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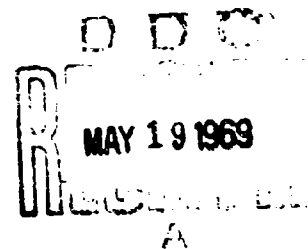
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TECHNICAL MANUSCRIPT 421

**THE CLINICAL ASPECTS OF
RIFT VALLEY FEVER VIRUS IN
HOUSEHOLD PETS: III. PATHOLOGIC CHANGES
IN THE DOG AND CAT**

John Q. Mitten
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APRIL 1969

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Process Development Division
AGENT DEVELOPMENT & ENGINEERING LABORATORIES

Project 1B562602A059

April 1969

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.

ACKNOWLEDGMENT

The senior author gratefully acknowledges the support of USPHS Fellowship 1-F2-FR-42,111-01 (PA).

ABSTRACT

A gross and histologic examination was performed on 13 kittens and 14 puppies that died after a subcutaneous inoculation of Rift Valley fever virus.

The significant gross lesions were confined to the liver and spleen. Both organs were congested, dark red, and very soft. On the serosal and cut surfaces of the liver, very faint gray to yellow foci with hyperemic edges were present. Petechiae and ecchymoses were observed on the serosal surfaces of the heart, mesenteric lymph glands, and mucosal surfaces of the gastrointestinal tract.

Focal hepatic necrosis was the most salient histologic change in the liver. The affected liver cells had swollen, eosinophilic, hyaline, or washed-out cytoplasm and pyknotic or fragmented nuclei. Contrary to observations in other species, there was a conspicuous absence of definite Councilman-like bodies and eosinophilic intranuclear inclusions.

Significant histologic changes were also found in the heart and brain. The lesions in the heart included acute and chronic myocarditis and necrosis. The histologic changes in the brain were essentially like those of other viral encephalitides and included focal gliosis and necrosis.

The problems encountered in diagnosing RVF in the canine and feline species are discussed, together with the important epidemiological aspects.

I. INTRODUCTION*

The pathologic changes that occur in natural and experimental Rift Valley fever (RVF) have been described for sheep, lambs, cattle, calves, and a variety of other species.¹⁻⁴ The primary lesion is in the liver and consists of focal necrosis, eosinophilic intranuclear granules, and hyaline eosinophilic cytoplasmic bodies very similar to the Councilman body in yellow fever. Experimental RVF as it occurs in the canine and feline species has now been characterized by Remmele et al.⁵ and Stephen et al.⁶ This report describes the pathologic changes that develop in these two species in response to inoculation of the RVF virus.

The pathology of the experimental disease in laboratory mice has been well documented.⁷⁻¹⁰ The pathologic changes occurring in Swiss-Webster mice are also reported here as control material for the disease in dogs and cats.

II. MATERIALS AND METHODS

A. VIRUS

The van Wyk¹¹ strain of RVFV was used for the inoculum. The virus was propagated in a monolayer tissue culture system, utilizing a permanent mouse cell line grown in 199 Peptone plus 10% calf serum at pH 7.8. All animals were inoculated subcutaneously with the appropriate dosage of the virus.^{5,6}

B. ANIMALS

The pathological materials used were derived from two epidemiological studies previously reported on RVF virus, that of Remmele et al.,⁵ who used 87 beagles of various ages and sex, and that of Stephen et al.,⁶ who observed 35 domestic cats of various ages. All of these animals were examined for pathology, and only those puppies and kittens that died 30 days postinoculation are subjects of the present study. All dogs older than 42 days were vaccinated for leptospirosis, infectious canine hepatitis, and rabies. The adult cats were vaccinated for infectious feline panleukopenia.

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Uninfected neonatal puppies and kittens housed under the same conditions as the experimental animals were the source of normal tissues for the pathologic studies. The tissues from one puppy and one kitten were collected at each of the following times: a few hours after birth, 24 hours, 72 hours, 1, 2, and 3 weeks of age. The control animals were sacrificed with sodium pentobarbital* and exsanguinated simultaneously.

Forty-five 10- to 12-g Fort Detrick strain Swiss-Webster mice, inoculated subcutaneously with $10^{5.89}$ MICLD₅₀ dose of the van Wyk wild uncloned strain of RVF, provided infected tissues for a control study of the pathologic morphogenesis of RVF. Five mice were killed by cervical fracture and examined immediately following the virus inoculation and at 12-hour intervals through 108 hours. The same pathologic methods described previously were employed. Mice that died naturally during this period were also examined.

C. PATHOLOGIC STUDIES

A complete gross examination was performed on each animal that died or was killed with intravenous sodium pentobarbital and simultaneously exsanguinated at the termination of each phase of the experiment. Blocks of liver, lung, spleen, kidney, adrenal glands, heart, gastrointestinal and reproductive tracts, visceral and peripheral lymph nodes, and brain were fixed in 10% buffered formalin and embedded in paraffin. Sections were cut at 1 to 5 μ and stained with hematoxylin and eosin.

* Nembutal, Abbott Laboratories, North Chicago, Illinois.

III. RESULTS

The tissues from the uninfected puppies and kittens were within normal limits for neonates and young animals of these two species.

The significant lesions in the mice were in the liver. The first hepatic changes were observed about 24 hours after inoculation and consisted of little more than swollen, very pale staining hepatocytes. By 60 hours the infection reached its peak both in numbers of spontaneous deaths and in severity of lesions. At this time the entire liver was extremely necrotic, together with large areas of hemorrhage, eosinophilic intranuclear inclusions, and Councilman-like bodies (Fig. 1-3).

Thirteen kittens and 14 puppies (Table 1) died within 10 days following the inoculation of the RVF virus. Three additional neonatal puppies were sacrificed over a 5-day period after receiving the virus. Lesions referable to the RVF virus were found only in these 30 animals. The tissues of all other dogs and cats used in these experiments and killed at various periods up to 30 days were unremarkable, although an occasional animal was infected with ascarids and/or cestodes. Henceforth, the results expressed will relate only to these 30 animals.

A. GROSS OBSERVATIONS

The extent and severity of the lesions varied greatly between animals. The livers and spleens were usually congested, dark red, and extremely soft. Very faint gray to yellow foci 1 mm in diameter, surrounded by thin hyperemic borders, were visible on the serosal and cut surfaces of the liver. Petechiae and ecchymoses were present on the serosal surfaces of the heart, abdominal lymph nodes, and mucosal surfaces of the gastrointestinal tract.

B. MICROSCOPIC OBSERVATIONS

The liver was the only organ consistently involved, and the lesions varied in degree and distribution. In general, the 30 animals demonstrating pathology showed congestion with early hemorrhage, individualization of hepatic cord cells, loss of hepatic cell cytoplasm, vacuolar change, peripheral margination of nuclear chromatin, and focal necrosis (Fig. 4). In some of the severely affected livers the cytoplasm was markedly acidophilic, cell margins were indistinct, and nuclei were disintegrated, leaving basophilic karyorrhectic particles intermixed in a background of homogenous eosinophilic debris (Fig. 5). All of this was in a collapsed reticulum network. In some animals the entire liver was necrotic, leaving very little normal parenchyma. In a few animals, scattered liver cells contained small, very poorly defined eosinophilic intranuclear bodies. In an occasional hepatic cell the cytoplasm was intensely eosinophilic with a displaced or absent nucleus, suggestive of Councilman-like bodies. In none of the cases was there inflammatory infiltrate, and fatty change was minimal.

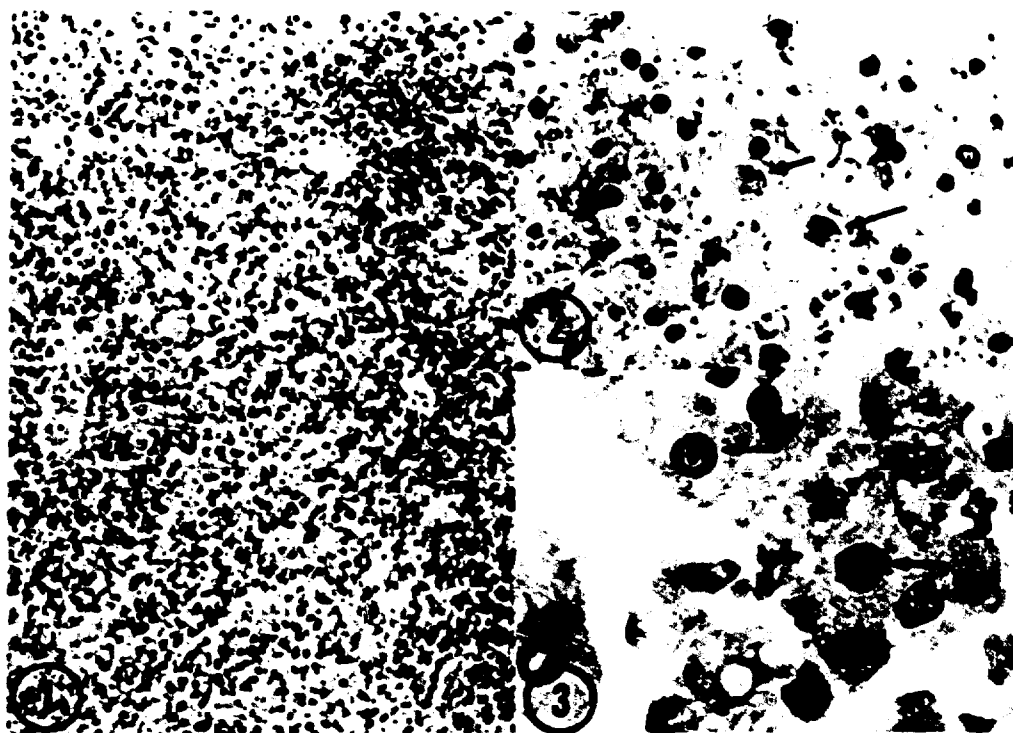


FIGURE 1. Severe Diffuse Necrosis in the Liver of a Mouse. Hematoxylin and eosin. 175X.

FIGURE 2. Higher Magnification of Figure 1, Showing Councilman-like Bodies (Arrows). 250X.

FIGURE 3. Intranuclear Inclusions (Arrows) in Hepatocytes of a Mouse Liver 48 Hours after Virus Infection. Hematoxylin and eosin. 900X.

TABLE 1. KITTENS AND PUPPIES WITH LESIONS REFERABLE TO RVFV

	Age in Days When Inoculated	Number of Animals	Virus Dose	Days Until Death
			Log ₁₀ MICLD ₅₀	
Kittens	2	2	8.2	1,2
		1	6.2	8
	7	3	8.2	7,8,8
		1	6.2	10
		2	2.2	10,10
	21	1	8.2	6
		1	6.2	4
		2	4.2	6,6
Puppies	1	2	8.2	1,1
		2	6.2	2,6
		1	4.2	3
		2	2.2	7,7
		2	0.2	8,8
	7	2	8.2	6,6
		2	4.2	6,6
		1	2.2	8
	14	3	8.2	<u>a/</u>

a. Nine animals were sacrificed, one on day 1, three on day 2, and five on day 5 following inoculation of virus.

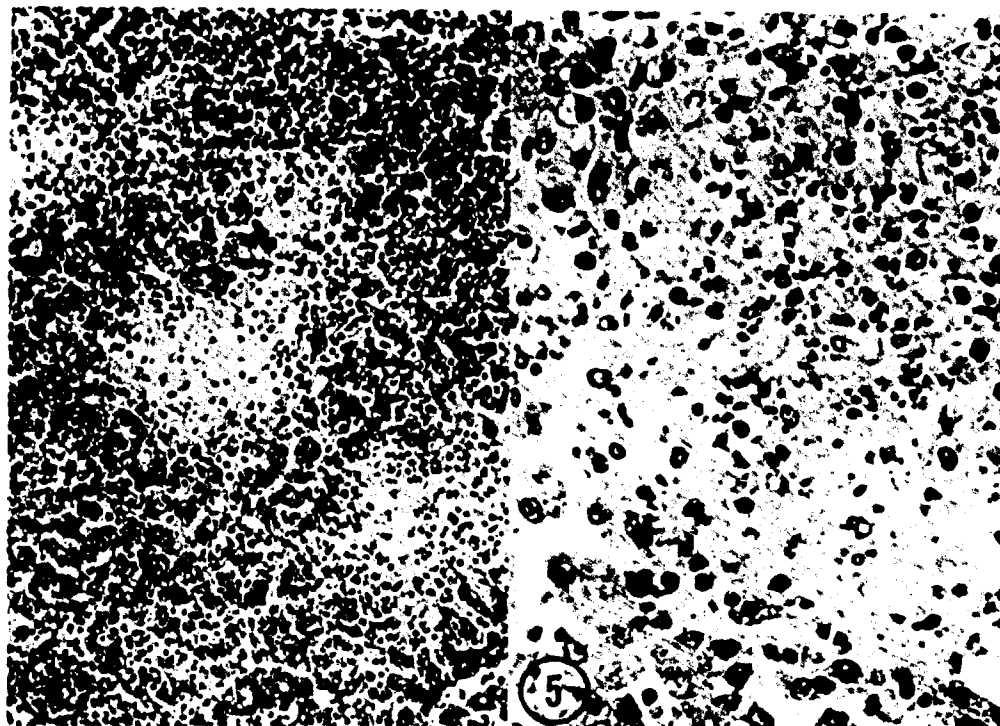


FIGURE 4. Focal Necrosis in the Liver of a Puppy. Hematoxylin and eosin. 155X.

FIGURE 5. From a Large Area of Focal Necrosis in a Puppy Liver with Severe Involvement. The cell margins are indistinct and many of the nuclei are disintegrated, leaving karyorrhectic nuclear particles intermixed in necrotic debris. Hematoxylin and eosin. 400X.

In one kitten and seven puppies, definite changes were found in the myocardium. The earliest lesion was focal ballooning degeneration of several adjacent cells. In the majority of animals, small focal areas of a mixed inflammatory cell infiltrate were present between hyalinized muscle fibers (Fig. 6). In several hearts multiple large focal areas of necrosis were seen. These were surrounded and infiltrated, predominantly by large numbers of lymphocytes, histiocytes, and a few neutrophils.

Moderate congestion and large perivascular cuffs (Fig. 7) were found predominantly in the cerebrum, but also in the cerebellum and medulla in three kittens and five puppies. There was no predisposition for either gray or white matter. The perivascular cuffs consisted mainly of lymphocytes with occasional histiocytes and plasma cells. Neutrophils were sometimes prominent (Fig. 8). Multiple areas of acute and chronic inflammation, together with gliosis, were also common findings. In some animals multiple focal areas of necrosis (Fig. 9) accompanied the vascular cuffs and gliosis. Meningitis was always present in the severely affected animals (Fig. 10). The cellular infiltrate in the meninges consisted primarily of lymphocytes, with a few plasma cells and large histiocytes.

Moderate congestion was evident in the spleen of most animals. In four puppies focal alveolar edema was seen in the lungs, and focal degeneration of renal tubular epithelial cells was visible in two kittens and five puppies.

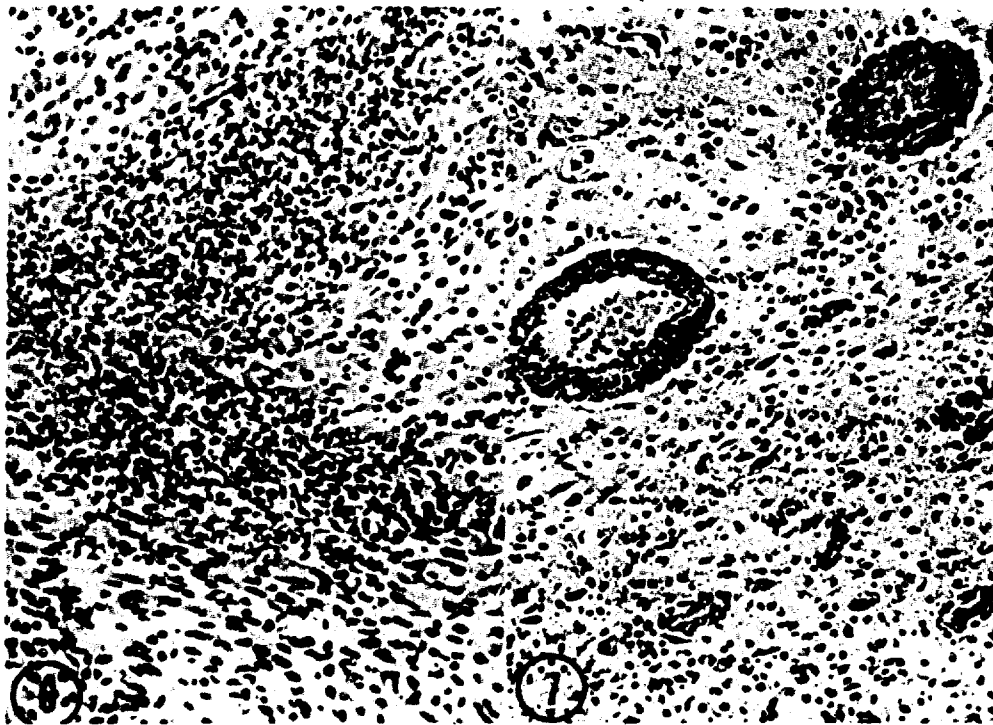


FIGURE 6. Irregular-Shaped Area of Necrosis with a Mixed Inflammatory Cell Infiltrate in a Kitten Heart. Hematoxylin and eosin. 250X.

FIGURE 7. Prominent Lymphocytic Perivascular Cuffs in the Cerebrum of a Puppy. Hematoxylin and eosin. 195X.

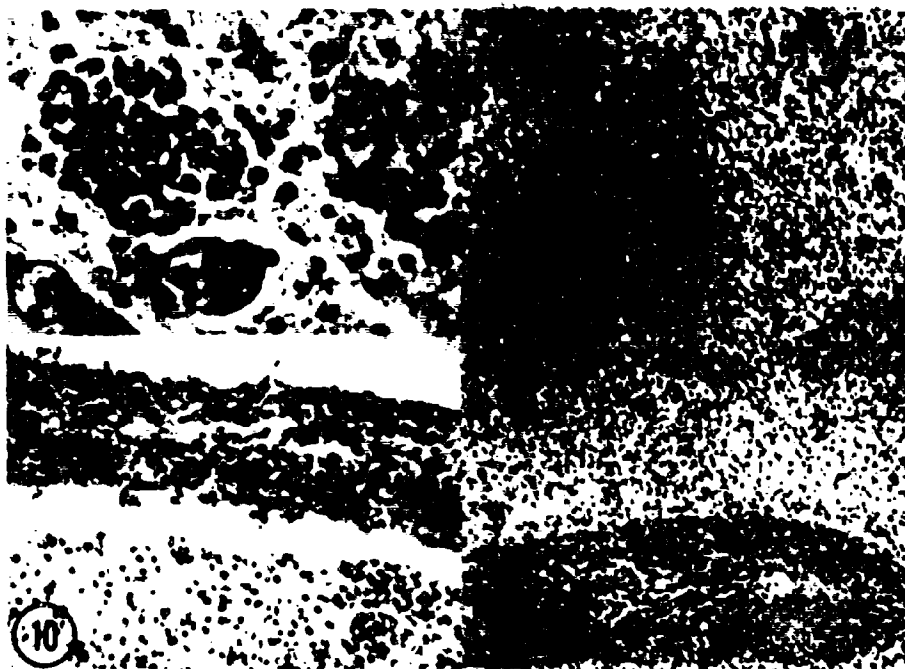


FIGURE 8. A Small Microabscess with Neutrophils in the Cerebrum of a Kitten Adjacent to a Blood Vessel. Hematoxylin and eosin. 250X.

FIGURE 9. A Necrotic Focus of Cerebral Tissue in a Puppy. Hematoxylin and eosin. 100X.

FIGURE 10. Meningitis as it Occurred in the Severely Affected Animals. Hematoxylin and eosin. 250X.

IV. DISCUSSION

The gross and histologic hepatic lesions of experimental RVF in the canine and feline species, although not pathognomonic, are characteristic of the disease and are almost identical to those found in natural and experimental RVF in other species.¹⁻⁴ The relative lack of definite Councilman-like bodies and eosinophilic intranuclear inclusions, in contrast to their presence in other animals studied, does not detract from the similarity of the liver lesions. This absence is very compatible with the present thoughts that these two changes are found early in the development of the lesion and are usually hard to find or are absent in liver sections taken from dead animals. No correlation between the age of the animals, the amount of virus inoculated, and the severity of the lesions could be established.

The findings of myocarditis and necrosis with RVF is significant in that it has not been reported before with either natural or experimental studies. The presence of a myocardial lesion is not surprising; it is seen in a number of viral infections such as poliomyelitis, varicella, influenza, mononucleosis, lymphocytic choriomeningitis, viral pneumonia, infectious hepatitis, mumps, and Coxsackie B. The pathogenesis of these viral lesions is unclear, but it has been explained on the basis of viral damage to essential energy systems in the muscle cells or an antigen-antibody interaction with resultant tissue damage.¹² It has been postulated that should the animal recover, these myocardial lesions might disappear after the necrotized cells had undergone digestion, or they might regress into minute scars as a consequence of local fibroblastic hyperplasia. In some of these diseases the myocardial lesion appears to be of little functional importance, for there is no evidence that any permanent disability remains.¹³ However, several epidemics of myocarditis in very young human infants, especially those with high case fatality rates, have been associated with meningoencephalitis.¹⁴ Coxsackie B has been recovered in many of these cases.¹⁵

Glial proliferation, focal necrosis, perivascular cuffs, and mononuclear meningitis are the major histologic lesions of most viral encephalitides, especially those due to arboviruses.¹⁶ These same lesions were present in eight animals in this study. Although these findings have not been observed in natural outbreaks of this disease, Findlay and MacKenzie¹⁷ have reported on experimental work in which the pantropic RVF virus has been converted to a strict neurotropism by intracerebral passage in mice. They described the brain lesions as vascular cuffing, mild meningitis, and occasionally as extensive necrosis of brain substance. They also reported the presence of intranuclear inclusions in nerve cells. The virus inoculum used in our studies was a pantropic strain and had not undergone an intracerebral passage at any time during its isolation or storage prior to use. Minor differences in the histology and distribution of the brain lesions have been found among the different types of arbovirus encephalitides, but have little or no diagnostic value. An accurate diagnosis can be made only by virus isolation and immunologic procedures.

Acute alveolar edema has been described in the lungs of sheep and lambs⁴ and of ferrets infected with RVF virus.¹⁸ Spleen and kidney lesions similar to those observed in this study have been reported in various animals with RVF.^{1,4,19}

The significance of RVF in the canine and feline species, especially the adult dog and cat with subclinical diseases has been suggested.^{5,6} It is therefore imperative that RVF be recognized quickly and without error, particularly in those countries where it does not currently exist, such as the United States. The difficulty of diagnosis in the bovine and ovine species has been adequately documented in the Union of South Africa in 1950 and 1951. The procedures for a definitive diagnosis of RVF have been discussed, and serologic techniques appear to be the most important.^{20,21} Easterday²² has listed the important features to be considered: (i) illness with a high mortality in lambs and calves; (ii) high abortion rates in cows and ewes; (iii) presence of typical liver lesions; (iv) presence of an influenza-like disease in humans handling infected tissues; and (v) high mortality rates among rodents, which might be concurrent with the epizootic in cattle and sheep. In light of the findings in the dog and cat, persons directly involved in establishing the diagnosis should also give close attention to any spontaneous disease in dogs and cats, especially the very young of these two species.

At this time one can only speculate as to the methods necessary to establish that a spontaneous disease in the dog or cat is RVF. The liver lesions without definite eosinophilic intranuclear inclusions and Councilman-like bodies are not unique for RVF. Also, the presence of central nervous system and myocardial lesions, which are not recognized in other animals with the natural disease, may hinder the efforts of the pathologist to make a diagnosis. The adult dog and cat provide the most perplexing problems, because RVF in these two animals appears to produce only an inapparent infection, with fluctuating viremia and serum-neutralizing antibodies being the only detectable pathophysiologic changes. Therefore, as with other species, serologic methods appear to be the most important diagnostic procedure. In cats, these methods are complicated by the possible existence of cross-reactions or interference between RVF virus and feline panleukopenia virus.²¹ Therefore, the clinical findings of high mortality in kittens and puppies, abortions in adult dogs and cats, serologic results, and pathologic changes must all be equally considered before an unequivocal diagnosis of RVF can be made. It is apparent that during an epizootic in cattle and sheep, adult dogs and cats should be seriously considered as possible reservoirs and disseminators of the disease.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D		
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)		
1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701		Unclassified
		2b. GROUP
3. REPORT TITLE		
THE CLINICAL ASPECTS OF RIFT VALLEY FEVER VIRUS IN HOUSEHOLD PETS: III. PATHOLOGIC CHANGES IN THE DOG AND CAT		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (First name, middle initial, last name)		
John Q. Mitten Richard C. Carter Norman S. Remmele Edward L. Stephen Jerry S. Walker Frederick (NMI) Klein		
6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
April 1967	19	22
8a. CONTRACT OR GRANT NO.		1a. ORIGINATOR'S REPORT NUMBER(S)
A. PROJECT NO 1B562602A059		Technical Manuscript 421
C.		9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
D.		
10. DISTRIBUTION STATEMENT		
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11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY
		Department of the Army Fort Detrick, Frederick, Maryland, 21701
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14. Key Words		
*Rift Valley fever	*Dogs (mammals)	Liver Pathology
*Rift Valley fever virus	Animal diseases	Viruses
*Cats	Animals	Spleen

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