The prevalence of *Toxoplasma gondii* in sheep in Maasai Mara, Kenya

- a One Health perspective

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Abstract

This study investigates the presence of antibodies against *Toxoplasma gondii* in sheep from villages near Mara Rianta in the Mara North Conservancy, Kenya, using a One Health perspective. The investigation aimed to identify potential links between the parasite, livestock abortions, and environmental factors in a region where humans and animals live in close proximity.

In total, 40 sheep were tested using an IgG/IgM rapid test, with all samples yielding negative results for *Toxoplasma gondii* antibodies. These findings contrast with previous research showing high seroprevalence (89.4%) in free-roaming cats in the same area, suggesting that cats are key hosts and disseminators of the parasite.

The study recorded high abortion rates among sheep, with a significant number of ewes becoming pregnant again after an abortion and successfully carrying to term. Various infectious agents, such as *Campylobacter* spp., *Chlamydia* spp., and *Listeria* spp., are known to cause abortion in sheep, but further research is needed to determine the specific cause in this region. The results highlight the importance of integrated veterinary and community-based efforts to manage zoonotic diseases, improve livestock health, and raise awareness of toxoplasmosis risks among the Maasai people.

Limitations, including the use of field-appropriate but less accurate diagnostic tests and a small sample size, underscore the need for further studies with robust diagnostic tools and larger datasets to confirm these findings and explore additional causative factors.

This study provides valuable insights into *Toxoplasma gondii* epidemiology and underscores the importance of One Health approaches in regions where human-animal-environment interactions are closely intertwined.

Keywords: Toxoplasma, toxoplasmosis, sheep, Kenya, Maasai Mara, One Health, seroprevalence, epidemiology

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1. Introduction

The Maasai people depend on sheep (*Ovis aries*) and goats (*Capra hircus*) for their sustenance and livelihood. The most prevalent breed of goats they keep are East African goats, (AU-IBAR, 2019, p. 19), while the most popular sheep breeds are Red Maasai and Black Head Somali sheep (Bekure, 1991, p. 95; AU-IBAR, 2019, pp. 26-27).

Their families are settled in small villages together with their animals. They live in huts made of mud and dung that are surrounded by a wide circular thorn-bush fence called a boma where they keep their animals. Each village can accommodate four to eight families and their herds. Their lifestyle is ancestral in their culture and goes back to the 15th century (Britannica, 2024). They let their animals roam free to graze during the day and keep them inside the bomas at night to protect them from predators (Bekure, 1991). The Maasai people´s livestock have always been important to them since their livelihood depends on them (Lamprey and Reid, 2004).

They have a nomadic lifestyle, mainly subsisting on meat, blood, and milk from their cattle (Britannica, 2024). The men are head of the family and responsible for the cattle while the women and the children tend to the smaller ruminants used for consumption. The cattle symbolize prosperity and wealth and are mostly utilized for their milk. Meanwhile, small ruminants are primarily used for meat consumption (Bekure, 1991).

Toxoplasmosis is a foodborne zoonotic disease (Tenter *et al.,* 2000) spread by *Toxoplasma gondii*, a unicellular protozoa parasite (Kim and Weiss, 2004). Toxoplasmosis affects both humans and animals globally, with an estimated one-third of the world's population afflicted (Tenter *et al.,* 2000).

The people in Africa have little knowledge of toxoplasmosis; thus, they can become infected and be silent carriers of the parasite (Tonouhewa *et al.,* 2017). It is mainly transmitted through the consumption of undercooked/raw meat, contaminated vegetables, and unpasteurized milk. People can also be infected by oocysts shed in cat faeces and spread in the environment. The disease can be life-threatening in immunosuppressed people and pregnant women (Tenter *et al.,* 2000).

The people in Maasai Mara live in close contact with their cattle (Britannica, 2024) which facilitates the transmission of *Toxoplasma gondii* between the Maasai and their animals. *Toxoplasma gondii* has a complicated life cycle, with the cat as the definitive host and warm-blooded animals, such as sheep and goats, as intermediate hosts for transmission of the parasite (Kochanowsky and Koshy, 2018).

This study aims to examine the potential presence of antibodies against the parasite *Toxoplasma gondii* in sheep in Mara North Conservancy from a One

Health perspective. Blood samples will be taken from sheep and analyzed using a rapid test. This topic is of great importance to investigate further, because of both public health reasons and economic losses.

2. Literature review

2.1 *Toxoplasma gondii* life cycle

Toxoplasma gondii is a ubiquitous zoonotic parasite. This unicellular eukaryotic parasite can infect, and proliferate in, almost all organisms. It is taxonomically categorized as phylum Apicomplexa with the subclass coccidia. The parasite can reproduce in two distinct ways, sexually and asexually (Kim and Weiss, 2004).

Toxoplasma gondii consists of three important infectious stages, the tachyzoites, the bradyzoites, and the oocysts. The cat is the only species able to shed oocysts and can become infected after intake of any of the infective stages (Dubey, 2022, p. 33).

The cat (*Felis catus*) is the final host and the only animal in which *Toxoplasma gondii* has both sexual and asexual cycles (Figure 1). As seen in figure 1 cats can become infected by ingesting tissue cysts from intermediate hosts or ingesting sporulated oocysts from the environment in soil, water, and food (Dubey, 2022, p. 34,49).

The sexual reproduction of *Toxoplasma gondii* occurs in the feline gut epithetlium when the cat ingests the encysted bradyzoites. The bradyzoites go through schizogony, converting into merozoites and dividing into male and female gametocytes (Kochanowsky and Koshy, 2018). After fertilization, a haploid zygote becomes an unsporulated oocyst excreted in cat faeces into the environment. The oocyst sporulates in the environment, each containing two sporocysts with four haploid sporozoites. The cat is the only animal able to shed oocysts. (Dubey *et al.,* 1998).

The oocyst phase is the only one that has extracellular development; the bradyzoites and tachyzoites have intracellular development. Sporulated oocysts exhibit greater environmental resistance than unsporulated oocysts because of the sporocyst generation process and the inner structural layers of both sporocysts and oocysts (Dubey, 2022, p. 60). A large number of oocysts are shed for about 1-2 weeks in the cat faeces (Marie and Petri, 2022). It takes approximately 1 to 5 days for the sporocyst to become infectious and the process is temperature-dependent (Jones and Dubey, 2010). Sporulated oocysts can survive for months to years in the environment (Marie and Petri, 2022).

All warm-blooded animals, i.e., sheep, goats, rodents, swine, birds, and humans, can be intermediate hosts (Figure 1). They become infected when they consume contaminated oocysts shed from the cat in soil and water, or on plants, or ingesting tachyzoites or bradyzoites in infected meat (Figure 1) (CAPC, 2024; Hide, 2024).

Shortly after ingestion, the oocysts develop into tachyzoites. The tachyzoites are mainly localized in muscle and neural tissue. Then the tachyzoites transform into bradyzoites where the cysts replicate (Hanafi *et al.,* 2014).

The bradyzoites encapsulate in the intermediate host's tissue cysts and can be ingested by cats and humans through infected/unpasteurized milk, uncooked/raw meat, and vegetables (Stanić and Fureš, 2020).

Two other less common transmission methods in humans are tachyzoites, which can be congenitally transmitted from the placenta to the foetus, and blood transmission from organ transplants and blood infusion (Figure 1) (Marie and Petri, 2022).

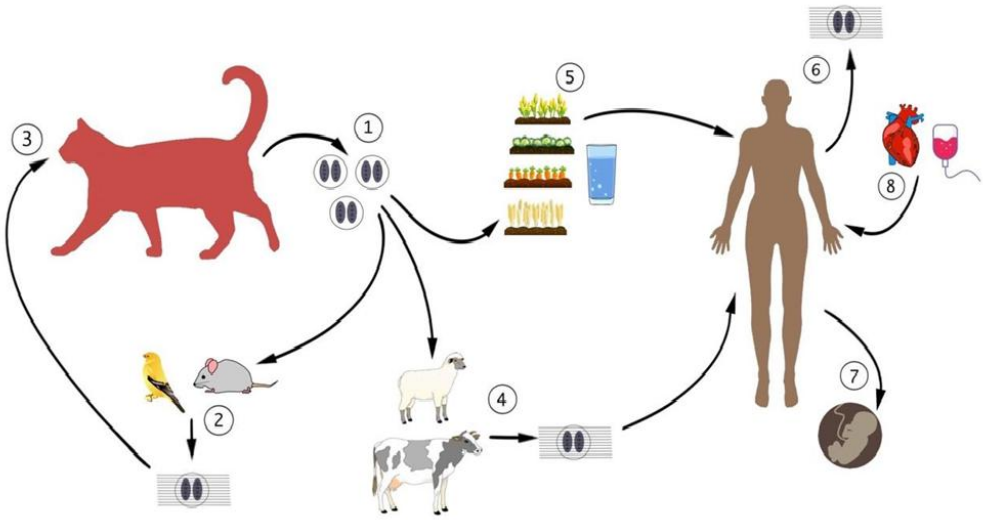

Figure 1. Toxoplasma gondii *life cycle as illustrated by Byström (2022). The figure is lent by permission from Ronja Byström.*

1. Oocysts shed in cat faeces. 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 soli and water. 6. Oocyst consumed by human either in vegetables or meat, forming tissue cysts. 7. Transplacental infection to foetus. 8. Infected transplantation organ.

2.1.1 Tachyzoites and bradyzoites

Both tachyzoites and bradyzoites proliferate asexually inside the host cell. The tachyzoite multiplies its haploid genome and divides into two daughter cells. This process is called endodyogeny and repeats multiple times. It differs from the usual eukaryotic cell division since two progenies are created inside the parent cell. When fully developed they will consume the parent cell and become independent organisms (Kochanowsky and Koshy, 2018).

Tachyzoite (tachos=fast) addresses the fast-growing phase of the cycle, as opposed to bradyzoite (brady=slow), being the slow-growing phase of the cycle. The tachyzoites transform into bradyzoites and can reconvert (Attias *et al.,* 2020). The cycle repeats itself in certain organs in the body (Dubey, Lindsay and Speer, 1998). The tissue cysts prominently grow in the brain, eyes, and skeletal and cardiac muscles, including the neural tissue, with the brain as the most common site (Kochanowsky and Koshy, 2018).

2.2 Prevalence and transmission pathway of *Toxoplasma gondii* in sheep

There are few studies on the prevalence of toxoplasmosis among animals and humans in Africa. There was variation in the data reported within species and country (Tonouhewa *et al*., 2017).

Tonouhewa *et al.* (2017) reviewed studies on the seroprevalence of toxoplasmosis in sheep between 1969 to 2016 in 24 African nations, reporting that it ranged from 4.30% to 68.00%, with an average value of 26.1%. The seroprevalence among goats varied from 3.6% to 74.8% with an average prevalence of 22.9%. Ethiopia is the country with the highest number of studies made on toxoplasmosis in East Africa.

The animals most impacted by abortions are sheep, which results in significant financial losses for humans (Hanafi, Ozkan and Bowman, 2014). The zoonotic transmission of the disease also affects public health. Most of the sheep population around the world are infected with *Toxoplasma gondii*. Cats are the most important source of toxoplasmosis infection among sheep (Innes *et al*., 2009).

Infection with *Toxoplasma gondii* primarily causes abortion in sheep but can also result in mummification and resorption of the foetus, stillbirth, embryonic death, neonatal death, and foetal death. Sheep that survive the first week of infection are healthy and produce healthy lambs (Edwards and Dubey, 2013). *Toxoplasma gondii* has three transmission routes in sheep: ingesting oocysts from cat faeces, bradyzoites capsulated within tissue cysts, and vertical transmission. Since sheep are strictly herbivores, the most common route of infection is from oocysts shed by cats in soil, water, or grass (Holec-Gąsior and Sołowińska, 2023).

The frequency of *Toxoplasma gondii* infection in sheep is dependent on several factors. The occurrence in farm animals mainly depends on environmental conditions, such as contamination of their crops, soil, and water. The prevalence of *Toxoplasma gondii* varies in every country depending on factors such as geography, climate, population of cats, age, breed, flock size, rainfall, grazing area, and farm management routines. The parasite has increased in all continents because of changing weather conditions. It prefers lower temperatures and humid weather conditions. The prevalence of *Toxoplasma gondii* is much higher in sheep living near homesteads and semi-intensive managed farms since it is more likely

that the cats are found in locations where food and shelter are available, as opposed to distant pastures and extensively managed farms situated further away with no access to food and shelter for the cats (Dubey *et al.,* 2020).

2.2.1 Hazard factors and prevention of *Toxoplasma gondii* in sheep

Sheep can become infected with *Toxoplasma gondii* at all ages. Seroprevalence of *Toxoplasma gondii* is higher in older sheep than in lambs. Sheep infected for the first time in the third trimester of their pregnancy usually give birth to healthy but infected lambs. Congenital transmission can also occur because of the recurrence of an endogenous infection (Innes *et al.,* 2009).

According to a study by Edwards and Dubey (2013), ewes that have aborted once have a lower risk of aborting again. Maiden ewes and females, in general, have a higher risk of vertical transmission of *Toxoplasma gondii*.

It is not possible to find one management routine for all species. In general, improvements can be made in livestock management to decrease the infection rate, i.e. providing veterinary care on time, and following hygiene routines to mitigate transmission risk. Genetically, sheep are inherently more sensitive to the parasite than goats, which is an uncontrollable factor (Tonouhewa *et al.,* 2017).

One way to protect ewes from transmission is to vaccinate them, or to allow them to become infected, since they then become naturally immune to the disease. Vaccination can reduce the risk of abortion but it does not kill the cysts (Innes *et al.,* 2009).

There are several ways to detect toxoplasmosis in small ruminants: serological tests (IgG and IgM antibodies), histology, cytology, and PCR. Serological tests are the most used in field tests, especially ELISA. For validation of the result, it should be followed up by PCR in a laboratory (WOAH, 2023).

2.3 Toxoplasmosis in humans

Approximately 30% of the global population is afflicted with *Toxoplasma gondii*, as the most common zoonotic foodborne parasite (CAPC, 2024; Hide, 2024).

The disease usually goes unnoticed since immunocompetent persons do not become infected. People with immunodeficiency are more likely to suffer from toxoplasmosis as a result of latent infection or reactivation of the infection (Dubey *et al.,* 2020).

2.3.1 General symptoms in humans

The symptoms of *Toxoplasma gondii* in the initial phase are fatigue, headache, nausea, extreme sweating, and muscle and joint pains. Fever and skin lesions with red flat areas and papules also occur in rare cases. These symptoms are diffuse and common for many diseases such as influenza and viral syndromes, and are therefore difficult to diagnose. For the disease to be toxoplasmosis, at least one of the symptoms mentioned above must be joined with lymphadenopathy. Lymphadenopathy is characteristic of *Toxoplasma gondii* and can appear in the angle of the jaw, the neck area, and behind the ear (Dubey, 2022, pp. 158–160).

2.3.2 Immunosuppressed people

In contrast to other microorganisms, intact tissue cysts persist for the host's whole life and can become activated when the person becomes immunodeficient (Jones and Dubey, 2010).

Immunosuppressed people under treatment for AIDS or malign tumors can develop toxoplasmosis due to latent reactivation of *Toxoplasma gondii*. Untreated AIDS patients are more prone to exhibit encephalitis with neurological symptoms. Transplantation with infected organs can have fatal consequences in immunodeficient people (Dubey, 2022, pp. 162–165).

2.3.3 Vertical transmission of *Toxoplasma gondii* in humans

Vertical transmission of *Toxoplasma gondii* occurs from the placenta to the foetus, resulting in congenital toxoplasmosis. The most common clinical signs are retinochoroiditis, hydrocephalus, and intracranial calcifications (Dubey, 2022, pp. 189–190).

The survivors of prenatally acquired toxoplasmosis show more severe clinical signs if they are infected during the first trimester of pregnancy than in the last trimester. Congenitally transmitted toxoplasmosis usually manifests in the last trimester of pregnancy. The earlier the illness is discovered and diagnosed, the easier it is to treat; the longer it is neglected, the worse it becomes (Dubey, 2022, p. 183).

The parasite may go undetected if it is contracted during pregnancy. Ultrasound screening for pregnant women is a useful method for the early detection of congenital toxoplasmosis although detection of parasite DNA from amnionic fluid with PCR can also be used (Dubey, 2022, p.195).

On the other hand, the American College of Obstetricians and Gynaecologists (ACOG) does not recommend screening for pregnant women since it can give false positive results and lead to redundant treatment (Jones *et al.,* 2001 see Stanić and Fureš, 2020).

If the woman has been infected once, the chance of becoming reinfected is low. Therefore, a positive result indicates that the woman has been infected before;

even if she is then infected during pregnancy, the child is not affected (Mittendorf *et al.,* 1992 see Stanić and Fureš, 2020).

2.3.4 Food and waterborne transmission

The main infective pathway route for humans is from the ingestion of tissue cysts from raw or undercooked meat, particularly pork and lamb, or oocysts from cat faeces that have been shed into the environment and can be found in plants, water or soil. The most common transmission method is still unknown (Tenter *et al.,* 2000). However, recent research by Stanić and Fureš (2020) concluded that cats are not the primary source of infection for the parasite. In humans, undercooked meat or raw uncooked vegetables are likely to be the main source of infection.

Water is one source of infection transmission that has received increased attention lately because of many outbreaks related to water. *Toxoplasma gondii* oocysts can live up to 54 days in unfiltered cold water and are naturally spread when handling soil (Jones and Dubey, 2010).

2.3.5 Prevention of *Toxoplasma gondii* in humans

According to EFSA recommendations (EFSA, 2007), the meat should be thoroughly cooked and reach a temperature of 67 ºC before consumption. Freezing the meat to -12 ºC can reduce the infection risk but is not enough alone (Kotula *et al.,* 1991; Dubey, 2000).

Precautions should be taken to lower the risk of infection, such as avoiding contact with cat faeces, washing hands thoroughly before handling fruits and vegetables, wearing gloves when handling dirt, and not eating raw or uncooked meat. Pregnant women and immunosuppressed people should be extra cautious and follow the advice to prevent infection (McLeod *et al.,* 2020, p. 172).

People in remote rural areas live in close contact with their animals, which, from a One Health perspective, contributes to the infection risk of *Toxoplasma gondii* to both people and animals. Poor hygiene routines, inadequate veterinary care, and poverty add to the problem. Vaccination of all cats, sheep, and humans could minimize the problem but there are no efficient vaccines for any species at present (Suijkerbuijk *et al.,* 2018).

Educational programs about the parasite and avoidance to protect oneself from the disease are important, particularly in rural areas where poverty is high, and people are uneducated. Communication of information is especially important for pregnant women, school children, health services and public authorities (Stanić and Fureš, 2020).

2.3.6 Epidemiological Challenges and Global Health Perspectives

Incidence, prevalence, and trends of toxoplasmosis differ in different countries. They even differ within the country´s regions and cities. The display of clinical signs of *Toxoplasma gondii* differs in all people depending on their genetic and immune condition. Therefore, there is not a single approach that works for everyone (Dubey, 2022, p. 211).

Epidemiological surveys are the best way to gather information about, and emphasize the importance of, *Toxoplasma gondii* and its diverse sources for humans. It is of significant matter for the country's public health and also for economic concerns. Since food, i.e. meat and vegetables are an important infection source for disease transmission, public surveys are also an important tool to collect facts and spread awareness of the disease among the public (Dubey, 2009).

Thiong'o *et al.* (2016) investigated the prevalence of *Toxoplasma gondii* among abattoir workers in slaughterhouses in Thika district, Kenya. Out of the 87 tested people, 39.1% were seropositive in the cow-sheep-goat slaughterhouses, whereas all the tested people were seropositive in the chicken slaughterhouses. The study indicated that workers in a high-risk group showed no symptoms, implying the need for public awareness.

Ogendi *et al.* (2013) conducted a study on farmers in the same area and came to the conclusion that cats roaming free amid the livestock are a big infection source.

According to a review made by Dubey (2021) on outbreaks of clinical toxoplasmosis from the five past decades, there is no information regarding whether toxoplasmosis is mostly transmitted through tissue cysts from infected meat or via environmental oocysts. Therefore, disease outbreaks can give us valuable knowledge about incubation time, infective doses, and transmission routes.

Food animals such as sheep and goats had a higher infection prevalence in the muscle tissue compared to the brain tissue. This is an important factor for the epidemiology and the infection risk for people consuming meat (Dubey, 2022, p. 44)

One study was made to detect the dispersion of tissue cysts in lamb and goat shoulders. Tissue was taken from different parts of the infected lamb shoulders and inoculated in mice. Different quantities of meat - 5,10 and 50 g were examined, and the result showed that 5g of uncooked or raw meat is enough to be infective, although larger sample sizes had higher infection rates (Rani *et al.,* 2020).

3. Material and methods

3.1 Literature search

Material concerning *Toxoplasma gondii* was acquired by searching the Swedish University of Agricultural Sciences (SLU) search engine "Primo". Other literature was found from cited articles.

The searches were obtained using the following terms, Toxoplasma* AND sheep* OR goat* AND Kenya, Toxoplasma* AND Africa, Toxoplasma*, Toxoplasma* AND epidemiology*, Toxoplasma* AND epidemiology* AND One health* and toxoplasma*AND risk* AND sheep* AND goat*.

3.1.1 Study area and climate

The study was carried out in Mara North Conservancy in Masai Mara, southwest of Kenya. The conservancy is within Narok County near a small town called Mara Rianta (Figure 2).

On average the climate in Kenya is warm and humid, and Masai Mara is no exception. During the daytime, the weather is hot and humid with an average temperature of 25 ºC, while the nights are cold with an average temperature of 15 ºC. Masai Mara has two rainy seasons, one longer with heavier rain from mid-March to May, and one shorter with lighter rainfalls from November to December. The annual rainfall is 1400 mm and the relative humidity is approximately 72%.

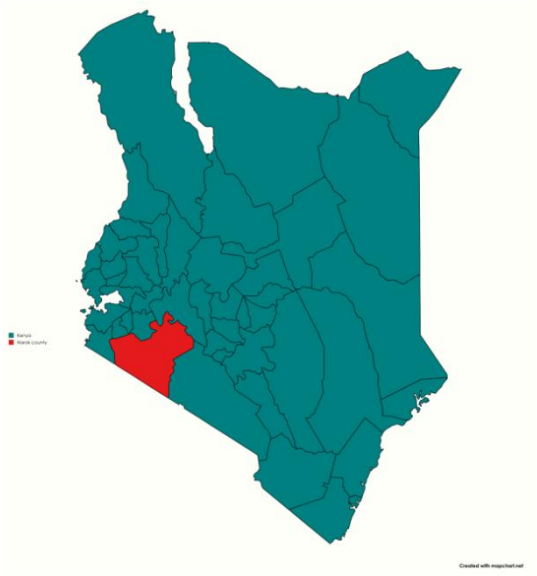

Figure 2. A topographical chart of Kenya with Narok County marked on the map. The map was made in 2024 using https://www.mapchart.net/.

3.2 Small ruminants sampled

In total, 40 sheep were tested, 36 females and 4 males (Table 1). The breeds were Red Maasai and Black Head Somali sheep, and a mix of the breeds.

The only information available beforehand was their age, ranging from one to five years old. Besides that, a randomized selection of the animals available in each boma was made.

The gender of the animal was checked before 1-2 mL of blood was taken from the jugular vein with a cannula into a syringe. The samples were tested in the field to avoid blood coagulation.

3.3 Test method and serological assay

A test kit (Toxoplasma IgG/IgM Antibody Rapid Test, Biopanda) was used to detect IgG/IgM antibodies against *Toxoplasma gondii* in whole blood samples. The rapid test is a qualitative test based on the sandwich lateral flow immuno-chromatographic assay technique, where antibodies against *Toxoplasma gondii* in the sample bind specifically to the target molecule. The sample pad absorbs the applied blood, which then flows laterally to the conjugate pad by capillarity. The conjugate pad is labeled with colloidal gold-labeled antibodies that bind to the antibodies in the solution and create immunological complexes. The antibodies are immobilized on a porous membrane, (usually nitrocellulose) to create a coloured test line (T) and a control line (C).

One drop of whole blood (approx. 40µL) was transferred from the test tube using a dropper into the sample well S of the cassette. Two drops of buffer (approx. 80µL) were also added. The test result was read after 5-10 minutes.

The result was interpreted as positive when one or twocoloured lines became visible in the test line region (IgM and/or IgG), and negative when no lines were visible. The control line region (C) should always have a one-coloured line whether the test result is negative or positive.

4. Results

In total, four families living in different villages, keeping sheep, goats, and cows in their bomas, were visited. Ten sheep from each family were tested, ranging in age from one to five years old. The period of gestation and abortions mentioned is during their whole lifetime.

Each family had two to three bomas where they kept sheep, goats, and cows. The bomas were situated at a distance of a few meters from each other and each boma had approximately 50 sheep and goats, mixed. The family also had around twenty cows that were kept in their boma. There were more sheep than goats; if they had lambs, they were kept separately in the boma. All the animals were let out to graze together close to the bomas during the day and were brought back to the bomas in the evening. Water was obtained from the dams in the area. The sheep and lambs were kept separated in the night and the ewes were milked in the morning before they were released to roam freely with their lambs.

Table 1. Results of antibody detection

Type of sheep	Positive (%)	Negative (%)	Total
Female	0 (0%)	36 (100%)	36
Male	0 (0%)	4 (100%)	4
Total	0 (0%)	40 (100%)	40

In the first boma, there was one ewe that had had two or three abortions, and two ewes that had each had one abortion and a normal gestation, 30% (3/10). The other seven had normal pregnancies and no abortions, 70% (7/10). None of the animals were pregnant now.

The second boma had an abortion storm from May to August with many losses. Eight of the 10 ewes aborted their foetus (80%), and two of them did not, (20%). Now all the ewes were pregnant.

None of the six ewes in the third boma had ever suffered an abortion and they were not pregnant.

In the fourth boma, 5/10 (50%) of the ewes had aborted and the rest had not. All of the ewes that had aborted were pregnant now, but only one of the ones that had not aborted was pregnant, 10% (1/10).

Figure 3. Left picture: Mix of a Blackhead Soomali sheep and Red Maasai together with her newborn lamb. Right picture: Sheep with their lambs in the boma. The pictures are published with consent from the owners and photographer Annika Seeliger.

5. Discussion

5.1 Serological assay

The aim of this study was to investigate the presence of antibodies against the parasite *Toxoplasma gondii* in sheep in the villages around Mara Rianta in Mara North Conservancy from a One Health perspective.

All the 40 blood samples from the sheep were negative (Table 1). In contrast to my findings, Ronja Byström's 2022 study on the seroprevalence of *Toxoplasma gondii* in free-roaming cats revealed that 42 out of 47 (89.4%) were seropositive (Byström, 2022).

The pattern seen in the ewes that had aborted was that the ewes give birth to healthy lambs in another pregnancy. According to studies, once the sheep has had one abortion due to toxoplasmosis, it does not occur in subsequent pregnancies (Innes *et al.,* 2009). Apparently, 80% (8/10) of the ewes in boma number 2 became pregnant after abortion. Also, in the fourth boma, only one ewe that had not had an abortion was pregnant, while all of the ones that had aborted were now pregnant. It would be interesting to follow up see if their next pregnancy is successful as well.

Red Maasai sheep are renowned for their resistance to parasites, especially *Haemonchus contortus*, and they are, therefore, a popular breed among the Maasai people. This was suggested in a study by Mugambi *et al.* (1996), which showed red Maasai sheep had higher resistance against parasites than Dorper sheep. The recommended dosages of antiparasiticides, according to veterinarian James Nayetuni are Ivermectin twice a year SC (2mL/sheep), Levamisole twice a year PO (8-15 mL/sheep), and Albendazole twice a year PO (10mL/sheep). All the bomas follow this scheme except the second boma, in which de-worming is done every three months. The sheep are being dewormed routinely without any diagnostic analyzes done beforehand. This has led to anthelmintic resistance against parasites, according to James Nayetuni (local veterinarian, Masai Mara, personal communication, Nov 2024).

Abortions were a common problem in all places, among sheep, goats, and cows, but especially among sheep. Sheep can become infected by a variety of infectious agents causing abortion, but the main ones include *Campylobacter* spp., *Chlamydia* spp., *Toxoplasma* spp., *Listeria* spp., *Brucella* spp., *Salmonella* spp., and border disease virus. The clinical signs are more or less similar regardless of which pathogen brought about the infection (Tibari, 2024). A suitable topic for additional research would be to conduct the same study to determine if another microorganism is causing abortions.

As mentioned before, the Maasai value their livestock since it is their main source of income. They take good care of them, in contrast to their cats and dogs,

and, for example, deworm them regularly. Therefore, projects such as the Mara North Conservancy Dog Project and the Mara Cat Project are important since the dogs and cats are spayed, dewormed and vaccinated against rabies free of charge. Free vaccination campaigns in the villages have led to most cats being dewormed. Although the local veterinarian James Nayetuni is highly respected in the area and tries to educate and advise the people as much as he can during his visits, they do not always follow his advice. The reasons can be many, for instance, lack of education and old habits. As highlighted previously, the cat is the definitive host for *Toxoplasma gondii* and the main disseminator of the parasite. Both the cats and the dogs are fed the placenta and foetus of aborted lambs, which may be responsible for the high seroprevalence of the parasite in cats.

The cats also lived in the same area as the families and their livestock. Most of the time they were roaming free, being both indoor and outdoor cats. They were not in close contact with the sheep since most of the time they were out hunting prey. They also might defecate far away from the bomas and therefore do not shed their oocysts close to the sheep.

The women both milked the cattle and fed the cats. They did it even during pregnancy, which is a great transmission hazard. Mulu Gelaw *et al.* (2024) analyzed seroprevalence of toxoplasmosis among pregnant women in Africa, reviewing articles published from 2020-2024. The pooled result showed that 42.89% of pregnant women in Africa are seropositive, specifically in Central and Eastern Africa.

Ronja Byström (2022) found, during an interview with the Maasai, that 26% of the households believed that cats could transmit disease to humans and seven households could name a disease in general. No person had ever heard of toxoplasmosis. As mentioned before, awareness and information are crucial to recognize the symptoms of the disease. Medical treatment should be sought at an early stage to mitigate the development of the disease. The information must be easy to understand and possible to apply in everyday life.

5.2 Limitations

It has been very challenging to find a suitable rapid test for sheep to use in the field. There was no test that was specifically designed for livestock; the test used is for small animals (dogs and cats) but the manufacturer said it could be used for sheep and goats as well. Rapid tests for antigens and antibodies were available; tissue sample DNA is needed for PCR detection of *Toxoplasma gondii*. The blood sample's high titer results indicate that the ewe has been exposed to infection, but not when. On the other hand, toxoplasmosis is most likely not the reason for the miscarriage if the ewe has very low or no titer levels (SVA 2024).

The IgG/IgM test was chosen for screening the presence of *Toxoplasma gondii* in sheep and goats.

It would have been desirable to conduct more than 40 tests to form a more representative picture of prevalence in the area. However, this was not possible due to the cost of the rapid test kits. With a bigger budget, it might have been possible to send the tests to an accredited laboratory for PCR, which would yield a more reliable result.

Another reason for choosing this test method was because the study was performed in a rural area far from the city. Therefore, regular transportation to a laboratory could not be organized and the cold chain could not be maintained until arrival. Consequently, other diagnostic methods, such as PCR could not be used to confirm the rapid test. Testing a cat sample with this kit would have been useful, to check that the kits were still functioning, but no samples had been kept from the previous study.

Many other factors could have affected the results. The test kit might have had a manufacturing defect. Depending on when the sheep was infected, the result can be false negative if the sheep had not developed immunity. Whole blood was used instead of serum because the coagulant tubes available were out of date. Serum is usually recommended over whole blood since it does not contain any blood cells that could interfere with the sample. Alternatively, there could be some substance in the sheep blood that blocks the rapid test reaction.

The result of this study shows no indication of *Toxoplasma gondii* among the sheep in the Mara Rianta area. The study needs to be further investigated since information regarding the disease is important for the Maasai people and their animals.

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Popular science summary

Toxoplasma gondii is a widespread zoonotic parasite that can infect nearly all warm-blooded animals, including humans. It is a unicellular eukaryotic organism classified in the phylum Apicomplexa. Its life cycle involves both sexual (in cats) and asexual (in intermediate hosts) reproduction, with three key infective stages: tachyzoites, bradyzoites, and oocyst.

Cats (*Felis catus*) are the definitive host, shedding oocysts in their faeces, which sporulate in the environment and become infectious. Cats become infected when they consume infected tissues (e.g., rodents), and sexual reproduction of the parasite occurs in the cat´s intestines. Intermediate hosts (e.g., sheep, humans, goats, pigs) ingest oocysts from contaminated soil, water, or food, or consume tissue cysts in undercooked meat. Oocysts develop into tachyzoites in the host, which then form tissue cysts (bradyzoites) in organs such as the brain and muscles.

Toxoplasmosis causes significant reproductive issues in sheep, including abortion, stillbirth, and neonatal death. Sheep primarily acquire the infection by ingesting oocysts from the environment, as they are herbivores. Prevalence varies based on environmental factors, management practices, and climate, with higher rates in intensively farmed systems. Vertical transmission can also occur, but infection through oocyst ingestion is more common.

Around 30% of the global population is infected, often without symptoms in healthy individuals. Immunocompromised people are at higher risk, with serious complications, such as encephalitis and retinochorditis, if the infection reactivates. Transmission to humans mainly occurs through ingestion of undercooked meat or contaminated water and soil, but cats can also contribute to infection via their faeces.

Pregnant women are particularly at risk of congenital toxoplasmosis, which can cause neurological damage in the foetus, or even miscarriage, especially if contracted early in pregnancy.

Safe food handling, such as thoroughly cooking meat, washing vegetables, and avoiding contact with cat faeces, can reduce the risk of infection.

Studies from Africa show varying prevalence rates in livestock, with higher rates in animals near farms. Transmission is complex, with both environmental oocysts and meat consumption contributing to the spread, making epidemiological tracking difficult.

In areas like Maasai Mara, where humans live closely with animals, improved veterinary care, hygiene practices, and awareness programs are essential to reduce the infection risk for both people and animals. However, there are no widely available vaccines for either cats or livestock at present.

This study investigates the presence of antibodies against *Toxoplasma gondii* in sheep from villages near Mara Rianta in Mara North Conservancy, with a focus on a One Health perspective.

The study found that all 40 sheep tested were negative for *Toxoplasma gondii* antibodies. This contrasts with a 2022 study by Ronja Byström, which found a high seroprevalence (89.4%) of *Toxoplasma gondii* in free-roaming cats in the same area, suggesting that cats may act as a significant host and disseminator of the parasite.

Abortion rates were notably high among sheep, with ewes that had previously aborted often giving birth to healthy lambs in subsequent pregnancies. According to existing studies, once an ewe has an abortion, it is less likely to have a subsequent abortion. The study observed that 80% of ewes in one boma became pregnant again after an abortion. Additionally, the local veterinarian recommended regular deworming for livestock, with the sheep in the study receiving more frequent treatments compared to the Swedish standard.

Various infectious pathogens are known to cause abortions in sheep, including *Toxoplasma* spp., *Campylobacter* spp., *Chlamydia* spp., *Listeria* spp., *Brucella* spp., *Salmonella*, and the Border disease virus. The results of this study suggest that further research is needed to investigate whether other microorganisms are responsible for the abortions.

The Maasai people value their livestock highly, as it is their primary source of income. However, despite the efforts of local veterinarians like James Nayetuni, not all farmers follow veterinary advice due to factors such as lack of education and established practices. Free vaccination campaigns for cats, which are the definitive hosts for *Toxoplasma gondii*, have reduced the presence of the parasite in the area. The study speculates that the seronegative results in the sheep may be due to these successful vaccination efforts.

The cats in the area were observed to be roaming freely, mostly indoors and outdoors, and were not in close contact with the sheep. However, they were fed placentas and aborted foetuses, which might explain the high seroprevalence of *Toxoplasma gondii* among the cats.

The study also highlights several limitations, including the challenges of using rapid tests in the field. The test used was designed for small animals, such as dogs and cats, but was suitable for sheep. While it provided results, it was not highly accurate, and a larger sample size and more advanced diagnostic methods, such as PCR, were not feasible due to budget constraints and logistical issues in the rural setting.

In conclusion, the study suggests that *Toxoplasma gondii* may not be the main cause of abortions in sheep in the area, although the high seroprevalence in cats points to the importance of controlling the parasite in the broader ecosystem.

Further research is needed to explore other potential causes of abortion and to confirm the findings using more reliable diagnostic methods.

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