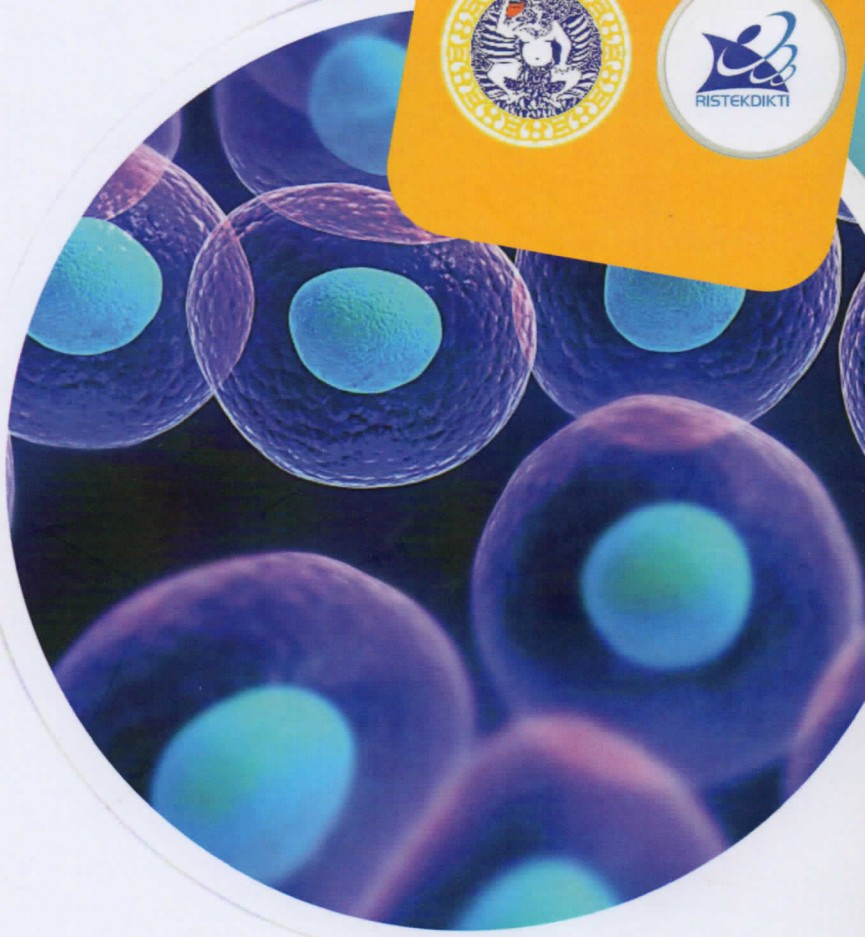
Internasional Seminar on

Molecular Biology in Veterinary Medicine



Host by:
Faculty of Veterinary Medicine
Universitas Airlangga

2 November 2016

Tandjung Adiwinata room, 2nd floor.
Faculty of Veterinary Medicine-Universitas Airlangga
Surabaya - Indonesia

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As Keynote Speaker

In The International Seminar
"Molecular Biology in Veterinary Medicine"

Surabaya, November 2nd 2016

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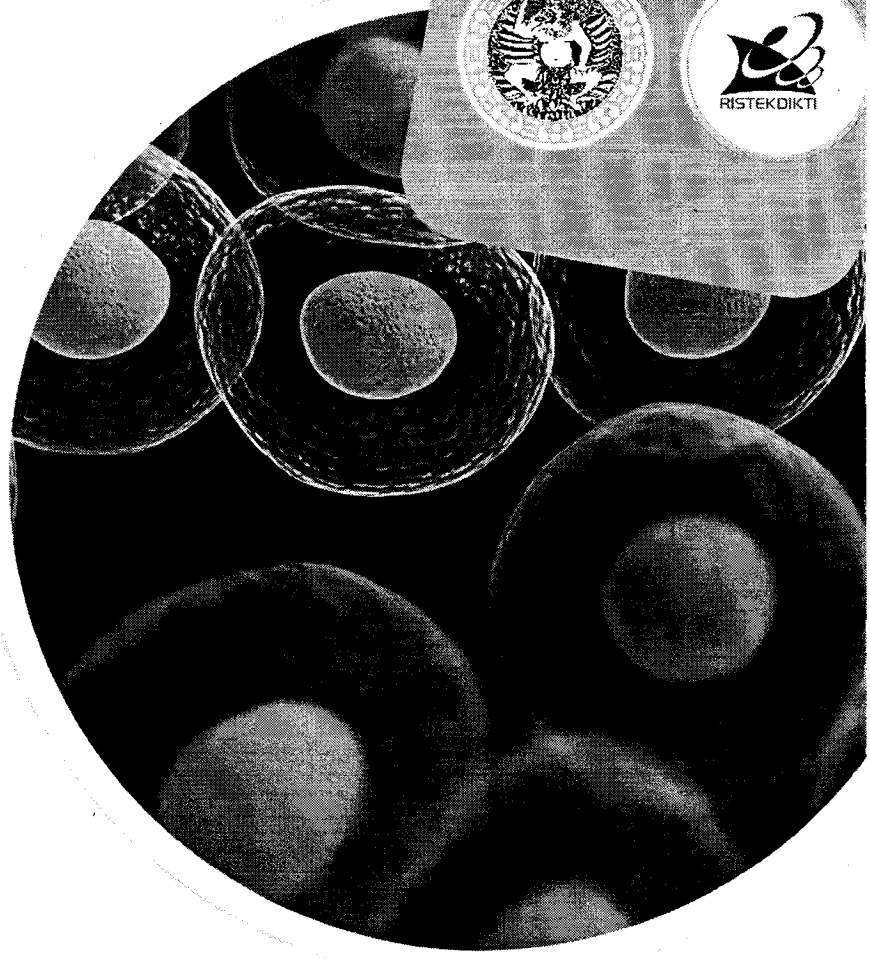

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CONTENTS:

A Challenge to The Diagnosis Feline Infectious Peritonitis Disease

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Abstract

Feline infectious Peritonitis (FIP) is a disease of cat with the high mortality rate. FIP caused by Feline Infectious Peritonitis Virus (FIPV) as a virulent biotype of Feline Corona Virus (FCoV). the essential prerequisites to the development of FIP lesions are a systemic infection with virulent FCoV (FIPV), FIPV replication in mononuclear cell, and activation of FIPV-infected mononuclear cell). There are two form of FIP namely dry (non effusive) form and wet (effusive) form. The clinical manifestation of FIP diseases will appear if there are a systemic infection of FIPV, replication of FIPV into monocytes, and activation of FIPV-infected monocytes. The obstacle diagnostic tool are due to the viruses responsible is thought to be a mutant strain with high variability and the genetic polymorphisms of the nonpathogenic feline enteric coronavirus. Treatments by supporting drugs just to slow down the coming death. Development of specific diagnostic method and therapy based on specific pattern of mutant viruses is a future prospect for animals with FIP disease.

Key words: Feline Infectious Peritonitis, Feline Coronavirus, Feline Infectious Peritonitis Virus, Feline Enteric Coronavirus Monocytes

Introduction

Feline infectious Peritonitis (FIP) is a disease caused by virulent Feline Corona Virus (FCoV) with immune-mediated pyogranulomatous vasculitis characteristic and often fatal consequence. FCoV can infect both wild and domestic felidae, Its cause often an infection of gastrointestinal tract (GIT) without clinical symptoms. The majority cat infected by FCoV shown no clinical symptoms, or only mild clinical symptoms of gastrointestinal and respiratory systems. Strains of FCoV that cause a mild intestinal disease in FIP is called Feline Enteric Coronavirus (FECV). The FECV would be mutated into the lethal FIPV in the affected cat and the specific mutations cause low-virulence FECV strains become high-virulence. All cats that have FIPV also have FECV, whereas not all cats that carry FECV develop FIP (Simons et al., 2005). Higher Antibodies titer against FCoV are found the animals living in catteries than the solitary cats; however only some less than 5 % of the seropositive cats eventually come down with FIP. The high seroprevalence obtained from no clinical symptoms infection cat caused by Avirulent FECV strains (Simons et al., 2005).

Patophysiology

Virus transmission via especially the fecal-oral route, an aerosol, contact with saliva or feces infected cats, share each other liters box, grooming are the important source of transmission or virus sheeding. A cat living in multiple household having greater risks infected by coronavirus and develop FIP, fomites

contamination (Food or water, personal equipment contaminated) are also sources of infection. Almost cats infected by FECV at 6–9 weeks of age (Hsieh and Burney, 2014). An animal become infected by ingesting FECV. Once ingested, FECV replicates in intestinal epithelial cells. FECV founded in tonsil and small intestinal tissues in 24 hours after ingestion. Most cats can eliminate the virus, and a few may become healthy carriers. The cecum, colon, mesenteric lymph nodes, and liver become infected within 2 weeks. FCoVs are primarily infect enterocytes and this animal will become persistently infected and continuously or intermittently shed virus with the feces without showing clinical symptoms. The animals appear remain healthy despite systemic infection, indicating that healthy (FECV) carriers play a key role in the transmission of FIP. FECV is spread via fecal shedding from the ileum, colon, and rectum. The same cat can obtain repeat infection with the same virus strains or with the different stains. The antibody titer found higher in the repeat infective cat then no repeat infection cat. Hence, colon is the main site of persistence FCoV, in this site the viral antigen has been found in differentiated enterocytes. The recurrent systemic spread occur when the virus was be present in other tissues and has been shown to infect tissue macrophages in the absence of viremia. An infected animal can develop FIP at any stage after initial viremia, even the virus is cleared from the intestine (Kipar et al., 2010). Hence the essential prerequisites to the development of FIP lesions are a systemic infection with virulent FCoV (FIPV), FIPV replication in monocytes, and activation of FIPV-infected monocytes. The Virulence increase of FCoV develop FIP are allegedly: 1) speed and sheeding virus replication fast enough 2) the increase of mutated virus in to the virulent FCoV biotype; and 3) both is not only once mutation is happening, some very simple changes on specific genes of FCoV that can provoque FIP manifestation (Kipar and Meli, 2014). FIP was actually caused by gravitational interactions among antibodies, viruses and the epithelial of various organs. compromised immune system allows cat infected by FIP. The closely related a cat to the FIP infection cat increase a risk to be infected.

Clinical Symptoms

The clinicical symptoms express by infected animal manifested in two forms, There are dry (non effusive) form and wet (effusive) form FIP. Dry form FIP occur when a partially protective cell-mediated immune response unable neutralize the virus. This dry form is characterized by multiple granulomas or pyogranulomas in various organs like intestines, liver lungs, kidneys, and central nervous system. fever, lethargy, anorexia, dehydration, weight loss, depression, ocular lesion neurologic signs , enlarged kidneys (uncommon), abnormality of ocular morpho-function likely changes of the iris , uveitis and the retina lesions, Signs of an organ failure especially kidney and liver, neurological changes (can include ataxia), changes in attitude and behavior. Wet form FIP occur when the cat able to produce humoral antibodies but fail to generate an effective cell-mediated immune response. In wet form FIP, the immune complexes aggregate in the vasculature and attract complement, causing a vasculitis and subsequent transudate. The wet for FIP is characterized by abdominal effusion with strands and mats of fibrin with multifocal serosal pyogranulomas, pleural effusion, hydrothorax and ascites caused by accumulation of protein fluid. Affected cats often have a combination of both the dray and wet forms, with one form predominating. The dry FIP form appears to be more common in older cats. Although clinically uncommon, both the wet form and the dry form can transform into the other (Pederson, 2014).

Diagnosis

The routine diagnosis of FIP performed by examination to the complete blood count, serum chemistry,

effusion fluid examination, titers in blood and effusion fluid. Measurement of Serum albumin/globulin ratio (Albumin: globulin ratio or Alb: Gl less than 0,8 indicate high probably FIP, and Alb : Gl ratio more than 0,8 likely negative FIP (Jeffrey et al., 2012) , Rivalta test on fluid, The Rivalta test is used in order to differentiate a transudate with an exudate.

histology/necropsy, the results obtain are often suspected FIP and must be confirm with IFA staining to observe if the FCoV found in macrophages. Measuremet of L-acid glycoprotein, Elevation of values of kidney or liver enzymes marker.

Histopathology examination to observe a lesion with perivascular granulomatous, pyogranulomatous vasculitis is until now is become a gold standard for diagnosing FIP (Giori et al., 2011). Examination with ultrasonography and radiography detect mesenteric lymphadenopathy, renomegaly with irregular renal contour, hypoechoic subcapsular echogenicity, Ascites, wall thickening, presence of a solitary mass at the ileocecocolic junction, include fungal granuloma, lymphoma, and adenocarcinoma. Although these examination sometimes showing no pathognomonic changes related with FIP disease, but normal abdominal feature cannot rule out FIP

Therapy

The way to prevent FIP disease to avoid FECV infection, cause there is no specific treatment that can prevent the progression of FIP once clinical signs arise, but a control of the immune-mediated vasculitis and reducing viral load are very crucial. Usage of anti-inflammatory and immunosuppressive drugs (the combinations of prednisolone and cyclophosphamide) to decrease the progression of inflammatory FIP lesions can make the animal more comfortable and may extend the survival time. Symptomatic drug is useful to treat coronavirus diarrhea. Giving A broad-spectrum antibiotics, adequate nutrition and fluid therapy, and high doses of ascorbic acid can be done. The stress condition should be controlled to minimize the chance of viral replication and mutation. The survival rate of cat with FIP disease is very poor hence the euthanasia of the suffering cat can be considered.

Molecular Biology-based Diagnostic for Feline Infectious Peritonitis.

There are many raisons that why development of molecular diagnosis or therapy for FIP disease is challenging. 1) The clinical signs can mimic many other systemic diseases; 2) The difficulties of diagnosis The virus responsible for causing FIP is thought to be a mutant strain of the nonpathogenic feline enteric coronavirus. The diagnosis has usually based on a high coronavirus antibody titers along with a strong clinical suspicion (based on review of history, clinical signs, and laboratory findings); 3) Until now, the specific diagnostic tests have been lacking and unable to differentiate between the less virulent FECV and the fatal FIPV biotypes of feline coronavirus; 4) there are 2 types of FCoV namely: type I (FIPV is the virulent FCoV biotype) is the most common cause of FIP and type II (FECV is the avirulent FCoV biotype). These types of viruses use the different cellular receptors and have different growth properties (Pederson, 2014); 5)The clinical manifestation of FIPV is caused by the host's immune response, hence FIPV is not usually transmitted between cats may the virus mutation can occur very quickly, and this mutation give impact with the development accelerated of FIP; 6) Weighs a consequence of medical decision taken by veterinarian and more accurate diagnosis is needed. If the FIP diagnosis perform based on the false positive result, so animals will be euthanized; If the FIP diagnosis FIP enforced based on the false negative result , the disease eradication will increase.

FIPV antibody titers test

There is no FIPV specific antibody titer tests. Most feline population is FCoV antibody positive, although high FCoV antibody titers can be found in healthy cats and never develop FIP. The measurement of FCoV antibody titers is useful to help narrow the probability of FIP, also to determine the possibility that a cat has been exposed to FCoV, and to maintain an animal free FCoV exposure. FCoV results need careful evaluation cause of FIP cannot be diagnosed by positive titer or ruled out by negative titer. Titers may drop terminally, because antibodies bind to large amounts of the virus, 10% of cats with FIP disease have negative titers (Meli et al, 2013). In the other hand, many healthy cats may have titers positive although never develop FIP and the examination results from different laboratories cannot be compared because they use often the different antigens.

ELISA test

The specific genetic ELISA tests to detect a 7b protein (to distinguish antibody to a particular protein epitope on the virus which has been developed (Greene, 2008). This protein is a one from the few specific protein for FIPV and lacking in enteric FCoV strains. Although there are more differences among this hypervariable protein between FIPV strains, FCoV strain and its FIPV mutant. Similarly, PCR has been used to differentiated between FCoV infection condition and mutant virus (FIPV during the FIP disease manifested). Its consider that the FCoV found in the systemic circulation represent FIPV mutant and its pathogenic.

Immunohistochemistry

Immunohistochemistry with Immunofluorecent staining (IFA staining) on effusion or tissue. IFA staining devoted to detects FCoV (Biotype I and II) antigen in cells, but FIPV is genetically difference from FECV. A genetic viral mutation occurs after infection causing the avirulent FECV to become virulent and present in the 3c gene but the tests based on viral genetics fail to distinguish FECV from FIPV consistently. FECV can spontaneously mutate in the enterocytes, changing the virus surface structure. The mutated virus then phagocytized and replicates in local macrophages and monocytes. The test have a 100 % positive predictive value if FCoV found in macrophages (Kipar and Meli, 2014).

PCR in blood, effusion fluid or feces

PCR test was developed to detect virus particles in whole blood based on the assumption that FIPV spreads via the bloodstream whereas feline enteric coronavirus remains in the intestinal epithelium. Unfortunately, the both healthy and FIP cats can show the positive blood tests. FIPV is able to replicate within mononuclear cells (monocytes and macrophages) within the blood, whereas FECV may be able to enter the bloodstream but is not able to replicate outside of the intestinal tract. The PCR can detect few viral genomic sequences that likely translocated from the portal circulation into the systemic circulation during enteric infection. RT-PCR on feces can indicate the shedding of FCoV. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) RT- PCR is Perform from blood, feces, effusions, and biopsy samples or fine-needle aspirates of affected organs. The genetic polymorphisms that convert FCoV to FIPV can diagnose ante-mortem FIP by PCR. a highly sensitive and specific real-time PCR assay for detection of sub genomic mRNA of the FCoV M gene (FIP matrix gene mRNA PCR), which identifies

replicating FCoV in extra-intestinal specimens that has characteristics specific as FIPV (Porter et al., 2014; Kipar et al., 2010; Gibson and Parry, 2007).

The other test has been done to detect the mutation in the 3c gene (as a virulent marker) and variable truncation of the 3c protein from feces and tissues samples of cats suffer with FIP disease. Almost all FECV carried an intact 3c gene and the FCoV in the feces of FIP cat generally also exhibited an intact 3c gene (indicate of FECV superinfection) and only in some cases also the mutated form. This indicated a role of 3c gene deletions in the viral switch from FECV to FIPV. Although 3c gene deletions in the FIP were not consistently observed suggested that additional factors are essential for the acquisition of the FIPV pathotype. But its can be interprete that a mutated 3c gene alone is not a distinctive characteristic of FIPVs, may FCoV need an intact 3c gene to be able replicate in the intestinal epithelium and be infective to other cats (Kipar and Meli, 2014). Hence evaluating this results compilate with clinical findings and other diagnostic tests are needed.

FIP Virus Real PCR Test on peritoneal fluid. If test is positive for FECV biotype, then FIP is unlikely. FIPV biotype indicates that the FCoV has mutated into the FIPV biotype. Since systemic antibodies are not protective, the disease progresses quickly, after pathogenic feline coronavirus infection. In a cat with clinical signs this supports the diagnosis of FIP.

Detecting active virus replication in the blood. The PCR test detects messenger RNA (mRNA) of the M gene of feline coronavirus. The M gene is a highly conserved viral gene that is only expressed during viral replication, The FIPV replicates in monocytes of peripheral blood and, thus, mRNA is produced, whereas FECV is incapable of replicating in peripheral blood, so there is no mRNA produced.

Recently, a conversion of FCoV to FIPV provoque by a mutations within the S1/S2 motif of the FCoV spike protein that reduce the ability of the cellular proteinase, furin, to cleave the spike protein. This mutation would alter the FCoV cell tropism towards monocyte/ macrophage infectivity, and thus convert FCoV to FIPV (Chang, 2010).

Conclusion

Feline Infectious Peritonitis (FIP) Disease is still a poorly understood fatal disease for for a cat. The diagnosis still perform based on the routine diagnosis confirm with some specific test. The development diagnostic methods still in progress especially in the molecular biology-based diagnostic. The PCR test availability and promise for a more accurate diagnosis of FIP, but the obstacle must be solved is the avaibility of the ease of use test instrument and applicative. There is still no specific and effective treatment that can prevent the progression of FIP disease. The survival rate of cat with FIP disease is very poor and the euthanasia suffering animals often considered.

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