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RABIES VIRUS AND ANTIBODY IN BATS IN GRENADA AND TRINIDAD

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Abstract: Rabies virus was detected by fluorescent-antibody and mouse inoculation tests in the brain of one bat, *Artibeus jamaicensis*, collected at La Tante, Grenada on 19 June 1974. No rabies virus was found in the brains and/or salivary glands of 411 other Grenadian bats of 6 species tested, including 56 *A. jamaicensis*. Rabies neutralizing antibody was detected by the rapid fluorescent focus inhibition test (RFFIT) in 27 of 353 Grenadian bats. Positives occurred in each of the 6 species sampled, with 40.5% prevalence in *A. jamaicensis*. In 11 of 86 Trinidadian bats of 4 species known to carry rabies, positive sera occurred only in *A. jamaicensis* (18.6%) and *A. lituratus* (8.1%). The potential use of the RFFIT in determining rabies activity is discussed.

INTRODUCTION

Rabies has hitherto been known in Grenada only as an endemic infection in the mongoose population of the island, with involvement of domestic animals and man.[4,6] Detection of Negri bodies in an insectivorous bat in Grenada is on record, but attempts to confirm rabies infection by mouse inoculation were inconclusive.[4] The present paper describes the first isolation of rabies virus from a Grenadian bat, and the first report of bat rabies serology from Grenada and Trinidad.

MATERIALS AND METHODS

Bats were collected alive with a long-handled dip net during the daytime in buildings and caves, and in Japanese mist nets set at ground level during the night. As soon as possible after capture bats were exsanguinated under ether anaesthesia by cardiac puncture, and brains and salivary glands were removed and stored at —60 C for virus studies.

Rabies fluorescent antibody (FA) tests were performed by the method of Dean and Abelseth.[3] Virus isolation attempts were made by intracerebral inoculation of 2-3 day old mice with supernates from centrifuged 10% suspensions of brains and salivary glands in phosphate buffered saline (pH 7.2).

The bat from which rabies virus was isolated was submitted for FA examination about 12 h. after being killed. Impression smears were prepared, and the remaining brain tissue was frozen at —60 C and used for mouse inoculation several days later; the bat carcass was then inadvertently discarded. Eventually the carcass was recovered but post mortem autolysis prevented virologic studies on other organs.

Rabies antibody was determined by the rapid fluorescent focus inhibition test (RFFIT) by the method of Smith *et al.*[12] Sera were screened at 1/5-1/6 dilution and positive sera titrated at further 5-fold dilutions. Serum antibody titers were calculated by the method of Reed and Muench[10] and expressed as the dilution. Only sera twice found to inhibit fluorescence at 1/5 dilution were considered as positive.

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RESULTS

Virus Isolation

On 19 June 1974, a bat which appeared to be disorientated and was flying into the walls of a house in La Tante, Grenada, was killed and submitted the next day to the rabies laboratory. The brain was rabies FA positive. Later a virus, Tr 132035, was isolated from this brain by mouse inoculation, and was rabies positive by mouse neutralization test (virus titer in immune mouse ascitic fluid prepared against Trinidad rabies strain Tr 5843: 1.7 dex,[b] control titer: 6.0 dex). The bat was identified as *Artibeus jamaicensis*.

The number of Grenadian bats examined for virus in 1973 and 1974 is given in Table 1. No additional FA positives were found nor virus isolations made from 411 bats of six species collected.

Rabies Antibody

The results of rabies RFFIT on Grenadian and Trinidadian bats are shown in Table 2. In Grenadian *A. jamaicensis*, 17 of 42 sera (40.5%) were positive for rabies antibody, with titers ranging from 1/8 to 1/280. Positive sera were found in each of the other 5 species

TABLE 1. Grenadian bats examined for the presence of rabies virus (1973-1974).

Species of bat	Fluorescent antibody test		Mouse inoculation test		
	Brain	Salivary glands	Brain	Salivary glands	Total bats
Anoura geoffroyi	48	48	56	73	121
Artibeus cinereus	—	—	6	6	6
Artibeus jamaicensis	26[a]	25	57[b]	56	57
Glossophaga longirostris	70	70	56	101	164
Molossus molossus	25	25	34	34	59
Sturnira lilium	—	—	5	5	5
Totals	169	168	214	275	412

[a] One rabies FA positive.

[b] One rabies virus isolation (same bat).

TABLE 2. Results of rabies RFFIT on bat sera from Grenada and Trinidad, 1974.

Species of bat	Grenada			Trinidad
	Individual sera	Pools of 2 sera	Pools of 3 sera	Individual sera
Anoura geoffroyi	4/68[a]	0/ 1	—	—
Artibeus cinereus	1/ 2	—	—	—
Artibeus jamaicensis	17/42	—	—	8/43
Artibeus lituratus	—	—	—	3/37
Desmodus rotundus	—	—	—	0/ 4
Diaemus youngi	—	—	—	0/ 2
Glossophaga longirostris	1/64	0/13	2/12	—
Molossus molossus	1/56	0/27	—	—
Sturnira lilium	1/ 1	0/ 1	—	—
Total Bats	25/233	0/84	2/36	11/86
	= 27/353 = 7.6%			12.8%

[a] Number positive at 1/5 dilution/number tested.

collected on Grenada with the highest titer (1/219) from a pool of 3 *Glossophaga longirostris* sera. In the four species of Trinidadian bats tested, rabies antibody positive sera were found in *A. jamaicensis* and *A. lituratus*, but the few vampire bat (*Desmodus* sp. and *Diaemus* sp.) sera tested were negative.

In Grenada 25 *A. jamaicensis* collected from a cave at Black Bay-Concord on 11 July 1974 included 7 non-flying infants, 6 of which were positive for rabies antibody. Eight of 18 adults in this collection also were positive. Two positive young were found attached to their presumptive mothers; the mothers also were positive. None of 13 *Glossophaga* collected in this cave were positive for rabies antibody.

DISCUSSION

Twelve species of bats have been recorded from Grenada,[9] and the addition of *Sturnira lilium* collected in the present study brings the total to 13. All of these species also occur on Trinidad, which is 12 km from Venezuela and 150 km south of Grenada. Vampire bats occur on Trinidad but are not known from Grenada or any other island in the West Indies. Evidence of rabies infection is here presented for the six species collected: *Anoura geoffroyi, Artibeus cinereus, A. jamaicensis, G. longirostris, Molossus molossus* and *Sturnira lilium*. *Anoura* sp., *A. cinereus*, and *Sturnira* sp. have not been reported infected with rabies, nor hitherto have they been reported to possess antirabies activity in their sera. The other three species, and *Carollia perspicillata, A. lituratus* and *Pteronotus davyi* which did not appear in the present collection, have been reported as rabies-infected in Trinidad.[7]

A. jamaicensis, now known from virus isolation and serology to be rabies-infected in Trinidad and Grenada, is widely distributed on every major island in the West Indies.[9] It is a fruit-eating bat of medium size (up to 50 gms weight), frequents gardens with fruit trees at night, and often roosts during the day under the eaves of houses. The potential for exposure of humans, pets and livestock to bat rabies therefore exists.

The relatively new rabies antibody test used in the present study merits comment. The RFFIT system is inexpensive once the capital equipment (carbon-dioxide incubator and fluorescent microscope) is available, rapid (results are obtained in 24 h. and a competent technician can test 50-60 sera per day), and relatively safe as the propagation of rabies virus in animals is not involved.

The present study shows that a test for the detection of rabies serum neutralizing antibodies such as the RFFIT promises to be a valuable adjunct to FA or virus isolation techniques and may assist in monitoring recent rabies virus activity in a host species of population. This is shown by comparisons of virus infection and antibody rates: in Grenadian *A. jamaicensis* bats 1 virus infection of 57 tested or 1.8%: vs an antibody rate of 40.5%; in Grenadian mongooses for 1974, infection rate 0.6%: antibody rate (by RFFIT) 43.5%.[5] Presence of antirabies activity in serum of adult bats suggests that exposure to the virus occurred at some time during the animal's lifetime. For small mammals, bats are remarkably long-lived—a longevity record of 7 years for a free-living *A. jamaicensis* has been reported.[14] The evidence of rabies antibody in suckling *Artibeus* presented here might indicate very recent virus activity in that population, passive transplacental acquisition of maternal antibody, or transference through the mother's milk.

It would be interesting to know whether bats (particularly *A. jamaicensis*) show evidence of rabies antibody in other West Indian islands as yet not investigated using the RFFIT.

The ecologic relationships of bat rabies and rabies of mongooses or other terrestrial carnivores are little known. The possibility of cross transmission of rabies infection from bats to carnivores has been demonstrated in a cave, by caging sentinel animals in a manner preventing direct bites.[1] Whether such transmission takes place naturally is not known. The situation in Trinidad and Grenada with respect to rabies infection is this: in Trinidad rabies is known in several species of bats and in livestock

to which it is transmitted by vampire bats, but it is not known from mongooses or other terrestrial carnivores; in Grenada rabies is present in bats, in mongooses, and in other animals where transmission from infected mongooses is usually apparent. It appears that rabies cycles can exist in bats for long periods, as in Trinidad, without transmission to an available carnivore host. The opinion has been expressed[2] that rabies cycles in different sympatric species of bats may be independent of one another.

Antigenic differences between rabies virus strains of bat and other origin have been little studied. Shope and Tignor[11,18] have reported such studies on rabies and African rabies-related viruses, including Lagos bat virus, using complement-fixation, neutralization and protection tests. Similarities and differences between the viruses were readily demonstrated. Careful serologic comparisons of the abundant rabies virus isolates from bats and other hosts in the western hemisphere are needed.

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