ESTONIAN UNIVERSITY OF LIFE SCIENCES
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INCIDENCE OF FELINE LEUKEMIA VIRUS AND FELINE IMMUNODEFICIENCY VIRUS IN WILD FELINES LIVING IN CAPTIVITY AT A NATURE RESERVE IN CENTRAL AFRICA

KASSIDE LEUKEEMIAVIIRUSE JA IMMUUNPUUDULIKKUSE VIIRUSE ESINEMINE KESK-AAFRIKA LOODUSKAITSEALAL VANGISTUSES ELAVATEL METSIKUTEL KASSLASTEL

Final Thesis
Curriculum in Veterinary Medicine

Supervisor: Professor Toomas Orro

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Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are among the most prevalent viral infections in domestic cats and they are both capable of causing serious harm to the health of the infected animals. These viruses have also been found to infect and cause disease in wild felids. Point of care (POC) tests for detecting FeLV antigen and FIV antibody are the most widely available tests for screening for these viruses and regular screening of domestic cats is recommended. This study aimed to find out the incidence of FeLV and FIV in caracals (*Caracal caracal*), cheetahs (*Acinonyx jubatus*), leopards (*Panthera pardus*) and lions (*Panthera leo*) living at a nature reserve by looking into the test results obtained from point of care tests for FeLV and FIV performed between the years of 2016-2022. The study found that three cheetahs out of the 54 animals in this study tested positive for FeLV, and no animals tested positive for FIV. This study result proves that FeLV is present in the study population and attention should be paid to the efficiency of the testing regime to ensure that the spread of the virus can be controlled, and the health of the infected animals is monitored sufficiently.

Keywords: FeLV, FIV, incidence

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Kasside leukeemiaviirus (FeLV) ja kasside immuunpuudulikkuse viirus (FIV) on kodukassidel ühed levinumad viirusnakkused ning mõlemad viirused põhjustavad tõsist kahju nakatunud loomade tervisele. Samuti on leitud, et need viirused nakatavad ja põhjustavad metsikute kasside haigustumist. *Poin-fo-care* (POC) testid FeLV antigeeni ja FIV antikehade tuvastamiseks on nende viiruste sõeluuringuks kõige laiemalt kättesaadavad testid ning soovitatav on kodukasside regulaarne testimine viiruste nakkuse avastamiseks. Selle uuringu eesmärk oli välja selgitada FeLV ja FIV esinemist looduskaitsealal elavatel karakalitel (*Caracal caracal*), gepardidel (*Acinonyx jubatus*), leopardidel (*Panthera pardus*) ja lõvidel (*Panthera leo*) aastatel 2016-2022 tehtud testide alusel. Uuring näitas, et kolmel gepardil kokku 54 uuritud loomast oli FeLV-i test positiivne ja ühelgi loomal polnud FIV-i test positiivne. Uuringutulemus viitab FeLV esinemisele uuringupopulatsioonis ja tähelepanu tuleks pöörata testimise tõhususele, et viiruse levikut oleks võimalik kontrollida ja nakatunud loomade tervist jälgida.

Märksõnad: FeLV, FIV, incidence

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

FeLV – feline leukemia virus

FIV – feline immunodeficiency virus

ELISA – enzyme-linked immunosorbent assay

PCR – polymerase chain reaction

POC – point-of-care

INTRODUCTION

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are retroviruses that are known to infect both domesticated and wild felids worldwide. These two viruses are among the most common pathogens in felines. They both hold the ability to cause serious health problems by affecting the immune system of the host. FIV suppresses the immune system and can cause acquired immunodeficiency syndrome, which predisposes the host to secondary infections. FeLV is more pathogenic than FIV and is capable of causing serious disease on its own (Hartmann, 2011). Point of care tests (POC) are available for both diseases and screening cats for these pathogens is recommended (Westman *et al.*, 2019). Both of these viruses have a relatively high prevalence in domestic cat populations around the world (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020). In recent years studies have also been carried out on other felids, both in captivity and in wild populations. Screening for these viruses also in other felids is important, especially in captive populations where the welfare of the animals is the responsibility of humans and preventative measures can be taken to stop the spread of diseases. In this retrospective survey study, POC test results from a seven-year period (2016-2022) for FeLV and FIV were collected from four species of felids kept captive at a nature reserve and analyzed to find out the incidence of both viruses in these populations. This study includes a literature review part that discusses the most important aspects of these two viruses and presents results from similar studies that have been done in the past. The second part of this study goes through the aim of the study, materials and methods used in this study, the results that were obtained, and a discussion bringing together the topics covered in the first part and the findings of this study. In the end, there is a brief chapter summarizing the conclusions that can be made based on this study.

1. LITERATURE REVIEW

1.1. Feline immunodeficiency virus

Feline immunodeficiency virus (Retroviridae: Lentivirus) referred to as FeLV, is a virus causing chronic, persistent infection in both domestic and wild felines worldwide. Together with the Feline leukemia virus, they are the two most common pathogens that affect the immune system of domestic cats. Data from recent years suggests that these pathogens are present and causing disease also in wild felids (Sykes, 2013).

1.1.1. Pathogenesis and transmission of FIV

After entering the host, the FeLV starts replicating in dendritic cells, macrophages, B cells, and CD4+ T and CD8+ T lymphocytes, with CD4+ T cells being the virus's main target. Targeting all these cells has a complex effect on the immune system of the host, but the whole mechanism is not well understood. What is known is that it seems to result in both immune suppression and immune activation. Viraemia can be seen already the second week after infection, and it reaches its peak between eight to twelve weeks post-infection. During this time the virus spreads through the whole organism and the numbers of CD4+ T and CD8+ T cells drop in the peripheral blood. This phase of the infection is called the acute phase. The acute phase can last anywhere from three to six months. After the acute phase the infected cats will develop both cellular and humoral immune responses. This is regarded as the onset of the asymptomatic phase (Sykes, 2013; Taniwaki *et al*., 2013).

In the asymptomatic phase, the numbers of the CD4+ T cells rebound and the levels of plasma virus load drop. Infected cats will show minimal to no clinical signs and this phase of the infection can last for years or even the whole life of the animal. Even though no clinical signs are apparent, there is a continuous reduction in the number of CD4+ T lymphocytes, and an inversion of the CD4+/CD8+ ratio, causing progressive immunodeficiency (Sykes, 2013; Taniwaki *et al*., 2013).

The next phase of the infection is the terminal phase, which is characterized by severe immunosuppression and a reduction in circulating antibodies. This allows the development of diseases caused by opportunistic or secondary chronic infections, neoplasia, and neurological disorders. The cats in the terminal phase of the FIV infection usually die of other causes, rather than the FIV infection itself. The course of the disease depends on the virus-host interaction and only some of the infected cats reach the terminal phase (Sykes, 2013; Taniwaki *et al*., 2013).

The virus is shed in large amounts in saliva but can only survive for short periods outside of the host and is very susceptible to disinfection. The major route of transmission of FIV is thought to be through bite wounds. A study (Litster, 2014) was conducted in the USA where they looked into the transmission of FIV among cohabiting cats at two cat rescues. The study found that no transmission between FIV-positive and FIV -negative cats occurred, despite the fact, that the cats lived together for months to years, shared food bowls, litter boxes, and bedding, and displayed both mutual grooming and mild aggression. They also found no vertical transmission from naturally infected mothers to their kittens at their other research site. Another study (Medeiros *et al*., 2012) found evidence of natural transmission between a queen and her kittens in a colony of naturally infected stray cats. The transmission occurred in utero and caused the infected queen to have two abortions and a small litter size in the litters that were born.

1.1.2. Clinical signs of FIV

The clinical signs of FIV infection depend on the phase of the disease. In the acute phase, non-specific clinical signs can be seen. These signs include lethargy, inappetence, and pyrexia. Neutropenia and generalized lymphadenopathy can also be found, and these may persist for several months. In the asymptomatic phase, the infected cats may present the same non-specific signs as in the acute phase, or there may be no clinical signs present. In the terminal phase, the clinical signs reflect the opportunistic infections. Some of the most common diseases seen with FIV infection are stomatitis, neoplasia, ocular inflammation, renal insufficiency, lower urinary tract disease, and endocrinopathies. In addition to these, also chronic bacterial skin and ear infections, persistent viral upper respiratory tract infections, dermatophytosis, mycobacterial infections, and fungal infections can be seen. Although the prevalence of many of these infections is actually the same in FIV-infected cats and cats without FIV infection, the secondary infections are more severe and less responsive to treatment in FIV-infected cats (Hartmann, 2011; Taniwaki *et al*., 2013; Bęczkowski *et al*., 2022).

1.1.3. Testing and diagnosing of FIV

The most used tests to screen cats for FIV are the serologic point-of-care (POC) tests that use lateral flow immunochromatography or bidirectional-flow enzyme-linked immunosorbent assay (ELISA) to detect FIV antibodies from whole blood. These tests are readily available, easy to use, and reliable. It is recommended to use a polymerase chain reaction test (PCR) in addition to a POC test in situations where the true FIV status of the cat is important. With a PCR test, it is possible to also detect seropositive cats that have been vaccinated against FIV, seropositive kittens that still have maternally derived antibodies against FIV, and seronegative

cats when not enough time has passed from the start of the infection for seroconversion to occur (Bęczkowski *et al*., 2022). It has been thought that POC tests are not able to differentiate between infected and vaccinated cats, but recently researchers have been able to prove that at least two commercially available POC tests were able to accurately distinguish between infected and vaccinated cats (Westman *et al*., 2019). One study (Levy *et al*., 2017) comparing the performance of four commercially available POC tests for FIV argued that especially in low-risk populations most positive test results obtained with these tests would be false negatives. The study also found that in a population with one percent FIV seroprevalence, approximately half of the positive results would be false-positive results when using three out of the four POC tests that they studied.

1.1.4. Prevalence and risk factors of FIV infection

FIV is present in cat populations worldwide and its seroprevalence is estimated to be between one and twelve percent in domestic pet cats (Sykes, 2013). A recent study (Westman *et al*., 2019) found the prevalence of FIV in Australia to be 15%. Their study consisted of pet cats more than two years old and with access to the outdoors. Age and outdoor access have been recognized as risk factors for FIV infection. Other risk factors include neuter status and health status. Adult, intact male cats with outdoor access and previous health problems seem to be at the highest risk for FIV infection (Bęczkowski *et al*., 2022). This is probably because these cats are more likely to get bite wounds which are the most important entry route of FIV (Sykes, 2013).

1.2. Feline leukemia virus

Feline leukemia virus (Retroviridae, Gammaretrovirus) referred to as FeLV, is one of the most common infectious agents causing mortality in domestic cats. FeLV causes immune suppression, bone marrow disorders, and hematopoietic neoplasia and it is known to cause disease also in wild felids (Sykes, 2013).

1.2.1. Pathogenesis and transmission

The most common route of entry for FeLV is the oral-nasal route. After entering the host, the virus infiltrates the local lymphoid tissue and infects lymphocytes and monocytes, which then carry the virus throughout the body. This initial viremia phase lasts up to two weeks. In cats with weak immunity to FeLV, the virus reaches the bone marrow, where it infects precursor cells. This leads to the release of infected neutrophils and platelets into the bloodstream and the second viremia phase around two to four weeks after infection. The virus is able to replicate in

mucosal and glandular tissues, including the gastrointestinal tract and salivary glands. The virus is mainly shed in saliva, but can also be present in feces, urine, and milk (Hartmann and Hofmann-Lehmann, 2020).

There are four disease courses, that have been experimentally characterized, that a FeLV infection can follow. These courses are progressive infection, regressive infection, abortive infection, and focal atypical infection. Which course the disease takes in each individual animal is determined by how well the animal's immune system can fight off the virus within the first 12 weeks of the infection. In some animals, the course of the disease can also be altered by different factors such as immunosuppression, coinfections, or change in environment later in the infection (Hartmann and Hofmann-Lehmann, 2020).

In the progressive infection course, the initial immune system response is not efficient, and the virus is able to reach the bone marrow. The viremia phase that follows is longer than 12 weeks and the animal can even remain viremic. Persistent replication of the virus occurs in the mucosal and glandular epithelial tissues and the animal sheds the virus in its saliva continuously and will be infectious to other animals for the rest of its life (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020).

In the regressive infection course, the immune system response is effective, and the virus is not able to reach the bone marrow. The viremia phase either never occurs or is cleared. These animals do not shed virus in their saliva, and they are not infectious to other animals. However, the virus can be re-activated in these animals if they become immunosuppressed. In this case, viremia will develop and the animal will shed the virus and can develop FeLV- associated disease (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020).

In the abortive infection course, the immune system response is also effective, and the replication of the virus is stopped already after the initial replication in the local lymphoid tissues. In this case, the viremia never develops. This infection course is likely caused by low-dose exposure to FeLV and the animals with this infection course can develop immunity against the virus (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020).

In the focal infection course, the infection is restricted only to certain tissues and persistent local viral replication occurs in these tissues. For example, mammary glands, bladder, eyes, spleen, lymph nodes, or small intestine can be infected. This infection course is considered rare under natural circumstances (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020).

1.2.2. Clinical signs of FeLV

Clinical signs in FeLV infection mostly only occur when active virus replication is present. For this reason, clinical signs are mainly seen in the progressive infection course. Some diseases such as lymphoma and bone marrow suppression have, however, also been reported in the regressive disease course (Hartmann and Hofmann-Lehmann, 2020). FeLV infection can cause several different clinical signs. They are classified into tumors, secondary infections caused by immunosuppression, hematologic disorders, immune-mediated diseases, and other syndromes (Hartmann, 2011).

The most common tumors caused by FeLV infection are lymphoma and leukemia. It has been found that animals with FeLV infection are more than 60-fold more likely to develop lymphoma than animals that do not have the infection (Shelton *et al*., 1990). Fibrosarcomas caused by FeLV infection have also been reported in younger cats (Sykes, 2013; Hartmann 2011).

Anemia caused by bone marrow suppression is the most common clinical syndrome associated with FeLV infection. It is caused by the infection of both hematopoietic stem cells and bone marrow stromal cells. (Hartmann and Hofmann-Lehmann, 2020). According to a recent study (Spada *et al*.,2018), anemia at the time of diagnosis of FeLV had a negative impact on the life expectancy of the animal.

Immune-related diseases related to FeLV infection are cytopenias, glomerulonephritis, uveitis, and polyarthritis. In cats, primary immune-mediated diseases are rare, so testing for FeLV and FIV is recommended if a cat is diagnosed with one of these disorders (Sykes, 2013).

Secondary opportunistic infections are commonly seen with FeLV infection due to immunodeficiency. These infections include bacterial infections of the urinary tract, hemoplasmosis, chronic stomatitis, toxoplasmosis, dermatophytosis, cryptococcosis, feline infectious peritonitis, and upper respiratory tract infections. Although the prevalence of these infections in FeLV-infected animals is similar to their prevalence in animals without a FeLV infection, the clinical signs are more severe, and the infections do not respond to treatment as well in FeLV-infected animals (Sykes, 2013). Most of the cats with FeLV arriving at clinics for treatment actually arrive because of the clinical signs caused by the secondary infections (Hartmann, 2011).

Other syndromes associated with FeLV infection are neuropathy, reproductive disorders, and fading kitten syndrome (Hartmann, 2011).

1.2.3. Testing and diagnosing of FeLV

Screening cats for FeLV is done using similar POC tests that are used for screening for FIV. The tests used to detect FIV detect antibodies in the blood, but because the antibody response is unreliable in FeLV infection, the tests used for FeLV screening detect the viral antigens. It is recommended that also FeLV test results from POC tests are confirmed with a PCR test (Westman *et al*., 2019). A study mentioned earlier (Levy *et al*., 2017) tested the performance of four POC tests for the detection of FeLV antigens. Their study found that only one out of the four tests was able to correctly identify the status of all the cats in the study. The other three tests produced both false negative and false positive results. They predicted that in a low-risk population with one percent seroprevalence for FeLV, 83-94% of the results would be erroneous using any of these three tests. They also found that the diagnostic accuracy of these tests did not improve in a population with higher seroprevalence. In a population with five percent seroprevalence, 49-76% of the results would still be erroneous. In another study (Westman *et al*., 2019) where three different POC tests were used, they reported 21-38% false-positive test results in a population with eight percent seroprevalence.

1.2.4. Prevalence and risk factors of FeLV infection

FeLV can be found worldwide. Prevalence of progressive FeLV infection has been reported to be 0.7% to 15.6% in Europe, 2.3% to 3.3% in the United States 3.0% to 28.4% in South America, and 0.5% to 24.5% in Asia, Australia and New Zealand (Hartmann and Hofmann-Lehmann, 2020). The testing and vaccination programs for FeLV have gotten much better over the years, and the overall prevalence of FeLV infections has declined over the past decades, however, the most recent data shows that the decrease has mostly stagnated (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020).

Many risk factors for FeLV infection have been studied. These risk factors include age, sex, outdoor access, aggressiveness, and the presence of other diseases. Male cats that have access to the outdoors and display aggressive behaviors seem to be more at risk. Cats that live in multi-cat households or otherwise have contact with other cats, as well as cats with previous health conditions, seem to also be at higher risk of FeLV infection. The median age of cats with FeLV infection is three years, and adult cats are more likely to get infected than cats less than six months of age (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020).

1.3. Prevalence of FIV and FeLV infections in wild and captive felids

Many studies have been made to look into the prevalence of FIV and FeLV in both free-roaming and captive felids. One study (Broughton *et al*., 2021) studied the effect of FIV and its co-morbidities on the morbidity of free-roaming lions (*Panthera leo*) in Kruger National Park in South Africa. Out of the 195 sampled lions 142 tested positive for FIV. They also found that the prevalence was similar between males and females but increased with the age of the host. Another study conducted in Chile (Mora et al., 2015) looked into FIV and FeLV infections in free-roaming guignas (*Leopardus guigna*) and domestic cats. They found that 13% of the guignas they studied tested positive for FIV, 20% for FeLV, and seven percent for both viruses. All the animals that tested positive had no clinical signs and were in good physical condition. One study conducted in Thailand (Tangsudjai *et al*., 2010) discussed the prevalence of FeLV in captive wild felids and they found that two out of 30 samples taken were positive for FeLV when using a POC test, but when using PCR testing, the number of positive test results was 20. A study (Villalba-Briones *et al*., 2022) where the prevalence of canine distemper virus, FIV, and FeLV were studied in captive wild ocelots (*Leopardus pardalis*) in Ecuador found that 87.5% of the tested animals tested positive for FeLV. None of the animals in this study tested positive for FIV. They hypothesized that the reason for this could be that FIV is not present in the area where the study was made or that the POC tests used, which were meant for domestic cats, were not able to detect it. They also found that two animals that initially tested FeLV positive, got negative test results in later tests, suggesting that ocelots could be able to recover from the infection. In one study, all of the nine animals tested negative for both FIV and FeLV. This study (Reyes *et al*., 2017) was conducted in the Philippines and looked into the prevalence of FIV, FeLV, and *Toxoplasma gondii* in captive tigers (*Panthera tigris*).

2. AIM OF THE THESIS

The aim of this study was to find out the incidence of FeLV and FIV between the years of 2016-2022 in caracals (*Caracal caracal*), cheetahs (*Acinonyx jubatus*), leopards (*Panthera pardus*) and lions (*Panthera leo*) kept in captivity at a nature reserve in Central Africa.

3. MATERIALS AND METHODOLOGY

3.1. The animals

For this study, data was collected from 22 cheetahs (*Acinonyx jubatus*), 18 lions (*Panthera leo*), nine caracals (*Caracal caracal*), and five leopards (*Panthera pardus*). In total 54 animals were included in the study. Out of these animals, 10 are deceased but had FeLV/FIV POC test results from the study period so the decision was made to include them in the study. The reasons for death included euthanasia due to medical issues and death due to natural causes such as deteriorating condition and accidents with other animals. Of the animals in this study, 52% (28 animals) were male and 48% (26 animals) were female. The sex distribution among study animals is seen in Figure 1. The highest difference between the numbers of females and males included in the study was in caracals, where there were twice as many males than there were females (six animals and four animals respectively).

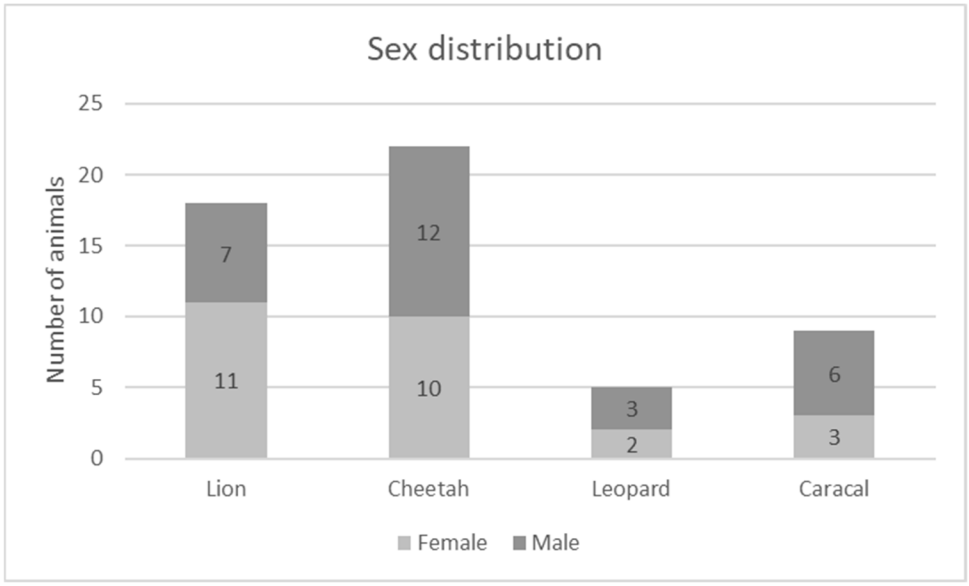

Figure 1. Sex distributions of animals included in the study. Total number of animals n=54 (18 lions, 22 cheetahs, 5 leopards, 9 caracals).

The age of the animals ranged from three to 20 years. The average age was 9.24 and the mode was 7. The age of nine animals was not known. The age distribution of study animals is seen in Figure 2.

Figure 2. Box and whiskers plots (median, interquartile range) for age distributions of the animals included in the study whose age was known. Total number of animals n=45 (13 lions, 22 cheetahs, 3 leopards, 7 caracals).

3.2. The living conditions

The animals arrive at this nature reserve either from other similar organizations or as rescues from the illegal wildlife trade or human-wildlife conflict. This organization aims to rehabilitate the animals and release them back into the wild, either on their land or at other locations. Some animals cannot be released due to different issues, and those animals are kept in captivity at the reserve. The animals kept in captivity are housed in large (several hectares), fenced enclosures either individually or in small groups of up to three animals. In addition to these animals, the reserve also houses multiple animals of other species, some of which are kept free-range on the reserve. There was a domestic stray cat living on the property, but it was euthanized in 2021 after it tested positive for FeLV. There are also wild animals living on the reserve. The animals are fed according to their dietary needs. The animals in this study are fed chicken meat with feathers, red meat with bones and skin, and organs. Supplements are added to the feed as needed

and the diets of individual animals are altered according to their individual needs. The meat is delivered to the reserve, stored in cold containers, and prepared in a dedicated area before it is fed to the animals. This meat preparation area is open to the outside and accessed by multiple different people daily. The same area is also used to prepare vegetables as feed for the other animals kept on the reserve.

3.3. The data and testing

Felines kept captive at this location used to undergo a yearly health check, but due to changes in the regulations of the country, changes are currently made to the testing regime at this location. For the health check, the animal is put under anesthesia in their enclosure using a dart gun to deliver the drugs safely. Once the animal is under anesthesia, an intravenous catheter is placed, intravenous fluids are administered, and a face mask is placed to cover the eyes of the animal. Any drugs that the animal needs as well as a rabies vaccine are administered. A team of professionals collects data that is relevant for the assessment of the health status of the animal on a pre-formatted sheet. The information collected includes different measurements (length, weight, size of paws, size of teeth, etc.) and acknowledgments about the general condition of the animal (body condition, condition of the teeth, possible wounds, or marks on the body, etc.). A blood sample is also collected for testing for FeLV and FIV or other tests depending on the needs of the individual animal. For FeLV and FIV testing, two different types of POC tests are used according to availability. These tests are The WITNESS® FeLV/FIV Rapid Test Kit produced by Zoetis and Anigen Rapid FIV Ab/FeLV Ag Test Kit produced by Bionote. The tests are done using whole blood. If the POC test result is positive, a sample is sent to a laboratory for PCR testing to confirm the test result. After all the necessary data is collected, the anesthesia is reversed, and the recovery of the animal is observed from a safe distance. If there are any complications with the recovery they are dealt with accordingly. The filled data sheet as well as possible blood work results and test results are added to folders that have the general information and health data for each animal organized according to their species.

The data for this study was collected individually for every animal from annual health report sheets. The data was collected from the period between 2016 to 2022. The data that was collected included species of the animal, sex of the animal, year of birth of the animal, any additional information that was written down about the animal, and information on which years a FeLV/FIV POC test was performed on that animal and if the test result was positive or negative. In case of a positive POC test result, information on further testing was also included.

For deceased animals, the reason or suspected reason for death was recorded. The data was combined and analyzed using Microsoft Office Excel.

3.4. Declaration of ethical considerations

The data for this study was collected retrospectively from already existing data. No samples were collected solely for the purpose of this study. All the data in this study is kept anonymous. There is no conflict of interest.

4. RESULTS

Between the years 2016-2022 altogether 62 FeLV/FIV POC tests were performed. The highest number of tests were performed in the years 2019 and 2021 with 19 and 22 tests performed respectively. The most tested species was cheetah with 29 tests performed and the least tested species was leopard with two tests performed. Some of the animals included in the study had not been tested at all within the study period. These animals included one caracal, two cheetahs, and four leopards. No tests were performed in the year 2016.

Out of the 62 tests performed, three (4.83%) were positive for FeLV, making the incidence of FeLV in this study population 5.55% (three out of 54 animals). Samples from all three positive animals were sent to the laboratory for PCR testing and the result for all three was confirmed positive for FeLV. None of the animals tested positive for FIV. All three positive FeLV test results are from cheetahs and from the year 2021. The positive test results are from two male cheetahs that live in an enclosure together with one more male cheetah. All three males are brothers and were born in 2016 in captivity and have remained in captivity all their life. The third male has tested negative in all tests. The third positive test result is from a female cheetah living in an enclosure next to the enclosure of the three brothers. The brothers moved to this enclosure in 2022 and the female has been in the same enclosure since 2016. The female cheetah was born in 2008 and arrived at this location in 2009. All the cheetahs that tested positive in 2021, had tested negative in 2019 and all the years before that. Out of these three animals, only the female cheetah has been tested in the year 2022. The POC test results for both viruses were negative in this test. Number of tests performed per animal species per year and the results of the tests are presented in Table 1.

Table 1. Tests performed on the animals in the study according to the species and year. The number of animals in total is n=54 and the number of performed tests in total was n=62.

Year		Species (n)				Total
		Caracal	Cheetah	Leopard	Lion	
2022	tests	3	3	0	6	12
	positives	0	0	0	0	0
2021	tests	2	11	1	8	22
	positives	0	3	0	0	3
2020	tests	0	1	0	0	1
	positives	0	0	0	0	0
2019	tests	3	10	1	5	19
	positives	0	0	0	0	0
2018	tests	3	4	0	0	7
	positives	0	0	0	0	0
2017	tests	0	0	0	1	1
	positives	0	0	0	0	0
2016	tests	0	0	0	0	0
	positives	0	0	0	0	0
Total	tests	11	29	2	20	62
	positives	0	3	0	0	3

5. DISCUSSION

In this study, all of the animals tested negative for FIV and all but three animals tested negative for FeLV using a POC test. With these results, it is worth discussing the reliability of the tests. It is a possibility that some of the animals that have been tested have gotten false negative test results. In a study (Levy *et al*., 2017) where different types of POC tests were compared, they compared also the two types of tests used in this study and found the sensitivity of these tests for FIV antibodies to be 94.7% with WITNESS and 96.8% with Anigen. For FeLV antigen they found the sensitivity to be 89.0% with WITNESS and 91.8% with Anigen. False negative test results were obtained with both tests and for both viruses in that study. Another factor that should be considered is the reliability of the testing regime. All the animals were not tested during the study period. This means that the actual disease status of these animals is not known. The animals that were tested, were not tested on a regular basis and some of the animals only have a few test results from several years ago, meaning that their current disease status is also not known. Three of the animals in this study tested positive for FeLV. The specificity of both of the POC tests for FeLV antigen was found to be high in the previously mentioned study (Levy *et al*., 2017), with 95.5% specificity for both tests. There were no false positive test results obtained with the WITNESS test and only one false positive test result was obtained with Anigen. The tests of all three animals that tested positive in this study were confirmed using PCR. This means that the possibility of false positive results can be considered very unlikely. Only one of the three animals has been tested again after obtaining a positive test result. This was the female cheetah. This cheetah was tested in 2022 and in that testing, a negative test result for both viruses was obtained, indicating that this cheetah has possibly cleared the infection. In another study with quignas (Mora *et al*., 2015) similar results were obtained, with three animals that originally tested positive for FeLV, getting negative test results six months later. For the other two positive animals in this study, there are no test results after the positive test results. Because of this, the current disease status of these animals can not be confirmed. The possibility remains that also these animals have cleared the infection.

It is known that both FeLV and FIV are secreted in large amounts in the saliva of the infected animals (Sykes, 2013). A study has shown that the main transmission route for FIV is through bite wounds and that the virus does not easily spread between cohabiting cats, even though they share the same food bowls and groom each other (Litster, 2014). Based on this information, the

spread of FIV in an environment like the one in this study would be unlikely, because the animals in this study are kept in enclosures. Only a few of the animals share an enclosure and hence could be able to bite each other. For FeLV, it has been shown that transmission is possible just through contact with salivary secretions (Sykes, 2013). For some of the animals in this study, it is possible to have contact with other animals through shared fences. Two out of the three animals that tested positive for FeLV live in the same enclosure and the third positive animal lives in the enclosure next to it. This means that the animals have contact with each other. However, the two animals living together moved to this enclosure only in the year 2022, and the positive test results are all from the year 2021, meaning that all three animals had tested positive already before they had contact with each other. There is also a third animal living with the two that have tested positive. This animal has tested negative in all POC tests, and the test result has been confirmed with PCR. These three animals living together are brothers and have lived together all their lives. Taking into consideration the way the virus spreads, it is likely that the third animal has also been infected with the virus. One possible explanation for the negative test result is that this one animal has gone through a different course of the disease than the other two. In the abortive, regressive, and focal infection courses of FeLV, the POC test result will be negative. A PCR done from a blood sample will also be negative in all three cases (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020). It is known that there was a stray cat that tested positive for FeLV living on the property up until the year 2021 when it was tested positive for FeLV and euthanized. The area where the meat fed to the animals is prepared is open to the outside and the cat has access to it. This could mean that the cat has brought the virus onto the property and has then contaminated the meat that was later fed to the animals creating an infection route. However, it is also known that the FeLV does not survive long periods in the environment (Sykes, 2013), leaving this theory questionable. Because of the structure of the nature reserve, it is also possible that the cat has had direct contact with the cheetahs through the fence. In a study (Sacristan *et al*. 2021) that looked into cross-species transmission of retroviruses among domestic cats and guignas in Chile, the results supported the theory that domestic cats can transmit FeLV to wild felids.

The majority of the animals in this study were males as well as two out of the three animals with a positive test result. The sex of the animal is known to be a risk factor for both of the viruses, with male cats being at a higher risk (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020). A study done with guignas (Mora *et al*., 2015) found the prevalence of both FeLV and FIV to be higher in males in their study population and that the animals who tested positive for

both viruses were male. Another study done with lions (Broughton *et al*., 2021) where only FIV was studied, found that the prevalence was similar among males and females. In both of these studies as well as other studies (Tangsudjai *et al*., 2010; Villalba-Briones *et al*., 2022) all the animals have been clinically healthy with no signs of disease. This was also the case in this study. The two male cheetahs with a positive test result were both 6 years old and the female was 14 years old. According to the literature the median age for domestic cats with FeLV is three years, with adults being more likely to get infected than kittens (Sykes, 2013; Hartmann and Hofmann-Lehmann, 2020). In the study with guignas (Mora *et al*., 2015) the age of the FeLV-positive animals was between one to two years, and in the study with lions (Broughton *et al*., 2021) the prevalence of FIV was found to be higher with higher age of the animal. For the most part, the findings of this study seem to be in accordance with the other studies.

CONCLUSIONS

This study found that the incidence of FeLV at the study location in the study population is 5.55% (three out of 54 animals) and the incidence of FIV is 0% (zero out of 54 animals). This result may not be accurate because of the fact that testing regime was not consistent, and data was lacking in some animals. The origin of the viruses on this property and the route of infection can only be speculated in this study based on the information available. It is suggested that more attention is paid to the effectiveness of the testing regime to ensure that animals carrying these viruses are detected and the spread of the virus can be controlled.

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APPENDICES

Appendix 1. Non-exclusive licence for depositing the final thesis and opening it for the public and the supervisor's (supervisors') confirmation for allowing the thesis for the defence

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