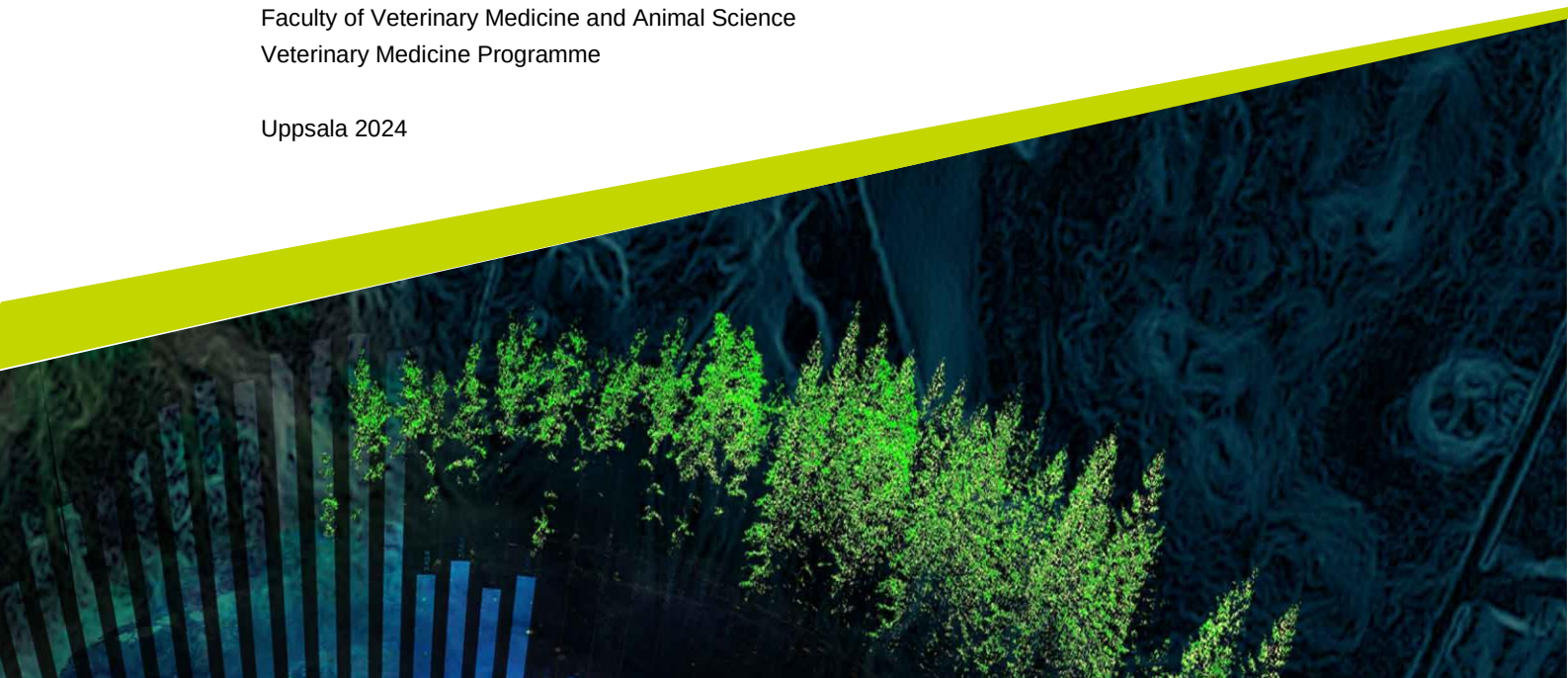
Seroprevalence of Canine Distemper Virus in Domestic Cats in Masai Mara, Kenya

A conservational approach

Signe Ahlbom

Independent Project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme

Uppsala 2024



Seroprevalence of Canine Distemper Virus in Domestic Cats in Masai Mara, Kenya - A conservational approach

Seroprevalens av valpsjukeviurs hos domesticerade katter i Masai Mara, Kenya – Ett bevarandeperspektiv

Signe Ahlbom

Supervisor:	**Eva Axnér, Swedish University of Agricultural Sciences, Department of Clinical Sciences**
Assistant supervisor:	Therése Hård, Knuthenborg Safaripark, Denmark
Examiner:	Jane Morrell, Swedish University of Agricultural Sciences, Department of Clinical Sciences

Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Independent Project in Veterinary Medicine
Course code:	EX1003
Programme/education:	Veterinary Medicine Programme
Course coordinating dept.:	Department of Clinical Sciences
Place of publication:	Uppsala
Year of publication:	2024

| **Keywords:** | Canine Distemper Virus, Domestic Cats, *Catus felis, Panthera leo,* Conservation, Masai Mara, Kenya |

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

Abstract

Canine Distemper Virus is a morbillivirus with high mortality, causing gastrointestinal and respiretory disease and in some cases neurological affection. The virus is able to infect many species, including all families in the order *Carnivora*. Big felids are especially susceptible, and the virus has resulted in deadly outbreaks in these species globally, with the outbreak in Serengeti's lion population in 1994 causing tremendous losses. Domestic dogs have been established to contribute to the transmission to wildlife, but additional sources and meta reservoirs are believed to be involved in the transmission to lions.

The domestic cat is not as susceptible to CDV and is usually subclinically infected. To the author's knowledge, no studies regarding the prevalence of CDV in domestic cats have been done in Kenya, nor the African continent. The purpose of this study was to investigate the seroprevalence of CDV in domestic cats living in Mararianda in Masai Mara, Kenya. The cats in the area roam free in close contact with dogs and wildlife and have been suspected to transmit other diseases to wild cats, a phenomenon that has been established in studies performed in other countries.

Serum samples were taken from 40 cats in the Mararianda area. The samples were then analysed for CDV antibodies using ELISA-kits performed in the field. The results of the study showed that 38 out of 40 cats were positive for CDV antibodies, resulting in a seroprevalence of 95%. To conclude, CDV is present in the cat population of Mararianda and likely to be highly prevalent, however further studies with more sample materials, identifying the virus itself as well as strain, is needed to draw additional conclusions.

Keywords: Canine Distemper Virus, Domestic Cats, *Catus felis*, *Panthera leo*, Conservation, Masai Mara, Kenya

Table of contents

Abbreviations

Ab	Antibody
CDV	Canine Distemper Virus
ELISA	Enzyme-linked Immunosorbent Assay
FeMV	Feline Morbillivirus
MMNC	Masai Mara North Conservancy
PCR	Polymerase Chain Reaction
PPRS	Peste de petit ruminants

1. Introduction

The purpose of this study was to investigate the seroprevalence of Canine Distemper Virus (CDV) antibodies in domestic cats in the village of Mara Rianta, Mara North Conservancy, Kenya. This highly contagious virus poses a threat to big felids, both in the wild and in captivity, and has caused outbreaks with high morbidity and mortality in the lion population in the area. Antibodies against the virus have also been found in hyena and African Wild Dog populations (Alexander & Appel 1994; Harder *et al.* 1995; Kock *et al*. 1998; Harrison *et al.* 2004).

The wildlife of the reserve lives in close contact with the Maasai people, which enables livestock and domestic cats and dogs to mix with the wild population. Additionally, households in the area often dispose of deceased cats in the bush (Shultz 2023). This enables the transfer of diseases between the groups, which in turn poses a threat to both human and animal health.

While the domestic dog is one key reservoir for transmission of CDV to felids and other wildlife, unknown factors and possible additional reservoirs may contribute to the spread of disease (Viana *et al.* 2015a; Wilkes 2023). To the authors knowledge, similar studies on the seroprevalence of CDV in domestic cats have not been done in Kenya or East Africa, something that potentially could add to the knowledge of virus transmission and demographic.

2. Literature study

2.1 Mara North Conservancy

Mara North Conservancy (MNC) is part of the Mara Ecosystem in Narok County, southeast Kenya. It borders the Masai Mara National reserve and is indirectly connected to the Serengeti Plain in Tanzania. Wildlife roams freely between neighbouring reserves and also has access to the villages and populated areas. This vast area is home to many threatened species in the family *Felidae* such as lion (*Panthera leo*), cheetah (*Acinonychinae acinonyx),* leopard (*Panthera pardus*) and African wildcat (*Felis silvestris lybica*) (Mara North Conservancy 2023).

Several zoonotic and epizootic diseases are present in MNC, many of them shared between wild and domestic animals (Harrison *et al.* 2004; Byström 2023; Prager 2012; Shultz 2023). Examples of diseases that have been confirmed in the area are rabies, Feline Coronavirus, Feline Parvovirus, Feline Herpesvirus, Feline Calicivirus, *Toxoplasma gondii* and Feline Leukemia Virus.

2.2 Canine Distemper Virus

2.2.1 Viral properties

Canine Distemper Virus (CDV) is an RNA morbillivirus in the family *Paramyxo-viridae*. The genus of morbillivirus also includes rinderpest in cattle and other cloven-hoofed animals (now extinct), peste-de-petit-ruminants in goats and sheep, measles in humans and other primates, and phocine and dolphin morbillivirus (Diallo 1990; Rima & Duprex 2006; Noyce *et al.* 2013). Many of these viruses are also a great cause of concern for public health, global economy, animal welfare and conservation.

The virus has a lipoprotein shell which envelopes a non-segmented, negative sense RNA genome, which codes for six different proteins: large protein (L), haemagglutinin (H), phosphoprotein (P), nucleocapsid protein (N) and fusion protein (F) (Diallo 1990; Noyce *et al.* 2013; Loots *et al*. 2017). There is only one subtype, but genetic drift has caused geographical variation among a protein called

H-protein and resulted in 11 different types. Immunity is thought to be related to the H-protein and can be used for studies in epidemiology.

The most important CDV host cell receptor is SLAM (Signalling Lymphocyte Activation Molecule), presented by mononuclear blood cells peripherally (Rendon-Marin *et al*. 2019). Another recognized host cell receptor is nectin-4 (PVRL4) in epithelial cells. Through these, CDV gains recognition of, and ability to enter, the host cell.

2.2.2 Transmission

The virus is highly contagious and is transmitted mainly by direct contact or through ocular, oral or respiratory fluids spread by aerosols (Karki *et al.* 2022; SVA 2023). The virus is also secreted with faeces and urine. Subclinically infected animals can also be infectious by shedding the virus. The duration of virus shedding through bodily secretions varies from a couple of weeks to as long as 90 days. Intrauterine transmission is also possible.

The outer layer of the virus particle is lipid, and through the effects of many cleaning agents on the lipid bi-layer, the virus can be inactivated. UV-radiation, heat and drought are also able to deactivate the virus. Survival time of the virus in colder climates exceeds that in warmer conditions.

2.2.3 Pathogenesis

Canine distemper virus has multiple cell tropism and can infect epithelia, lymphoid and neurological tissue (Loots *et al*. 2017). After introduction of the virus to the host, CDV recognizes SLAM expressed on dendritic cells and alveolar macrophages in the respiratory tract and these are the first to be infected. The infected cells travel to the regional draining lymph node where activated T- and B-lymphocytes become infected. After infection of the lymphocytes, the virus replicates and disseminate through lymph and blood to hematopoietic tissues – this comprises the first viraemic phase and results in infection of spleen, thymus, lymph nodes, bone marrow and mucosa-associated tissues (Beineke *et al.* 2009; Rendon-Marin *et al*. 2019). After this initial stage, clinical signs and shedding of the virus develops.

Time of incubation varies, depending on species, strain and the immune status and age of the affected animal, but is usually around 1-4 weeks.

The second viremia occurs days later and leads to the infection of parenchyme and tissue; consequently the virus can be found in cells throughout the entire body.

The described pathogenesis is that of the domestic dog, variation in pathogenesis may occur depending on genotype and host animal.

If the disease does not prove fatal, a humoral immune response persists for many years or even for life, but cell mediated immunity remains only for a few days after having undergone infection (Beineke et al. 2009).

2.2.4 Manifestation and clinical signs

The severity of disease with CDV varies between asymptomatic infection to clinical illness with high morbidity and mortality (SVA 2023). How the disease presents depends on genotype as well as species, age and immunological defence of the host (Deem *et al.* 2000; Beineke *et al.* 2009).

In dogs that develop clinical signs, the disease usually presents initially with conjunctivitis, which develops into coughing, rhinitis and eventually pneumonia (SVA 2023). High, biphasic fever is also common, as well as clinical signs from the gastrointestinal tract and various secondary infections following the immune-suppression. In some cases, the virus causes encephalitis and neurological signs. This usually presents after systemic disease but can also be seen without other clinical signs. Individuals with neurological manifestation can display myoclonus, postural reaction deficits, ataxia, tetraparesis, plegia or nystagmus (Koutinas *et al.* 2002).

Other possible clinical signs consist of ocular damage, such as uveitis, optical neuritis and necrosis of the retina, hyperkeratosis of planum nasale and pawpads as well as hypoplasia of teeth enamel in younger individuals who has not yet developed adult teeth. Intrauterine vertical transmission usually results in abortion or weak puppies (Evermann & Kennedy 2011).

2.2.5 Diagnosis

Diagnosis is ideally made by detecting the virus itself, either by PCR or immunofluorescence (SVA 2023). Both analyses can be performed either with samples taken from conjunctiva or tonsils or tissue biopsies, while PCR also can be performed with urine, blood, cerebrospinal fluid and other bodily fluids.

Another option is to identify antibodies, either IgG or IgM, in blood samples. The antibodies can, for example, be detected with ELISA-assays. This presupposes that the patient has not been vaccinated and the method is not able to determine when the infection has taken place, since many individuals develop long-standing immunity.

To diagnose wildlife with CDV can be more challenging, partly due to the need for cool storage of samples. Postmortem histopathology is usually necessary. (Loots *et al*. 2017)

2.2.6 Treatment and prophylaxis

There is currently no specific treatment for CDV-infection (SVA 2023). In a clinical setting, supportive care with IV-fluids, anti-seizure medication and treatment for secondary infections caused by immunosuppression is often applied. The mortality rate in dogs is around 50% (Wilkes 2023).

The best preventative measure against the virus is vaccination, and is recommended as a core vaccine in domestic dogs globally by the World Small Animal Veterinary Association (Day *et al.* 2016; Georoff *et al*. 2020).

Vaccination drives for domestic and feral dogs have been implemented in several areas where CDV has caused outbreaks in wildlife in an effort to diminish potential spread to the wild animals, although their effectiveness has been debated (Georoff *et al*. 2020; Wilkes 2023). For example, cases of vaccine-induced distemper have been described in carnivorous species that had been given live attenuated vaccine. Vaccination has proved a successful tool in the eradication processes of measles and rinderpest, but the continuously expanding host range of canine distemper impedes reduction and eradication of the disease (Beineke *et al*. 2015)

2.2.7 Epidemiology

Canine Distemper Virus is present worldwide with varying prevalence in wild, zoo and domestic populations (Duque-Valencia *et al*. 2019; Karki *et al.* 2022; Wilkes 2023). As can be guessed from its name, Canine Distemper Virus was originally only known to cause disease in domestic dogs, for which the virus remains an important pathogen. However, it is now established that CDV can infect a wide variety of families and genus, including all families of genus *Carnivora*. Many of them are still found in the family of *Canidae*, such as dingo, fox, coyote, jackal and wolf, but also *Procyonidae, Mustelidae, Ursidae, Ailuridae* and *Hyaenidae* (Harrison *et al.* 2004; Rendon-Marin *et al.* 2019). Some studies have also found CDV to infect other mammalian families outside of the carnivorous order, such as *Cricetidae, Cercopithecidae, Suidae* and *Elephantidae* (Duque-Valencia *et al.* 2019).

The family of *Felidae* was long thought not to be susceptible to the virus, but a fatal outbreak of CDV in the lion population (*Panthera leo)* in Serengeti National Park in 1994 proved otherwise. The virus has since been found in many species of big cats (*Panthera tigris, Panthera pardus, Panthera onca),* both wild and captive (Ikeda *et al.* 2001; Guiserix *et al.* 2007; Terio & Craft 2013; Beineke *et al*. 2015).

The virus is not zoonotic and there is no evidence that it can infect humans (Rima & Duprex 2006). However, it has caused fatal outbreaks in non-human primates, and the ability of the virus to readily spill over into new species and populations may be cause for continued surveillance on its zoonotic potential (Qiu *et al*. 2011).

2.2.8 CDV in the family *Felidae*

In felids, CDV has the potential to either remain silent or result in severe and fatal illness (Terio & Craft 2013; Weckworth *et al*. 2020). Big felids in captivity, such as lions and leopards (*Panthera pardus*), have presented with a variety of the common clinical signs, such as gastrointestinal and respiratory distress, while systemic disease in large felids in the wild usually presents with neurological symptoms, with encephalitis and pneumonia as clinical findings (Appel *et al.* 1994; Evermann & Kennedy 2011). One of the most severe outbreaks was the one in the Serengeti in 1994, killing one third of the entire lion population in the area (Harder *et al.* 1995; Guiserix *et al.* 2007).

What determines the difference in pathogenicity is not yet well understood. One explanation for the varying mortality rates might be co-infection with other pathogens because of the immunodeficiency brought on by CDV. For example, simultaneous infection with *Babesia* was connected to increased mortality during the 1994 outbreak in Serengeti (Munson *et al.* 2008).

Unlike its larger relatives, the domestic cat does not seem to be as susceptible to the virus (Ikeda *et al*. 2001). Studies have shown that domestic cats can be seropositive for CDV, although they do not develop symptoms. When experimentally exposed or inoculated, seroconverted cats did not show signs of disease or viral shedding in two studies, but in another study ab-positive cats did show signs of illness in some cases (Loots *et al*. 2017; Wilkes 2023).

Cheetahs have tested positive for CDV antibodies in both the wild and in captivity, but, like the domestic cats, do not seem to develop clinical signs (Thalwitzer *et al*. 2010).

2.2.9 CDV in Masai Mara

The Serengeti outbreak in 1994 also spilled over into the Masai Mara lion population, since there are no physical barriers between the two reserves and they form one ecosystem (Harrison *et al*. 2004; Guiserix *et al.* 2007). Antibodies against the virus have since been found in jackals, spotted hyenas, African wild dogs and bat-eared fox in and around the reserve.

Outbreaks of CDV have also occurred in the domestic dog population around the park, and vaccination programmes have been in place since 2007 (Mara Conservancy n.d.; Karen Blixen Camp Trust n.d.).

2.2.10 Reservoirs and host expansion – a threat to wildlife

Domestic dogs are considered to be one of the key reservoirs for virus spread to wildlife, (Alexander *et al*. 2010) and was believed to be the responsible population for transmission to the lion tribes in Serengeti after the fatal outbreak in 1994. Further studies, however, have shown that the persistence of CDV in the ecosystem

is more likely to be of multi-host character (Viana *et al.* 2015b). The virus has not been found to be capable of persistence in the dog populations examined, based on seroprevalence and population size (Prager *et al.* 2012; Wilkes 2023), and outbreaks in the dog and lion populations have not always been synchronous.

The high mortality caused by the virus in already vulnerable wild species can expedite the extinction of these decimated populations, wherefore CDV epidemiology and an understanding of the continuously expanding host range is of great importance for conservational efforts. (Duque-Valencia *et al.* 2019)

3. Materials and Method

3.1 Sample collection and castration project

The cats in the study were collected from households in Mara Rianta village in Mara North Conservancy (see fig. 1). The background of the cats varied from household pets to semi-feral; however further anamnestic information was not possible. One of the cats in the study was semi-free roaming in the area of Karen Blixen Camp and was caught in a trap before being brought to the clinic, as part of a trap-neuter-return project.

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

Figure 1: Map of Kenya. Narok county in blue, Mara Rianta marked with red dot. (Schultz 2023)

The collection of samples was done in conjunction with the Mara Cat Projects castration drive, where 29 females and 11 males were spayed or castrated, respectively and neutered while also undergoing clinical examinations and being vaccinated against rabies. The aim of the castration project was to prevent the further growth of the rapidly expanding population of free-roaming cats in the area, and therefore, to diminish the spread of infectious diseases to wildlife and humans, and prevent genetic mixing with wild felids as well as increasing the health status

of the cat population. In total 40 blood samples were collected from the jugular or brachiocephalic vein.

After collection the samples were centrifuged and the serum was transferred to Eppendorf tubes and kept frozen until the analyses were made.

3.2 Sample analysis and CDV IgG antibody ELISA

To investigate whether the cats had undergone an infection with CDV, the collected samples were analysed using CDV IgG antibody ELISA kits. The kits were provided by European Veterinary Laboratory "Canine Distemper Virus IgG Antibody ELISA", adapted for use with feline samples. The method used was qualitative, aiming to decide whether antibodies were present or not. Specific instructions and a protocol were provided by European Veterinary Laboratory (2020).

Samples were thawed 15 minutes before the start of the analysis and 10 microlitres of each sample was diluted to 1:150 with ELISA buffer. A negative and a positive control were used, as well as two substrate controls, consisting of only ELISA buffer. After following the provided protocol, the absorbency values were read with the ELISA-reader SpotXL, using an iPhone 14 camera.

According to the instructions provided with the ELISA kit, results were considered valid if the following criteria were met:

- The mean value (MV) of the measured optical density (OD) value for the positive control, diluted to 1:50, must be >/ 0.850.
- The MV of the measured OD value for the negative control, diluted 1:50, must be </0.400.

The MV of the measured OD of the negative and positive control was calculated.

The ratio of sample OD to mean OD (S/P) was calculated according to the following equation:

$$S/P = \frac{ODsample - MV\ OD\ nc}{MV\ OD\ pc - MV\ OD\ nc}$$

The analysis was repeated twice, once with three dilutions of each sample and once with two dilutions of each sample.

4. Results

Out of the 40 cats that were tested, 38 were positive for antibodies against CDV (see table 1).

Table 1: Seroprevalence of CDV in 40 Mararianda cats

Gender	Positive	Negative	Seropositivity
Female	28	1	96%
Male	10	1	90%
Total	38	2	95%

5. Discussion

The aim of this study was to investigate the seroprevalence of CDV antibodies in domestic cats included in Mara Cat Project and evaluate whether this information can be used to aid conservational efforts of big felids in the area. The results of the study show that CDV is prevalent in the Mararianda cat population.

In the sampled population of N=40, the calculated seroprevalence of CDV-antibodies was 95%. No significant difference between male and female cats was detected. There are few comparable studies, but in the study by Ikeda (2001) the highest seroprevalence recorded was 88% in a Taiwanese shelter, with N=11. Why the seroprevalence was so high in the Mararianda population is uncertain. It is possible that the local cats are more susceptible to infection, either because of inherent traits or that the CDV strain circulating in the area has mutated and now is more capable of infecting domestic cats. It could also be that the CDV circulation in the area is high in general.

Regarding the conservational relevance, the high seroprevalence of the virus in the cat population could potentially indicate the prevalence of the virus in other populations in the area, for example wildlife and domestic dogs. In the study by Ikeda (2001) it was suggested that the main cause of CDV transmission to cats was domestic dogs. Since the domestic dog population in Mararianda is routinely vaccinated against CDV, they cannot be used to evaluate the prevalence of the disease in the area. If the dogs are the main cause of disease transmission to cats, it could indicate that either the vaccination programme is not sufficient, that vaccinated dogs are able to transmit the disease or that another source of infection is present. It can also be discussed whether the unvaccinated dog population is big enough to be able to maintain a steady source of transmission to the cats, or if the virus is able to persist in the cat population without additional transmission from dogs. Understanding how the virus spreads through different populations, both wild and domestic, will aid in determining which method for preventing outbreaks is most effective.

It cannot be ruled out that the strain of CDV present in the Mararianda cat population is not the same as the one circulating in the dog population and wildlife. To determine if this is the case, virus isolation and genome analyses would be needed, something that also would be required to confirm the results of CDV infection in the Mararianda cats. Genome sequencing or serum neutralization tests

to identify the virus itself would be a natural continuation of the findings of this study. It is possible that a more cat-specific strain of the virus has a different predilection to spread to big felids and wildlife. Considering the rapidly expanding host range, the fact that cats are not currently considered to spread the disease further nor develop clinical signs, should not be a reason to discard the importance of the high CDV seroprevalence. This is especially the case in this area, considering the additional mortality the virus poses to already threatened species.

Regarding animal welfare for the cats, it is not fully established whether CDV infection has a negative effect on cat health. Several studies have not seen an association between CDV-infection and health status. The overall health in the study population of this article was deemed good, but since viral antibodies, and not the virus itself, were identified, it is not possible to say whether CDV infection can cause disease in cats. However, the high seroprevalence in this small population suggests that infection is seldom fatal. Further studies regarding whether the high seroprevalence of CDV is affecting the animal welfare in the Mararianda cat population would be necessary to reach a conclusion on this subject.

There are studies describing the effectiveness of trap-neuter-return projects on population size. In a study by (Kreisler *et al.* 2019), a long term TNR-project was performed on feral/stray cats in Florida, USA, which resulted in a 55% decrease of the population. In the same population, the prevalence of retroviruses, including FeLV and FIV, decreased with 0.32% per year. The decrease was thought to be due to the elimination of risk factors such as mating, vertical transmission and fighting, as well as vaccination. Decreasing the population size would be beneficial when aiming to diminish disease transmission, since it is one of the factors affecting the dynamics of infectious diseases (Anderson & May 1979). The long-lasting immunity that follows CDV infection further adds to the need for bigger populations. For example, measles virus, a morbillivirus similar to CDV, can only persist within human populations when the community size is significantly large, surpassing a critical threshold (Bartlett 1960). Similarly to what has been established regarding the dog population, the Mararianta cat population would need to be big enough to maintain the transmission of CDV on its own (Prager *et al.* 2012).

5.1 Limitations

The study was conducted in the field, with several limitations. Frequent power outages and transportation from the clinic to storage resulted in the fact that an un-broken cold chain cannot be ensured. Freeze-thaw processes increase the risk of non-specific reactivity in the ELISA-kit used (European Veterinary Laboratory 2020). The lack of potential reactions with other viruses therefore cannot be guaranteed.

Another possibility is that the positive reactions of the ELISA were due to reactions with another morbillivirus. In an unpublished study by Berg an ELISA for PPRS, made on samples from Zambian goats, cross-reacted with CDV, which was confirmed with serum neutralization tests. Unknown morbilliviruses, or morbilliviruses not considered present in cats, could therefore potentially interfere with the results.

Feline Morbillivirus

Another morbillivirus present in cats is Feline Morbillivirus (FeMV) (De Luca *et al*. 2021). The virus was first discovered in house cats in Hong Kong in 2012, and has since been detected in cats globally. However, no studies have yet been performed on the African continent nor in wild felids. The seroprevalence in different studies range from 17.32% in Italian household cats to 63% in Chilean colony cats (De Luca *et al*. 2020; Busch *et al.* 2021), and has been found to be higher in multi-cat environments in comparison to household cats. It has been suggested that the virus is associated with kidney disease, such as tubulointerstitial nephritis (Woo *et al.* 2012).

Feline Morbillivirus has been shown to cross react with anti-CDV neutralizing antibodies, which means that FeMV-infection of the cats participating in this study could show up as positive for CDV-antibodies. As of now, there is no commercially available detection assay, and virus isolation remains reference standard for diagnosis. This would be true especially when aiming to differentiate CDV and FeMV infections from each other. Further studies on prevalence of FeMV and the possibility of transmission to wildlife would be needed to evaluate whether FeMV would be a possible explanation for the results in this study, and if it could be relevant from a conservational viewpoint.

Vaccination

When using viral antibodies to evaluate the presence of disease in an area, it is essential that the animals from which the samples are collected have not been vaccinated against the virus in question. Since cats in Mararianda generally are not id-chipped or tattooed and several veterinary projects are present in the area, it is difficult to discard with certainty the possibility that vaccinated cats could have been included in the study. However, there are no records of any CDV-vaccination campaign in cats in the area. Also, the majority of cats in this study were estimated to be around a year old, which would mean that a possible vaccination drive would need to have taken place recently.

Bias

The collection of cats for this study was made by a local veterinary technician, some members of the project and the staff of Karen Blixen Camp. The temperament of

the cats therefore affected whether they could be collected or not, providing a selection bias. It is possible that feral cats who have a greater potential for interaction with wildlife were under-represented in the study population. It is also possible that sick cats were more likely to be caught because of slowness, or that, on the contrary, sick cats were not included in the population.

5.2 Conclusion

The result of this study shows that CDV is prevalent in the Mararianda cat population. The 95% seroprevalence in this study indicates that the overall frequency is high. However, to be able to draw conclusions regarding the degree of prevalence, studies with larger number of participating cats are needed.

A high seroprevalence in the cats suggests circulation of the virus and a high prevalence in other, more susceptible, populations as well, such as domestic dogs and wildlife. However, isolation and genetic analysis of the virus itself would be necessary to confirm this speculation.

References

Alexander, K. & Appel, M. (1994). African wild dogs (Lycaon pictus) endangered by a canine-distemper epizootic among domestic dogs near the Masai-Mara National Reserve, Kenya. *Journal of Wildlife Diseases*, 30 (4), 481–485. https://doi.org/10.7589/0090-3558-30.4.481

Alexander, K.A., McNutt, J.W., Briggs, M.B., Standers, P.E., Funston, P., Hemson, G., Keet, D. & van Vuuren, M. (2010). Multi-host pathogens and carnivore management in southern Africa. *Comparative Immunology, Microbiology and Infectious Diseases*, 33 (3), 249–265. https://doi.org/10.1016/j.cimid.2008.10.005

Anderson, R.M. & May, R.M. (1979). Population biology of infectious diseases: Part I. *Nature*, 280 (5721), 361–367. https://doi.org/10.1038/280361a0

Appel, M.J.G., Yates, R.A., Foley, G.L., Bernstein, J.J., Santinelli, S., Spelman, L.H., Miller, L.D., Arp, L.H., Anderson, M., Barr, M., Pearce-Kelling, S. & Summers, B.A. (1994). Canine distemper epizootic in lions, tigers, and leopards in North America. *Journal of Veterinary Diagnostic Investigation*, 6 (3), 277–288. https://doi.org/10.1177/104063879400600301

Bartlett, M.S. (1960). The critical community size for measles in the United States. *Journal of the Royal Statistical Society. Series A (General)*, 123 (1), 37–44. https://doi.org/10.2307/2343186

Beineke, A., Baumgärtner, W. & Wohlsein, P. (2015). Cross-species transmission of canine distemper virus—an update. *One Health*, 1, 49–59. https://doi.org/10.1016/j.onehlt.2015.09.002

Beineke, A., Puff, C., Seehusen, F. & Baumgärtner, W. (2009). Pathogenesis and immunopathology of systemic and nervous canine distemper. *Veterinary Immunology and Immunopathology*, 127 (1), 1–18. https://doi.org/10.1016/j.vetimm.2008.09.023

Busch, J., Sacristán, I., Cevidanes, A., Millán, J., Vahlenkamp, T.W., Napolitano, C. & Sieg, M. (2021). High seroprevalence of feline morbilliviruses in free-roaming domestic cats in Chile. *Archives of Virology*, 166 (1), 281–285. https://doi.org/10.1007/s00705-020-04882-2

Byström, R. (2023). *The seropositivity of Toxoplasma gondii in free-roaming domesticated cats in Masai Mara, Kenya*. Swedish University of Agricultural Sciences. The Veterinary Programme. http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-18620

Day, M.J., Horzinek, M.C., Schultz, R.D. & Squires, R.A. (2016). WSAVA Guidelines for the vaccination of dogs and cats. *The Journal of Small Animal Practice*, 57 (1), E1–E45. https://doi.org/10.1111/jsap.2_12431

De Luca, E., Crisi, P.E., Marcacci, M., Malatesta, D., Di Sabatino, D., Cito, F., D'Alterio, N., Puglia, I., Berjaoui, S., Colaianni, M.L., Tinelli, A., Ripà, P., Vincifori, G., Di Teodoro, G., Dondi, F., Savini, G., Boari, A. & Lorusso, A. (2020). Epidemiology, pathological aspects and genome heterogeneity of feline morbillivirus in Italy. *Veterinary Microbiology*, 240, 108484. https://doi.org/10.1016/j.vetmic.2019.108484

De Luca, E., Sautto, G.A., Crisi, P.E. & Lorusso, A. (2021). Feline morbillivirus infection in domestic cats: What have we learned so far? *Viruses*, 13 (4), 683. https://doi.org/10.3390/v13040683

Deem, S.L., Spelman, L.H., Yates, R.A. & Montali, R.J. (2000). Canine distemper in terrestrial carnivores: A review. *Journal of Zoo and Wildlife Medicine*, 31 (4), 441–451. https://doi.org/10.1638/1042-7260(2000)031[0441:CDITCA]2.0.CO;2

Diallo, A. (1990). Morbillivirus group: genome organisation and proteins. *Veterinary Microbiology*, 23 (1–4), 155–163. https://doi.org/10.1016/0378-1135(90)90145-L

Duque-Valencia, J., Sarute, N., Olarte-Castillo, X.A. & Ruíz-Sáenz, J. (2019). Evolution and interspecies transmission of canine distemper virus-an outlook of the diverse evolutionary landscapes of a multi-host virus. *Viruses*, 11 (7), 582. https://doi.org/10.3390/v11070582

European Veterinary Laboratory (2020). *Canine Distemper Antibody ELISA 96 wells.* https://evlonline.eu/product/canine-distemper-virus-antibody-elisa-96-wells/ [2023-11-14]

Evermann, J. & Kennedy, M. (2011) Chapter 16: Viral infections. In: Peterson, M., Kutzler, M. (eds.). *Small Animal Pediatrics*. Elsevier. 119-129. https://doi.org/10.1016/B978-1-4160-4889-3.00016-4

Georoff, T.A., Ramsay, E.C., Gyimesi, Z.S., Kilburn, J.J. & Sykes, J.M. (2020). Review of canine distemper vaccination use and safety in North American captive large felids (Panthera spp.) from 2000 to 2017. *Journal of Zoo and Wildlife Medicine*, 50 (4), 778–789. https://doi.org/10.1638/2018-0163

Guiserix, M., Bahi-Jaber, N., Fouchet, D., Sauvage, F. & Pontier, D. (2007). The canine distemper epidemic in Serengeti: are lions victims of a new highly virulent canine distemper virus strain, or is pathogen circulation stochasticity to blame? *Journal of the Royal Society Interface*, 4 (17), 1127–1134. https://doi.org/10.1098/rsif.2007.0235

Harder, T.C., Kenter, M., Appel, M.J.G., Roelke-Parker, M.E., Barrett, T. & Osterhaus, A.D.M.E. (1995). Phylogenetic evidence of canine distemper virus in Serengeti's lions. *Vaccine*, 13 (6), 521–523. https://doi.org/10.1016/0264-410X(95)00024-U

Harrison, T.M., Mazet, J.K., Holekamp, K.E., Dubovi, E., Engh, A.L., Nelson, K., Van Horn, R.C. & Munson, L. (2004). Antibodies to canine and feline viruses in spotted hyenas (Crocuta crocuta) in the Masai Mara National Reserve. *Journal of Wildlife Diseases*, 40 (1), 1–10. https://doi.org/10.7589/0090-3558-40.1.1

Ikeda, Y., Nakamura, K., Miyazawa, T., Chen, M.-C., Kuo, T.-F., Lin, J.A., Mikami, T., Kai, C. & Takahashi, E. (2001). Seroprevalence of canine distemper virus in cats. *Clinical and Diagnostic Laboratory Immunology*, 8 (3), 641–644. https://doi.org/10.1128/CDLI.8.3.641-644.2001

Karen Blixen Camp Trust (n.d.). *Mara North Conservancy Dog Project.* https://karenblixencamptrust.org/program/dog-project/ [2023-10-31]

Karki, M., Rajak, K.K. & Singh, R.P. (2022). Canine morbillivirus (CDV): a review on current status, emergence and the diagnostics. *VirusDisease*, 33 (3), 309–321. https://doi.org/10.1007/s13337-022-00779-7

Kock, R., Chalmers, W.S.K., Mwanzia, J., Chillingworth, C., Wambua, J., Coleman, P.G. & Baxendale, W. (1998). Canine distemper antibodies in lions of the Masai Mara. *Veterinary Record*, 142 (24), 662–665. https://doi.org/10.1136/vr.142.24.662

Koutinas, A.F., Polizopoulou, Z.S., Baumgaertner, W., Lekkas, S. & Kontos, V. (2002). Relation of clinical signs to pathological changes in 19 cases of canine distemper encephalomyelitis. *Journal of Comparative Pathology*, 126 (1), 47–56. https://doi.org/10.1053/jcpa.2001.0521

Kreisler, R.E., Cornell, H.N. & Levy, J.K. (2019). Decrease in population and increase in welfare of community cats in a twenty-three year trap-neuter-return program in Key Largo, FL: The ORCAT Program. *Frontiers in Veterinary Science*, 6. https://www.frontiersin.org/articles/10.3389/fvets.2019.00007 [2023-12-26]

Loots, A.K., Mitchell, E., Dalton, D.L., Kotzé, A. & Venter, E.H. (2017). Advances in canine distemper virus pathogenesis research: a wildlife perspective. *The Journal of General Virology*, 98 (3), 311–321. https://doi.org/10.1099/jgv.0.000666

Mara Conservancy (n.d.). *Dog Vaccination Project.* https://www.maratriangle.org/about-us/work-projects/canine-distemper-rabies-vaccinations [2023-10-31]

Mara North Conservancy (n.d.). *Our role in conservation.* https://maranorth.org/conservation/ [2023-11-01]

Munson, L., Terio, K.A., Kock, R., Mlengeya, T., Roelke, M.E., Dubovi, E., Summers, B., Sinclair, A.R.E. & Packer, C. (2008). Climate extremes promote fatal co-infections during canine distemper epidemics in African lions. *PLoS ONE*, 3 (6), e2545. https://doi.org/10.1371/journal.pone.0002545

Noyce, R.S., Delpeut, S. & Richardson, C.D. (2013). Dog nectin-4 is an epithelial cell receptor for canine distemper virus that facilitates virus entry and syncytia formation. *Virology*, 436 (1), 210–220. https://doi.org/10.1016/j.virol.2012.11.011

Prager, K.C., Mazet, J.A.K., Dubovi, E.J., Frank, L.G., Munson, L., Wagner, A.P. & Woodroffe, R. (2012). Rabies virus and canine distemper virus in wild and domestic carnivores in Northern Kenya: Are domestic dogs the reservoir? *EcoHealth*, 9 (4), 483–498. https://doi.org/10.1007/s10393-013-0815-9

Qiu, W., Zheng, Y., Zhang, S., Fan, Q., Liu, H., Zhang, F., Wang, W., Liao, G. & Hu, R. (2011). Canine distemper outbreak in rhesus monkeys, China. *Emerging Infectious Diseases*, 17 (8), 1541–1543. https://doi.org/10.3201/eid1708.101153

Rendon-Marin, S., da Fontoura Budaszewski, R., Canal, C.W. & Ruiz-Saenz, J. (2019). Tropism and molecular pathogenesis of canine distemper virus. *Virology Journal*, 16 (1), 30. https://doi.org/10.1186/s12985-019-1136-6

Rima, B.K. & Duprex, W.P. (2006). Morbilliviruses and human disease. *The Journal of Pathology*, 208 (2), 199–214. https://doi.org/10.1002/path.1873

Schultz, E. (2023). *The occurrence of FeLV, FIV and FeCoV in free-roaming cats in Mara North Conservancy, Kenya*. Swedish University of Agricultural Sciences. The Veterinary Programme. http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-18619

SVA (2023). *Valpsjuka, Canine distemper virus (CDV).* Statens veterinärmedicinska anstalt. https://www.sva.se/amnesomraden/djursjukdomar-a-o/valpsjuka-canine-distemper-virus-cdv/ [2023-10-30]

Terio, K.A. & Craft, M.E. (2013). Canine distemper virus (CDV) in another big cat: Should CDV be renamed carnivore distemper virus? *mBio*, 4 (5), 10.1128/mbio.00702-13. https://doi.org/10.1128/mbio.00702-13

Thalwitzer, S., Wachter, B., Robert, N., Wibbelt, G., Müller, T., Lonzer, J., Meli, M.L., Bay, G., Hofer, H. & Lutz, H. (2010). Seroprevalences to viral pathogens in free-ranging and captive cheetahs (Acinonyx jubatus) on Namibian farmland. *Clinical and Vaccine Immunology : CVI*, 17 (2), 232. https://doi.org/10.1128/CVI.00345-09

Viana, M., Cleaveland, S., Matthiopoulos, J., Halliday, J., Packer, C., Craft, M.E., Hampson, K., Czupryna, A., Dobson, A.P., Dubovi, E.J., Ernest, E., Fyumagwa, R., Hoare, R., Hopcraft, J.G.C., Horton, D.L., Kaare, M.T., Kanellos, T., Lankester, F., Mentzel, C., Mlengeya, T., Mzimbiri, I., Takahashi, E., Willett, B., Haydon, D.T. & Lembo, T. (2015a). Dynamics of a morbillivirus at the domestic–wildlife interface: Canine distemper virus in domestic dogs and lions. *Proceedings of the National Academy of Sciences*, 112 (5), 1464–1469. https://doi.org/10.1073/pnas.1411623112

Viana, M., Cleaveland, S., Matthiopoulos, J., Halliday, J., Packer, C., Craft, M.E., Hampson, K., Czupryna, A., Dobson, A.P., Dubovi, E.J., Ernest, E., Fyumagwa, R., Hoare, R., Hopcraft, J.G.C., Horton, D.L., Kaare, M.T., Kanellos, T., Lankester, F., Mentzel, C., Mlengeya, T., Mzimbiri, I., Takahashi, E., Willett, B., Haydon, D.T. & Lembo, T. (2015b). Dynamics of a morbillivirus at the domestic–wildlife interface: Canine distemper virus in domestic dogs and lions. *Proceedings of the National Academy of Sciences*, 112 (5), 1464–1469. https://doi.org/10.1073/pnas.1411623112

Weckworth, J.K., Davis, B.W., Dubovi, E., Fountain-Jones, N., Packer, C., Cleaveland, S., Craft, M.E., Eblate, E., Schwartz, M., Mills, L.S. & Roelke-Parker, M. (2020). Cross-species transmission and evolutionary dynamics of canine distemper virus during a spillover in African lions of Serengeti National Park. *Molecular Ecology*, 29 (22), 4308–4321. https://doi.org/10.1111/mec.15449

Wilkes, R.P. (2023). Canine distemper virus in endangered species: Species jump, clinical variations, and vaccination. *Pathogens*, 12 (1), 57. https://doi.org/10.3390/pathogens12010057

Woo, P.C.Y., Lau, S.K.P., Wong, B.H.L., Fan, R.Y.Y., Wong, A.Y.P., Zhang, A.J.X., Wu, Y., Choi, G.K.Y., Li, K.S.M., Hui, J., Wang, M., Zheng, B.-J., Chan, K.H. & Yuen, K.-Y. (2012). Feline morbillivirus, a previously undescribed paramyxovirus

associated with tubulointerstitial nephritis in domestic cats. *Proceedings of the National Academy of Sciences of the United States of America*, 109 (14), 5435–5440. https://doi.org/10.1073/pnas.1119972109

Popular science summary

The purpose of this study was to evaluate whether a virus called Canine Distemper Virus (CDV) was prevalent in the population of household- and free roaming cats in the village Mararianda, Masai Mara, Kenya.

Initially only recognized as a disease present in dogs, causing symptoms from the digestive, respiratory, and neurological systems, it is now known to be able to infect many species, mainly from the carnivorous order. CDV poses an especially big threat to already endangered species of big cats, like lions and leopards. Outbreaks of disease have caused deaths in populations of these species globally, in the wild as well as in captivity. One of the biggest outbreaks occurred in 1994 in Serengeti National Park, Tanzania, part of the same ecosystem as neighbouring Masai Mara, Kenya. The outbreak killed 30% of the local lion population.

It is known that domestic dogs have contributed to transmission of the disease to the lions and other wildlife, and dogs in the Serengeti/Mara ecosystem are now included in vaccination programs to try and reduce the circulation of CDV. However, CDV is still present in the wildlife, and more information about the virus, and how and by which species it is transmitted, is needed to be able to further contain the disease.

The domestic cat does not seem to be as vulnerable to infection as its larger relatives. Cats infected by the virus do not seem to become sick or transmit the virus to others. However, CDV is a virus that is constantly mutating and expanding its range of possible hosts, which means that what has been known about CDV in cats could change. By investigating whether the cats in the area become infected with CDV, it is possible to get a better understanding of the virus and how we can protect wild cats.

In this study, 40 free roaming house cats were collected from Masai residents in the village of Mararianda. Blood samples were collected and they were also castrated and vaccinated against rabiesas a part of Mara Cat Project. The blood samples were analysed for CDV antibodies, which are produced by the body to fight the virus infection. Detection of CDV antibodies means that the cats have been infected with CDV or vaccinated against the virus.

Out of the 40 tested cats, 38 of them carried CDV antibodies, meaning that 95% of them at some point have had the disease. This indicates that CDV is circulating

in the area to a high degree. It is possible that this information could be used to monitor the CDV situation in the area; however further studies are needed.

Acknowledgements

I want to extend my warmest thanks to everyone at Karen Blixen Camp in Masai Mara for their tireless effort to help us with everything from dead car batteries to ant attacks. You made our stay in Masai Mara feel like a home away from home.

I also want to thank Mara Cat Project and everyone who participated in it – Dr. Ann-Marie Appel, Dr. Esther Macharia, Dr. Mathew Murket Nchoko, Francis Samunyak Ketuyio, Maina, Louise Fjord and especially my dear friends Gillian Nyambura Muiruri and Silas Kirwa Lagat. I also want to thank the families in Mararianta for trusting us with their cats and participating in this study.

Of course, a warm thank you to my supervisors Therese Hård, Eva Axnér and Jane Morrell for all of your support and help.

This project would not have been nearly as fun without my project colleagues Agnes Diamanti Barredal and Clara Björkholm, you are the best.

I also want to thank Mikael Berg and Sara Lysholm for your help on viruses and ELISAs, as well as Rob from European Veterinary laboratory for your help regarding the ELISA assays.

Lastly, I want to extend my gratitude to the foundation Nilsson-Aschans scholarship fund and the Faculty of Veterinary Medicine and Animal Science, without whose contributions this study would not have been possible.

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. Read about SLU's publishing agreement here:

- https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.