Seroprevalence of feline panleukopenia virus in domesticated cats in Maasai Mara, Kenya

A possible threat of transmission to wildlife?

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Independent Project • 30 credits
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Uppsala 2024

Seroprevalence of feline panleukopenia virus in domesticated cats in Maasai Mara, Kenya – a possible threat of transmission to wildlife?

Seroprevalens av felint panleukopenivirus hos domesticerade katter i Maasai Mara, Kenya – ett möjligt hot för smittspridning till vilda djur?

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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
Copyright:	All featured images are used with permission from the copyright owner.

Keywords:	Feline panleukopenia virus, FPV, domestic cat, seroprevalence, Kenya, Maasai Mara, Mara North Conservancy, Mara Cat Project

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Abstract

Feline panleukopenia virus (FPV) is a highly contagious virus that can stay stable in the environment for a long period of time. The virus can infect both domesticated cats, wild felids, and other wild animals. It has the highest morbidity and mortality rate in new-born and young kittens. The virus leads to viremia and mostly affects the dividing cells in the body, i.e. bone marrow, lymphoid tissues, intestinal epithelium, cerebellum in young animals, retina, and embryonic and foetal cells. Common clinical signs of infection with FPV in adult cats are lethargy, vomiting and diarrhoea. In young or new-born kittens, neurological signs are common. Infection with FPV can vary in its severity, from subclinical to per acute with little to no clinical signs before a sudden death.

This study was conducted in the Mara North Conservancy (MNC) within the nature reserve Maasai Mara in Kenya. Mara North Conservancy holds a large wildlife with lions, cheetahs, leopards, hyenas, zebras, elephants and many more. This study aimed to investigate the sero-prevalence of FPV in domesticated cats in the area and to see if there would be a possibility of transmission of FPV between domesticated cats and wild animals.

In total, 40 cats were sampled, 29 females and 11 males. 85% of the sampled cats had antibodies against FPV. There was a difference in the seroprevalence between genders, 93.1% of the females had antibodies against FPV and 63.6% of the males.

The results of this study showed a high prevalence of FPV antibodies in domesticated cats within the Mara North Conservancy. Theoretically there is a risk of domesticated cats spreading FPV to wildlife in the area, but a combined study that would look at both domesticated cats and wild animals and if they share FPV antibodies with the same genome would be needed to make that conclusion.

Keywords: Feline panleukopenia virus, FPV; domestic cat, seroprevalence, Kenya, Maasai Mara, Mara North Conservancy, Mara Cat Project

Sammanfattning

Felint panleukopenivirus (FPV) är ett mycket smittsamt virus som kan förbli stabilt i miljön under lång tid. FPV kan infektera både tamkatter, vilda kattdjur och andra vilda djur. Nyfödda och unga kattungar har högst morbiditet och mortalitet. Infektion med FPV leder till viremi och påverkar framför allt de snabbt delande cellerna i kroppen, dvs celler i benmärg, lymfoida vävnader, tarmepitel, lillhjärnan hos unga djur, näthinnan samt embryonala- och fosterceller. Vanliga symptom på infektion med FPV hos vuxna katter är letargi, kräkningar och diarré. Hos unga eller nyfödda kattungar är neurologiska symptom vanliga. Infektion med FPV kan variera i svårighetsgrad, från subklinisk till perakut med få eller inga kliniska symtom före en plötslig död.

Denna studie genomfördes i Mara North Conservancy (MNC) som ligger i naturreservatet Maasai Mara i Kenya. Inom MNC finns det många olika vilda djur t.ex. lejon, geparder, leoparder, hyenor, zebror, elefanter och många fler. Studiens syfte var att undersöka seroprevalensen av FPV hos domesticerade katter i området och om det skulle finnas en möjlighet för överföring av FPV mellan domesticerade katter och vilda djur.

Totalt provtogs 40 katter, 29 honor och 11 hanar. 85 % av de provtagna katterna hade antikroppar mot FPV. Det fanns en skillnad i seroprevalensen mellan könen, 93,1 % av honorna hade antikroppar mot FPV och 63,6 % av hanarna.

Resultatet av denna studie visade en hög förekomst av FPV-antikroppar hos domesticerade katter i Mara North Conservancy. Rent teoretiskt finns det en risk för att domesticerade katter sprider FPV till vilda djur i området, men en kombinerad studie som tittar både på domesticerade katter och vilda djur och om de delar antikroppar mot FPV med samma genom behövs för att dra den slutsatsen.

Nyckelord: Felint paleukopenivirus, FPV, domesticerad katt, seroprevalens, Kenya, Maasai Mara, Mara North Conservancy, Mara Cat Project

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Abbreviations

CPV	Canine parvovirus
FPV	Feline panleukopenia virus
MDA	Maternally derived antibodies
MNC	Mara North Conservancy
SLU	Swedish University of Agricultural Sciences
TNR	Trap-Neuter-Return
TR	Trap-Remove

1. Introduction

Feline panleukopenia virus (FPV) is a highly contagious virus which can infect domesticated cats (*Felis catus*) and other wild felids (Stuetzer & Hartmann 2014). Recent studies showed that other species can be infected (Calatayud *et al.* 2019). The virus can lead to the diseases feline panleukopenia, feline infectious enteritis and feline distemper (Payne 2023), with clinical signs such as fever, depression, anorexia, leukopenia, vomiting and diarrhoea (Little 2012). Infection with FPV is associated with both high mortality and high morbidity (Stuetzer & Hartmann 2014), especially in kittens (Little 2012). Per acute cases can lead to death within 12 hours with few or no clinical signs before death.

A study made in the Serengeti ecosystem between 2002-2011 showed that FPV antibodies could be found in lions, hyenas, African wild dogs, civets, genets, jackals and white-tailed mongoose (Calatayud *et al.* 2019). Another study made in 2004 on spotted hyenas in Maasai Mara showed that there was a higher proportion of hyenas with FPV antibodies the closer they lived to humans (Harrison *et al.* 2004). This indicates there could be a risk of FPV spreading between domestic animals and wildlife.

This study is part of the Mara Cat project that focuses on conservation, research, and animal welfare. This is done by neutering, vaccination, and sample collection of cats in the Mara North Conservancy (MNC) in Kenya. Mara North Conservancy is a part of the Greater Maasai Mara national reserve and holds a large wildlife population consisting of leopards, lions, cheetahs, hyenas, elephants, zebras, African wild dogs and many more. Within the MNC there are multiple villages and a large population of domesticated cats. The cats included in this study were free-roaming domesticated cats and one feral cat. The cats live closely together with wildlife, and they share both home and feed. Risk of transmission of diseases are greater the more closely domesticated animals and wildlife live (Harrison *et al.* 2004; Duarte *et al.* 2013; Chaiyasak *et al.* 2020; Guerrero-Sánchez *et al.* 2022).

The aim of this study was to investigate the prevalence of FPV antibodies in domesticated cats in MNC and Maasai Mara by looking at antibodies against FPV using an ELISA test. No known study has investigated this in domesticated cats in the area. By investigating the prevalence of FPV in these cats this study might be able to determine if the domesticated cats could be a potential threat to spreading the virus to wild animals in the area.

2. Literature Review

2.1 Feline panleukopenia virus

Feline panleukopenia virus (FPV) is a parvovirus and a member of the family *Parvoviridae* (Sykes 2014). It is a small and non-enveloped virus with a single-stranded DNA genome (Payne 2023). The virus is extremely stable and can survive long periods of time in the environment and resist disinfection (Sykes 2014; Payne 2023). Feline panleukopenia virus is spread worldwide and can infect both domesticated cats and wild felids and some members of related families e.g., minks, foxes, and racoons. Infection with FPV can lead to the diseases feline panleukopenia, feline distemper and feline infectious enteritis (Payne 2023). Vaccination against FPV is considered a core vaccination for cats and is therefore recommended for all domestic cats (Stuetzer & Hartmann 2014; Stone *et al.* 2020). This has led to FPV infection being rarely diagnosed by veterinarians in developed countries, but it is still widespread in feral cats (Washabau & Day 2013).

Feline panleukopenia virus is closely related to the canine parvoviruses (CPVs) and these viruses can lead to similar disease in felids (Washabau & Day 2013; Sykes 2014). Feline panleukopenia virus has been known since the 1920s, while CPVs emerged in the 1970s and now also have a worldwide distribution. It is understood that CPVs emerged from the feline panleukopenia virus, and currently the predominant genotypes of CPVs are CPV-2b and CPV-2c (Washabau & Day 2013). Canine parvoviruses mainly infects dogs but can also infect cats and lead to disease.

2.1.1 Pathogenesis

Feline panleukopenia virus is transmitted through the faecal-oral route (Sykes 2014). Indirect contact through contaminated fomites is believed to be the most important means of infection. The virus can be shed in faeces, saliva, urine, vomit and blood and for long periods of time (Payne 2023). In the acute phase of infection, the highest number of viral particles are shed and can be detected in faeces for weeks (Washabau & Day 2013).

Feline panleukopenia virus cannot infect humans (Sykes 2014), but humans can transmit virus by handling an infected animal and not practicing proper hygiene before coming into contact with a non-infected animal.

The virus enters actively dividing cells, such as cells in the bone marrow, lymphoid tissues, intestinal epithelium, cerebellum in young animals, retina, embryonic and foetal cells (Washabau & Day 2013). The virus enters these cells by using transferrin receptors and replicates when the mitotically active cell is in the S-phase (Washabau & Day 2013; Stuetzer & Hartmann 2014; Sykes 2014). The

virus initially replicates in oropharyngeal lymphoid tissue (Steinel *et al.* 2001; Sykes 2014) and after 2-7 days´ viremia leads to dissemination of the virus to all tissues in the body (Stuetzer & Hartmann 2014). Infection of the bone marrow is associated with leukopenia, and the circulating white blood cells can decrease by up to 90% (Payne 2023). The replication of FPV in the intestinal crypt epithelial cells leads to shortening of villi, increased intestinal permeability and mal-absorption (Sykes 2014).

Infection during pregnancy and in neonates

Feline panleukopenia virus replicates in a variety of tissues in the developing foetus or neonate (Sykes 2014). Infection during early pregnancy can lead to foetal death, resorption, congenital abnormalities, abortion and mummified foetuses (Stuetzer & Hartmann 2014; Sykes 2014).

Later in pregnancy and in neonates up to 1 week of age, infection can damage neuronal tissue in the cerebellum and lead to cerebellar hypoplasia. Infection during this time period can also affect other parts of the central nervous system (CNS) such as cerebrum, retina and optic nerves (Stuetzer & Hartmann 2014). Less common CNS abnormalities that have been reported due to FPV infection are hydrocephalus, porencephaly (cystic lesions within the cerebral hemispheres) and hydranencephaly (complete replacement of the cerebral hemispheres with cystic lesions) (Sykes 2014). These abnormalities can be seen together with signs of forebrain damage, such as behavioural changes and seizures.

2.1.2 Clinical signs

Infection with FPV can lead to the diseases feline panleukopenia, feline distemper and feline infectious enteritis (Payne 2023). Recently weaned kittens are most at risk to these diseases as levels of maternally derived antibodies (MDA) decline.

The disease can come in different forms; subclinical infection, per acute, subacute and acute form (SVA 2023). The severity of the disease depends on age, immune status and any concurrent infections (Sykes 2014). Subclinical infection is believed to be widespread and especially in adult immune-competent cats, but the actual prevalence of subclinical infections of FPV are unknown (Stuetzer & Hartmann 2014). The per acute form can lead to death within 12 hours with little to no clinical signs before death (Little 2012).

The most common form of FPV infection is the acute form with the initial clinical signs of fever, depression and anorexia (Stuetzer & Hartmann 2014). Vomiting is unrelated to eating and is usually bile-coloured (Little 2012). Later in the disease the cats can develop watery to haemorrhagic diarrhoea. Vomiting and diarrhoea can lead to extreme dehydration with progressive weakness and depression (Stuetzer & Hartmann 2014). Death is usually associated with complications such as septicaemia, dehydration and disseminated intravascular

coagulopathy (DIC). If infected cats survive the first week, they usually recover within days or weeks (Little 2012; Stuetzer & Hartmann 2014). Cats with the acute form of the disease have a mortality of 25-90% and peracute cases have a mortality up to 100% (Stuetzer & Hartmann 2014).

Infection during pregnancy and in neonates

The main clinical signs of an FPV infection in new-born kittens are neurological (Stuetzer & Hartmann 2014). The kittens can have hypermetric movements, ataxia and be blind. They can also show problems with incoordination and tremor although being bright and alert, which is a sign of cerebellar dysfunction (Stuetzer & Hartmann 2014; Sykes 2014). Infected kittens can also have retinal degeneration with or without neurological signs (Stuetzer & Hartmann 2014). Infection with FPV can in some cases also cause damage to the forebrain with signs such as seizures, abnormal behaviour, and postural reaction deficits (Stuetzer & Hartmann 2014; Sykes 2014).

Kittens in the same litter can show a variation of severity of the disease, and some littermates can be clinically healthy (Stuetzer & Hartmann 2014; Sykes 2014). This is believed to be due to maternally derived antibodies (MDA) or innate resistance. Clinically healthy kittens within an affected litter can still harbour virus for up to 2 months after birth (Stuetzer & Hartmann 2014).

2.1.3 Epidemiology and vaccination

Feline panleukopenia virus is spread worldwide and has been known since the 1920s (Allison & Parrish 2014; Jiao *et al.* 2020). In the 1930s and 1940s the first outbreaks of disease related to FPV in captive felids were reported (Allison & Parrish 2014). In 1947 a park in London, "Zoological Society of London", had an outbreak of FPV-related diseases and multiple feline species were infected, e.g., tigers (*Panthera tigris*), leopards (*Panthera pardus*), cheetahs (*Acinonyx jubatus*), wild cats (*Felis sylvestris*), lynx (*Lynx lynx*), servals (*Leptailurus serval*), tiger cats (*Felis tigrine; Felis aurata*) and ocelots (*Leopardus pardalis*). It is generally assumed that all felids can become infected with FPV.

Outbreaks of disease related to FPV in domesticated cats have also been reported. Australia had multiple epizootic outbreaks with high mortality between 2014-2018 (Jenkins *et al.* 2020). A case report from Italy in 2021 showed that an outbreak of severe haemorrhagic diarrhoea in a group of 12 cohabiting adult cats originated from an FPV infection (Pacini *et al.* 2023). Three out of 12 cats died in the peracute form, and the remaining cats survived thanks to isolation, disinfection, and vaccination.

Another study made in Florida in 2007 showed that 33% of 61 feral cats in a TNR-project had antibodies against FPV (Fischer *et al.* 2007), suggesting that the virus is circulating in the area.

Vaccination

Vaccination against FPV is considered a core vaccine according to the American Association of Feline Practitioners (AAFP) and the European Advisory Board on Cat Disease (ABCD) (Stuetzer & Hartmann 2014; Stone *et al.* 2020). This means that it is recommended for all domesticated cats.

When vaccinating kittens against FPV the first dose should be given earliest at 6 weeks of age and then in an interval of 3-4 weeks until 16-20 weeks of age (Stone *et al.* 2020). Revaccination should be done at 6-12 months of age depending on whether the kitten had maternally derived antibodies (MDA) or not. After this, revaccination should be made every 3 years (see table 1).

Kittens are not born with an effective immune system with antibodies; however, they absorb MDA through colostrum from their mother which provides protection during early life (Stone *et al.* 2020). Although MDA play an important role in the kittens' early life, they can interfere with active immunization and neutralize vaccine antigens (Jakel *et al.* 2012; Stone *et al.* 2020). Maternally derived antibodies will then decline with time, but there is considerable individual variation in the rate of decline. Some kittens maintain a high concentration of MDA for months, which will interfere with the effectiveness of the vaccination protocol. One of the most common reasons for vaccine failure is the persistence of MDA (Stone *et al.* 2020).

Table 1. Vaccination guidelines from AAFP (Stone et al. 2020) .

Type of vaccine	<16 weeks of age first dose administered	>16 weeks of age first dose administered	Revaccination
Attenuated live	Earliest 6 weeks of age, and then q 3-4 weeks until 16-20 weeks of age	One or two doses	6-12 months of age depending on the MDA status Revaccinate every 3 years
Inactivated	Earliest 6 weeks of age, and then q 3-4 weeks until 16-20 weeks of age	Two doses 3-4 weeks apart	6-12 months of age depending on the MDA status Revaccinate every 3 years

Vaccination against FPV can be made with inactivated or attenuated live vaccines (Yang *et al.* 2008; Stone *et al.* 2020). Inactivated vaccines contain a “killed” target pathogen and is unable to replicate within the host. This will produce a weaker immune response with a shorter duration time compared to attenuated live vaccines (Stone *et al.* 2020). Inactivated vaccines are considered safer to use during pregnancy and in immunosuppressed animals. However, they have the potential to contain incompletely inactivated virus (Yang *et al.* 2008). Attenuated live vaccines contain modified microorganisms and replicate within the host (Stone *et al.* 2020). These vaccines will stimulate a more natural immune response and have a faster onset of immunity. Live vaccine organisms that are shed from the vaccinated animal can potentially also immunize other animals in a population. There is, however, a risk of vaccine organism-induced disease, and these vaccines are also more likely to give false positive results when using diagnostic tests to detect antigens or nucleic acid.

The vaccination program for domesticated cats is recommended for captive wild felids, meaning that captive wild felids should be vaccinated against FPV (Lamberski 2012). Steinel *et al.* (2001) recommends vaccination against FPV for all captive carnivores and if possible, also to free-ranging carnivores, and before reintroduction of captive-born animals to the wild. Allison and Parrish (2014) suggest that in areas with a small population of wild animals and a big population of domestic animals, vaccination of domestic animals may reduce the level of exposure to the virus for wildlife. By vaccinating the domestic cats in the area, it might delay the infection in wild animals until they are less susceptible to the most severe disease.

2.1.4 Diagnosis and treatment

Physical examination findings

Common findings during a physical examination are weakness, lethargy and dehydration (Sykes 2014). During early illness, the animal may present with fever (39.5-42.5°C). A history of anorexia, vomiting and diarrhoea are common (Washabau & Day 2013). During abdominal palpation the cat might show pain and discomfort.

Infected kittens can show different types of neurological symptoms such as intention tremors, ataxia, hypermetria and absence of a menace response during clinical examination (Sykes 2014).

Laboratory findings

Panleukopenia is not pathognomonic for FPV infection (Little 2012), but is the most commonly seen abnormality during infection (Sykes 2014). The panleukopenia is caused by neutropenia and lymphopenia. Even though panleukopenia is

common in infected cats with FPV, one study showed that only 65% of 187 cats were leukopenic, therefore FPV infection cannot be ruled out even if the cat does not have panleukopenia.

Other blood-related laboratory findings that are common are thrombocytopenia and mild anaemia. The reason FPV infection leads to thrombocytopenia may be due to damage to the bone marrow or because of disseminated intravascular coagulation (DIC).

It is also common that FPV infection leads to hypoalbuminemia, hypochloraemia, hyponatremia and hypoproteinemia.

Diagnostic tests

Feline panleukopenia virus antigen can be detected with commercially available ELISA tests for canine parvovirus in faeces (Little 2012). The sensitivity of these tests varies both with the assay used and the timing of the test (Sykes 2014). Diagnosis can also be confirmed by PCR, faecal electron microscopy, virus isolation or histopathology (Washabau & Day 2013; Sykes 2014). Recent vaccination with attenuated live vaccines can give false positive results; therefore vaccination history needs to be considered in the interpretation of the test results (Washabau & Day 2013).

When identifying antibodies against FPV, hemagglutination inhibition is the gold standard (Stone *et al.* 2020) for diagnosis. However, an ELISA test can also be used to identify FPV antibodies (Egberink *et al.* 2022). The identification of antibodies can be used to diagnose infection, to indicate previous exposure to pathogens, especially in unvaccinated cats, and to assess the immunity prior to vaccination (Stone *et al.* 2020). The disadvantage in looking for antibodies is that it is not possible to determine when the individual was exposed to the pathogen, only that exposure has occurred.

Treatment

Treatment for affected cats consists primarily of supportive care with intravenous fluid therapy (Little 2012; Washabau & Day 2013; Sykes 2014). For cats with persistent vomiting, antiemetics are often used (Washabau & Day 2013; Sykes 2014), and oral intake of food and water should be retained until the vomiting has stopped because of the risk of aspiration pneumonia (Sykes 2014). When at risk for secondary bacteraemia or sepsis parenteral antibiotics are given (Little 2012; Washabau & Day 2013; Sykes 2014). During the recovery period probiotics and GI supportive food are often prescribed (Washabau & Day 2013).

2.2 FPV in wild animals

Multiple studies show that FPV antibodies can be found in wild animals around the world, and Maasai Mara and the Serengeti ecosystem is no exception (Harrison *et al.* 2004; Calatayud *et al.* 2019). Harrison *et al.* (2004) found antibodies against FPV in spotted hyenas (*Crocuta crocuta*) in Maasai Mara and saw in their study that there was a higher risk for hyenas to have antibodies for FPV if they lived close to humans. This increased their suspicion that the primary source of hyena exposure to FPV is domesticated animals that live with humans.

Calatayud *et al.* (2019) did their research on carnivore parvoviruses including FPV in the Serengeti ecosystem in Tanzania with samples from 2002-2011. They found FPV antibodies in three lions, two spotted hyenas (*Crocuta crocuta*), two African wild dogs (*Lycaon pictus*), two civets, one genet (*Genetta genetta*), one white-tailed moongose (*Ichneumia albicauda*) and in one black-backed jackal (*Canis mesomelas*). This data is shown in table 2 below. They could not, however, see any association between FPV infection in wild animals and closeness to human habitation. Calatayud *et al.* (2019) also investigated the prevalence of the parvovirus CPV in domesticated dogs in the Serengeti ecosystem and found that the prevalence was 42.9%. There was a higher prevalence of FPV in wild animals than of CPV, even though domesticated dogs had a high prevalence of CPV, which could indicate that domesticated cats with FPV infection could play a role in transmission to wild animals.

Table 2. Data from Calatayud et al. (2019) study in Serengeti, showing tested species, number of samples and number of positive FPV samples.

Species	Total no. of samples	No. of positive FPV samples
African civet	5	2
Genet	3	1
Mongoose	7	1
Lion	44	3
Cheetah	6	0
Leopard	1	0
Serval	1	0
Black-backed jackal	15	1
African wild dog	20	2
Spotted hyena	32	2

A study made in eastern Africa with samples from Tanzania's national parks, including the Serengeti National Park, showed a prevalence of 68% of antibodies to FPV in 309 tested lions (Hofmann-Lehmann *et al.* 1996). The samples were collected between 1984 and 1991. There was a higher titre of FPV antibodies in lions within Serengeti National Park compared to lions in other national parks

within Tanzania. The authors suggested that the high seroprevalence in Serengeti National Park was caused by lions moving over larger distances and encountering more lions compared to those living in other areas.

Other studies in different parts of the world also showed that wild animals have antibodies against FPV. A study made in Borneo found FPV antibodies in Sunda clouded leopards (*Neofelis diardi*) and Malay civet (*Viverra tangalunga*) (Guerrero-Sánchez *et al.* 2022). These animals lived close to domesticated cats and human habitation, which also could indicate the importance of domesticated cats in the transmission of FPV to wild animals.

Another study made in Eastern Thailand in 2013 described an outbreak of FPV among 55 captive small Indian civets (*Viverricula indica*) farmed for perfume production and living close to domesticated cats and dogs (Chaiyasak *et al.* 2020). The civets showed typical signs of FPV infection with acute bloody diarrhoea, anorexia, vomiting and having seizures. 38 out of 55 civets died within a month. The authors suggested that unvaccinated free-roaming domesticated cats and dogs might serve as a potential risk factor for transmission.

A study made in Portugal tested for FPV in hunted or accidently road-killed animals between 2008 and 2011 (Duarte *et al.* 2013). The species in the study were red fox (*Vulpes vulpes*), Egyptian mongoose (*Herpestes ichneumon*), stone marten (*Martes foina*), Eurasian badger (*Meles meles*) and common genet (*Genetta genetta*). The study found DNA from parvovirus in 63.3% out of the tested animals. Out of these 63.3% animals, 90% of them had CPV/FPV antibodies. The authors suggested that domestic cats serve as a reservoir and source of infection to other animals and highlight the risk of this event, especially in areas with endangered wild species.

All these studies show that FPV is widespread around the world, and many species of wild animals are affected by the virus. It is known that domesticated cats that are infected with FPV can serve as reservoirs whether they have clinical signs or not (Duarte *et al.* 2013). Multiple authors suggests that domesticated animals, and especially cats, that live in close proximity to wildlife could be a risk factor for transmission of FPV to wild animals. This is especially worrying regarding conservation, and specifically for endangered species that could contract the virus from domesticated cats.

3. Material and Methods

3.1 Literature search

Information and articles regarding feline panleukopenia virus were obtained from searches in Web of Science and the Swedish University of Agricultural Sciences (SLU) library search function "Primo". Cited articles was also used in the literature search.

The following words were used for the searches: "feline panleukopenia virus" OR "feline parvo*" AND prevalence*, "feline panleukopenia virus" OR "feline parvo*" AND "wild animal*", "feline panleukopenia virus" OR "feline parvo*" AND outbreak*, "feline panleukopenia virus" OR "feline parvo*" AND Africa OR Kenya OR Maasai Mara OR Serengeti.

3.2 Study area

The study was conducted within the Mara North Conservancy (MNC) in Maasai Mara, Kenya. Mara North Conservancy is located in Narok county in the southwest of Kenya. It is a not-for-profit organization that started in 2009 to support conservation efforts within the Maasai Mara ecosystem. This study took place in a small village called Mara Rianta within the MNC.

The average rainfall in Maasai Mara is 1400 mm per year and the temperature is between 12-28°C depending on the month.

Figure 1. Map of Kenya and Narok county highlighted. Map made by the author in 2023 merging two custom maps made in Mapchart. https://www.mapchart.net/

3.3 The sampled cats

The cats included in this study were free-roaming owned cats and one feral cat. The cats were collected from their homes in the morning and brought to the clinic by a local veterinary assistant. All cats lived within or nearby Mara Rianta. All owned cats were included in this project and study because their owners wanted their cats

to be spayed or castrated. All cats were considered as non-vaccinated since there is no regular veterinary clinic in the area and this study is the first time that the cats in this area were vaccinated.

The feral cat was caught using a trap that was checked regularly. This cat was brought to the clinic in the morning and returned to the same area where it was trapped at the end of the day. This was the only cat that was part of the TNR-project.

3.3.1 Blood sampling

Almost all blood samples were taken from the cephalic vein using a needle and a serum tube. Two cats had blood collected from the jugular vein. A small number of cats had to be sedated to be able to take the blood sample. The blood was collected in serum tubes of 500 µl. After clotting, the serum tubes were centrifuged at 3000 RPM for 10 minutes before transferring the supernatant serum to an Eppendorf tube with a disposable plastic pipette. The Eppendorf tube was then kept cold, and frozen at the end of each day.

3.3.2 Vaccination, spaying and neutering

After blood sampling all cats in this study were vaccinated against rabies and were neutered. Sedation was made with Medetomidine. All cats were given Meloxicam as analgesia and all females were given Buprenorphine before spaying. All surgery took place under dissociative anaesthesia with Ketamine. All cats were also given Atipamezole after surgery. Females were spayed with either flank- or midline procedure. All males were castrated with auto ligation.

3.4 Serological test

Immunoglobulin G (IgG) antibodies against FPV was detected using an indirect ELISA test kit (Feline Panleukopenia Virus Antibody ELISA, ref F1004-AB01, European Veterinary Laboratory, the Netherlands). Briefly, the test principle is that a monoclonal antibody specific to the Panleukopenia virus is immobilized on a solid phase and serves as a capture antibody anchoring the virus to the solid surface. The plate is then incubated for 75 minutes in 37°C and washed, and a sample is added. Any antibodies in the sample that can bind to the attached Panleukopenia virus antigen will do so. The plate is then incubated for 60 minutes in 37°C and washed, and a second antibody, a monoclonal HRPO conjugate, is added. This antibody will bind to any unoccupied binding sites on the antibodies that have attached to the Panleukopenia virus antigens. The plate is incubated a third time in 37°C for 60 minutes and washed, and a substrate is added which reacts with HRPO and a colour change will occur. This colour change is proportional to the amount of antibodies present in the sample that reacted with the immobilized Panleukopenia virus

antigens. The plate is incubated for 10 minutes at room temperature in the dark and washed. A stop-solution is then added, and the plate is read.

The test was performed according to the instructions of the manufacturer for a qualitative test, and each sample was analysed in duplicate. Two negative controls, four positive controls in different solutions and two substrate controls were also used. The plates were read using the Spotxel® Reader app on iPhone 13 Pro. The OD value was then calculated for each sample and interpreted either positive or negative for FPV antibodies.

To validate the test the mean value (MV) of the measured OD value for the positive control (PC) diluted 1:100 must be ≥0,850, and the mean value of the measured OD value for the negative control (NC) diluted 1:250 must be ≤0,400. To calculate the OD value for each sample the following equation was used:

$$\frac{S}{P} = \frac{OD_{sample} - MVOD_{NC}}{MVOD_{PC} - MVOD_{NC}}$$

A sample with the S/P ratio <0.22 is negative and specific antibodies to FPV could not be detected. A sample with the S/P ratio ≥0.22 is positive and specific antibodies to FPV were detected.

3.4.1 Statistical analysis

The statistical analysis was made using Minitab 21.4.1 (Minitab LLC 2023). The confidence interval for each gender and the total sample size was calculated. Fisher's exact test was used to assess whether there was a statistically significant difference in the results of FPV antibodies based on gender.

4. Results

This study tested 40 individual cats; 39 of them were free-roaming domesticated owned cats and one was a feral cat. 11 male cats and 29 female cats were sampled. The feral cat was a female.

4.1 Seroprevalence of FPV antibodies

Antibodies could be found in 85% of the tested cats (34 out of 40). In the female population 27 out of 29 had antibodies (93.1%), and amongst the male 7 out of 11 had antibodies against FPV (63.6%). The distribution is showed in table 3.

Table 3. Prevalence of antibodies against FPV.

Gender	Positive (%)	Negative (%)	Total
Female	27 (93.1%)	2 (6.9%)	29
Male	7 (63.6%)	4 (36.4%)	11
Total	34 (85%)	6 (15%)	40

The mean value of the measured OD value for the positive control was 17.47 and the mean value of the measured OD value for the negative control was 0.045. These mean values are within the criteria for a validated test. A list of the S/P ratio for each tested cat is shown below in table 4.

Table 4. S/P ratio for each individual cat, if they had FPV antibodies or not, and their gender.

Sampled cat no.	S/P ratio	FPV antibodies	Gender
1.	0.704	+	Female
2.	0.688	+	Female
3.	0.666	+	Female
4.	0.615	+	Male
5.	0.721	+	Female
6.	0.657	+	Female
7.	-0.047	-	Female
8.	-0.040	-	Male
9.	-0.388	-	Male
10.	0.662	+	Female
11.	0.472	+	Female
12.	0.094	-	Male
13.	0.625	+	Female

14.	0.076	-	Female
15.	0.529	+	Female
16.	0.547	+	Female
17.	0.747	+	Female
18.	0.561	+	Female
19.	0.501	+	Female
20.	0.792	+	Male
21.	0.818	+	Male
22.	0.820	+	Male
23.	0.697	+	Female
24.	0.465	+	Female
25.	0.806	+	Male
26.	0.886	+	Male
27.	0.911	+	Female
28.	0.608	+	Female
29.	0.753	+	Female
30.	0.832	+	Female
31.	0.729	+	Female
32.	0.727	+	Female
33.	0.123	-	Male
34.	0.853	+	Female
35.	0.824	+	Female
36.	0.733	+	Female
37.	0.822	+	Female
38.	0.769	+	Female
39.	0.818	+	Female
40.	0.731	+	Male

4.1.1 Statistical analysis

With a confidence interval of 95% the prevalence of FPV antibodies in the sampled females were 77.2-99.2%, and in the sampled males 30.8-89.1%. The sero-prevalence for all sampled cats were 70.2-94.3%.

There was a significant difference between the genders (p=0.039).

5. Discussion

5.1 Seroprevalence of FPV antibodies

This study aimed to determine the prevalence of antibodies against FPV in a group of domesticated cats in Mara North Conservancy, Kenya. In this study, a total of 85% of tested cats (34 individuals) had antibodies against FPV. This area is remote, and the cats have not been vaccinated before, therefore it is highly unlikely that the antibodies derive from vaccination. It is more likely that FPV circulates in the area and that the cats have developed antibodies from an infection, either a subclinical or a clinical infection that they have survived.

Of 40 sampled cats, 29 were female and 11 were male. Due to the males more often going out roaming early in the morning, it was harder to collect them for sampling. 93.1% of the females and 63.6% of the males had antibodies against FPV.

The sample sizes of each gender were very different but using a Fisher's exact test for statistical analysis suggests that there is a significant difference between the genders. Female cats spend more time in their household, compared to male cats that more often go out wandering, which leads to the females spending more time in an eventually contaminated area where the virus survives for long period of times. This could be one reason why the females have a higher seroprevalence than the males. A study made in 2022 from the Mara Rianta area, where this study also took place, showed that 69 of 100 households of interviewed Maasai households had at least one cat, and that 21 households had more than one cat (Byström 2023). The genders of these cats are not known. However, this suggests that interaction between cats occur in their households, and since the female cats spend more time at home there could be a higher chance of them contracting FPV compared to males.

5.2 Risk of transmission of FPV to wild animals

A study made in 2022 in the same location, interviewed local Maasai household regarding their cats' living conditions (Schultz 2023). This study showed that 7 out of 100 households had seen domestic cats interact with wild felids. Some of the wild felids mentioned were African wild cat and leopard. However, the author and the households interviewed suggested that since most activity of domestic cats occur during night-time when people are asleep, interaction might actually occur more frequently than stated. The same study stated that 90 out of 100 households threw their dead cats out in the bush for wild animals to eat and 7 households left the dead cats where they were found. This could also be a possible mode of transmission of virus from domestic cats to wildlife, since virus may survive in cat

carcasses for some time after death and thus infect wild animal when ingesting the carcass.

Studies on FPV antibodies made on wildlife in the Maasai Mara and Serengeti (Harrison *et al.* 2004; Calatayud *et al.* 2019) showed that FPV antibodies could be found in different types of wild animals, such as hyenas and lions. There are, however, different opinions whether domesticated animals could play a role in the transmission of FPV to wildlife. Harrison *et al.* (2004) suggests that there is a higher risk of FPV antibodies in wildlife if they live in close contact with domesticated animals. Calatayud *et al.* (2019) did not find any association between the presence of FPV antibodies in wildlife and distance to human habitation. Guerrero-Sanchéz *et al.* (2022) found FPV antibodies in leopards and civets in Borneo and argued that the domesticated animals in the area could play a role in the transmission of FPV. Chaiyasak *et al.* (2020) and Duarte *et al.* (2013) also highlights the risk of domesticated animals playing a key role in transmission of FPV to wild animals. To get more answers to this question a study must be made which investigates FPV in domesticated animals and wildlife in the same area, and a comparison of the genome of the virus has to be made to see if it is the same in both domesticated animals and wild animals.

However, FPV is a very contagious virus that stays stable in the environment for a long period of time, and it is well known that domesticated cats and wild felids can become infected and sick with FPV. Recent studies have also shown that other wild animals can become infected with FPV (Calatayud *et al.* 2019). With the knowledge of how this virus survives in the environment, what its hosts are, and the infection route, there is no reason why a transmission between domesticated cats and wild animals, and vice versa, would not be possible.

As late as in August and September this year (2023), seven leopard cubs between three and 10 months of age at the Bannerghatta National Park in India died from infection with FPV (Ramesh 2023; The Times of India 2023). All the cubs had received their initial vaccinations, but not their second dose. Even though the exact cause of the outbreak is unknown, it is suggested that the virus might have been spread through pet keepers who have unvaccinated domesticated cats at home or by stray cats that roam freely in the park. The cubs were rescued from the wild and could also have been already infected when they came to the park. This outbreak caused 15 infected leopard cubs, and seven deaths. This also highlights the risk of FPV being spread through different populations and, since leopards are on the IUCN's red list of vulnerable species, it is a catastrophe from a conservation point of view.

A part of this study was to spay and neuter the sampled cats. This will in time help to reduce the number of domesticated and feral cats in the area. By reducing the number of susceptible cats, it will also reduce the number of interactions between cats and wildlife and could therefore also minimize the risk of transmission

of disease. Young kittens and newborns are the most susceptible for FPV infection and by reducing that population there is a chance of reducing FPV in the area altogether. A review article from 2020 looked at current global cat population management practices for owned, free-roaming cat populations, including Indigenous communities in Australia (Kennedy *et al*. 2020). The cat populations in remote Indigenous communities in Australia live a similar life to this studied cat population in Maasai Mara, Kenya. The cats live in environments with a high biodiversity and a lot of wildlife which they can both hunt and spread disease to. Kennedy *et al.* (2020) reviewed both articles of in-situ field cat populations and studies simulating computer modelled cat populations in different communities. All socialized owned, free-roaming cats were surgically neutered or spayed and the most common practice for unsocialised cats was the TNR-method. The authors also found that a version of TNR was used, i.e. trap-remove (TR), meaning that unwanted, socialised cats are adopted out of the community, and that ill or injured cats are euthanised. Kennedy *et al.* (2020) found that long term projects of neutering and spaying cats, supplemented with TR, is the best and most ethical method to manage cat populations. However, spaying or neutering the cats in a population will not have an immediate impact on the current cat population, but it will have a long-term impact on the population by decreasing the chance of reproducing, and by that also decreasing the risk of diseases spreading in the cat population and transmit to wildlife.

Mara North Conservancy (MNC) holds a lot of wildlife and some of them are endangered. There are many reasons why a species is endangered, for example: human-animal conflict, climate changes, loss of habitat, and diseases. Feline panleukopenia virus is at the highest risk to new-born kittens, including cubs of lions, leopards, cheetahs and so on. There are also many reasons why not all cubs survive to adulthood, but if there is a chance to lower the risk of death in these species by vaccinating domesticated cats in the area against FPV, this is a measure that should be strongly considered.

5.3 Limitations

This study was conducted in a remote area with uncertain electricity power supply, and it was not possible to keep the cold chain uninterrupted the whole time. The risk of freezing and thawing the samples more than necessary is that non-specific reactivity increases, which could explain the high percentage of cats with antibodies against FPV in this study. The ELISA test was also made in the field and not in a laboratory with high-end equipment, which increased the risk of human errors.

The cats included in this study were picked up by a veterinary assistant in the morning before being brought to the clinic. Some of the cats that were supposed to be in the study were not sampled, since they had gone out roaming when the

veterinary assistant came to pick them up. This led to a convenience bias being present in the study. The cats in the area are not registered in any sort of list, which made a random selection of the cats impossible.

No known studies on FPV antibodies in domesticated cats in the area have been made; therefore, it is hard to say if the high percentage of FPV antibodies found in these studied cats are likely to be true since there are no comparable studies. A study made in Germany on sheltered cats showed that the prevalence of FPV antibodies was as high as 87.7%; however 55.4% of these cats had been vaccinated (Rehme *et al.* 2022). A study made in France in 2011 on owned and not owned cats living in a rural environment, showed that FPV antibodies could be found in 36.41% of owned cats and in 15.61% of unowned cats (Hellard *et al.* 2011). A study made in Florida in 2007 on feral cats in a TNR-project showed that 33% of sampled cats had antibodies against FPV (Fischer *et al.* 2007). These studies were not made in the same circumstances as the present study, according to both studied area and cat ownership; however, it suggests that the result of 85% of cats having FPV antibodies is unusually high. It is not known if this high percentage is due to FPV circulating a lot in this study area, or if the circumstances surrounding the ELISA test or the ELISA test itself somehow was faulty.

5.4 Conclusion

The aim of this study was to determine the seroprevalence of FPV in domesticated cats in a part of Maasai Mara, Kenya and to see if there could be a risk of transmission of FPV to wildlife. This study sampled 40 cats, 85% of which were found to have antibodies against FPV. The study took place under field circumstances, meaning that there were limitations with convenience sampling, resulting in bias for the sample size, and limitations regarding an uninterrupted cold chain for the samples, and with lab facilities. However, this study showed that domestic cats in this area had a high seroprevalence of FPV, resulting in a risk of transmission between domesticated cats and wildlife. In interviews, the Maasai households in the area had confirmed seeing a small number of interactions between their domesticated cats and wildlife, and they also said that the most common way of disposing of dead cats is to leave them out in the bush for wild animals to eat (Schultz 2023). Eating a dead carcass that is infected with FPV could be a possible route of transmission.

The FPV virus is also highly contagious and stays stable in the environment for a long period of time. This means that since the domesticated cats in the area have a high seroprevalence of FPV, there is a high chance for them to spread the virus to wildlife. Other studies regarding FPV antibodies in the area, and around the world, suggest that domesticated animals are a risk for transmission of disease to wildlife. Multiple authors suggests that wild animals that live in close proximity to

domesticated animals have a higher percentage of FPV antibodies than wildlife that does not have this close contact (Harrison *et al.* 2004; Duarte *et al.* 2013; Chaiyasak *et al.* 2020; Guerrero-Sánchez *et al.* 2022). This is highly worrying, especially regarding conservation for endangered species. Further studies need to be done in this topic, and preferably comparing the genome of FPV to see if domesticated animals and wildlife share the same virus.

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Popular science summary

Feline panleukopenia virus (FPV) is a highly contagious virus that can infect domesticated cats, wild felids, and other wildlife. FPV infects via the mouth and is shed in faeces, vomit, saliva, urine, and blood for long periods of time. The virus is very stable in the environment and occurs worldwide. Vaccination against FPV is recommended to all domesticated cats and is considered a core vaccine. This has led to a decline in infection with FPV in places where domesticated cats are vaccinated. However, outbreaks still happen in some parts of the world and in groups of feral cats where vaccination is not as common.

Infection with FPV is most severe in new-born kittens and can lead to death within 12 hours with few or no signs beforehand. Infection in older cats usually shows with signs such as lethargy, diarrhoea, and vomiting. The infection also leads to a lower count of white blood cells and it has a poor prognosis if left untreated. Cats with the acute form of the disease have a mortality rate of 25-90% and per acute cases have a mortality rate up to 100%. Infection during pregnancy or early in life can lead to different neurological symptoms.

Wild animals around the world can contract FPV and it is argued that domesticated cats can transmit FPV to wild animals. This is supported by multiple studies showing a higher percentage of antibodies against FPV in wild animals living close to humans and their domesticated cats, than wild animals that live further away from human habitation.

This study investigated the presence of antibodies against FPV in domesticated cats in Mara North Conservancy, Kenya. 85% (34/40) of the sampled cats had antibodies against FPV. This means that they have been infected with FPV at some point in their lives. Studies on wild animals in the area show that they also have antibodies against FPV. This suggests that FPV is a virus that is existing in the area and that it is spread both in the domesticated cat population and amongst wild animals. To say if these domesticated cats transmit FPV to wildlife in the area a study must be made that looks on both domesticated cats and wildlife and compare the genome of FPV to see if it is the same. However, knowing the transmission route, the virus stability in the environment and that both domesticated cats and wild animals in the area have antibodies against FPV, suggests that it is highly likely that a transmission between domesticated cats and wild animals occurs.

Acknowledgements

Firstly, I would like to thank the Mara Cat Project and everyone at Karen Blixen Camp in Maasai Mara for making this study possible. A special thanks to my supervisors Therese Hård, Eva Axnér and Jane Morrell for all their help making this project possible and for taking such good care of us during this project. I would also like to give a big thank you the Maasai people for trusting us with their cats.

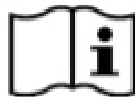
A big thank you to Dr. Ann-Marie Appel, Dr. Esther Macharia, Gillian Nyambura Muiruri, Silas Kirwa Lagat, Dr. Mathew Murket Nchoko, Francis Samunyak Ketuyio, Maina and Louise Fjord for the great teamwork during the week of sampling and treating the cats. Thank you Agnes Diamanti Barredal and Signe Ahlbom for doing this project with me and making this a trip of a lifetime.

Thank you to the European Veterinary Laboratory (EVL) for selling the ELISA test kit for a reduced price.

Lastly, I would like to thank Veterinärmedicinska fakultetens stipendiesamfond, Kungliga skogs- och lantbruksakademin and Stiftelsen Nilsson-Aschans stipendiefond for the scholarships that made this project possible.

Asante sana.

Appendix 1



Userś Manual

Feline Panleukopenia Virus Antibody ELISA

An ELISA to detect IgG antibodies against Feline Panleukopenia Virus in serum or plasma samples

F1004-AB01

96

December 2020

Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.
Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Test kit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1. Introduction

For diagnosis of Feline Panleukopenia Virus (FPV) infection or vaccination control, demonstration of antibody titer is the most commonly used method. The virus that is attached to the solid phase by use of monoclonal antibodies catches antibodies induced through infection or vaccination. IgG antibody titers above dilutions of 1:900 are considered protected.

2. Intended use of the test kit

The principle of the FPV test kit is based on the detection of antibodies against Panleukopenia virus. The Panleukopenia virus is attached to the solid phase by use of a monoclonal antibody. After the attachment of the antigen (Panleukopenia virus virus) sera or plasma containing antibodies are able to react with the antigen. After the antigen/antibody reaction, the attached antibodies can be detected by use of a monoclonal HRPO conjugate.

3. Principle of the test kit

The test is based on the reaction of FPV proteins with dog antibodies. To this end FPV proteins have been coated to a 96-wellmicrotiter plate by use of monoclonal antibodies.

- **Qualitative**
  The cat sample is added (diluted 1:250) to the wells of the coated plate.
- **Quantitative**
  The cat sample also can be titrated using a 3-step dilution, starting with a dilution 1:100 (→ 1:300→ 1:900 →1:2700).

After washing the bound cat antibodies are detected by a HRPO conjugated anti-species conjugate. The color reaction in the wells is directly related to the concentration of FPV antibodies in the serum/plasma sample.

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4. Contents

- 12 x 8 Microtiter strips
- 1 x Strip holder
- 1 x 18 ml ELISA buffer (green cap)
- 1 x 12 ml Inactivated Feline Panleukopenia virus antigen (lilac cap)
- 1 x 12 ml HRPO conjugated anti-species antibodies (red cap)
- 1 x 0,5 ml Positive control (freeze dried) (purple cap)
- 1 x 1,0 ml Negative control (freeze dried) (silver cap)
- 1 x 20 ml Wash solution (200x concentrated) (black cap), dilute in de-ionized water before use!
- 1 x 8 ml Substrate A (white cap)
- 1 x 8 ml Substrate B (blue cap)
- 1 x 8 ml Stop solution (yellow cap)
- 1 x Plastic cover sea
- 1 x User's manual

Supplies needed (not included)

- Round-bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes (EVL)
- ELISA plate reader

5. Handling and storage of specimens

The kit should be stored at 4°C.
An open packet should be used within 10 days.
Samples may be used fresh or may be kept frozen below -20°C before use.
After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20°C.
Avoid repeated freezing and thawing as this increases non-specific reactivity.

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6. Wash protocol

In ELISA's, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better result.

Manual washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl wash solution.
3. This washing cycle (step 1 and 2) should be carried out at least 5 times.
4. Turn the plate upside down and empty the wells with a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual wash solution in the wells.
6. Take care that none of the wells dry out before the next reagent is added.

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the wash solution is correctly added, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

7. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min. at room temperature (± 21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

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8. Test protocol **qualitative**

Before starting this test read "**preparations**"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C. and use them within 10 days.

   Wash microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Add **100µl** of **inactivated Feline Panleukopenia Virus antigen** to all wells of the coated microtiter strips to be used.
3. Incubate 75 min. at 37°C.

4. Reconstitute directly before use the **positive control** (purple cap) **in 0,5 ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
5. Reconstitute directly before use the **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

6. Dilute the **positive control** (purple cap) **starting 1:100 → 1:300 → 1:900 → 1:2700 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - A **pre-dilution** is needed:
   - Add 90µl ELISA buffer to **row 1A**, add 10µl of the positive control to the **well 1A** and mix well.
   - Add 180µl ELISA buffer to **row 2A**,
   - And 120µl to **2B, 2C, 2D**
   - Add 20µl of pre-dilution **well 1A** in the well **2A** and mix well
   - Mix well and transfer 60µl to the well **2B**
   - Mix well and transfer 60µl to the well **2C**
   - Mix well and transfer 60µl to the well **2D**
   - Mix well and discard 60µl.
7. Dilute the **negative control** (silver cap) **1:250 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 45µl ELISA buffer to **row 1E**, add 5µl of the negative control to the **well 1E** and mix well.
   - Add 96µl ELISA buffer to **row 2E**, add 4µl of pre-dilution **well 1E** in the well **2E** and mix well.
8. Dilute the **sample 1:250 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - A pre-dilution is needed:
   - Add 45µl ELISA buffer to **row 1F**, add 5µl of the sample to the **well 1F** and mix well.
   - Add 96µl ELISA buffer to **row 2F**, add 4µl of pre-dilution **well 1F** in the well **2F** and mix well.

9. Take 2 wells as **substrate controls** add only **120µl ELISA buffer** (green cap) to these wells.

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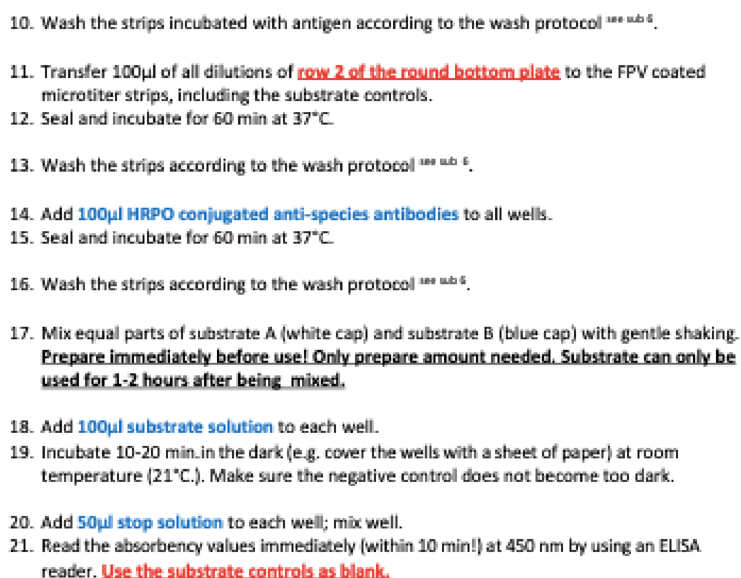

10. Wash the strips incubated with antigen according to the wash protocol [see sub 6].
11. Transfer 100µl of all dilutions of **row 2 of the round bottom plate** to the FPV coated microtiter strips, including the substrate controls.
12. Seal and incubate for 60 min at 37°C.
13. Wash the strips according to the wash protocol [see sub 6].
14. Add **100µl HRPO conjugated anti-species antibodies** to all wells.
15. Seal and incubate for 60 min at 37°C.
16. Wash the strips according to the wash protocol [see sub 6].
17. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**
18. Add **100µl substrate solution** to each well.
19. Incubate 10-20 min. in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21°C.). Make sure the negative control does not become too dark.
20. Add **50µl stop solution** to each well; mix well.
21. Read the absorbency values immediately (within 10 min!) at 450 nm by using an ELISA reader. **Use the substrate controls as blank.**

NB: if you pipet directly into the coated ELISA plate with only a small number of samples (<6), make sure the first dilution is done in round bottom microtiter plate second step can be done directly in the coated Elisa plate.

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9. Test protocol **quantitative**

Before starting this test read "**preparations**"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C. and use them within 10 days.

   Wash microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Add **100µl** of **inactivated Feline Panleukopenia Virus antigen** to all wells of the coated microtiter strips to be used.
3. Seal and incubate 75 min. at 37°C.

4. Reconstitute directly before use the **positive control** (purple cap) **in 0,5 ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.
5. Reconstitute directly before use the **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

6. Make a pre-dilution of the **positive control** (purple cap) **in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 90µl ELISA buffer to **well 1A** and add 10µl of the positive control to **well 1A** and mix well.
7. Make a pre-dilution of the **negative control** (silver cap) **in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 90µl ELISA buffer to **well 1B** and add 10µl of the negative control to **well 1B** and mix well.
8. Make a pre-dilution of the **each sample in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 90µl ELISA buffer to **well 1C** and add 10µl of the sample to **well 1C** and mix well.
9. Take 2 wells as **substrate controls**, add only **120µl ELISA buffer** (green cap) to the wells.

10. Wash the strips incubated with antigen according to the wash protocol [see sub 6].

11. Add for dilution of the **positive control 135µl ELISA buffer** to **well 1A**. And 100µl buffer to the **wells 1B, 1C, 1D** of the coated microtiter strip.
12. Add for dilution of the **negative control 135µl ELISA buffer** to **well 1E**. And 100µl buffer to the **wells 1F, 1G, 1H** of the coated microtiter strip.
13. Add for dilution of **the samples 135µl ELISA buffer** to **well 2A and 2E** (depending on the number of samples). And 100µl buffer to the **wells 2B, 2C, 2D** and **wells 2F, 2G, 2H** of the coated microtiter strip.

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14. Make 3-step dilution of the **positive control** in the coated microtiter strip **starting 1:100 → 1:300→ 1:900 →1:2700.**
    Example: - Add 15µl positive control from step 6 to **well 1A** of the microtiter strip.
    - Mix well and transfer 50 µl to the well **1B**
    - Mix well and transfer 50µl to the well **1C**
    - Mix well and transfer 50µl to the well **1D**
    - Mix well and discard 50µl.
15. Make 3-step dilution of the **negative control** in the coated microtiter strip **starting 1:100 → 1:300→ 1:900 →1:2700.**
    Example: - Add 15µl negative control from step 7 to **well 1E** of the microtiter strip.
    - Mix well and transfer 50µl to the well **1F**
    - Mix well and transfer 50µl to the well **1G**
    - Mix well and transfer 50µl to the well **1H**
    - Mix well and discard 50µl.
16. Make 3-step dilution of **each sample** in the coated microtiter strip **starting 1:100 → 1:300→ 1:900 →1:2700.**
    Example: - Add 15µl of each sample from step 8 to the well **2A and/or 2E** of the microtiter strip.
    - Mix well and transfer 50 µl to the well **2B and/or 2F**
    - Mix well and transfer 50µl to the well **2C and/or 2G**
    - Mix well and transfer 50µl to the well **2D and/or 2H**
    - Mix well and discard 50µl.
17. Add **100µl** of the **substrate control** of step 9 to the last 2 wells of the microtiter strip.
18. Seal and incubate for 60 min at 37°C.

19. Wash the strips according to the wash protocol [see sub 6].
20. Add **100µl HRPO conjugated anti-species antibodies** to all wells.
21. Seal and incubate for 60 min at 37°C.

22. Wash the strips according to the wash protocol [see sub 6].
23. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking.
    Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.

24. Add **100µl substrate solution** to each well.
25. Incubate 10-20 min. in the dark (e.g. cover the wells with a sheet of paper).at room temperature (21°C.). Make sure the negative control does not become too dark.

26. Add **50µl stop solution** to each well; mix well.
27. Read the absorbency values immediately (within 10 min!) at 450 nm by using an ELISA reader. **Use the substrate controls as blank.**

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10. Precautions

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal, results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

11. Validation of the test

Qualitative:

- The results are valid if the following criteria are met:
  - The mean value (MV) of the measured OD value for the Positive Control (PC), diluted 1:100, must be ≥0.850.
  - The MV of the measured OD value for the Negative Control (NC), diluted 1:250, must be ≤0.400.

  In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

  Calculation
  Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

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

  $S/P = \frac{OD_{sample} - MVOD_{NC}}{MVOD_{PC} - MVOD_{NC}}$

Quantitative:

In order to confirm appropriate test conditions the OD of the positive control, diluted 1:100, should be ≥ 1.000 OD units (450nm).
The negative control, diluted 1:100, should be ≤0.400 OD units (450nm) and give an endpoint titer of ≤ 50.

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12. Interpretation of the test results

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/P ratio <0.22 is negative.
    - Specific antibodies to Panleukopenia virus could not be detected.
- A sample with the S/P ratio ≥0.22 is positive.
    - Specific antibodies to Panleukopenia virus were detected.

Quantitative: End point titre

- The ELISA titre can be calculated by constructing a curve and using cut-off line(dilution 1:100-300-900 -2700-8100 -24300 etc. total 8 dilutions of 3 steps) OD on Y-as and Titre on X-as
  Elisa titres can be calculated using as cut-off 2,5 times OD value of negative control at 1:100.

13. Symbols used with EVL ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructionsforuse	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	Forresearchuseonly	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Containsufficient for <n> tests/	Ausreichendfür "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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