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Morbidity, Mortality and Coronavirus Antigen in Previously Coronavirus Free Kittens Placed in Two Catteries with Feline Infectious Peritonitis

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Hök, K.: Morbidity, mortality and coronavirus antigen in previously coronavirus free kittens placed in two catteries with feline infectious peritonitis. Acta vet. scand. 1993, 34, 203-210. – Serologically coronavirus free kittens were placed in 2 catteries with a history of feline infectious peritonitis (FIP), each cattery representing 1 of the 2 different predominant clinical characteristics of FIP - effusive and granulomatous. The kittens were clinically observed for 100 days. A 100% morbidity and a 90% mortality was observed. The first signs were observed after 14 and 27 days respectively. The clinical pattern of the disease was similar in all kittens and showed a pattern of recurrent periods of conjunctivitis, upper respiratory and gastrointestinal signs. Once developed, wasting and signs of CNS disturbances were consistent. The "effusive strain" had a 2 weeks earlier onset of signs and death, and a 40% outcome of effusive FIP. Mean survival times during the observation period were 57 ±26 and 57 ±16 (mean ±SD in days), respectively. The death rates were similar in both groups. Feline coronavirus (FCoV) antigen was immunohistochemically detected using indirect immunofluorescence and was present in all kittens and in 93% of the 5 investigated organs (lung, liver, spleen, kidney, and mesenteric lymph node).

FIP; feline corona-virus antigen.

Introduction

Feline infectious peritonitis (FIP) is a coronaviral disease causing a variety of signs in Felidae (*Neu & Pfeifer* 1984, *Ward* 1970, *Ward et al.* 1974). As the virus causing FIP can not be distinguished antigenically from feline enteric coronavirus, it will be referred to as feline coronavirus (FCoV) in this paper. However, when referring to publications where the authors have used the expression feline infectious peritonitis virus (FIPV), FIPV will be used.

Experimentally FCoV has been transmitted by several routes. Utilizing FIPV propagated in cell culture, to inoculate susceptible kittens, *Pedersen et al.* (1981) found that "intraperitoneal inoculation caused seroconversion and effusive peritonitis in 100% of the kittens. Intratracheal inoculation produced disease in 60% of the kittens, and oral inoculation produced disease in 20%". *Weiss & Scott* (1981) found a mortality of 83%, when exposing FIPV-seronegative kittens to an aerosol of an organ suspension prepared from a FIP diseased cat. Subsequently *Pedersen* (1989) expressed: "it is very difficult to induce FIP in susceptible cats by exposing them to cats that are clinically ill with the disease". In line with this statement *Weiss & Scott* (1981) claimed that "SPF kittens had naturally seroconverted during prolonged contact exposure to FIPV-

seropositive minimal-disease cats" and that "the seropositive kittens remained asymptomatic when they seroconverted". Thus there seems to be a contradiction in the development of FIP in the experimental infections and the cat to cat contact transmission.

In order to study the development of the disease, FCoV-seronegative kittens were exposed to asymptomatic FCoV-seropositive cats. Two groups of FCoV-seropositive cats were used in simultaneous experiments, each representing the effusive and granulomatous forms of FIP, respectively. The cats were observed for 100 days. After death/euthanasia they were screened for FCoV antigen in selected organs.

Materials and Methods

Animals

Twenty healthy coronavirus free kittens, 10 queens and 10 tomcats from 5 litters, barrier bred, 12 weeks old, of CIBA-GEIGY's Abyssinian type x European, from The National Veterinary Institute, Uppsala, Sweden, served as recipients. Initial antibody titres against FCoV antigen were below detectable values (<1:10) using a heterologous immunofluorescence assay (IFA), (The European Veterinary Laboratory, The Netherlands). They were also antigenically free from feline leukemiavirus, and serologically from calici and herpes virus (The National Institute, Sweden). Before leaving the breeding unit, they were vaccinated against feline panleukopenia (Nordpan Vet.®, NordVacc, Stockholm, Sweden).

Within each sex group, the kittens were randomly divided into 2 groups, and were placed, 5 of each sex, in 2 catteries 150 km apart.

Cattery A had a clinical history of 23 kittens out of 88 kittens born dying of effusive FIP. Cattery A housed Recipient Group A (RG-A). RG-A was kept in a room (12m^2) together with an asymptomatic breeding pair as the source of infection. At the start of the study, the antibody titres of the breeding pair against FCoV antigen were 1:300 and 1:2000 (IFA, The European Veterinary Laboratory, The Netherlands).

The RG-A and the breeding pair were housed in the same building but separate from the breeding nucleus of the cattery, that was asymptomatic but FCoV-seropositive. No case of FIP, or any other disease, occurred among the other cats in the cattery during the study.

Cattery B had lost 9 cats to granulomatous FIP over 7 months and housed Recipient Group B (RG-B). RG-B was kept together with all cats in the cattery (10 queens and 5 tomcats) that were seropositive - the range of the antibody titres of the breeding nucleus was 1:40 - 1:1280 (IFA, The European Veterinary Laboratory, The Netherlands) - and served as the source of infection. The cats were roaming free and had access to the owner's living quarters. After 1 month, during the first relapse, 1 cat in the cattery developed the same symptoms as the kittens and at the final relapse died, aged 6 months old, of granulomatous FIP. No other incidence of disease was recorded during the time of the study.

Both catteries were serologically negative for herpes and calicivirus and antigenically negative for feline leukemia virus, when tested before and after the observation period (The National Institute, Sweden).

Experimental design

The experiment was performed simultaneously in the 2 catteries from September through December. Neither light, temperature, nor humidity were standardized. All cats were kept indoors and fed a commercial canned food (Mjau, Tre Kök, Solna, Sweden) and tap-water ad lib.

When dead, cats were mailed for autopsy at a

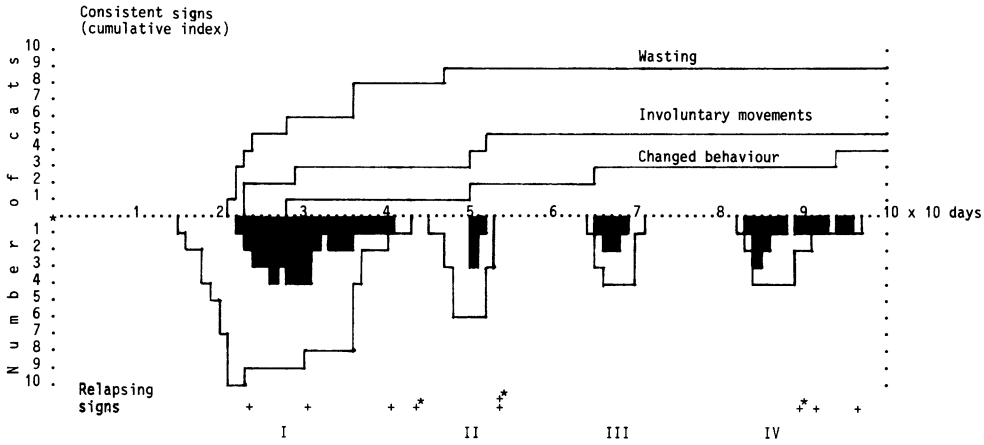

Figure 1 A. Recipient Group A, start of consistent signs are shown cumulatively over the time axis and recurrent signs (mainly conjunctivitis/upper respiratory signs) in actual numbers below. Inserted shadowed areas are numbers of cats with diarrhea. Crosses beneath mark times of death. Periods of signs are marked with Roman numbers. Numbers in the horizontal axis mark tenths. Asterisk marks effusive FIP.

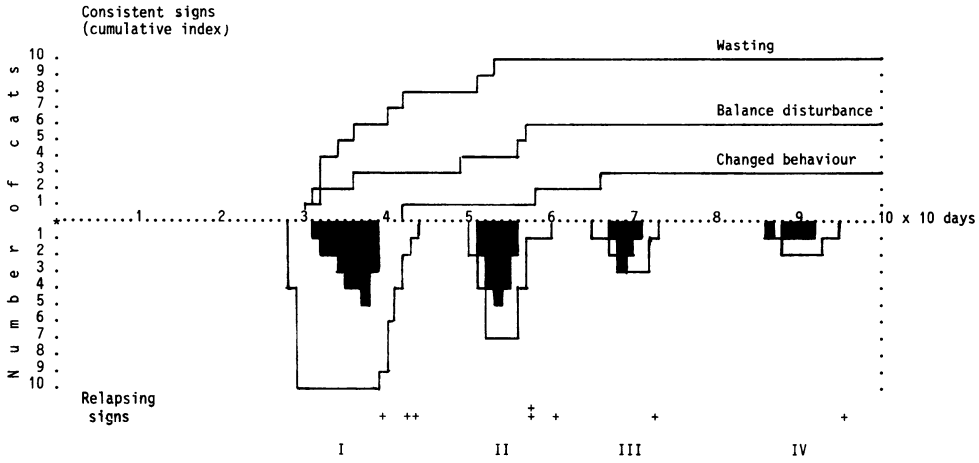

Figure 1 B. Recipient Group B, for further explanation see Fig.1 A.

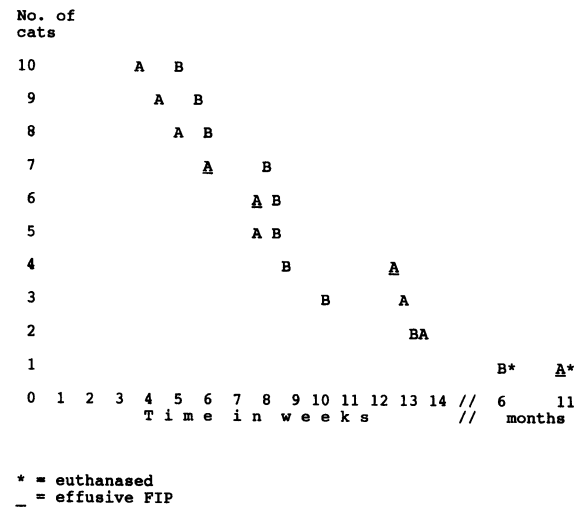

Figure 2. Survival time in the two Recipient Groups A and B during 100 days. Death date is marked with A and B for the two groups, respectively.

routine pathological laboratory. Samples for indirect immunofluorescence assay were collected from 5 organs, spleen, kidney, lung, liver, and mesenteric lymph node and were stored at -70°C for later cryosection.

Clinical picture

The clinical picture was followed for 100 days. The general appearance was registered daily by the cattery owners, who were instructed to report every alteration observed directly to the author by telephone. Temperature was measured when fever was suspected. A rectal temperature above 39.0°C was considered as fever. For practical reasons the kittens were not weighed. The catteries were visited frequently by the author.

Immunohistochemistry

Indirect immunofluorescence assay (IIFA) was used on sampled material as originally described by *Hök* (1989). Briefly, rabbits were immunized using a feline coronavirus strain that was obtained from a spontaneous case of effusive FIP, propagated in tissue culture, purified by means of gradient centrifugation and verified by EM. The cryosectioned samples were stained using rabbit pre-immune sera and anti-FCoV-rabbit sera, followed by an anti-rabbit-FITC-conjugated sera (Dacopatts A/S, Denmark). Fluorescing cytoplasma in cells were considered positive.

Results

Clinical observations

Morbidity was 100% in the recipients. The first sign observed was registered in RG-A after 14 days and in RG-B after 27 days, and all kittens were sick after 20 and 28 days, respectively.

Mortality

The first death was registered in RG-A after 22 days and in RG-B after 38 days (Fig.1 A and B, and Fig.2). In both groups there was a 90% mortality. Mean survival time (mean

Table 1. The average number of periods with signs that all 10 kittens in each group lived to experience during the study. Mean length in days as measured for the 3 relapses with symptoms common to all kittens; intervals between primary signs and relapses, and intervals between relapses. Mean lengths and standard errors in survival days as calculated for the 9 kittens in each group dying within 100 days.

Kittens	Average number periods/cat	Mean ±SD length in days		
		Periods	Intervals	Survival
Group A	2.4	4.3±0.8	13.3±0.9	56.7±26.2*
Group B	2.2	4.3±0.8	13.3±1.9	57.1±16.2*

*9 kittens.

±SD in days) in the kittens dying was for RG-A 57 ±26 (min 22 and max 96 days) and for RG-B 57 ±16 (min 38 and max 94 days). In both groups, 1 kitten survived the study and was euthanised 11 and 6 months, respectively, after first contact with the infection.

Periods with signs

The periods during which all kittens showed clinical signs were measured and considered as the duration of the period. Only the first period differed in length (15 and 10 days in RG-A and RG-B, respectively). The relapses were the same length in both groups (4,4, and 5 days). The onset and fading of the signs varied individually in all periods of signs and appeared at intervals of about 2 weeks (Table and Fig.1). For kittens dying in connection with the relapses, the signs lasted 7-11 days.

Recurring signs

During 4 periods of time, all kittens, that were still alive, displayed similar signs. They had conjunctivitis and upper respiratory signs. They looked sick and febrile, had a tufted, greasy fur, and slept more than normal, but were alert when awake.

Additionally some kittens had diarrhea, but the diarrhea was not consistent during the period of signs. (Fig.1). Regardless of signs, all kittens were reported to show an interest in food and water until they died. However, the exact food intake was not measured.

Consistent signs

The time for onset of consistent signs is demonstrated in Fig.1 A and B.

Wasting, starting as a sudden onset of a leanness over backbones and shoulders, occurred in all kittens except the one euthanised after 11 months.

CNS dependent signs were of 3 types.

Involuntary movements, such as head shakes and waving the front paw around the ear, were seen in 5 kittens in RG-A.

Balance disturbances were observed in 6 kittens in RG-B. They consisted of a loss of balance in the hind limbs for some sec and could be noticed several times a day.

Changed behaviour, in form of mental changes, were observed in 6 kittens - 3 in each group - of which 4 kittens became shy, and 2 kittens became aggressive.

Pathological observations

Emaciation was seen in all kittens except the one last euthanised. Effusive FIP (4/20, kittens with effusive FIP/all kittens in both

Table 2. Organs screened for FCoV antigen employing indirect immunofluorescence (IIFA), number of positive versus total number.

Organs	Recipient Group A	Recipient Group B
Spleen	10/10	9/10
Kidney	9/10	10/10
Lung	10/10	9/10
Liver	9/10	8/10
Mesenteric Lymph Node	8/9	9/9
Total in per cent	94	92

Table 3. Inflammatory changes of FIP-character in different organs from 20 cats divided in the 2 groups A and B.

Organs	Recipient Group A	Recipient Group B
Respiratory system	6	6
Digestive system	2	4
Occular system	4	4
Spleen	1	6
Liver	7	3
Kidney	3	0
Brain	6	2

groups) was only registered in RG-A, in kittens from 3 different litters, and spread in time from the first period till the last euthanised kitten (Fig. 1 A, Fig.2). Inflammatory changes of FIP-character in different organs are displayed in Table 3.

Immunohistochemistry

FCoV antigen was found in almost all investigated organs, 94% and 92% in RG-A and RG-B, respectively (Table 2). No kitten had FCoV antigen in less than 3 of the 5 organs investigated. FCoV antigen was found in all kittens in a similar pattern for each type of organ, regardless if the kitten died or was euthanised as in the case of the 2 survivors.

Discussion

A remarkable similarity was noticed between the 2 groups regarding the signs, pattern of disease, mean survival time, and death-rate (Table 1 and Fig.1). Thus the acute development of FIP was the same, irrespectively of the earlier observed disease pattern in the cattery - effusive or granulomatous. In main the signs were in accordance with other observations (*Addie & Jarrett* 1992, *Hayashi et al.* 1982, *Neu & Pfeifer* 1984).

Some of the signs observed, such as ocular and nasal discharge are also described during feline chlamydial infection. It differs clinically from FIP in starting as an unilateral conjunctivitis which persists if untreated (*Povey & Jarrett* 1984).

Balance disturbance has also been reported by several authors (*Doherty* 1971, *Kornegay* 1978, *Krieglieder & Geyer* 1984, *Neu & Pfeifer* 1984, *Pedersen* 1987) as well as a wavering gait (*Lutz et al.* 1986). The balance disturbances were only observed in RG-B, and differed from those seen in Sweden in a syndrome referred to as staggering disease ("vingelsjuka"), that is characterised by nonsuppurative meningoencephalomyelitis (*Kronevi et al.* 1974). Contrary to FIP, the majority of these cats have been FCoV-seronegative, with a stiff and staggering gait that progressed to posterior paresis, and an inability to withdraw the claws. (*Kronevi et al.* 1974, *Lundgren* 1992, *Ström* 1992).

The clinical character of FIP has been claimed to depend on the host's genetic variation, individual resistance, and immune status, as well as route of infection, and virus strain (*Pedersen et al.* 1984, *Pedersen & Floyd* 1985, *Pedersen* 1987, 1989). Regarding the congruency of the kittens, it can be assumed that factors other than a strain variation of the virus are of minor importance as the cause of the differences seen - the time for onset of symptoms and death, as well as the 40% outcome of effusive FIP.

Almost all organs (93%) investigated with IIFA were positive for FCoV antigen (Table 2). The result was in line with other investigations utilizing immunohistochemical techniques, where the initial spread of FCoV antigen has been demonstrated (*Stoddart et al.* 1988, *Weiss & Scott* 1981 a, 1981 b) as well as the terminal presence of FCoV antigen (*Hök* 1990,1991, *Walter* 1987, *Walter & Rudolph* 1988, 1989).

Conclusion

A similar disease pattern developed in 2 groups of seronegative cats, one exposed to the effusive form and the other to the granulomatous form of FIP. All cats developed conjunctival/respiratory signs. The small differences seen - a slightly earlier onset of signs and death, as well as a 40% outcome of effusive FIP in the first group - suggest that the diversity is caused by a strain variation of the virus rather than other factors.

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Sammanfattning

Sjuklighet, dødlighet och Coronavirus antigen i tidigare Coronavirusfria kattungar placerade i två katterier med flelin infektiös peritonit.

Serologiskt coronavirusfria kattungar, placerade i 2 katterier med tidigare förekomst av våt respektive torr felin infektiös peritonit (FIP).Under en observationstid av 100 dagar registrerades 100% morbiditet och 90% mortalitet. De första sjukdomssymptomen uppträdde efter 14 respektive 27 dagar. Sjukdomens kliniska mönster var likartat hos samtliga katter nämligen återkommande symptom på konjunktivit, respirationslidande och gastroenterit, medan avmagring och symptom från CNS kvarstod då de en gång uppträtt. Våt FIP sågs hos 4 katter och endast från katteriet som tidigare haft våt FIP, där även första sjukdomssymptom och död inträdde 2 veckor tidigare än i det andra katteriet. Genomsnittlig överlevnadstid var 57 ±26 dagar respektive 57 ±16. Felint coronavirus (FCoV) antigen påvisades immunohistokemiskt med indirekt immunofluorescens hos samtliga katter och till 93% i samtliga undersökta organ (lunga, lever, mjälte, njure och mesenteriallymfknuta).

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