Jurnal Sain Veteriner, Vol. 42. No. 1. April 2024, Hal. 121-128
DOI: 10.22146/jsv.79590
ISSN 0126-0421 (Print), ISSN 2407-3733 (Online)
Tersedia online di https://jurnal.ugm.ac.id/jsv

Diagnosis of Feline Panleukopenia Based on Clinical Signs and Polymerase Chain Reaction in Various Ages of Cats

Diagnosis Feline Panleukopenia berdasarkan Gejala Klinis Polymerase Chain Reaction pada Kucing berbagai Usia

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Naskah diterima: 25 Agustus 2023, direvisi: 30 November 2023, disetujui: 23 Februari 2024

Abstrak

Feline panleukopenia (FPL) adalah penyakit infeksi virus yang disebabkan oleh *feline panleukopenia virus* (FPV) yang menyerang kucing pada segala usia. Gejala klinis yang muncul pada setiap individu kucing sangat bervariasi, bergantung pada usia, status imun, dan ada tidaknya infeksi sekunder. Penelitian ini bertujuan untuk menentukan diagnosis FPL berdasarkan gejala klinis dan *polymerase chain reaction* (PCR) pada kucing berbagai usia. Penelitian ini menggunakan 15 ekor kucing yang menunjukkan salah satu gejala klinis antara lain letargi, anoreksia, demam, diare, dan muntah. Semua kucing diperiksa secara fisik dan PCR menggunakan sampel darah, kemudian dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa 10/15 (66,7%) kucing berusia <7 bulan, 4/15 (26,7%) berusia 7-12 bulan, dan 1/15 (6,6%) berusia >1 tahun. Identifikasi dengan PCR menunjukkan bahwa 100% sampel positif, sehingga semua kucing mengalami FPL. Gejala klinis FPL yang sering muncul dalam penelitian ini antara lain anoreksia (80%), demam (80%), muntah (73,3%), letargi (66,7%), dan diare (40%). Kucing muda <7 bulan umumnya menunjukkan gejala anoreksia, demam, muntah, dan letargi, kucing usia 7-12 bulan umumnya menunjukkan anoreksia, demam, diare dan muntah, dan kucing usia >12 bulan mengalami anoreksia dan muntah. Disimpulkan bahwa gejala klinis yang dominan pada kasus FPL pada kucing kelompok usia muda (<7 bulan) adalah demam, lesu, diikuti anoreksia, dan muntah, sedangkan pada kucing kelompok usia 7 bulan hingga dewasa, gejala anoreksia dan muntah lebih banyak terjadi. Gejala klinis dapat digunakan untuk skrining awal FPL, tetapi diagnosis penyebab perlu ditentukan dengan *polymerase chain reaction*.

Kata kunci: Feline panleukopenia virus; gejala klinis; PCR; usia

Abstract

Feline panleukopenia (FPL) is a viral infectious disease caused by the feline panleukopenia virus (FPV) that affects cats of all ages. Clinical symptoms that appear in each individual cat vary greatly, depending on age, immune status, and the presence or absence of secondary infection. The aim of this research was to

diagnose the FPL based on clinical signs and polymerase chain reaction (PCR) in cat with various ages. This study used 15 cats that showed one of clinical symptoms including lethargy, anorexia, fever, diarrhea, and vomiting. All cats were examined physically and by PCR of blood, then analyzed descriptively. The results showed that 10/15 (66.7%) cats were <7 months, 4/15 (26.7%) were 7-12 months, and 1/15 (6.6%) was >1 year old. Identification by PCR showed that 100% of the samples positive, so that all of cats diagnosed FPL. Clinical signs that commonly appeared in this study included anorexia (80%), fever (80%), vomiting (73.3%), lethargy (66.7%), and diarrhea (40%). Young cats <7 months commonly showed anorexia, fever, vomiting, and lethargy, cats aged 7-12 months commonly showed anorexia, fever, vomiting, and diarrhea, cat aged >12 months experienced anorexia and vomiting. Concluded that the predominant clinical symptoms in cases of FPL in the young age group (<7 months) were fever, lethargy, followed by anorexia, and vomiting, whereas anorexia and vomiting were more common in the age group from 7 months to adult. Clinical symptoms can be used for initial screening of FPL, but the causative diagnosis needs to be determined by polymerase chain reaction.

Keywords: Age; clinical signs; feline panleukopenia virus; PCR

Introduction

The feline panleukopenia virus (FPV), which belongs to the *Parvoviridae* family, is the cause of feline panleukopenia (FPL) infection (Abd-Eldaim *et al*., 2009; Mosallanejad *et al*., 2009; Decaro *et al*., 2010). Feline panleukopenia virus can be transmitted from one cat to another through direct or indirect contact by faecal-oral, transplacental, mechanical vector, and oral-nasal routes (Mosallanejad *et al*., 2009; Kruse *et al.,* 2010; Stuetzer and Hartmann, 2014; Awad *et al.,* 2019; Mahendra *et al.,* 2020). The impact of cat-to-cat illness transmission is significant and happens very quickly (Abd-Eldaim *et al*., 2009). This virus can infect other cats in the same environment because it can persist there for a long enough period of time (Pfankuche *et al.,* 2017).

Hematological examination, rapid immunochromatographic test for qualitative detection of antigens, polymerase chain reaction (PCR) for viral DNA detection, direct erythrocyte hemagglutination, hemagglutination-inhibition test to determine the presence of virus-specific antibodies, enzyme-linked immunosorbent assay (ELISA) for viral antigen detection, immunofluorescence antibodies, virus isolation, and monoclonal antibodies are some of the diagnostic methods that can be used to detect FPV (Mosallanejad *et al*., 2009; Raj and Haryanto, 2020). Polymerase chain reaction (Abd-Eldaim *et al*., 2009; Awad *et al*., 2018; Jacobson *et al*., 2021) and hemagglutination-inhibition (Mende *et al*., 2014; Miller *et al*., 2021) are the gold standards for the diagnosis of FPV patients.

All cat ages are affected by the virus, although cats under a year old are the most vulnerable (Kruse *et al.,* 2010). The virus will predominantly target cells that are actively dividing, such as lymphoid tissue, bone marrow, and the crypts of the small intestine epithelium (Awad *et al.,* 2018). Studies done in Iran, Germany, Egypt, and Indonesia showed a higher prevalence of infection in cats less than six months old compared to those over six months old (Mosallanejad *et al*., 2009; Kruse *et al.,* 2010; Awad *et al.,* 2018; Purnamaningsih *et al.,* 2020). In cats up to 12 months old, the highest morbidity and mortality rates were recorded. The morbidity rate is 100% (Awad *et al.,* 2019), while the mortality rate is 25-90% in acute infections, and 100% in peracute infections (Kruse *et al.,* 2010). The higher incidence of infection in young cats is associated with rapidly dividing active cells and the absence of an active antibody response against the virus (Larry and Francis, 2011).

Clinical signs that appear in cases of FPL are very diverse, and the clarity of clinical signs that appear depends on age, immune status, and the presence or absence of the secondary infection. Clinical illness ranges from subacute infection to peracute infection with sudden death (Miller *et al*., 2021). Early signs include the presence of leukopenia, fever, lethargy, anorexia,

and dehydration (Kruse *et al.,* 2010), later manifesting into severe leukopenia, vomiting, diarrhea, to severe depression (Abd-Eldaim *et al*., 2009; Awad *et al.,* 2018). Infections in fetuses and neonates cause clinical signs in the form of ataxia and eye disorders (Zhang *et al.,* 2019), whereas infections in cats older than 4-6 weeks of age mainly cause gastrointestinal disturbances and leukopenia (Abd-Eldaim *et al*., 2009). Infection in adult cats causes clinical signs such as fever, lethargy, vomiting, diarrhea to dehydration (Kruse *et al.,* 2010; Jacobson *et al*., 2021).

Field challenges that frequently take the form of delays in case handling, leading to a high mortality rate, particularly in young animals. Clinical signs can be used as an initial screening for disease diagnosis since they are information that can be instantly collected when the animal is examined. It is also the fastest and simplest method of diagnosis. As a result, a retrospective study of the clinical signs of infected cats at different ages is required. This study's objective was to assess the clinical signs of FPV-infected cats at different ages. The findings of this study are expected to give a general overview of laboratory examination, diagnosis, and treatment for FPV-infected cats.

Materials and methods

This research has received ethical approval from the Ethical Clearance Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada with number 033/EC-FKH/ Int./2022, so that the owner knows and gives permission when sampling is carried out. Fifteen cats of various sexes and ages were used in this study. Cats are then grouped into young cats (aged <7 months), cats aged 7–12 months, and adult cats aged (>12 months). Cats are patients treated at several veterinary clinics in Yogyakarta, Indonesia. The cat was physically examined to detect clinical symptoms of feline panleukopenia, such as anorexia, lethargy, dehydration, fever (>39,5^{o}C), vomiting, diarrhea, stomatitis, halitosis, hypersalivation, abdominal pain, lacrimation, conjunctivitis, eye lesions, epistaxis, otitis, ataxia and abortion. Blood samples from cats exhibiting clinical signs of FPL were then collected for PCR analysis. The primers used in the study were Forward 5'-CATACATGGCAAACAAATAGAGCA-3' and Reverse 5'-TGTTTTAAATGGCCCTTG TGTAGA-3' (Zhang *et al.,* 2019). The DNA electrophoresis process was carried out on a 2% agarose gel, and the electrophoresis process was run for 45 minutes at 70 volts. The gel results were then visualized on a UV transilluminator. The results were then analyzed descriptively.

Results and discussion

The results of FPV molecular identification using PCR with blood samples showed that all of patients tested positive for the virus. Electrophoresis results showed that all samples DNA amplification represented DNA fragmentation, with a product size of 237 bp (Figure 1). The intensity of the DNA bands in this study was quite varied; samples D5, D6, and D7 had the highest band intensities, while samples D8 and D9 had the lowest band intensities. The different band intensities indicate the DNA concentration in the sample. The greater the number of viruses detected, the clearer the color intensity of the band image will be. The subjectivity of the band appearance will affect the interpretation of the results, especially in samples with small amounts of virus, so that false negative results may be found. Walter-Weingartner *et al.* (2021) stated that the lower the number of virus copies per gram of feces, the lower the probability of detection. The correlation between false-negative results and low viral load was proven in the study.

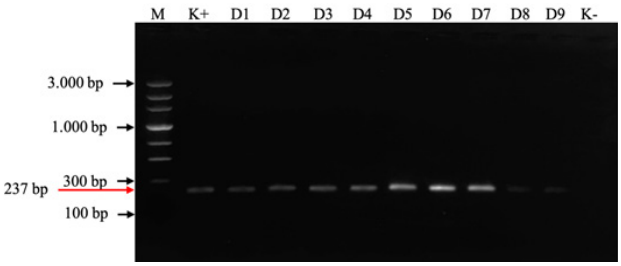

Figure 1. PCR-amplified DNA analysis on an agarose gel using blood samples. M: marker, K+ and K-: positive and negative controls, and a sample range of D1 to D9. All wells except the K- wells showed DNA bands. The DNA bands that manifested had a size of 237 bp, as measured by M wells with a range of 100–3,000 bp.

PCR examination is a sensitive test method for Protoparvovirus, which has been

used as a diagnostic standard reference for FPV examination. Conventional PCR methods as used in this study, have been widely used to diagnose infectious disease causative agents (Awad *et al.,* 2018; Raj and Haryanto, 2020; Jacobson *et al*., 2021). The PCR method is able to detect a lower number of viruses, so that infections in the early and late stages can be detected with this method (Sykes, 2014).

The age of the cats in this study was quite varied, including 10/15 (66.7%) were cats less than seven months old, 4/15 (26.7%) were 7-12 months old and 1/15 (6.6%) were adult cat or older than 1 year (Table 1). These results are in agreement with several studies that have previously reported that the majority of infected cats were under 6 months old (Mosallanejad *et al*., 2009; Kruse *et al.,* 2010; Awad *et al.,* 2018; Purnamaningsih *et al.,* 2020).

Table 1. Age of cats infected with FPV (n=15)

Age (months)	Quantity (head)	Percentage (%)
<7	10	66.7
7-12	4	26.7
>12	1	6.6
Total	15	100

Due to the fact that this virus will mostly replicate in tissues with active cells that are undergoing rapid division, namely cells that are in the S-phase of the cell cycle, the condition of young cats with FPV is likely (Jakel *et al.,* 2012). The pathogenesis of this viral infection is influenced by the need for viral DNA replication when cells undergo mitosis, but only in lymphoid cells, intestinal epithelial cells and bone marrow cells (Awad *et al.,* 2018). As a result, this virus will primarily infect young cats between three and six months old (Arslan *et al.,* 2021). Cats at that age are still undergoing active cell development, making them more prone to contracting FPV.

Adult cats have better immunity than young cats, especially cats that have received vaccinations and cats that have acquired immunity from previous infections, which may be acquired from the environment during their development. Cats without adequate antibodies to FPV will find it difficult to fight off viral infections and tend to be fatal, causing death (Jacobson *et al*., 2021). Maternal derived antibodies (MDA) is a type of passive immunity that is transmitted from mother to kitten during the pregnancy phase and during lactation through colostrum (Larry and Francis, 2011). Generally will provide protection against disease for at least 12 weeks or longer in some cats (Mosallanejad *et al*., 2009; Jakel *et al.,* 2012). The period between the loss of passive immunity and the ability to respond to vaccination with active immunity is said to be the critical phase or immunological gap (Zenad and Radhy, 2020), during which the incidence of FPV infection is quite high.

The clinical symptoms that appeared in this study are shown in Table 2, including anorexia (80%), fever (80%), vomiting (73%), lethargy (67%), diarrhea (40%), dehydration (20%), halitosis (20%), rhinitis (13%), hypersalivation (7%), hemorrhagic diarrhea (7%), anemia (7%), stomatitis (7%) and otitis (7%). These clinical symptoms are as reported before, showing clinical symptoms of lethargy, anorexia, thirst, dehydration, vomiting, fever, diarrhea, accompanied by nerve symptoms, oral lesions and eye lesions (Litster and Benjanirut, 2014; Awad *et al.,* 2018) in the results of a study on clinical examination of cats infected with FPV.

The relationship between the main clinical symptoms in this study and the age of the infected cats is summarized in Table 3. The main clinical symptoms in this study included anorexia, fever, vomiting, lethargy and diarrhea. The most common clinical symptoms experienced

Table 2. Clinical signs of FPV-infected cats (n=15).

Clinical signs	Quantity (head)	Percentage (%)
Anorexia	12	80
Fever	12	80
Vomiting	11	73
Lethargy	10	67
Diarrhea	6	40
Dehydration	3	20
Halitosis	3	20
Rhinitis	2	13
Hypersalivation	1	7
Hemorrhagic diarrhea	1	7
Anemia	1	7
Stomatitis	1	7
Otitis	1	7

Table 3. Number of cats infected with FPV based on clinical signs and age.

Age (months)	Anorexia	Fever	Vomiting	Lethargy	Diarrhea
<7 (n= 10)	7	9	7	8	3
7-12 (n= 4)	4	3	3	2	3
>12 (n= 1)	1	0	1	0	0
Total	12	12	11	10	6

by young cats <7 months of age were anorexia, fever, vomiting and lethargy, while those of 7-12 months old cats were anorexia, vomiting, fever and diarrhea. Adult cat in this study showed symptoms of anorexia and vomiting.

Anorexia and lethargy are the earliest common symptoms found in almost all patients. Animals that experience discomfort in their bodies tend to look lethargic, decrease their level of activity and appetite. Pain and stress can reduce appetite and cause anorexia (Zhang *et al.,* 2019). Study before reported the same thing in their research, namely anorexia and lethargy were the most common early symptoms of FPV infection, both in young and adult cats (Levy *et al.,* 2015; Awad *et al.,* 2018). Lethargy can be associated with the effects of decreased bone marrow cell count, anorexia, and fever (Truyen *et al.,* 2009). Cats infected with FPL will experience a decrease in the number of bone marrow cells because most of these viruses will replicate in tissues with cells that are actively dividing rapidly, one of which is the bone marrow. Young cats (<6 months) have more actively dividing cells compared to older cats. The relatively high incidence of lethargy in young cats can be caused by a decrease in the number of bone marrow cells, which is more likely due to viral replication, although further studies need to be carried out on the number of bone marrow cells in the age group of cats infected with FPL.

The results of body temperature measurements in this study showed that 80% of patients had records of body temperature >39.5^{o}C, with details of 9 cats <7 months old and 3 cats 7-12 months old. The mechanism of fever is associated with the body's response to several things, namely infection, inflammation, neoplasia, exercise conditions, stress and the body's response to heat. The body's response in the form of fever at the time of infection is usually beneficial, because the immunological mechanism will be accelerated. An increase in body temperature will also harm some microorganisms, but if the febrile condition is maintained for a long time, it will have a detrimental impact (Graneto, 2010; Batchelor *et al.,* 2013).

Fever was the most common clinical symptom in this study. Some studies showed similar results, namely that the majority of cats in their study had fever (Litster and Benjanirut, 2014; Awad *et al.,* 2018; Raj and Haryanto, 2020). Younger children experience higher and longer fevers than adults (Batchelor *et al.,* 2013). Fever condition accompanied by abnormal leukocyte count, caused by microorganisms, both bacteria and viruses. FPV infection is characterized by an increase in body temperature which is accompanied by a decrease in the number of leukocytes (Jakel *et al.,* 2012). Hypothermia also occasionally occurs in the later stages of FPV disease (Mosallanejad *et al*., 2009). This condition is in accordance with the results in this study, so that fever can be used as the main parameter of FPV infection at the initial examination, followed by other physical examinations and other supporting examinations.

Vomiting is a complex reflex that involves the digestive system, respiration, abdominal muscles and changes in posture. Vomiting can be triggered by peripheral stimuli such as afferent nerves from the gastrointestinal tract or other visceral organs, as well as central stimuli. The most common causes of vomiting include a response to food, the presence of infectious agents such as FPV, or acute vomiting of unknown cause, but which resolves on its own (Riya *et al.,* 2020). Stimuli in the vomiting center due to gastritis suffered by FPV patients are manifested as vomit with the color of bile, which is yellow or frothy (Riya *et al.,* 2020).

The clinical symptoms of vomiting were quite common in this study, as the research of (Litster and Benjanirut, 2014; Pfankuche *et al.,* 2017; Awad *et al.,* 2018; Raj and Haryanto, 2020), that vomiting is the main clinical symptom found in the study.

There were 6 (40%) patients who showed clinical symptoms in the form of diarrhea, one of the six patients had hemorrhagic diarrhea. Shortening of intestinal villi caused by viral replication in intestinal crypt epithelial cells accompanied by impaired enterocyte regeneration, causes malabsorption and increased permeability (Pfankuche *et al.,* 2017; Miller *et al*., 2021). Diarrhea in young cats is quite health threatening and can be fatal. Viral diarrhea is quite common in young cats, and FPV is the main cause of the virus (Stuetzer and Hartmann, 2014). Various studies (Abd-Eldaim *et al.,* 2009; Kruse *et al.,* 2010; Awad *et al.,* 2018; Jacobson *et al.,* 2021) mention that the main clinical symptoms of FPL infection include vomiting and diarrhea, while in this study, the incidence of diarrhea only occurred in 6 patients, 3 of which were cats in the young age group and the other 3 were cats aged >7 months to adult. When compared with other clinical symptoms, the incidence of diarrhea is quite low. This study proves that not all cases of FPL infection show symptoms of vomiting and diarrhea. Although not all cats in this study had diarrhea, the virus can still be spread through the feces of infected cats. Cats that do not experience vomiting and diarrhea are included in subclinical infections, so cats do not show significant clinical symptoms, although they are still capable of being a source of infection.

Lethargy, anorexia and fever are clinical signs with the highest percentage in cats aged <6 months and 6-12 months. Uncharacteristic clinical symptoms can be associated with four forms of infection in FPV, namely subacute, peracute, acute and perinatal. Subclinical infection can also occur in cases of FPV (Miller *et al*., 2021). Subacute clinical symptoms generally show only mild clinical features such as fever, lethargy and anorexia. This condition usually occurs in animals infected with the virus without co-infection from bacteria or other diseases.

Conclusion

The predominant clinical symptoms in cases of FPL in the young age group (<7 months) were fever, lethargy, followed by anorexia, and vomiting, whereas anorexia and vomiting were more common in the age group from 7 months to adult. Clinical symptoms can be used for initial screening of FPL, but the causative diagnosis needs to be determined by polymerase chain reaction.

Acknowledgments

The authors are grateful to Department of Internal Medicine, Faculty of Veterinary Medicine, Universitas Gadjah Mada and some veterinary clinics in Yogyakarta, Indonesia, for providing the data and lab resources that made this research possible. Thank to Universitas Gadjah Mada, Indonesia for funding the research through Penelitian Tesis Magister, Universitas Gadjah Mada, 2022 with contract number 1950/UN1/DITLIT/Dit-Lit/PT.01.03/2022.

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