

Natural Occurrence of *Leptospira ballum* in Rural House Mice and in an Opossum. (20721)

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Borg-Peterson(1) first isolated the serotype strain (mus 127) of *Leptospira ballum* from the urine of a rural house mouse, *Mus musculus specilegus*, trapped in Southern Jutland, Denmark, and established its distinct leptospiral serologic relationship. Subsequently, Wolff, *et al.*(2) traced the source of an accidental human laboratory infection with *L. ballum* to the natural occurrence of these organisms in the urine of the laboratory white mice. The inapparent infectivity of these leptospires for white mice and the persistence of the urinary carrier stage during the entire life span of the host have been noted(1,2).

In the Western hemisphere, leptospires of the serotype *ballum* have been recovered from naturally occurring infections in human beings and Norway rats in Puerto Rico(3,4), Norway rats trapped in British Columbia(4), stock laboratory white mice at the Army Medical Service Graduate School, Washington, D. C. (5) and the Rocky Mountain Laboratories of the U. S. Public Health Service(6).

In this paper, the authors report naturally occurring infection with *L. ballum* in domestic mice (*Mus musculus*) and in an opossum (*Didelphis virginiana*), trapped in a rural area near Middleburg, Virginia, coincident to an epidemiological investigation of possible wild life carriers of *L. pomona* in an endemic area of bovine leptospirosis.

Methods. Animals were trapped by the Virginia Cooperative Wildlife Research Unit, Virginia Polytechnic Institute Biology Department, Blacksburg, in cooperation with Dr. Wilson C. Bell, in and around pastureland and housing facilities frequented by domestic ruminants. Trapped animals were delivered to the Veterinary Division, Army Medical Service Graduate School, where they were bled for serum specimens. The animals sacrificed, were then autopsied and appropriate tissues cultured for leptospires. Fletcher's and Schufner's leptospiral media(7) were inoculated

with 1 drop (ca .03 ml) of a 10% kidney suspension in physiological saline solution. Urine or saline washings were aspirated aseptically from the bladder, examined by dark field microscopy and cultured for leptospires. Cultures were incubated at 30°C for 30 days. *Identification* of the isolated strains was based upon the cross agglutination-lysis pattern of these organisms employed as antigens against 18 type-leptospiral immune rabbit serums, selected on the basis of previous serologic classification studies. Similarly the serums from all host animals except house mice were tested for the presence of leptospiral antibodies against a screening battery of type-leptospiral antigens(4). The technic of the agglutination-lysis test employed is described elsewhere(7).

Results. The following species and numbers of wild animals were trapped and studied: 1 skunk (*Mephitis mephitis*); 3 squirrels (*Sciurus carolinensis*); 2 wild rabbits (*Sylvilagus floridanus*); 1 muskrat (*Ondatra zibethica*); 1 deer mouse (*Peromyscus sp.*); 2 opossums (*Didelphis virginiana*); 27 house mice (*Mus musculus*).

Post mortem examination of tissue revealed no gross pathology, except for occasional tapeworm cysts in the livers from several house mice. Examination of sampled serums from all animals but the house mice did not disclose leptospiral antibodies, except for 1 opossum which showed an agglutination-lysis titer of 1:400 against *L. ballum*.

Dark-field microscopic examinations of urine or bladder saline washings of 10 house mice disclosed the presence of leptospires. Positive leptospiral cultures of kidney suspensions or urine were obtained from 9 of these 10 house mice, and from 6 additional house mice. A leptospiral isolate was also obtained from the opossum which had an agglutinin titer against *L. ballum*. The cross agglutination pattern of all the isolates were identical within the limits of the methods employed. The results of the

TABLE I. Cross Agglutination-Lysis Reactions of Isolates.

Antiserum	Homologous titer	Antigens	
		<i>L. ballum</i> ^f	Isolates
<i>L. andaman</i> ^a	102400	—	—
<i>semarang</i> ^a	409600	—	—
<i>djasiman</i> ^a	25600	—	—
<i>ballico</i> ^a	25600	—	—
<i>australis</i> B ^a	25600	—	100
<i>pomona</i> ^b	25600	—	—
<i>canicola</i> ^b	6400	400	400
<i>djatzi</i> ^{b,h}	102400	—	—
<i>alexii</i> ^{b,i}	6400	100	400
<i>hyos</i> ^c	102400	—	—
<i>sentot</i> ^d	409600	—	—
<i>medanensis</i> HC ^d	102400	—	—
<i>autumnalis</i> ^e	25600	—	—
<i>ballum</i> ^f	25600	25600	25600
<i>grippotyphosa</i> ^f	102400	—	—
<i>icterohemorrhagiae</i> AB ^g	25600	—	—

^a Received from G. W. Hooper Foundation, San Francisco, Calif. ^b Isolated by Veterinary Division, AMSGS, Walter Reed Army Medical Center, Washington, D. C. ^c Received from the Inst. Bacteriologica Malbran, Buenos Aires. ^d Received from the Indisch. Inst., Amsterdam. ^e Received from Nat. Inst. of Health, Bethesda, Md. ^f Received from Pasteur Inst., Paris. ^g Received from the Inst. Trop. Med., Leiden. ^h Member of the serogroup *bataviae*. ⁱ Member of the serogroup *pyrogenes*.

serologic typing of the isolates are shown in Table I. Titers are expressed as the reciprocals of the highest dilution showing a positive reaction. The strains show the typical cross-agglutination reactions peculiar to *L. ballum*.

Summary. In a search for wildlife carriers of *L. pomona*, various species of mammals were trapped in an endemic area of bovine leptospirosis. Direct dark-ground microscopic

examinations of urine or bladder saline washings disclosed the presence of leptospires in 10 of the 37 house mice examined. Leptospiral cultural isolates were obtained from 9 of these 10 house mice. The recovery of leptospires from 6 additional house mice and from 1 of 2 opossums emphasizes the importance of cultural methods in any survey for leptospirosis. All isolates, serologically typed on the basis of a cross agglutination-lysis pattern, employing 18 type leptospiral immune rabbit serums, belonged to the serotype *L. ballum*. The failure to detect *L. pomona* in the rodent and other wild life population in an endemic area of bovine leptospirosis affords ancillary evidence that the natural hosts of *L. pomona* leptospirosis are to be found primarily in the livestock population. To the authors' knowledge, this is the first report of leptospirosis in the opossum.

1. Borg-Peterson, C., *Acta Pathol. Microbiol. Scand.*, 1944, v21, 594.
2. Wolff, J. W., Bohlander, H., and Ruys, A. C., *Antonie van Leeuwenhoek. J. Microbiol. Serol.*, 1949, v15, 1.
3. Yager, R. H., and Gochenour, W. S., Jr., *Am. J. Trop. Med. Hyg.*, 1952, v1, 457.
4. Gochenour, W. S., Jr., and Yager, R. H., *Proc. A.V.M.A.*, 1952, 178.
5. Unpublished data from the Army Medical Service Graduate School.
6. Wolff, J. W., personal communication.
7. Gochenour, W. S., Jr., Yager, R. H., Wetmore, P. W., and Hightower, J. A., *Am. J. Pub. Health*, 1953, v43, 405.

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