosts' immune system.

2.2.2 Clinical signs and diagnosis in cats

Although cats are definitive hosts for the parasite and clinical toxoplasmosis is rare in this species, it does occur occasionally (Dubey 2022 pp. 150).

According to Dubey (2022 pp 150), the most common clinical finding is pneumonia. Other symptoms include fever, anorexia, jaundice, dyspnea, and an indication of abdominal pain during abdominal palpation attributable to hepatitis or pancreatitis. Neurological symptoms have also been observed, for example, hypothermia, partial or total blindness, stupor, incoordination, circling, head bobbing, anisocoria, and seizures. The infection can be fatal in any age, gender or breed.

The diagnosis is not specific ante-mortem, but antibody tests, combined with fecal examination for detection of oocysts can be used as a tool for clinicians to obtain a probability diagnosis and initiate treatment protocols (Dubey & Prowell 2013). The diagnosis is confirmed post-mortem via histology on samples from macroscopically affected organs (Dubey 2022 pp. 150-155). Histological examination confirms the diagnosis by revealing active tachyzoites in the tissues. In research, tissues from affected organs can be used for inoculation in mice to further confirm the diagnosis.

2.2.3 Toxoplasma in feral and free-roaming cats in Africa

The prevalence of *Toxoplasma* in domestic cats varies in different countries and the geographical area within the country. In addition, the analysis methods and the number of cats vary between studies. This makes it challenging to determine the seroprevalence in a whole country and to compare results between countries (Dubey 2022 pp. 135).

A systematic review made by Montazeri *et al.* (2020), using data from 217 published articles from 1967 to 2017, attempted to find the global seroprevalence of *Toxoplasma gondii* in both domesticated and non-domesticated felids. For domesticated cats, the global seroprevalence was found to be 35% (95%CI). In addition, the review indicated the seroprevalence for each continent. In Africa this ranged from 20-80% (95%CI), with a mean of 51%, making Africa the continent with the second highest seroprevalence for domesticated cats, after Australia. However, the authors point out that there are not many published articles regarding the seroprevalence of *Toxoplasma* in domesticated cats from Africa, thus more research is required to establish a more assured prevalence. The difference and similarities between studies performed in Africa are summarized in Table 1.

To my knowledge, there is only one study in Kenya regarding *Toxoplasma gondii* in free-roaming domesticated cats (Nyambura Njuguna *et al.* 2017). The study was performed in the Thika region with an article being published 2017. The authors found that 7.8% (8/103) of the cats had oocysts in their faeces, confirmed by inoculation in mice. This study did not use antibody detection.

Table 1. Articles investigating seroprevalence of Toxoplasma gondii *in Africa.*

Reference	**Country**	**Type of cat**	**Number of cats**	**Analyse method**	**Sero-positivity***
Al-Kappany *et al.* (2010)	Egypt	Feral	158	MAT	97.4%
Al-Kappany *et al.* (2011)	Egypt	Feral	172	MAT	95.5%
Hammond-Aryee *et al.* (2015)	South Africa	Feral	159	IFAT	37.1%
Tiao *et al.* (2013)	Ethiopia	Feral	48	MAT	85.4%
Yekkour *et al.* (2017)	Algeria	Stray	96	MAT	50%

*Seropositivity presented as IgG- antibody detection.
MAT= Modified Agglutination Test
IFAT= Indirect Fluorescent Antibody Test

2.3 Toxoplasmosis in humans

2.3.1 Transmission route and infection avoidance.

Humans contract toxoplasmosis either from the environment, such as via contaminated water or vegetables or by consuming animals harbouring the bradyzoite cysts i.e. intermediate hosts (Centers for Disease Control and Prevention 2020). Thus, animals raised for human consumption or wild game are transmission hazards (Frenkel *et al.* 1970; Centers for Disease Control and Prevention 2020).

To prevent transmission via meat it needs to be heated properly (Frenkel *et al.* 1970), as the bradyzoite tissue cysts are killed if the meat is heated to at least 67°C (Dubey *et al.* 1990). Avoiding consuming undercooked or raw meat is the most secure way to avoid transmission of *Toxoplasma* via meat, and is a recommendation from authorities in Sweden, America and the United Kingdom, among others. (Folkhälsomyndigheten 2013; Centers for Disease Control and Prevention 2020; National Health Service 2020)

Toxoplasma can also infect the human fetus by congenital infection and cause miscarriage or brain damage to the fetus (Kochanowsky & Koshy 2018). In an immunocompetent woman, congenital infection of the fetus occurs if she is infected during pregnancy. If infected 4-6 months or earlier before conception, acquired

immunity will prevent vertical transmission to the foetus if the woman is exposed to the parasite once again during pregnancy (Tenter *et al.* 2000). The exception is in immunocompromised women, where vertical transmission has occurred even though the person was infected before pregnancy.

Another route of infection is through organ transplantation (Derouin & Pelloux 2008). The recipient can contract toxoplasmosis either by receiving an infected organ or by reactivation of latent toxoplasma because of the immunosuppressive medicine used before and after the transplantation. Because of this, several countries screen both donor and recipient for toxoplasma.

2.3.2 Symptoms in humans.

Infection in humans usually goes unnoticed or it can cause influenza-like symptoms in the acute phase of infection. The infection then becomes latent in tissue cysts but can cause symptoms later in life if the immune system is compromised. (Kochanowsky & Koshy 2018).

People with acquired immunodeficiency syndrome (AIDS) are one group that is especially at risk to develop severe symptoms due to their highly suppressed immune status (Kochanowsky & Koshy 2018). Up to 40% of AIDS patients world-wide develop severe encephalitis due to *Toxoplasma gondii* infection, and 10-30% of AIDS patients die from toxoplasmosis (Tenter *et al.* 2000). To suppress the infection and avoid severe symptoms, people with AIDS need lifelong medication (Kochanowsky & Koshy 2018). However, the available treatments are associated with adverse side effects and toxicity, which can lead to discontinuation of therapy (Smith *et al.* 2021).

Children born with congenital acquired toxoplasmosis are usually asymptomatic at birth (Dubey 2022 pp.122-129). If symptoms appear in the neonatal period, they are often severe, and the child rarely recovers without sequela. The most common symptom later in childhood is retinochoroiditis with compromised vision, even if treated. Other signs of congenital toxoplasmosis are microphthalmia and hydro-cephalus.

3. Material and Methods

3.1 Literature search

To gain information about *Toxoplasma gondii*, articles were found using the following search words: Toxoplasma AND cat* AND Kenya, Toxoplasma AND cat* AND environment, Toxoplasma AND oocyst, Toxoplasma AND structure, Toxoplasma AND seroprevalence, Toxoplasma in humans, Toxoplasma AND Africa.

The literature was found by searching the Swedish university of agricultural sciences (SLU) library search function "primo", which in turn directs to different journals. Cited articles were also used in the literature search.

3.2 Study area and climate

The study was conducted in Mara North Conservatory (MNC) in the southwest of Kenya. MNC is located in Narok county, highlighted in orange in Figure 2. The study originated from Karen Blixen Camp (latitude 1°11'14.2''S, longitude 35°03'26.6''E), close to a small town called Mara Rianta.

Masai Mara has an annual rainfall of 1400 mm with mean relative humidity of 58.9%. The daytime average annual temperature is 27°C, while the average annual night time temperature is 15°C. The temperature, rainfall, and relative air humidity fluctuate depending on the month. The warmest month is February and the coldest is May.

Figure 2. A map with Kenya and Narok county highlighted. Created 2022 using mapchart.net. Two custom maps have been merged into one picture. https://www.mapchart.net/index.html

3.3 The domesticated cats sampled

Both free-roaming owned cats and feral cats were included in the study. Consent was obtained from the cat owners to collect blood samples and the cats were collected from their homes in the morning and brought to the clinic by a local veterinary assistant. All cats lived in Mara Rianta area, within an estimated radius of 10 km^2, calculated using freemapstool.com.

To catch the feral cats a trap was set and checked regularly. Once trapped the cat was sedated, and samples were taken in the field so that the animal could be released again in the same place where it was trapped.

3.3.1 Sedation protocol

Each cat was weighed before handling and the cats that were aggressive/scared or prone to escape were sedated with 0.1mg/kg of medetomidine. After blood collection and a health examination (reported by another student), the sedation was reversed with 0.5mg/kg atipamezole.

3.3.2 Sample collection

Approximately 1.5-2.0 ml of blood was drawn from the cephalic or jugular vein with a needle and collected in a serum tube.

3.4 Test method and serological assay

Immunoglobulin (IgG) against *Toxoplasma gondii* was detected using a commercial test kit (FASTest® TOXOPLASMA g, Megacor). The test uses a rapid immunochromatic technique, where antibodies against *Toxoplasma gondii* in the sample react with mobile monoclonal antibodies conjugated to colloidal gold particles. These complexes then migrate along the cellulose membrane and bind to fixed antibodies, forming a pink-purple test line (T) and a control line (C). The test has a 98% sensitivity and 97% specificity.

The obtained blood was centrifuged at 3000 RPM for 10 minutes and a disposable plastic pipette was used to transfer the serum to an Eppendorf tube before analysing.

Ten µl of serum was taken from the Eppendorf tube using a plastic pipette included in the test kit and placed in the sample window S in the test cassette. Immediately after that, 2 drops (ca 80-100µl) of buffer diluent were added to the sample window. The test result was read 15 minutes after the buffer diluent was added. All procedures were according to the manufacturer's instructions.

A positive result was indicated by a pink-purple line of any intensity at the test line (T) accompanied by a pink-purple control line (C). If only the control line appeared the result was interpreted as negative.

3.4.1 Statistical analysis

The statistical analysis was made using Stata/IC 16.1, United States of America. A chi-2 test was performed to investigate if there were statistically significant differences between the seropositivity rates in males and females. A $p \leq 0.05$ was considered statistically significant.

3.5 Human interaction and transmission hazards

To get a better understanding of the cats' living conditions, visits were made to the Masai people's houses. A questionnaire with open and closed questions was used to interview them via an interpreter, as most people did not understand or speak English. Data from the interview regarding the transmission of Toxoplasma is included in this study. The whole questionnaire is shown in Appendix 1. The questions included in this report are numbers 1, 2, 6, 7, 8, 11, 11.1, 11.2, 12, 12.1, 19, 19.1, 19.2, 20, and 20.1.

4. Results

4.1 The domesticated cats sampled

In total 47 adult cats were sampled, of which 27 were females and 20 males. One cat was brought to the clinic by the veterinary assistant, but at clinical examination was found to be younger than 6 months and excluded from the study due to the likelihood of maternal antibodies. The age estimate was made based on the presence of deciduous teeth. All cats, except one were estimated to be older than 1 year and younger than 5 years, based on oral examination and an overall clinical examination. Since the cat estimated to be younger than 1 year had all its adult teeth, it was judged to be >8 months and was included in the study.

Of the 47 cats, 45 were owned by the local Maasai people, and 2 feral cats were obtained around Karen Blixen Camp using the trap. Fifteen cats could be handled awake for the blood sampling. Both feral cats were sedated.

The feral cats lived close to the camp, with staff testifying that the cats were eating trash from the kitchen and staff canteen. These cats were described as wild and not friendly. Nobody in the camp had physical interactions with these cats.

4.2 Serological assay

In total, antibodies against *Toxoplasma gondii* were found in 42 (89.4%) of 47 cats. Gender distribution is shown in Table 2. Calculation of the sample size indicates that the true prevalence is likely to be 89.4 +/- 10% with 80% confidence interval. The presence of antibodies was not different between males and females (90% and 88.9% respectively); P=0.903.

Table 2. Results of antibody detection

Type of cat	Positive (%)	Negative (%)	Total
Female	24 (88.9%)	3 (11.1%)	27
Male	18 (90%)	2 (10%)	20
Total	42 (89.4%)	5 (10.6%)	47

4.3 Human interaction and transmission hazards

During the visits to the villages, the cats' habitat was observed. The free-roaming owned cats lived close to livestock such as cows, goats, hens, and sheep as these species lived close to the houses (Figure 3). The cats were seen both outside and inside the houses and seemed to come and go as they pleased. Some of the children and women were observed touching the cats, with a few children carrying them.

Figure 3. Pictures showing the home state of cats, with interactions between different species. In the pictures are cats, sheep, cows, goats, and a rooster. One of the pictures is showing a cat inside a house. Consent for taking the pictures and using them was obtained from the owners.

In total, 100 households answered the questionnaire. The majority of interviews (90%) were carried out with one person being the respondent; the other 10% were group interviews with several people from the same household. Because of this, the results are presented per household. In addition, the gender distribution was skewed, with 95% percent of the respondents being women. The reason for this is that the women are the ones who own and take care of the cats in the Maasai culture. Only 2% percent were male respondents; 3% percent included both men and women as a group.

Sixty-nine households reported that they had one or more cats, 69.5% (48/69) had one cat, 17.4% (12/69) had two, 5.8% (4/69) had three, and 7.2% (5/69) had four cats. In total there were 104 cats in the 69 households, with a mean SD of 1.5 ± 0.9 cats per household.

All cat owners allowed the cat inside the house, and 86.2% (25/29) of non-cat owners allowed other peoples' cats inside their homes. All of the households with cats reported that they gave them food, and 77.4% (24/31) of non-cat owners answered that they gave food to other people's cats.

The most common food was milk, followed by meat. Another common answer was leftovers or the same food as the family ate, which include milk, meat, rice, vegetables, and ugali depending on the day (Table 3).

Table 3. Types of food given to the cats.

Type of food	**Number of households**	**Percentage (%)**
Only milk	32	34.4
Meat and milk	30	32.2
Leftovers/same food as family	27	29
Ugali*	1	1.1
Milk and vegetables	1	1.1
Ugali, milk and vegetables	1	1.1
Meat and ugali	1	1.1
Total	93	100%

**A traditional East African food made from maizemeal and water.*

Regarding the cats hunting habits, 86% (86/100) of the respondents had seen a cat hunt small animals. The cats were reported to hunt rats, snakes, lizards, birds, frogs, grasshoppers, and chameleons in descending frequency (Table 4). The majority of households reported seeing cats hunt more than one type of prey.

Table 4. Prey observed being hunted by cats

Prey observed being hunted by cats	**Number of households**	**Percentage (%)**
Rats	75	87.2
Snakes	64	74.4
Lizards	28	32.6
Birds	10	11.6
Frogs	7	8.1
Grasshoppers	2	2.3
Chameleons	1	1.2

Only 26% (26/100) of households believed that cats can transmit disease to humans, 22% answered "I don't know" and 52% answered "no". Seven households of the ones answering "yes" named a disease that they thought could be transmitted to

humans. Other replies were tuberculosis (one person, because she had heard a cat cough); tetanus (one person); rabies (three people); diarrhoea (one person), and two households said that because cats eat snakes the venom from the snake transfers to the cat, making the cat venomous. The rest who thought that cats can transmit diseases to humans could not name any diseases in particular.

Question number 19, 19.1, and 19.2, regarding the actions taken if the cat defecated near or inside the house, were only asked if the household reported that they owned cats. Of these, 20.3% (14/69) said that the cat had never defecated inside or close to the house and 79.7% (55/69) said that they would take the faeces outside. When asked how the faeces was taken outside, 83.6% (46/55) of them reported using a broom to sweep it outside of the house. The remaining nine people had different ways of taking it outside, but all of them used a utensil of some kind and not their bare hands; for example, stick, shovel, tissue, or water and soap. One person used a container to collect the faeces in and then threw them in the toilet.

To get a better understanding regarding the cleaning procedure the cat defaecated indoor, two households were asked to show the broom that was used. One of them agreed to a picture being taken of the broom (Figure 4). According to those households and the interpreter, this was the typical type of broom used in households in the area.

Question number 7 and 8, (do people pet their cat), was also only asked if the household had cats. Thirty-six households reported petting their cats (52.2%) and 85.5% (59/69) answered that the children pet the cat. Of the 33 households answering that they did not pet the cat 82% (27) of them said that the children did.

Figure 4. The broom commonly used in Maasai homes.

5. Discussion

5.1 Serological assay

This study aimed to establish the seroprevalence of *Toxoplasma gondii* in domesticated cats in the surroundings of Mara Rianta in Masai Mara North Conservatory. As the number of cats that could be obtained for sampling was limited and the total population size was unknown, a reliable true seroprevalence could not be obtained. Based on sample size calculation the true seroprevalence can be estimated to be in the range of 89.4% ± 10 within an 80% confidence interval. The results of this study are therefore best interpreted as a high presence of antibodies against *Toxoplasma gondii*; further research would be necessary to obtain a more reliable prevalence.

The seropositivity in this study aligns with the findings in Ethiopia (Tiao *et al.* 2013), where 85.4% of the sampled feral cats had antibodies against *Toxoplasma gondii*. The results are also supported by the review by Montazeri *et al.* (2020), where the continental seroprevalence of *Toxoplasma gondii* in Africa ranges between 20 and 80%. Taking into consideration that the results of this study can vary between 79.4-99.4% with an 80% confidence interval it is also supported by the results Al-Kappany *et al.* (2010 and 2011) found in Egypt, where the seropositivity was 97.4% (2010) and 95.5% (2011) respectively.

However, in the study in South Africa (Hammond-Aryee *et al.* 2015) the seropositivity was 37.5% and Yekkour *et al.* (2017) in Algeria found a seropositivity of 50%. The difference in these findings can depend on several factors, including the test method and the habitat of the cats. Dubey (2022 pp.135) states that seropositivity varies between countries, within countries, and even within cities and that the reasons for the variations are numerous which makes a conclusion regarding the most important one impossible. However, seroprevalence tends to be higher in cat populations that hunt prey for food. All sampled cats in this study were outdoor cats and 86% of the households had observed the cats hunting. In the studies made by Hammond-Aryee *et al.* (2010 and 2011) and Yekkour *et al.* (2017) the cats sampled were obtained from more urban areas, which could indicate that

those cats do not hunt as much. In addition, Yekkour *et al.* (2017) had a confidence interval of 40-60%, which could further contribute to differences between these findings and theirs. The other studies in Africa did not state a confidence interval, but the number of cats sampled and their decision not to use the word seroprevalence makes interpretation of their data difficult.

Yekkour *et al.* (2017) found no difference in seropositivity between genders, which aligns with the results in this report. This is also supported by other reports from around the world (Haddadzadeh *et al.* 2006; Pena *et al*. 2006; Bolais *et al.* 2017). However, Miró *et al.* (2004) found a higher prevalence in male stray cats compared to females, which could be explained by males having bigger territories and therefore a higher chance of being exposed to *Toxoplasma*. In contrast, Besné-Mérida *et al.* (2008) found a higher prevalence in owned female cats.

One cat with remaining deciduous teeth was excluded from the study because its exact age could not be determined. The eruption of adult teeth usually starts around 4 months of age and completes around 7-8 months of age. (Fleming *et al.* 2021). Maternally transferred antibodies have been shown to disappear after 12 weeks of age (Dubey 2022 pp. 135). There was therefore a possibility that the cat was younger than 12 weeks, and that maternal antibodies would interfere with the result.

The cat which was <1 year old, but had all its adult teeth, was seronegative. This can either indicate that it had not been infected or it was newly infected and had not yet developed IgG antibodies against the disease. It would have been interesting to test this cat for IgM antibodies, or to repeat the test at later date.

5.2 Human interactions and transmission hazards

The area is more cat dense and the Maasai people have more interactions with the cat than previously assumed. The results from the interview show that it is generally the women who take care of the cats and that children of any gender have the most physical contact with the cats. The women are usually the person feeding them and are also the person who cleans the home if a cat defaecates inside. This places women in a more exposed position compared to men regarding the risk of transmission, but it can also be regarded as a protecting factor against miscarriages and fetal damage as the likelihood of infection before pregnancy is advanced. In future studies, it would be interesting to investigate the seroprevalence in the human population and compare those results in women and men and examine the rate of miscarriages and/or fetal damages in the area and possible connection to toxo-plasmosis.

Only 26% believed that cats can transmit disease to humans and 7 households were able to name a disease. Not one mentioned toxoplasmosis and when told about it no one could recall hearing about the disease before. During the interviews and other interactions with the local population, it also became obvious that there is an interest in gaining information about toxoplasmosis and zoonotic diseases in general.

To minimize the risks of transmission of toxoplasmosis in this area it is necessary to inform the population about the disease and how to minimize the risks of contraction. As Smith *et al.* (2021) emphasize, it is important to consider that the precautions should be easily implemented in everyday life and that people understand the risks of disease in order to enhance compliance.

As a first step in order to educate about toxoplasmosis in this area, information regarding the parasite and its transmission route could be introduced in pregnancy care. Other means of education are through schools, other healthcare services, and at governmental level in the future. The government in several countries has website resources to educate their population about toxoplasmosis (Folkhälso-myndigheten 2013; Centers for Disease Control and Prevention 2020) but toxoplasmosis is not mentioned on the Kenyan ministry of health website, neither is the word "zoonosis" if one searches the word in the search function. This further highlights the importance of increasing access to information regarding toxoplasmosis and zoonotic diseases in the country, as the information does not appear to be easily attainable by the population.

Several of the recommendations given by authorities in other countries, including Sweden, are not practical in this area. For example, training the cats to use a litter box, keeping the cat indoors, or freezing meat (Centers for Disease Control and Prevention 2018). Precautions, such as avoiding eating raw or undercooked meat, using gloves while in contact with soil, and washing hands after cooking, are examples of safeguards that I consider could be implemented with relative ease.

Another method of reducing the risk of transmission is to decrease the number of cats in the area. As the majority of people appreciated the cat and kept them as rodent control, total elimination of cats is not the right approach. Instead, castration programs could be implemented to control the population size or to encourage people to share one cat within several households. An increase in the cats' overall health would also extend their lifespan, which in turn would decrease the contamination of oocysts in the environment, as the re-shedding of oocysts is rare in cats if infected with the same strain of *Toxoplasma gondii* (Zulpo *et al.* 2018).

The feral cats included in the study lived close to the camp and although never touched, have indirect contact with humans through soil and water as they defaecates outside. Faeces from cats is a crucial transmission route of toxoplasmosis to human, as the oocysts are the reason for meat, vegetables, and water becoming contaminated. This supports the importance of controlling the cat population as a whole, both tame and feral, to decrease the risk of indirect transmission of *Toxoplasma.*

5.3 Limitations

The test method was chosen because of resource limitations. As this study was conducted in a rural area, far from cities, it was not possible to arrange frequent transportation to a laboratory and the cold chain could not be guaranteed. This is the reason that an additional test method was not used to confirm the FASTtest. In addition, the test only detects IgG antibodies and not IgM antibodies, therefore newly infected cats could have been missed.

Faecal examination was not performed, partly because of the difficulties of collecting faeces. But also, because it has little epidemiological value (Dubey 2022 pp 55). Consequently, there is no knowledge regarding if these cats were shedding oocysts at the time of sampling. According to Dubey (2022 pp. 135) cats usually contract *Toxoplasma* after weaning and then shed the oocysts after a period of weeks. As all cats were adults, with most of them >1 year, it is likely that these cats were not shedding oocysts at the time they were sampled. Nonetheless, the seroconverted cats have been shedding oocysts at some point and have thereby contaminated the environment.

As the cats were taken to the clinic by the veterinary assistant, the decisions regarding which cats to bring is not available. However, he said that some of the promised cats ran away or were too hard to catch, which implies that the sampling has a convenience bias. On the other hand, the assumption that the cats included in the study were easy to catch could represent a population that has closer contact with humans, making the result even more important for human health. In future studies on the cats in this area, a more randomized sample is desirable, which would be facilitated by a register of cat owners.

Another limitation is the language barrier, as words can be lost in translation and can cause misunderstandings. To decrease that risk, additional questions or clarifications were made if the answers seemed to contradict previous answers. The interviews were conducted by two interpreters, which could have affected the

answers. However, the questions that could have been affected are not included in this report.

On the other hand, the possibility of carrying out this study at all was exceedingly rewarding and I am very grateful for the opportunity to participate in such an important Public Health initiative. The results of this report are important and indicate that more research and initiatives regarding Public Health are necessary in this area.

6. Conclusions

The results of this report found a high seropositivity to *Toxoplasma gondii* and highlights that information regarding the disease, in combination with actions to reduce the parasite burden, is desirable in the area.

References

Al-Kappany, Y.M., Lappin, M.R., Kwok, O.C.H., Abu-Elwafa, S.A., Hilali, M. & Dubey, J.P. (2011). Seroprevalence of Toxoplasma gondii and concurrent Bartonella spp., feline immunodeficiency virus, feline leukemia virus and Dirofilaria immitis infections in Egyptian cats. *The Journal of Parasitology*, 97 (2), 256–258. https://www.jstor.org/stable/23019070 [2022-10-07]

Al-Kappany, Y.M., Rajendran, C., Ferreira, L.R., Kwok, O.C.H., Abu-Elwafa, S.A., Hilali, M. & Dubey, J.P. (2010). High prevalence of Toxoplasmosis in cats from Egypt: Isolation of viable Toxoplasma gondii, tissue distribution, and isolate designation. *The Journal of Parasitology*, 96 (6), 1115–1118. https://www.jstor.org/stable/40962045 [2022-10-06]

Besné-Mérida, A., Figueroa-Castillo, J.A., Martínez-Maya, J.J., Luna-Pastén, H., Calderón-Segura, E. & Correa, D. (2008). Prevalence of antibodies against Toxoplasma gondii in domestic cats from Mexico City. *Veterinary Parasitology*, 157 (3), 310–313. https://doi.org/10.1016/j.vetpar.2008.06.019

Bolais, P.F., Vignoles, P., Pereira, P.F., Keim, R., Aroussi, A., Ismail, K., Dardé, M.-L., Amendoeira, M.R. & Mercier, A. (2017). Toxoplasma gondii survey in cats from two environments of the city of Rio de Janeiro, Brazil by Modified Agglutination Test on sera and filter-paper. *Parasites & Vectors*, 10 (1), 88. https://doi.org/10.1186/s13071-017-2017-8

Centers for Disease Control and Prevention (2018). *Toxoplasmosis - Prevention & Control*. https://www.cdc.gov/parasites/toxoplasmosis/prevent.html [2022-12-04]

Centers for Disease Control and Prevention, (2020) *Toxoplasmosis - Biology*. https://www.cdc.gov/parasites/toxoplasmosis/biology.html [2022-09-02]

Davis, S.W. & Dubey, J.P. (1995). Mediation of immunity to Toxoplasma gondii oocyst shedding in cats. *The Journal of Parasitology*, 81 (6), 882–886. https://doi.org/10.2307/3284034

Derouin, F. & Pelloux, H. (2008). Prevention of toxoplasmosis in transplant patients. *Clinical Microbiology and Infection*, 14 (12), 1089–1101. https://doi.org/10.1111/j.1469-0691.2008.02091.x

Dubey, J.P. (1995). Duration of immunity to shedding of Toxoplasma gondii oocysts by cats. *The Journal of Parasitology*, 81 (3), 410–415. https://doi.org/10.2307/3283823

Dubey, J.P. (1998). Toxoplasma gondii oocyst survival under defined temperatures. *The Journal of Parasitology*, 84 (4), 862–865. https://doi.org/10.2307/3284606

Dubey, J.P. (2006). Comparative infectivity of oocysts and bradyzoites of Toxoplasma gondii for intermediate (mice) and definitive (cats) hosts. *Veterinary Parasitology*, 140 (1), 69–75. https://doi.org/10.1016/j.vetpar.2006.03.018

Dubey, J.P. (2022). *Toxoplasmosis of Animals and Humans*. 3rd ed., Boca Raton, Florida: CRC Press.

Dubey, J.P., Kotula, A.W., Sharar, A., Andrews, C.D. & Lindsay, D.S. (1990). Effect of high temperature on infectivity of Toxoplasma gondii tissue cysts in pork. *The Journal of Parasitology*, 76 (2), 201–204. https://doi.org/10.2307/3283016

Dubey, J.P. & Prowell, M. (2013). Ante-mortem diagnosis, diarrhea, oocyst shedding, treatment, isolation, and genetic typing of Toxoplasma gondii associated with clinical toxoplasmosis in a naturally infected cat. *The Journal of Parasitology*, 99 (1), 158–160. https://www.jstor.org/stable/23355033 [2022-09-27]

Dumètre, A., Dubey, J.P., Ferguson, D.J.P., Bongrand, P., Azas, N. & Puech, P.-H. (2013). Mechanics of the Toxoplasma gondii oocyst wall. *Proceedings of the National Academy of Sciences*, 110 (28), 11535–11540. https://doi.org/10.1073/pnas.1308425110

Fleming, P.A., Crawford, H.M., Auckland, C. & Calver, M.C. (2021). Nine ways to score nine lives – identifying appropriate methods to age domestic cats (Felis catus). *Journal of Zoology*, 314 (3), 211–226. https://doi.org/10.1111/jzo.12869

Folkhälsomyndigheten (2013). *Sjukdomsinformation om toxoplasmos*. https://www.folkhalsomyndigheten.se/smittskydd-beredskap/smittsamma-sjukdomar/toxoplasmos-/ [2022-09-02]

FreeMapTools (n.d.). *Area calculator*, https://www.freemaptools.com/area-calculator.htm [2022-11-06]

Frenkel, J.K., Dubey, J.P. & Miller, N.L. (1970). Toxoplasma gondii in cats: Fecal stages identified as coccidian oocysts. *Science*, 167 (3919), 893–896. https://www.jstor.org/stable/1728441 [2022-10-03]

Haddadzadeh, H.R., Khazraiinia, P., Aslani, M., Rezaeian, M., Jamshidi, S., Taheri, M. & Bahonar, A. (2006). Seroprevalence of Toxoplasma gondii infection in stray and household cats in Tehran. *Veterinary Parasitology*, 138 (3), 211–216. https://doi.org/10.1016/j.vetpar.2006.02.010

Hammond-Aryee, K., Esser, M., van Helden, L. & van Helden, P. (2015). A high seroprevalence of Toxoplasma gondii antibodies in a population of feral cats in the Western Cape province of South Africa. *Southern African Journal of Infectious Diseases*, 30 (4), 141–144. https://doi.org/10.1080/23120053.2015.1107295

Kochanowsky, J.A. & Koshy, A.A. (2018). Toxoplasma gondii. *Current Biology*, 28 (14), R770–R771. https://doi.org/10.1016/j.cub.2018.05.035

Lepczyk, C.A., Lohr, C.A. & Duffy, D.C. (2015). A review of cat behavior in relation to disease risk and management options. *Applied Animal Behaviour Science*, 173, 29–39. https://doi.org/10.1016/j.applanim.2015.07.002

Mara North Conservancy (2017) *Community*. http://maranorth.org/community/ [2022-10-13]

Mara North Conservancy (2017). *Conservation*, http://maranorth.org/conservation/ [2022-10-13]

Miró, G., Montoya, A., Jiménez, S., Frisuelos, C., Mateo, M. & Fuentes, I. (2004). Prevalence of antibodies to Toxoplasma gondii and intestinal parasites in stray, farm and household cats in Spain. *Veterinary Parasitology*, 126 (3), 249–255. https://doi.org/10.1016/j.vetpar.2004.08.015

Montazeri, M., Mikaeili Galeh, T., Moosazadeh, M., Sarvi, S., Dodangeh, S., Javidnia, J., Sharif, M. & Daryani, A. (2020). The global serological prevalence of Toxoplasma gondii in felids during the last five decades (1967–2017): a systematic review and meta-analysis. *Parasites & Vectors*, 13 (1), 82. https://doi.org/10.1186/s13071-020-3954-1

National Health Service (2020) *Toxoplasmosis*. https://www.nhs.uk/conditions/toxoplasmosis/ [2022-10-19]

Nisbet, A.I., Omuse, G., Revathi, G. & Adam, R.D. (2018). Seroprevalence data at a private teaching hospital in Kenya: An examination of Toxoplasma gondii, cytomegalovirus, rubella, hepatitis A, and Entamoeba histolytica. *PLoS ONE*, 13 (10), e0204867. https://doi.org/10.1371/journal.pone.0204867

Nyambura Njuguna, A., Kagira, J.M., Muturi Karanja, S., Ngotho, M., Mutharia, L. & Wangari Maina, N. (2017). Prevalence of Toxoplasma gondii and other gastrointestinal parasites in domestic cats from households in Thika Region, Kenya. *BioMed Research International*, 2017, e7615810. https://doi.org/10.1155/2017/7615810

Pena, H.F.J., Soares, R.M., Amaku, M., Dubey, J.P. & Gennari, S.M. (2006). Toxoplasma gondii infection in cats from São Paulo state, Brazil: Seroprevalence, oocyst shedding, isolation in mice, and biologic and molecular characterization. *Research in Veterinary Science*, 81 (1), 58–67. https://doi.org/10.1016/j.rvsc.2005.09.007

Smith, N.C., Goulart, C., Hayward, J.A., Kupz, A., Miller, C.M. & van Dooren, G.G. (2021). Control of human toxoplasmosis. *International Journal for Parasitology*, 51 (2), 95–121. https://doi.org/10.1016/j.ijpara.2020.11.001

Speer, C.A., Clark, S. & Dubey, J.P. (1998). Ultrastructure of the oocysts, sporocysts, and sporozoites of Toxoplasma gondii. *The Journal of Parasitology*, 84 (3), 505–512. https://doi.org/10.2307/3284713

Spotte, S. (2014). *Free-Ranging Cats: Behavior, Ecology, Management*. Hoboken, United Kingdom: John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1742829 [2022-09-06]

Suss-Toby, E., Zimmerberg, J. & Ward, G.E. (1996). Toxoplasma invasion: The parasitophorous vacuole is formed from host cell plasma membrane and pinches off via a fission pore. *Proceedings of the National Academy of Sciences of the United States of America*, 93 (16), 8413–8418. https://www.jstor.org/stable/39876 [2022-09-28]

Taylor, M.A., Coop, R.L. & Wall, R.L. (2007). *Veterinary Parasitology.* 3rd ed., Oxford, United Kingdom: Blackwell Publishing Ltd. [2022-09-09]

Tenter, A.M., Heckeroth, A.R. & Weiss, L.M. (2000). Toxoplasma gondii: from animals to humans. *International Journal for Parasitology*, 30 (12), 1217–1258. https://doi.org/10.1016/S0020-7519(00)00124-7

Tiao N., Darrington, C., Molla, B., Saville, W.J.A., Tilahun, G., Kwok, O.C.H., Gebreyes, W.A., Lappin, M.R., Jones, J.L & Dubey, J.P. (2013). An investigation into the seroprevalence of Toxoplasma gondii, Bartonella spp., feline immunodeficiency virus (FIV), and feline leukaemia virus (FeLV) in cats in Addis Ababa, Ethiopia. *Epidemiology and Infection*, 141 (5), 1029–1033. https://www.jstor.org/stable/23462769 [2022-09-23]

Watts, E., Zhao, Y., Dhara, A., Eller, B., Patwardhan, A. & Sinai, A.P. (2015). Novel approaches reveal that Toxoplasma gondii bradyzoites within tissue cysts are dynamic and replicating entities in vivo. *mBio*, 6 (5), e01155-15. https://doi.org/10.1128/mBio.01155-15

Yekkour, F., Aubert, D., Mercier, A., Murat, J.-B., Khames, M., Nguewa, P., Ait-Oudhia, K., Villena, I. & Bouchene, Z. (2017). First genetic characterization of Toxoplasma gondii in stray cats from Algeria. *Veterinary Parasitology*, 239, 31–36. https://doi.org/10.1016/j.vetpar.2017.04.013

Yilmaz, S.M. & Hopkins, S.H. (1972). Effects of different conditions on duration of infectivity of Toxoplasma gondii oocysts. *The Journal of Parasitology*, 58 (5), 938–939. https://doi.org/10.2307/3286589

Zulpo, D.L., Sammi, A.S., dos Santos, J.R., Sasse, J.P., Martins, T.A., Minutti, A.F., Cardim, S.T., de Barros, L.D., Navarro, I.T. & Garcia, J.L. (2018). Toxoplasma gondii: A study of oocyst re-shedding in domestic cats. *Veterinary Parasitology*, 249, 17–20. https://doi.org/10.1016/j.vetpar.2017.10.021

Popular science summary

Toxoplasma gondii is a parasite that can affect almost all animals, including humans. The disease it causes is referred to as toxoplasmosis, which is an important zoonotic disease, e.i. it is a disease that can be transmitted from animals to humans. It can cause life-threatening symptoms in immune-compressed people, such as human immunodeficiency virus (HIV) patients, and cause miscarriages or fetal damages if infection occurs during pregnancy.

Toxoplasma gondii has a complex lifecycle and cats play an important role in its transmission. The parasite uses the cats' intestines to form oocysts, which are then spread to the environment through the faeces of cats to the environment. Within approximately a week after infection, the cats shed oocysts in their faeces, but after that, the immune system protects the cat from reinfection for up to 36- 77 months. Cats are termed as final hosts because they are the only animal in which *Toxoplasma gondii* can reproduce sexually, whereas other animals serve as intermediate hosts.

When the oocysts are ingested by either animals or humans the parasite makes its way from the intestines to the bloodstream and spreads throughout the body. The parasite then forms cysts in an attempt to avoid being recognized by the immune system of the host. These cysts containing parasites can form anywhere in the body. In humans, brain tissue is the most common site for cysts formation, but they can also occur in muscle tissue or in the eyes.

The tissue cysts are also a method for the parasite to transmit to new hosts. When an infected animal is eaten, the cysts are digested in the stomach and the parasites within are released, infecting the new host. Rats and other prey are particularly important for transmission to cats, as cats are more easily infected from tissue cysts rather than by ingesting oocysts in the environment.

Toxoplasma gondii can therefore be transmitted to humans via tissue cysts from animals produced for meat or from wild game in form of tissue cysts, as well as from the environment via oocyst contaminated vegetables and water.

In a healthy person, the immune system suppresses the infection and the disease usually goes unnoticed by the patient, although in a few cases, the infection can

cause flu-like symptoms during the acute phase. Therefore, most people are not aware that they have the disease. After the acute phase, the parasite is dormant in the host's body for the rest of its life, due to the ability to form these tissue cysts. If the immune system is weakened the infection can become active once again. The importance of avoiding infection is therefore to avoid severe symptoms later in life and especially to avoid women contracting the disease while pregnant.

To minimize the risks of transmission, it is recommended not to eat raw or undercooked meat, to wash hands or to use gloves while in contact with soil, to teach cats to use litterboxes and for pregnant women not to handle cat faeces. It is also recommended to wash vegetables before eating and to washing cutting boards with soap and water after usage.

This study investigated the seropositivity of *Toxoplasma gondii* in domesticated cats from Mara North Conservancy in Kenya. This is a measurement of how many of the cats have produced antibodies after being infected with the parasite. This measurement cannot tell if the cat is newly infected or was sick at the time of sampling, nor can it show if the cat is shedding oocysts. The antibody measured in this study is IgG, which develops later in the course of the infection and persists for a long period of time. This measurement is used to indicate the parasite burden in the area and to draw conclusions whether or not action is needed to reduce that burden.

The results revealed a high seropositivity in the cat population in the area, with 89.4% (42/47) of the cats being positive. This means that these cats have been shedding oocysts in their faeces at some point in their life, and as these cats are outdoor cats the environment has been contaminated. All cats were estimated to be younger than 5 years, which implies that none of them had been shedding oocysts more than one time during their life.

The study also investigated the relationship and interaction between humans and cats in the area. In total 100 households were interviewed using a questionnaire with the help of an interpreter. The results showed that 69% of the households owned a cat and 86.2% of non-cat owners allowed other peoples' cat inside their homes. The wife of the household was the one responsible for the cat and was also at most risk to come in contact with cat faeces, as they cleaned the house if the cat defecated inside. Only 26% of the households thought that cats can transmit disease to humans, and nobody could recall hearing about toxoplasmosis.

In conclusion, the results of this report found a high seropositivity to *Toxoplasma gondii* in the cats in this area, which highlights the importance of providing information regarding the disease, in combination with actions to reduce the parasite burden. Such actions could be to castrate the cats in order to control the

population or to improve the overall health of the cats in an effort to increase their lifespan. A longer lifespan reduces the need for new cats and thereby reduces the population, which in turn decreases the oocysts in the environment. A first step to inform people about the risks and how to avoid them could be via pregnancy care, as it is important to protect women from infection, especially at this time.

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Appendix 1

Questionnaire Masai Mara

1. **Do you or someone in your household have a cat?**
   Yes
   No
   Do not know

2. **How many cats do you have?**
   _____ Number of cats

   Do not know

3. **What gender is the cat or cats living in or close to your house?**
   Male
   Female
   Both females and males
   Do not know

4. **Do you know how many of them are female and how many are males?**
   _____ Number of females
   _____ Number of males

5. **Do you prefer one gender over the other?**
   Yes
   No

   5.1. **If yes: Which gender do you prefer?**
   Female
   Male
   5.2. **Why do you prefer that gender?**
   _____
   _____
   _____

6. **Are the cats allowed inside your house?**
   Yes
   No
   Do not know

7. **Do you or someone in your household pet the cats?**
   Yes
   No

8. **Do children play and pet the cats?**
   Yes
   No
   Do not know

9. **Can you catch the cats and hold them?**
   Yes
   No
   Some of them

10. **When the cats are outside, where do they go?** e.g around the village, with the children or far away.

    _____
    _____
    _____
    _____

11. **Do you feed the cats in your household?**
    Yes
    No
    Do not know
    11.1. If yes: What do you give them?
    11.2. If yes: Who gives them food?
    11.3. If no: Do you know what the cats are eating?

12. **Have you seen a cat hunt or eat small animals?**
    Yes
    No
    12.1. If yes: What animals have you seen the cat eat or hunt?

13. **Have you seen a cat being hunted or eaten by other animals?**
    Yes
    No
    13.1. If yes: Do you know which animal it was?

14. **Have you seen a cat interact with other wild cats?** For example geopard, servals, african wild cat, lions etc.
    Yes
    No
    Do not know
    14.1. If yes: which wild felides have you seen interact with the cats?

15. **If a cat that lives around your house gets sick, what happens to the cat?**

    _____
    _____
    _____
    _____

16. **If you find a dead cat, what do you do with it?**

_____
_____
_____

17. **What is your opinion of the cats in the village?**

_____
_____
_____
_____

18. **In some countries, black cats are considered unlucky, do you have something similar**?

_____
_____
_____
_____

19. **Do you do something with the cat's feces if they defecate near the house?** For example, bury it in the ground or take it away from the house.

Yes
No
Do not know

19.1. If yes: What do you do with it?
19.2. If yes: How do you do it? for example using hands, a showel or a bag

20. **Do you think that cats can transmit disease to humans?**

Yes
No
Do not know

20.1. If yes: Which disease do you think can be transmitted from a cat to a human?

21. **Has there been a case where you think a person has caught a disease from a cat?**

Yes
No

21.1. If yes: Can you tell me about that case?

22. **Can we use your cat for research and neuter it?**

Yes, both
Yes, only neuter
Yes, only research
No

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