The designation Leptospira mini georgia is proposed for a new subserotype of L. mini isolated from wild mammals. A case of human infection with L. mini georgia is reported on pages 922–924 and the first isolation of Leptospira pomona from a woodchuck, on page 925.

A New Leptospiral Subserotype in the Hebdomadis Group

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INVESTIGATIONS concerning possible wild animal reservoirs of leptospires were conducted by the Communicable Disease Center of the Public Health Service from 1953 to 1958 at Newton, Ga. (*1–3*). Leptospires isolated between March 1956 and May 1957 from 15 raccoons (*Procyon lotor*), 4 opossums (*Didelphis marsupialis*), and 1 striped skunk (*Mephitis mephitis*) appeared to be identical and to belong to the hebdomadis serogroup. Serologic characterization of these strains, recorded in this paper, indicate that they are a new subserotype of *Leptospira mini*.

L. mini AB Sari was isolated by Mino in 1941 from the blood of an Italian ricefield worker with leptospirosis and described by Babudieri (*4*). *L. mini A* Szwajizak was isolated first in Queensland in 1952, also from the blood of a human patient, and reported by Smith and coworkers in 1954 (*5*). Several years later Van der Hoeden (*6*) isolated the Szwajizak strain from the kidneys of a hedgehog in Israel. The Barthélémy strain was isolated by Van Riel and Szpajshendler (*7*) from the blood of a patient with a fatal case of leptospirosis in the mining region of the Belgian Congo.

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Methods

Source of strains. All the animals from which leptospires were isolated were trapped in Georgia, 11 in Calhoun County, 5 in Dougherty County, and 2 each in Baker and Seminole Counties. These counties are in the southwestern part of the State between the Flint and Chattahoochee Rivers. The leptospiral strains were isolated by direct culture of a kidney tissue suspension into Fletcher's semisolid medium (*8*).

Antiserums. Immune serums were prepared according to previously described methods (*3*) with the exception of *L. wolffii A*, *L. borincana*, *L. worsfoldi*, *L. hemolyticus*, *L. ricardi*, *L. jules*, *L. mini A* Szwajizak and *L. kabura*, which were prepared by inoculation of live Fletcher's cultures into rabbits. These eight antiserums were supplied by the Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C.

Antigens. Leptospiral antigens were pre-

pared as previously described (*3*) except they were not formalinized. If the antigens appeared too dense, cultures were diluted with sterile Stuart's medium (*9*).

Microscopic agglutination test procedure. Serial twofold dilutions were prepared in buffered 0.85 percent saline to provide serum dilutions of 1:25 through 1:102,400. To 0.2 ml. of each serum dilution, 0.2 ml. of antigen was added. The tubes were shaken, incubated at 30° C. for 3 hours, and examined. A drop from each dilution was placed on a slide and examined by dark ground microscopy, using low-power objective and 15× oculars without a coverslip. The degree of agglutination or "lysis" or both were read as 1+, with at least 25 percent of the leptospires agglutinated or "lysed"; 2+, approximately 50 percent; 3+, about 50–75 percent; and 4+, 75–100 percent. The end point was taken as the last dilution showing a 1+ reaction.

Agglutinin-absorption procedure. Antigens for absorption studies were prepared from 5- to 7-day-old cultures grown in Stuart's medium in 500 ml. amounts. Cultures were killed by the addition of formalin to provide a concentration of 0.3 percent. The cultures were centrifuged at 5,000 × gravity in a Servall for 25 minutes. The remainder of the agglutinin-absorption procedure was essentially the same as that described by Alexander (*10*). Microscopic agglutination tests with the absorbed serums were performed with antigens prepared as mentioned above but killed by the addition of formalin to provide a concentration of 0.3 percent. The absorbed serums were diluted twofold to provide final serum dilution of 1:100 to 1:51,200 after the addition of antigen. The tubes were incubated in a waterbath at 52° C. for 2 hours, refrigerated for 1 hour, and read as described above.

Findings

The first isolate in this group of cultures, LT117, grew very slowly in liquid medium for the first 10 months and was carried in both Stuart's and Chang's (*11*) media. Frequently the culture failed to grow in one of these liquid media and it was necessary to continue to subculture in Fletcher's medium and transfer repeatedly from these to the liquid media before the culture became adapted. However, after about 12 months the culture began to grow quite well in Stuart's medium and has continued to do so.

Initial screening of antigen prepared from strain LT117 with antiserums against *L. aus-*

Table 1. Cross agglutination studies on leptospiral strain LT117 and related isolates

Antiserum	Homologous titer	Antigen [1]										LT117 serum + 1–18 antigens
		LT117	LT138	LT146	LT153	LT164	LT172	LT185	LT186	LT188	LT196	
hebdomadis	12, 800	800	400	------	------	------	400	800	800	800	800	400
medanensis	12, 800	–	–	–	–	–	–	50	–	–	–	64
wolffii	12, 800	200	256	128	128	32	200	200	50	100	100	32
hardjo	51, 200	–	–	–	------	------	–	–	–	–	–	32
sejroe	6, 400	50	–	128	128	–	–	100	50	–	100	–
saxkoebing	25, 600	200	200	------	------	------	100	400	200	100	800	50
wolffii A	51, 200	200	256	512	128	64	–	100	–	–	400	512
kremastos	6, 400	3, 200	1, 024	1, 024	1, 024	512	800	1, 000	800	800	800	256
borincana	51, 200	12, 800	16, 384	4, 096	4, 096	8, 192	6, 400	25, 600	6, 400	6, 400	6, 400	32
worsfoldi	102, 400	12, 800	6, 400	2, 048	4, 096	2, 048	12, 800	25, 600	6, 400	6, 400	6, 400	128
hemolyticus	51, 200	400	200	1, 024	256	128	100	400	200	200	400	512
mini A Szwajizak	51, 200	25, 600	------	------	------	------	------	------	------	------	------	3, 200
mini AB Sari	6, 400	400	512	512	512	256	400	400	400	400	400	1, 600
jules	25, 600	12, 800	12, 800	------	------	------	3, 200	6, 400	3, 200	3, 200	12, 800	200
kabura	102, 400	3, 200	1, 600	------	------	------	1, 600	3, 200	1, 600	1, 600	6, 400	400
barthélémy	12, 800	6, 400	------	------	------	------	------	------	------	------	------	800
ricardi	51, 200	–	–	–	–	–	–	–	–	–	–	128
LT117	6, 400	6, 400	3, 200	2, 048	------	------	6, 400	12, 800	6, 400	6, 400	12, 800	6, 400

[1] Live antigen.

NOTE: – indicates no reaction in a 1:50 dilution.

tralis, LT95; *L. autumnalis* (AB), Akiyama A; *L. bakeri; L. ballum*, S102; *L. grippotyphosa*, Moscow V; *L. pomona*, LT91; and *L. sejroe* Mallersdorf, the battery used to test isolates from this Communicable Disease Center field station, yielded agglutination only with *L. sejroe* serum in a dilution of 1:50. Subsequent testing with antiserums for the remaining 28 serotypes recognized in the Wolff-Broom schema (*12*) revealed similar low titers with other members of the hebdomadis serogroup. Antiserum against LT117 was then prepared and cross agglutination studies performed with live antigens and antiserums of serotypes of the hebdomadis serogroup (*13*). Results of these studies indicated that LT117 was closely related to the hebdomadis serogroup, as shown in table 1.

In agglutinin-absorption studies with *L. borincana*, *L. worsfoldi*, *L. kremastos*, *L. hemolyticus*, *L. wolffii A*, *L. mini AB* Sari, *L. mini AB* Barthélémy, *L. mini A* Szwajizak, *L. jules*, and *L. kabura* antiserums, LT117 failed to reduce the homologous titer significantly except in the *L. mini* antiserums. The serologic characteristics of the Barthélémy strain were described recently by Wolff and Bohlander (*14*), and it was shown to be very closely related to Sari. For practical reasons they considered Barthélémy as a complete biotype of *L. mini*. To confirm the apparent serologic relationship between LT117, Sari, and Szwajizak, a box titration of the cross agglutinin-absorption reactions among these three strains was performed by A. D. Alexander and L. B. Evans, Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C. These findings, as shown in table 2, indicate that LT117 is a subserotype of *L. mini*. Therefore, the subserotype designation *L. mini georgia* is proposed for strain LT117.

During these studies, 19 additional isolates were obtained from raccoons, striped skunks, and opossums that reacted to the homologous titer with LT117 antiserum. Nine of these cultures were tested against antiserums for other members of the hebdomadis serogroup and showed a cross agglutination pattern similar to LT117 as shown in table 1.

Blood was collected from each of the animals the same day the kidney suspensions were cultured. The serum from 16 animals was available for agglutination studies. Of these significant antibodies against LT117 were detected in dilutions ranging from 1:50 to 1:800 in eight animals. Four of the samples were tested with antigens of all serotypes of the hebdomadis group except *L. ricardi*, and no antibodies were detected in two. A titer of 1:200 to Sari was observed in the serum from one skunk that showed only a plus minus reaction (at the lowest dilution, 1:50) with LT117 antigen. These findings are shown in table 3.

Discussion

L. mini georgia represents the second new leptospiral strain that has been isolated in the United States. Since 1952 (*15*) increasing serologic evidence has suggested that infections with another member of the hebdomadis group, *L. sejroe*, occur in cattle. While the etiological significance of these bovine *sejroe* reactors has not been determined completely, infection with *L. hardjo*, a closely related serotype, has been

Table 2. Results of cross agglutinin-absorption test with L. mini AB Sari, L. mini A Szwajizak, and LT117 [1, 2]

Antiserum	Antigen		
	Sari	Szwajizak	LT117
L. mini AB Sari:			
Unabsorbed	25, 600	25, 600	6, 400
Absorbed with:			
Sari	—	—	—
Szwajizak	1, 600	—	—
LT117	6, 400	400	—
L. mini A Szwajizak:			
Unabsorbed	6, 400	25, 600	6, 400
Absorbed with:			
Sari	—	—	—
Szwajizak	—	—	—
LT117	400	400	—
LT117:			
Unabsorbed	1, 600	25, 600	25, 600
Absorbed with:			
Sari	—	100	1, 600
Szwajizak	—	—	1, 600
LT117	—	—	—

[1] Titer expressed as reciprocal of serum dilution.

[2] The techniques employed in these tests are described by A. D. Alexander, L. B. Evans, H. Jefferies, C. A. Gleiser, and R. H. Yager in "Serologic Characterization of the Fort Bragg Leptospire," Proc. Soc. Exper. Biol. & Med. 86: 405–408, June 1954.

NOTE: — indicates no reaction in a 1:100 dilution.

established in Louisiana (*16*). The possibility that *L. mini georgia* may be involved in leptospirosis in cattle should be considered.

Serums from 6 of the 11 raccoons and 1 of 4 opossums tested were serologically negative against LT117. Serum from two of these six raccoons and the opossum showed a very weak reaction in a 1:50 dilution to Sari antigen. Another raccoon was positive at 1:50 with *kabura* antigen, but no antibodies to other members of the hebdomadis group were detected in serum from three of the raccoons. This is not unusual as similar findings were observed by Broom and Coghlan (*17*) in Scotland in serums from mice that were infected with *L. ballum* and with an unidentified member of the hebdomadis group. These authors pointed out the apparently misleading picture that serologic surveys may give concerning the prevalence of leptospirosis in small rodents. It is obvious that a similar situation exists also in the larger wild mammals, such as raccoons.

Subsequent to the isolation of the LT117 cultures from wild animals, serologic evidence of human infection with this serotype was seen in a patient in the Phoebe Putney Memorial Hospital, located in the same county in which several isolates were obtained. The initial serum sample from this patient showed a positive slide agglutination test with leptospiral pooled antigens but no reaction by microscopic agglutination when tested with live antigens for the routine battery of 12 leptospiral serotypes (*18*). A second sample requested a week later showed a microscopic titer of 1:200 against LT117 antigen.

Investigation of this suspected human case of leptospirosis by Dr. L. E. Starr, Georgia Department of Public Health, revealed an interesting history. The patient's physician stated that she had experienced an acute febrile illness of about 1 week's duration, accompanied by muscle aches, chills, and nausea. Tetracycline therapy was commenced about the fourth day after the onset of illness and continued for 6 days. Several weeks prior to onset of her illness the patient had experienced one partial and one complete immersion in the Flint River while fishing. About the time she entered the hospital, several rats discovered in her kitchen and attic were trapped and destroyed. The patient had also had contact with two dogs, but serums from both animals showed titers to *L. pomona* and *L. autumnalis*.

Table 3. Results of agglutination tests on serum from 16 animals infected with LT117

Animal No.	Species	Date collected	County	Antigens					
				LT117	Sari	Szwajizak	Kabura	Jules	Hebdomadis
		1956							
LT117	Raccoon	March 19	Baker	—	—	—	—	—	—
LT138	do	June 6	Dougherty	—	±50	—	—		
LT146	do	July 12	Seminole	—	±50	—	—		
LT153	do	December 5 [1]	do	—	—	—	—	—	—
LT179	Opossum	October 24	Calhoun	—	±50	—	—		
LT185	Raccoon	October 30	do	—	—	—	—		
LT186	do	October 31	do	800	400	200	400	400	800
LT188	do	November 1	do	50	±50	—			
LT196	do	November 7	do	50	±50	—	—		
N-196	do	November 8	do	800					
LT222	do	October 19	do	—	—	—	50		
		1957							
LT224	Opossum	January 10	Dougherty	400	800	200	100		
LT250	do	January 30	do	100	400	—	200		
LT252	Raccoon	January 30	do	100	—	—	—		
LT259	Striped skunk	February 20	Calhoun	±50	200	—	—		
LT282	Opossum	May 17	Dougherty	800					

[1] Received culture.

NOTE: — indicates no reaction in 1:50 dilution.

A third serum sample taken from the patient 2½ months after onset of illness showed a titer of 1:200 to both LT117 and *L. sejroe* antigens. After absorption with LT117 cells, this serum showed no reaction with LT117 or *L. sejroe* antigen but absorption with *L. sejroe* only reduced the original titer of LT117 by 50 percent. This serologic evidence together with the clinical and epidemiological history is at least suggestive of human infection with LT117. More recently, the infectivity of *L. mini georgia* for man was conclusively demonstrated through an accidental infection that occurred in another laboratory. This infection is reported by Goley and co-workers on pages 922–924.

Summary

A new strain of leptospires belonging to the hebdomadis serogroup is described. This strain is represented by 20 isolates from raccoons, opossums, and a striped skunk. Cross agglutinin-absorption studies indicate that the new strain is a subserotype of *L. mini*, and the designation *L. mini georgia* is proposed.

Agglutination tests with serum from 16 of 20 animals revealed antibodies against LT117, Sari, Szwajizak, or *kabura* antigen in 10 animals.

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