# NEW LEPTOSPIRAL SEROTYPE IN THE PYROGENES SEROGROUP

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THE pyrogenes serogroup of the genus *Leptospira* is presently composed of eight serotypes. *Leptospira pyrogenes*, the first serotype found, was recognized by Vervoort (*1*) in Indonesia. Cotter (*2*) detected illness among sugarcane cutters in North Queensland which later proved to be caused by *L. zanoni*=*L. australis* B of Lumley (*3*). *L. abramis*, *L. biggis*, and *L. hamptoni* were isolated in Malaya and characterized by Alexander and associates (*4*). Alexander and associates reported studies on *L. alexi* which had been isolated from a person with leptospirosis in Puerto Rico (*5*). *L. robinsoni* was isolated in North Queensland and reported by Alexander and Smith (*6*). Galton and coworkers (*7*) have studied a strain isolated in Manila and designated it provisionally as *L. manilae*.

Leptospirosis among nutria was first reported by Anchezar and associates (*8*). They reported that *L. icterohaemorrhagiae* (*L. bonariensis*) occurred naturally among nutria in Argentina. Roth and associates (*9*) isolated six strains of *L. paidjan* from nutria in Louisiana.

This report describes the identification of a single leptospiral strain, LSU 1551, isolated from the renal tissue of a nutria, *Myocastor coypus*, captured in a lake on the campus of Louisiana State University, Baton Rouge. On antigenic analysis, the strain was found to be a subserotype of *L. zanoni*, strain Zanoni. This finding constitutes the first bacteriological evidence of a member of the pyrogenes serogroup in the United States. The designation *L. zanoni myocastoris* is proposed for the new strain, and the designation *L. zanoni zanoni* is proposed for the Zanoni strain.

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## Methods

*Cultural and serologic procedures.* The LSU 1551 leptospiral strain was isolated from the renal tissue of the nutria in Fletcher's and Stuart's semisolid mediums by methods previously described (*10,11*).

All antiserums were prepared with live cultures as described by Alexander and associates (*12*), except that 10-day-old cultures were used. Living 5- to 7-day-old cultures in Stuart's medium were used as antigens in the microscopic agglutination test. Dense formalinized suspensions of leptospires in phosphate buffered saline were used as antigen in the agglutinin-absorption test. One part of a 1:10 dilution of antiserum was added to four parts of absorbing antigen, incubated at 37° C. for 4 hours and overnight at 30° C. The cells were removed by centrifugation, and the serum was absorbed a second time by adding the supernatant fluid from the first absorption to packed cells. The interlocking tenfold dilution scheme of Wolff (*13*) was used to determine the results of agglutinin-absorption tests. Living antigen was used to determine these results. A fourfold dilution scheme was employed in all other studies. Separate pipettes were used to prepare each dilution. Details of these procedures have been described elsewhere (*11,14,15*).

*Leptospiral serotypes.* The type strains recommended by the Taxonomic Subcommittee on Leptospira were employed in these studies. They were obtained from the WHO/FAO Leptospirosis Reference Laboratory, Division

of Veterinary Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D.C.

## Results

Antigen prepared from strain LSU 1551 was tested against antiserums of *L. ballum*, Mus 127; *L. canicola*, Hond Utrecht; *L. icterohaemorrhagiae*, M 20; *L. bataviae*, Swartz; *L. grippotyphosa*, Moskva V; *L. pyrogenes*, Salinem; *L. autumnalis*, Akiyami A; *L. pomona*, Pomona; *L. hyos*, Mitis Johnson; *L. hardjo*, Hardjoprajitno; *L. australis*, Ballico; and *L. mini georgia*, LT 117, which are used routinely in our laboratory for initial screening in the typing of leptospiral strains. Strain LSU 1551 was agglutinated by antiserum against *L. icterohaemorrhagiae* to 25 percent of its homologous titer and by antiserum against *L. pyrogenes* to 10 percent of its homologous titer. No agglutination at a dilution of 1:100 was noted with the remaining antiserums.

**Table 1. Results of cross-agglutination studies between strain LSU 1551 and members of the pyrogenes and icterohaemorrhagiae serogroups**

| Antileptospiral serum | Reciprocal of titer against antigen [1] [2] | | |
|---|---|---|---|
| | Homologous titer of antiserum | Titer against antigen of LSU 1551 | Titer of LSU 1551 antiserum against respective antigen [3] |
| *L. pyrogenes* | 6, 400 | 400 | 100 |
| *L. zanoni* | 6, 400 | 6, 400 | 6, 400 |
| *L. abramis* | 25, 600 | 6, 400 | 400 |
| *L. biggis* | 6, 400 | 6, 400 | 1, 600 |
| *L. hamptoni* | 6, 400 | 100 | Negative |
| *L. alexi* | 6, 400 | 100 | Negative |
| *L. robinsoni* | 25, 600 | 1, 600 | 100 |
| *L. manilae* | 25, 600 | 6, 400 | 100 |
| *L. icterohaemorrhagiae*, M 20 | 25, 600 | 1, 600 | 100 |
| *L. icterohaemorrhagiae*, RGA | 25, 600 | 25, 600 | 100 |
| *L. icterohaemorrhagiae*, Mankarso | 25, 600 | 400 | Negative |
| *L. naam*, Naam | 25, 600 | 100 | Negative |
| *L. sarmin* | 25, 600 | 6, 400 | 400 |
| *L. birkini*, Birkin | 6, 400 | 100 | 400 |
| *L. birkini*, Smith | 6, 400 | 1, 600 | 1, 600 |
| *L. ndambari* | 1, 600 | 400 | 1, 600 |
| LSU 1551 | 25, 600 | 25, 600 | 25, 600 |

[1] Living antigen was used.
[2] Positive—50 percent agglutination of "lysis" or both at 1:100 dilution.
[3] Negative—less than 50 percent agglutination or "lysis" or both at 1:100 dilution.

**Table 2. Results of additional cross-agglutination studies between strain LSU 1551 and members of the genus Leptospira**

| Antileptospiral serum | Reciprocal of titer against antigen [1] [2] | | |
|---|---|---|---|
| | Homologous titer of antiserum | Titer against antigen of LSU 1551 [3] | Titer of LSU 1551 antiserum against respective antigen [3] |
| *L. malaya* | 6, 400 | 6, 400 | 6, 400 |
| *L. sumneri* | 25, 600 | 6, 400 | 1, 600 |
| *L. jonsis* | 6, 400 | Negative | 400 |
| *L. broomi* | 6, 400 | Negative | 100 |
| *L. sentot* | 25, 600 | Negative | 100 |
| *L. djasiman*, Djasiman | 25, 600 | 100 | Negative |
| LSU 1551 | 25, 600 | 25, 600 | 25, 600 |

[1] Living antigen was used.
[2] Positive—50 percent agglutination or "lysis" or both at 1:100 dilution.
[3] Negative—less than 50 percent agglutination or "lysis" or both at 1:100 dilution.

NOTE: No cross-reactions were noted between strain LSU 1551 and *L. javanica, L. poi, L. coxus, L. celledoni celledoni, L. celledoni whitcombi, L. canicola, L. schueffneri, L. benjamin, L. ballum ballumensis, L. ballum castellonis, L. cynopteri, L. butembo, L. autumnalis autumnalis, L. autumnalis rachmati, L. autumnalis fortbragg, L. bangkinang, L. mooris, L. pomona pomona, L. australis, L. muenchen, L. grippotyphosa, L. hebdomadis hebdomadis, L. kremastos, L. worsfoldi, L. jules, L. borincana, L. kabura, L. mini mini, L. mini szwajizak, L. mini georgia, L. hardjo, L. wolffii, L. medanensis, L. sejroe sejroe, L. saxkoebing saxkoebing, L. haemolyticus haemolyticus, L. haemolyticus ricardi, L. bataviae, L. paidjan, L. djatzi, L. hyos hyos, L. hyos bakeri, L. hyos guidae, L. atlantae, L. kisuba, L. semaranga.*

Based on these initial findings, the serologic relationship of strain LSU 1551 was determined for the other members of the icterohaemorrhagiae and pyrogenes serogroups. In these studies antiserum against LSU 1551 was tested against antigens of the type strains employed. These results (table 1) revealed a closer serologic relationship between strain LSU 1551 and *L. zanoni* than for any of the other serotypes employed.

Since strain LSU 1551 possessed a serologic pattern which was not consistent with other leptospiral serotypes, its cross-reactions with

most of the remaining serotypes of the genus *Leptospira* were determined. Although strain LSU 1551 was not agglutinated by antiserum of *L. canicola* in the screening tests, high cross-reactions were found for *L. malaya* and *L. sumneri* which are members of the canicola serogroup (table 2). No other significant cross-agglutination reactions were noted.

Based on the results of cross-agglutination studies, reciprocal agglutinin-absorption studies were conducted with three members of the pyrogenes serogroup, two members of the canicola serogroup, and one member of the icterohaemorrhagiae serogroup. Results of the agglutinin-absorption studies (table 3) showed that strain LSU 1551 is a subserotype of *L. zanoni* and distinctly heterologous to the other serotypes employed. The agglutinin-absorption tests between *L. zanoni* and strain LSU 1551 were repeated three times. Although minor differences occurred, the results repeatedly showed a subserotype relationship. Furthermore, formalinized antigen was used in place of living antigen to determine the results of one agglutinin-absorption trial between strain LSU 1551 and *L. zanoni*. This absorption trial also showed a subserotype relationship.


## Summary

A new leptospiral subserotype of the pyrogenes serogroup was isolated from the renal tissue of a nutria, *Myocastor coypus*, captured in a lake on the main campus of Louisiana State University. Cross-agglutination and agglutinin-absorption studies showed the new strain to be a subserotype of *Leptospira zanoni*. The designation *Leptospira zanoni myocastoris* is proposed. This finding constitutes the first bacteriological evidence of a member of the pyrogenes serogroup in the United States.


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**Table 3. Cross agglutinin-absorption studies on strain LSU 1551 with indicated members of the pyrogenes, canicola, and icterohaemorrhagiae serogroups**

| Antileptospiral serum | Absorbed with— | Reciprocal of titer against antigen [1] [2] | | | |
|---|---|---|---|---|---|
| | | Homologous strain | | Absorbing strain | |
| | | Before | After [4] | Before | After [4] |
| LSU 1551 | LSU 1551 | 300, 000 | Negative | 300, 000 | Negative |
| | *L. zanoni* [3] | 300, 000 | 100, 000 | 30, 000 | Do. |
| | *L. abramis* | 300, 000 | 300, 000 | 1, 000 | Do. |
| | *L. biggis* | 300, 000 | 300, 000 | 3, 000 | Do. |
| | *L. sumneri* | 300, 000 | 300, 000 | 3, 000 | Do. |
| | *L. malaya* | 300, 000 | 300, 000 | 30, 000 | Do. |
| | *L. icterohaemorrhagiae*, RGA | 300, 000 | 300, 000 | 3, 000 | Do. |
| *L. zanoni* | *L. zanoni* | 100, 000 | Negative | 100, 000 | Do. |
| | LSU 1551 [3] | 100, 000 | 100 | 100, 000 | Do. |
| *L. abramis* | *L. abramis* | 300, 000 | Negative | 300, 000 | Do. |
| | LSU 1551 | 300, 000 | 100, 000 | 100, 000 | Do. |
| *L. biggis* | *L. biggis* | 100, 000 | Negative | 100, 000 | Do. |
| | LSU 1551 | 100, 000 | 1, 000 | 100, 000 | Do. |
| *L. sumneri* | *L. sumneri* | 300, 000 | Negative | 300, 000 | Do. |
| | LSU 1551 | 300, 000 | 300, 000 | 100, 000 | Do. |
| *L. malaya* | *L. malaya* | 100, 000 | Negative | 100, 000 | Do. |
| | LSU 1551 | 100, 000 | 100, 000 | 30, 000 | Do. |
| *L. icterohaemorrhagiae*, RGA | *L. icterohaemorrhagiae*, RGA | 300, 000 | Negative | 300, 000 | Do. |
| | LSU 1551 | 300, 000 | 300, 000 | 300, 000 | Do. |

[1] Living antigen was used.
[2] Positive—50 percent agglutination or "lysis" or both.
[3] Repeated three times with no major differences in results. Results determined in one trial with killed antigen revealed the same subserotype relationship.
[4] Negative—less than 50 percent agglutination or "lysis" or both at 1:100 dilution.

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## FDA to Increase Information to Medical Professions

Under the authority of the Kefauver-Harris Drug Amendments of 1962, the Food and Drug Administration will increase the flow of scientific information about new drugs to the medical professions through professional organizations and journals and, occasionally, letters to individual members, FDA Commissioner George P. Larrick stated at the commencement exercises of the University of Tennessee Medical Units.

The Kefauver-Harris amendments aim to improve the reliability of drugs by strengthening regulations on clinical investigation (see March 1963 issue of *Public Health Reports*, p. 194) and to improve communication of information about new drugs. Commissioner Larrick predicted that the legislation will also contribute to better and more effective drug research. Under the new regulations, the drug sponsor must be sure trials on man are justified before he begins them. He must advise the FDA of adverse reactions during trials and after the drug is marketed under an approved new drug application.

Larrick warned, however, that "the most careful premarket testing cannot be expected to reveal as much about a drug as does widespread use of the product in general practice." While the manufacturer or sponsor must advise the FDA promptly of adverse reactions that come to his attention, he may not receive reports of some significant observations on a new drug. Doctors, dentists, pharmacists, and nurses would be performing a valuable service by reporting significant adverse effects or unusual effects of drugs and devices to professional associations and to the FDA, the commissioner stated.