

USE OF DOXYCYCLINE FOR LEPTOSPIROSIS AFTER HIGH-RISK EXPOSURE IN SÃO PAULO, BRAZIL

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SUMMARY

A clinical trial pilot study, double-blinded, randomized, and controlled with a placebo to assess the effectiveness of oral doxycycline (200 mg, single dose) in preventing leptospirosis after high exposure to potentially contaminated water was performed in São Paulo, SP, Brazil. Confirmed cases were defined as those with leptospira IgM antibody and symptoms; asymptomatic cases were those presenting with IgM antibodies but no symptoms; and suspected cases were individuals with symptoms but no IgM antibody. Forty subjects were given doxycycline and 42 were given placebo. In the drug-treated group there were 2 confirmed cases, 11 asymptomatic cases, and 6 suspected cases. In the placebo group there were 5 confirmed, 6 symptomatic, and 5 suspected cases. Even though we found a protective association of doxycycline for confirmed leptospirosis cases (RR = 2.3) and seroconversion only (RR = 2.0), the association was not statistically significant because of the small number of individuals enrolled in this pilot study. We observed that the 22% of the volunteers already had IgM antibodies to leptospirosis at the first sampling. Finally, the attack rate to confirmed, asymptomatic, and suspected cases of Leptospirosis was 8.5%, 22%, and 13%, respectively, in this population.

KEYWORDS: Leptospirosis control; São Paulo; Brazil; Doxycycline; Incidence.

INTRODUCTION

Leptospirosis is a spirochetal zoonosis that may cause a wide spectrum of clinical manifestations in humans. The principal syndromes include subclinical infection, self-limited anicteric febrile illness and a severe illness known as Weil's syndrome².

The causative agent, *Leptospira interrogans*, is distributed worldwide but is more common in wetter climates. *Leptospira* infects rats and other rodents which excrete the pathogen by urination. The organisms can survive in water for long periods of time, such as 15 days. Pathogenic leptospires rapidly invade the human bloodstream after penetrating skin or mucous membranes. The incubation period in humans ranges from two to twenty-six days, with an average of ten days³, and the attack rates may range from 2 to 8 percent⁵.

TAKAUFUGI et al. demonstrated unequivocally that doxycycline is both an effective and acceptable prophylaxis for leptospirosis before exposure, and suggested that it might also be effective therapeutically⁸.

The Surveillance Service at São Paulo City reported an incidence rate of leptospirosis of 1.56 to 4/100,000 people from

1991 to 1994 in some areas of the city (KATZ, 1995, CVE São Paulo, personal communication). The main route of transmission was due to flooding of homes during the summer rainy season when the rainwater catchment system is insufficient. In this study, we attempted to determine the usefulness of doxycycline for conferring protection against leptospirosis in an endemic area in São Paulo, Brazil after high-risk exposure.

MATERIAL AND METHODS

Study population

Residents of a small community in Cabuçu District São Paulo region (approximately 1500 habitants) who lived in an area at high risk for flooding were invited to participate. Two visits prior to enrollment were used to explain about leptospirosis transmission routes, how to avoid infection, and they were aware to look for the investigators after the flooding in the pre-determined public space. Inclusion criteria for the study: older than 18-years and no use of antibiotics before and during the study. Subjects with allergy to tetracycline or a high-exposure risk to leptospirosis in the last 6 months were excluded. Informed consent was obtained from all participants. Until 48 hours of exposure of the area to potentially contaminated water from a flood on March 29, 1992, the

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researchers went to the study site and collected 10 ml of peripheral venous blood from the subjects, and a similar collection occurred 45 days later. On both occasions house-to-house search were done to advice the community. During the first bleed, 200 mg of doxycycline or a placebo was administrated orally to each individual, using randomization criteria.

Study Design

Clinical trial, double-blinded, randomized and controlled with a placebo. The Fisher's Exact Test and relative risks with 95% confidence intervals with approximation of Woolf were calculated to compare treatment and placebo groups with regard to the effectiveness.

Enzyme immunoassay (EIA)

IgM antibodies to leptospirosis were measured by EIA employing as antigen a pool of leptospira (*L. canicola*, *L. cynopteri*, *L. hebdomadis*, *L. brasiliensis*, *L. icterohaemorrhagiae*) obtained from the same population as previously described¹. Briefly, the volunteers' sera were diluted 1:100 and incubated at 37°C for one hour with the antigen. Five washes with PBS were performed and 100 ul of anti-human IgM-peroxidase conjugate diluted 1:16,000 in PBS was added and incubated for one hour at 37°C. After washing, 100 ul of chromagen solution was added and the reaction was stopped with addition of 100 ul of 2 N H₂SO₄ solution. Samples giving optical density above 0.470 at 409 nm, were considered positive. Parallel tests using PBS instead of antigen were carried out as a reaction control.

Case definition: a confirmed case of leptospirosis presented with Weil's syndrome or anicteric leptospirosis with at least 3 of the following symptoms: fever, chills, myalgia, nausea, vomiting, abdominal pain, conjunctivitis and headache accompanied by neck stiffness in the period immediately after exposure to the flooding^{4,5,6,8} and demonstration of IgM antibody to leptospirosis.

Subjects presenting with symptoms (at least three) but negative serology were considered suspect cases; individuals presenting with IgM antibodies to leptospira and absence of symptoms were considered asymptomatic cases.

RESULTS

106 subjects were enrolled and eighty-two volunteers of them qualified for analysis. The age ranged from 18 to 74 years old (mean 39 years) and thirty-four (41.4%) were men.

The results as summarized in Figure 1 showed that 24 volunteers possessed IgM antibodies for leptospirosis, 18 at the first bleeding and 6 cases more at the second bleeding. Two of these in the doxycycline group and 5 in the placebo group were also symptomatic and were considered confirmed cases (RR = 2.3; CI = 0.4-11.5). Seventeen were asymptomatic but demonstrated IgM antibody (11 in doxycycline group and 6 in placebo group). Eleven subjects had clinical symptoms compatible with leptospirosis but were serologically negative and were considered suspected cases of leptospirosis (6 were from the doxycycline group and 5 from the placebo group). Six subjects presented seroconversion during the study, 4 from placebo group and 2 from doxycycline one (RR = 2.0; CI = 0.3-11.5). One individual case from the placebo group presented the Weil's Syndrome. Myalgia was the most common symptom observed either during the first sampling as well in the second bleeding.

DISCUSSION

Leptospirosis is a waterborne disease that occurs as two clinically recognizable syndromes. The most common syndrome is anicteric leptospirosis, a self-limited systemic illness that occurs in 85%-90% of the cases. However, Weil's syndrome, which may occur in 5%-10% of all cases, is a more serious,

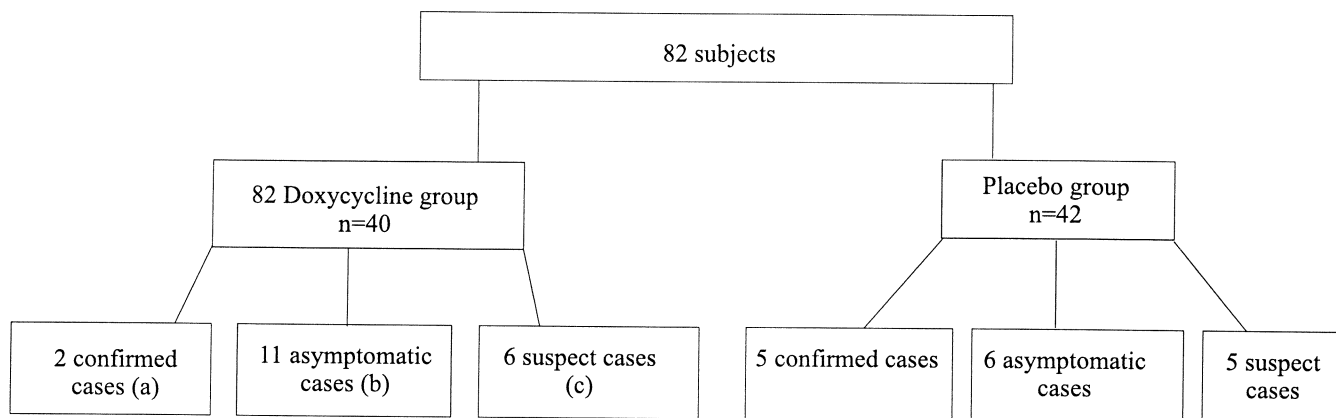


Fig. 1 - Evaluation of doxycycline for preventing leptospirosis among individuals after high risk exposure in São Paulo, Brazil.

a) Confirmed cases: Individuals with Weil's syndrome or anicteric leptospirosis with at least 3 of the following symptoms: fever, chills, myalgia, nausea, vomiting, abdominal pain, conjunctivitis or headache accompanied by neck stiffness in the period immediately after a flood and IgM antibodies to leptospira.

b) Asymptomatic cases: Individuals presenting IgM antibodies to leptospira and absence of symptoms.

c) Suspected cases: Individuals with symptoms (at least three) but negative serology.

Note: 2 seroconversion cases in doxycycline group among the asymptomatic cases and 4 seroconversion cases in placebo group among the asymptomatic cases.

usually fatal illness that presents as hemorrhage, renal failure, and jaundice^{2,3}.

We found that 24 of 82 (29%) subjects possessed anti-leptospira IgM antibodies following the exposure. The attack rate of confirmed cases was 8.5% overall and JACKSON et al. found similar rates in an outbreak associated with swimming⁴. However, both rates were 3-4 fold higher than observed in another study⁵.

We found protective association of doxycycline for confirmed cases of leptospirosis (RR = 2.3) and for seroconversion (RR = 2.0). However, neither association was statistically significant, and this study did not have statistical power to give more precise estimates of the magnitude of the potential protective associations.

MCLAIN, et al.⁶ demonstrated efficacy of doxycycline in reducing the duration of leptospiruria as well as symptoms related to leptospiremia phase, such as fever, malaise, headache and myalgia. The possible mechanism of doxycycline in preventing leptospirosis may be the ability to destroy the organisms soon after their invasion of the bloodstream, thus decreasing the amount of antigens and leading to a low activation of the immune system during the early immune response process. It seems that the immune response is implicated in the pathogenesis of leptospirosis, such as immune complex formation, release of cytokines, and autoimmune vasculitis, as observed in Weil's syndrome³.

IgM antibodies for leptospira were detected in 18 (22%) individuals at the first sampling. The area studied is considered to be endemic for leptospirosis, and IgM anti-leptospira antibodies have been measured over one year after infection⁷. Although we did exclude volunteers who presented with any high risk exposure within 6 months before of the study enrollment, it is not possible to rule out the possibility of pre-existing IgM antibody in some of the population.

Leptospirosis prevention is directly related to improvement of the social-economic conditions of the population. However, some high risk exposures are unavoidable and use of a drug with high efficacy would be very useful, especially in tropical countries, where leptospirosis is still endemic. Although the number of individuals enrolled in this study was small, this is the first report of a drug evaluation for preventing leptospirosis after exposition but before onset of symptoms.

RESUMO

Uso da doxiaciclina para prevenção de leptospirose após exposição de alto risco em São Paulo, SP, Brasil

Um ensaio clínico, duplo-cego, ao acaso, e controlado com placebo para aferir a eficácia da doxiciclina (200 mg, dose única)

em prevenir leptospirose após exposição de alto risco com água potencialmente contaminada foi realizado em São Paulo, SP, Brasil. Casos confirmados foram definidos como aqueles que apresentavam anticorpos IgM anti-Leptospira e sintomas; casos assintomáticos eram aqueles que apresentavam somente anticorpo IgM, casos suspeitos apresentavam sintomas, porém sem anticorpo IgM. 40 indivíduos tomaram doxiaciclina e 42 tomaram placebo. No grupo tratado houve 2 casos confirmados, 11 assintomáticos e 6 casos suspeitos. No grupo placebo houve 5 casos confirmados, 6 assintomáticos e 5 casos suspeitos. Apesar de haver uma associação protetora da doxiaciclina nos casos confirmados (RR = 2,3) e soroconversão (RR = 2,0), a associação não foi estatisticamente significativa devido ao pequeno número de indivíduos envolvidos no estudo. Foi observado que a taxa de ataque de casos confirmados, casos assintomáticos e casos suspeitos de leptospirose foi 8,5%, 22%, e 13%, nessa população.

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