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A Comparison between Immunofluorescence staining on Smears from *Membrana nictitans* (M3 Test), Immunohistopathology and Routine Pathology in Cats with Suspected Feline Infectious Peritonitis (FIP)

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Hök, K: A comparison between immunofluorescence staining on smears from *Membrana nictitans* (M3 test), immunohistopathology and routine pathology in cats with suspected feline infectious peritonitis (FIP). Acta vet. scand. 1991, 32, 171–176. – An indirect immunofluorescence method using smears from *membrana nictitans* (M3 test) to diagnose feline corona virus (FCV) infection was compared with immunohistopathology (using indirect immunofluorescence assay (IFFA) performed on organs (IFO)), and routine pathology (RP) in cats with suspected feline infectious peritonitis (FIP).
A close correlation between the 2 immunofluorescence methods (IFO and M3) was observed. Although the M3 test requires samples from only 1 organ per animal, both the sensitivity and specificity were high (80 %), when compared to IFO (using samples from an average of 5 organs per animal). In 21 % of the cats with suspected FIP typical pathological lesions were found. As the M3 test is relatively easy to perform, it could reduce work-load of pathology laboratories and provide valuable data for clinical and epidemiological use.

Membrana nictitans; feline corona virus; feline infectious peritonitis; FIP; indirect immunofluorescence assay.

Introduction

Feline infectious peritonitis virus (FIPV) infects both domestic and wild *Felidae* worldwide (*Robinson et al.* 1971, *Horzinek & Osterhaus* 1979, *Lutz et al.* 1986). The disease is caused by a coronavirus (*Ward* 1970) belonging to a group of antigenically related viruses (*Pedersen et al.* 1978). It has been suggested that the cat has 2 different viruses (feline infectious peritonitis virus (FIPV) and feline enteric corona virus (FECV)) within this group, that are indistinguishable by methods clinically used today (*Pedersen* 1987).

Diagnosis of FIP is carried out by evaluation of history, clinical signs, plus the results of supportive laboratory procedures (biopsy, serological, histopathological and immunohistopathological methods) (*Weiss & Scott* 1980, *Barlough* 1985).

In this paper FIP diagnosis is divided into 2 categories: feline corona virus (FCV) infection and the clinical disease FIP. FCV infection, which is diagnosed using the 2 indirect immunofluorescence methods, indicates the presence of FCV antigen. FIP is diagnosed by means of routine pathology, and is only

detected when typical lesions have developed.

This study was carried out to evaluate the immunofluorescence staining on smears from *membrana nictitans,* M3 test (*Hök* 1989), when compared with immunohistopathology and routine pathological methods in cats suspected of having FIP disease.

The M3 test like all other routine laboratory tests, can not distinguish between FIPV and the antigenically related FECV. However, unless FECV infects the *membrana nictitans,* which at this moment has not been investigated, the M3 test presumably is detecting FIPV.

Materials and methods

The routine pathology (RP) work was carried out in the Department of Pathology at the National Veterinary Institute, on cats obtained from all over Sweden. The cats were either euthanized because of poor health or dead due to unknown causes. Of these cats, 76 were suspected of having FIP and were selected for this study. The selection of these cats was based upon the evaluation of anamnesis (positive serology or FIP diagnosed earlier in the house/cattery) and gross pathological observations. Only cats with typical lesions were considered positive, although all cats had pathological lesions, these were not all specifically correlated with FIP.

Four cats served as negative controls (Ico: Fec Eur, Tif obtained from IFFA-CREDO, L'Arbresle, France). They were barrier-bred, health-monitored, serologically free from FCV antigen and clinically and histopathologically free from FIP. IFFA was performed on 16 organs from these cats. Organs, sampled for this study from the 76 cats suspected of having FIP, were selected by the pathologist, who carried out the autopsy, besides a smear taken from *membrana nictitans* from each animal for the M3 test (*Hök* 1989). An average of 5 organs per cat was sampled. The organs most frequently sampled were spleen, kidney, lung, liver, brain and mesenterial lymphnode. From each organ 2 samples were taken, 1 for routine histopathology and the other for IFO. The sample for IFO was stored at – 70°C for later cryosectioning. Smears from *membrana nictitans* and cryosections of the frozen organ samples were stained with IFFA using rabbit preimmunization sera and anti-FCV-rabbit sera (*Hök* 1989). A feline corona virus strain, obtained from a spontaneous case of effusive FIP, propagated in tissue culture was used for immunization. The virus was purified, both from the initial organ suspension of the diseased animal and after propagating on tissue culture, by means of gradient centrifugation and each time varified by EM (*Hök* 1989). Routine histopathological, M3 test and IFO investigations were all carried out in a blind fashion without prior knowledge of each test's results. The sensitivity (true-positives/true-positives + false-negatives) and specificity (true-negatives/true-negatives + false-positives) were calculated for each test.

Results

Using routine pathology (RP) 16 cats out of the 76 suspected cases revealed lesions typical of FIP. In these 16 cats FCV infection was demonstrated with both the immunofluorescence methods (IFO and M3 test. Fig. 1). IFO detected 75 of the 76 selected cats to have FCV antigen present in at least 1 organ (of an average of 5 organs sampled per cat) (Table 3). M3 test detected FCV antigen in 61 of these 75 FCV positive cats (using IFO). The only suspected cat which was negative for IFO was however positive in the M3 test, i.e. a total of 62 cats positive for M3 test out of the 76 suspected animals cho-

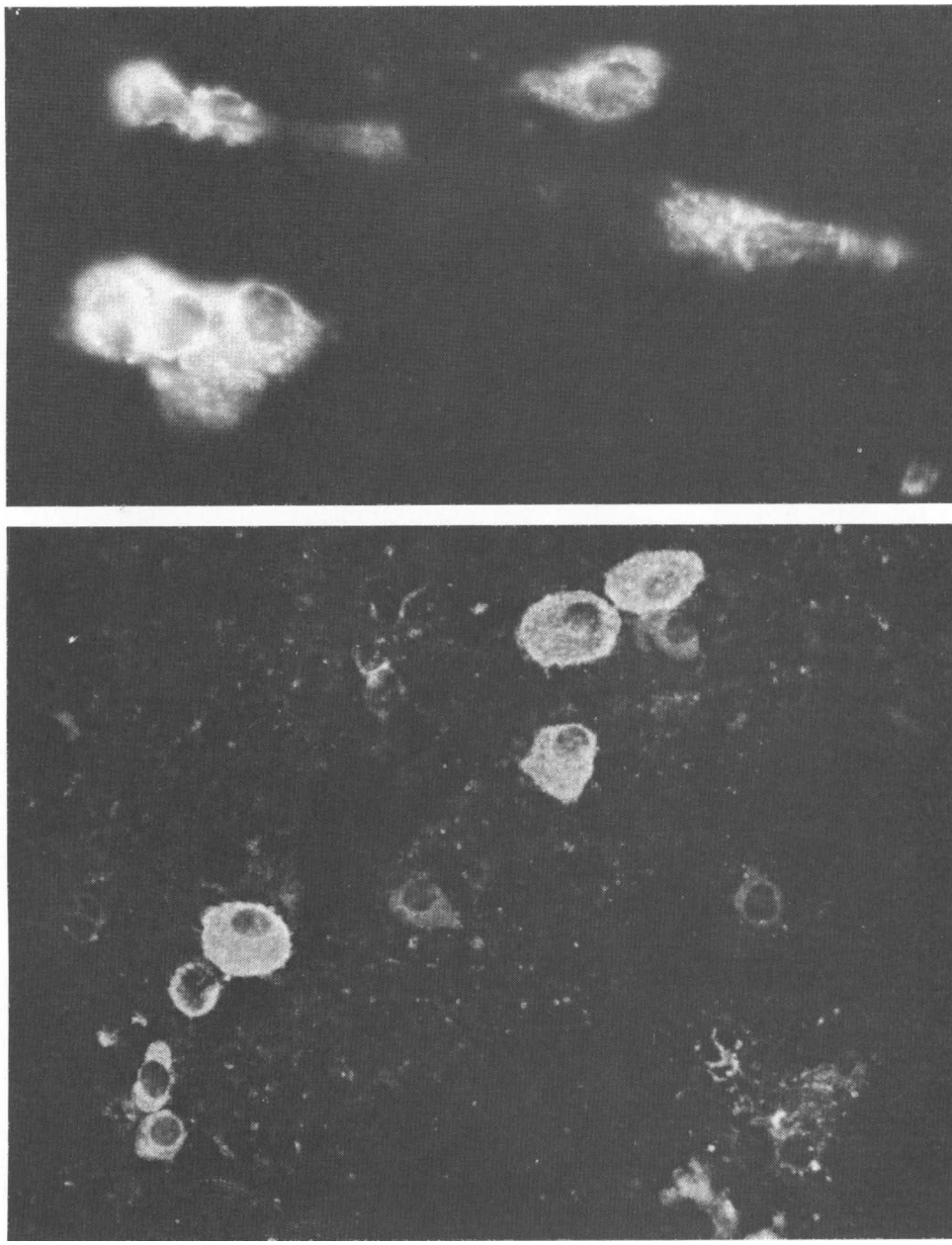

Figure 1. A: A positive smear from *membrana nictitans.* B: A positive smear from the lung. Immunofluorescence staining was carried out as outlined in the Methods section. Magnification 1200 ×.

Table 1. Immunofluorescence assay performed on *membrana nictitans* (M3 test) and on organs (IFO) compared with routine pathology (RP) as reference.

	RP+	RP–	Total	Sensitivity (%)	Specificity (%)
M3+	16	46	62		
M3–	0	18	18	100	28
Total	16	64	80		
IFO+	16	59	75		
IFO–	0	5	5	100	8
Total	16	64	80		

The 80 cats consist of 4 control cats and 76 cats suspected of having FIP.

sen (Tables 1 and 2). RP failed to pick out 60 of the FCV infected animals.

The results in Table 1 shows M3 test and IFO data in relation to RP data (reference method in this table). Both M3 test and IFO have a sensitivity of 100 %, but low specificity. When either IFO or M3 test (demonstrating the presence of the virus) was used as reference method and compared to RP

Table 2. Immunofluorescence assay performed on *membrana nictitans* (M3 test) and routine pathology (RP) compared with immunofluorescence assay performed on organs (IFO) as reference.

	IFO+	IFO–	Total	Sensitivity (%)	Specificity (%)
M3+	61	1	62		
M3–	14	4	18	81	80
Total	75	5	80		
RP+	16	0	16		
RP–	59	5	64	21	100
Total	75	5	80		

The 80 cats consist of 4 control cats and 76 cats suspected of having FIP.

Table 3. Immunofluorescence assay performed on organs (IFO) and routine pathology (RP) compared with immunofluorescence assay performed on *membrana nictitans* (M3 test) as reference.

	M3+	M3–	Total	Sensitivity (%)	Specificity (%)
IFO+	61	14	75		
IFO–	1	4	5	98	22
Total	62	18	80		
RP+	16	0	16		
RP–	46	18	64	26	100
Total	62	18	80		

The 80 cats consist of 4 control cats and 76 cats suspected of having FIP.

(displaying FIP) (Tables 2 and 3), a close correlation between the 2 immunofluorescence methods was observed. When the M3 test was compared with the IFO (Table 2) both the sensitivity and specificity reached 80 %. All 4 control cats were found to be negative with all 3 methods used.

Discussion

Routine pathological investigation has recently been the method of choice in diagnosing FIP. Immunohistopathological methods are now increasingly being used as they provide the best assessment to obtain a definitive diagnosis of FIPV infection (*Barlough* 1985). The most common immunohistochemical method employed is immunofluorescence performed on organ sections.

In an earlier paper, the M3 test was described and compared with serology (*Hök* 1989). In this study an assessment was made to find out how suitable the M3 test is in diagnosing FIP compared with routine histopathology and IFO. The 2 immunofluorescence methods (IFO and M3 test) provide evidence that the virus is present in the tissue, while RP demonstrates damages in the

tissue caused by virus and/or other agents. In this investigation, the RP method (detecting FIP) has been reliable with no false positives, but compared to the IFO (detecting the antigen) it missed out 59 FCV infected cats and 46 compared to the M3 test. When the 2 immunofluorescence methods were compared (Table 2), a sensitivity and a specificity of around 80 % was obtained. It must be noted that the M3 test here is compared with IFO performed on several organs, and only 1 positive organ is needed to classify a positive FCV infection. Because the IFO method has been used to screen several organs (on average 5) in each cat, the possibility of detecting a FCV positive animal is most likely higher compared to when 1 organ from each animal is used, i.e. the M3 test. The M3 test, however, detected 81 % of the FCV positive cats (using the IFO as reference test), the result indicating its practical value.

Information regarding the infection by FCV is vital for taking preventive measures to protect healthy cats from becoming infected. This can only be made possible if epidemiological screenings are carried out. Based on today's methodology this will involve a large amount of time and money. The M3 test with its simplicity could thus play an important role in epidemiological screening programs. It superceedes the IFO method in which a biopsy is required to obtain samples from live animals as the risk involved with biopsy sampling are eliminated when using the M3 test. The ease in sample preparation (no cryosection required) would reduce the work-load of many pathology laboratories. Samples are easily stored and can be sent to routine laboratories where the results obtained can be used for confirming diagnosis made during necropsies under field conditions. These merits together with those mentioned in a previous study and the advantage of the M3 test compared to the serological test (*Hök* 1989) would make the M3 test a valuable tool in routine investigation of FCV infection.

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References

Barlough JE: Cats, coronaviruses and coronavirus antibody tests. J. small. Anim. Pract. 1985, *26,* 353–362.

Horzinek MC, Osterhaus ADME: Feline infectious peritonitis: A worldwide serosurvey. Amer. J. vet. Res. 1979, *40,* 1487–1492.

Hök K: Demonstration of feline infectious peritonitis virus in conjunctival epithelial cells from cats. Acta Path. Microb. Immun. Scand. 1989, *97,* 820–824.

Lutz H. Hauser B, Horzinek MC: FELINFO No. 1. Feline infectious peritonitis (FIP) – the present state of knowledge. J. small Anim. Pract. 1986, *27,* 108–116.

Pedersen NC, Ward J, Mengeling WL: Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species. Arch. Virol. 1978, *58,* 45–53.

Pedersen NC: Coronavirus diseases. Diseases of the cat. Vol. 1. Ed. J Holtzworth. Philadelphia, W. B. Saunders 1987, 193–214.

Robinson RL, Holzworth J, Gilmore CE: Naturally occurring feline infectious peritonitis. Signs and clinical diagnosis. J. Amer. vet. med. Ass. 1971, *158,* 981–986.

Ward JM: Morphogenesis of a virus in cats with experimental feline infectious peritonitis. Virology 1970, *41,* 191–194.

Weiss RC, Scott FW: Laboratory diagnosis of feline infectious peritonitis. Feline Pract. 1980, *10,* 16–22.

Sammanfattning

En jämförelse av immunofluorescensfärgade utstryk från Membrana nictitans (M3 test), immunohistopatologi och rutinpatologi på katter med misstänkt felin infektiös peritonit (FIP).

Katter, med FIP-sjukdom som misstänkt dödsorsak, undersöktes med användande av 3 metoder: utstryk från *membrana nictitans* infärgad för indirekt immunofluorescence (M3 test) för att påvisa kattcoronavirus- (FCV-) infektion, immunohistopatologi utförd på i genomsnitt 5 immunofluorescensfärgade organsnitt (IFO) och rutinpatologi (RP). De 2 immunofluorescensbaserade metoderna stämde väl överens, specificitet och sensitivitet cirka 80 %, trots att M3 testen är baserad på endast ett organ, medan endast ett positivt organ av i genomsnitt 5 undersökta krävdes för att ge ett positivt IFO-svar. Rutinpatologin bekräftade diagnosen i 21 % av fallen. M3 testen som är lätt att utföra, medför minskat arbete, samtidigt som den ger värdefull information vid klinisk, epidemiologisk och patologisk undersökning. Dessutom erhålls en säkrare diagnos genom att påvisa virus istället för antikroppar.

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