

LEPTOSPIROSIS IN THE NON-HUMAN PRIMATE MODEL :
CHEMOPROPHYLAXIS AND EARLY DIAGNOSIS OF
INFECTION (1 OCT. '81 - 30 SEPT. '82)

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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 disease prophylaxis for the acute infection.
3. To determine if an ELISA method for detecting leptospira antibody or antigenemia is a useful means 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 antisero var 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

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. Temperature was measured, blood cultures, and CBC's, and observation for signs of illness were done daily. In addition, CSF and urine samples were cultured at less frequent intervals. Cultures were done in EMJH media and observed by dark phase microscopy weekly. Cultures were negative if no growth was observed after six weeks. Serum and CSF samples were also obtained for antibody measurement and the detection of antigen by the ELISA method.

RESULTS : In the pilot study, four rhesus and four cynomolgus all developed a bacteremia during a period of one to seven days after infection (Table 1). One other cynomolgus monkey which was not included in this table, did not develop a bacteremia after infection. Although no positive blood cultures were obtained after day 7, positive CSF cultures were found on days 8, 12, and 14. Positive urine cultures were found in monkeys as early as the first week but more commonly in samples taken three and four weeks after infection (data not shown). No obvious signs of illness (anorexia, weakness, etc.) were seen although a mild fever ($1-2^{\circ}\text{F}$) was present 2 to 5 days after infection. Serum antibody was always detectable by microscopic agglutination testing one week after infection and titers remained positive throughout the 8 week period serum samples were tested (Table 2). CSF samples were negative for leptospira antibody for the first 8 days although low titers (1:2-1:32) were found in some monkeys 10 or more days after infection. There was no difference in CSF titers from the monkeys with positive and negative CSF cultures.

Experiments are now in progress to determine the effect of doxycycline prophylaxis on preventing bacteremia and bacteruria in the primate model. Because all rhesus monkeys developed a bacteremia after infection, this species was chosen for doxycycline studies. Doxycycline will be given at a human dose equivalent for monkeys based upon body surface area. Samples of CSF, serum and urine are being collected and stored for future testing for antigenemia or antigenuria by the ELISA method as a means for early diagnosis of acute infection.

Table 1. Blood and cerebrospinal fluid (CSF) cultures of monkeys infected with *Leptospira bataviae*.

		Days Post Infection												
		0	1	2	3	4	5	6	7	8	10	14	21	28
Cynomolgus	blood	0/4 ^a	3/4	3/4	4/4	3/4	2/4	1/4	0/4	0/4	0/4	0/4	0/4	0/3
	CSF	0/4	ND ^b	ND	ND	0/2	ND	ND	ND	0/4	1/4	0/4	0/3	0/3
Rhesus	blood	0/4	2/4	3/4	4/4	4/4	1/4	1/4	1/4	0/4	0/4	1/4	0/4	0/2
	CSF	0/4	ND	ND	ND	0/4	ND	ND	ND	1/4	0/4	1/4	0/4	0/2

^a Number positive/number cultured.

^b Not done.

Table 2. MAG titers to *Leptospira bataviae* in cynomolgus and rhesus monkeys.

	DAY POST INFECTION						
	0	4	8	14	21	28	56
<u>MONKEYS :</u>							
Cynomolgus							
AF-1	0	0	512	2048	1024	1024	1024
AF-18	0	8	512	2048	2048	2048	1024
AF-20	0	2	32	512	1024	2048	1024
Rhesus							
H-87	0	16	1024	2048	2048	2048	2048
H-115	0	2	16	512	512	512	512
H-2	0	0	128	ND	1024	ND	1024
G-346	0	0	64	512	256	256	2048

ND = not done

REFERENCES :

1. Faine, S. : Leptospirosis-Here, now. Pathology 13:1, 1981.
2. Christmas, B.W., Tennant, R.B., and Lindsay, P. Dairy farm fever in New Zealand. N.Z. Med. Jour. 79:901, 1974.
3. Heath, C.W., Alexander, A.D., and Galton, M.M. Leptospirosis in the United States. NEJM. 273:915, 1965.
4. Brown, G.W., Lee, D.L., Huxsoll, T.S. *et al.* Leptospirosis in Malaysia. Southeast Asian J. Trop. Med. Pub. Hlth. 7:200, 1976.
5. Berman, S.J., Tsai, C., Holmes, K., *et al.* Sporadic anicteric leptospirosis in South Vietnam. Ann. Int. Med. 79:167, 1973.
6. Anonymous, Army Fact Sheet, Leptospirosis, 24 Nov. 81 and 29 Dec. 81.
7. Minette, H.P., Leptospirosis in primates other than man. Am. J. Trop. Med. Hyg. 15:190, 1966.