

LEPTOSPIRA PAIDJAN (BATAVIAE SEROGROUP)
ISOLATED FROM NUTRIA IN LOUISIANA



Immature nutria captured in Louisiana

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LEPTOSPIROSIS in nutria, *Myocastor coypus*, was first reported by Anchezar and associates (1,2). They reported the isolation of *Leptospira bonariensis* from this rodent in Argentina. Erroneously designated as *L. bonariensis* by Savino and Anchezar (3), this serotype was later found by Savino and Rennella (4) to be *Leptospira icterohaemorrhagiae*.

Leptospira paidjan, first isolated from a Javanese laborer in Sumatra by Wolff (5), was closely related to *Leptospira bataviae*, but antigenic analysis proved it to be heterologous, and it was therefore designated as a new serotype within the bataviae serogroup. McCrumb and associates (6), during a study of leptospirosis in Malaya, isolated numerous strains of lep-

tospire from military and civilian personnel. Eleven of the strains were subsequently identified as *L. paidjan* by Alexander and associates (7). No previous isolations of *L. paidjan* from an animal host are known to us.

This report describes the isolation and identification of six strains of leptospire recovered

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from nutria in Louisiana. On antigenic analysis, these strains proved homologous with *L. paidjan*, a member of the *bataviae* serogroup.

Materials and Methods

Collection and processing of animals. The nutria were collected from a marsh in St. Charles Parish in south-central Louisiana, primarily a hunting and trapping area. The methods employed in processing the animals have been reported in detail (8).

Cultural procedures. Fletcher's (9) and Stuart's (10) semisolid mediums and the solid medium described by Cox and Larson (11) were inoculated with kidney suspensions. Semisolid mediums were inoculated with 3 to 5 drops of 10 percent kidney suspension prepared in Stuart's liquid medium (12). Three plates of solid medium were inoculated with 0.1 ml. of 1:10, 1:100, and 1:1,000 dilutions of 10 percent kidney suspension. Remaining details of cultural procedures have previously been reported (8, 13).

Serologic procedures. All antisera were prepared as described by Alexander and co-workers (14), except that 10-day-old cultures were used. Antigens employed in the microscopic agglutination test and in the agglutinin-absorption test were prepared and used essentially as previously described (10, 13). The interlocking tenfold dilution scheme of Wolff (15) was used to determine the results of agglutinin-absorption tests. 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 (8, 10, 13).

Leptospiral serotypes. Strains of the following serotypes were employed to produce antigens and antisera:

<i>ballum</i> , MUS 127	<i>pomona</i> , Pomona
<i>canicola</i> , Hond Utrecht	<i>hyos</i> , Mitis Johnson
<i>icterohaemorrhagiae</i> , M 20	<i>hardjo</i> , Hardjoprajitno
<i>bataviae</i> , van Tienen	<i>australis</i> , Ballico
<i>grippityphosa</i> , Moskva V	<i>mini georgia</i> , LT117
<i>pyrogenes</i> , Salinem	<i>djatzi</i> , HS-26
<i>autumnalis</i> , Akiyami A	<i>paidjan</i> , Paidjan

All strains were supplied by the Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C., except *L. mini georgia* (16), which was supplied by the Veterinary Public Health Laboratory, Communicable Disease Center, Atlanta, Ga.

Results

Eight strains of leptospire were isolated from 26 nutria collected from the same vicinity. All strains were isolated in Fletcher's and Stuart's semisolid mediums; however, two strains, LSU 2578 and LSU 2590, were not isolated on solid medium. No differentiating colonial characteristics were noted. No difficulty was encountered upon subculturing into Stuart's liquid medium for antigen production.

In initial screening studies, antigens prepared from each isolate were tested against

Table 1. Cross-agglutination reactions of *bataviae* isolates with members of the *bataviae* serogroup

Antileptospiral serum	Reciprocal of titer against antigen ^{1 2}								
	<i>L. bataviae</i>	<i>L. paidjan</i>	<i>L. djatzi</i>	LSU 2564	LSU 2569	LSU 2577	LSU 2578	LSU 2588	LSU 2590
<i>L. bataviae</i>	25, 600	6, 400	1, 600	1, 600	1, 600	1, 600	1, 600	1, 600	1, 600
<i>L. paidjan</i>	6, 400	102, 400	6, 400	25, 600	25, 600	25, 600	25, 600	6, 400	25, 600
<i>L. djatzi</i>	1, 600	6, 400	6, 400	1, 600	1, 600	1, 600	1, 600	400	1, 600
LSU 2564.....	1, 600	25, 600	1, 600	6, 400	6, 400	6, 400	25, 600	6, 400	6, 400
LSU 2569.....	6, 400	25, 600	6, 400	6, 400	6, 400	6, 400	6, 400	1, 600	6, 400
LSU 2577.....	1, 600	25, 600	1, 600	6, 400	6, 400	6, 400	6, 400	1, 600	6, 400
LSU 2578.....	1, 600	25, 600	1, 600	6, 400	6, 400	6, 400	6, 400	1, 600	6, 400
LSU 2588.....	6, 400	25, 600	1, 600	6, 400	6, 400	6, 400	6, 400	1, 600	6, 400
LSU 2590.....	1, 600	25, 600	6, 400	6, 400	6, 400	6, 400	25, 600	1, 600	6, 400

¹ Living antigen was used.

² Positive—75 percent agglutination or "lysis" or both.

Table 2. Results of cross-agglutinin-absorption studies between strains LSU 2564, *L. paidjan*, *L. bataviae*, and *L. djatzi*

Antileptospiral serum	Absorbed with—	Reciprocal of titer against antigen ^{1 2}			
		Homologous strain		Absorbing strain	
		Before	After	Before	After
<i>L. bataviae</i>	<i>L. bataviae</i>	10,000	Negative.....	10,000	Negative.
	LSU 2564.....	10,000	10,000.....	10,000	Do.
<i>L. paidjan</i>	<i>L. paidjan</i>	30,000	Negative.....	30,000	Do.
	LSU 2564.....	30,000	do.....	30,000	Do.
<i>L. djatzi</i>	<i>L. djatzi</i>	10,000	do.....	10,000	Do.
	LSU 2564.....	10,000	10,000.....	1,000	Do.
LSU 2564.....	LSU 2564.....	30,000	Negative.....	30,000	Do.
	<i>L. bataviae</i>	30,000	10,000.....	3,000	Do.
	<i>L. paidjan</i>	30,000	Negative.....	30,000	Do.
	<i>L. djatzi</i>	10,000	3,000.....	3,000	Do.

¹ Living antigen was used.

² Positive—50 percent agglutination or “lysis” or both.

antisera prepared from *L. ballum*, *L. canicola*, *L. icterohaemorrhagiae*, *L. bataviae*, *L. grippotyphosa*, *L. pyrogenes*, *L. autumnalis*, *L. pomona*, *L. hyos*, *L. hardjo*, *L. australis*, and *L. mini georgia*. The results of these tests indicated that six strains were related to the *bataviae* serogroup, one strain to the *australis* serogroup, and one strain could not be categorized by the antisera employed. This report is concerned with strains LSU 2564, LSU 2569, LSU 2577, LSU 2578, LSU 2588, and LSU 2590, all of which showed serologic affinity for the *bataviae* serogroup.

Additional cross-agglutination studies showed the serologic affinity of the six strains with *L. bataviae*, *L. paidjan*, *L. djatzi*, and with each other (table 1). On the basis of these results, serotype identity could not be assessed. Reciprocal agglutinin-absorption tests, conducted between strain LSU 2564, *L. bataviae*, *L. paidjan*, and *L. djatzi*, showed that strain LSU 2564 is homologous with *L. paidjan* and heterologous to *L. bataviae* and *L. djatzi* (table 2). The results of agglutinin-absorption tests between the five remaining strains and *L. paidjan* show their homologous relationship (table 3).

No antibodies were detected at a dilution of 1:25 in the sera of the 26 nutria when tested against all the leptospiral serotypes, except *L. djatzi* and *L. paidjan*, listed under “Methods.” When tested in retrospect against *L. paidjan*, three sera of the six nutria from which *L.*

paidjan was isolated were negative at 1:25, whereas three sera were positive at a dilution of 1:25.

Seven of the 18 sera obtained from nutria culturally negative for *L. paidjan* possessed antibodies only against *L. paidjan*; 4 were positive at 1:25, and 3 were positive at a dilution of 1:100. Sera of the remaining two nutria from which leptospire other than *L. paidjan* were isolated have not been submitted to complete serologic analysis.

Discussion

Only serologic evidence of a member of the *bataviae* serogroup has previously been reported in the United States. Gochenour and associates (17) reported the finding of antibodies against *L. bataviae* in the serum of a trapper from Louisiana. In view of the high cross-reactions between *L. paidjan* and *L. bataviae*, the infecting serotype could well have been *L. paidjan*.

From these limited observations it appears premature to evaluate the importance of *L. paidjan* in the overall picture of leptospirosis in the United States. Nutria were introduced into Louisiana from Argentina about 20 years ago. They have become widespread in Louisiana, and they have migrated to other States as well. Furthermore, their distribution has been enhanced by the sale of domesticated stock for fur production. However, their value as a fur-

Table 3. Results of agglutinin-absorption tests between *L. paidjan* and strains LSU 2569, LSU 2577, LSU 2578, LSU 2588, and LSU 2590

Antileptospiral serum	Absorbed with—	Reciprocal of titer against antigen ^{1 2}			
		Homologous strain		Absorbing strain	
		Before	After	Before	After
<i>L. paidjan</i>	<i>L. paidjan</i>	30,000	1,000	30,000	Negative. ³
	LSU 2569	30,000	1,000	10,000	Do.
	LSU 2577	30,000	1,000	10,000	Do.
	LSU 2578	30,000	1,000	30,000	Do.
	LSU 2588	30,000	1,000	10,000	Do.
	LSU 2590	30,000	1,000	10,000	Do.
LSU 2569	LSU 2569	10,000	Negative	10,000	Do.
LSU 2577	<i>L. paidjan</i>	10,000	300	10,000	Do.
	LSU 2577	10,000	Negative	10,000	Do.
LSU 2578	<i>L. paidjan</i>	10,000	do	10,000	Do.
	LSU 2578	30,000	do	30,000	Do.
LSU 2588	<i>L. paidjan</i>	30,000	300	30,000	Do.
	LSU 2588	30,000	Negative	30,000	Do.
LSU 2590	<i>L. paidjan</i>	30,000	300	30,000	Do.
	LSU 2590	10,000	Negative	10,000	Do.
	<i>L. paidjan</i>	10,000	300	30,000	Do.

¹ Living antigen was used.

² Positive—50 percent agglutination or “lysis” or both.

³ Negative—less than 50 percent agglutination or “lysis” or both at 1:300 dilution.

producing animal has never reached expectations. Instead, nutria cause considerable destruction to agricultural crops in Louisiana.

Summary

Isolation of *Leptospira paidjan* from nutria, *Myocastor coypus*, in Louisiana established the first bacteriological proof of the presence of a member of the bataviae serogroup in the United States.

Six strains of leptospires, recovered from 26 nutria, were isolated in both Fletcher's and Stuart's semisolid mediums. Four of these strains were also isolated by direct inoculation of Cox's solid medium with kidney suspensions. All strains adapted to Stuart's liquid medium easily, which facilitated antigen production.

Cross-agglutination studies of the six strains revealed an antigenic relationship with *L. bataviae*, *L. paidjan*, and *L. djatzi*. Agglutinin-adsorption studies showed that they were homologous with *L. paidjan*, but heterologous with *L. bataviae* and *L. djatzi*.

Serums of the nutria from which *L. paidjan* was isolated did not agglutinate antigen prepared from *L. bataviae*, and only three of the

six serums agglutinated antigen prepared from *L. paidjan*.

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Day Care Services

A report of the National Conference on Day Care for Children, held November 17-18, 1960, by the Children's Bureau of the Department of Health, Education, and Welfare and the Women's Bureau of the Department of Labor, has been published under the title, "Day Care Services, Form and Substance." The conference was attended by 400 representatives of volunteer women's associations, labor, management, national social agencies, and health and welfare groups.

In announcing publication of the report, the conference sponsors stated that according to a recent study at least 400,000 children in the United States under 12 years old are without any kind of care while their mothers are at work. One conference speaker described the changes in the working patterns of women that are responsible in great part for the increased need for day care. According to Department of Labor statistics, the number of married women in the labor force who have children under 6 years old increased from 1¼ million in 1948 to 2½ million in 1959. Working women with children 6 to 17 years old increased from 2 to 4 million during the same period.

Besides the children whose mothers must be away at work, many other children need day care because of behavior problems, physical handicaps, or illness in the home or because

the home is overcrowded and the mother constantly overworked.

The National Conference on Day Care for Children recommended that:

- Day care services be an integral part of the child welfare services in every community and that these services be provided for all children who need them from infancy to adolescence.
- A comprehensive day care program should include a variety of services: family day care homes, day care centers, counseling services.
- Communities make a special effort to develop family day care services to meet the needs of children under 3 years old and of those older children who cannot adjust to group care.
- Emphasis be placed on the development of more adequate licensing and consultation services, including establishment of goals, guides on minimum standards, special training of licensing personnel, and recruitment.
- A concentrated effort be made to obtain local, State, and Federal funds for establishing a broad range of day care services of good quality in every community.

Copies of "Day Care Services, Form and Substance" may be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington 25, D.C., for 25 cents each.

100th Anniversary in Sacramento

March 6, 1962, was the 100th anniversary of the establishment of the Sacramento Board of Health in Sacramento, Calif.

The first major attempts to form a board of health in Sacramento were made in 1850 in the wake of a great cholera epidemic. During the epidemic, 188 deaths were recorded in 1 week, and four-fifths of the population fled the city. Of about 50 physicians in the city, 17 died of cholera. Moreover, a high incidence of typhoid fever, dysentery, smallpox, and tuberculosis plagued Sacramento. Nevertheless, despite the urgent pleas of physicians, the board of supervisors decided in November 1850 that a board of health was not necessary because (a) the cholera epidemic was over and (b) further taxation was unadvisable at that time.

In the meantime two voluntary groups, the fraternity of the Odd Fellows and the Howard Benevolent Association, were attempting to provide for the medical wants of the needy. The State legislature provided funds to the Howard group on several occasions in sums of \$2,000 to \$6,000, perhaps the first public monies appropriated for medical care of the needy in the county except for funds provided for building and operating a pesthouse.

Demands for action by various groups could not overcome such reluctance until a smallpox epidemic developed in the midst of great floods during the winter of 1861-62. Seventy-one smallpox cases were reported and two deaths were recorded. In March 1862 the board of supervisors at last instructed five physicians, including men who had helped organize the Boston Board of Health, to form a board of health in Sacramento.

After their first meeting, the board requested and received authority to post signs on houses that contained smallpox patients, and two members of the board proceeded with this task. Many people, however, removed the signs from their homes soon after they were posted. Although one board member favored a "mild conciliatory course" in dealing with such persons, another member "was adamant, swore to complaints, and sought the serving of warrants of arrest." He was opposed to "succumbing to the prejudices, or yielding to the whim or caprices of the people in a matter of such vital importance—he

wanted the Ordinance enforced when it became necessary to do so in his judgment." On this stand were built the most effective health laws of the city and county of Sacramento and the State of California.

Besides smallpox, the new board's problems included typhoid fever, malaria, cholera, tuberculosis, and wretched sanitary conditions. The floods set the stage for many health problems: "The waters, which swept with destructive violence over the city, were still standing upon more than half its surface; stagnant pools, the receptacle of filth and debris of a superficial drainage, lay exposed in the heart of the city; and depositions of decaying animal and vegetable matter, steaming under the influence of the warm sun, diffused their exhalations about them. . . . If ever the recognized conditions necessary to the production and propagation of diseases were present, they were thought to exist here."

After such inauspicious beginnings, the public health program of Sacramento County has developed rapidly over the years to keep pace with a growing and changing community. Whereas in the 1860's members of the Howard association made home visits to the sick, today public health nurses and visiting nurses provide professional services. Then, doctors had the sole responsibility for educating the public about health and urging people to improve sanitary conditions; today various units of the health department take on these tasks. The first budget of the board of health included a minimum of expenses for records and other supplies and probably amounted to less than \$1,000 a year. Today the health department operates on a million-dollar budget and employs about 140 people, including representatives of at least 16 professions. The department's program includes a wide variety of activities in disease prevention, environmental health, education, laboratory testing, and other fields.

To improve the historical record of health conditions and medical efforts before 1900 in the Sacramento area, a search is being conducted for publications, pictures, instruments, and other materials that have historical interest.—D. J. HANSEN, M.P.H., chief, health education services, Sacramento County Public Health Department, Sacramento, Calif.