

LEPTOSPIROSIS IN THE NON-HUMAN PRIMATE MODEL :
CHEMOPROPHYLAXIS AND EARLY DIAGNOSIS OF
INFECTION (1 OCT. '82 - 30 SEPT. '83)

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OBJECTIVES :

1. To characterize clinical leptospirosis in the non-human primate model.
2. To determine the efficacy of antibiotic treatment as a prophylaxis for the acute infection.
3. To determine if an ELISA method for detecting leptospira antibody may be useful for obtaining rapid early diagnosis of leptospirosis.

BACKGROUND : Leptospirosis is a common zoonotic disease found throughout the world. The clinical features in man range from an influenza-like illness to a more severe disease form manifested by continued fever with meningitic symptoms and signs (1,2). In some cases infection can lead to renal and hepatic failure, jaundice, and even death (1,3). Leptospirosis is frequently found in the tropical areas of the world (4,5) and recent attention has focused on several outbreaks in soldiers training in jungle areas (6). One of these incidents resulted in the hospitalization of 25 of 490 men on a jungle training exercise; an additional 10 men were ill with a "flu-like" illness but not hospitalized. Symptomatic treatment and antibiotic therapy is used in the acute illness. However, once symptoms are evident the beneficial effect of antibiotics is questionable. In a study of an outbreak of leptospirosis where patients were treated with penicillin within 48 hours of diagnosis, they were not able to return to work for 2-3 weeks after treatment began (2). The potential morbidity in the jungle outbreak and the relatively long recovery period, even with treatment, suggest that prevention is the practical approach in solving the problem of leptospirosis. It is difficult to prevent direct contact with leptospira contaminated water in a tropical environment, especially during military maneuvers. Immunization against specific serovars of leptospira can protect animals but immunization of man is not practical unless the serovar endemic to the area is identified or a vaccine with broad antiserovar activity is developed. Antibiotic prophylaxis may be a useful means of protection for troops in an endemic area. Doxycycline is presently being tested for its prophylactic benefit effect on scrub typhus and malaria. This antibiotic will be tested in primates as a prophylaxis for acute leptospirosis infection. The serological incidence for natural leptospira infection in old world non-human primates is low. There are few studies of experimental leptospirosis in non-human primates. One review article reported

attempts to infect *Macaca sp* resulted in mild febrile response with thirst and vomiting (7). One *M. sinensis*, infected with a massive amount of infected tissue developed a typical clinical case of leptospirosis with icterus (7).

M. mulatta (Rhesus) and *M. fascicularis* (Cynomolgus) monkeys were screened for antibody to 21 different serovars of leptospira including *L. bataviae*, the most common human isolate from our laboratory. Seronegative monkeys were infected ip with 10^7 organisms of the *bataviae* strain. Last year we reported that the experimental infection of monkeys with a local human isolate of the *bataviae* serovar produced a bacteremia of 1 to 6 days, a variable infection of the CSF, and a bacteruria for up to 4 weeks. An antibody response was detected by microagglutination by 1 week and peak titers were reached by 3-4 weeks.

MATERIALS & METHODS : Adult male (3.5-5.5 kg) rhesus monkeys were used in this study. Monkeys were negative for leptospira antibody (21 serovars) prior to ip infection with 10^7 or 10^8 organisms of the *bataviae* serovar (LC0475). Antibody titers were determined using the microagglutination method. On days specified for each experiment 2-3 drops of each specimen was placed in a culture tube containing 5 cc of modified EMJH media incubated at 30°C, and observed for 6 weeks for growth. A doxycycline suspension in water was given via nasogastric tube to monkeys as a single dose or as a daily dose for 10 days. The single dose was based upon a 200 mg dose for man adjusted for body surface and the daily doses were equivalent to a 100 mg human dose. Sham treated controls were given only the water diluent. The *in vitro* inhibitory effect of 4 antibiotics and *bataviae* serovar isolates were determined by addition of specified concentrations of an antibiotic to tubes containing 10^3 organisms and observed at 4 weeks for growth. Two monkeys were given a single oral dose of doxycycline and serum was collected at specific intervals to determine if leptospira inhibitory effects of this antibiotic were detectable in the blood. For ELISA testing, 7 antigens and monkey immune serum to each has been obtained. A solid phase immunoglobulin in capture test is being developed to find an antigen or group of antigens that can detect a variety of serovar antibodies.

RESULTS : The effect of doxycycline prophylaxis for acute leptospirosis infection with the *bataviae* serovar was studied. The inhibitory effect of different antibiotics on the growth of leptospira was first tested *in vitro* (Table 1). Doxycycline was more effective than minocycline, tetracycline, or dicloxacillin against three isolates of *L. bataviae*. Doxycycline also completely inhibited growth of another rodent isolate, the *javanica* serovar, at 0.25 µg/ml (data not shown). The LC 0475 isolate of the *bataviae* serovar from a patient with leptospirosis was chosen for most experiments because it was the most resistant isolate tested. It was more difficult to determine the inhibitory concentration level of doxycycline in monkeys given a single oral dose. Because serum alone has an inhibitory effect on leptospira growth in culture, samples were diluted in a 2 fold series in media. By this manner, the dilution where the first growth of leptospira occurred in the 0 hr (pre-treatment) sample was compared to samples taken at later times and similarly diluted (Table 2). After an oral dose of doxycycline (5 mg/kg), inhibition of leptospira growth was detected in the serum as long as 72 hrs after treatment.

Monkeys were infected with 10^8 leptospira and treated daily with a single oral dose of doxycycline based on body surface to an equivalent dose of 2-3 mg/kg for man. When compared to sham treated control monkeys, the days of leptospiremia were reduced. Infection of the CSF and kidney that occurred in the control monkeys was not detected in any given prophylactic doxycycline treatment for 10 days. Eight other monkeys were treated only on the day of infection with doxycycline or water (Table 4). This single day doxycycline treatment prevented CSF and renal infection in 3 of 4 monkeys while all sham treatment controls had renal or renal and CSF infection. This experiment was repeated in 8 more monkeys with the same results.

An IgM capture ELISA was developed for detecting antibody to the bataviae serovar in the monkey and man. Presently specific antibody to 7 different serovars is being produced in monkeys. These sera will be tested to find a single antigen or antigen combination that is effective for detecting antibody to a variety of serovars.

FUTURE OBJECTIVES :

1. Further develop and refine the ELISA to detect low antibody titers to leptospira.

2. Test sera from monkeys immunized with different serovar antigens by the ELISA method to find an antigen or combination of antigens that will detect the variety serovar infections that are present in Thailand.

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Table 1. Concentration of antibiotic ($\mu\text{g/ml}$) required to inhibit leptospira growth in EMJH media

	<i>bataviae</i> serovar isolate		
	LC 0475	LC 1443	LC 0118
Antibiotic Minocycline	4.00 ^a	4.00	4.00
Doxycycline ^c	4.00; NE ^b	0.25; 0.50	4.00
Tetracycline	NE	NE	0.50
Dicloxacillin	NE	NE	NE

^a Concentration ($\mu\text{g/ml}$) of antibiotic at which no growth of leptospira occurred in culture.

^b No effect at concentrations up to 4 $\mu\text{g/ml}$.

^c Inhibitory concentrations obtained from two experiments using doxycycline.

Table 2. Inhibition of leptospira by serum from monkeys given oral doxycycline.

Monkey #	Serum Dilution	Time (hrs) serum taken after doxycycline					
		0	4	8	24	48	72
11103	1:2	0 ^a	0	0	0	0	0
	1:4	++ ^b	0	0	0	0	0
	1:8	+++	0	+	+++	++	+++
	1:16	+++	++	++	+++	++	+++
	1:256	+++	+++	+++	+++	+++	+++
11013	1:2	0	0	0	0	0	0
	1:4	0	0	0	0	0	0
	1:8	+++	0	+++	+	+++	0
	1:16	+++	0	+++	+++	+++	+++
	1:256	++++	++++	++++	++++	++++	++++

a 0 = no growth

b +, ++, +++, +++++; 25, 50, 75, 100% respectively of leptospira concentration observed in EMJH media without addition of serum or doxycycline.

Table 3. Days of isolation of leptospira from blood, CSF and urine of infected monkeys.

Treatment ^a	Blood ^b	CSF ^c	Urine ^b
Sham			
18126	1,2,3,4,5,6	7,14	5,6,9,10,14,21,28
H-17	1,2,3,4,5,6	-	5,8,9,10,21,28,56
G-183	1,2,3	-	28
G-425	-	21	-
Doxycycline			
B-299	1,2	-	-
H-162	1,2	-	-
H-163	-	-	-
H-168	1	-	-

^a Single oral dose daily : -1 to +9 days. Infection with 1.2×10^8 *L. bataviae*. Sham treatment with water diluent.

^b Cultured daily from 0 - 10 days, 14, 21, 28, 56.

^c Cultured on days 0, 4, 7, 10, 12, 14, 21, 28, 56.

Table 4. Days of Isolation of *Leptospira* from blood, CSF, and urine of infected monkeys.

Treatment ^a	Blood ^b	CSF ^c	Urine ^b
Sham			
11105	1,2,3	-	7,12,14,18,21,28,35
11093	1,2,3	18	7,10,14,18,21,28,35
11139	1,5	12,14	10,12,14,18,21,28,35
H-129	1,2,3	-	14,18,21,28
Doxycycline			
11103	-	-	-
B-300	-	-	-
B-432	1,2,3	-	4
B-612	1	-	-

^a Single oral dose given 2 hours before ip infection with 1.1×10^7 *L. bataviae*.

^b Cultured daily from 0 - 8 days, 10, 12, 14, 18, 21, 28, 35, 42, 49, 56.

^c Cultured days 0, 4, 7, 10, 12, 14, 18, 21, 28, 35, 42, 49, 56.