This is a study of feral rodents in Georgia to determine their importance as reservoirs of leptospiras. It focuses on the significance of habitat in perpetuating natural infections.

THE OCCURRENCE OF LEPTOSPIRAL INFECTIONS IN FERAL RODENTS IN SOUTHWESTERN GEORGIA

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THE OCCURRENCE of leptospirosis in feral rodents in southwestern Georgia was investigated in an effort to determine the importance of these animals as reservoirs of leptospiras transmissible to man and to evaluate the importance of habitat in the perpetuation of infections in nature. These studies were conducted between October, 1953, and July, 1955, after which date emphasis was shifted from rodents to larger feral mammals.[1]

The importance of leptospirosis in the southeastern states has increased greatly in recent years. While it is most commonly associated with domestic animals, recent outbreaks of the disease in human beings in Alabama,[2] North Carolina,[3] and Georgia[4] have demonstrated the need of additional information on possible wildlife reservoirs and methods of transmission.

Rodent reservoirs of leptospiras have been demonstrated in various parts of the world.[5-9] The first Leptospira isolation in the United States was Leptospira icterohemorrhagiae from wild rats.[10] Leptospira ballum has been isolated from rural house mice in Virginia and from stock laboratory white mice.[11]

Methods

Rodents were collected with Young's No. 15A live animal traps. Usually 25 traps spaced 20 feet apart were used in each line. In a few instances 50 or 100 traps were used in a single line, but these instances were so few that for purposes of analyses they were considered to be two or four lines placed end to end. Lines were placed wholly within recognized habitat types so that no part of any line was within 50 feet of other adjacent habitat borders. Areas to be trapped were chosen for proximity to cattle in which leptospirosis had been diagnosed. Because of the abundance and distribution of infected herds it was possible to sample by trapping the majority of stages in the ecological succession of the area. These stages and the habitats representing them are shown in Table 1. The stages in primary succession labeled moist lowland, hydric hammock, and streambank forest are heavily wooded and appear to be fairly permanent. The grassy limesink ponds are also communities of long duration. None of these areas are considered climax. The geographic area included in the studies covers approximately 500 square miles in Baker, Dougherty, and Mitchell Counties in southwestern Georgia.

Traps were baited with a mixture of rolled oats and peanut butter. With few exceptions, lines were run for three nights. Captured rodents were identi-

fied and transferred from the traps to cloth bags. A record was made immediately of the date, line number, trap number, species, and sex of each specimen.

At the laboratory the identification and sex of each specimen was checked again. They then were anesthetized with ether, weighed, measured, and killed by exsanguination. Kidneys were removed aseptically, ground in a small amount of physiological saline, and three drops inoculated into each of four tubes of Chang's[12] semisolid culture medium fortified with rabbit sera. Cultures were incubated for 30 days at 28° C and examined for the presence of leptospiras using darkfield microscopy. Positive cultures were subcultured into additional tubes of the medium to obtain maximum growth of the organism. These subcul-

Table 1—Number of Specimens Collected from Various Habitats, Number Cultured for Leptospiras, and the Number Positive by Culture

Stage of Ecological Succession and Representative Habitats	Number Specimens Collected	Number Cultured	Number Positive	Per cent Positive by Culture
Primary Succession				
Dry pineland (fire)	66	18	0	
Mixed pine-oak woodland	85	7	1	14.0
Dry oakland (lumbered for pine)	131	41	0	
Moist lowland	8	8	0	
Hydric hammock	6	6	0	
Streambank forest	36	22	0	
Grassy limesink pond	60	49	1	2.0
Secondary Succession Cultivation				
Barnyard	5	3	0	
Pastureland	9	2	0	
New oatfield	1	1	0	
New cornfield	51	24	2	8.3
Lupine field	14	12	1	8.3
Harrowed pecan grove	10	4	0	
Planted cover for birds	15	15	0	
Oldfields				
First-winter cornfield	203	49	4+9 pools	26.0
1-year-old cornfield	44	9	0	
2-year-old cornfield	194	76	10+1 pool	14.0
3-year-old cornfield	89	66	5+3 pools	12.0
1-year-old cottonfield	62	12	0	
1-year-old peanutfield	60	43	8	19.0
2-year-old peanutfield	111	23	1	4.4
3-year-old-peanutfield	68	18	0	
3-year-old unclassified field	5	4	0	
Broomsedge fields (over 3 years old)	1,024	337	6+5 pools	3.3
Miscellaneous				
Young planted pines	36	9	0	
Burned oldfield	23	12	0	
Cover strip unplanted	53	2	0	
Dry fence row	137	15	4	27.0
Moist fence row	15	0	0	
Unknown	52	46	4+1 pool	11.0

Table 2—Species and Number of Specimens Collected, Number Cultured for Leptospiras, and Number Positive by Culture

Species	Number Collected	Number Cultured	Number Positive	Per cent Positive by Culture
Peromyscus gossypinus gossypinus	190	90	0	
Peromyscus polionotus polionotus	1,053	390	2+1 pool	0.78
Peromyscus nuttalli	5	2	0	
Sigmodon hispidus komareki	450	104	2	1.9
Mus musculus	875	284	43+18 pools	21.0
Microtus (Pitimys) pinetorum	5	1	0	
Reithrodontomys humulis	43	22	0	
Oryzomys palustris palustris	11	10	0	
Rattus norvegicus	34	30	0	
Cryptotis parva	5	0		
Blarina brevicauda	1	0		
Total	2,673	933	47+19 pools	7.1

tures were submitted for typing to the Leptospira Research Laboratory of the Communicable Disease Center Laboratory Branch.

Sera were harvested from blood drawn from individual specimens and sent to the Diagnostic Research Laboratory of the CDC Laboratory Branch, where complement-fixation tests for leptospirosis were performed.

Results

From October, 1953, to July, 1955, 2.673 animals representing eight species of rodents and two species of shrews were taken in 16,375 trap nights. Species taken in greatest numbers were the house mouse, Mus musculus, 875; the oldfield mouse, Peromyscus polionotus, 1,053; the cotton mouse, Peromyscus gossypinus, 190; and the cotton rat, Sigmodon hispidus, 450. The six other species were collected in numbers ranging from one to 43 (Table 2). Of the specimens collected, 933 were tested to determine the occurrence of leptospiral infections. Death in traps (weather, ants, and so forth), lack of facilities during periods of heavy trapping, and the difficulties of handling the small blood samples prevented testing a greater percentage of the total catch. Leptospiras were cultured from 47 individual specimens and from 19 pools which contained a total of 64 specimens, each pool including one specie only. The 66 isolations obtained were all identified as L. ballum. Considering each positive pool as one infected specimen, isolations of leptospiras were obtained from three species, as follows: 0.8 per cent of the P. polionotus, 1.9 per cent of the S. hispidus, and 21 per cent of the M. musculus. None of the positive rodents demonstrated apparent evidence of infection.

Leptospiral isolations were obtained from 11 of 30 types of habitat from which specimens were tested (Table 1) and, with two exceptions, from each habitat from which 20 or more specimens were tested. These exceptions were the streambank forest from which 22 P. gossypinus were the only specimens tested and dry oakland from which 38 of 41 specimens tested were P. polionotus. Two P. gossypinus and one S. hispidus from dry oakland were also

tested. More than 10 per cent of specimens were infected in six habitats. These were: first-winter cornfields, 27 per cent; two-year-old cornfields, 14 per cent; three-year-old cornfields, 12 per cent; one-year-old peanutfields, 19 per cent; dry fence rows, 27 per cent; and mixed pine-oak woodland, 14 per cent.

Infected house mice as determined by culture were found in 10 of the 11 habitats from which positive specimens were collected. The exception, a grassy limesink pond, gave one isolation from two cotton rats tested. Five house mice from this area were tested but gave negative results. The second isolation from a cotton rat was obtained from a specimen collected in an oldfield. One infected oldfield mouse was collected from an oldfield, one from a one-year-old peanutfield, and one pool of specimens of this species collected in a first-winter cornfield was positive.

Positive specimens of all three species were obtained only from oldfields (Table 3). In this habitat, all the isolations were obtained from rodents collected on five of 35 trap lines run. From these positive lines, one cotton rat of 35 tested was positive, one of 33 oldfield mice was positive, and nine isolations were obtained from 58 house mice. Included in the nine isolations from house mice were five positive pools containing a total of 18 specimens, so the infection rate may have been higher than is indicated by the data.

Collections from two habitats contained infected house mice and infected oldfield mice. In first-winter cornfields all isolations were obtained from specimens collected on five of 12 trap lines run. The line giving one positive pool of oldfield mice also gave three positive pools of house mice. Here again the one pool of oldfield mice and the three pools of house mice contained the six and 12 specimens, respectively, collected on the line, so the rate of infection may have been greater than is indicated. In one-year-old peanutfields, all isolations were obtained from specimens collected on four of nine trap lines run. The infected oldfield mouse was the only posi-

Table 3—Number of Isolations of Leptospiras Obtained from Three Species of Rodents Collected in Various Habitats

Habitat	Species		
	Peromyscus polionotus	Sigmodon hispidus	Mus musculus
New cornfield			2
First-winter cornfield	1 pool		4+8 pools
2-year-old cornfield			10+1 pool
3-year-old cornfield			5+3 pools
1-year-old-peanutfield	1		7
2-year-old peanutfield			1
Oldfield	1	1	4+5 pools
Dry fence row			4
Mixed pine-oak woodland			1
Grassy limesink pond		1	
Lupine field			1
Unknown			4+1 pool
Total	2+1 pool	2	43+18 pools

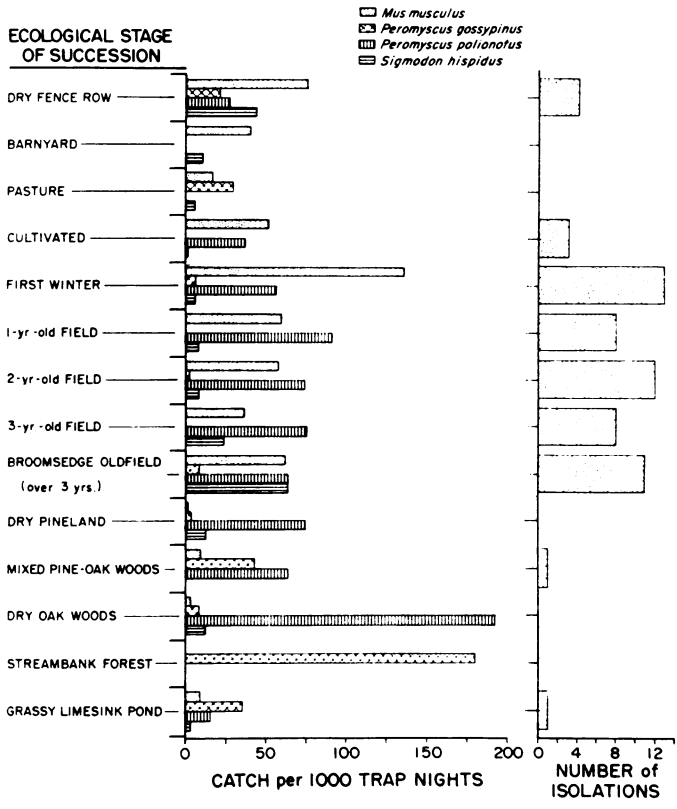

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Figure 1—Population Density of More Abundant Species of Rodents in Various Habitats as Indicated by Tray Success, and Number of Isolations of Leptospiras from Each Habitat

tive specimen collected on that particular trap line; however, no house mice from this line were tested. A second line run simultaneously in the same field collected five positive house mice out of the eight tested. Six oldfield mice on this second line were negative.

The isolations of leptospiras obtained from all of the rodents tested are shown together with the population density as indicated by trap success in Figure 1. It is apparent from the figure that isolations were obtained almost exclusively from habitats supporting significant house mouse populations. This would be expected since nearly all isolations came from house mice. The data indicate that sizable populations of the rodent species studied, other than house mice, probably do not perpetuate L. ballum infections.

The prevalence of infections in house mice in relation to population density is shown in Figure 2. Approximately 30 per cent of this species was infected throughout most of the range of habitats ecologically suited to its development. In oldfields, however, only 8.2 per cent of house mice were infected even though these fields supported sizable house mouse populations.

Infections isolated from rodents col-

lected from a particular type habitat always were concentrated in particular areas and absent in other similar areas supporting as large or even larger populations. For example, in oldfields all positive specimens were collected from no more than four restricted areas of 25 acres or less, and were collected with only 18 per cent of the trap nights used to collect specimens from this habitat. In first-winter cornfields, positive specimens were restricted to two areas and were collected with less than 15 per cent of the trap nights. Results from other habitats were similar. In positive habitats, a total of 12,175 trap nights was utilized. However, all positive specimens were collected on lines which utilized only 2,900 trap nights.

None of the sera from the specimens collected gave positive complement-fixation tests for leptospirosis, including those from animals infected with L. ballum despite the use of specific L. ballum antigen in the tests.

Discussion

Results of these investigations indicate that L. ballum is firmly established in house mouse populations in southwestern Georgia. Other serotypes, if they occur

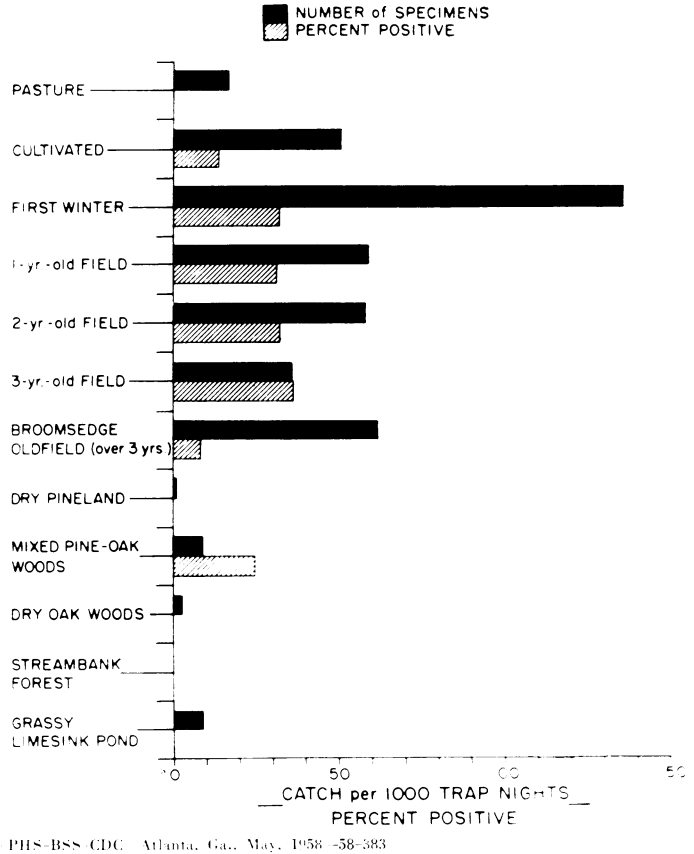

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Figure 2—Population Density of Mus musculus in Various Habitats and Per cent of Specimens with Leptospiras

in rodents, apparently are rare unless the technics used were selective with regard to the serotypes which could be cultured. This possibility must be considered since modified technics were used when detecting the various other serotypes cultured from larger feral mammals collected in the same areas.[1]

From the data obtained it appears that L. ballum infections in other species of rodents are unusual, since almost without exception, infected specimens of species other than house mice were closely associated with large house mouse populations in which infection rates were high.

The failure to obtain positive complement-fixation tests for leptospiras on any of the specimens collected plus the lack of visibly apparent evidence of infection in any of the specimens indicate that in house mice this Leptospira may be a commensal parasite afforded a place of survival with stimulation of a low level of complement-fixing antibodies by the host.

Summary

Between October, 1953, and July, 1955, 2,673 rodents representing ten species were collected from 28 habitats in southwestern Georgia, and 933 were tested for leptospiras by culturing kidney tissue. L. ballum was the only serotype isolated. Positive cultures were obtained from 22 per cent of the house mice, 0.8 per cent of the oldfield mice, and 1.9 per cent of the cotton rats. Positive specimens, regardless of species, were collected almost exclusively from habitats supporting large house mouse populations. Infection rates in house mice ranged between 30 and 35 per cent in most habitats ecologically suited to sizable house mouse populations.

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REFERENCES

1. McKeever, S.; Gorman, G. W.; Chapman, J. F.; Galton, M. M.; and Powers, D. K. Incidence of Leptospirosis in Wild Mammals from Southwestern Georgia, with a Report of New Hosts for Six Serotypes of Leptospirae. Am. J. Trop. Med. & Hyg. 7:646–655, 1958.
2. Schaeffer, M. Leptospiral Meningitis. Investigations of Water-Borne Epidemic Due to L. pomona. J. Clin. Invest. 30:670–671, 1951.
3. Gochenour, W. S., Jr.; Smadel, J. E.; Jackson, E. B.; Evans, L. B.; and Yager, R. H. Leptospiral Etiology of Fort Bragg Fever. Pub. Health Rep. 67: 811–813, 1952.
4. Williams, H. R.; Murphy, W. J.; McCroan, J. E.; Starr, L. E.; and Ward, M. K. An Epidemic of Canicola Fever in Man with the Demonstration of Leptospira canicola Infection in Dogs, Swine and Cattle. I. Clinical and Epidemiological Studies. Am. J. Hyg. 64:46–69, 1956.
5. Babudieri, B., and Bianchi, L. Untersuchungen Uber Ein Epidemisches Vorkommen Der Reisfelderleptospirose in Der Provinz Pavia. Ztschr. f. Immunol. 98:37–75, 1940.
6. Borg-Petersen, C., and Fennestad, K. L. A Field Rodent (Apodemus agrarius) as Carrier of Leptospira pomona in Denmark. J. Am. Vet. M. A. 128: 204–205, 1956.
7. Doherty, R. L.; Emanuel, M. L.; and Moore, N. An Outbreak of Leptospirosis in North Queensland. M. J. Australia (Feb. 18), 1956, p. 276.
8. Fraga de Azevedo, J.; Valente, J. S.; and Novos Queiros, J. J. de S. Dados Sobre as Leptospiras de Portugal. An. Inst. med. trop. 8:621, 1951. Cited by Borg-Petersen in Ref. 6.
9. Kmety, E. Leptospirosenherde in der Slowakei. Zentralbl. Bakt. 163:464–476, 1955.
10. Noguchi, H. Spirochaeta icterohemorrhagiae in American Wild Rats and its Relation to the Japanese and European Strains. J. Exper. Med. 25:755–763, 1917.
11. Yager, R. H.; Gochenour, W. S., Jr.; Alexander, A. D.; and Wetmore, P. W. Natural Occurrence of Leptospira ballum in Rural House Mice and in an Opossum. Proc. Soc. Exper. Biol. & Med. 84:589–590, 1953.
12. Chang, S. L. Culture Medium for Leptospiras. J. Infect. Dis. 81:28–34, 1947.

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