Pantropism of Rabies Virus in Free-Ranging Rabid Red Fox Vulpes fulva

Authors: DEBBIE, JOHN G., and TRIMARCHI, CHARLES V.

Source: Journal of Wildlife Diseases, 6(4) : 500-506

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-6.4.500

Pantropism of Rabies Virus in Free-Ranging Rabid Red Fox *Vulpes fulva*

JOHN G. DEBBIE and CHARLES V. TRIMARCHI

Division of Laboratories and Research
New York State Department of Health, Albany 12201

Abstract

The fluorescent antibody and mouse inoculation tests were employed to study the pantropism of rabies virus in tissue from free-ranging red fox *(Vulpes fulva)* submitted for rabies diagnosis. Viral antigen was found in various organs and tissues of the body. The distribution of antigen within tissues is discussed in the light of the pathogenesis and potential excretion of the virus.

Introduction

Numerous investigators have studied the pathogenesis of rabies virus in man and animals. The virus is generally considered to have a special affinity for the central nervous system (CNS) and salivary glands, but it has also been demonstrated in many tissues of the body by the mouse inoculation test (MIT)[5-8,10,12,14,18] and by the fluorescent antibody test (FAT)[3,9,16] in one or more species of animals.

This paper reports the use of the FAT in an attempt to demonstrate rabies virus antigen in tissues from naturally infected red fox *(Vulpes fulva).*

Materials and Methods

The fox were presented to the Rabies Laboratory, New York State Department of Health, for routine rabies diganosis. The transit time to the laboratory was 24 to 48 hours. The organs and tissues were collected with instruments sterilized by flaming alcohol before each collection. Tissue, approximately 20 mm. square, was taken at random from designated locations.

The tissues were placed in individual petri plates and frozen at —20° C. Portions of each tissue were mounted on tissue holders with cryoform* and placed on the freezer bar of an International Cryostat Model CTI* set at a cutting temperature of —20° C. The microtome blade was set at 8 microns. A minimum of four sections per tissue was cut and transferred to warm microscope slides. The microtome blade was removed and sterilized after the sectioning of each tissue.

Sections to undergo the FAT were fixed in acetone at —20° for 2 hours. The FAT performed was essentially that described by Johnson.[11] The rabies antisera were derived from hamsters and the titration of conjugated antiserum was 1/28. When photography was employed a section was exposed for an additional hour to increase the background staining. Examinations were made using a Zeiss fluorescent microscope fitted with a dark-

* International Equipment Co., Needham Heights, Mass.

field condenser and a pressure mercury vapor lamp (OSRAM HBO 200W.) A Ug-2 (Zeiss) exciter filter and barrier filter 41 (Zeiss) were employed. Examinations were made using a planachromat 10X, 25X and 40X objectives and 12.5X oculars.

Duplicate sections of all FAT positive tissues were fixed and stained with hematoxylin and eosin (H & E) following the technique of Russell et al.[15]

Representative tissues found positive by the FAT were ground with buffered physiological saline containing 10% horse serum with 500 I.U. of penicillin and 0.5 mg. streptomycin to yield a 20% suspension. Ten- to twelve-gram NYLAR mice were inoculated intracerebrally (IC) or intramuscularly (IM) with the suspension. Brains of mice that died were examined for rabies virus by the FAT. Known rabies-free fox were examined and appropriate tissues were used as controls.

Results

A total of twelve fox were studied. Table 1 gives the tissues collected from each fox and the presence of specific rabies fluorescence is recorded as (+) or (—). The findings represent randomly chosen locations. The only exception is that brain tissue was examined by the routine technique for examination for rabies virus, in which two slides are used, one an impression smear of hippocampus

TABLE 1. *The occurrence of rabies antigen in tissues of red fox as demonstrated by the fluorescent antibody test.*

	1	2	3	4	5	6	7	8	9	10	11	12	Total
Brain	+	+	+	+	+	+	+	+	+	+	+	+	12/12
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	12/12
Salivary gland	+	—	+	+	+	+	+	+	+	+	+	+	11/12
Skeletal muscle	ND	ND	ND	ND	—	—	+	+	—	—	—	—	2/8
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	12/12
Stomach	—	—	—	—	+	—	+	+	+	+	—	+	6/12
Small intestine	+	—	—	—	—	—	—	+	+	—	—	—	3/12
Large intestine	—	—	—	—	—	—	—	+	—	—	—	—	1/12
Pancreas	ND	—	ND	ND	—	—	—	+	+	—	—	—	2/9
Omentum	ND	ND	ND	ND	—	—	—	—	ND	—	—	—	0/7
Liver	—	—	—	—	—	—	—	—	—	—	—	—	0/12
Gall bladder	—	—	—	—	—	—	—	—	—	—	—	—	0/12
Spleen	—	—	—	—	—	—	—	—	—	—	—	—	0/12
Lymph node	+	—	—	—	—	—	—	—	—	—	—	—	1/12
Thyroid	+	—	ND	+	—	+	+	+	—	—	—	—	5/11
Thymus			—			+	—				—	—	1/5
Lung	+	—	—	—	+	+	—	+	+	+	—	+	7/12
Heart	+	—	+	+	+	+	+	+	—	—	—	+	8/12
Adrenal	+	—	+	+	+	+	+	+	+	+	+	+	11/12
Kidney	+	+	+	+	+	+	+	—	+	+	—	+	10/12
Kidney capsule	+	—	+	+	+	—	+	—	+	+	—	+	8/12
Ureter	+	—	+	+	—	—	—	—	+	—	—	—	4/12
Bladder	+	+	+	—	+	+	+	+	+	+	—	+	10/12
Prostate	+		+	—	+	+	—		+	+	+		7/9
Urethra	+		+	—	+	—	—		—	+	+		5/9
Uterus		—						—				—	0/3
Ovary		—						—				—	0/3
Testicle	—		—	—	—	+	—		—	+	—		2/9
Fetus												—	0/1

and the second an impression smear of a 20% suspension of combined hippocampus, cerebellum, and brain stem.

All animals had demonstrable rabies antigen in the brain and lumbar spinal cord. Although the parotid gland has been reported as not being as reliable a focus for the presence of rabies antigen as the submaxillary gland it was examined. All but one fox had positive parotid glands.

The liver, gall-bladder, spleen, uterus and ovary were the only tissues studied in all the fox in which rabies virus was not found. Antigen was not observed within lymph nodes but was present in the connective tissue surrounding the gland in 6 animals and present in the capsule of the gland in 1 fox (Figure 1). No virus was detected in the tissues of fetuses of the 1 pregnant fox submitted.

The esophagus of every animal studied contained antigen. The antigen was discernible in the epithelial cell lining of 4 fox (Figure 2) and in the connective tissue of the submucosa and/or between the muscle layers in all. The virus was primarily in the submucosa and serosal layers in the stomach, and small and large intestine. Rabies virus was found in the large intestine of only one fox.

Extensive antigen deposition was never noted in the lung except in nerve fibers. However, antigen was present in the epithelial layer of bronchioles in two fox (Figure 3).

Most animals had rabies virus in the kidney and adrenal gland. The distribution indicated its presence within tubular cells of the kidney and it was consistently observed in nerve fibers associated with renal blood vessels. Antigen was pronounced in the adrenal medulla with the

FIGURE 1. *A mesenteric lymph node with rabies antigen dispersed along the capsule. 100X.*

FIGURE 3. *Rabies antigen present in the bronchiolar epithelium. The elastin fibers appear conspicuous due to bright staining. 100X.*

FIGURE 2. *Rabies antigen (arrows) present in the epithelial lining of the esophagus. 40X.*

FIGURE 4. *Capsule of the adrenal gland showing rabies antigen. 40X.*

cortex occasionally having small foci of antigen. Although the virus was not demonstrated in the kidney capsule as frequently as in the kidney proper, when antigen was present in the capsule it was extensive. The same was true for the adrenal capsule (Figure 4). Antigen was observed along the endothelial layer of a vein associated with the adrenal in one fox (Figure 5).

The virus distribution in the heart coincided with areas between muscle bundles where one would expect nerve fibers to be present. In a few instances, H & E preparations confirmed nerve tissue presence. This was also the case with the positive striated muscle (diaphragm and semitendinosus) examined. In no case was antigen present within muscle cells.

The urinary bladder was positive in all but two fox. The virus distribution included the epithelial lining as well as submucosal cells (Figure 6). The prostate gland was positive in seven fox and the urethra through the gland was positive in five of these individuals. The distribution in the urethra was along the epithelial lining (Figure 7) while the prostate antigen was interspersed throughout the gland. The distribution of antigen in the ureters was similar to that of the urethra.

Rabies antigen was present in both semitendinosis and diaphragm in 2 of 8 fox. Except for ovaries, rabies antigen was present in all glandular tissue studied. The distribution and character of the fluorescence indicated dispersion throughout the tissue with the exception of lymph node.

Mice were inoculated IC with a 20% suspension of brain, spinal cord, salivary gland, urinary bladder, gall bladder and bile. All but the last two, found negative by FAT, killed inoculated mice with rabies within 14 days. Animals inoculated intramuscularly with pancreas, kidney capsule and adrenal gland died with rabies within 20 days.

FIGURE 5. *Arteriole adjacent to the adrenal gland. The arrows indicate rabies antigen in the endothelial cells. 100X.*

FIGURE 6. *Rabies antigen in epithelial cells of the urinary bladder. 100X.*

FIGURE 7. *Rabies antigen on the epithelial lining of the urethra. 100X.*

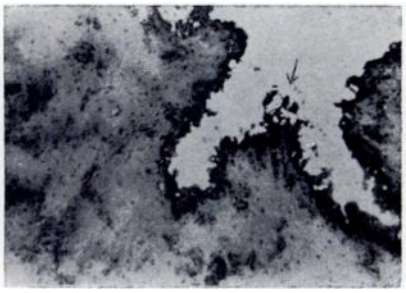

FIGURE 8. *An H & E section of the urethra with the arrow pointing to the fluorescing area shown in figure 7. 40X.*

Discussion

To the authors' knowledge, this is the first attempt to use the FAT to demonstrate rabies antigen in various body locations during the naturally occurring disease of red fox. da Silva and Souza[5-7] found evidence of rabies virus in a number of tissues of naturally infected vampire bats and in one dog by IC inoculation of adult mice, while Schneider and Hamann[16] using FAT and MIT demonstrated the virus in numerous tissues of experimentally infected mice. The employment of the FAT on frozen sections has the advantage of determining the approximate location of the antigen within a tissue. Although the usual route of invasion of rabies virus following peripheral inoculation occurs via the Schwann cell endoneurium or associated tissue spaces to the central nervous system,[1,4,16] Dean et al.[4] who were able to infect mice with CVS virus by the intravenous route suggested that blood-borne infection is possible especially in animals such as fox, cattle and hamsters which are known to be highly susceptible to rabies. Hronovsky and Benda[9] found early occurrence of FAT rabies antigen in kidneys of guinea pigs exposed to inhalation rabies, which further suggests a possible hematogenous spread of rabies virus.

Schneider and Hamann[16] found that the infectious dose, the length of nervous connection to the CNS and the abundance of nerves of the organs determined the time and intensity of the infection of non-nervous organs. These authors reported that extraneural multiplication sites were the epithelial tissues of the salivary glands, the brown fat and the cornea. All the fox in this present study were collected at the time of clinical signs and with unknown incubation periods, so the nature and duration of spread are not known. The focal and linear distribution of viral antigen as observed with FAT, along with comparative sections stained by hematoxylin-eosin, indicates that much of the spread in various organs could be by the neural pathway. However, hematogenous spread cannot be ruled out.

Rabies infection by inhalation[2,9] and by ingestion of infected tissue[8,12,17] suggests a route other than the neural route of invasion although neuroepithelial cell entrance of the virus may be the explanation. Our observance of rabies antigen in the epithelial layer of the bronchioles, esophagus, ureter, urinary bladder and urethra may be a result of its presence in neuroepithelial cells, but these are potential pathways of virus excretion by epithelial sloughing. Mice inoculated with urine from one of five rabid fox died with rabies.

The observance of rabies virus in the submucosa, musclaris and serosal layers of the small and large intestine, with associated findings by H & E of nerve tissue present in these areas, suggests neural spread, as does the observance of antigen in connective tissues of other organs. Antigen was prominent in the capsules of the kidney and adrenal but these organs also had virus within the tissue proper. The reports of focal degenerative lesions in the adrenal medulla, tubular epithelium of the kidney and acinar epithelium of the pancreas[12] could be the direct result of antigen observed in these areas. Degeneration of cells of the adrenal medulla was noted in our studies.

Rabies antigen in lung tissue was generally sparse except when present in nervous tissue of the organ, an observation which is in agreement with the finding of small amounts of virus in guinea pigs which were exposed by inhalation.[9] However, the presence of antigen in the bronchial epithelium causes consideration of the respiratory tree as a potential source of virus excretion. Our observations indicate that quantitation of virus using mouse inoculation techniques may be difficult to interpret. The amount of nervous tissue present in the organ suspension inoculated may determine the virus titer demonstrated.

Rabies virus appears to have an affinity for glandular tissue since it was found in all glands studied except the ovaries; its presence in the lymph node was confined to the capsular connective tissue.

The observance of rabies antigen in the endothelial layer of a vein associated with the adrenal gland of one fox suggests the possibility of a viremia during some portion of the disease.

Rabies antigen in tissues and organs of secretion and excretion, *i.e.*, intestines, urinary tract, lung, testicles and salivary glands, suggests numerous potential pathways as the possible source of airborne infection. In this laboratory films of brain tissue left at room temperature for one week contained viable virus; therefore, rabies virus in tissue or excrement could remain viable, at least for a limited time, in small amounts of tissue.

The presence of virus in various tissues, as well as in the excreta, in quantities large enough for titration in mice should alert public health officials to the potential hazards of rabies-infected animals. Slaughtered rabid animals, even those which do not demonstrate visible signs of disease, could result in the distribution of infected and infectious meat products. Medical personnel assigned to the care of animals and humans infected with rabies should take the necessary precautions.

Literature Cited

1. BAER, G. M., T. R. SHANTHAVEERAPPA, and G. H. BOURNE. 1965. Studies on the pathogenesis of fixed rabies virus in rats. Bull. WHO 33: 783-794.
2. CONSTANTINE, D. G. 1962. Rabies transmission by non-bite route. Public Health Rep. 77: 287-289.
3. CORREA-GIRON, E. P., R. ALLEN, and S. E. SULKIN. 1970. The infectivity and pathogenesis of rabies virus administered orally. Amer. J. Epid. 91 (2): 203-215.
4. DEAN, D. J., W. M. EVANS, and R. C. McCLURE. 1963. Pathogenesis of rabies. Bull. WHO 29: 803-811.
5. DA SILVA, R. A., and A. M. SOUZA. 1968. Rabies virus in the tissue of bats. I. In various tissues of vampire bats. Pesquisa agropec. bras. 3: 291-301.
6. DA SILVA, R. A., and A. M. SOUZA. 1968. Occurrence of rabies virus in different tissues of the dog in the natural disease. Pesquisa agropec. bras. 3: 317-318.
7. DA SILVA, R. A., and A. M. SOUZA. 1968. Rabies virus in the tissue of bats. III. In the testicle, foetus and uterus of vampire bats. Pesquisa agropec. bras. 3: 365-368.
8. FISCHMAN, H. R., F. E. WARD. 1968. III. Oral transmission of rabies virus in experimental animals. Amer. J. Epid. 88: 132-138.
9. HRONOVSKY, V., and R. BENDA. 1969. Development of inhalation rabies infection in suckling guinea pigs. Acta virol. 13: 198-202.
10. JOHNSON, H. N. 1959. The role of the spotted skunk in rabies. Proc. 63rd Ann. Meet. U.S. Livestock San. Assoc: pp. 267-274.
11. JOHNSON, H. N. 1964. Rabies virus pp. 369-372. In E. H. Lennette and N. J. Schmidt (eds.). *Diagnostic Procedures for Viral and Rickettsial Diseases.* 3rd Ed. American Public Health Assoc. Inc., New York, New York.
12. JOHNSON, H. N. 1965. Rabies virus. p. 818. In F. L. Horsfall and K. Tamm (eds): *Viral and Rickettsial Infections of Man.* 4th Ed. J. B. Lippincott Co., Philadelphia and Toronto.
13. MACRAE, A. D. 1969. Rabies in England. Lancet 7635: 1415-1417.
14. PARKER, E. L., and R. E. WILSNACK. 1966. Pathogenesis of skunk rabies virus: quantitation in skunks and foxes. American J. Vet. Res. 27 (116): 33-38.

15. RUSSELL, W. O., I. P. CHANG, and M. L. IBANEZ. 1961. *Cryostat Frozen Section and Freeze Substitution Techniques Workshop Manual.* American Society of Clinical Pathologists.

16. SCHNEIDER, L. G., and I. HAMANN. 1969. Die Pathogenese der tollwut bei der maus. II. Die zentrifugale virusausbreitung und die virusgeneralisierun im organismus. Zentralblatt fur Bakteriologie, Parisitenkunde, Infektionskrankheiten und Hygiene 212 (1): 1-13.

17. SOAVE, O. A. 1966. Transmission of rabies to mice by ingestion of infected tissue. Am. J. Vet. Res. 27 (116): 44-46.

18. SULKIN, S. E., P. H. KRUTYSCH, R. ALLEN, and C. WALLIS. 1959. Studies on the pathogenesis of rabies in insectivorous bats. I. Role of brown adipose tissue. J. Exp. Med. 110: 369-388.