Rabies in New Mexico Cavern Bats

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SEVERAL thousand Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, died at Carlsbad Cavern, N. Mex., in August 1955. Since circumstances suggested the deaths were caused by rabies, an epidemiologic study was done at the cavern during the next 2 years, and related studies were conducted in Texas and Mexico. The investigations were oriented to bat ecology, nonrabies mortality, and monthly rates of rabies morbidity, rabies infection, and serum-rabies antibodies. Experiments in bat rabies transmission to Carnivora were done, and potential sources of bat infection in Mexico were investigated.

Materials and Methods

Initial rabies virus isolations from bats picked up in the cavern in 1955 were made by Lt. Col. Kenneth F. Burns, Brooke Army Medical Center, Fort Sam Houston, Tex. (*1*). Subsequent work was conducted by Communicable Disease Center personnel. Brief studies were made at the cavern in November 1955 and in May 1956, and a continuous investigation was conducted at the cavern from July 1956 through early 1958. A few brief trips were made to the cavern to collect bats in the years that followed. Related work was performed in Mexico in 1957 and in Texas in 1962 and 1963.

Ecologic techniques used are described in another paper (*2*). Bats were collected in flight in an automatic bat trap installed in the entrance of Carlsbad Cavern (*3*). Dead or ill bats were located by a careful search of the floor of the bat roost in the cavern and the area between there and the entrance.

From 1955 until early 1958, tests for viruses were done at the Communicable Disease Center's Newton Field Station, Newton, Ga., and its Virus and Rickettsial Laboratory, Montgomery, Ala. Later tests were performed at the center's Southwest Rabies Investigations Station, University Park, N. Mex. Tests for insecticides were done at its Technical Development Laboratories, Savannah, Ga.

Mouse inoculation tests, serum-virus neutralization tests, and fluorescent antibody tests for rabies were made by conventional methods

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Dr. L. N. Locke, U.S. Fish and Wildlife Service, assisted in fieldwork, Dr. R. E. Kissling, National Communicable Disease Center, performed neutralization tests on serums collected in 1955 and 1956, and Dr. W. J. Hayes, Jr., National Communicable Disease Center, provided insecticide assays of bat tissues.

(*4–8*), with the following variations. Bats and Carnivora used in this survey were bled and partially dissected in the field before being tested in Georgia and Alabama. Heads were removed, individually wrapped in aluminum foil, and labeled. Blood specimens were collected from bats by cardiac puncture, using a 20-gauge needle through which blood was drawn directly into a small serum tube, fitted with a sleeved rubber stopper. A mild vacuum was maintained in the serum tube by attaching another needle and length of rubber tubing to a faucet attachment vacuum. The large gauge needle rapidly removed the blood, which tended to clot quickly. After dislodging the clot from the sides of the tube and allowing it to shrink overnight in a refrigerator, the serum was poured into another tube. Blood was collected from bat fetuses by allowing cut umbilical vessels to bleed directly into serum tubes. Blood specimens were obtained from Carnivora by cardiac puncture, and specimens were kept frozen and shipped periodically to the laboratory. Animals tested in New Mexico were bled and dissected in the laboratory.

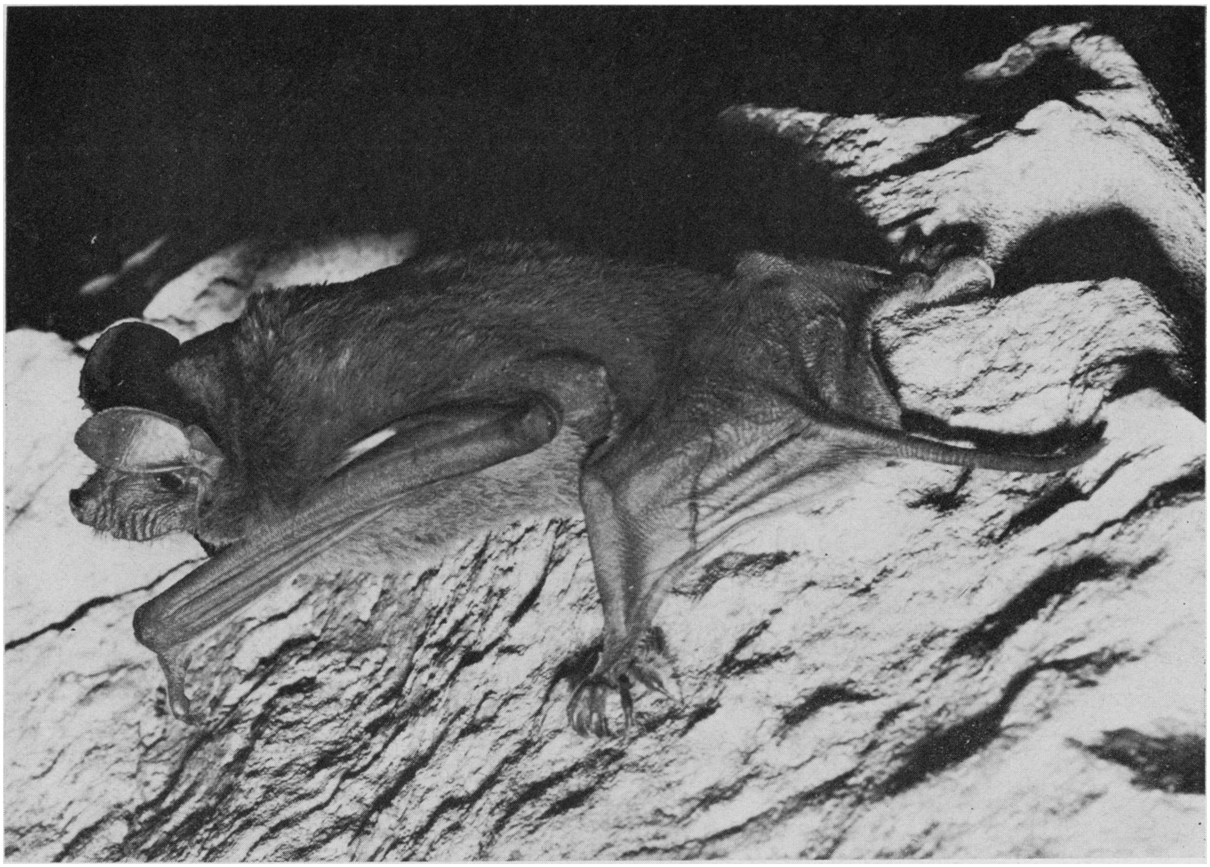
Procedures and some reagents used in the mouse inoculation tests varied at the different laboratories. Albino Swiss mice, about 3 weeks of age, were used. Tissues were ground with mortar, pestle, and Alundum, an abrasive agent. The diluent used in testing specimens collected during 1955–57 was 10 percent normal horse serum in a physiological salt solution. The horse serum was replaced by 10 percent egg yolk in 1958–60. After 1960 the diluent was a 0.75 percent solution of bovine albumin (Armour fraction V) in phosphate-buffered saline, pH 7.2 to 7.5.

From 1955 through June 1957 bat brains were tested in pools of three to four, and if a pool of brains showed positive for virus, the remaining samples of brains were tested individually. A similar procedure was followed in testing salivary glands. However, pooling was discontinued after June 1957, and thereafter bat tissues were tested individually. Bat tissues constituted different percentages by weight of suspensions prepared by the two methods. By the pooling method, brains were 7 percent and salivary glands were 1 percent. By individual tests, brains were 25 percent; salivary glands were 4 percent. The individual test procedure was continued during the period 1958–62, except that brains tested were reduced to 10 percent, and salivary glands to 5 percent. Two milligrams of dihydrostreptomycin sulfate and 1,000 units of crystalline penicillin were included per milliliter of tissue suspension. Saliva samples were collected by swabbing the oral cavity with small cotton swabs. The cotton was rinsed in 0.5 ml. of 10 percent normal horse serum. The only bat or Carnivora glands tested were submaxillary glands.

During 1956, rabies diagnoses were based only on a positive mouse inoculation test and the presence of Negri bodies in the brains of dead mice. The remaining diagnoses were based on positive mouse inoculation tests, with positive serum-virus neutralization tests or positive fluorescent antibody tests, or both.

Serum-virus neutralization tests to detect antibody were done by the virus dilution method except in 1959, when the serum dilution method was used to test bat serums. Bat serums collected in 1955 were pooled in groups of five or fewer for testing, and the numbers of individual bats with rabies-neutralizing antibodies were estimated statistically. All other bat serums were individually tested.

Rabies-virus inoculums used to infect bats and Carnivora in transmission experiments were suspensions of submaxillary salivary glands from free-tailed bats. The first inoculum, D25S, was from a bat found dead in the cavern entrance, and it had a titer of $10^{6.8}$ 50 percent mouse lethal doses (MLD_{50}) per gram of salivary gland tissue. Volumes of 0.03 ml. and 0.075 ml. of a 10^{-2} dilution provided doses of 2,000 for bats and 5,000 MLD_{50} for certain Carnivora. The inoculum sample used to inoculate the bats, however, was shipped frozen to New Mexico after titration, so simultaneous titration in mice was not performed. The second inoculum, XD102S, was taken from one of the bats inoculated with D25S. It had a titer of $10^{7.0}$ MLD_{50} per gram of salivary gland tissue. A 0.60 ml. volume of a 10^{-2} dilution provided a dose of 64,000 MLD_{50} for Carnivora. A third inoculum, XD101S, was from another inoculated bat, and its titer was identical to the second inoculum. Volumes of 0.09 ml. and 0.90 ml. of a 10^{-2} dilution provided MLD_{50} doses of 10,000 and 100,000 for Carnivora.



Mexican free-tailed bat, *Tadarida brasiliensis mexicana*

Chemical analyses of bat tissues for insecticide content were performed by the method of Schechter and co-workers (*9*) as modified by Mattson and co-workers (*10*). Bioassay was achieved using 4-day-old flies (*Musca domestica*).

Wild Carnivora used in the virus and serum investigations and the bite transmission experiments were captured in New Mexico. Species included the gray fox, *Urocyon cinereoargenteus;* ringtail, *Bassariscus astutus;* raccoon, *Procyon lotor;* striped skunk, *Mephitis mephitis;* and spotted skunk, *Spilogale putorius.* Domestic dogs and cats were born in the laboratory and raised in isolation. Carnivora inoculated with virus included gray foxes, a red fox, *Vulpes fulva*, and striped skunks, all captured in southern Georgia. Carnivora were acquired a minimum of 2 months before the experiment and held in isolation from acquisition until death. Mexican free-tailed bats used in experiments were captured at the cavern, kept in isolation, and cared for as described earlier (*11*). Vampire bats, collected in Mexico, were kept locked in separate cages and fed each evening with defibrinated bovine blood, supplied in petri dishes.

Experimental Carnivora were inoculated subcutaneously or intramuscularly, and experimental bats were inoculated intracranially. Experiments in which bats bit Carnivora or other bats were accomplished when bats and Carnivora were held together at biting sites. Each carnivore was bitten by each bat once on the labial mucosa and once on the nose. Each bat used in the experiment was bitten once on the nose by the biting bat. Blood serum specimens were collected from these animals just before exposure and after they were sacrificed.

Results

Patterns of Bat Activity

The population of Mexican free-tailed bats at the cavern varied interseasonally and intraseasonally in sex and age and in numbers of transient and resident bats. To relate results of investigations in virology, serology, and morbidity to the bat population, it was necessary to measure the population and to maintain surveillance. In addition, it was necessary to determine the behavior pattern of the species throughout its range to understand how the bats in Carlsbad Cavern fitted into the overall pattern of behavior. The population study has been described in detail in another paper (*2*) and is briefly summarized here.

Although some of these bats may be found in warmer parts of the United States in winter, most fly to tropical areas of Mexico and possibly farther south. Some share caves with vampire bats while in Mexico. After mating in March, the sexes separate and the bats migrate to the United States or to higher elevations in Mexico. After a gestation period of about 3 months the females bear and rear the young bats in hot, relatively low areas, whereas the males spend the summer at higher elevations. At about 6 weeks of age the young bats begin to fly, and the southward migration begins. Carlsbad Cavern, located on the border between ecologic ranges of males and females, may shelter either or both sexes in summer. The cavern also accommodates transient bats during periods of migration.

Mortality in Bats

In some years the beginning of the southward migration coincided with great numbers of bat deaths. Epizootics occurred at Carlsbad Cavern in 1955 and in 1956, but few deaths were observed in 1957. Epizootics also occurred after 1957, but few details were available. Similar dieoffs occurred at these times at bat caves in Texas.

After several thousand bats died at the cavern in August 1955, tissues of six dead bats were tested for insecticide poisoning, and a retrospective survey was made of insecticides used in the area in 1954 and 1955. Results did not support the hypothesis that the deaths were caused by insecticides. Chemical analyses showed 2 ppm of 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDT) and 9 ppm of 1,1-dichloro-2,2-bis (*p*-chloro-phenylethylene) (DDE, a DDT breakdown product) in the bat tissues. Bioassay of tissues showed a toxicity equivalent to 1.3 ppm of DDT. Chemical analyses of tissues of 11 control bats, collected several weeks after the epizootic, however, indicated 5 ppm of DDT and 11 ppm of DDE, evidently ruling out insecticide poisoning as the cause of death. Twenty ill or dead bats, picked up at the cavern between 2 and 8 days after the majority of the deaths had occurred, were tested for rabies, and 11 were infected with the virus. Thus, rabies was suspected as the cause of the bat deaths.

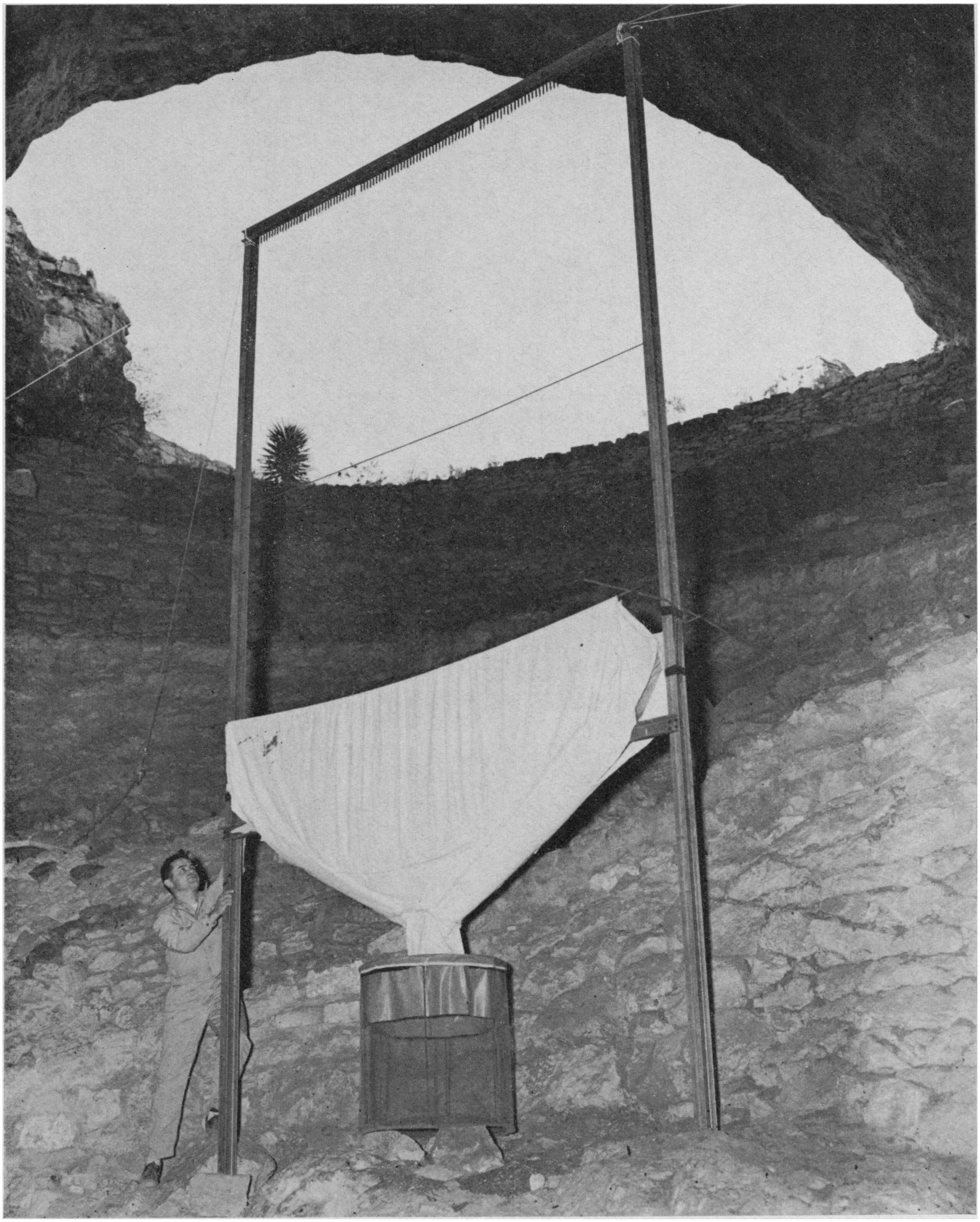
Another epizootic occurred the following August, and 2,228 fallen bats were recovered. Morbidity was characterized by epileptiform convulsions and at times by hemorrhaging in the lungs and thorax. Although rabies was isolated from 3.9 percent of a sample of the symptomatic bats (table 1), rabies could not be considered the cause of the 1956 epizootic. Circumstances attending this dieoff were compared with circumstances in 1957, when little mortality occurred (*2*). The 1956 epizootic was associated with migration during inclement weather outside and unfavorably cool temperatures in the cavern due to the presence of relatively few bats in the roost.

Similar mortality was seen in late July and early August 1962 in two Texas bat caves (*2*). Rabies was detected in 0.94 percent (one of 106) of a sample of bats picked up at one Texas cave and in 3 percent (three of 100) of bats picked up at the other cave. The senior author has observed apparently identical mortality in this species of bat in California.

Rabies Infections in Bats

Monthly samples of cavern bats were obtained to determine rates of rabies-virus infection and, when possible, rates of rabies neutralizing antibody in samples of blood. Both clinically normal bats, trapped in flight at the cavern entrance, and dead or moribund bats, picked up from the cavern floor, were collected and tested. Turnover in the cavern bat population occurred (*2*), so data on rabies obtained during a given month were applicable only with reservations to bats in preceding or succeeding months.

The number of rabies-virus positive bats in samples of clinically normal bats was low (table 2), and no significant differences in monthly rates of infection were detected. Rates in monthly samples were consistently no greater



Photograph by Tex Helm

Automatic bat trap, designed by Dr. Constantine, installed in the entrance of a cave

Table 1. Rabies infections in dead or moribund bats found at Carlsbad

Year and month	Average number of bats in cavern [1]	Total found dead or moribund	Adult males		Adult females		Immature males	
			Tested	Infected	Tested	Infected	Tested	Infected
1956								
August	98,000	2,228	100	4	28	1	------	--------
September	90,000	128	10	2	7	2	1	0
October	94,500	8	------	--------	1	1	2	2
1957								
May	15,000	0	------	--------	------	--------	------	--------
June	38,000	7	0	--------	0	--------	0	--------
July	55,000	11	1	1	2	2	------	--------
August	71,000	8	0	--------	1	1	0	--------
September	127,000	18	1	1	6	3	1	1
October	98,000	22	3	2	3	2	4	3

[1] Estimated on basis of 300 bats per square foot of ceiling area used for roosting. SOURCE: Reference 1.

[2] Based on clinical evidence only; figure represents maximum possible.

[3] Collective rate given because individual rates do not differ significantly.

NOTE: nd means not determined.

than 1 percent; the overall rate for combined samples of asymptomatic bats was 0.23 percent (five of 2,192). From May to October, between 15,000 and 127,000 bats took shelter in the cavern (*2*). Application of the 0.23 percent rate of infection to the population figures indicated that the number of asymptomatic, rabies-virus infected bats varied from 32 to 292. By comparison, samples of bats from another roost had infection rates of 1.6 percent or lower (table 3).

Sampling of dead or moribund bats for rabies indicated that only about 4 percent of the symptomatic bats in the epizootic of August 1956 were infected with the rabies virus (table 1). Evidently, at least 96 percent of the symptomatic bats were downed by another cause. A rabies-associated morbidity rate of 0.089 percent was calculated for August by dividing the estimated 87 cases of rabies among the dead or moribund bats with the estimated cavern population of 98,000. This rate was more than seven times greater than the relatively constant rabies morbidity rate observed at other times. By October 1956 the rate had dropped to 0.008 percent. The 0.008 percent rate and rates for each month in 1957 did not differ significantly, and collectively they indicated a constant rabies morbidity rate of 0.012 percent. Estimated numbers of ill or dead rabies-virus infected bats found on the cavern floor decreased from about three each day in August 1956 to one every 2 to 4 days in October 1956 and throughout 1957. Thus, the epizootic of bat deaths, evidently not caused by rabies, included a greater proportion of rabies-virus infected bats than the proportion usually seen in samples of clinically normal bats. It was not clear whether infected bats picked up during the epizootic were killed by rabies or by something else. Individual bats may have died of either cause or both.

Knowledge of rabies in symptomatic bats, based on the 32 infected bats picked up in 1956 and 1957, was supplemented by data on 45 additional infected bats picked up in the cavern between 1958 and 1962. Virus titers were as high as $10^{8.7}$ MLD_{50} per gram of brain tissue and $10^{6.8}$ MLD_{50} per gram of salivary gland tissue. Rabies virus was detected in the brains of each of the 77 bats, and the salivary glands in 37 (48 percent) of these bats were infected.

The only signs of rabies observed in the rabies-virus positive bats were signs of paralysis. No furious rabies was seen, and there were no unprovoked attacks on persons. Retaliatory bites, normal in all wild mammals, usually were attempted when the bats were picked up.

Serum Rabies-Neutralizing Antibodies

Monthly samples of bats tested for rabies virus also had their serums tested for rabies antibodies (table 4). The rate of rabies antibody appeared to be relatively constant in

Cavern, 1956 and 1957

Immature females: Tested	Immature females: Infected	Percent of total infected	Estimate of symptomatic bats infected: Numbers found	Estimate of symptomatic bats infected: Percent of population
------	--------	3.9	87	0.089
------	--------	22.2	28	.031
------	--------	100.0	8	
------	--------	--------	0	
0	--------	nd	[2] 7	[3].012
------	--------	100.0	11	
1	1	100.0	8	
3	3	73.0	13	
2	0	58.3	13	

adults, except for a high rate in adult females during July 1956. The antibody rate in all bats was higher in the spring and autumn of 1956 than it was during these seasons in other years.

In June 1957, pregnant females and their fetuses were separated, bled, and tested individually. Results of serums of 88 adult females paired with their fetuses were as follows: 57 pairs had rabies-negative serum, 15 pairs had rabies-positive serum, nine adults had rabies-positive serum and their fetuses had rabies-negative serum, and seven adults with rabies-negative serum had fetuses with rabies-positive serum.

A chi-square test for independence produced a probability of less than 0.0005, demonstrating that a highly significant association existed between maternal and fetal antibody. Thus, it appears that these bats receive antibodies prenatally. The evident loss of serum rabies-virus antibody in young bats after August (table 4) is consistent with the expected course of passive immunity.

Rabies-virus antibodies evidently were present in similar proportions of rabies-free, symptomatic bats and rabies-free, asymptomatic bats collected during the August 1956 epizootic. Antibodies were found in 21 percent (six of 23) of the symptomatic bats and in 25 percent (44 of 174) of the asymptomatic bats. Therefore, no association was observed between rabies-free morbidity and rabies antibody.

Serums were collected from only two symptomatic, rabies-virus infected bats, and one of the two bat serums had demonstrable rabies antibodies. None of four serums collected from asymptomatic, rabies-virus infected bats contained neutralizing antibodies. Thus, a positive association between subclinical rabies and rabies antibodies was not observed.

Table 2. Rabies infections in asymptomatic bats collected in flight at Carlsbad Cavern

Year and month	Asymptomatic bats tested: Adult males	Adult females	Immature males	Immature females	Total	Total positive [1]: Number	Percent	Virus in—
1955								
November [2]	nd	nd	nd	nd	177	0	--------	------------------
1956								
May	103	109	--------	--------	212	0	--------	------------------
July	101	89	--------	--------	190	0	--------	------------------
August	158	117	56	51	382	0	--------	------------------
September	101	123	[3] 118	79	421	1	0.2	Brain and salivary glands.
October	135	[3] 230	41	40	446	1	.2	Brain only.
November	26	36	1	1	64	0	--------	------------------
1957								
May	[3] 100	100	--------	--------	200	1	.5	Brain only.
June	------	[3] 100	[4] 52	[4] 48	100	1	[4] 1.0	Do.

[1] Virus isolates identified as rabies by Negri bodies in dead test mice (1956 isolations) or by serum-virus neutralization test (1957 isolations). Not included in table, although salivary gland suspensions killed mice, were the following bats: 2 in November 1955, 1 in May 1956, 3 in May 1957, and 1 in June 1957. Agents could have been Rio Bravo virus, detected in salivary glands of 2 bats in September 1957. (Source: Constantine, D.G., and Woodall, D.F.: Latent infection of Rio Bravo virus in salivary glands of bats. Public Health Rep 79: 1033–1039, December 1964.)

[2] Captured at rest on the cavern ceiling.

[3] 1 bat in sample positive.

[4] Fetuses were taken from the adult females cited. They are excluded from the totals.

NOTE: nd means not determined.

Transmission from Bats to Carnivora

Mexican free-tailed bats are preyed upon by many species of mammals, birds, and reptiles that capture them at cave entrances during the dense flights. Many species enter the caves to eat fallen bats or to remove them from accessible ceiling areas. Foxes, coyotes, skunks, and raccoons were abundant in the cavern area. Striped skunks, ringtails, and raccoons entered the cavern and ate fallen bats. Although pets were confined while in the park, feral cats of the domestic species were common. It appeared, therefore, that some fallen, rabies-virus infected bats would be attacked by hungry or curious Carnivora and that the bat would bite in retaliation. This subject was investigated by a virus and serum survey of local Carnivora and by exposure of captive Carnivora to the bat rabies virus. Exposures were by bat bite, ingestion, and intramuscular or subcutaneous inoculations of the virus.

Carnivora were collected near the entrance of Carlsbad Cavern in November 1955. Brain and salivary gland tissues of 33 animals (five gray foxes, seven ringtails, six raccoons, and 15 striped skunks) were negative to the tests for

Table 3. Rabies in asymptomatic bats collected while at rest under a bridge, 24 miles east of Carlsbad Cavern

Year and month	Brains and salivary glands tested				Totals			Antibody tests of bat serums		
	Adult males	Adult females	Immature males	Immature females	Number tested	Number infected	Percent infected	Number tested	Total with antibody	Percent with antibody
July 1956	0	0	98	88	186	[1] 3	1.6	118	36	[2] 30.5
October 1958	nd	nd	nd	nd	66	[3] 1	1.5	0	------	------
July 1959	0	100	50	0	150	[4] 0	------	150	36	24
September 1960	50	50	50	50	200	[5] 1	.5	0	------	------
June 1962	50	------	------	------	50	0	------	0	------	------

[1] Three females were infected in brains and salivary glands. These bats were too young to fly.

[2] Serums were obtained from 2 of the 3 rabies-virus positive bats; neither had detectable rabies-neutralizing antibodies.

[3] Only salivary glands tested.

[4] Mammary glands of females also tested.

[5] Brain of 1 immature female infected, salivary glands negative.

NOTE: nd means not determined.

Table 4. Blood serum rabies-neutralizing antibodies in asymptomatic bats collected at Carlsbad Cavern [1]

Year and month	Adult males		Adult females		Immature males		Immature females		Total	
	Number tested	Percent with antibody	Number tested	Percent with antibody	Number tested	Percent with antibody	Number tested	Percent with antibody	Number tested	Percent with antibody
1955										
November	nd	------	nd	------	nd	------	nd	------	154	15
1956										
May	99	22	95	22	------	------	------	------	194	22
July	98	25	65	40	------	------	------	------	163	31
August	72	22	40	20	34	32	28	32	174	25
September	32	28	28	29	20	5	21	14	101	21
October	52	25	113	26	12	8	11	9	188	23
1957										
May [2]	91	12	96	18	------	------	------	------	187	15
June [2]	0	------	100	26	[3] 44	30	[3] 44	27	100	26
September	50	18	49	16	49	12	50	10	198	14

[1] Serums were obtained from the bats in table 2 except for bats collected in September 1957.

[2] 1 bat rabies-virus positive in each month. Neither had rabies-neutralizing antibodies.

[3] Fetuses taken from the adult females cited. They are excluded from the total.

NOTE: nd means not determined.

rabies virus, but serums of one raccoon and two skunks had rabies-neutralizing antibodies. Serum-virus neutralization tests indicated neutralization indices of 63 for the raccoon and 63 and 40 for the two skunks.

During the August 1956 epizootic of bat deaths two striped skunks and two raccoons were exposed to bites of four to 15 fallen bats, which were tested for rabies after they died. A spotted skunk was fed 28 symptomatic bats and another raccoon 45 such bats. None of the bats could be tested for rabies. None of the Carnivora died of rabies, but a raccoon, bitten by a rabies-virus positive bat, had developed a serum-neutralization index of 100 when sacrificed, 8 months after exposure. A striped skunk, bitten by another rabies-virus positive bat, developed an index of 40 in the same period. Serums of the remaining animals were negative.

In another experiment, Carnivora and bats were exposed to bites of bats that had been intracranially inoculated with rabies virus originating in the salivary gland of a naturally infected bat. Each of 12 bats received an estimated 2,000 MLD_{50} (0.03 ml.) of virus. Thirty days later, when the bats had signs of rabies, nine bats capable of biting were made to bite 12 other bats on the nose and 33 Carnivora (six dogs, four cats, six gray foxes, six ringtails, six raccoons, three striped skunks, and two spotted skunks) on the nose and labial mucosa. Saliva samples were taken from the inoculated bats just before biting, and saliva from three of the biting bats contained rabies virus. None of the bitten animals died of rabies, and their brains and salivary glands were negative for the virus when killed, 3 months after exposure. Neutralization tests were made on Carnivora serums collected before exposure and at necropsy; one raccoon had developed a neutralization index of 200.

In a third experiment, Carnivora were exposed to the bat rabies virus by inoculation. Suspensions of infected bat salivary glands were inoculated in dorsal neck muscles, muscles of the posterior limb, or subcutaneously in the lips of 11 animals (table 5). All six foxes died of rabies; each had virus in brain tissue, and three also had virus in salivary gland tissue. One of two striped skunks died of rabies, and the virus was detected in its brain and salivary glands. One skunk and three raccoons did not develop the disease. Paralysis was the predominant sign observed, although affected animals were vicious.

Vampires as a Potential Source of Rabies

The discovery of cohabitation of Mexican caves by vampire bats and free-tailed bats (*2*) suggested that vampires may be a source of rabies for the migratory, insectivorous species. Bats banded earlier at Carlsbad Cavern and

Table 5. Transmission of rabies virus of bat origin by inoculation of Carnivora

Species and number	Inoculum (MLD_{50})	Inoculation route	Course of disease: Incubational days	Course of disease: Clinical syndrome	Course of disease: Days ill	Killed: days after inoculation	Rabies virus detection: Brain tissue	Rabies virus detection: Salivary glands
Gray fox:								
F1	5, 000	Neck muscles	10	Paralysis	2	--------	Positive	Positive.
F4	10, 000	Leg muscles	17	do	<1	--------	do	Negative.
F6	10, 000	Lips subcutaneously.	18	do	1	--------	do	Do.
F7	10, 000	do	13	do	1	--------	do	Positive.
F2	64, 000	Neck muscles	11	do	2	--------	do	Negative.
Red fox: F3	100, 000	do	10	do	2	--------	do	Positive.
Raccoon:								
C1	5, 000	do	--------	----------	------	120	Negative	Do.
C2	64, 000	do	--------	----------	------	90	do	Negative.
C3	64, 000	do	--------	----------	------	90	do	Do.
Striped skunk:								
S30	100, 000	do	28	nd	nd	--------	Positive	Positive.
S31	100, 000	do	--------	----------	------	56	Negative	Negative.

NOTE: nd means not determined.

in Arizona were found in Mexican caves. Observation of great numbers of chilled, lethargic, free-tailed bats, roosting near fully active vampires, was consistent with the hypothesis that similarly incapacitated insectivorous bats might constitute a source of blood for the hematophagous vampire. The vampire, reported as a rabies carrier (*12, 13*), might thus transmit the disease. In an experiment, each of 10 individually caged vampires was given a free-tailed bat companion, and the regular daily feeding of bovine blood was withheld. The vampires attacked the free-tailed bats and licked their wounds free of blood, demonstrating that hungry captive vampires will feed on available bats.

Discussion

Rabies in the bats of Carlsbad Cavern is part of a widespread problem, since the disease is known in the Mexican free-tailed bat in California (*14*), Arizona (*15, 16*), Texas (*1, 17–23*), and Oklahoma (*24*) as well as in the southeastern subspecies (*Tadarida brasiliensis cynocephala*) in Louisiana (*18*), Georgia (*25*), and Florida (*26*). The migratory habits of the species makes this perspective particularly important.

It is not clear why the rate of rabies-associated deaths increased during the August 1956 epizootic over the rate observed at other times, since rabies evidently was not the cause of the epizootic. The estimated 87 rabies virus-positive bats picked up that month were not in excess of the 225 clinically normal, rabies-infected bats estimated by the usual 0.23 percent infection rate, so the bat population evidently had a potential of producing more than 87 rabies deaths. At least two possible explanations exist.

One explanation would be that an increase in rabies morbidity detection was made possible because, as observed, the bats suspended a great deal of foraging activity during the epizootic and remained in the cavern at night. As a result, bats that might have developed signs of rabies while foraging outside might have developed signs of the disease in the cave instead, increasing the rate detected there.

A second explanation would be that the dead bats were part of a migrant group with an exceptionally high infection rate. The 3.9 percent rate of infection detected in the dead bats was similar to rates among flying bats collected in Texas during migration; however, those bats were held captive, and infected individual bats developed rabies signs as long as 3 months after capture (*27*).

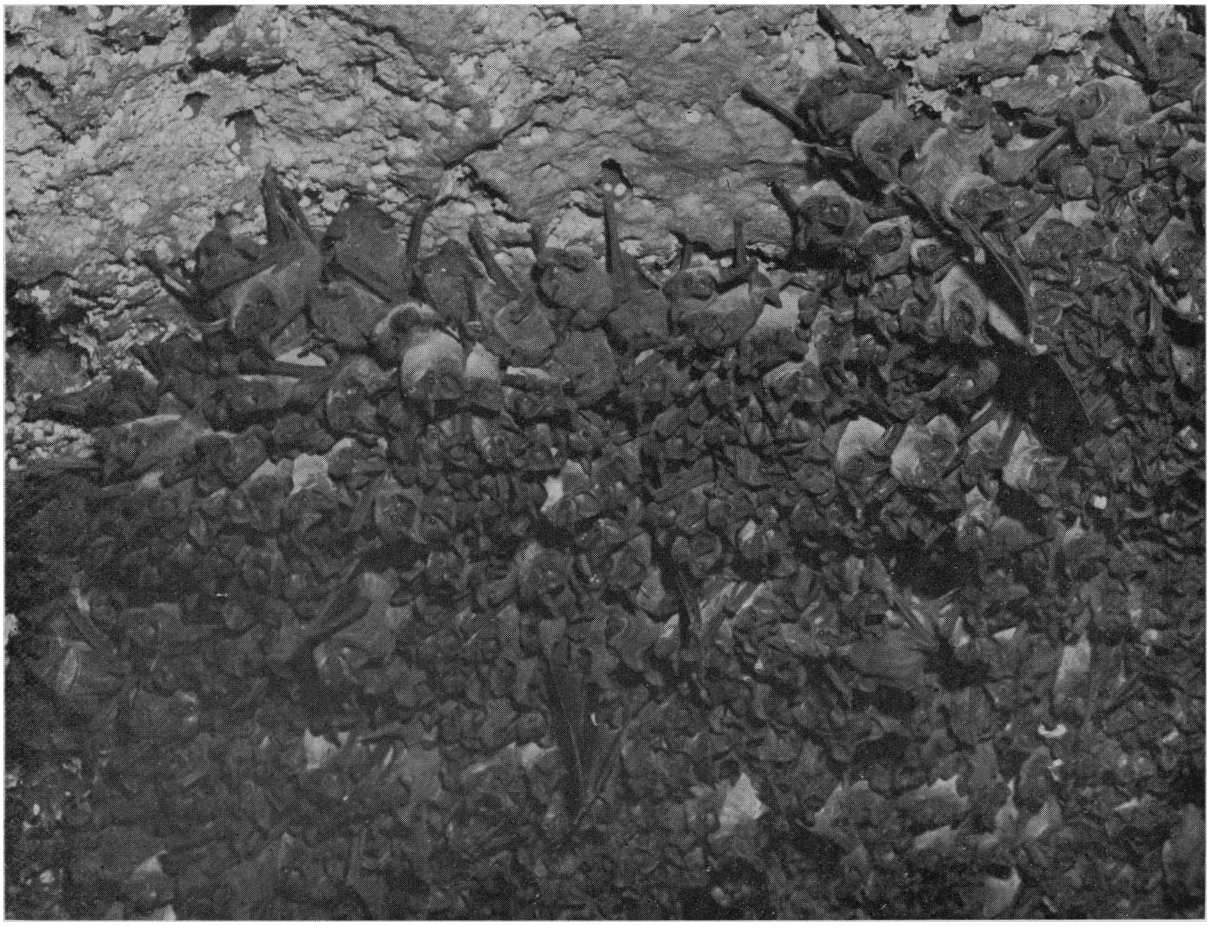
The association observed between relatively high rates of rabies infection and migration may be more than coincidental. The work of Soave and co-workers (*28–30*) with guinea pigs demonstrates two possibilities—the existence of latent infections or of prolonged incubation periods, with the animals developing clinical rabies when subjected to stress. Stress experienced by bats in migration may have a similar effect.

Apparently many bats survived exposure to the virus, since a high proportion had serum-rabies neutralizing antibodies, and the virus was not detected in their tissues. Conceivably, the clinically normal, infected bats could have (*a*) been incubating the disease, (*b*) been recovering from infection, or (*c*) had latent infections.

A difference was observed between the usual rates of rabies-virus infection in asymptomatic bats (0.23 percent) and rabies morbidity (0.012 percent). The 0.012 percent rate must have been lower than the true rate, because it represented morbidity only in the cavern. Additional bats probably developed signs of rabies outside the cavern.

Monthly differences in antibody rates in adult females coincided with developments in the local breeding pattern, but they were also coincident with changes in the adult female population. The antibody rate was low in spring, peaked just before weaning, and then dropped. However, in spring and after weaning transient female populations contained high proportions of aged bats (*2*), so the significance of the monthly differences in antibody rate is unknown. Similar population changes occurred in adult males, but the antibody rates were less variable.

Evidently, prenatal transfer of antibody occurs in this species. The drop in antibody rate in immature bats in September was relatively large and may have been due to loss of passive immunity. Clinical rabies and subclinical rabies were detected in immature bats about the same time. But the drop in antibody rate and the rabies morbidity coincided with the arrival of



Mexican free-tailed bats roosting in Frio Cave, Uvalde County, Tex.

many transient immature bats, making interpretation difficult.

It is a remote possibility that the rabies-virus inactivating substance in bat serum (referred to in this paper as a neutralizing antibody) is a heat-stabile, nonspecific virus inactivator. Burns and Farinacci (*17*), who first detected the substance in bat serum, favored the interpretation that it was a specific rabies-neutralizing antibody. Schneider and co-workers (*26*) found no evidence of rabies-neutralizing activity in serums of 245 bats (predominantly *T.b. cynocephala*).

It is conceivable that the trap method of collecting bats introduced bias by favoring trapping of rabies-virus infected bats. Infected bats that were well enough to fly but ill enough to be trapped more readily could have been taken in numbers exceeding their proportions in the population. This seems improbable, however, because rabies morbidity rates did not show a direct relation to rabies-virus infection rates in samples of trapped bats. Samples of bats collected by net when the bats were at rest had rates of infection similar to or higher than rates usually detected in bats trapped in flight (tables 2 and 3).

Carnivora were infected with airborne rabies-virus in a free-tailed bat cave roost, where two men could have been infected in the same way (*31*). Carnivora died of rabies after inoculation with rabies virus from free-tailed bats, and similar results were obtained in another experiment (*32*). Although experiments in rabies transmission by bat bites reported in this paper did not cause any deaths, another experiment did cause the deaths from rabies of one coyote and two foxes when they were bitten many times by free-tailed bats (*33*). Moribund, rabies-infected Mexican free-tailed bats, frequently found on the ground, will bite inquisitive persons and animals, and the hazard of infection by bat bite to carnivores and possibly man must be acknowledged.

Free-tailed bats may be infected in nature by vampires, since they were observed occupying the same caves in Mexico. The vampires obtained blood from the insectivorous species in the laboratory, although vampires may not react this way in nature. Wimsatt reported that captive vampires fed on each other when confined in the laboratory (*34*). Vampires as a source of rabies for the free-tailed bat evidently would supplement an autogenous source, since infected suckling bats, too young to fly, were found in a bat roost in New Mexico (table 3).

It seems improbable that the relatively small numbers of bats in Carlsbad Cavern during recent years would constitute a danger of rabies transmission to human beings or Carnivora by air. The bat roost of the cavern is huge in relation to the numbers of bats using it, and it is ventilated by several shafts which penetrate its roof. But persons should avoid places crowded with great numbers of bats, such as the cavern entrance during the dense flights in and out of the cavern. How bats transmit rabies virus other than by biting is unknown, but conceivably the virus is liberated in saliva or urine. Rabies virus in the kidneys of these bats make it advisable to avoid contact with the urine. Rabies virus was isolated from kidneys of 30 percent (seven of 23) of a sample of rabies-infected adult free-tailed bats collected in five Texas caves in 1962.

If a person picks up a bat, he can expect to be bitten, even though the bat appears to be dead; fallen bats usually appear lifeless. Evidently these bats do not experience furious rabies or engage in unprovoked attacks. In our studies, pregnant bats occasionally collided with persons in the cavern entrance, because it is difficult for these bats to navigate around unexpected obstacles. This lack of maneuverability was true of other bats in the cave entrance during very heavy flights. One might be tempted to conclude that lack of recognized deaths of human beings from rabies at the cavern constitutes convincing evidence that there is no cause for concern. The fact that five persons have died after exposure to bats in the United States in recent years (*35*), at least two after exposure to Mexican free-tailed bats (*36*), should constitute convincing proof of the current hazard.

Summary

An epizootic of deaths of Mexican free-tailed bats at Carlsbad Cavern, N. Mex., in August 1955 at first appeared to be caused by rabies, stimulating epidemiologic studies to define the extent of the disease, its origin, and its public health significance.

Excessive bat mortality occurred in 1956 and in certain subsequent years. The deaths of the bats were associated with migration during inclement weather and unfavorably cool temperatures in the cavern due to the presence of relatively few bats. Rabies virus was detected in 3.9 percent of the fallen bats, a rate similar to that subsequently observed in clinically normal migrating bats. During nonepizootic periods, relatively few moribund or dead rabies-virus infected bats were found.

Migratory and other bat population movements made rates of rabies infection and the presence of rabies antibody in bat serums difficult to interpret. In monthly samples, rabies virus was detected in less than 1 percent of the bats collected in flight, and serum rabies-neutralizing antibody usually was found in 15 to 30 percent. Evidently, prenatal transfer of antibody occurs in the free-tailed bat; a decrease of antibody rate in young bats was followed by clinical rabies in bats of similar ages.

Naturally infected bats were not observed to develop furious rabies, and there were no unprovoked attacks by bats, but moribund bats bit when handled. Certain native Carnivora developed rabies after intramuscular or subcutaneous inoculations of the bat rabies virus. Rabies-virus transmission to Carnivora by bat bite was attempted and failed, but it has since been achieved.

Bats banded at the cavern migrated into Mexico, where in winter they shared caves with vampire bats. In an experiment, captive vampires got blood from free-tailed bat cagemates. Rabies virus might be transmitted to free-tailed bats in this manner in nature. Rabies-infected

suckling free-tailed bats, however, were found in New Mexico, which indicates an intraspecies source of infection.

Mexican free-tailed bats appear to be a potential source of rabies infection for man and Carnivora, and direct contact with bats should be avoided. Ventilation in the cavern appears adequate to prevent airborne rabies-virus transmission, but a remote hazard of infection from this source may exist in corridors crowded with great numbers of flying bats entering or departing from the cavern.

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Conference Calendar

May 1–2, 1968. American Pediatric Society, Atlantic City.

May 1–3, 1968. Operations Research Society of America, San Francisco.

May 5–10, 1968. American Society for Microbiology, Detroit.

May 6–9, 1968. Canadian Public Health Association, Vancouver, Totem Park Complex, University of British Columbia.

May 8–11, 1968. National Geriatrics Society, Toronto.

May 10–11, 1968. Association of University Radiologists, Columbus, Ohio.

May 10–12, 1968. American Academy of Psychoanalysis, Boston.

May 12–17, 1968. American Psychiatric Association, Boston.

May 13–17, 1968. American College of Psychiatrists, Boston.

May 13–17, 1968. American Industrial Hygiene Association, St. Louis.

May 13–17, 1968. American Nurses Association, Dallas.

May 13–17, 1968. Society for Applied Spectroscopy. Chicago, Sheraton-Chicago Hotel.

May 19–22, 1968. National Conference of Tuberculosis Workers and the National Tuberculosis Association, Houston.

May 20–27, 1968. World Congress on Fertility and Sterility, Tel Aviv.

May 26–31, 1968. National Conference on Social Welfare, San Francisco.

May 28–31, 1968. American Public Health Association's Southern Branch, Roanoke, Va., Hotel Roanoke.

May 30–31, 1968. Sanitary and Water Resources Engineering Conference, Nashville, Tenn., Noel Hotel.

June 9–13, 1968. Tenth Annual National Industrial Pharmaceutical Research Conference, Land O' Lakes, Wis., Gateway Hotel.

June 2–7, 1968. American Water Works Association, Cleveland.

June 9–14, 1968. Medical Library Association, Denver.

June 14–15, 1968. American Rheumatism Association, Seattle, Olympic Hotel.

June 15–16, 1968. American Diabetes Association, San Francisco.

June 16–20, 1968. American College of Preventive Medicine, San Francisco.

June 16–20, 1968. American Medical Association, San Francisco.

June 23–28, 1968. Air Pollution Control Association, St. Paul, Minn.

June 23–28, 1968. Educational Conference in Environmental Health, Washington, D.C., Sheraton-Park Hotel.

June 24–29, 1968. American Association for the Advancement of Science, Logan, Utah.

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