J. Hyg., Camb. (1971), **69**, 627
Printed in Great Britain

Study of an attenuated strain of feline infectious enteritis (panleucopaenia) virus

I. Spread of vaccine virus from cats affected with feline respiratory disease

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(*Received* 7 *June* 1971)

SUMMARY

In the course of developing a living attenuated feline infectious enteritis (panleucopaenia) vaccine, it was found that respiratory disease-infected cats newly inoculated with this vaccine spread vaccine virus to respiratory disease-infected in-contact controls. These in-contact controls were able to infect other cats with which they were placed in contact so that after five natural transmissions in this way and two oral administrations and subsequent re-isolations, reversion to virulence became evident. It is clear that before general release of a new living feline infectious enteritis vaccine, there must be satisfactory evidence that concurrent infection will not affect the safety of the modified antigen.

In cats infected with feline infectious enteritis there appears to be a short period, coinciding with the onset of leucopaenia, during which they are highly infectious. It seems possible that some infected animals may become immune carriers because virus has been recovered from the small intestine of two of four cats with significant antibody titres 22–24 days after exposure to infection.

INTRODUCTION

During preliminary investigations with a living attenuated feline infectious enteritis (FIE) vaccine prepared in a feline embryonic cell line (O'Reilly & Whitaker, 1969), no spread of virus from 11 vaccinated cats to 21 in-contact controls occurred. In later work, however, evidence of contact spread was obtained at a time when a moderately severe outbreak of respiratory disease was also present. This paper reports the results of investigations into the role of feline respiratory disease in the spread of an attenuated FIE vaccine virus and the possibility of reversion to virulence by successive cat to cat passage.

MATERIALS AND METHODS

Cats

Details of the cat colonies, method of obtaining sera and measurement of neutralizing antibody have been described by O'Reilly, Paterson & Harriss (1969).

FIE vaccine

A wild strain of FIE virus, recovered from the kidneys of an affected cat, was adapted to grow in a feline embryonic cell line (O'Reilly & Whitaker, 1969) and, after serial subculturing, was shown to have lost its pathogenicity, although it was still immunogenic for cats. In Expt. 1 the vaccine consisted of undiluted virus-infected tissue culture fluid and cells. In Expt. 2, similar material was diluted with stabilizer in the proportion of 3:2 and freeze-dried.

Virus isolation

Cat tissues for examination were ground with sterile sand in a mortar and a 10% suspension prepared in sterile phosphate buffered saline containing 2000 units of penicillin and 1000 μg. of streptomycin per ml. After centrifuging at 2000 *g* for 30 min., the supernatant was collected and stored at $-20°$ C. until required. Tenfold dilutions in 0·25 ml. volumes were inoculated into each of six test tubes containing a coverslip and $2{\cdot}0 \times 10^5$ freshly versenized feline embryonic cells in 2·0 ml. of medium. The cells were incubated in the stationary position at 37° C. One coverslip per dilution was removed each day, stained and examined for evidence of FIE infection (O'Reilly & Whitaker, 1969).

Respiratory disease viruses

The feline picornavirus (K-3/C1) and the feline herpes virus (F-62/C1/1) used in these experiments had been plaque purified and were free of contaminating FIE virus. Infection of cats was done under general anaesthesia by spraying 0·5 ml. of a 1/2 dilution of virus into each nasal passage.

Experimental procedures

Experiment 1. *Contact spread.* Twenty-four kittens, aged from 8 to 10½ weeks, from six litters bred at the Wellcome Veterinary Research Station, Frant, were randomly divided into four equal groups. Group A was infected with the feline picornavirus and group B with the feline herpes virus. These two groups were kept in separate rooms in strict isolation. The attendants wore protective rubber clothing which was washed down with 'Tego'* before entry and after leaving each of the rooms. Group A kittens were always visited before group B kittens.

Groups C and D, which were housed in another isolation building, were not infected with respiratory disease and there was no contact between personnel attending these two groups and groups A and B. Three kittens in each of groups A, B and C were inoculated subcutaneously once with attenuated FIE vaccine. The remaining kittens were unvaccinated and served as controls to the vaccine. Three days after vaccination, three group D kittens were placed in contact with group B, and 5 days after vaccination the other three group D kittens were placed in contact with group A. Rectal temperatures were recorded daily.

Experiment 2. *Reversion to virulence by cat passage.* Cats used in this experiment

* Tego MHG, Hough, Hoseason Company Ltd., Manchester.

had been bred at the Wellcome Research Laboratories and were aged 14–26 weeks. Five cats, immediately after inoculation with freeze-dried attenuated FIE vaccine, and two unvaccinated cats, were infected with the feline herpes virus and housed together in one room of an isolation building. A further two unvaccinated cats also infected with this feline herpes virus were introduced every 5th day until the 35th day of the experiment. On the 10th and 15th days two cats, unvaccinated and not infected with respiratory disease, were also added.

The vaccinated cats were killed on the 15th day and the unvaccinated cats were killed after they had been in the experiment for periods varying from 16 to 27 days. Whenever possible, serum was obtained from all cats at the time of death when bone marrow, mesenteric lymph node and portions of duodenum, ileum and spleen were also collected for recovery of virus.

RESULTS

Experiment 1. *Contact spread*

Group A. Only one of the six kittens developed a temperature exceeding 105° F. and this persisted from the 3rd to the 7th day. The three kittens from group D became pyrexic 5–8 days after being introduced. Evidence of respiratory disease was seen in three of the six group A kittens and in two of the three group D in-contact kittens. Symptoms were mild although little food was eaten by the group A kittens during the first few days of the experiment. Some of the kittens had slight inflammation of the borders of the tongue.

Group B. One kitten died within 24 hr. of infection. The remaining five developed temperatures of about 105° F. on one or more occasions between the 3rd and 9th day. Two of the group D kittens developed temperatures between the 8th and 11th day after being placed in contact.

The group B kittens showed no interest in food for the first 5 days and became severely affected with respiratory disease. One was destroyed on the 13th day because of its poor condition and two had glossitis. Symptoms in group D kittens were milder.

Clinical respiratory disease caused by the herpes virus was more severe than that produced by the picornavirus. However, all kittens infected with either of the two viruses lost weight.

Group C. These kittens remained free of respiratory disease and maintained normal temperatures.

Post-mortem findings. All the surviving kittens were bled and destroyed on day 21 of the experiment. Nothing of significance was seen in the group A kittens or in those in contact with them. In group B, foci of red hepatization were found in the cardiac lobes of the lungs of three of the four remaining kittens. Similar lesions were seen in the lungs of the in-contact group D animals. No abnormalities were seen in the group C kittens.

Antibody to FIE virus. At the start of the experiment, no kitten had demonstrable FIE antibody. When the surviving kittens were bled on the 21st day, antibody was found in five of the eight vaccinated kittens in groups A, B and C,

in four of the five animals in groups A and B which were unvaccinated but infected with respiratory disease, and in two of the three group D kittens in contact with group A. There was no rise of FIE antibody in the three unvaccinated kittens in group C which were not infected with respiratory disease nor in the three group D kittens in contact with group B (Table 1).

Table 1. *The titres of FIE antibody found in both vaccinated and in-contact unvaccinated cats infected with feline respiratory disease (Expt. 1)*

Group	Treatment	Antibody titres	
		Day 0	Day 21
A	Vaccine plus feline picornavirus	<8*	128
		<8	128
		<8	<8
	Feline picornavirus only	<8	512
		<8	512
		<8	128
D	In-contact with group A from the fifth day after vaccination	<8	512
		<8	128
		<8	<8
B	Vaccine plus feline herpes virus	<8	128
		<8	128
		<8	Died
	Feline herpes virus only	<8	128
		<8	<8
		<8	Died
D	In-contacts with group B from the third day after vaccination	<8	<8
		<8	<8
		<8	<8
C	Vaccine	<8	128
		<8	<8
		<8	<8
	No vaccine	<8	<8
		<8	<8
		<8	<8

* Reciprocal of serum dilution.

Experiment 2. Reversion to virulence by cat passage

Only mild signs of respiratory disease were observed during the first four weeks; thereafter the symptoms increased in severity.

No cat had antibody to FIE virus at the beginning of the experiment. With two exceptions, all had developed significant titres by the time they were re-examined (Table 2). The exceptions were a cat that died of a *Pasteurella* pericarditis after 13 days exposure, and another that died from pulmonary oedema after only four days. From the latter (cat 27), FIE virus was recovered from the mesenteric lymph node, spleen and bone marrow. It was also recovered from either the small intestine or mesenteric lymph node of five other unvaccinated cats fouund ead or killed 13–24 days after introduction to the experiment (Table 3).

Table 2. *The day of introduction, duration of exposure and final antibody titre of cats used in Expt. 2: reversion to virulence by cat passage*

Cats (number)	Day of introduction	Duration of exposure	Final antibody titre†
Vaccinated			
3	0	15	512‡
5	0	15	512
7	0	15	512
8	0	15	512
10	0	15	512
Unvaccinated			
1	0	20	512
6	0	20	512
11	5	20	128
12	5	13	Died
13	10	20	128
16	10	20	128
14*	10	25	128
15*	10	25	128
17	15	20	128
18	15	20	512
20*	15	27	128
23*	15	27	128
25	20	16	128
26	20	22	512
27	25	4	Died
29	25	24	32
30	30	19	128
35	30	19	512
41	35	22	512
43	35	22	512

* These cats were not deliberately infected with feline respiratory disease.
† All cats were devoid of antibody at the beginning of the experiment.
‡ Reciprocal of serum dilution.

Table 3. *Recovery of FIE virus from the tissues of vaccinated and in-contact cats and their antibody titres at the time of death (Expt. 2)*

	Days after vaccination or exposure									
Tissue	4	13	15	16	19	20	22	24	25	27
	(1)	(1)	(5)*	(1)	(2)	(7)	(3)	(1)	(2)	(2)
Duodenum	0/1†	0/1	0/5	0/1	0/2	0/7	1/3	0/1	0/2	0/2
Ileum	—	—	—	0/1	—	0/1	—	1/1	—	—
Mesenteric lymph node	1/1	1/1	—	1/1	1/2	0/2	0/3	—	0/1	—
Spleen	1/1	0/1	—	0/1	0/1	0/2	0/1	—	0/2	—
Bone marrow	1/1	0/1	0/5	0/1	0/2	0/7	0/3	0/1	0/2	0/2
Antibody titre-range	NA	NA	512‡–512	128	128–512	128–2048	512–512	32	128–128	128–128

NA = *Not available.*
( ) = *number of cats examined.*
* Vaccinated cats.
† Numerator = number of isolations of virus; denominator = number of cats providing tissue.
‡ Reciprocal of serum dilution.

https://doi.org/10.1017/S0022172400021902 Published online by Cambridge University Press

One ml. of the pooled supernatants from the suspensions of spleen, bone marrow and mesenteric lymph node of cat 27 was administered orally to one of the two cats infected with the feline herpes virus and sharing the same cage. Daily leucocyte counts were done and both cats were killed on the 15th day. The orally

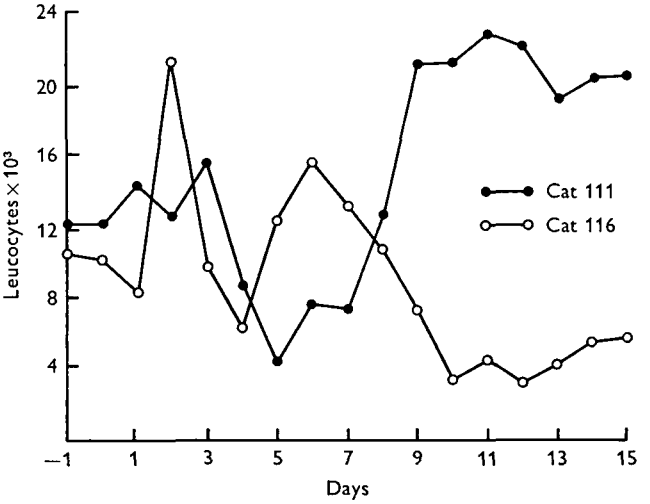

Fig. 1. Daily leucocyte counts of cat 111, dosed orally with a suspension of virus-infected tissues from cat 27, and of cat 116, the in-contact control (Expt. 2).

Table 4. *Isolations of virus from, and antibody titres of, cats* 111 *and* 116 (*Expt.* 2)

	Cats	
Tissues	111	116
Ileum	0	+
Faeces	0	+
Mesenteric lymph node	0	+
Spleen	NT	+
Bone marrow	NT	0
Antibody titres		
Day 0	<8*	<8
15	512	32

+ = virus isolated.
0 = no virus isolated.
NT = Not tested.
* Reciprocal of serum dilution.

dosed cat (111) was leucopaenic on the 5th day and its in-contact cage-mate (116) was similarly showing leucopaenia on the 10th to 13th days (Fig. 1). While no virus was recovered from tissues taken after death from cat 111, virus was isolated from the mesenteric lymph node, ileum, faeces and spleen of cat 116. Both cats developed antibody (Table 4).

Two cats (70 and 73) infected with the herpes virus were each infected orally with 1 ml. of a suspension prepared from the tissues of cat 116. On each of days

https://doi.org/10.1017/S0022172400021902 Published online by Cambridge University Press

5 and 11, two cats, not infected with respiratory disease, were introduced. Cats 70 and 73 were killed on the 10th day and the in-contact animals between the 16th and 28th days. Daily leucocyte counts showed that all the cats developed leucopaenia (Fig. 2) and they lost both appetite and weight, shortly after entry

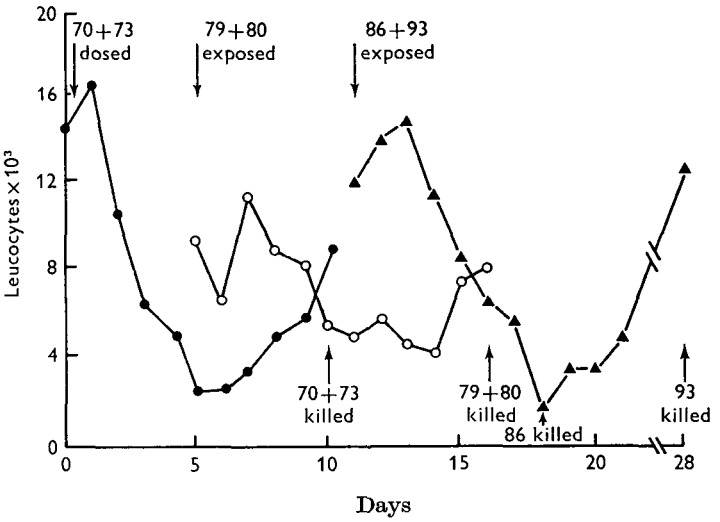

Fig. 2. Geometric mean daily leucocyte counts of two cats dosed orally with a suspension of virus-infected tissues from cat 116, and of four cats in which the virus was passaged naturally (Expt. 2).

Table 5. *Antibody titres of two cats after oral infection with a suspension of tissues from cat* 116 *and of the four cats in which the virus was subsequently passaged naturally* (*Expt.* 2)

	Days after dosing or exposure				
	0	7	11	12	17
Dosed cats					
70	<8*	—	128	—	—
73	<8	—	8	—	—
Exposed cats					
79	<8	—	—	32	—
80	<8	—	—	32	—
86	<8	<8	—	—	—
93	<8	—	—	—	32

* Reciprocal of serum dilution.

into the experiment. The weight loss, although appreciable in cats 70 and 73, became more noticeable in each new entry even though none of the cats were clinically affected with respiratory disease. The last pair of cats added were vomiting on the 5th day of exposure and one (cat 86) was killed on the 7th day to provide tissues for recovery of virus. FIE virus was isolated from its mesenteric lymph node, jejunum, spleen and urine. No attempts were made to recover virus from the tissues of the other cats. With the exception of cat 86, all developed neutralizing antibody (Table 5).

https://doi.org/10.1017/S0022172400021902 Published online by Cambridge University Press

DISCUSSION

The findings reported in this paper add a further requirement to the tests used in the development of a living FIE vaccine. Before general release, there must be satisfactory evidence that concurrent infections will not affect the safety of the modified antigen.

Expt. 1 showed quite clearly that the FIE attenuated vaccine virus used in these studies spread to in-contact control kittens in the presence of concurrent respiratory disease, despite the fact that some animals showed no evidence of clinical respiratory symptoms. In the absence of deliberate concurrent infection with one or other of the respiratory viruses, this did not occur (group C).

Table 6. *Proportion of* 8- *to* 10½*-week-old kittens developing FIE antibody* 3 *weeks after either one dose of living attenuated vaccine or being in contact with vaccinated kittens* (*Expt.* 1)

Age in weeks at time of vaccination	Proportions developing antibody	
	Vaccinated	Unvaccinated
10½	1/2	2/6
10	1/3	2/5
9½	2/2	2/3
8	1/1	—

Only 11 of the 22 kittens developed antibody. This is not surprising since none were older than 10½ weeks when vaccinated (Table 6). Lack of antibody conversion in a proportion of kittens given one dose of vaccine at this age or younger has been demonstrated by O'Reilly *et al.* (1969) who were able to obtain a 100% response only in animals vaccinated at 12 weeks or older.

The results of Expt. 2 confirmed the spread of the attenuated virus from vaccinated cats to unvaccinated in-contact cats experimentally infected with respiratory virus, whether or not clinical symptoms of respiratory disease developed. Natural transmission continued over the seven contact passages carried out. When virus isolated from the fifth contact passage (cat 27) was passed twice more by oral administration and re-isolation from the recipients, clear evidence of reversion to virulence was seen in cats not infected with respiratory disease and placed in contact (cats 86 and 93; Fig. 2).

These experiments suggest that cats are highly infectious within a few days of infection and that some animals may possibly develop into immune carriers. In cats infected with FIE virus, the first indications of leucopaenia usually occur between the 4th and 6th days (Lawrence & Syverton, 1938; Hammon & Enders, 1939; O'Reilly, 1970). Presumably viraemia has preceded this event (Lawrence & Syverton, 1938) and, from Figs. 1 and 2 and O'Reilly (1970), it would appear that cats are most infectious at the onset of leucopaenia. Because antibody has been found in cats as early as 7 days after inoculation with living attenuated vaccine (O'Reilly, unpublished), it seems likely that the highly infectious period of the disease is short-lived. On the other hand, virus was recovered from the small

intestine of cats on the 22nd and 24th days after exposure (Table 3). One of these cats had an antibody titre of 32 and the other 512. However, it still remains to be confirmed that there is a 'carrier state' similar to that reported in mink infected with the related mink enteritis virus (Bouillant & Hanson, 1965).

The attenuated vaccine used in these experiments has been extensively tested in breeding catteries known to be infected with FIE where all the young kittens are vaccinated after weaning and the queens are boosted annually. No problems related either to the vaccine or to subsequent vaccination failures have been observed. However, it might not be suitable for use in boarding catteries where there is a continually changing cat population and high risk of respiratory disease, and work continues in an effort to achieve further modification and remove the propensity to spread in the presence of intercurrent infection.

I wish to thank Mr J. Prydie, M.R.C.V.S., for supplying the feline respiratory disease viruses and Mr W. F. Matchett, A.I.M.L.T., A.I.S.T., and Mrs L. M. Hitchcock for their valued technical assistance.

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