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

II. Removal of the spread factor by further passaging in tissue culture

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SUMMARY

The propensity of an attenuated strain of feline infectious enteritis (panleucopaenia) virus to spread from vaccinated cats affected with intercurrent feline respiratory disease to unvaccinated in-contact cats was eradicated by further passaging of the vaccine virus in tissue culture. No virus was recovered from, and no antibody was found in the sentinel cats in contact with seven vaccinated animals. Thus, a further 27 passages of the vaccine virus in tissue culture has eliminated the spread factor.

INTRODUCTION

In the previous paper (O'Reilly, 1971) it was reported that an attenuated strain of feline infectious enteritis (FIE) virus, believed to be safe for use as a vaccine, spread from vaccinated cats to in-contact unvaccinated cats during an outbreak of respiratory disease. Later, it was established that the virus which was shed reverted to virulence after serial cat-to-cat passage.

This paper records the eradication of the spread factor by further modification of vaccine virus.

MATERIALS AND METHODS

Serology

Collection of blood in Expt. 1 was by heart puncture (O'Reilly, Paterson & Harriss, 1969) and in Expt. 2 from the jugular vein (Hovell, O'Reilly, Calder & Povey, 1970): neutralizing antibody was measured by the technique of O'Reilly *et al.* (1969).

FIE vaccine

The tissue culture adapted strain of virus used in the previous experiments (O'Reilly, 1971) was serially subcultured a further 27 times in a feline embryonic cell line (O'Reilly & Whitaker, 1969). In Expt. 1, vaccine consisted of undiluted virus-infected tissue culture fluid and cells, the dose being 1·0 ml. In Expt. 2, similar material was diluted with stabilizer in the proportion of 3:2 and given as a 2·0 ml. dose.

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Respiratory disease virus

The feline herpes virus used (F-62/Cl/l) was plaque purified and free of contaminating FIE virus.

Virus isolations

The method used for attempted recovery of virus from feline tissues has been described (O'Reilly, 1971).

Challenge virus

Cats were challenged with a 10^{-2} dilution of virulent virus given orally; the characteristics of this virus pool have been described (O'Reilly, 1970).

Experimental procedures

Experiment 1. The cats, bred at the Wellcome Veterinary Research Station, Frant, were aged 17–18 weeks. Three cats were placed in contact with a donor group showing clinical evidence of respiratory disease. One week later the three experimental animals, now showing signs of respiratory disease, were moved to another isolation room where they were bled and inoculated subcutaneously with 1·0 ml. of FIE vaccine. Three healthy cats were bled, and then added to the group of vaccinated cats. Eight days later, another three healthy cats were placed in contact with the respiratory disease-infected donor group and, after 2 days, were bled and added to the experimental animals.

The animals were challenged with virulent virus on the 21st day after vaccination. White blood cell counts were done and temperatures recorded daily throughout the experiment.

Experiment 2. The cats used in this experiment were bred either at the Wellcome Veterinary Research Station (W.V.R.S.) or at these laboratories (W.R.L.). Four W.V.R.S. cats and four W.R.L. cats were placed in an isolation room; daily leucocyte counts were started 2 days later, and on the following day all eight animals were bled and infected intranasally with a feline herpes virus (O'Reilly, 1971). Two cats from each of W.V.R.S. and W.R.L. were inoculated subcutaneously with 2·0 ml. of vaccine. On each of the 5th, 10th, 15th, and 20th days after the start of the experiment, four more cats – two from W.V.R.S. and two from W.R.L. – were introduced into the environment. Only those animals added on day 5 were experimentally infected with feline herpes virus.

On the 25th day one cat introduced on the 15th day and the four introduced on the 20th day were killed and their tissues taken for recovery of virus.

On the 29th day of the experiment, all the surviving cats were bled and challenged with virulent virus.

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RESULTS

Experiment 1

Clinical

Throughout the duration of this experiment most cats had slightly elevated temperatures which seldom exceeded 104° F. Most cats became severely affected with respiratory disease during the first week of exposure, but thereafter the symptoms subsided and the condition became chronic with periodic recurrences of ocular and nasal involvement.

No evidence of leucopaenia or depression of the white blood cell counts was

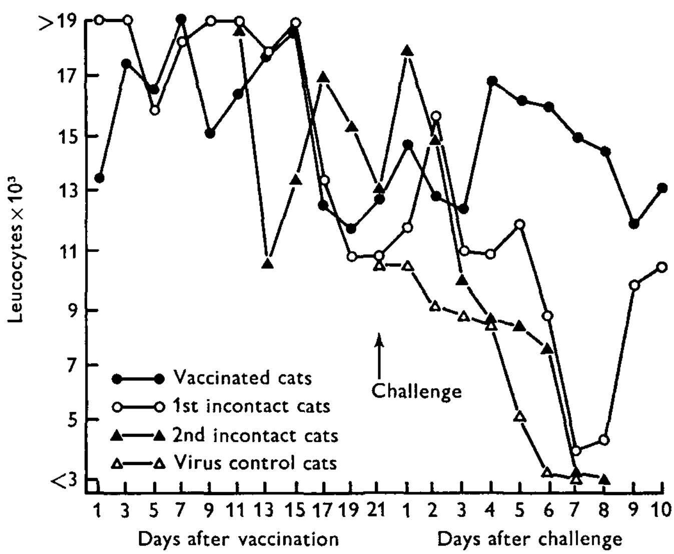

Fig. 1. The geometric means of the leucocyte counts of vaccinated, in-contact unvaccinated and virus control cats. Expt. 1.

Table 1. *SN antibody titres* (*Expt.* 1)

Treatment	Cat number	After vaccination (days) −14	0	3	7	10	16	21	14th day after challenge 14
Vaccinated	152	—	< 8*	NB	32	NB	128	512	128
	160	—	< 8	< 8	8	—	—	—	—
	162	—	< 8	< 8	8	NB	128	128	32
In-contact controls									
1st group	153	< 8	< 8	NB	< 8	NB	< 8	< 8	—
	158	< 8	< 8	NB	< 8	NB	< 8	< 8	—
	159	< 8	< 8	NB	< 8	NB	< 8	< 8	128
2nd group	155	—	—	—	—	< 8	< 8	< 8	—
	157	—	—	—	—	< 8	< 8	< 8	—
	161	—	—	—	—	< 8	< 8	< 8	—
Virus control	154	—	—	—	—	—	—	< 8	—
	168	—	—	—	—	—	—	< 8	—

* Reciprocal of serum dilution.
NB = not bled.

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observed between the beginning of the experiment and challenge (Fig. 1). After challenge, frank leucopaenia was seen by the 5th day in the virus control cats; both died, one on the 7th day and the other one the next day. The first group of in-contact cats did not show a drop in the leucocyte counts until the 7th day – any tendency towards an impending leucopaenia was masked by cat 153 having cell counts in excess of 19,000 cells per mm³ during the first 5 days. Nevertheless, two of the cats in this group died on the 8th day and the 3rd cat survived. The white blood counts of the second group of in-contact controls dropped steeply from the 2nd to the 7th day and all three cats were dead by the 8th day. Neither of the two vaccinated cats showed any evidence of leucopaenia.

Antibody to FIE virus

Antibody was detected in the vaccinated cats by the 7th day and reached its peak titre by the 16th day (Table 1). Vaccinated cat 160 failed to recover from the anaesthetic after the 7th day bleeding.

Only the vaccinated cats had antibodies at the time of challenge; 14 days later, the sole surviving control cat (159) also had antibody.

Experiment 2

Clinical

Although the respiratory disease was clinically more severe than in Expt. 1 only cats bred at W.V.R.S. were affected. Two cats were killed because of the severity of the disease (cat 256 on day 13 and cat 257 on day 19), and cat 264 died of pneumonia on day 26. Vaccinated cat 254 was destroyed because it developed a physical disability suggestive of iliac thrombosis.

Between vaccination and challenge (day 29) there were no depressions of the

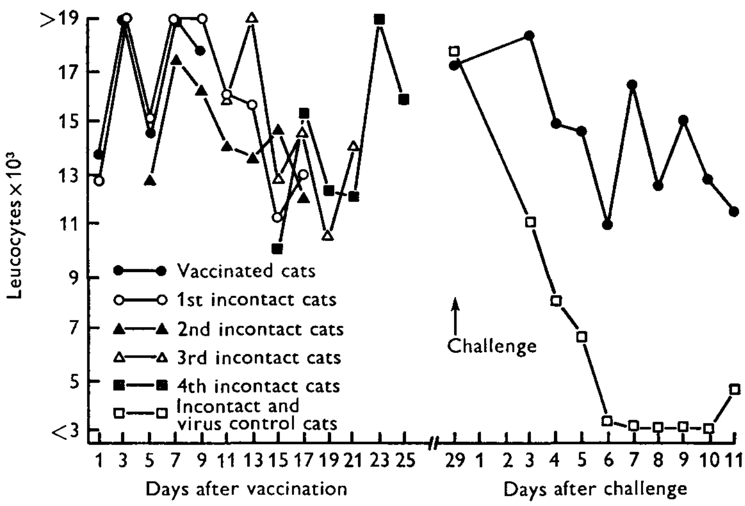

Fig. 2. The geometric means of the leucocyte counts of vaccinated, in-contact unvaccinated and virus control cats. Expt. 2.

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Table 2. *SN antibody titres* (*Expt.* 2)

Treatment	Cat number	After vaccination (days) 0	5	10	11	15	20	25	29	13th day after challenge
'accinated	54	< 8*	NB	NB	128	128	NB	NB	128	128
	69	< 8	NB	NB	128	512	NB	NB	512	128
	253	< 8	NB	NB	128	128	NB	NB	128	128
	254	< 8	NB	NB	32	—	—	—	—	—
n-contact controls										
1st group	56	< 8	NB	NB	< 8	< 8	< 8	< 8	< 8	512
	57	< 8	NB	NB	< 8	< 8	< 8	< 8	< 8	512
	256	< 8	NB	NB	< 8	—	—	—	—	—
	257	< 8	NB	NB	< 8	< 8	—	—	—	—
2nd group	62	—	< 8	NB	< 8	< 8	< 8	< 8	< 8	512
	66	—	< 8	NB	< 8	< 8	< 8	< 8	< 8	512
	258	—	< 8	NB	< 8	< 8	< 8	< 8	< 8	—
	259	—	< 8	NB	< 8	< 8	< 8	< 8	< 8	—
3rd group	64	—	—	< 8	NB	NB	< 8	< 8	< 8	—
	66	—	—	< 8	NB	NB	< 8	< 8	< 8	512
	260	—	—	< 8	NB	NB	< 8	< 8	< 8	—
	261	—	—	< 8	NB	NB	< 8	< 8	< 8	128
4th group	80	—	—	—	—	< 8	NB	< 8	< 8	—
	81	—	—	—	—	< 8	NB	< 8	—	—
	263	—	—	—	—	< 8	NB	< 8	< 8	—
	264	—	—	—	—	< 8	NB	< 8	—	—
5th group	65	—	—	—	—	—	< 8	< 8	—	—
	82	—	—	—	—	—	< 8	< 8	—	—
	262	—	—	—	—	—	< 8	< 8	—	—
	265	—	—	—	—	—	< 8	< 8	—	—
'irus controls	271	—	—	—	—	—	—	—	< 8	512
	272	—	—	—	—	—	—	—	< 8	512
	273	—	—	—	—	—	—	—	< 8	512
	274	—	—	—	—	—	—	—	< 8	—

* Reciprocal of serum dilution.
NB = not bled.

Table 3. *Attempts at recovery of FIE virus from the tissues of cats in-contact with vaccinated cats* (*Expt.* 2)

Treatment	Cat number	Exposure in days	Tissue: Mesenteric lymph node	Bone marrow	Duodenum	Spleen	Ileum	Reason for cat death
1st group	256	13	—	—	—	—	NT	Respiratory disease
	257	19	NT	—	—	—	—	
4th group	81	10	—	NT	—	—	NT	Recovery of virus
	264	11	—	NT	—	—	NT	Pneumonia
5th group	65	5	NT	NT	—	—	NT	Recovery of virus
	82	5	—	—	—	NT	NT	
	262	5	—	NT	—	—	NT	
	265	5	—	NT	—	—	NT	

NT = not tested.
— = no virus recovered.

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leucocyte counts in either the vaccinated or in-contact cats (Fig. 2). However, during the 11 days on which white cell counts were done after challenge, leucopaenia was seen in all the in-contact and virus control cats but not in the vaccinated cats.

Two specific FIE deaths occurred in each of the 2nd and 3rd in-contact control groups and one death in each of the 4th in-contact control and virus control groups; no obvious reason was found for the death of cat 263 on the 14th day after challenge.

Antibody to FIE

Antibody was found in the vaccinated cats on day 11 (first bleeding after vaccination) but none was detected in any of the other cats at challenge (Table 2). Those animals in the control groups surviving challenge all developed antibody.

Recovery of virus

Attempts to recover virus from the tissues of eight in-contact cats were unsuccessful. Four of these cats had been exposed to the experimental environment for 5 days, and the other four from 10 to 19 days. Two of the cats, destroyed because of respiratory disease, were from the first group of in-contact animals (Table 3).

DISCUSSION

The previous paper (O'Reilly, 1971) stated that FIE virus passaged in tissue culture might appear to be fully attenuated because there was no spread of vaccine virus from healthy vaccinated cats to healthy in-contact cats. However, cats suffering from intercurrent feline respiratory disease were capable of shedding vaccine virus which reverted to virulence after several cat-to-cat passages.

This paper reports that the vaccine strain of virus referred to above has, after further passaging in tissue culture, become fully attenuated without loss of antigenicity. Intercurrent infection of cats with respiratory disease did not provoke spread of vaccine virus since there was no development of antibody in in-contact cats, no isolation of virus from cats either severely affected with respiratory disease or killed shortly after potential exposure to virus and no depression of the leucocyte counts.

After challenge, none of the five vaccinated cats showed any signs of ill health whereas 58 % (14/24) of the in-contacts and virus control cats died of the disease; the surviving 42 % of these cats all had antibody conversions. It has been demonstrated by O'Reilly (1970) that cats with detectable antibody do not show leucopaenia or depression of leucocytes after challenge with virulent virus. Conversely, he showed that cats without antibody at the time of challenge become leucopaenic or exhibit a depression in the number of circulating white blood cells and the surviving animals develop antibody.

This paper confirms the necessity for testing new living vaccines for contact spread of vaccine virus to animals afflicted with some intercurrent infectious disease. In such conditions, the test animals are likely to be under greater strain

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than healthy laboratory animals, a situation more closely resembling the natural environment.

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