

LEPTOSPIROSIS IN THE HAMSTER MODEL AND CHEMOPROPHYLAXIS
IN THE ACUTE INFECTION (1 OCT. '82 - 30 SEPT. '83)

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OBJECTIVES :

1. To characterize the acute *Leptospira bataviae* infection in the hamster model as to virulence and infectivity of the organism.
2. To determine the effect of doxycycline prophylactic treatment in experimental infection.
3. To obtain serovar specific antisera for developing as ELISA method to detect the acute infection.

BACKGROUND : Leptospirosis is a worldwide zoonotic illness that is common in tropical areas and recently has been a cause of outbreaks of acute "flu-like" illness in troops in jungle training exercise (1). Presently the nonhuman primate is being studied in our laboratory as a model for acute leptospirosis and chemoprophylaxis. The hamster model for acute leptospirosis has been previously reported (2,3). A clinical, often fatal illness results in hamsters infected with some leptospira serovars (4). This animal model will be a useful addition to the primate model for testing potential prophylactic drug treatment for leptospirosis. Also the hamster is an ideal laboratory animal for isolating strains of leptospira from samples contaminated with other bacterial or fungal organisms (5). Proposed studies on the epidemiology of leptospirosis will necessitate the use of hamsters and familiarity with this model for isolation of leptospira from infected water sources.

MATERIALS & METHODS : Leptospira isolate of the bataviae serovar were used to infect hamsters. These isolates were cultured from the blood of febrile patients at Children's Hospital. Golden hamsters (LVG Outbred, Charles Rivers) were used in all experiments. An intraperitoneal infectious dose of 10^5 leptospira organisms was used. Doxycycline was given orally at 28.8 mg/kg for the 1st day and 14.4 mg/kg on subsequent days. These are based on body surface for equivalent doses of 200 mg and 100 mg for man. Cultures were done on ground kidney (in saline) using modified EMJH media and were observed for growth for up to 6 weeks. Antibody titers to bataviae were determined using a microagglutination test.

RESULTS : Several leptospira isolates from human infections in Thailand have been found to be virulent in hamsters up to 40 grams. In hamsters of over 50 grams, death from acute infection does not always occur. However, in older hamsters chronic renal infection is generally produced as well as antibody to the infecting serovar. A titration of a local bataviae isolate in hamsters is

shown in Table 1. Intraperitoneal injection (0.2 cc) of dilutions a stock inoculum containing 10^7 organisms/ml caused death and/or renal infection in hamsters given even the 10^0 dilution (1-10 organisms) of the inoculum. Three of six hamsters at this high dilution were infected (positive kidney culture) suggesting that injecting a group size of 4 hamsters should be sufficient to detect the presence of small numbers of leptospires in a water sample. The reduced virulence of the two highest infectious doses is attributed to an enhanced immune response to injection of excess soluble or particulate antigen present in the media of the less diluted cultures. In a similar experiment, freeze-thaw killed antigen was injected with an infectious dose of 10^5 organisms; mortality was reduced and survival time increased when compared to hamsters given the same infectious dose without the killed antigen. For this reason the challenge dose was reduced to 10^5 organisms for future experiments testing doxycycline prophylaxis in acute leptospirosis. Doxycycline treatment for prophylaxis of leptospirosis in hamsters was tested using different dose schedules. This antibiotic was given as a single oral dose of 28.8 mg/kg; if additional daily doses were given they were reduced to 14.4 mg/kg. This antibiotic produced a fatal enterocolitis in hamsters only at high doses (60-240 mg/kg) but never at 30 mg/kg or less. The effect of single and multiple days of treatment before and after infection are shown in Table 2. A single oral dose of doxycycline near the day of infection prevented deaths in hamsters. However, unless multiple days of treatment were given, a chronic renal infection usually resulted. Treatment beginning as late as 4 days after infection prevented any deaths and reduced the incidence of renal infection if given daily through day 10. Doxycycline was also effective in eliminating established renal infections when hamsters were treated daily for 1 week with an oral dose of 14.4 mg/kg (Table 3). A single experiment to detect antigenemia in acute infection by an ELISA technique was not successful. Blood from hamsters infected on day 0 was tested on 4 consecutive days when a leptospiremia was detected by culture in 20/25 samples. ELISA test results were not different between bacteremic and noninfected hamsters.

FUTURE OBJECTIVES :

1. Serum from hamsters immunized with different strains will be tested using an ELISA method for the diagnosis of leptospirosis. An antigen of broad cross reactivity for a many serovars will be sought to use in this test.
2. Hamsters will be used for checking water from field locations for leptospira infection.

Table 1. Virulence and infectivity of the bataviae serovar for 40g hamsters.

Dilution of Inoculum ^a	Deaths ^b		<u>Kidney cultures</u> ^c		Titers of Survivors ^d
			dead	survivors	
10 ⁰	3/6	(6.0 ± 0.0)	2*/3	3/3	2048, 2048, 8192
10 ¹	3/6	(9.3 ± 0.6)	2*/3	3/3	512, 512, 2048
10 ²	6/6	(14.3 ± 4.1)	6/6	NA ^e	NA
10 ³	5/6	(11.0 ± 1.2)	3**/5	1/1	512
10 ⁴	3/6	(11.0 ± 1.0)	3/3	1/3	0, 0, 1024
10 ⁵	4/6	(11.3 ± 0.5)	4/4	0/2	0, 0
10 ⁶	1/6	(15.0)	0*/1	2/5	0, 0, 0, 512, 2048
10 ⁷	0/6	NA	NA	0/6	All negative
10 ⁸	0/6	NA	NA	0/6	All negative

^a All dilutions through 10⁶ were positive on culture by 4 weeks; 10⁷ and 10⁸ were negative.

^b Number of deaths over the number injected. (Mean day of death ± LSD).

^c Number with positive cultures over number tested. Each* indicates a contaminated culture in group.

^d Reciprocal titers 4 weeks after infection.

^e Not applicable

Table 2. Effect of doxycycline prophylaxis on acute leptospira infection of hamsters.

Days of Treatment ^a	Infection strain	Deaths	Chronic Renal infection
None	<i>bataviae</i>	9/10	1/1
-1	<i>bataviae</i>	0/6	6/6
-1, 0	<i>bataviae</i>	0/6	6/6
-1 to 1	<i>bataviae</i>	0/6	0/6
-1 to +2	<i>bataviae</i>	0/6	0/6
-1 to +4	<i>bataviae</i>	0/6	0/6
-1 to +6	<i>bataviae</i>	0/6	0/6
-1, +6, +13	<i>bataviae</i>	0/6	3/6
-1 to +6	none	0/6	0/6
none	none	0/6	0/6
+4 to +10	<i>bataviae</i>	0/6	3/6

^a Single oral dose of 28.8 mg/kg followed by daily single dose of 14.4 mg/kg.

Table 3. Effect of doxycycline treatment on chronic renal infection of hamsters.

Infection serovar (isolate)	Treatment ^a	Kidney Culture ^b
<i>bataviae</i> (LC0475)	none	3/3
<i>bataviae</i> (LC0475)	doxycycline	0/3
<i>bataviae</i> (LC0118)	none	2/2
<i>bataviae</i> (LC0118)	doxycycline	0/2
none	none	0/2
none	doxycycline	0/3

^a Doxycycline 14.4 mg/kg given daily from day 29-35 after infection.

^b On day 42 post infection.

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