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Determination of an optimal dilution of virulent feline infectious enteritis (panleucopaenia) virus for challenge purposes

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SUMMARY

When ten cats were infected orally with undiluted or a 10^{-1} dilution of virulent feline infectious enteritis (panleucopaenia) virus, all developed severe leucopaenia followed by the development of demonstrable antibody, but none died. Eighteen of 29 cats given a 10^{-2} dilution of virus died of the disease. Three of the survivors had white blood cell counts of less than 4000 and three had counts between 4000 and 6000 cells. Although the remaining five animals never had individual counts of less than 6000 cells, the geometric means of these counts showed that a marked depression in the leucocyte counts had occurred. All surviving cats developed antibody.

Among the ten cats dosed with either 10^{-3} or 10^{-4} dilution of virus, four died of feline infectious enteritis and three developed antibody after falls in the leucocyte counts. It is suspected that low dilutions of feline infectious enteritis virulent virus in cats produce a phenomenon similar to that reported by von Magnus (1954) with influenza virus in eggs.

Leucopaenia is commonly defined as less than 4000 white blood cells/mm.^{3} of blood. Counts lower than this are usual in cats which either die of the disease or have received large doses of virus; they are less common in cats surviving after administration of diluted virus. Challenge of cats with pre-existing antibody did not provoke a depression in the leucocyte counts.

INTRODUCTION

Over a number of years experimental infection of young cats with a 1/10 suspension of feline tissues infected with virulent feline infectious enteritis (FIE) (panleucopaenia) virus has produced leucopaenia in about half of the animals and death in less than one-quarter. These cats, aged between 10 and 16 weeks, were bred and reared in isolation, and were believed to be fully susceptible to the disease. Recently, O'Reilly, Paterson & Harriss (1969) have shown that, even in the absence of detectable antibody, not all cats will respond to a single dose of living attenuated FIE vaccine inoculated before the age of 12 weeks. Although these findings provide a partial explanation for the poor infection rate recorded above, they do not satisfactorily explain the low mortality rate. It was decided, therefore, to investigate the possibility that there is an optimal dilution at which virulent FIE virus should be used in order to cause the greatest number of deaths among antibody-free cats aged 12 or more weeks.

MATERIALS AND METHODS

Cats

The cats used in these experiments were bred in isolation at the Wellcome Veterinary Research Station, Frant, and all were 12 or more weeks old at the time of infection. Blood for leucocyte counts was obtained from the peripheral vein of the ear. When sera were required, the cats were bled from the jugular vein, using the method of restraint described by Hovell, O'Reilly, Calder & Povey (1970).

Experimental

Groups of five cats were used in the series of experiments to determine the dilution of virulent FIE virus that caused the highest mortality rate. Each cat was infected orally with 1·0 ml. of the appropriate dilution of virus, group A with 10^0, group B with 10^{-1}, group C with 10^{-2}, group D with 10^{-3} and group E with 10^{-4}. The groups were maintained in separate rooms and strict precautions taken to preserve isolation (O'Reilly, 1970).

Neutralization test

Sera were inactivated at 56° C. for 30 min., filtered and then stored at −20° C. until tested for neutralizing antibody by the method of O'Reilly *et al.* (1969).

Challenge virus

Small intestine, spleen and faeces from a naturally infected case of FIE were ground in a mortar with sterile sand and a 10 % suspension prepared in phosphate-buffered saline (PBS) containing 2000 units of penicillin and 1000 μg. of streptomycin/ml. The suspension was centrifuged at 3000 rev./min. for 15 min. and the supernatant stored at −20° C. Dilutions of virus were prepared in PBS.

RESULTS

The geometric means of the daily leucocyte counts of group A and group B cats showed a similar pattern of fall and rise after infection and have been combined (Fig. 1). During the first 4 days, the counts fell from 9000 to 4100. After a further 4 days, during which the counts did not exceed 5100 cells, there was a rapid increase. Although there were no deaths from FIE, three cats were destroyed on the 11th day because of severe respiratory disease and sera from these and the remaining animals (killed on the 15th day) had antibody to FIE virus (Table 1).

In group C a fall from 12,900 to 5300 in the geometric means of the leucocyte counts was seen between the 1st and 6th days after infection. The count then rose to 11,900 by the 10th day. On the next day, however, there was a drop to 7500 followed by a gradual rise during the next 2 days (Fig. 2). One cat died of FIE on the 7th day. When the four surviving cats were killed on the 15th day, three possessed significant amounts of antibody. The 4th cat's antibody titre ($\pm$ 1/8) was of doubtful significance (Table 1).

In group D the geometric means of the leucocyte counts of the cats (Fig. 3) showed the same biphasic fluctuation as seen in group C. The initial fall by 5900

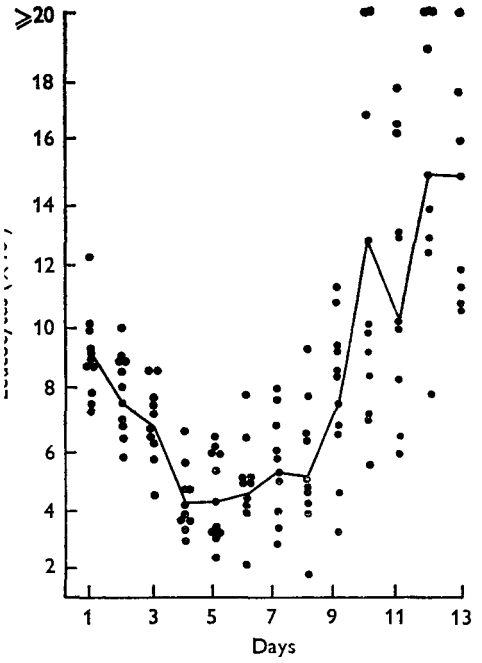

Fig. 1

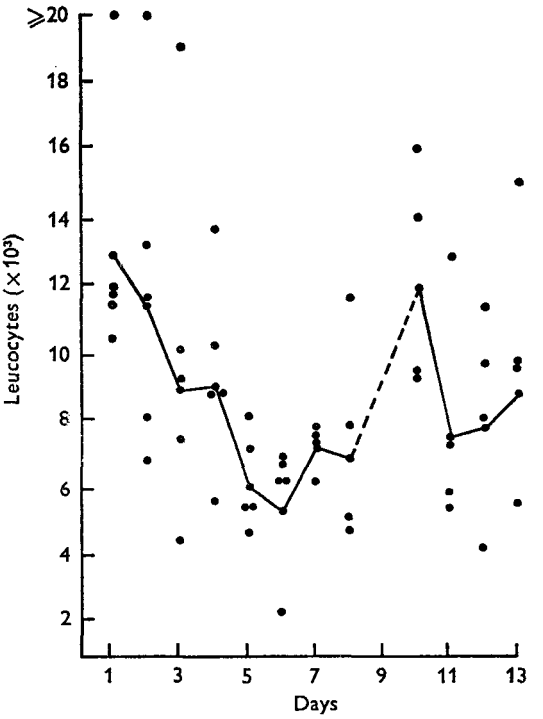

Fig. 2

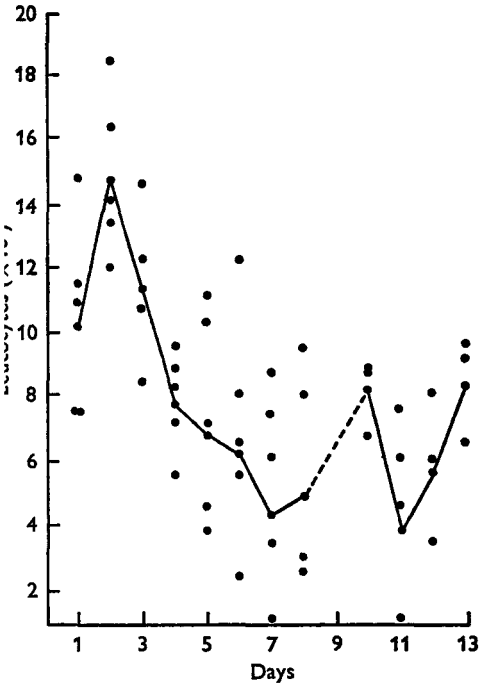

Fig. 3

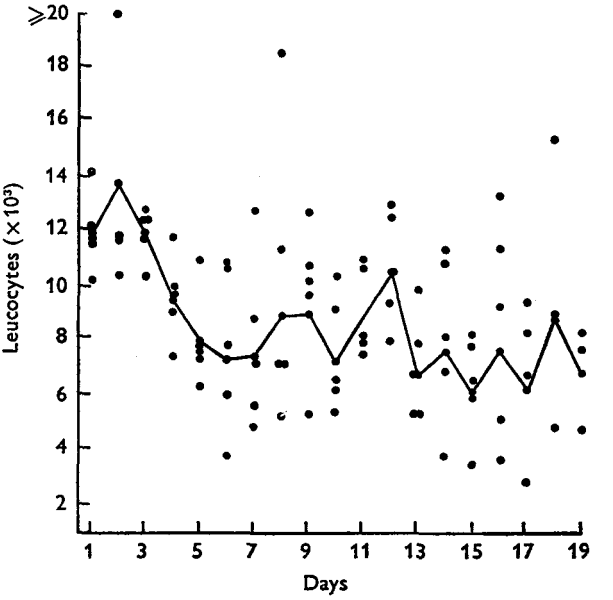

Fig. 4

Figs. 1–4. The individual and geometric means of daily leucocyte counts of cats infected orally with a suspension of tissues taken from a naturally infected case of feline infectious enteritis or a dilution of this suspension. Fig. 1. The pooled results of those given undiluted and 10^{-1} dilution (groups A, B). Fig. 2. 10^{-2} dilution (group C). Fig. 3. 10^{-3} dilution (group D). Fig. 4. 10^{-4} dilution (group E).

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to 4300 cells during the first 7 days was followed by a rise to 8100 on the 10th day, after which another drop was observed. During the next 3 days, the count increased from 3800 to 8400. One cat died of FIE on the 8th day and another on the 12th day, and two of the three surviving cats had antibody when bled on the 15th day (Table 1).

Table 1. *Antibody responses of cats infected orally with different dilutions of virus*

Group	Dilution of virus suspension	Antibody titres after infection (days) 0	11	15	20	Remarks
A	10^{0}	< 8	—	128	—	—
		< 8	—	128	—	—
		< 8	—	128	—	—
		< 8	128	—	—	Killed—respiratory disease
		< 8	128	—	—	Killed—respiratory disease
B	10^{-1}	< 8	—	32	—	—
		< 8	—	32	—	—
		< 8	—	128	—	—
		< 8	—	128	—	—
		< 8	128	—	—	Killed—respiratory disease
C	10^{-2}	< 8	—	± 8	—	—
		< 8	—	32	—	—
		< 8	—	128	—	—
		< 8	—	512	—	—
		< 8	—	—	—	Died FIE day 7
D	10^{-3}	< 8	—	< 8	—	—
		< 8	—	128	—	—
		< 8	—	128	—	—
		< 8	—	—	—	Died FIE day 8
		< 8	—	—	—	Died FIE day 12
E	10^{-4}	< 8	—	—	< 8	—
		< 8	—	—	< 8	—
		< 8	—	—	8	—
		< 8	—	—	—	Died FIE day 17
		< 8	—	—	—	Died FIE day 18

In group E during the first 6 days after infection, the geometric means of the leucocyte counts fell from 12,000 to 7300 cells; thereafter, the count fluctuated between 6000 and 9000 (Fig. 4). Specific FIE deaths occurred on the 17th and 18th days and only one of the remaining three cats had antibody on the 20th day (Table 1).

Among the cats infected with undiluted or a 10^{-1} dilution of virus, there were no FIE deaths, but specific deaths did occur after the 10th day in cats infected with 10^{-3} or 10^{-4} dilution of virus. Since deaths were confined to the higher dilutions, the 10^{-2} dilution of virus was tested further to establish the suitability of this dilution for challenge purposes. The results now recorded were accumulated from a series of experiments and include the five cats in group C above. Twenty-nine susceptible cats were infected orally and 18 (62 %) died of FIE after showing clinical symptoms of the disease. Seventy-two per cent (13/18) of the deaths

occurred on the 7th and 8th days (Table 2). One cat died of pneumonia on the 14th day. When bled 11–18 days after infection, nine of the survivors had demonstrable antibody titres and the 10th cat (from group C) an indication of antibody (Table 3).

Table 2. *Distribution of specific deaths among* 29 *susceptible cats infected orally with* 10^{-2} *dilution of virulent FIE virus*

Days after infection	⩽ 5	6	7	8	9	10	⩾ 11
Number of cats dying	0	2	8	5	2	1	0

Table 3. *Number of days on which the leucocyte counts fell below* 6000 *cells/mm.*^{3} *in* 11 *cats surviving more than* 10 *days after infection with* 10^{-2} *dilution of virus, and the antibody titres*

Number of days on which leucocyte counts were			Post-challenge antibody*	
< 4000	4000–< 5000	5000–< 6000	Day of bleeding	Titre
4	1	1	11	128†
4	1	0	—	Died‡
3	0	0	18	128
0	1	2	15	± 8
0	1	1	15	128
0	1	1	15	512
0	0	0	15	32
0	0	0	12	32
0	0	0	15	128
0	0	0	12	128
0	0	0	12	8

* All cats were devoid of detectable antibody at challenge.
† Reciprocal of serum dilution.
‡ Died of pneumonia on the 14th day after infection.

Of the 11 cats which survived this challenge, three recorded leucocyte counts of less than 4000 and three between 4000 and < 5000. Fewer than 4000 leucocytes were seen most commonly in cats immediately before death and less frequently in those which survived. The five cats (Table 3) with counts in excess of 6000 all showed a depression in the number of circulating cells between the 4th and 7th days after challenge with the lowest mean count of 8200 on the 6th day (Fig. 5). The lowest single count recorded was 6200 on the 4th day and over the next 4 days, two other cats had counts in the region of 7000.

Twelve cats with antibody resulting from vaccination were challenged with 10^{-2} dilution of virus. Two of the cats had each received one dose of a live attenuated vaccine 21 days before challenge, and the remainder 2 doses of inactivated vaccine 42 and 21 days before challenge. Challenge of these cats with virulent virus produced neither leucocyte depression (Fig. 6) nor an anamnestic serological response (Table 4).

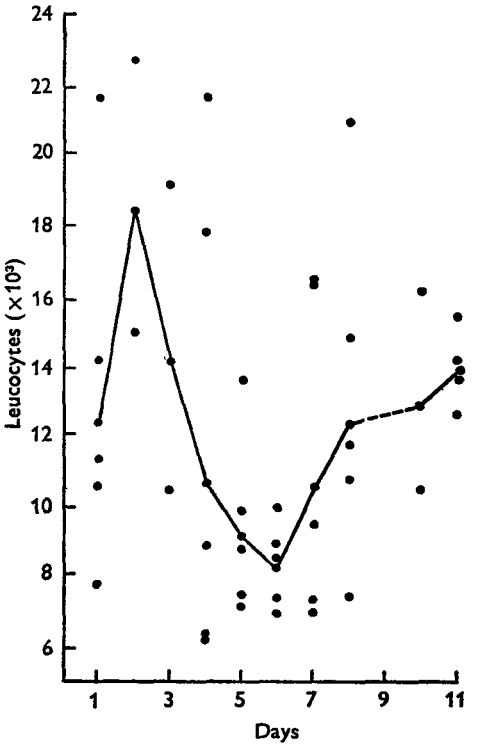

Fig. 5

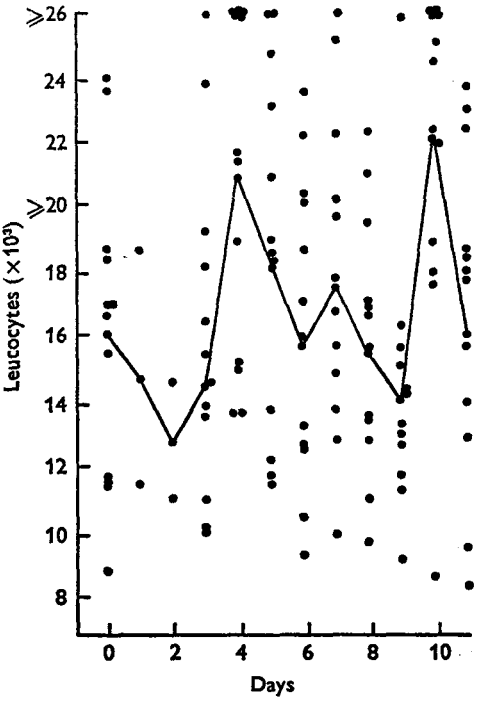

Fig. 6

Fig. 5. The individual and geometric means of leucocyte counts of five of eleven antibody-free cats whose leucocyte counts after challenge with 10^{-2} dilution of the suspension were depressed, but did not fall below 6000 cells/mm³.

Fig. 6. The individual and geometric means of leucocyte counts of 12 cats with pre-existing antibody before challenge with 10^{-2} of the suspension. Note that there was no depression of the leucocyte counts.

Table 4. *Antibody titres of 12 vaccinated cats before and after challenge with 10^{-2} dilution of FIE virus*

Type of vaccine	Time in days between pre- and post-challenge sera	Antibody titres	
		Pre-challenge	Post-challenge
1 dose of attenuated	14	512	128
		128	32
2 doses of inactivated	18	8	8
		8	32
		32	32
		32	128
		128	128
		128	128
		128	128
		128	128
		128	128

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DISCUSSION

The oral administration of either undiluted or a 10^{-1} dilution of virus caused cats to become infected with FIE, as shown by the severe falls in the numbers of circulating white blood cells and the development of antibody. However, none of them died. On the other hand, the higher dilution of virus, which failed to produce antibody in all dosed cats, did cause some specific FIE deaths. These findings suggest that low dilutions of virus are less likely to cause mortality, possibly resulting from 'auto-interference'. Von Magnus (1954) first described a similar phenomenon when he injected undiluted influenza virus into the allantoic cavity of embryonating hens' eggs and found the harvested fluid had low infectivity but high haemagglutinating activity. Inoculation of high dilutions of influenza virus, however, yielded viral particles of high infectivity and haemagglutinin.

Many cats clinically ill with FIE will have leucocyte counts below 4000 cells; some will die and the others recover. Seventy-three per cent (8/11) of the survivors in the 10^{-2} dilution of virus challenge experiments never had leucocyte counts below 4000. Nevertheless, all survivors must have been infected, for they exhibited a depression in the white blood cell counts and subsequently developed antibody. Thus, it appears that infection of susceptible cats with FIE always causes a depression in the leucocytes, whereas challenge of cats with antibody does not provoke a drop in the total of the circulating white blood cells. Since a number of cats may show a rise in leucocytes on the 2nd day after infection (Figs. 3–5) (Riser, 1947), any subsequent depression should be related to the cell count on the 1st day after infection.

Among the ten cats dosed with 10^{-3} or 10^{-4} dilution of virus, there were three specific deaths which occurred after the 11th day. Lawrence & Syverton (1940) found virus present in experimentally infected cats on the 2nd, 3rd and 5th days but not on the 9th day after infection. O'Reilly (1970) has shown that virus was present in the tissues of a cat 4 days after exposure to infection and that an in-contact cage mate to another deliberately infected cat had a leucocyte count of 3800 five days after the infected cat was leucopaenic. He has also found antibody as early as 7 days after vaccination (unpublished results). Presumably, viraemia, as in most other virus infections, is of short duration and coincides with the fall in the leucocyte counts. It is reasonable, therefore, to assume that those cats which died 12, 17 and 18 days after dosing were secondary and not primary cases of FIE. Among the cats dosed with a 10^{-2} dilution of virus, that which had an antibody titre of ± 8 (Table 1) had its lowest leucocyte count on day 12 (Fig. 3). This cat may well have been a secondary case of FIE which would explain the low antibody titre when it was bled 3 days later.

In these experiments the optimal dilution of FIE virus giving an effective challenge was 10^{-2}. It caused depression of the leucocyte counts, specific deaths of more than 50% of the animals within the first 9 days and the development of antibody in the survivors.

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