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Source: Bulletin of the Wildlife Disease Association, 4(2) : 37-40

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-4.2.37

Airborne Rabies Virus Isolation

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Received for Publication 12 December, 1967

ABSTRACT

Airborne rabies virus was isolated from Frio Cave, Texas, using a mechanical air sampler. Several types of samplers have been used, but only an electrostatic precipitation device has collected measurable quantities of virus. Prior to this, the only known reported isolations of airborne rabies virus had occurred when susceptible animals were confined in bat caves for long periods of time. The use of a mechanical sampler can facilitate quantitation of virus in caves.

INTRODUCTION

The transmission of rabies virus by non-bite route was demonstrated experimentally in 1960. Constantine succeeded in infecting susceptible animals by exposing them to the atmosphere in Frio Cave, near Uvalde, Texas.[2] The presence of rabies virus in Frio Cave has been demonstrated in subsequent years by the successful infection of introduced susceptible animals.

Frio Cave is a large multichambered limestone cavern, a part of which is utilized by the Mexican free-tailed bats, *Tadarida braziliensis mexicana,* during the summer months as a nursery. Millions of bats, primarily lactating females and suckling, flightless young, may be found from June into early August in the nursery chamber. Most of the experimental studies to demonstrate airborne rabies virus have been done in this nursery chamber. Among the species which have been experimentally infected with rabies through cave exposure are: red and grey fox, coyote, opossum, and ring-tailed cat. Exposure times and techniques have varied but the basic principle of encaging a suspectible animal within the bat nursery room for 7 to 30 days has been utilized each year since 1960.

In the summers of 1962, 1964, and 1965, besides the successful demonstration of rabies in the cave environment using susceptible animals, attempts were made to isolate rabies virus directly from the cave atmosphere using mechanical air samplers such as the Ace All Glass Impinger (AGI-4)[2], the Andersen Sampler[3], and a homemade device which collected frozen condensate from the atmosphere.[1,3] No virus was isolated from the air with these types of equipment, although animals exposed to the atmosphere simultaneously did develop rabies.

In the summer of 1966 sentinel animals were again successfully infected with rabies using techniques previously mentioned. In addition, rabies virus was isolated for the first time directly from the atmosphere utilizing a mechanical air sampler. This report describes the air sampling techniques used for the isolation of airborne rabies virus.

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MATERIALS AND METHODS

Two types of air samplers were used. One was the Ace All Glass Impinger (AGI-4). This sampler utilizes a vacuum to draw air through a capillary tube at high velocity and impinge airborne particles on the blass bottom of the flask containing the collecting fluid. A vacuum pump connected in parallel with a mercury manometer and in series with a variable diameter flowmeter and the AGI-4 provided a measured air flow.

All samples were collected using a negative pressure of 180 mm. of mercury which processed 10 liters of air/minute through the sampler. Air was passed through 20 ml. of collecting fluid which consisted of 64% Eagles' Minimum Essential Medium, 16% normal newborn calf serum, 19.5% phosphate buffered water (0.1 M, pH 6.3), 0.5% Tympanol[4] antifoam Agent, and 1000 units Potassium procaine penicillin/ml., 2 mgm. dihydrostrepotomycin/ml. and 10. ugm. amphotericin B/ml.

Samples were collected in the bat nursery room, moving the sampler continuously throughout the ten-minute sampling period. The air intake of the sampler was moved approximately 5 feet vertically, from 6 inches below the bat covered ceiling to 24 inches above the cave floor, and horizontally throughout a circle of 6-foot radius.

The second air sampler used was a Litton Industries Large Volume Air Sampler, Model L[5] (LVAS-L), which utilizes electrostatic precipitation to concentrate airborne particulate matter. Air is drawn through an 18-mesh screen filter through a 6-inch hole at the top of the sampler and passes through a high voltage, 10-25 kilovolt, 0.5-4.0 milliamp discharge corona where the particulate matter is charged. Air then passes in a thin layer over the surface of a grounded, horizontal rotating disc upon which the charged airborne particles precipitate. The rate of airflow can be regulated from zero up to 10,000 liters per minute. Collecting fluid flowing from the hollow central spindle moves centrifugally to the disc rim, where it is collected along with precipitated particulate matter. Composition of collecting fluid is similar to that already described for use in the AGI-4 except that the 0.5% Tympanol is replaced by an equal volume of phosphate buffered water (pH 6.3).

Because of the size and weight of this sampler it was left in one position during sampling procedures. The air intake, a one-foot length of metal tubing 6 inches in diameter, was located approximately 3 feet above the floor of the cave and 4 feet below the ceiling. Sampling times varied from 10 minutes to 30 minutes. In addition to operating this sampler in the usual manner with constantly flowing collecting fluid, a modified technique was also used in which the machine was operated "dry" for a given period of time and then fluid was permitted to wash off collected precipitate for a brief period. This modified technique permitted increased concentration of particles in a smaller fluid volume.

All samples collected with either the AGI-4 or the LVAS-L were placed in a wet ice bath (4°C) immediately following collection, and were either inoculated into test animals within 90 minutes of collection, or were placed in storage at —70°C until tested.

Two species of test animal were inoculated with each collected sample. One young adult red fox *(Vulpes fulva)* was inoculated with each sample fluid, the dose divided into two equal aliquoits and administered intramuscularly into the semimembranosus muscle area of each rear leg (See Table 1). Six white Swiss mice, Albany strain, three weeks old, were also inoculated intracerebrally with 0.03 ml. of each sample. All rabies virus isolations were identified by fluorescent antibody technique and serum virus neutralization tests.[6,7] Confirmation of isolation from original material and first passage material was performed in another laboratory by different personnel using similar techniques.

RESULTS

Rabies virus was isolated from four of eight samples collected with the LVAS-L. All isolations were made in foxes inoculated with 20-30 ml. of sample fluid. No isolations were made from the five samples collected with the AGI-4 (See Table 1).

One fox died of unknown causes three days after inoculation and there was insufficient air sample fluid to permit reinoculation of this material into another fox. No isolations were made in intracerebrally inoculated mice. At least 50% of the mice inoculated intracerebrally died within 48 hours. Repeat inoculations into mice produced similar results so intracerebral mouse inoculation was abandoned.

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[5] Litton Industries, Applied Science Division, Minneapolis, Minnesota

DISCUSSION

The AGI-4 and the LVAS-L have been used successfully for some time in sampling airborne bacteria and larger particulate matter. Virus isolation utilizing these two devices is a relatively new field. The recovery of airborne rabies virus with a mechanical sampler demonstrates another use for these devices and may add materially to our knowledge of rabies virus ecology.

Laboratory studies have shown that the AGI-4 is one of the most efficient devices currently available for sampling airborne particles.[9] The LVAS-L is considered to be equally as efficient for some viruses.[6] Successful recovery of rabies virus in Frio Cave with the LVAS-L and not with the AGI-4 is probably a result of factors other than the mechanical filtering efficiency of the two devices. The probable explanation lies in the greater capacity of the LVAS-L to process air through a given volume of collecting fluid in a given period of time. The LVAS-L yields a 100-fold greater concentration of particulate matter than the AGI-4. In sampling where airborne virus titers are very low as they presumably are in Frio Cave atmosphere, this 100-fold concentration could mean the difference between recovery and non-recovery of the virus.

No attempt was made to quantitate the amount of virus present in Frio Cave atmosphere. This study was conducted to establish the presence of airborne rabies virus only. It is, however, apparent that quantitation of airborne rabies virus is now practical through the use of mechanical samplers.

Rabies in insectivorous bats of the United States was first reported in 1953. Since that time considerable effort has been devoted to determining the importance of bats in wildlife rabies epidemiology. Rabies transmission from bat to terrestrial mammal by bite has been demonstrated experimentally, but appears to be a difficult transmission and not likely to occur with any degree of regularity in nature.[8,4] However, because of airborne rabies transmission, it is possible that bat caves rather than bats themselves may serve as reservoirs for infection of terrestrial mammals. To finally determine the importance of cave bats and their environment in the ecology of wildlife rabies several areas of study must be pursued. These include work to determine the amount of airborne rabies virus in bat caves, to de-

TABLE 1. *Rabies isolations from cave aerosols inoculated into red foxes* (Vulpes fulva)

Sample No	Date	Sample Time	Kilovolt	Milliamp	Liter/min Airflow	Inoculum Quant of	Isolation Rabies
AGI-1	14 Jul 66	10 min	—	—	10^1	10 ml	Neg.
LVAS-1	14 Jul 66	10 min	22	.5-1.2	10^4	20 ml	Pos.
LVAS-2	15 Jul 66	10 min	13	.5-1.0	10^4	20 ml	Neg.
LVAS-3	15 Jul 66	10 min	13	2.0-4.0	10^4	20 ml	Pos.
AGI-2	15 Jul 66	10 min	—	—	10^1	5 ml	Neg.
LVAS-4	17 Jul 66	30 min D*	20	2.5	10^4	20 ml	Pos.
LVAS-5	17 Jul 66	10 min	20	2.5	10^4	20 ml	Neg.
LVAS-6	17 Jul 66	30 min D*	19	3.0	10^4	20 ml	N.T.**
LVAS-7	17 Jul 66	10 min D*	19	3.0	10^4	30 ml	Pos.
LVAS-8	18 Jul 66	15 min	17	2.0	10^4	20 ml	Neg.
AGI-3	18 Jul 66	10 min	—	—	10^1	8 ml	Neg.
AGI-4	19 Jul 66	10 min	—	—	10^1	10 ml	Neg.
AGI-5	19 Jul 66	10 min	—	—	10^1	10 ml	Neg.

*D = Dry run

**N.T. = not tested. Fox died of unknown causes three days after inoculation.

te mine aerobone rabies LD_{50}s for certain terrestrial mammals, and finally, to determine the extent to which these terrestrial mammals utilize bat caves for shelter or as food sources.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Peter Gerone and Mr. Bill Roberts, Department of the Army, Ft. Detrick, Maryland, and Mr. George F. Mallison, Biophysics Section, National Communicable Disease Center, Atlanta, Georgia, for their assistance and suggestions.

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