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TECHNICAL MANUSCRIPT 484

THE CLINICAL ASPECTS
OF RIFT VALLEY FEVER VIRUS
IN HOUSEHOLD PETS:
II. SUSCEPTIBILITY OF THE CAT

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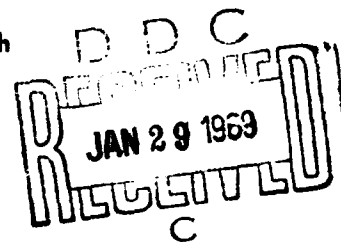
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DECEMBER 1968

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

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THE CLINICAL ASPECTS OF RIFT VALLEY FEVER VIRUS IN
HOUSEHOLD PETS: II. SUSCEPTIBILITY OF THE CAT

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AGENT DEVELOPMENT & ENGINEERING LABORATORIES

Project 1B533001D426

December 1968

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Challenge with Rift Valley fever virus (RVFV) resulted in an 81% mortality in kittens 21 days of age or younger, whereas it produced only a subclinical infection in 84-day-old kittens and adult cats. Infection in the latter groups was demonstrated by the presence of serum neutralizing antibodies. Thus, kittens 3 weeks of age or younger are as susceptible as lambs and more susceptible than calves to RVFV. Other data indicated (i) the possible existence of cross-immunity between RVFV and some other, as yet unknown, entity and (ii) the possibility of both horizontal (kitten-to-kitten) and ascending (kitten-to-adult) transmission of RVFV. The epidemiological implications of the study are discussed.

I. INTRODUCTION

A previously published paper describes the effects of Rift Valley fever virus (RVFV) infection in the dog.¹ The dog proved to be highly susceptible to RVFV and capable of acting as a reservoir and carrier of the disease; puppies up to 14 days old died following challenge with RVFV. Accordingly, a similar study was undertaken with cats to (i) more fully document the susceptibility of common household pets to RVFV and (ii) further elucidate their role as carriers or transmitters of the disease.

II. MATERIALS AND METHODS

A. ANIMAL PROCUREMENT, HANDLING, AND PARTURITION

The cats used in this study were procured through the Fort Detrick Animal Farm, a central animal-receiving area. All adult cats were immunized against feline panleukopenia virus prior to delivery to the laboratory. Each adult was placed in an individual wire cage to prevent direct contact between animals.

The 14 adult cats procured (Table 1) included the five pregnant females that bore all of the kittens used in the 2-, 7-, and 21-day-old studies. These kittens were born in the laboratory so their exact ages could be accurately recorded. Cat No. 13 bore the 2-day-old litter (kittens 1 through 4, Fig. 1). The 7-day-old kittens (Fig. 1) included two (No. 5 and 6) borne by Cat No. 6, two (No. 7 and 8) borne by Cat No. 7, and four (No. 9 through 12) borne by Cat No. 14. Cat No. 11 bore the 21-day-old litter (No. 13 through 16, Fig. 2).

The kittens used in the 84-day-old study (No. 17 through 21, Fig. 2) were procured just after weaning, so they could be received, processed, and caged in the laboratory sufficiently in advance of the time required for use.

B. VIRUS PROPAGATION

The van Wyk strain² of RVFV was used for all challenges and serum neutralization studies. The virus was propagated in a monolayer tissue culture system, using a mouse cell line grown in medium 199 peptone plus 10% bovine serum at pH 7.8. Test serum and blood samples were routinely diluted in a medium composed of 60% Hanks balanced salt solution, 30% medium 199 peptone, and 10% bovine serum (v/v) at pH 7.8.

C. BIOASSAY METHODS

1. Viremia

Tenfold dilutions (10^{-1} , 10^{-2} , and 10^{-3}) were made on whole heparinized blood (1,000 USP units per ml of Na heparin, Upjohn Co.), and eight 0.03-cc samples of each dilution were injected intracranially into 10- to 12-g Swiss-Webster mice. The Spearman-Kärber method³ was used to calculate mouse intracranial lethal doses (MICLD₅₀).

2. Serum Neutralization

Serum neutralizing antibody to RVFV was demonstrated by combining equal volumes of undiluted, inactivated serum (56 C for 30 minutes) and serial tenfold dilutions of virus. The inactivated serum-virus mixture was incubated in a 37 C water bath for 1 hour before mouse inoculation. The log serum neutralization index (LSNI) was calculated as the difference between the MICLD₅₀ titer of RVFV in the presence of known negative serum and the test serum. The LSNI was considered positive for values of 1.0 or greater. Known negative and positive sera for RVFV antibody were used in each assay as negative and positive control samples.

D. EXPERIMENTAL PROCEDURES

All animals were challenged subcutaneously with 0.5 ml of virus at concentrations of 2.2, 4.2, 6.2, or 8.2 log₁₀ MICLD₅₀. Controls were injected with 0.5 ml of diluent.

Prior to and at 21 days postchallenge, blood (20 cc) was collected from each adult cat. The blood was allowed to clot, the serum was collected, and the LSNI was determined. Heparinized whole blood (2.5 cc) was collected from each test animal twice during the 1st week postchallenge, and viremias were determined. Rectal temperatures were recorded prechallenge and for 7 to 10 days postchallenge. All animals were necropsied, and vital tissues were examined grossly and histopathologically.

III. RESULTS

A. 2-, 7-, AND 21-DAY-OLD KITTENS

Virus challenge caused death in 13 (81%) of 16 kittens 21 days of age or younger. The mean time to death was 6 days, with a range of 1 to 10 days.

Three of four 2-day-old kittens died, two within 2 days postchallenge and one at 8 days postchallenge. No particular temperature response pattern or trend was noted for this age group (Fig. 1), but all three animals that died were hypothermic at death, and the lone survivor was hyperthermic on the last day that temperature was recorded.

Six of eight 7-day-old kittens died. One died at 1 day postchallenge, the other five from 7 to 10 days postchallenge. Generally, this age group exhibited a febrile response that tended to peak about 6 to 7 days postchallenge (Fig. 1). However, the six animals that died were hypothermic at death, and the temperatures of the two survivors were about normal on the last day that temperature was recorded.

All four 21-day-old kittens that were challenged died from 4 to 6 days postchallenge. Three of these were hypothermic at death, while the temperature of the fourth was essentially normal (Fig. 2).

Death in the 2-, 7-, and 21-day-old groups was preceded by a typical progressive clinical syndrome. The kittens first became ataxic about 24 hours prior to death. As the infection progressed, the kittens lost the righting reflex and began to paddle. Pathological examinations showed central nervous system lesions consistent with these clinical manifestations.*

One 7-day-old kitten that died (No. 12, Fig. 1) was the only one among these three age groups with a demonstrable viremia. It had a virus concentration of $2.77 \log_{10}$ MICLD₅₀ per ml of blood at 7 days postchallenge.

B. 84-DAY-OLD KITTENS AND ADULT CATS

Five 84-day-old kittens and 14 adult cats were used (Fig. 2-4; Table 1).

Four of the 84-day-old kittens were challenged, and one was not. All five were caged together following challenge, and the unchallenged kitten died after 7 days of contact with its challenged age-group mates. The unchallenged kitten (No. 17, Fig. 2) was the only one in this age group that died, was slightly hypothermic throughout the 7-day observation period, and showed histopathological evidence at necropsy similar to that found in kittens dying of RVFV.* The circumstances of this kitten's death indicate possible horizontal (kitten-to-kitten) transmission of the virus.¹

* Mitten, J.Q., personal communication.

Age, Days

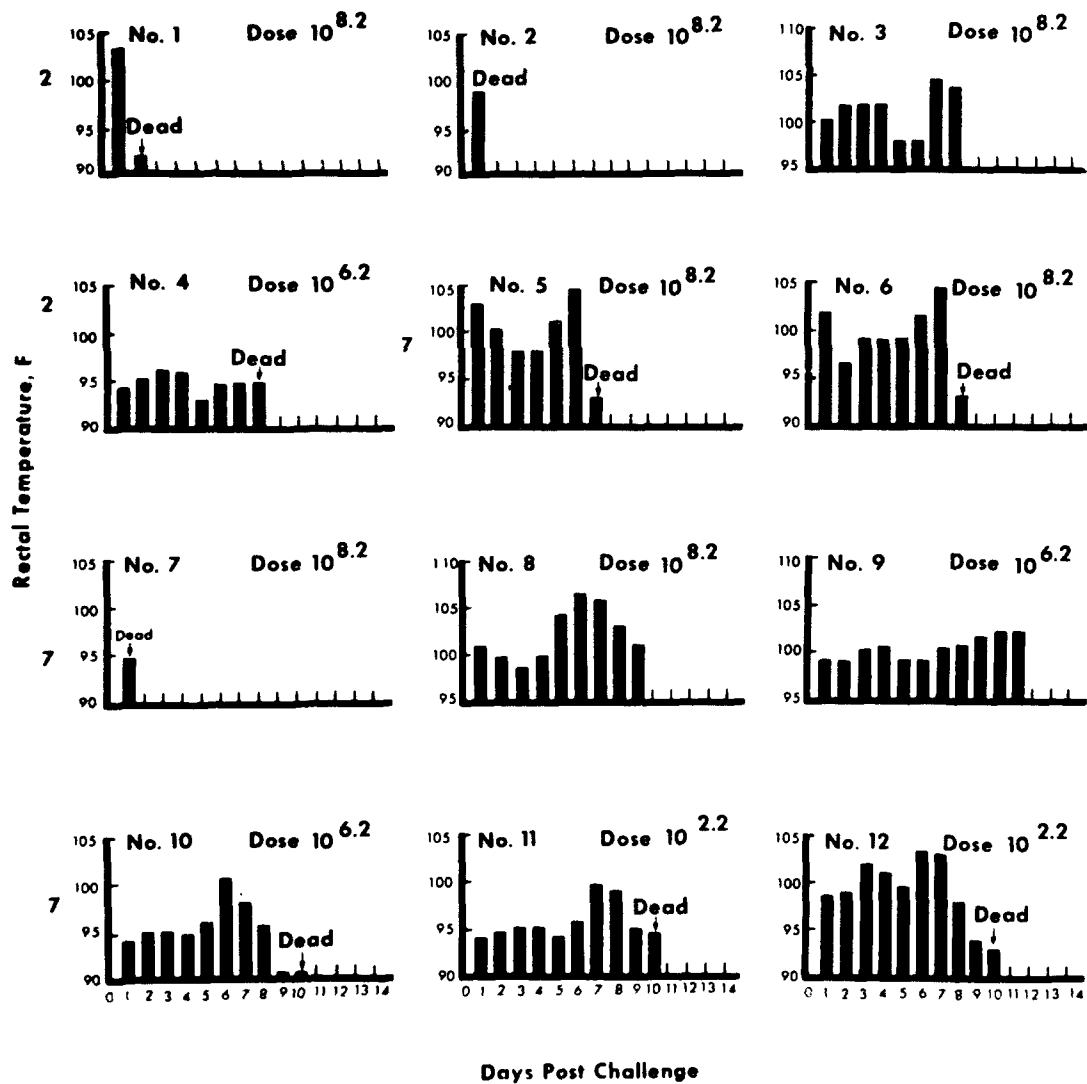


FIGURE 1. Temperature versus Time in 2- and 7-Day-Old Kittens Challenged with Various Doses of Rift Valley Fever Virus.

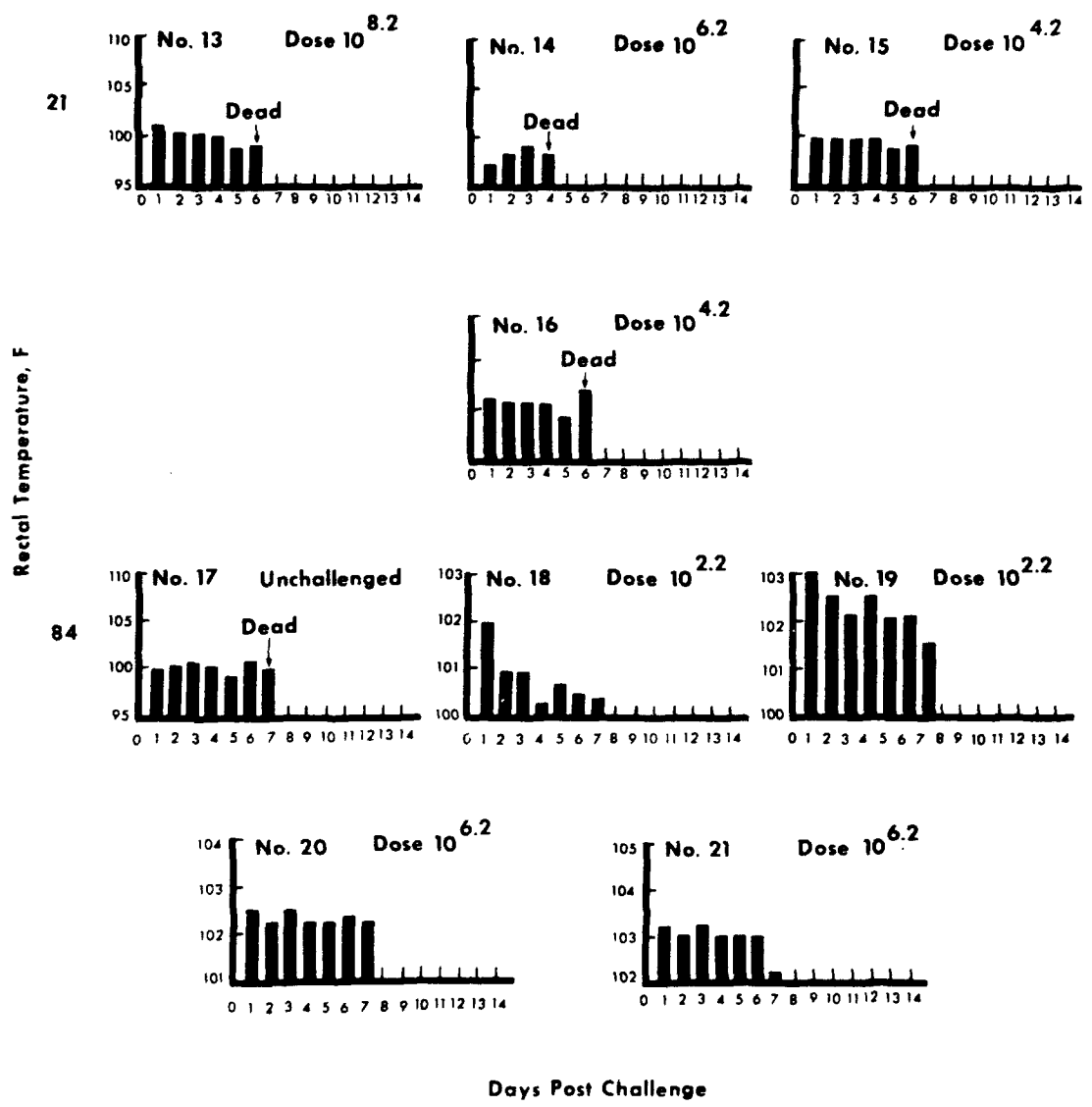


FIGURE 2. Temperature versus Time in 21- and 84-Day-Old Kittens Challenged with Various Doses of Rift Valley Fever Virus.

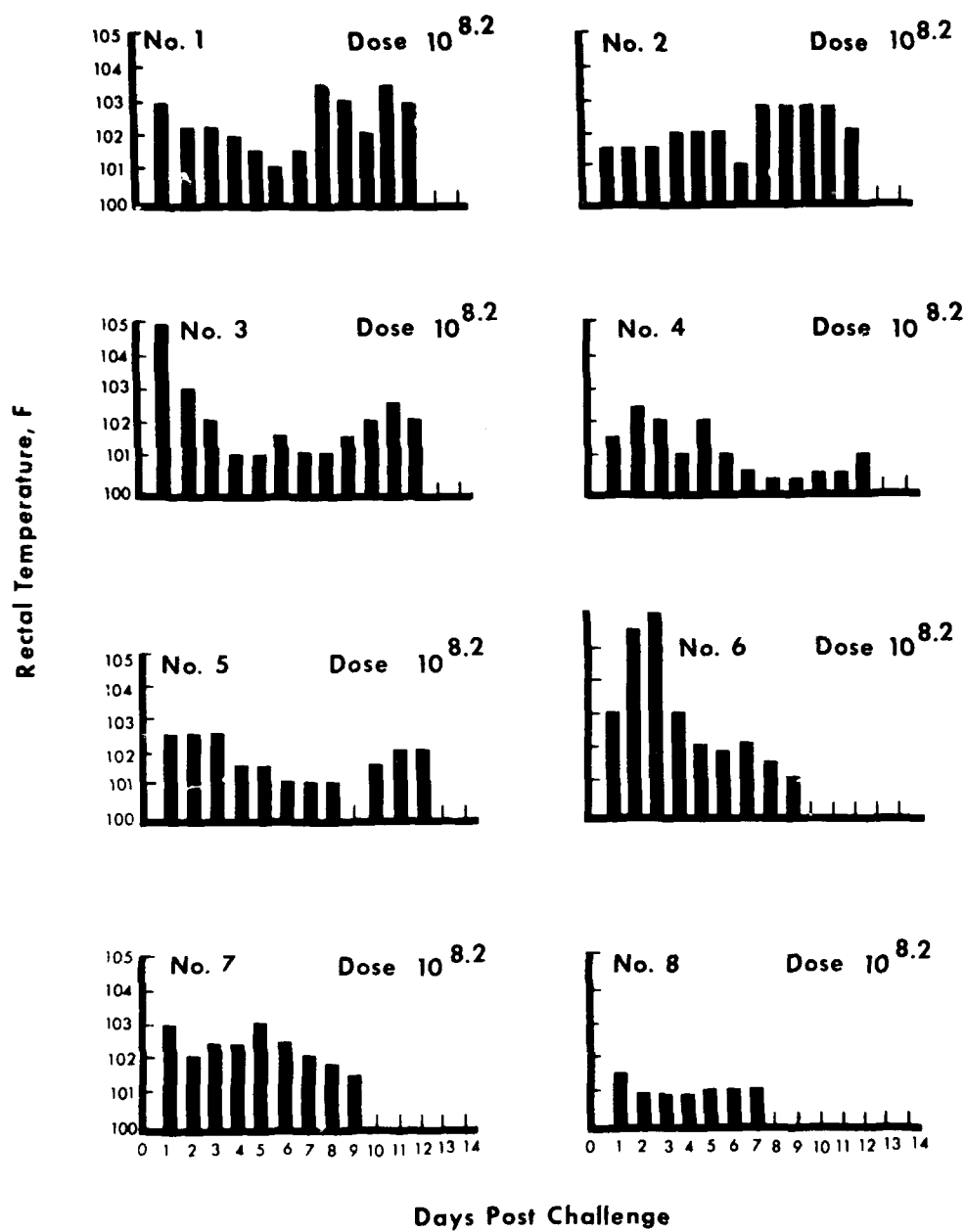


FIGURE 3. Temperature versus Time in Adult Cats Challenged with High Doses ($10^{8.2}$) of Rift Valley Fever Virus.

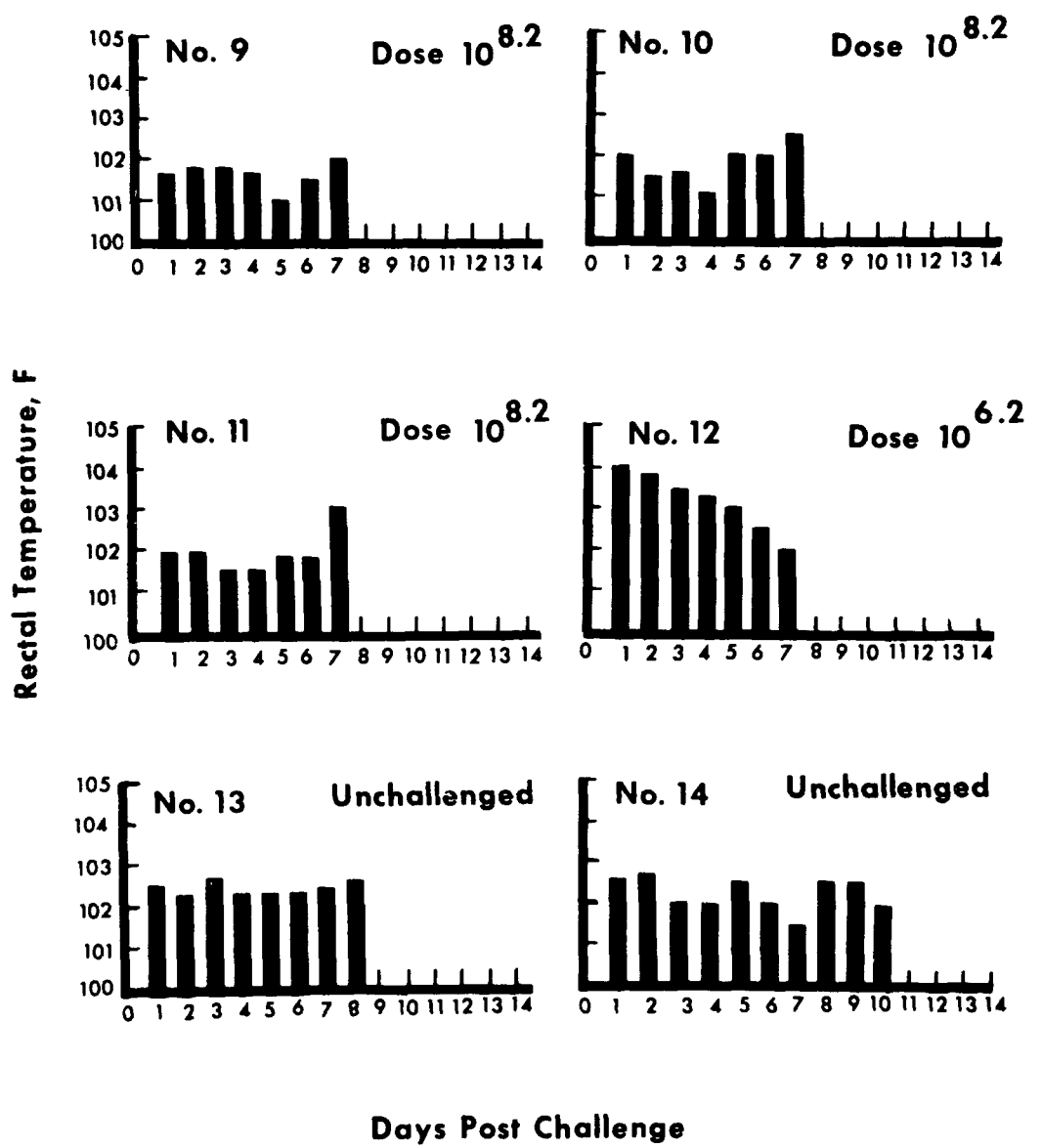


FIGURE 4. Temperature versus Time in Adult Cats Challenged with High Doses ($10^{6.2}$, $10^{8.2}$) of Rift Valley Fever Virus and in Two Unchallenged Adults.

TABLE 1. SERUM NEUTRALIZATION RESPONSES IN 84-DAY-OLD KITTENS
AND ADULT CATS AFTER SUBCUTANEOUS CHALLENGE
WITH RIFT VALLEY FEVER VIRUS

Animal	Challenge Dose, $\log_{10}$ MICLD ₅₀	Log Serum Neutralization Index	
		Prechallenge	21 Days Postchallenge
Kitten No. 17	0.0	0.4	1.40
18	2.2	0.4	ND ^a /
19	2.2	0.8	ND
20	6.2	0.4	ND
21	6.2	0.8	ND
Adult No. 1	8.2	- ^b /	2.37
2	8.2	-	1.37
3	8.2	-	2.00
4	8.2	-	2.25
5	8.2	-	1.87
6 ^c /	8.2	ND ^a /	2.57
7 ^c /	8.2	ND	2.29
8	8.2	ND	1.12
9	8.2	ND	0.50
10	8.2	ND	-
11 ^c /	8.2	1.63	-
12	6.2	2.13	-
13 ^c /	0.0	1.63	ND
14 ^c /	0.0	1.20	0.88

a. No data.

b. - = negative.

c. Litter mothers.

The temperature responses of the four surviving 84-day-old kittens showed no particular pattern, but the temperatures of three were somewhat above, and of the fourth somewhat below, normal during the 7 days that temperatures were recorded (Fig. 2).

Twelve of the 14 adult cats were challenged, and two were not. None of the adults died. Generally, the temperature response among adults exhibited no marked pattern, although there was a tendency toward slightly elevated temperatures. Except for increasing levels of specific neutralizing antibodies, as evidenced by pre- and postchallenge serum neutralization studies, neither the 84-day-old kittens nor the adult cats showed any frank clinical symptoms of disease.

Five adult cats (No. 1 through 5, Table 1) showed a definite rise in LSNI between the pre- and postchallenge serum samples. The postchallenge LSNI values for these five ranged from 1.37 to 2.37, indicating that virus infection had occurred. Postchallenge antibodies were not demonstrated in at least three of four adult cats (No. 11 through 14) that had exhibited prechallenge LSNI values ranging from 1.20 to 2.13.

Two adult cats (No. 6 and 14, Table 1) had demonstrable viremias and also had positive LSNI either pre- or postchallenge. However, complete and reasonably accurate interpretation of the data for these two animals is impossible because: (i) both were females that bore litters used in previous portions of this study, (ii) data were incomplete, and (iii) cross-immunity between RVFV and some other unknown entity was possible.

Cat No. 6 bore two of the kittens used in the 7-day-old study, and, unfortunately, data on viremia and LSNI were not obtained for this animal prior to its challenge in the adult study. This adult was challenged with $108.2 \log_{10}$ MICLD₅₀ per ml of virus and had a virus concentration of $4.19 \log_{10}$ MICLD₅₀ per ml of blood on the 2nd day postchallenge. However, because virus clearance studies were not done, it is impossible to state whether this viremia was the result of (i) ascending transmission, (ii) challenge, or (iii) a combination of both of the preceding.

Cat No. 14 bore four of the kittens used in the 7-day-old study. Seven days postchallenge of its litter, this adult had a virus concentration of $2.77 \log_{10}$ MICLD₅₀ per ml of blood, indicating a possible case of ascending (kitten-to-adult) transmission.¹ This cat also was one of the two unchallenged animals in the adult study, and it had an LSNI of 1.20 and 0.88 (the latter considered negative) at the pre- and postchallenge times, respectively, of the adult study.

IV. DISCUSSION

The limited data resulting from this study do indicate several findings either not previously or only incompletely documented. The response of kittens 3 weeks of age or younger to RVFV challenge corresponded to 4+ (100% or nearly 100% fatal) in Findlay's classification.⁴ Thus, kittens in this age group apparently are as susceptible as lambs and more susceptible than calves. On the other hand, 84-day-old kittens and adult cats appear to be susceptible to infection, as indicated by the presence of serum neutralizing antibody, but resistant to the subsequent development of the disease, although these assumptions must be considered quite tentative in view of the limitations in the data previously noted.

The prechallenge LSNI in some of the adult cats (No. 11 through 14) indicates possible explanation for the apparently low degree of correlation among age, dose, and time to death in the kittens. Three of these adults bore 12 of the kittens used in the 2-, 7-, and 21-day-old studies, and it is interesting to note that two of the three surviving kittens in those three age groups were borne by two of these adults. The data may indicate the possible existence of cross-immunity between RVFV and some other, as yet unknown, entity. Here again, unfortunately, the lack of LSNI data for five of the adults leaves the question of virus interaction and cross-immunity unanswered. If the possibility of cross-immunity is borne out by further work, it will complicate the diagnosis of RVFV infection by serum neutralization tests. Some preliminary data concerning this question of cross-immunity are presented in the Appendix.

As noted in the results, one unchallenged kitten and one unchallenged adult exhibited evidence of RVFV infection as a probable result of direct transmission from other infected animals. Remmele et al.¹ demonstrated direct transmission in dogs. The data presented here, although limited, indicate that direct transmission may occur in cats, and this possibility warrants further investigation.

The fact that manifestations of RVFV infection in 84-day-old kittens and adult cats are inapparent or subclinical assumes importance when one considers the possible epidemiological role of cats as reservoirs or carriers in association with arthropods and susceptible populations of sheep, cattle, and humans. Again, the data presented here are limited, and this potential role of the cat also warrants further investigation.

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2. Kaschula, V.R. 1953. The propagation and modification of strains of Rift Valley fever viruses in embryonated eggs and their use as immunizing agents for domestic ruminants. Thesis, Doctor of Veterinary Science, University of Pretoria, South Africa.
3. Finney, D.J. 1952. Statistical methods in biological assay, p. 524-530. Hafner Publishing Company, New York.
4. Findlay, G.M. 1932. Rift Valley fever or enzootic hepatitis. Trans. Roy. Soc. Trop. Med. Hyg. 26:157-160.

APPENDIX

A PRELIMINARY STUDY OF CROSS REACTIONS IN RIFT VALLEY FEVER VIRUS INFECTION

The limited studies on susceptibility of the cat to Rift Valley fever virus (RVFV) indicated the possible existence of cross-immunity between RVFV and some other unknown entity. Because all adult cats used in that study were routinely immunized against feline panleukopenia virus (FPV) prior to delivery to our laboratory, the FPV vaccine seemed the most likely cause of this cross reaction. Thus, a limited investigation was undertaken to evaluate this possibility.

A. ANIMALS

Swiss-Webster strain mice were used. They weighed 6 to 8 g at the beginning of immunization and 16 to 18 g at the time of challenge.

B. IMMUNIZATION PROTOCOLS

The mice were injected intraperitoneally (IP) with one of the following three vaccines:

- 1) Rift Valley fever virus vaccine (RVFVV) prepared in monkey kidney tissue culture and inactivated with formalin, obtained from the Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.
- 2) Feline panleukopenia virus vaccine (FPVV-1) prepared as feline tissue homogenate, inactivated with formalin, and preserved in oil, obtained from Philips Roxane, Inc., St. Joseph, Missouri.
- 3) Feline panleukopenia virus vaccine (FPVV-2) prepared in tissue culture and inactivated with formalin, obtained from Corn States Laboratories Inc., Omaha, Nebraska.

The different immunizing protocols and challenge procedures are described in Table 1. Control animals were given injections of sterile distilled water. Two tests were run with FPVV-1, the homogenized-feline-tissue product, and one test with FPVV-2, the tissue-culture product.

C. CHALLENGE

All animals were challenged IP with the wild pantropic van Wyk strain of Rift Valley fever virus.* Dose levels usually covered six logs of virus and were adjusted when necessary to compensate for resistance induced by the different vaccines.

D. MEASUREMENT OF VIRULENCE AND IMMUNITY LEVEL

All animals were observed for 10 days postchallenge, and the response to challenge was measured in mouse intracranial lethal doses (MICLD₅₀) calculated by the Spearman-Kärber method.** The levels of immunity were described in terms of a Resistance Index, which represented the log₁₀ difference (protection) in the MICLD₅₀ for immunized and control animals. Comparisons between such Resistance Indexes for RVFV and the two FPV's indicated whether and to what degree the two FPV's may have provided cross-immunity against RVFV.

E. RESULTS

The Resistance Indexes of groups of mice given the three vaccines by different immunizing protocols are shown in Table 2. Both RVFV immunizing protocols gave comparable protection against RVFV. A similar degree of protection against RVFV was afforded mice by both protocols using undiluted FPV-1 as the immunogen. However, when FPV-2 was used, no cross-immunity against RVFV was evident.

The difference in the efficacy of the two FPV vaccines undoubtedly is due to differences in vaccine strains and preparation methods used by the two companies. Therefore, until the necessary serological studies are done to determine the effects of these differences in antigens and their preparation, little can be said concerning the role each plays in the cross reaction against RVFV. From this limited data, however, it is apparent that FPV vaccine does cross-react against RVFV in some cases, thus making dose-response data highly questionable.

* Kaschula, V.R. 1953. The propagation and modification of strains of Rift Valley fever viruses in embryonated eggs and their use as immunizing agents for domestic ruminants. Thesis, Doctor of Veterinary Science, University of Pretoria, South Africa.

** Finney, D.J. 1952. Statistical methods in biological assay, p. 524-530. Hafner Publishing Company, New York.

TABLE 1. SUMMARY OF INTRAPERITONEAL IMMUNIZATION AND CHALLENGE PROTOCOLS

Protocol Code ^a /	Vaccine Administered ^b , ml, on Indicated Day						Day of Challenge ^c /	
	1	3	7	9	11	Total	21	22
5RVFVV	0.008	0.008	0.008	0.008	0.008	0.04	X	
2RVFVV	0.02		0.02			0.04		X
5FPVV-1 & 5FPVV-2	0.08	0.08	0.08	0.08	0.08	0.4	X	
2FPVV-1 & 2FPVV-2	0.2		0.2			0.4		X
2RVFVV	0.02		0.02			0.04		X
+ 2FPVV-1	0.2		0.2			0.4		X
2RVFVV	0.02		0.02			0.04		X
+ 2FPVV-2	0.2		0.2			0.4		X

- a. The initial number in each code indicates the number of injections of vaccine given and is used for convenient identification in Table 2.
- b. All RVFVV was administered as a 1:10 dilution; all FPVV was administered undiluted.
- c. Indicates days after the first injection of vaccine.

TABLE 2. RESPONSE OF MICE IMMUNIZED BY DIFFERENT PROTOCOLS
TO CHALLENGE WITH RVFV

Vaccines Compared	Immunization Protocol Code ^a /	Test	Log ₁₀ MICLD ₅₀ per ml ^b /	Resistance Index ^c /
RVFVV vs. FPVV-1	5RVFVV	1	5.40	1.60
		2	<2.70	
	2RVFVV	1	5.20	1.80
		2	4.80	2.40
	5FPVV-1	1	5.40	1.60
		2	5.80	1.40
	2FPVV-2	1	5.40	1.60
		2	5.80	1.40
	2RVFVV + 2FPVV-1	1	4.00	3.00
		2	5.20	2.00
	Controls	1	7.00	
		2	7.20	
RVFVV vs. FPVV-2	5RVFVV		4.00	2.80
	2RVFVV		5.00	1.80
	5FPVV-2		7.20	-0.40
	2FPVV-2		7.00	-0.20
	2RVFVV + 2FPVV-2		6.60	0.20
	Controls		6.80	

a. For descriptions of the various protocols, see Table 1.

b. All data are means for 30 animals.

c. Resistance Index = log₁₀ MICLD₅₀ of controls minus log₁₀ MICLD₅₀ of immunized group.

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13. ABSTRACT		
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