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Placebo-controlled evaluation of a modified live virus vaccine against feline infectious peritonitis: safety and efficacy under field conditions

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A modified live virus vaccine against feline infectious peritonitis (FIP) was evaluated in a double blind, placebo-controlled field trial in two high-risk populations. The vaccine was found to be safe and efficacious in one population of cats that had low antibody titre against feline coronavirus (FCoV) at the time of vaccination. Although clinically healthy at the time of vaccination, retrospectively some vaccinees that later came down with FIP were found to be RT-PCR positive for FCoV in plasma and showed changes in blood parameters consistent with early stage of FIP. It is concluded that vaccination can protect cats with no or low FCoV antibody titres and that in some cats vaccine failure was probably due to pre-existing infection. © 1997 Elsevier Science Ltd.

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Feline infectious peritonitis (FIP) is a normally fatal disease of cats caused by infections with feline coronaviruses (FCoV) which are antigenically related to a respiratory coronavirus strain of man (HCoV 229E), transmissible gastro-enteritis virus (TGEV) of swine and canine coronaviruses^{1,2}. In Switzerland, infections with FCoV in domestic cats are widespread. About 80% of the cattery cats and 50% of all cats with access to outdoors were found to be seropositive³. Five to 12% of these develop lethal FIP⁴. Certain cat populations seem to be more susceptible to FIP. Young cats are especially prone: 54% of all FIP cases affected cats younger than 12 months of age and 70% cats younger than 4 years⁵. A genetic disposition in certain breeds and in cheetahs was described⁵⁻⁷, and cats living in multiple-cat-households such as catteries or cat shelters and cats with access to outdoors are more likely to get exposed to FCoV and develop FIP than animals from single-cat-households⁸.

Clinical signs include the effusive or the non-effusive, granulomatous form of FIP; both can also appear together. Characteristic laboratory findings in FIP

are anaemia, neutrophilia, lymphopenia, increase of total serum protein, hyperglobulinemia and decreased albumin⁵.

In 1981, a low virulent FCoV type, called feline enteric coronavirus (FECV), which caused only mild gastrointestinal and respiratory diseases mainly in kittens, was described⁹. Antibodies to these FECV and the virulent FIP-causing viruses (feline infectious peritonitis virus: FIPV) do crossreact. These authors formulated the hypothesis that most of these seropositive cats are actually infected with FECV and that FIPV is just a mutant of FECV which has the ability to infect macrophages. At this time, no molecular or immunological differences are known between FECV and FIPV which can explain the difference of virulence between these coronaviruses¹⁰. Therefore, it appears to be justified to generally designate them as FCoV and to consider every FCoV infection in cats as a potential risk^{4,11}.

Several observations point out the important role of the cell mediated immunity (CMI) in FIP pathogenesis¹²⁻¹⁶, but the detailed immune mechanisms for controlling FCoV infection remain unknown. Under experimental conditions humoral immunity does not lead to protection. On the contrary, after experimental FIP infection seropositive cats develop FIP after a much shorter incubation period than seronegative control cats¹⁷⁻¹⁹. This antibody dependent enhancement (ADE) is thought to occur when virus-antibody complexes are formed and bound to the Fc receptors of macrophages. Macrophages are then more efficiently infected by the Fc receptor-mediated endocytosis than by the virus alone²⁰⁻²².

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When we initiated this study, this modified live virus vaccine (Primucell FIP[®]) was already commercially available in the USA, but many questions concerning safety and efficacy under field conditions were still unanswered. The safety of the vaccine was confirmed under experimental and field conditions²³, but vaccinated cats showed ADE when challenged with a high dose of heterologous virus strain²⁴. The efficacy of the vaccine was assessed only under experimental conditions. With this trial the safety and efficacy of the vaccine was evaluate under field conditions in two high risk populations. A preliminary report has been presented at the FECV/FIPV-Workshop in Davis, CA in 1994 and published in the proceedings²⁵.

MATERIALS AND METHODS

Experimental design

The study was performed as a placebo-controlled double blind assay. Neither the investigators, nor the cat owners, knew which of two colour coded vials contained the vaccine. The code was not opened to the investigators, veterinarians and cat owners, until all cats terminated the 12 month observation period. Two populations with a high risk for FCoV infection and FIP were included in this trial. The first population consisted of 138 cats from 15 catteries with FIP problems. In all of these catteries, FIP cases had occurred in the last 18 months prior the beginning of this trial either in the cattery itself or in kittens which had been re-homed to new owners. We expected that some of these cats had been already exposed to FCoV. The second population consisted of 609 cats <12 months of age, which were vaccinated by veterinarians in Switzerland. As already mentioned, this age group is more susceptible to FIP than older cats^{5,26}. The cats of each population were further subdivided into two groups, vaccine and placebo, respectively, which were comparable regarding age, sex, breed and living conditions. Only clinically healthy cats older than 16 weeks of age were vaccinated and pregnant queens were excluded from the study. In week 0 and 3–4 weeks later the cats were vaccinated intranasally with either the coded vaccine or the placebo. After the vaccination the two coded groups were kept separately for 48 h to prevent spread of the vaccine virus to cats of the placebo group.

In both populations a blood sample was collected before vaccination (week 0) and tested for FeLV and FCoV-antibodies. In cattery cats only, haematology and clinical chemistry were done in week 0, 8 and 30 and in 20 cats each of the vaccinated and of the placebo group. CD4+/CD8+-T-cells were measured in week 0, 8 and 30. FIV-tests were carried out in week 0 in the cattery cats. Of sick cats, a blood sample was collected and haematology, clinical chemistry, FeLV and FCoV-antibodies were determined.

Vaccine

The modified live virus vaccine was developed by Gerber *et al.*²⁷ Briefly, FIPV-DF2 was attenuated in 99 cell culture passages on the Norden Laboratories Feline Kidney (NLFK) cell line. Passages 61–99 were propagated at 31°C. The 99th passage was exposed to ultraviolet irradiation. The vaccine has been shown to induce

Table 1 Characteristics of cats included in the vaccine study

Cattery cats	Total n=138	Vaccine group n=68	Placebo group n=70
<i>Age</i>			
16 weeks to 1 year	44 (31.9%)	21 (30.9%)	23 (32.9%)
1–4 years	65 (47.1%)	34 (50.0%)	31 (44.3%)
4–10 years	24 (17.4%)	11 (16.2%)	13 (18.6%)
10 years	5 (3.6%)	2 (2.9%)	3 (4.3%)
<i>Breed</i>			
Persian	67 (48.6%)	34 (50.0%)	33 (47.1%)
British Shorthair	24 (17.4%)	11 (16.2%)	13 (18.6%)
DSH and DLH ^a	20 (14.5%)	10 (14.7%)	10 (14.3%)
Others	27 (19.6%)	13 (19.1%)	14 (20%)
<i>Sex</i>			
Queen	86 (62.3%)	41 (60.3%)	45 (64.3%)
Tom Cat	26 (18.8%)	12 (17.6%)	14 (20.0%)
Neutered	26 (18.8%)	15 (22.1%)	11 (15.7%)
<i>Young pet cats</i>			
Total n=609		Vaccine group n=300	Placebo group n=309
<i>Age</i>			
Median	23 weeks	22 weeks	23 weeks
<i>Breed</i>			
DSH and DLH ^a	466 (76.5%)	231 (77.0%)	235 (76.1%)
Pure-bred	143 (23.5%)	69 (23.0%)	74 (23.9%)
<i>Sex</i>			
Female	290 (47.6%)	144 (47.2%)	146 (48.0%)
Male	315 (51.7%)	154 (52.2%)	161 (51.3%)
<i>Living conditions</i>			
Single cat	172 (28.2%)	79 (26.3%)	93 (30.1%)
Free roaming	313 (51.4%)	151 (50.3)	162 (52.4%)

^aDomestic short hair, domestic long hair

IgA antibodies in the mucosa and to stimulate the cell mediated immune response²⁸.

The serial of the vaccine used in this study was a commercial batch (serial number 54851020) with a titre of 10^{6.2} TCID₅₀. The placebo consisted of supernatant of non-infected NLFK cell culture. The vaccine and placebo were provided by the manufacturer in identical vials coded with coloured labels. The code was not broken to the veterinarians and the cat owners until all cats had finished the 12 months observation period.

Animals

The characteristics of the cattery cats and young pet cats are summarized in *Table 1*. Animals of the placebo and the vaccine groups in both, the cattery cats and the young pet cats, did not differ significantly with respect to age, sex, breed and living conditions.

Diagnosis of FIP

Final diagnosis of FIP was done by necropsy which was performed by the Department of Veterinary Pathology at the University of Zurich. Macroscopic and histopathologic examinations were performed to diagnose FIP.

Laboratory parameters

Haematology. Total white blood cell count, red blood cell count and haemoglobin were determined

using an electronic cell counter (Autolyzer 820, ALV AG, 8200 Schaffhausen, Switzerland). Determinations of haematocrit, fibrinogen, plasmaprotein and white blood cell differentiation were performed by standard techniques.

Clinical chemistry

The following parameters were determined using a Cobas Mira analyser (Cobas Mira, Roche Diagnostica, Basel, Switzerland) under conditions defined by the International Federation of Clinical Chemists: AP, AST, ALT, bilirubin, urea, creatinin, calcium, potassium, sodium, phosphor, albumin, protein, cholesterol, and glucose.

Antibody titres to FCoV were measured by indirect immunofluorescence using PD-5 cells of swine origin infected with TGEV as antigen. Plasma dilutions of 1:25, 1:100, 1:400 and 1:1600 were tested. Plasma samples of all cats were examined for circulating feline leukemia virus (FeLV) p27 antigen²⁹ and plasma samples of the cattery cats were also examined for antibodies to feline immunodeficiency virus (FIV) by indirect immunofluorescence using FIV-infected FL-4 cells as antigen³⁰. Samples with positive fluorescence results were subjected to Western blotting for confirmation³¹. In 20 cattery cats CD4+/CD8+-T-cells were measured by flow cytometry as described³². Of all cats dying of FIP, 100 µl of plasma samples taken at the time of first vaccination were retrospectively examined for presence of FCoV-RNA by polymerase chain reaction (PCR)³³.

Statistical methods

The mean of the haematological and clinical chemistry parameters between the vaccine and placebo group were analysed for significant differences by the Mann-Whitney U test, changes of laboratory values obtained from different cats over time were examined by the Wilcoxon test. Frequencies of FCoV antibody titres in the placebo and vaccine group were compared using the χ^2 test. To determine differences in the frequencies of FIP in the vaccine and placebo group, the exact test of Fisher was performed³⁴.

RESULTS

The results are presented separately for the cattery cats and the population of the young pet cats.

Cattery cats

The side-effects reported after the vaccination in the cattery cats are summarized in *Table 2*. During the 12–21 months of observation, 13 cats of the vaccine group and 11 of the placebo group died due to various causes. Five cats of the vaccine group and six cats of the placebo group died due to non FIP-related causes. All cases, except one cat of the vaccine group which died 14 months after the vaccination with liver problems and two cats of the placebo group which died 12 and 22 months after vaccination due to an accident and joint problems in a 14-year-old cat, respectively, were submitted to necropsy and FIP was excluded.

FIP cases occurred in six catteries. The characteristics of all cattery cats which died of FIP are summarized in

Table 2 Side-effects reported after vaccination

Cattery cats (n=138)	Vaccine group n=68	Placebo group n=70
Fatigue for 1–2 days	5	4
Diarrhoea after second vaccination	1	1

Young pet cats (n=609)	Vaccine group n=300	Placebo group n=309
Total No. of reported side-effects	18	18
Sneezing	8	5
Fatigue for 1–2 days	5	8
Diarrhoea and/or vomiting	4	4
Others	1	1

Table 3. Some of these cats, though clinically healthy, showed changes in blood parameters at the time of vaccination.

To our knowledge, the safety of the vaccine in breeding cats has not been investigated so far neither under experimental nor under field conditions. Therefore, all data collected from queens which had kittens after the vaccination are summarized in *Table 4*. No differences were found between the parameters evaluated.

With respect to the laboratory parameters no differences were found between those in the vaccine group and the placebo group at the different time points (haematology, clinical chemistry, CD4+/CD8+ lymphocytes). However, in both the vaccine and placebo groups, changes in some of the laboratory parameters were observed at the different time points. Both groups showed a decrease in albumin in weeks 8 and 30 compared to week 0 and an increase in plasmaprotein in week 30 compared to weeks 0 and 8 ($P<0.05$) (data not shown).

At the beginning of this trial, all cattery cats had tested negative for FeLV and FIV-antibodies, but 98.6% and 95.6% of the cats in the vaccine and placebo group showed FCoV antibody titres of 25 or higher. The frequency of the FCoV titres in cats of the vaccine and placebo group at different time points after vaccination (weeks 0, 8 and 30) is shown in *Figure 1*. There was no statistically significant difference in the distribution of the FCoV antibody titre in the vaccine and placebo group at the different time point, but the vaccine group as well as the placebo group showed a transient increase of titres in week 8 compared to week 0 ($P<0.05$) followed by a decrease in week 30 compared to week 8 ($P<0.05$).

Retrospectively, plasma samples collected from cats at the time of first vaccination, which were stored frozen, were submitted for RT-PCR for FCoV (*Table 3*). Of 13 plasma samples tested, three were positive.

Young pet cats

The side-effects reported in the population of the young pet cats are summarized in *Table 2*. The observation period in this population was between 12 and 19 months. The health condition of the cats at the end of the observation period is summarized in *Table 5*. Thirteen cats of the vaccine group and 18 cats of the placebo group died from FIP. All, except one in each group, were confirmed by necropsy (*Table 6*). Two cats in the vaccine group were already ill at the time of

Table 3 Compilation of clinical and laboratory findings in cattery cats that died from FIP

No.	Age (at time of death)	Gender	Breed	Time of death: after first vaccination ^a	Necropsy performed	FCoV-titre (at first vaccination)	PCR for FCoV ^b	Changes in haematology and clinical chemistry (at first vaccination)
<i>Vaccine group</i>								
2-F-5	4.3 months	Female	DSH	12 days	Yes	100	Positive	Anaemia, protein ↑, leukocytes ↑
7-F-1	10 months	Female	Brit. SH	6 weeks	Yes	1600	Negative	Protein ↑
6-F-14	1 year	Female	Persian	7 months	Yes	25	Negative	Bilirubin ↑
6-M-5	1 year	Male	Persian	7 months	Yes	100	Negative	Anaemia
6-F-6	1.8 years	Female	Persian	7 months	No	400	Negative	Anaemia
16-MK-2	2.2 years	Male	Somali	10 months	No	1600	Negative	Protein ↑
8-F-2	1.9 years	Female	Persian	15.5 months	No	100	Positive	Anaemia, albumin ↓
7-F-6	1.7 years	Female	Brit. SH	17 months	Yes	400	Negative	Bilirubin ↑
<i>Placebo group</i>								
6-F-7	8 years	Female	Persian	5 weeks	Yes	1600	Negative	Anaemia, protein ↑, albumin ↓
7-M-4	5 months	Male	Brit. SH	5 weeks	Yes	400	Positive	(Anaemia)
9-F-1	5.1 years	Female	Birma	8 weeks	Yes	1600	Negative	Anaemia, protein ↑, albumin ↓
2-F-1	5.5 years	Female	DSH	5 months	No	100	Negative	Anaemia, albumin ↓
7-F-3	2.6 years	Female	Brit. SH	14 months	Yes	100	Negative	Bilirubin ↑

^aThe average time of death after vaccination was 35 weeks in the vaccine and 20 weeks in the placebo group

^bDone in retrospect at the time of death of the cat

Table 4 Population of the cattery cats: data collected to study the effect of the vaccine on the fertility of the queens and the health condition of kittens of queens, which had litters after the vaccination

	Vaccine group (30 queens with litters)	Placebo group (31 queens with litters)
Number of litters/queen	1.6	1.4
Number of kittens/litter	3.8	4.0
Deformities in kittens	5	6
<i>FIP-cases in kittens</i>		
FIP suspected/ FIP confirmed by necropsy	5/2	2/1

first vaccination. These cats died 5 and 7 days after vaccination because of FIP (Table 6).

Plasma samples collected at the time of first vaccination of the cats which later developed FIP were submitted for RT-PCR for FCoV (Table 6). Of 30 samples tested, 10 were found positive.

In one cat shelter with high FIP incidence, 25 cats were vaccinated (placebo 13 cats, vaccine 12 cats), of which 15 cats developed FIP (placebo 9, vaccine 6).

The frequency and distribution of antibody titres to FCoV at the time of first vaccination is presented in Figure 2. More than 50% of these clinically healthy young cats had already been exposed to FCoV in the first year of life. The distribution was identical in the vaccine and placebo group. Domestic shorthair cats showed statistically significantly lower FCoV antibody titres than pure-bred cats of the same age ($P < 0.001$) (Figure 3).

Cats of the placebo group, which showed a titre of 100 or higher, had a significantly higher risk for developing FIP in the next 12 months (10.7%) than cats with a titre of 25 or lower (3.3%) ($P = 0.016$) (Figure 4).

Though the vaccinated cats showed less FIP cases ($n = 13$) than the placebo group ($n = 18$), this difference was not significant. However, vaccinated cats with a titre of 100 or lower at the time of first vaccination showed significantly less FIP cases (four out of 201) than cats of the placebo group with a titre of 100 or lower (14 out of 219) ($P = 0.030$) (Figure 5).

To determine if vaccinated cats developed an accelerated form of FIP, the FIP cases in the vaccine and placebo group were displayed as a function of time of death after vaccination (Figure 6). In the first 150 days after vaccination 12 cats of the vaccine group and 11 of the placebo group developed FIP. After this time period, there were significantly less FIP cases in the vaccine group ($n = 1$), than in the placebo group ($n = 7$) ($P = 0.046$).

Seven cats of the vaccine group and six of the placebo group were FeLV positive at the time of first vaccination, three more cats of each group tested positive during the observation period. One of these FeLV-positive cats of the vaccine group and three of the placebo group died due to FIP which was confirmed by necropsy.

DISCUSSION

The aim of this study was to evaluate the efficacy and safety of a modified live virus vaccine in a double-blind study under field conditions in two cat populations with higher risk for FIP. Not all cats infected with FCoV develop lethal FIP and the incidence of FIP in a cat population can hardly be predicted⁸. In this study, the placebo and vaccine group were indistinguishable regarding distribution of age, sex and living conditions and no statistically significant difference in laboratory parameters (haematology, clinical chemistry, serology, CD4+/CD8+-lymphocyte subsets) were observed at the time of first vaccination. Therefore, cats in the vaccine and placebo group had about the same probability to become infected with FCoV and to develop FIP, and differences in the frequency of FIP cases or changes in laboratory parameters must be attributed to the vaccine.

The vaccinated cats did not show an accelerated form of FIP (Tables 3 and 6, Figure 6). One cattery cat aged 4.3 months was euthanized 12 days after vaccination, but this cat, though clinically inconspicuous, showed growth retardation compared to litter mates, anaemia (PCV: 27%), increase of total serum protein and viremia with FCoV at the time of first vaccination. Two young pet cats were euthanized 5 and 7 days after vaccination. Cat 1, which died 5 days after vaccination had shown

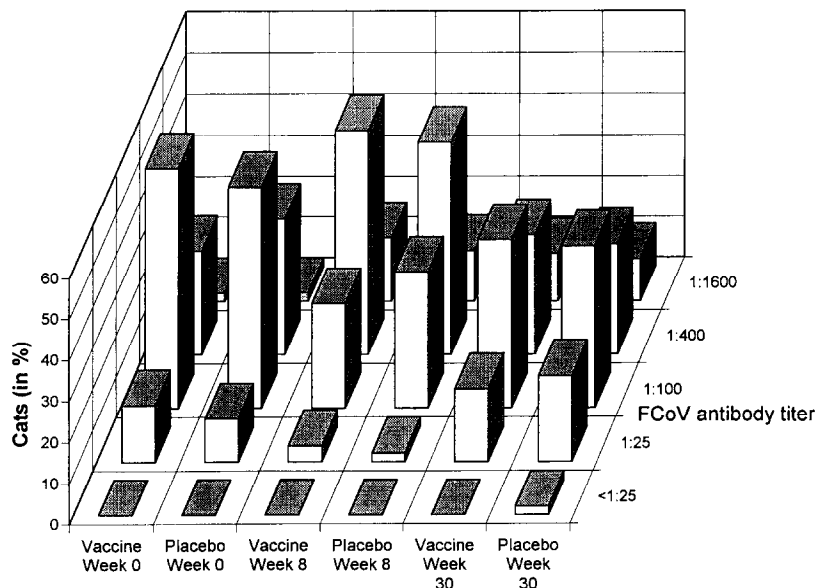


Figure 1 Distribution of FCoV antibody titres in the placebo ($n=48$) and vaccine group ($n=51$) of the cattery cats. Only cats from which blood samples were obtained from three different time points are included in this figure

Table 5 Health condition and FIP cases of the population of the young pet cats at the end of the observation period

	Vaccine group ($n=300$)	Placebo group ($n=309$)
Cat healthy	237 (79.0%)	249 (80.6%)
Not known (cat run away or owner moved)	32 (10.7%)	26 (8.4%)
Cat dead	31 (10.3%)	34 (11.0%)
Accident total	9	13
Necropsy performed	1	2
Presumed FIP total	13	18
Confirmed by necropsy	12	17
FIP cases: RT-PCR positive for FCoV at the time of first vaccination	4	6
Other causes of death	9	3
Necropsy performed	7	2
No necropsy	2	1

chronic nasal discharge and anorexia and had been treated with antibiotics and corticosteroids the last 2 weeks before vaccination. The FCoV antibody titre was high (1600) at the time of vaccination and in retrospect this cats was positive for FCoV in the RT-PCR. Cat 2, which died 7 days after vaccination had also been anorectic for some time and had been treated by the veterinarian. This cat too had a high titre at the time of vaccination (1600) and was PCR positive. These two cats were vaccinated by mistake and should not have entered the study in the first place. They remained in the study for the sake of completeness and because our study represent the real situation in the field where cats with obvious clinical signs may be vaccinated by mistake. It can be argued that in these three cats vaccine induced acceleration of FIP may have happened. However, if the incidence of FIP cases during the first 150 days after vaccination were compared in vaccinated vs placebo cats, there is absolutely no difference in that 12 cats in the vaccine group and 11 cats in the placebo group died during this time. In the population of the cattery cats where prevalence of FCoV antibodies was especially high (95%) the average time of death after

vaccination was 35 weeks in the vaccine group and 20 weeks in the placebo group. From this, the lack of side-effects and effect on fertility it was concluded that the vaccine is safe.

As expected, most of the cattery cats showed antibodies to FCoV, indicating prior infection. Not only the vaccine group, but also the placebo group showed an increase of titres in week 8 compared to week 0 and a decrease in week 30 compared to week 8 (Figure 1). Increase of antibodies could be explained by vaccination in the vaccine group, but not in the placebo group. As animals of the vaccine and placebo groups were separated for 48 h after the vaccination, spreading of the vaccine virus to cats of the placebo group seems unlikely. During the 8 weeks between vaccination and the collection of a second blood sample, antibody titres increased in cats of catteries, where FIP cases occurred. A similar rise of antibody titres was observed in cattery cats where no FIP cases were seen during this time period. It is well known, that stress can influence the immune system. Therefore, we speculate that the stress caused by the handling during vaccination and the collection of blood samples led to a weakening of the CMI and to a reactivation of a persistent FCoV infection and an increase of the antibody titres.

Surprisingly 50% of the clinically healthy pet cats younger than 12 months showed antibodies to FCoV (Figure 2). Pure-bred cats showed higher titres and were more frequently seropositive for FCoV than domestic cats (Figure 3), which is probably due to different living conditions. Pure-bred cats are normally raised in multiple-cat-households with close contact of the cats to each other, whereas domestic cats are often kept in single-cat-households or in small groups.

When vaccination was performed according to the recommendations of the manufacturer, the vaccine showed no overall efficacy in these cattery cats. In view of the fact that in all catteries FIP cases had occurred during the last 18 months prior this study, it has to be concluded that some of the cattery cats, which later died of FIP during the observation period, had already been

Table 6 Compilation of clinical and laboratory findings in young cats that died from FIP

No.	Age (at the time of death)	Living conditions	Time of death (after first vaccination)	Necropsy performed	FCoV-titre (at first vaccination)	PCR for FCoV ^a
<i>Vaccine group</i>						
103	11.2 months	Single cat, free running	5 ^b days	Yes	1600	Positive
363	13.2 months	Single cat, free running	7 ^b days	Yes	1600	Positive
33	4.9 months	Shelter with FIP	15 days	Yes	400	Negative
56	8.4 months	Multiple-cat-household	26 days	Yes	25	Positive
31	10.2 months	Shelter with FIP	56 days	Yes	400	Positive
37	8.5 months	Shelter with FIP	64 days	Yes	1600	Negative
516	7.6 months	Multiple-cat-household	86 days	Yes	100	Negative
318	13.8 months	Single cat, free running	88 days	Yes	<25	Negative
69	6.9 months	Multiple-cat-household	98 days	No	400	Negative
257	8.1 months	Shelter with FIP	129 days	Yes	1600	Negative
35	12.7 months	Shelter with FIP	130 days	Yes	100	Negative
246	8.1 months	Multiple-cat-household	140 days	Yes	1600	Negative
34	15.9 months	Shelter with FIP	199 days	Yes	400	Negative
<i>Placebo group</i>						
40	11.0 months	Shelter with FIP	18 days	Yes	<25	Negative
41	11.2 months	Shelter with FIP	24 days	Yes	25	Positive
88	10.9 months	Multiple-cat-household	29 days	Yes	1600	Negative
237	10.1 months	Multiple-cat-household	31 days	No	1600	Negative
43	8.5 months	Shelter with FIP	34 days	Yes	100	Positive
45	8.9 months	Shelter with FIP	46 days	Yes	100	Positive
23	7.3 months	Multiple-cat-household	62 days	Yes	400	Negative
491	6.3 months	from cattery with FIP	68 days	Yes	100	Negative
44	9.7 months	Shelter with FIP	71 days	Yes	100	Negative
62	10.8 months	Shelter with FIP	93 days	Yes	100	Negative
42	11.9 months	Shelter with FIP	137 days	Yes	100	Negative
426	9.9 months	From cattery with FIP	180 days	Yes	100	Negative
259	17.9 months	Shelter with FIP	211 days	Yes	100	Positive
46	16.8 months	Shelter with FIP	226 days	Yes	100	Positive
482	12.9 months	Multiple-cat-household	256 days	Yes	<25	Positive
590	12.8 months	Single cat, free running	275 days	Yes	N.D. ^c	N.D. ^c
485	13.5 months	Multiple-cat-household	275 days	Yes	<25	Negative
424	15.4 months	Multiple-cat-household	285 days	Yes	<25	Negative

^aDone in retrospect at the time of death of the cat

^bThese cats showed clinical signs of FIP at the time of first vaccination

^cN.D., not done

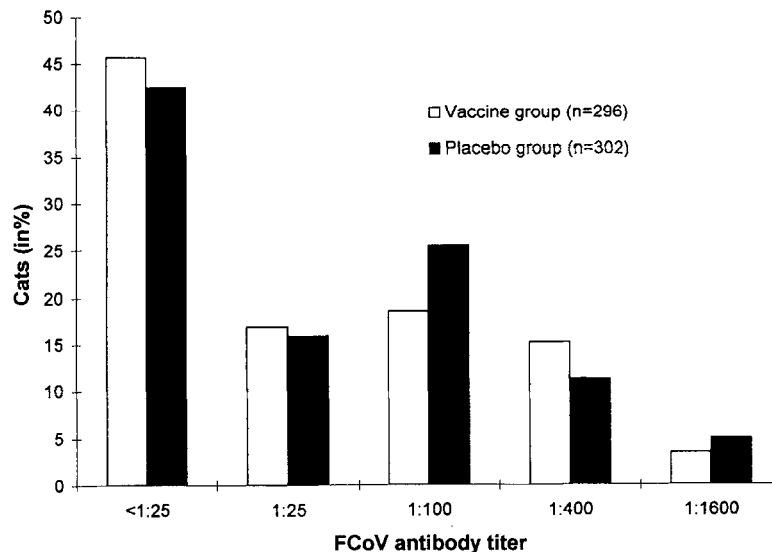


Figure 2 Young pet cats: distribution of FCoV antibody titres in the placebo and vaccine group at the time of first vaccination

infected with virulent FCoV and were vaccinated during the incubation period. This conclusion is also supported by the facts that all cats showed antibodies to FCoV, that some cats showed changes in blood parameters consistent with FIP (anaemia, low serum albumin, high serum protein, high bilirubin), and that retrospectively some cats were RT-PCR positive for FCoV at the time of first vaccination (Table 3). Vaccination itself did not

influence laboratory parameters such as haematology, clinical chemistry or CD4+/CD8+-lymphocyte subsets (data not shown).

In the population of the young pet cats no reduction of FIP cases in the vaccinated cats was observed in the first 150 days after infection (Figure 6). However, after this time point until the end of the observation period, the vaccinated cats showed significantly less FIP cases

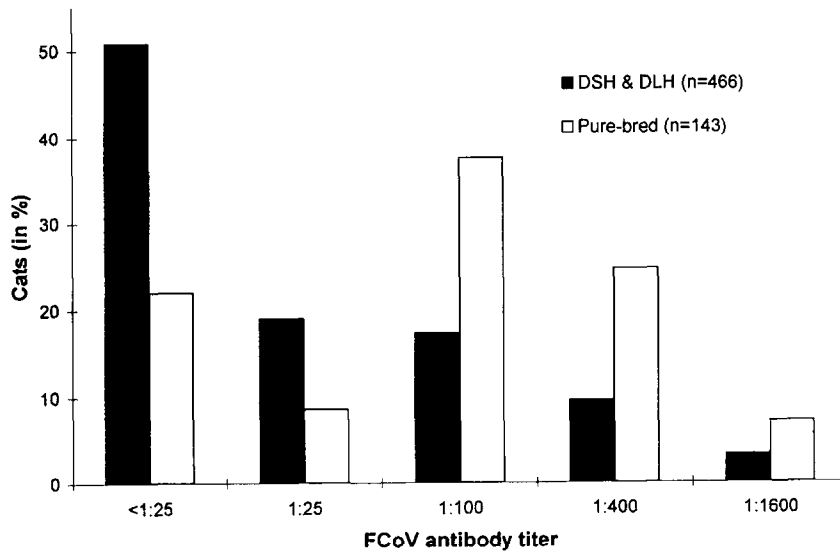


Figure 3 Young pet cats: comparison of the distribution of FCoV antibody titres at the time of first vaccination between domestic and pure-bred cats

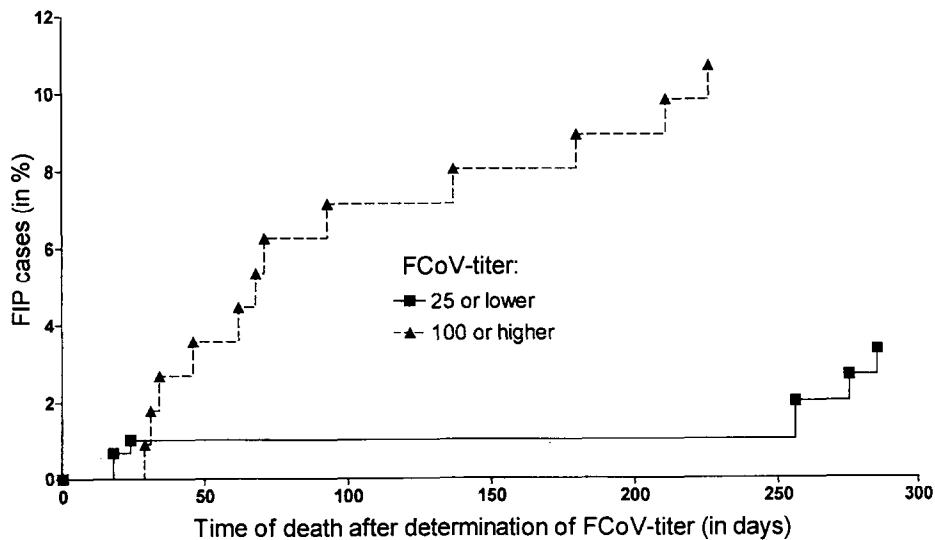


Figure 4 Placebo group of young pet cats: cumulated death cases from FIP during the observation period of 12 months

than the placebo group ($P=0.046$). As in the cattery cats, more than half of all cats had antibodies to FCoV at the time of first vaccination, indicating prior exposure. Retrospectively, some cats which later developed FIP were already viremic with FCoV at the time of first vaccination.

In one cat shelter the incidence of FIP was 50% in the vaccine group (six out of 12 cats) and 69% in the placebo group (nine out of 13 cats). It can be concluded that the exposure to FCoV was extremely high for these cats. As these animals were vaccinated after they had already been housed in the shelter for some time, it can be concluded that most of the vaccine failures in this population were due to previous infection with FCoV.

If cats with high FCoV antibody titres at the time of first vaccination (400 or higher) were excluded and only cats with titres of 100 or lower at the time of first vaccination were examined in our study, vaccination significantly decreased the incidence of FIP ($P=0.030$) (Figure 5). This observation suggests that low antibody titres are related with some degree of immunity,

probably through a strong cell-mediated immune response via the Th1 pathway^{35,36} and presumably with a lower virus load compared to cats with titres of 400 or higher. This interpretation would also be in agreement with the finding that high titres (in the placebo group) are associated with a higher risk for the development of FIP (Figure 4).

It can be concluded that vaccination of cats, which were already infected with FCoV, cannot prevent or alter the course of the disease. However, vaccination of cats with no or low antibody titres seems to be efficient. These findings confirm the results of a placebo-controlled trial in which seronegative animals were vaccinated, before they were entered into a cat shelter with FIP problems³⁷. To increase the efficacy of the vaccine changes in husbandry and management conditions may be initiated that lead to a decrease in coronaviral load in the kittens^{4,11}.

Under experimental conditions, vaccination of FCoV naive cats was also found to reduce the incidence and the severity of infections with the low virulent viruses called

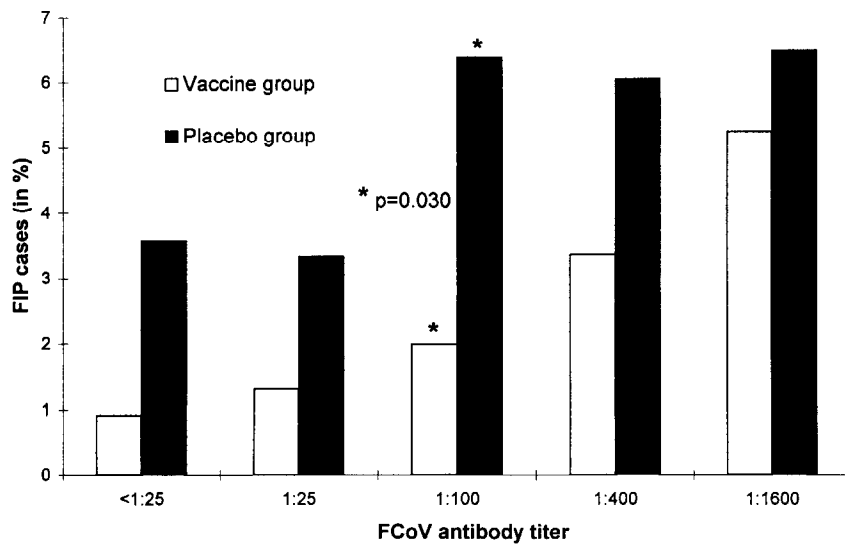


Figure 5 Young cats: cumulated death cases from FIP during the observation period of 12 months depending on the different FCoV-antibody titres the cats had at the time of first vaccination

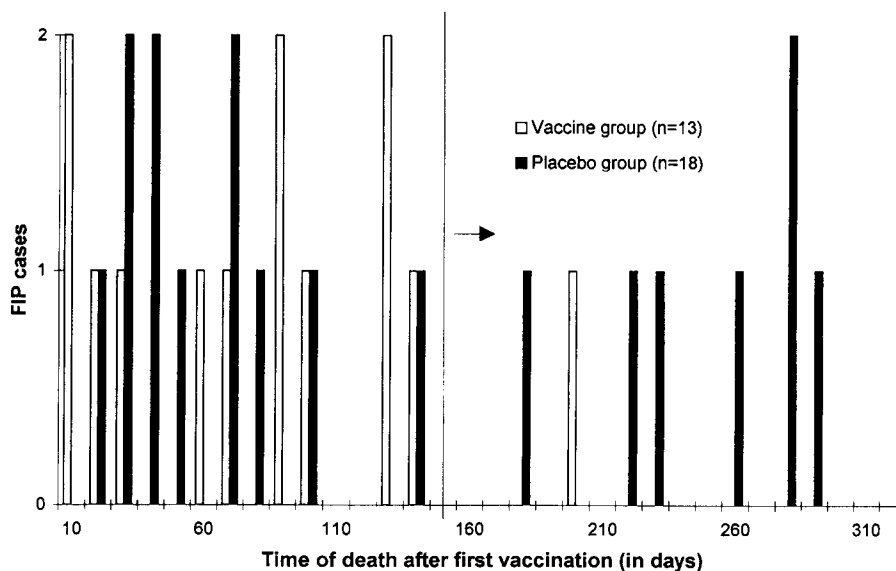


Figure 6 Occurrence of FIP cases in the vaccine and placebo group of the young cats displayed as a function of time after first vaccination (in days)

FECV³⁸. Since every FCoV infection has to be considered a potential risk for FIP^{4,11}, reducing these infections by vaccination may also help to reduce the FIP incidence.

Cats of the placebo group with a titre of 25 showed a significantly higher risk for developing FIP in the following 12 months (10.7%), than cats with lower titres (3.3%) ($P=0.016$, Figure 4). This finding and the observation that many of these cats were PCR positive at the time of first vaccination suggest that they were already infected with FCoV and were vaccinated during the incubation period. This would explain why vaccination showed a low efficacy in the young pet cats. However, it is difficult to apply the classic term of an incubation period to FIP. According to Pedersen and co-workers more virulent mutants of FCoV capable of infecting macrophages can emerge spontaneously any time during an FCoV infection³⁹. As mutations in viruses occur randomly, it can be concluded that a high virus load increases the probability that such mutants arise. A

functioning immune system can control the virus load at a low level. Therefore, all factors which suppress the immune response, may increase the virus load and the genesis of virulent mutants. Thus, a cat can develop FIP many months or even years after an FCoV infection.

In conclusion, the use of a live modified vaccine against FIP was found to be safe in high risk populations under field conditions. Efficacy was clearly shown in cats with low FCoV antibody titre, but overall was rather low in high risk populations. To optimize its efficacy, changes in management and husbandry should help to prevent FCoV exposure prior to vaccination.

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