The occurrence of FeLV, FIV and FeCoV in free-roaming cats in Mara North Conservancy, Kenya – A possible threat to wild felids?

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Abstract

The domestic cat serves an important purpose to households in the Mararienda district in Mara North Conservancy as they protect the house from snakes, rats and other small animals. No previous studies have been performed on the cats in this area and little is known about the populations size and health status. The aim of this study was to investigate the occurrence of the feline viruses feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and feline coronavirus (FeCoV) in domestic free-roaming cats in Mararienda district in Mara North Conservancy, Kenya. In addition to disease occurrence, another aim was to investigate if the cats, if they carry the diseases, can transmit them to wild felids. These viruses can potentially infect wild felids and lead to the development of disease and even death which makes this kind of research important in a conservational point of view.

Feline leukemia virus is a retrovirus and can cause clinical disease in both domestic cats and wild felids. Transmission between domestic cats and wild felids have been suggested. Feline immunodeficiency virus is also a retrovirus that infects domestic cats and closely related viruses can infect wild felid species. FIV causes a similar disease as HIV in humans with severe immunosuppression (AIDS) in the end stage. Feline coronavirus is a common virus in the domestic cat population but usually don't cause clinical disease. However, mutation can occur in specific genes of the virus and cause the fatal disease feline infectious peritonitis. Apart from FeLV and FIV, several wild felids are also susceptible to FeCoV and FIP.

100 households were interviewed and 47 cats from different households were sampled and tested using FASTests detecting antibodies for FIV and FeCoV and antigens for FeLV. Of the 47 cats, 27 were females and 20 were males, all estimated to be over 6 months old but younger than 5 years. None of the cats tested positive for either FeLV or FIV while 6 cats (12.8%) tested positive for FeCoV antibodies. Regarding sex distribution, 4 out of 27 females (14.8%) and 2 out of 20 males (10%) tested positive. Regarding interactions and potential disease transmission 7 out of the 100 households interviewed stated that they have seen domestic cats interact with other wild felid species such as African wildcat and leopard. In addition, 90/100 households informed that when a cat died, they threw them out in the bush for wild animals to eat, posing a possible route for transmission to wild animals.

Keywords: Feline infectious peritonitis, Feline immunodeficiency virus, Feline coronavirus, Feline infectious peritonitis, Domestic cat, Conservation, Masai Mara, Wild felids, Kenya

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
BBB	Blood-brain-barrier
CNS	Central nervous system
FeCoV	Feline corona virus
FIV	Feline immunodeficiency virus
FIP	Feline infectious peritonitis
FeLV	Feline leukaemia virus
HIV	Human immunodeficiency virus
LTR	Long terminal repeats

1. Introduction

The purpose of this study was to investigate the possible presence of the important feline viral pathogens feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and feline coronavirus (FeCoV) in feral cats in Mararienda, Mara North Conservancy, Kenya. The national reserve is home to many different kinds of animal species including several wild species from the family *Felidae*. The selected viruses can cause severe disease in domestic cats and in wild felids, and free-roaming domestic cats may introduce these in the vulnerable wild population making this research important for conservational purpose (Reviewed in O'Brien *et al.* 2012).

Feline leukemia virus causes clinical disease in both domestic cats and different wild felids and transmission between species has been reported (Brown *et al.* 2008; Meli *et al.* 2009; Sacristán *et al.* 2021). The virus is spread via bites or even friendly contact and can stay latent in the body after viral RNA, after being converted to DNA, has been incorporated in the hosts cell genome (Maclachlan 2017 pp. 273).

Feline immunodeficiency virus is endemic in domestic cat population all over the world and can also cause disease in wild felids (Maclachlan 2017 pp. 292) and even infect hyenas (Troyer *et al.* 2005). FIV causes similar disease as human immunodeficiency virus (HIV) does in humans with severe immunodeficiency in the terminal stage (Miller *et al.* 2018).

Feline corona virus usually only causes mild symptoms in infected cats but a fatal disease known as feline infectious peritonitis (FIP) can develop after spontaneous mutations occur within special genes of the virus (Tekes & Thiel 2016 pp. 203). Stout *et al.* (2021) stated in their review that FIP has been reported in several wild felids and has been reported to cause outbreaks with high mortality in cheetah populations (Evermann *et al.* 1988).

No studies have previously been performed on the cats in the Mararienda district and this study will be the first of its kind. To our knowledge, no previous prevalence studies about these viruses has been conducted in Kenya.

2. Literature Review

Mara North Conservancy is a private nature reserve in south-western Kenya and borders the Masai Mara National Reserve. The conservancy is a partnership between tourism partners and Masai landowners, which focus on being sustainable regarding environment, wildlife and local residents.

The conservancy is home to several wild felids, for example lions, servals, African wildcat, leopards and cheetahs. A review article by Obrien *et al.* (2012) stated that the majority of these felids are susceptible to some of the same viruses domestic cats are. Besides felids, hyenas and black backed jackals might also be susceptible to some of the viruses that cats may carry. Harrison *et al.* (2004) showed that hyenas had antibodies against both FIV and FeCoV while another study by Thalwitzer *et al.* (2010) showed antibodies against FeCoV in a black backed jackal. Both species are found in Mara North Conservancy.

Several studies have shown that the domestic cat play a role in transmission of viruses to wild felids (Brown *et al.* 2008; Meli *et al.* 2009; Sacristán *et al.* 2021). In addition, domestic dogs has played a vital role in canine distemper virus outbreaks in African lions in Serengeti resulting in high mortality in the lion population (Carpenter *et al.* 1998). Because of this, investigation of the prevalence of a number of selected viruses is important as they can potentially pose a threat for conservation of vulnerable species.

As local people, and their dogs and cats, live in close proximity to the wild animals that are susceptible to viruses that the domestic animals may carry, it is important to get an overview of the possible presence, and prevalence of these pathogens. The domestic animals might interact with wild carnivores, directly or indirectly, and thus pose as a possible source of infection.

Some of these wild carnivores in Mara North Conservancy are vulnerable (IUCN 2022) and conducting this study might be of importance for the purpose of presservation and might motivate further research in the area. In addition to conservation, this study can also help to improve the welfare of the domestic cats in Mararienda district.

Figure 1. Pictures of houses and cats in Mararienda. Photos by author Emilia Schultz and Ronja Byström.

2.1 Feline Leukemia Virus

2.1.1 The Virus

Feline leukemia virus (FeLV) belongs to the family *Retroviridae* in the Genus *gammaretrovirus (*Maclachlan 2017 pp. 285*)*, and is an enveloped RNA virus which is unstable in the environment (Francis *et al.* 1979). The virus is endemic in most parts of the world and cause clinical disease in domesticated cats and free ranging wildcats (*Felis silvetris),* possibly because wild cats and domesticated cats have more interactions than other wild felids (Duarte *et al.* 2012). The virus has been associated with clinical disease in other wild felids such as Florida panthers (*Puma concolor coryi*) (Brown *et al.* 2008), leopards (*Panthera pardus),* and cheetahs (*Acinonyx jubatus*), among others (Maclachlan 2017 pp. 285). Similarities in viral sequences has been found in Iberian lynx (*Lynx pardinus*) (Meli *et al.* 2009), Florida panthers (Brown *et al.* 2008) and kodkod (*Leopardus guigna*) (Sacristán *et al.* 2021). These three authors all suggested that viral transmission between species could have occurred (Brown *et al.* 2008; Meli *et al.* 2009; Sacristán *et al.* 2021).

The virus has been divided into different subgroups; FeLV-A, B, C and T which are associated with different diseases depending on the subgroup, e.g. FeLV-C with anemia, FeLV-B with neoplasia and FeLV-T with immunosuppression as a result of destruction of T-lymphocytes (Maclachlan 2017 pp. 286). Endogenous FeLV has been identified in domestic cats and wildcats (Polani *et al.* 2010). Endogenous virus occurs when FeLV proviral DNA has been integrated into the germ cells and is inherited by their offspring. Exogenous, via transmission, and endogenous forms of FeLV can create recombinants and form hybrids with particular pathogenic properties (Maclachlan 2017 pp. 275, 286).

2.1.2 Clinical Signs

Infection with FeLV often has a chronic course with a long asymptomatic phase before any clinical signs is observed. Diseases associated with FeLV are neoplastic disorder, bone marrow suppression, neurological disorders, immunosuppression and stomatitis. FeLV can also have reproductive effects e.g. embryonic death and fading kitten syndrome. Common signs or symptoms are anemia, lymphomas, other cytopenias, secondary infections, leukemia or myeloproliferative disorders. (Maclachlan 2017 pp. 286)

2.1.3 Pathogenesis

After infection, FeLV infects hematopoietic precursors and continues to replicate in lymphatic and hematopoietic tissues (Boes & Durham 2017 pp. 761). The disease can be divided into four types: progressive, regressive, abortive and focal depending on various host and viral factors. For example, the age of the cat when infected, presence of other infectious agents, dose of the virus, viral strain and subgroup along with genetic variation between individual cats (Maclachlan 2017 pp. 286).

The pathogenicity of the different viruses is determined within the viral long terminal regions (LTR) and *env* gene. Within the LTR region the viral promoter is encoded, and different sequence motifs have been related to cell-specific expression of viral genes. The *env* gene, which encodes the envelope protein, decides cellular and tissue tropism and therefore can affect the development of the disease (Maclachlan 2017 pp. 286).

Like all retroviruses, the replication of the virus starts with transforming RNA to DNA, which is conducted by the enzyme reverse transcriptase (RT), thereafter the enzyme integrase incorporates the DNA as a provirus into the cells' genome. The provirus will stay integrated in the genome until the cell dies or the cell divides. The later will result in expression of the provirus and virus shedding (reviewed in Willett & Hosie 2013).

Progressive infection

Progressive infection occurs when the infected cats' immune system is unable to contain the virus in an early stage, which will result in a persistent viremia. The infection usually commences in lymphoid tissue in the oropharyngeal region and is spread to peripheral tissue and via monocytes and lymphocytes (Maclachlan 2017 pp. 286). When the bone marrow becomes involved a secondary viremia will occur due to infection of granulocytes and platelets (Hoover *et al.* 1977).

Because of the persistent viremia, FeLV antigen can be detected in peripheral blood for the remainder of the cats' life. Progressively infected cats will stay infected and shed virus for their entire lifetime and usually dies within a few years

due to FeLV associated diseases (Hartmann 2012b; Maclachlan 2017 pp. 286). Because of their continuous shed of virus these cats pose the biggest threat regarding the spread of the infection to other cats (Reviewed in Hofmann-Lehmann & Hartmann 2020).

Regressive infection

Regressive infection is characterized by a transient viremia that persists for weeks to month after initial infection (Maclachlan 2017 pp. 286). These cats have an effective immune system and are able to clear the infection either prior to, or shortly after, bone marrow involvement. If precursor cells (platelets or granulocytes) in the bone marrow get infected, some cats may still be able to clear the viremia but proviral DNA will still be present in stem cells in the bone marrow. If proviral DNA get incorporated in cellular chromosomal DNA the infection can reactivate. For example in case of immunosuppression, cells might start to produce virus again (Reviewed in Hartmann 2012b; Hofmann-Lehmann & Hartmann 2020). This phase where the viral DNA is present in the cells' DNA but does not produce any virus is called latency.

Regressively infected cats only shed virus during the viremic phase and mainly through saliva, during this time viral antigen will also be present in peripheral blood. Repeated testing for FeLV antigen is required to distinguish progressive infection from regressive infection where cats with progressive infection will stay positive while regressively infected cats eventually will turn negative (Hartmann 2012b; Hofmann-Lehmann & Hartmann 2020). FeLV associated diseases are not commonly developed in regressively infected cats (reviewed in Hofmann-Lehmann & Hartmann 2020). However, cytopenias has been reported in a few cases and it is unclear if regressive FeLV-infection might increase the risk of developing lymphoma or leukemia (Sykes & Hartmann 2014 pp. 227).

Abortive infection

Cats that develop an abortive infection either have a very effective cell-mediated and humoral immune response (reviewed in Hofmann-Lehmann & Hartmann 2020) or was exposed to a low viral dose (Major *et al.* 2010) or both and are therefore able to clear the virus before viremia occurs (Maclachlan 2017 pp. 286). Viral antigen or provirus DNA are not present in blood and FeLV-antibodies are the only evidence of FeLV infection (reviewed in Hartmann 2012b; Hofmann-Lehmann & Hartmann 2020).

Focal infection

Focal or atypical infection has been observed in experimentally FeLV- infected cats where the immune system has been able to constrict viral replication to specific tissues (Maclachlan 2017 pp. 286), e.g. spleen, small intestine and mammary glands

(reviewed in Hofmann-Lehmann & Hartmann 2020). Free viral antigen will be produced in these tissues and can therefore be found intermittently in peripheral blood. Infectious virus is rarely released but can be found in the specific tissues (reviewed in Hartmann 2012b; Maclachlan 2017; rewieved in Hofmann-Lehmann & Hartmann 2020).

2.1.4 Epidemiology

FeLV is widely spread around the world in domestic cat populations and prevalence varies between geographical areas, health status and if they are kept indoors or free roaming (Levy *et al.* 2006). The prevalence varies in different continents: Middle East-Africa 7.1-42.9%, North America 1.6-4.1%, Southern Europe 5.6-20.2% and Northern Europe 1.6-19.4% (Buch *et al.* 2017). A study performed by Bande *et al.* (2012) concluded that some risk factors for FeLV-infection includes being male, aggressive behavior and outdoor access. The virus is shed from mucosal sites and can therefore spread via bites, sexual interactions, grooming each other, sharing dishes, vertically and through milk (Caney 2000; Maclachlan 2017 pp. 285). Kittens appear to be more susceptible to the virus compared to adult cats. A study (Hoover *et al.* 1976) has shown 100% of newborn kittens developed persistent viremia whereas only 15% of the kittens over 4 month developed persistent viremia (Luckman & Gates 2017).

2.1.5 Vaccines and Treatment

Treatment

Treatments for FeLV-infected cats are very limited, partly because of severe side effects along with high cost for the owner (Greggs *et al.* 2012). There is no cure for FeLV but a symptomatic treatment based on clinical signs can help prolong the cats' lifetime. Immune modulators are often used in cats with retrovirus infection with varying results. In some uncontrolled studies, significant improvement was shown but in controlled trials clear evidence of efficacy was absent for some of the immune modulating drugs (Hartmann 2012a). In some special cases, antiviral chemotherapy was indicated, however in most cases the toxicity and lack of evidence of efficacy outweighs the potential positive effects (Hartmann 2015).

Vaccines

There are several vaccines commercially available for FeLV but the duration of immunity and level of efficacy can vary (Maclachlan 2017 pp. 285). Vaccination with adjuvanted killed, whole-virus can prevent infection between 90-100% (Patel *et al.* 2015).

2.2 Feline Immunodeficiency Virus

2.2.1 The Virus

Feline immunodeficiency virus is an enveloped RNA-virus in the family *Retroviridae* in the genus *Lentivirus* (Maclachlan 2017 pp. 290). The virus infects domestic cats all over the world, and closely related viruses can also infect wild felid species (Maclachlan 2017 pp. 292) for example lion, pallas cat and pumas (Brown *et al.* 2010). Harrison *et al.* (2004) showed that even hyenas can have antibodies against FIV which suggests that they also can get infected.

The virus is divided in six different subtypes, A-F, depending on sequence diversity of the *env* gene and the most common subtypes are A, B and C. Different recombinant subtypes have also been identified for example A/B, B/D and A/B/C (Sykes 2014 pp. 209).

When viral DNA, generated via RT, is integrated to the cell genome they can affect genes that are important for cell growth and differentiation, called proto-oncogenes. The viral DNA can also carry oncogenes and then mutate, which is incorporated in the cells' genome. These events can result in abnormal growth or differentiations and subsequently formation of tumors (Sykes 2014 pp. 209).

2.2.2 Clinical Signs

Feline immunodeficiency virus has different stages comparable to HIV in humans. In experimentally infected cats three stages have been described: *acute phase*, *chronical asymptomatic phase (subclinical)* and *terminal stage* (feline acquired immunodeficiency syndrome "AIDS") but not all stages have been documented in naturally infected cats. Most naturally infected cats do not develop severe disease and usually live as long as uninfected cats (Maclachlan 2017 pp. 292).

The first phase (*acute*) can often go unnoticed, but if noticeable it is characterrized by transient and mild clinical signs, for example lethargy, fever, lymphadenopathy, stomatitis, signs of enteritis, respiratory tract infection etc. The acute phase is followed by the subclinical/chronical asymptomatic phase where the duration varies but most often last for years. The length of the subclinical phase is influenced by the pathogenicity of the viral strain, the age of the cat when infected, and presence of secondary infection. In the last stage of infection, the terminal phase, clinical symptoms are characterized by opportunistic infections, myelosuppression, neoplasia and neurological disease (Hartmann 2011; Sykes 2014 pp. 210-211). Common clinical signs in the terminal stage are periodontal disease, tooth resorption, stomatitis, skin infections, upper respiratory infection and ear infections (Sykes 2014 pp. 211-212). Common neurological signs are for example increased aggression (Azadian *et al.* 2020), stereotypic behaviors (Steigerwald *et al.* 1999) and changes in sleep pattern (Prospéro-García *et al.* 1994).

2.2.3 Pathogenesis

Like all retroviruses, the FIV-RNA strand is transformed to DNA by the enzyme reverse transcriptase. After this, the DNA enters the nucleus and the double-stranded DNA is incorporated into the host cell genome, which is performed by the enzyme integrase. The DNA stays in the cells genome and can either be latent or become transcriptionally active and create new virions depending on the cell environment and various other factors of the host. (Maclachlan 2017 pp. 272-274)

The virus primarily targets CD4+ T-cells but can also infect CD8+ T-cells, B-cells, dendritic cells, macrophages, microglia and astrocytes. After inoculation the virus replicates in lymphoid tissue and after two weeks, high levels of virus can be found in blood. (Sykes 2014 pp. 210). Productive infection can also be seen in mucosal and systemic lymphoid tissue, thymus, central nervous system (CNS), and bone marrow (Burkhard & Dean 2003).

Acute phase

During the acute phase the infection causes an inversion of CD4+/CD8+ T-cell ratio, the levels of CD4+ t-cell will then progressively decrease while CD8+ cells will increase (Fletcher *et al.* 2011; Maclachlan 2017 pp. 293), this results in transient illness which persists for 3 to 6 months (Sykes 2014 pp. 210). The infected cat can also have neutropenia possibly because the neutrophils undergo apoptosis (Sykes 2014 pp. 210) but the mechanism behind neutropenia in FIV-infected cats are not yet fully understood (Sprague *et al.* 2010). The cytokine support also declines, which will result in increase in CD8+ programmed cell death, spontaneous apoptosis and a decline in thymic regeneration (Miller *et al.* 2018). Dendritic cells can also be affected. The changes in these cells will result in further immuno-suppression. Even with marked immunosuppression most infected cats survive this phase because of a robust humoral immune response and a recovery of CD8+-T cell levels (Sykes 2014 pp. 210).

Asymptomatic phase

The acute phase is followed by an asymptomatic phase where circulating viral levels remain stable and a reservoir of target cells infected with integrated provirus will be established. The CD4+ T-cells along with other leukocytes will continue to decrease resulting in further immunodeficiency. This phase can last for years, however with progressive suppression of the immune system, the cats will be more prone to opportunistic infections (Hosie *et al.* 2009; Miller *et al.* 2018). The subclinical phase can last for the remainder of the cats' life or can progress to the terminal phase (AIDS). The subclinical phase continues beyond the studies' timespan in many studies performed in a specific pathogen-free environment (Fletcher *et al.* 2011). However, one long-term study showed that 6/24 cats

progressed to the terminal phase between 3.8 and 5.8 years after initial infection (Mathiason-DuBard *et al.* 1998).

Terminal phase

The terminal phase is characterized by opportunistic infections due to the immunodeficiency, resulting in inability to react to recall antigens together with the inability to organize a primary immune response to secondary pathogens (Tompkins & Tompkins 2008).

Neuropathogenesis

FIV is also neurotropic and can cause neurological damage (Power *et al.* 2004). The virus enters the CNS in the acute phase (Ryan *et al.* 2003) and causes neuropathology both in early and late stages of the disease (Boche *et al.* 1997). In the acute phase studies have shown that lymphocytes plays an important role in transporting the virus through the blood-brain-barrier (BBB) and blood-choroid plexus-barrier to the choroid plexus, brain parenchyma and meninges (Ryan *et al.* 2003, 2005). Studies have also shown that disruption of the BBB might occur which can facilitate viral entry into the CNS (Ryan *et al.* 2005). The primary target cells for FIV in the CNS is astrocytes followed by glia cells (Dow *et al.* 1992).

2.2.4 Epidemiology

Feline immunodeficiency virus is spread all over the world in domestic cats and the prevalence varies depending on geographical area and the populations health status (Sykes 2014 pp. 210). The prevalence in Middle East- Africa varies between 9.224.1%, North America 3.5-5.3%, Southern Europe 5.6-20.2% and Northern Europe 1.6-19.4% (Buch *et al.* 2017).

Feline immunodeficiency virus occurs in high concentrations in the saliva and the main route of transmission is through bites (Sykes 2014 pp. 209). The virus can also be transmitted from an infected queen to the kittens either via transplacental transmission, during parturition or through milk, however these transmission routes are not common in naturally infected cats. The virus has also been found in semen (Jordan *et al.* 1998) but sexual transmission has not been confirmed. Outside the cat the virus can only survive for a few minutes and indirect transmission does not occur (Sykes 2014 pp. 209).

2.2.5 Vaccines and Treatment

Treatment

Similar to FeLV there is no cure available once infection is established. However, many cats can live for years before any symptoms occur, and with symptomatic treatment the infection can be managed. FIV-infection can also be treated with antiviral therapy and immune modulators even though significant evidence of their efficacy is lacking (Sherding 2006a pp. 129-130).

Vaccine

To date there is no efficient vaccine available against FIV, some studies show that FIV vaccine (Fel-O-Vax FIV) has a protective rate of 56%. There is no significant difference in infection rate between vaccinated and un-vaccinated cats there might be a tendency of protection for vaccinated cats. More studies are required to determine the protective rate of the vaccine against all subtypes of FIV (Westman *et al.* 2016).

2.3 Feline Corona Virus / Feline Infectious Peritonitis Virus

2.3.1 The Virus

Feline corona virus is a positive-stranded RNA-virus which belongs to the family *Coronaviridae* and the genus *Alphacoronavirus*. Feline enteric coronavirus (FeCV) is a common virus in domestic cats which causes a relatively harmless persistent infection resulting in mild diarrhea (Tekes & Thiel 2016 pp. 199). According to the established hypothesis, FeCV can mutate into feline infectious peritonitis virus (FIPV) resulting in a fatal disease due to changing the patho-genicity and tropism of the virus (Tekes & Thiel 2016 pp. 203; Maclachlan 2017 pp. 446).

Feline enteric corona virus has two distinctive serotypes, type I and II, both serotypes have the ability to cause FIP. Serotype I is most common, whereas type II is relatively rare. Type II seems to be a recombinant virus between feline and canine CoV (Tekes & Thiel 2016 pp. 197; Maclachlan 2017 pp. 446).

The virus infects domestic cats along with wild cats in the family *Felidae* and FIP has been described in Cheetahs (*Acinonyx jubatus)* (Evermann *et al.* 1993), Lions (*Panthera leo)* (Mwase *et al.* 2015), Serval (*Felis serval)* (Juan-Sallés *et al.* 1998) and European wildcat (*Felis silvestris)* (Watt *et al.* 1993) among others (reviewed in Stout *et al.* 2021). There has also been one alleged case in leopard (*Panthera pardus pardus*) in a zoo in Germany (Tuch *et al.* 1974).

2.3.2 Clinical Signs

Feline corona virus infection

Most cases of feline corona virus infection are benign and often asymptomatic but FeCoV can sometimes cause vomiting, mild transient diarrhea or more rarely severe enteritis (Pedersen *et al.* 1981; Kipar *et al.* 1998). Apart from gastrointestinal impact, infected cats can also show mild clinical signs from the upper respiratory tract. Kittens are more prone to develop clinical symptoms compared to adults (Hoek 1993; Hartmann 2005).

Feline infectious peritonitis

After FeCoV mutates to FIPV it causes the fatal disease feline infectious peritonitis (FIP) which is divided into wet and dry form depending on the clinical characteristics. In addition, a combination of wet and dry form can also occur (Hartmann 2005; Sherding 2006b pp. 134).

The wet (or effusive) form is characterized by ascites caused by fibrinous peritonitis, pleural effusion as a result of pleuritis along with pericarditis which can cause effusion in the pericardium (Hartmann 2005; Sherding 2006b pp. 134-135). This manifests clinically as a distended abdomen, respiratory distress such as dyspnea (Riemer *et al.* 2016) and tachypnea and muffled heart sound during auscultation (Hartmann 2005). The typical symptoms of wet FIP are often preceded by nonspecific symptoms such as weight loss, fever, anorexia or increased appetite. If the liver is involved icterus can also be seen (Hartmann 2005).

The dry (or non-effusive) form is more subtle compared to the wet form (Hoskins 1993). The first clinical signs may consist of weight loss, fever and often occur weeks before any organ specific signs follow (August 1984; Foley *et al*. 1998; Hartmann 2005). Organs that may be involved in dry FIP are the eyes, kidneys, pancreas, liver and CNS (Sherding 2006b pp. 135). Common organ specific signs is uveitis (Andrew 2000), ataxia, hyperesthesia, seizures (Foley *et al.* 1998), vomiting and diarrhea (Harvey *et al.* 1996). Even in the dry form effusions are present to a smaller extent and dry form can convert to wet form and vice versa (Hartmann 2005).

2.3.3 Pathogenesis

Feline enteric corona virus

Feline enteric corona virus primarily infects and replicates in epithelial cells of the intestinal villi from the lower part of the duodenum to the caecum (Pedersen *et al.* 1981). More recent studies reveal that FeCoV can infect the entire intestine along with the majority of parenchymal organs, for example mesenteric lymph nodes, liver, kidney and spleen (Meli *et al.* 2004; Kipar *et al.* 2010). The widespread

distribution of the virus outside the intestines is possibly caused by infected monocytes which spreads systemically via the blood (Meli *et al.* 2004). The virus is believed to start replicating in the intestine, which cause viral shedding in the feces, before spreading systemically to lymph nodes and other organs, resulting in seroconversion. Even after the FeCoV has spread systemically no clinical signs are observed except for the potential mild clinical signs in the beginning (Meli *et al.* 2004).

In another study by Kipar *et el.* (2010), FeCoV was most often, and in the highest levels, detected in the colon. However viral shedding was most common and in higher loads when both the small intestine and colon contained virus. This study also concluded that most likely, the colon is where the virus stay persistent and can re-infect the small intestine which cause recurrent viral shedding.

Feline infectious peritonitis

FIP is developed after spontaneous mutations in certain genes of the FeCoV occurs (Herrewegh *et al.* 1995; Emmler *et al.* 2020) resulting in changes in the surface structures subsequently leading to the ability to replicate at a higher rate in monocytes/macrophages, compared to feline enteric coronavirus, therefore enhancing the pathogenicity (Dewerchin *et al.* 2005; Rottier *et al.* 2005). The likelihood for mutations to occur increases if viral replication in the intestines increases. Different factors can influence the replication rate in the intestines for example the age of the cat (Worthing *et al.* 2012) and the immune status which can be affected by infections (Poland *et al.* 1996), stress (Riemer *et al.* 2016) or glucocorticoid treatment as well as infection dose and viral virulence (Hartmann 2005).

The fatal outcome of FIP is not mainly caused by the virus but it is rather the hosts' own immune response that causes severe damage. The pathogenesis revolves around immune complexes consisting of virus or viral antigen together with antibodies and complement factors (Jacobse-Geels *et al.* 1982).

There are two different theories on what happens after virus is spread from the intestines. The first is that infected macrophages are spread to different tissues where the virus attracts antibodies subsequently activating complement factors which in turn attracts more macrophages and neutrophils to the site which results in granulomatous changes. The other mechanism is that the granulomatous changes develops as a result of circulating immune complexes that migrate to the walls of the blood vessels and there activates the complement system (Hartmann 2005).

In addition to the virus and immune complexes, chemotactic substances such as complement factors and inflammatory cytokines are released from dying or damaged macrophages which will lead to subsequently vasoconstriction and increased vascular permeability. The increased permeability allows proteins to exit the circulation resulting in protein-rich exudate characteristically seen in FIP (Hartmann 2005).

What determines whether dry or wet form of FIP develops is not clearly understood but seems to depend on the type of immune response the host develops (Tekes & Thiel 2016 pp. 201).

2.3.4 Epidemiology

Feline enteric corona virus

Feline coronavirus is spread worldwide both in housed cats, feral cats and wild felids. The virus has the highest prevalence in households with more than one cat because they defecate in the same place which is the most important role of transmission. Free-roaming cats have lower prevalence, most likely because they do not interact much with each other, and particularly not defecating at the same place (Hartmann 2005). The prevalence varies between less than 10% to greater than 50% depending on geographical area (Horzinek & Osterhaus 1979) .

The main route of infection is oronasally from feces shed from a FeCoV-infected cat although indirect transmission via clothes and toys etc. can also occur (Pedersen *et al.* 1981). Transmission via saliva, for example when grooming or sharing bowls, is also possible but occurs very rarely. Transplacental transmission is possible but rarely occur in natural infection, most kittens are infected at 5-16 weeks of age when maternal antibodies begin to decline (Sherding 2006b pp. 133). The virus inactivates in 24-48 h in room temperature but in dry environment the virus can survive for up to 7 weeks (Hartmann 2005; Sherding 2006b pp.133).

Feline infectious peritonitis

After FeCoV mutates causing FIP, the mutated virus does not seem to be shed in secretions or feces, therefore transmission of FIP between cats is considered unlikely (Hartmann 2005). However, FIP can be transmitted experimentally (Watanabe *et al.* 2018). Although the prevalence of FeCoV is relatively high, only approximately 5% of persistently FeCoV-infected cats develop FIP (Addie *et al.* 1995; Hartmann 2005).

2.3.5 Vaccines and Treatment

Treatment

There are currently no available cure for FIP and typically the treatment of choice is symptomatic with for example corticosteroids to suppress the immune response, however there are no controlled studies to support the efficacy of treatment with immunomodulators (Sherding 2006b pp. 140). Recent studies however have shown promising results using GS-441524 as a treatment for FIP, which completely prevents replication of the virus in cells from naturally infected cats *in vitro and in vivo.* This pharmaceutical is currently not commercially available (Murphy *et al.*

2018; Pedersen *et al.* 2019), but will likely be available soon. The efficacy of this treatment in shown in a study where 25/31 cats with naturally occurring FIP was treated with GS-441524 and stayed in remission. In addition, eight cats relapsed in this study but 7/8 cats responded to retreatment with a higher dosage of GS-441524 (Pedersen *et al.* 2019).

Vaccine

There is only one available vaccine against FIP, an intranasal vaccine that contains a heat-sensitive mutant of FeCoV type II, however there is no clear evidence of its efficacy (Addie *et al.* 2009); in addition type I FeCoV is more common than type II. There has been many attempts to develop an efficient vaccine but because of the involvement of antibodies in the pathogenesis many attempted vaccines resulted in a higher rate of FIP development (Addie *et al.* 2009). In general, vaccination is not recommended.

2.3.6 Study Aim

As previously mentioned, the goal of this study is to investigate the presence of FeLV, FIV and FeCoV/FIP in free ranging domestic cats in Mararienda, Mara North Conservancy, as they can cause disease in wild felid species. These viruses can potentially pose a threat to other wild felids and carnivores and by studying their potential presence one might contribute to the conservation of wild carnivores as well as to improve the welfare of the domestic cat in Mararienda.

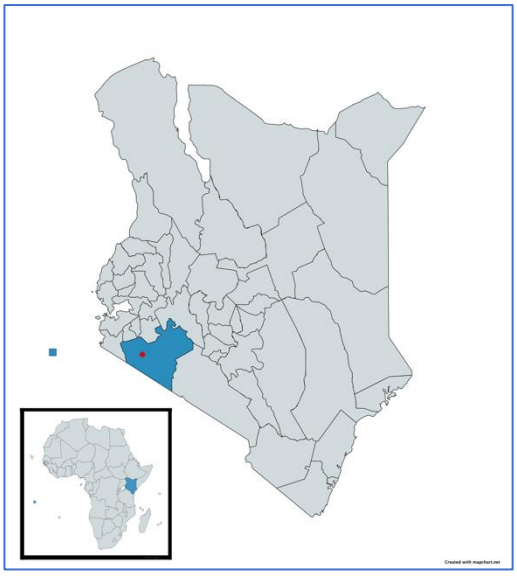
3. Material and Methods

3.1 Data Collection

The study was performed in the Mararienda district in Masai Mara national reserve, Kenya (see Figure 1 & 2). This district is located in the Mara North Conservancy and there is nothing that prevents wild felids from entering this area, which enables a domestic – wildlife interface. In addition, the area was suspected to have a population of free-roaming or feral cats. To obtain the cats for the study, different households in the Mararienda district was visited to collect information about which households owned a cat and wanted to participate in the study. To conduct the interviews, an interpreter was used because of the language barrier. A total of 100 households were asked to accomplish the goal of 60 cats to collect samples from. It was only possible to sample 47 cats, of which two cats were feral and the remaining were free roaming house cats.

The Maasai households were also interviewed regarding how they take care of the cats, their opinion about cats, and interactions between the domestic cat and wild felids (see appendix 1 for the questionnaire).

The cats that were sampled and part of the study were the cats that either the owner or the participants in the study was able to catch. To collect samples the cats were taken to a temporary clinic to possibly be sedated, reducing stress, before blood were taken. Before blood sampling we estimated the age of the cat based on dental formula, oral status and overall clinical examination. Cats in the study needed to be over 6 months of age for maternal antibodies not to interfere with the testing.



Coordinates (Karen Blixen Camp in Mara North Conservancy): 1°11'14.2"S 35°03'26.6"E

Figure 2. Map over Kenya: Narok county marked with blue, Mararienda marked with red dot. Map done with Mapchart.net

3.2 Blood Sampling

The blood was sampled from the jugular or cephalic vein using either a vacutainer or and open needle. Some of the cats were sedated depending on their temperament and stress level. The blood was then centrifuged, after 30 min- 3.5 h, to separate and collect the serum which was used for the FASTests.

If sedation was necessary, Domitor (1mg/ml) was used in the dose 0.1 mg/kg, after blood sampling and examination, the Domitor was reversed with Antisedan (5 mg/ml) 0.25 mg/kg. Some cats were sedated after blood sampling to maintain a good blood pressure and some did not need sedation at all.

3.3 FASTest for FeLV, FIV and FeCoV

To detect antibodies or antigens three different lateral flow tests (FASTest, MEGACOR Diagnostik, Austria) were used. One test was a combination-test to detect antigen from FeLV and antibodies for FIV in blood called FASTest FeLV/FIV. To detect antibodies from FeCoV/FIP a test called FASTest FIP was used. All FASTest was from the manufacture MEGACOR Diagnostik.

The FASTests are based on rapid immunochromatographic technique also known as lateral flow immunoassay. The test contains different zones; sample pad, conjugate pad, reaction matrix (a porous membrane, nitrocellulose membrane) where test line and control line will appear and lastly absorption pad. The sample

is added to the sample pad and will then migrate by capillary flow to the next zone, conjugate pad, where specific conjugated monoclonal detector antibodies (conjugate) is immobilized that will bind to the target antigen or antibody. When the sample reach this zone, the conjugates will re-mobilize and react with potential analyte and then migrate to the reaction matrix. In the reaction matrix antibodies or antigens have been arranged in two bands, one test line and one control line. The test line consists of specific antibodies (for antigen detection) or antigen (for antibody detection) that will capture the targeted antigens or antibodies. The control line consists of antibodies that will capture the conjugate (see figure 3 & 4). If the targeted antigen or antibody is present in the sample a colored line will appear in the test line area, the control line will appear whether the analyte is present or not and ensures that the test procedure is correct (figure 5). Excess reagent will be captured in the absorption pad (Wong & Tse 2009).

The test was performed on serum according to the instructions from the manufacture.

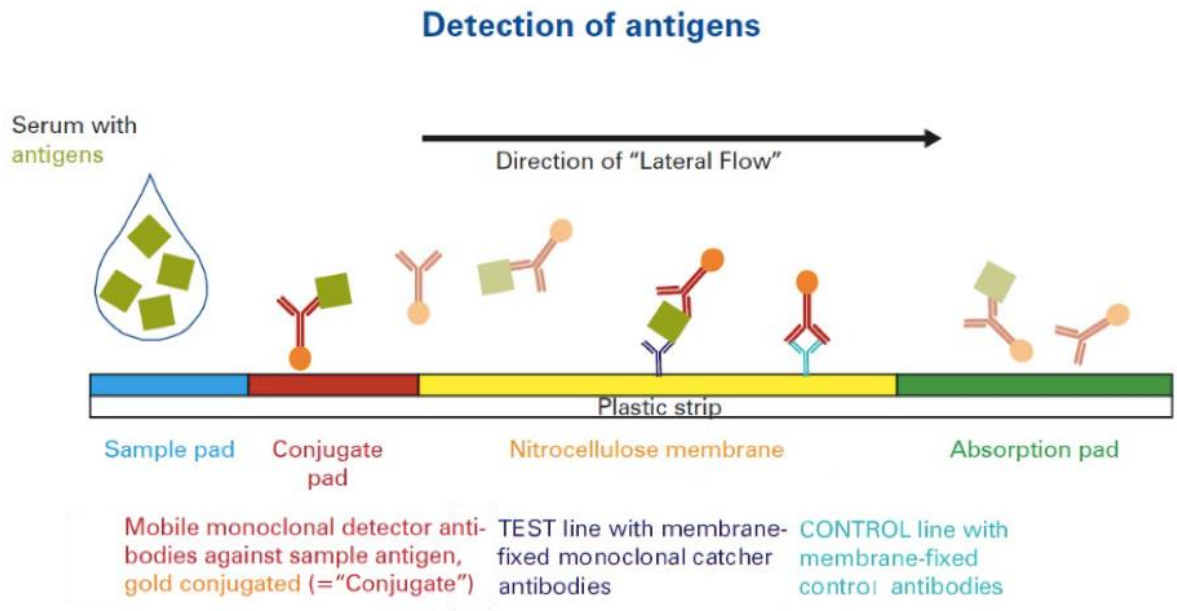


Figure 3. Test principle for the detection of antigens. Edited picture from MEGACOR Diagnostik.

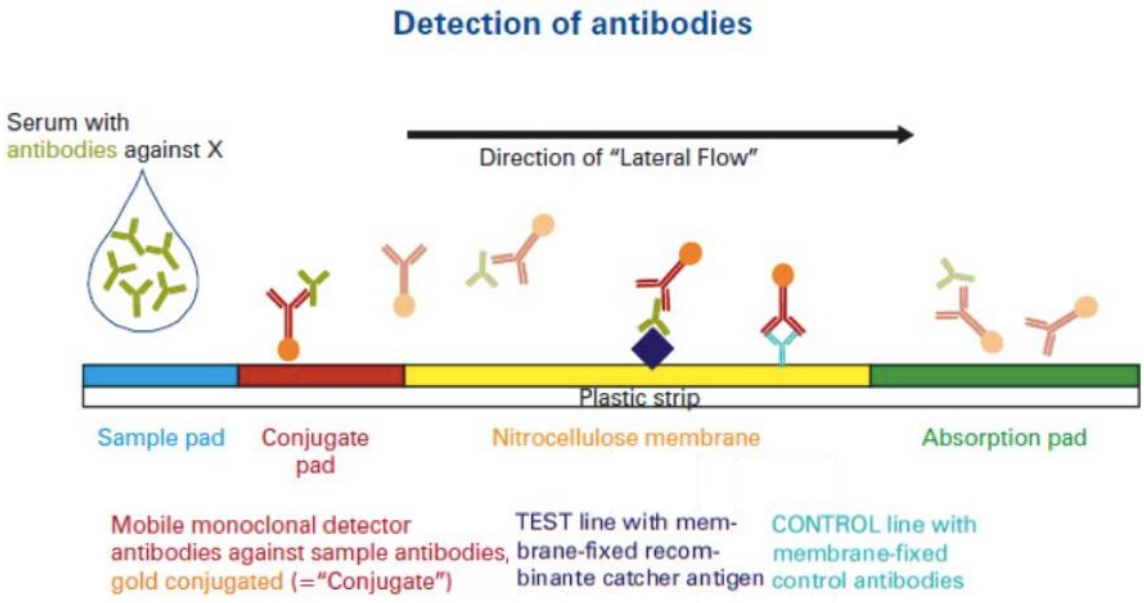


Figure 4. Test principle for the detection of antibodies. Edited picture from MEGACOR Diagnostik.

Figure 6. Positive test to the left, negative test to the right. Figure by Megacor.

FASTest FIP

According to the manufacturer, the test has a sensitivity of 97.4% and a specificity of 94.6%. Another study calculated the Se to 84.6% and Sp to 100% (Addie *et al.* 2015).

FASTest FeLV-FIV

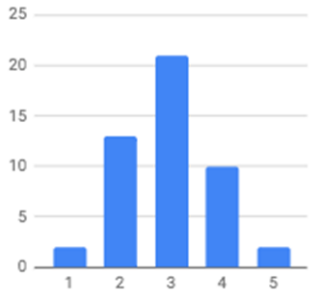
According to the manufacturer, the test has a sensitivity of 95% for FeLV and 96.4% for FIV, while the specificity is 99% for FeLV and 99.2% for FIV. Another study calculated the sensitivty to 94.7% for FeLV and 96.4% for FIV and the specificty to 98.8% for FeLV and 99.2% for FIV (Hartmann *et al.* 2007).

Data analyses

Data was handled in excel. All prevalences are calculated with binomial proportion confidence interval with Wilson score interval using epitools.ausvet.com.

4. Results

In total 47 cats were managed to be tested, of which two cats were feral and the remaining were free roaming house cats. Of the 47 cats 27 were female and 20 were male and all cats were estimated to be older than 6 months but younger than five years old based on the dental formula, oral status and overall clinical examination. All cats collected were unneutered. See table 1 for distribution of gender and feral or free roaming owned.



In general, the cats were underweight with a mean BCS of 3/10 (2.94) (See Figure 6). In addition, many of the cats had ectoparasites for example fleas and ticks along with fractured or missing teeth. However, none of the cats showed obvious clinical signs of systemic diseases.

Figure 6. Distribution of body condition score

Table 1. Distribution of female and male and feral or free roaming owned cats

	Female	Male
Free roaming owned	27	18
Feral cat	0	2
Total	27	20

4.1 Seroprevalence

Of the 47 cats sampled, 6 were positive for FeCoV/FIP antibodies. 1 of the 6 seropositive cats was a feral cat while the other 5 were owned free-roaming cats. The prevalence of FeCoV/FIP is 12.77% (4.83–25.74% with a binomial confidence interval of 95%).

See table 2 for the proportion of FeCoV/FIP-positive cats related to gender and overall FeCoV/FIP-positive proportion of the sampled population.

None of the sampled cats were positive for FeLV or FIV. The prevalence of FeLV and FIV is between 0–7.56% with a binomial confidence interval of 95%. See table 3 for prevalence for the viruses.

Table 2. Prevalence of antibodies against FeCoV/FIP in sampled population

Gender	Positive	Negative	Seropositivity (%)
Female	4	23	14.8
Male	2	18	10
Total	6	41	12.77

Table. 3 Prevalence of the viruses

Virus	Prevalence (%)	95% CI*
FeCOV/FIP	12.77	5.98-25.17
FeLV	0	0-7.56
FIV	0	0-7.56

*Confidence interval

4.2 Interview with Maasai Households

Summarized from the 100 interviews, the domestic cats in Mararienda serves an important purpose as they chase away rats, snakes and other small animals from the houses and the area around them. Of the 100 households we interviewed, 69% had a cat, most of them had 1 cat, but it varied between 1-4 cats per household with a mean value of 1.08 cats/ household.

The cats were owned by the women of the household who took care of the cat and fed them. The cats were usually fed with meat and milk, along with ugali (a dish made of maize flour and water) and vegetables. The cats roam around free during the day but usually stay inside during the night according to the owners. Most owners did not know where the cats went when they were outside, but most people stated that the cat did not go so far from the house. If the cats got sick most of the people interviewed said that they could not do anything, while a few people stated that they treated the cat with antibiotics used for livestock. One person informed that she treated her cat with chili mixed with water if it got sick.

Regarding domestic cat – wildlife interactions only 7 of 100 household stated that they have seen a domestic cat interact with wild felids species such as African wildcat and leopard. One stated that leopards have caught cats, four stated that they have seen domestic cats interact with African wildcat and two informed that they have seen a domestic cat interact with another bigger felid species but did not know the name of the species. However, 90 out of the 100 interviewed households

informed that if the cats died they threw them into the bush for wild animals to eat, 7 households did nothing and left them where they found them, 2 of them buried the cats and 3 had never seen a dead cat or didn’t know what to do.

5. Discussion

The purpose of this study was to investigate whether FeLV, FIV and FeCoV/FIP occur in domestic cats in Mararienda district in the Mara North Conservancy and whether there is a risk of these viruses transmitting to wild felid species, posing a threat to wildlife conservation in the area. FeLV-antigen and FIV-antibodies was not detected in the sampled cats while antibodies against FeCoV/FIP occurred with a positive proportion of 12.8% in the sampled population. As relatively few cats were sampled, it is not possible to calculate a prevalence in the cat population in Mararienda, but it is possible to say that FeCoV is present in the population. It is not possible to say that FeLV or FIV do not occur but based on this study these two viruses might not be widely spread in the population and based on epidemiological calculation the prevalence is under 7.5% with a 95% binomial confidence interval. However, further studies are needed to evaluate the prevalence or occurrence of FeLV and FIV in this part of Kenya.

Domestic cats in Mararienda district

The domestic cats serve an important purpose to households because they keep small animals such as rodents and snakes away. Almost every household has a cat and since the households are in close proximity to each other, interactions between domestic cats might be common. In addition, the cats in the area are unneutered which subsequently might lead to more frequent aggressive interactions, further increasing the risk of disease transmission.

Only 7 out of 100 households stated that interactions between domestic cats and wild felids occurred with one stating that leopards have caught domestic cats, four stating that they have seen interactions between African wildcat and domestic cats and two informing that they have seen interaction with another felid species that is bigger than the domestic cat but they don't know what kind. In addition, two people answered that they have not seen domestic- wildlife interactions but it might occur during the night. However, since domestic cats are most active during the night or dusk/dawn, these interactions may be occurring at times when humans are not awake and therefore occur more frequently than the owners stated.

Another potential risk for disease transmission from domestic cats to wild animals is that dead cats are thrown into the bush to be eaten by wild animals.

Viruses may survive in cat carcasses for some time after death and subsequently be transmitted to wild carnivores after ingesting the carcasses.

Feline leukemia virus and feline immunodeficiency virus

Even though none of the cats sampled were positive for FeLV, it doesn't mean the virus is not present, only a small proportion of the cat population was sampled, and infected cats might have been missed. FeLV and FIV are more common in males than females ((Luckman & Gates 2017; Maclachlan 2017 pp. 283,293) and in this study more females (57.5%) than males (42.5%) were sampled.

Contrary to FeLV, which is more prevalent in younger animals, the risk of testing positive for FIV increases if the cat is over 5 years of age according to (Luckman & Gates 2017). All cats in this study were estimated to be younger than 5 years and therefore might reduce the chances of finding FIV positive individuals in the sampled population.

None of the sampled cats were neutered in this study, neutering status impacts various behaviors in cats such as decrease in aggressivity (Finkler & Terkel 2010; Cafazzo *et al.* 2019). FIV and FeLV can be transmitted via bites (Lutz *et al.* 2009), particularly FIV (Sykes 2014 pp. 209), which makes unneutered cats a possible risk factor since aggression is more common. However, none of the cats sampled were positive, but if FIV or FeLV was to be introduced to the population, if not already present, the transmission rate might be higher if the cats are unneutered.

Feline corona virus/ feline infectious peritonitis

The proportion of cats that tested positive for FeCoV/FIP-antibodies was 12.8% which is similar to prevalence studies investigating single-cat households (15%) and feral cat populations (12%) (Hartmann 2005). The prevalence of FeCoV is higher in multi-cat households possibly because of cats sharing litterboxes (Addie *et al.* 2009), a free-roaming population does not defecate in the same area, minimizing the risk of transmission since it is a fecal-oral route (Hartmann 2005). This could explain the relatively low proportion of positive cats in Mararienda since they roam around freely, defecates outside and multi-cat household are not common. In addition cats in single cat-household seems to have a decreased risk of developing FIP compared to cats in multi-cat-household, shelters or catteries possibly because of reduced stress and less disease burden compared to multi-cat environment (Addie *et al.* 2009; Drechsler *et al.* 2011).

FIP is most common in younger animals <1 years (Hartmann 2005; Yin *et al.* 2021) and is more prevalent in purebred cats, possibly because of confounding factors, mentioned previously, such as sharing litterboxes subsequently leading to higher exposure of FeCoV together with more stressors associated with living in a multi-cat environment (Addie *et al.* 2009). Some studies also show a higher prevalence of FIP in some breeds for example ragdoll and rex, possibly explained

by genetic factors or environmental factors (Pesteanu-Somogyi *et al.* 2006). The cats sampled were all mix-breed cats potentially minimizing the risk of developing FIP, but more studies are needed to determine if the predisposition only occurs in some family lines or in the breed in general. If present in certain family lines the cats in Mararienda might not be less likely to develop FIP.

It is not possible to distinguish FeCoV and FIP antibodies with the test used, but none of the cats that tested positive for FeCoV showed any obvious clinical signs of FIP. However, since the disease can occur in both dry and wet form with varying degree of obvious symptoms, clinical signs might not be obvious enough to notice on a general clinical examination under sedation.

Cheetahs seem to be highly susceptible to FIP, where outbreaks of coronavirus in cheetah populations have resulted in a morbidity of up to 60% as a result of FIP (Heeney *et al.* 1990). It is speculated that it might be because of a more homozygous genome with low diversity in alleles due to a previous decrease in the wild population leading to a reduced gene pool with subsequently inbreeding (O'Brien *et al.* 1985; Evermann *et al.* 1988). Since cheetahs are present in Mara North Conservancy, domestic cats might be a possible source of infection even though cheetahs usually do not come close to the village according to interviews with local people.

Regarding potential risk of developing FIP in certain family lines or if inbreeding occurs, it is hard to estimate the risk of inbreeding since the population size of domestic cats in Mararienda is unknown, the breeding is also not controlled, which could lead to a higher degree of inbreeding possibly increasing the risk of developing FIP. On the other hand, since the cats roam around free and the population could possibly be big since every household has a mean value of 1.08 cats the diversity in alleles might be high.

Wild felids and other susceptible carnivores in Mara North Conservancy

There are several species in Mara North Conservancy that are susceptible to diseases that domestic cats in the area can carry. As previously mentioned, cheetahs are present in the conservancy, with potential higher risk since they seem to be more susceptible to developing FIP. In addition, other felids such as lions, leopards, African wildcat and serval along with hyena occur in the area with the potential risk of contracting disease from the domestic cat. However, the risk of FIV and FeLV-infection might be low since these viruses have species-specific strains that usually don't transmit between species even if it has been proposed to have occurred both for FeLV (Brown *et al.* 2008; Meli *et al.* 2009; Sacristán *et al.* 2021) and FIV (Nishimura *et al.* 1999; Troyer *et al.* 2005). Further studies are needed to conclude the threat domestic cats may pose to wild animals since this study is not enough to determine which viruses that circulate in the population. Additionally, studies on domestic – wildlife interaction would be of value to determine the risk of trans-

mission for different diseases as well as which species are at risk of contracting diseases from domestic cats.

Disease status in the population

As previously mentioned, the sampled population was quite small, resulting in that conclusions cannot be drawn regarding the presence of FIV and FeLV in the population but it is possible to conclude that FeCoV is present. The seropositivity was low for FeCoV and non-existent for FIV and FeLV, which might indicate that these diseases are not a big problem in the population and thereby not a big threat to wild species. Therefore, there might not be necessary at present time to try to reduce the risk of infection since the occurrence of the diseases are very low. If FIV and FeLV would emerge in the population, castrations might be a way to reduce the risk of infection. Further research is needed to determine the prevalence of these diseases in the domestic cat population in Mararienda district by expanding the sampling. Regarding the risk of disease transmission between domestic cats and wild felids there seems to be interactions between both living animals as well as cat carcasses and wild animals, which are both possible ways of disease transmission and a possible threat to the conservation of vulnerable species. More research is needed to determine how great the risk is for wild species to become infected and which species that are at risk.

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Popular Science Summary

The aim of this study was to investigate if three different viruses were present in the domestic cat population in Mararienda district in Mara North Conservancy, Kenya and if they might pose a threat to the wild felids in the conservancy.

Feline leukaemia virus, feline immunodeficiency virus and feline corona virus are all viruses that can affect domestic cats along with other wild feline species. These viruses are spread worldwide in the domestic cat population and can cause severe disease and even death. Since the viruses can be transmitted to other wild felids and cause disease and sometimes death these diseases could be of importance in conservational purpose. Mara North Conservancy is home to several species from the *Felidae* family and no previous studies have been conducted regarding diseases in the domestic cat population in the area. In addition, little is known about interactions between domestic cats and wild animals in Mara North Conservancy.

Feline Leukaemia Virus

Feline leukaemia virus (FeLV) is a retrovirus that can cause several different diseases such as tumours, weakened immune system and neurological symptoms. Once infected the virus will always stay in the cats' DNA. The virus is transmitted via direct contact and is shed via mucous membranes.

Feline Immunodeficiency Virus

Feline immunodeficiency virus (FIV) is also a retrovirus comparable to HIV in humans and can cause compromised immune system in infected cats with a final stage of the disease similar to AIDS with severe immunosuppression and opportunistic infections. The virus is shed in high concentration in the saliva and the major route of transmission is via bites.

Feline Coronavirus

Feline coronavirus (FeCoV) is a coronavirus that infects domestic cats along with other wild felids. The virus appears in two different forms, the benign feline enteric coronavirus which can cause mild symptoms, such as diarrhea, or can occur without any symptoms. The other form is feline infectious peritonitis which develops after the virus mutates inside the cat and subsequently causes severe symptoms that

results in death without treatment. Cheetahs seem to be particularly susceptible to this disease.

100 households in the Mararienda district was interviewed to find out more about how the cats are taken care of, how they live and if they might have interaction with wild animals. Blood samples were also collected from 47 cats to investigate if antibodies against FIV and FeCoV and antigen (part of the virus) from FeLV were present in the population.

Out of the 47 cats only 6 cats (12.8%) tested positive for antibodies against FeCoV, none of the cats tested positive for FeLV or FIV. Since the size of the population of domestic cats in Mararienda district is unknown and only a small proportion of the whole population was tested it is not possible to say that FeLV or FIV does not occur, but it is possible to say the FeCoV is present in the population.

The interviews showed that only 7 out of 100 households had seen domestic cats interact with wild felid species for example leopard and African wildcat. However, the vast majority of household stated that when a cat dies, the owners throw it out in the bush for wild animals to eat. This might be a possible transmission route for diseases to spread between domestic cats and wild animals.

In conclusion, feline coronavirus appears to the be present in domestic cat population in Mararienda district, however, even though no cat tested positive for FeLV or FIV it is not possible to say that these diseases are not present. Further research is needed to determine if FeLV and FIV occur and how common all of the three diseases are in the population. Additional studies are also needed to investigate the risk of diseases transmitting from domestic cats to wild felids or other wild animals.

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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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