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## Natural Occurrence of *Leptospira icterohaemorrhagiae* in an Opossum. (27440)

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Shortly after *Leptospira icterohaemorrhagiae* was shown to be the cause of Weil's disease(1), the Norway rat was found to be the principal host carrier(2). The strain was subsequently found sporadically in infections in domestic animals and in low incidence in few other rodent hosts. In the past decade, there has been a broader recognition of the host distribution of pathogenic leptospiras, particularly in larger mammalian wildlife. In addition to rats, mice, dogs, cattle and swine, *L. icterohaemorrhagiae* has been isolated from skunks, foxes, mongooses, raccoons, muskrats and nutria(2,3,4,5,6).

This paper reports the natural occurrence of *L. icterohaemorrhagiae* in opossums. This study also afforded the opportunity to reexamine the serological interrelationship of several members of the *icterohaemorrhagiae* serogroup.

*Materials and methods.* Animals were live-trapped on a military reservation at Aberdeen, Md. from 5 May 1961 to 18 July 1961. Animals were killed by exposure to chloroform and carbon dioxide (Dry Ice) in a closed 20-gallon can. Heart blood was obtained for serologic studies. Bladder urine and a 10% suspension of triturated kidney, as well as one in 10 dilutions thereof were cultured in Fletcher's semisolid medium. The employed diluent was a phosphate-buffered (pH 7.4) physiological salt solution. Each preparation was cultured in 4 tubes of medium employing minimal inocula; *e.g.,* 1 drop per 5 ml medium.

All cultures were incubated at 28°-30°C and were examined for growth at 2-week intervals by darkground microscopy. All cultures negative after 6 weeks incubation were discarded.

Serums were examined for leptospiral agglutinins by the microscopic-agglutination technic with live antigens. The following 18 screening antigens were used: *L. andamana, L. butembo, L. celledoni, L. bataviae, L. pomona, L. djasiman, L. hyos, L. autumnalis, L. ballum, L. canicola, L. icterohaemorrhagiae, L. pyrogenes, L. alexi, L. grippotyphosa, L. borincana, L. wolffi, L. javanica* and *L. australis.*

For the preliminary culture typing of the isolates, rabbit antiserums of known serotypes were used. The procedures for the microagglutination and agglutinin-adsorption tests, and the method for preparing antiserums have been described(7).

*Results and conclusion.* During the specified interval, 37 opossums were studied. Isolates were cultured from the kidney and/or urine of 2 opossums (strains Op-3 and Op-6). Both of these animals were serologically positive, one had a titer of 1:25 versus *L. icterohaemorrhagiae,* the other a titer of 1:100 versus *L. ballum.* Three additional animals had

TABLE I. Cross-Agglutination Reactions of Leptospiral Isolates.

| Antiserum | Homologous titer | Antigen Op-3 | Antigen Op-6 |
|---|---|---|---|
| andamana | 25,600 | —* | — |
| semaranga | 25,600 | — | — |
| hyos | 25,600 | — | — |
| pomona | 25,600 | 400 | — |
| djasiman | 25,600 | — | — |
| sentot | 25,600 | 100 | — |
| autumnalis | 25,600 | 400 | — |
| australis | 25,600 | — | — |
| ballum | 6,400 | — | 6,400 |
| canicola | 25,600 | 1,600 | 1,600 |
| icterohaemorrhagiae | 25,600 | 25,600 | — |
| zanoni | 25,600 | 1,600 | 1,600 |
| alexi | 25,600 | — | — |
| grippotyphosa | 25,600 | — | — |
| medanensis | 25,600 | — | — |
| javanica | 25,600 | — | 100 |
| pyrogenes | 25,600 | 400 | 100 |

* Negative at 1:100 dilution.

Titers are expressed as reciprocals of the highest dilutions showing positive reactions.

low-titer agglutinins, 1:25 against *L. autumnalis*(2) or *L. butembo* and *L. grippotyphosa* (1). Partial or questionable reactions were seen in 9 other samples.

The 2 isolates employed as antigen were tested for cross-agglutination reactions with 17 type leptospiral antiserums (Table I). The cross-agglutination reactions of strain Op-6 were indistinguishable from those of *L. ballum* strains and this strain presumptively identified to be a *L. ballum* serotype. This serotype had repeatedly been disclosed in opossums(4,8). Strain Op-3 showed serologic affinities with members of the icterohaemorrhagiae group. The preliminary culture typing for both isolates was consistent with serologic findings in their respective hosts. For definitive identification of strain Op-3, cross-agglutination adsorption studies were conducted with the various members of the icterohaemorrhagiae serogroup. On the basis of test results (Table II), strain Op-3 was antigenically indistinguishable from strain M 20, the type strain of *L. icterohaemorrhagiae*. To the authors' knowledge, *L. icterohaemorrhagiae* had not heretofore been isolated from an opossum.

Studies of the antigenic interrelationship among the "complete" (M 20) and "incomplete" (RGA) biotypes of *L. icterohaemorrhagiae* and *L. mankarso* were prompted by previous serologic findings and indicated antigenic interrelationships that differed from other published reports. The cross-agglutinin adsorption tests indicate that the incomplete biotype, strain RGA, contains antigenic components not shared by the type strain M 20, contrary to the findings of Borg-Petersen(9). Moreover, *L. mankarso* on the basis of test results could be reclassified according to currently employed taxonomic criteria(10) as a biotype of *L. icterohaemorrhagiae*. These divergent findings in this and other laboratories may reflect differences in the respectively employed technics or minor antigenic changes in the lines maintained in this laboratory. A reevaluation of the antigenic interrelation-

TABLE II. Results of Cross-Agglutination Adsorption on Strain Opossum 3 with Members of *L. icterohaemorrhagiae* Serogroup.

| Antiserum Against | Antiserum Adsorbed with | Reciprocal of titer* with Homologous strain after adsorption | Reciprocal of titer* with Adsorbing strain before adsorption |
|---|---|---|---|
| Opossum 3 (30,000)† | ictero. M 20 | — | 30,000 |
| | " RGA | 3,000 | 10,000 |
| | mankarso | 10,000 | 10,000 |
| | naam | 10,000 | 10,000 |
| | sarmin | 10,000 | 1,000 |
| | birkini | 3,000 | 10,000 |
| | ndambari | 10,000 | 1,000 |
| Ictero. M 20 (10,000) | opossum 3 | — | 3,000 |
| | ictero. RGA | 3,000 | 1,000 |
| | mankarso | 1,000 | 3,000 |
| Ictero. RGA (30,000) | opossum 3 | 3,000 | 30,000 |
| | ictero. M 20 | 3,000 | 30,000 |
| | mankarso | 30,000 | 30,000 |
| Mankarso (30,000) | opossum 3 | 300 | 30,000 |
| | ictero. M 20 | 300 | 30,000 |
| | " RGA | 30,000 | 1,000 |
| Naam (30,000) | opossum 3 | 3,000 | 10,000 |
| Sarmin (30,000) | " 3 | 30,000 | 3,000 |
| Birkini (30,000) | " 3 | 30,000 | 3,000 |
| Ndambari (30,000) | " 3 | 30,000 | 3,000 |

* Following adsorption with homologous antigen, serum agglutinins were undetectable at 1:100 dilution. Cross-agglutinins against heterologous (adsorbing strain) antigens were undetectable at 1:100 dilution following adsorption with respective heterologous antigens.

† Homologous titers of unadsorbed serum shown in parentheses.

ships among strains M 20, RGA and Mankarso is indicated.

*Summary.* Serological and cultural studies for leptospirosis were conducted on 37 opossums trapped at a military reservation at Aberdeen, Md. Five of the 37 animals were serologically positive. Questionable reactions were seen in serums from 9 other animals. Two isolates were obtained and identified as *Leptospira ballum* and *L. icterohaemorrhagiae,* respectively. *L. icterohaemorrhagiae* in an opossum had not heretofore been reported. The antigenic interrelationships among the "complete" and "incomplete" biotypes of *L. icterohaemorrhagiae* and *L. mankarso,* disclosed in the serologic studies, differed from previously reported findings.

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## Amino Acid Composition and Nutritive Value of Goat Milk Casein.* (27441)

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Much attention has been directed to the amino acid composition of various proteins and to the relationship between amino acid composition and nutritive value. However, there is relatively little information on the casein of goat milk. This is surprising in view of the extensive literature(1,2,3,4) on the casein of cow's milk and the frequent use of goat milk as a therapeutic agent.

The information available concerning the amino acid composition of goat milk was published in 1933(5,6) and was obtained by fractional solubility and steam distillation technics. No values are available for glycine, isoleucine, methionine, or threonine. Published values for arginine and histidine vary greatly.

In this study, we have calculated the nutritive value of goat milk casein by the rat growth method of Miller and Bender(7) and have compared it with that of cow's milk casein. The same preparation of goat milk casein was separated into its component amino acids by ion exchange chromatography by the method of Moore and Stein(8) and measured by quantitative ninhydrin assay(9).

*Experimental.* I. *Preparation of casein.* Commercially available evaporated goat milk† was employed as a source of casein. Casein was isolated by isoelectric precipitation, as described by Jacobs(10). It was redissolved and reprecipitated 2 more times before use. The final precipitate was filtered on coarse filter paper and dried *in vacuo* at 50°C. The dried protein was extracted 3 times with boiling diethyl ether and dried *in vacuo* at room temperature. It was then ground to a very fine powder in a Wiley Mill and used for rat

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