LEPTOSPIRA ICTEROHAEMORRHAGIAE SUBSEROTYPE INCOMPLETA ISOLATED FROM WILDLIFE IN PENNSYLVANIA

E. A. Carbrey, V.M.D., M.S., L. G. Clark, D.V.M., and J. I. Kresse, B.S.

THE FIRST STRAIN of *Leptospira icterohaemorrhagiae* recovered in Europe was isolated in 1915 from a man and identified by the initials RGA (*1*). Additional isolations of the serotype followed. The different strains of *L. icterohaemorrhagiae* were believed to be similar until 1938 when it was shown that two subtypes existed (*2*). Through cross absorption studies it was determined that strain RGA lacked an antigenic component possessed by the M20 strain of *L. icterohaemorrhagiae*. The word biotype was coined to cover this situation (*3*). The different strains were classified as belonging to the complete biotype, *L. icterohaemorrhagiae* AB, or to the incomplete biotype, *L. icterohaemorrhagiae* A. In 1958, the Joint World Health Organization/Food and Agriculture Organization Expert Committee on Zoonoses recommended the adoption of a subserotype classification (*4*). The designation, *L. icterohaemorrhagiae* A for the incomplete biotype was changed to *L. icterohaemorrhagiae* subserotype *incompleta*.

Numerous isolations of *L. icterohaemorrhagiae* from various animal species have been reported in the literature with no differentiation made as to which subserotype was found (*5–17*). A notable exception was a survey made in Britain of the leptospiral infection rates of wild rats (*18*). From 219 rats trapped in English cities, 65 strains were isolated. Of the 50 strains which were typed, 20 were identified as belonging to the incomplete biotype, *L. icterohaemorrhagiae* A. The remaining 30 strains belonged to the complete biotype.

During routine cross-agglutination studies performed on leptospiral strains submitted to the Animal Disease Eradication Diagnostic Laboratory of the U.S. Department of Agriculture, Ames, Iowa, for identification, five strains were found which reacted to high titers with *L. icterohaemorrhagiae* M20 antiserum but produced unusual cross-reactions with other serotype antiserums. Agglutinin-absorption studies were performed to identify the cultures.

Dr. Carbrey, a research veterinarian with the Department of Agriculture's Animal Disease Eradication Division, is assigned to the Plum Island Animal Disease Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Greenport, Long Island, N.Y. Dr. Clark is an assistant professor in the University of Pennsylvania's School of Veterinary Medicine, Philadelphia. Mr. Kresse is a bacteriologist at the National Animal Disease Laboratory, Agriculture Research Service, U.S. Department of Agriculture, Ames, Iowa.

Materials and Methods

The cultures studied were isolated at the Leptospirosis Laboratory, New Bolton Center, University of Pennsylvania, Kennett Square, Pa. The wildlife had been trapped on farms in southeastern Pennsylvania where leptospirosis had been detected in cattle. Results in this study and the methods employed for trapping animals and obtaining cultures were described previously (*19*). The animals from which the cultures were obtained were as follows:

Culture number	*Animal*
1177	mouse (*Mus musculus*)
1244	skunk (*Mephitis mephitis*)
1277	raccoon (*Procyon lotor*)
1315	raccoon (*Procyon lotor*)
1353	gray fox (*Urocyon cinereoargenteus*)

Antiserums were prepared by intravenous inoculation of rabbits with successive doses of

Table 1. Cross-agglutination patterns of five isolates with leptospiral antiserums prepared with type cultures

Leptospiral antiserum	Titer of culture [1]				
	1177	1244	1277	1315	1353
L. pomona	[2] Negative	50	Negative	200	400
L. icterohaemorrhagiae	6, 400	6, 400	3, 200	6, 400	6, 400
L. canicola	6, 400	3, 200	3, 200	6, 400	6, 400
L. ballum	50	25	Negative	25	25
L. australis	Negative	Negative	Negative	25	Negative
L. pyrogenes	12, 800	6, 400	6, 400	6, 400	6, 400
L. celledoni	1, 600	1, 600	800	1, 600	800
L. sentot	Negative	Negative	Negative	50	50
L. javanica	1, 600	1, 600	800	1, 600	1, 600
L. cynopteri	50	50	Negative	50	25
L. schuffneri	800	800	800	1, 600	800
L. benjamin	6, 400	3, 200	1, 600	6, 400	3, 200
L. zanoni	25, 600	25, 600	12, 800	25, 600	12, 800
L. bangkinang	1, 600	1, 600	1, 600	1, 600	1, 600
L. sarmin	6, 400	3, 200	6, 400	6, 400	3, 200
L. icterohaemorrhagiae subserotype *incompleta*	25, 600	25, 600	25, 600	25, 600	25, 600

[1] Less than 50 percent agglutination at 1 : 25 against *L. hardjo, L. autumnalis, L. hebdomadis, L. hyos, L. grippotyphosa, L. bataviae,* and *L. djasiman.*
[2] Negative indicates less than 50 percent reaction in the 1 : 25 dilution.

Table 2. Cross agglutinin-absorption studies with L. icterohaemorrhagiae subserotype incompleta and isolates

Antiserum	Reciprocal of titer against antigen [1]					
	L. icterohaemorrhagiae subserotype *incompleta*	1177	1244	1277	1315	1353
L. icterohaemorrhagiae subserotype *incompleta:*						
Unabsorbed	10, 000	300, 000	100, 000	10, 000	30, 000	10, 000
Absorbed with—						
Bolton Farm 1177	300	[2] Negative	----	----	----	----
Bolton Farm 1244	300	----	Negative	----	----	----
Bolton Farm 1277	300	----	----	Negative	----	----
Bolton Farm 1315	100	----	----	----	Negative	----
Bolton Farm 1353	300	----	----	----	----	Negative
Bolton Farm 1177:						
Unabsorbed	10, 000	30, 000	----	----	----	----
Absorbed with *incompleta*	Negative	300	----	----	----	----
Bolton Farm 1244:						
Unabsorbed	10, 000	----	100, 000	----	----	----
Absorbed with *incompleta*	Negative	----	100	----	----	----
Bolton Farm 1277:						
Unabsorbed	10, 000	----	----	30, 000	----	----
Absorbed with *incompleta*	Negative	----	----	100	----	----
Bolton Farm 1315:						
Unabsorbed	10, 000	----	----	----	30, 000	----
Absorbed with *incompleta*	Negative	----	----	----	300	----
Bolton Farm 1353:						
Unabsorbed	10, 000	----	----	----	----	10, 000
Absorbed with *incompleta*	Negative	----	----	----	----	100

[1] Living antigens were used.
[2] Negative indicates less than 50 percent reaction in the 1:100 dilution.

1.0, 2.0, 4.0, and 6.0 ml. of 7-day cultures of each leptospiral strain grown in Fletcher's medium (*20*). The injections were given at 6-day intervals, and the rabbits were bled from the heart 6 days after the last injection. After the addition of 50 percent glycerine the serums were stored at −20° C.

Leptospiral strains were maintained in Stuart's medium at 30° C. and transferred twice a week. Antigen for the microscopic agglutination test was prepared by centrifuging 3- to 5-day cultures at 500 g. on a horizontal head for 10 minutes.

For cross-agglutination studies serial twofold dilutions of the serums in buffered saline, with separate pipettes for each transfer, were prepared so that the final dilutions after mixing with antigen were 1:25, 1:50, 1:100, and so on. After these dilutions were incubated at room temperature for 2 hours, a drop from each dilution was examined by dark-field microscopy at 150 x magnification. The end-point titer was the last tube with agglutination of the leptospires of at least 50 percent.

For the agglutinin-absorption studies, cultures were grown in 200 ml. of Stuart's medium in screw-capped flasks for 7 to 9 days and killed by the addition of 0.25 percent formalin. The cultures were centrifuged at 20,000 g. for 10 minutes, and the cell mass was resuspended to 1 percent of the original culture volume in buffered saline containing 0.25 percent formalin. A 1:10 dilution of the immune serum was prepared and mixed with four parts by volume of the absorbing antigen. After overnight incubation at 37° C., the cells were removed by centrifugation, and the serum was absorbed a second time. To avoid further dilution of the serum, it was added to a tube containing centrifuged leptospires. For this second absorption, the serum-cell mixture was incubated 2 hours at 37° C. Absorptions were considered adequate when the agglutinins were completely removed by the homologous antigen. Reabsorption was performed when necessary.

Microscopic agglutination tests with absorbed serums were performed with living antigens. The first dilution was 1:100 after the absorbed serum had been mixed with an equal volume of antigen. With a separate pipette for each transfer, the absorbed serum was diluted serially in the following manner to make final dilutions of 1:300, 1:1,000, 1:3,000, 1:10,000, 1:30,000, and so on:

Transfer	*Diluent*	*Final dilution*
1.0	2.0	1:300
.9	2.1	1:1,000
1.0	2.0	1:3,000
.9	2.1	1:10,000

The end-point titer was considered the last dilution having at least 50 percent agglutination.

Results

The cross-agglutination patterns of the five cultures were not typical of *L. icterohaemorrhagiae* M20 (table 1). However, the titer patterns of the five cultures were similar. Agglutinin-absorption studies with *L. icterohaemorrhagiae* M20 and one of the cultures indicated a subserotype relationship.

The agglutinin-absorption studies comparing *L. icterohaemorrhagiae* subserotype *incompleta* RGA and the five cultures revealed less than 10 percent of the homologous titer remaining after adequate cross absorptions (table 2). The cultures were identified as *L. icterohaemorrhagiae* subserotype *incompleta.*

Summary

Five leptospiral cultures isolated respectively from a mouse, a skunk, a gray fox, and two raccoons in Pennsylvania were submitted for typing. Cross-agglutination patterns with type antiserums were unusual but indicated a relationship with *Leptospira icterohaemorrhagiae.* Through agglutinin-absorption studies, the cultures were all identified as *Leptospira icterohaemorrhagiae* subserotype *incompleta.*

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Institutes in Michigan

Two institutes, one on administration of medical care programs for the needy and the other on administration of arthritis and metabolic disease programs, will be sponsored by the University of Michigan School of Public Health in Ann Arbor during July 1963.

Medical care administration. At the third Institute on the Administration of Medical Care for the Needy, scheduled for July 8–19, State and local public welfare and public health personnel, both medical and nonmedical, will study planning, administration, and evaluation of medical care programs for the needy, discuss the relationships of these programs to community health services and medical care programs for the entire population, and explore ways by which health and welfare departments may work together and consider new approaches to the provision of medical care. Collaborating in this institute are the Public Health Service, the Bureau of Family Services of the Welfare Administration, Department of Health, Education, and Welfare, the American Public Welfare Association, and the School of Social Work of the University of Michigan.

Arthritis and metabolic diseases. Objectives of the Institute of Arthritis and Metabolic Diseases, scheduled for July 22–26, are to review the current status of community approaches to arthritis and metabolic diseases, consider possible new approaches to control of these conditions, and develop a manual for use of chronic disease program administrators based on material presented at the institute. This institute, the fourth in a series of annual conferences on various aspects of chronic disease, is planned primarily for those responsible for the administration of chronic disease programs, but others interested in or concerned with such programs are eligible to attend.

Further details about both institutes are available from the Director of Continued Education, School of Public Health, University of Michigan, Ann Arbor, Mich.