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Authors: SHOTTS, EMMETT B., ANDREWS, CHARLES L., SULZER, CATHERINE, and GREENE, ELLEN

Source: Journal of Wildlife Diseases, 7(2) : 115-117

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-7.2.115

LEPTOSPIROSIS IN COTTONTAIL AND SWAMP RABBITS OF THE MISSISSIPPI DELTA

EMMETT B. SHOTTS, JR.,[1] CHARLES L. ANDREWS,[2], CATHERINE SULZER,[3] ELLEN GREENE[4]

Abstract: Fifty rabbits, 44 cottontails *(Sylvilagus floridanus)* and six swamp rabbits *(Sylvilagus aquaticus)*, were collected from the Mississippi Delta Area. Serum and tissue from these animals were studied for evidence of leptospirosis. *Leptospira interrogans* antibodies were demonstrated in 77% (37/48) of th serums collected, of which 21% (10/48) had significant titers. Serotypes most frequently encountered were *ballum, australis, icterohaemorrhagiae, canicola,* and *grippotyphosa.* Focal nephritis was observed histologically in 92% (46/50) of the kidneys. Isolation of *grippotyphosa* was made from 8% (4/50) of the kidneys collected. These studies have assisted in establishing the importance of cottontail and swamp rabbits as reservoirs for leptospires and have also identified two new host-serotype relationships.

INTRODUCTION

Prior to this investigation, cottontail and swamp rabbits have been considered of little or no importance as reservoirs of pathogenic leptospires. A previous attempt to isolate leptospires from rabbits in the United States resulted in the recovery of a single isolate of *Leptospira interrogans,* serotype *ballum* from 245 cottontails.[8] Canadian workers have reported the recovery of a single isolate of *pomona* from this same species.[7] A limited serologic study of nine cottontail rabbits revealed one animal with a titer against *canicola.*[6] No data were found regarding the status of leptospirosis in swamp rabbits.

The current study was undertaken when focal nephritis was observed histologically in a large number of cottontail rabbits collected from the southeastern nited States for parasitologic investigations. Further studies of kidney tissue stained with fluorescent antibody revealed the presence of leptospires.[1]

MATERIALS AND METHODS

Rabbit kidneys collected from the Mississippi Delta Area had yielded the highest percentage of lesions. Therefore the two collection sites chosen for the study reported here were in Bolivar County, Mississippi. Each of these sites were private hunt clubs of 12,500 and 20,000 acres, respectively, situated between the levee and the Mississippi River. The land was intensively managed for hardwood timber production with food plots interspersed throughout the acreage.

Rabbits were collected by shooting, at dawn and dusk during 14-18 July 1969. Shotguns were used to minimize trauma. Fifty rabbits (44 cottontail, and six

[1] Department of Medical Microbiology, College of Veterinary Medicine, University of Georgia, Athens 3001.

[2] Department of Biology, Brenau College, Gainesville, Georgia 30501.

[3] Epidemiology Program, Center for Disease Control, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia 30333.

[4] Southeastern Cooperative Wildlife Disease Study, Department of Pathology and Parasitology, college of Veterinary Medicine, University of Georgia, Athens 30601.

This study was supported by an appropriation from the Congress of the United States. Funds were administered and research coordinated under the Federal Aid in Wildlife Restoration Act (50 Stat. 917) and through Contract No. 14-16-0008-676, Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior.

The authors are very grateful for the invaluable assistance and cooperation of the Mississippi Game and Fish Commission and the Catfish Point and Merigold Hunt Clubs of that state. Many thanks also are extended to Conservation Officer Matt Dakins, for his untiring efforts on behalf of this study. Gratitude is expressed to the Science Department, Delta State College, Cleveland, Mississippi, for providing laboratory facilities.

swamp) consisting of 27 adults (10 males, 17 females) and 23 juveniles (11 males, 12 females) were collected.

Blood collected by cardiac puncture was allowed to clot. Serum was aspirated, centrifuged to remove cellular components and held frozen at —20 C until serological tests were done.

Serums were examined for leptospiral antibodies by the macroscopic slide agglutination test. All serums which reacted were examined further by the microscopic agglutination test.[9]

After collection of blood, animals were identified by number and placed in plastic bags. Collection groups were staggered so that kidneys were cultured within three hours of the rabbit's death. Kidneys were removed aseptically and cut transversely. A portion of each kidney was ground with a sterile mortar and pestle in Sorenson's buffered saline to yield a 10% suspension.[9] This suspension was diluted 10-fold to 10^{-3} in buffered saline. A series of four tubes of Fletcher's semi-solid medium were inoculated with these suspensions. One drop of suspension from a 20 gauge needle was used as the inoculum in each case. The first two tubes contained 5-fluorouracil, 200 micrograms/ml.[4] Cultures were incubated at 30C for nine weeks with weekly observations by darkfield microscopy. Identification of isolated leptospires was made by agglutinin-absorption techniques. Two of the isolates were further studied by absorption with serum recovered from the animal that had harbored the isolant.[9]

A portion of kidney from each rabbit was fixed in 10% buffered formalin and examined histologically.[5]

RESULTS

Serums suitable for testing were collected from 43 cottontail and five swamp rabbits. Thirty-four cottontail and three swamp rabbits had leptospiral antibodies as indicated by the macroscopic slide agglutination test of Galton (Table 1). Of the 37 serums reacting to the slide agglutination test, only ten reacted to the microscopic agglutination test. The majority (eight of ten) of these reactions were against *australis*, which is a common serologic cross pattern noted in *grippotyphosa* infections.

Leptospires were isolated from two immature cottontail and two mature

TABLE 1. Leptospiral serologic response of Mississippi Delta Rabbits by esrotype : macroscopic agglutniation test (Galton)

Serotype	Cottontail — 43		Swamp — 5	
Ballum	28*	(65**	4	(80)
Canicola	7	(16)	2	(40)
Icterohemorrhagica	8	(19)	2	(40)
Grippotyphosa	7	(16)	2	(40)
Pyrogenes	1	(2)		
Autumnalis	1	(2)		
Pomona	1	(2)		
Sejroe	1	(2)		
Australis	12	(28)	3	(60)

* Number of animals reacting
** Percentage of serotype reactions

TABLE 2. Relation of nephritis, serologic response, and isolation in rabbits collected from the Mississippi Delta area.

	Cottontail	Swamp	Total	Percent
Nephritis	41/44	5/6	46/50	92
Serologic Response	34/43	3/5	37/48	77
Isolation	2/44	2/6	4/50	8

swamp rabbits. These isolants were identified as *grippotyphosa.*

Histologic studies of kidneys demonstrated focal nephritis in 46 (92%) of the 50 rabbits examined (Table 2).

DISCUSSION

The demonstration of leptospiral antibodies in both adults and juveniles indicates that rabbits are susceptible to leptospiral infection at an early age. The resulting disease is probably not an important cause of mortality, as evidenced by the ratio of age groups and sexes collected. The high prevalence of antibodies in these animals (77%) is surprising however, in view of the low rate of isolation of leptospires from rabbits in the past. This reactor rate probably is related to the repeated exposure of the rabbit to leptospires in swampy areas.

These findings indicate that while the slide test (Galton) may be used to screen rabbit serums for leptospiral antibodies, its extreme sensitivity limits its value compared to that of the microscopic agglutination test. This sensitivity may be directly related to some factor(s) found in rabbit serums.

Previous isolations of *grippotyphosa* have been reported from various wild and domestic animals.[2,5] However, recovery of this serotype has not been reported rom either the cottontail or swamp rabbit. The relative ease with which this organism was recovered from juvenile and adult rabbits suggests that a carrier state may exist in these animals.

Focal nephritis apparently was a good index of past or present leptospiral infection in the rabbits. While associated lesions were not indicative of the presence of leptospires, they indicated renal malfunction and suggested leptospirosis as a possible cause. The close correlation of leptospiral serologic responses with nephritis supports this assumption.

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Received for publication December 15, 1970