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Leptospirosis as a cause of Pyrexia of Unknown Origin in Chiang Mai Hospital

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Abstract

Agglutination-lysis test was employed in detecting leptospiral antibodies in 262 patients which had mostly been diagnosed as "pyrexia of unknown origin". Seventy cases or 26.7 % were positive and the predominant serotype was Leptospira wolffii, 30%, secondary was Leptospira icterohaemorrhagiae, 27.1 %. Most of the seropositive patients were in the 16 - 45 age group.

INTRODUCTION

Leptospirosis is one of the most important and cosmopolitan of the zoonoses, diseases transmitted among animals and from animal to man (1,2,3,4,5). It is a major economic and public health problem. For example, abortion in animals due to leptospiral infections is a serious economic problem. The etiologic agent is the organisms in the genus Leptospira. These organisms comprise two major groups, the so-called "saprophytic" and the "pathogenic" leptospires. The "saprophytic" or "water" leptospires are omnipresent in fresh surface waters, and are rarely associated with mammalian infection. Pathogenic leptospires are usually found in a wide veriety of wild and domesticated mammals, as carrier hosts, and cause acute, febrile, systemic disease of man and other animals (6,7,8)

Humans or animals may be infected by direct contact with urine or infected tissue of carriers, or by indirect contact. Water is important in the transmission of leptospires. Contact with water contaminated by carrier's urine is thought to be usual mode of transmission of the disease, the leptosires gaining entrance to the body

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through the alimentary or respiratory tracts or perhaps through abraded skin. General transmission from man to man is very rare, usually from animal to animal and from animal to man (5,9).

The disease in man is found most commonly among farmers, cane cutters, veterinarians and other people particularly exposed because of their occupation (10, 11). The disease exhibits many degrees of severity, varying from symptoms so mild as to be ignored by the patient to serious illness (7, 12, 13, 14, 15, 16). The clinical symptoms alone are not only variable but are non-specific for leptospirosis and definitive diagnosis must be based on demonstrating or isolating **Leptospira** and upon serological findings. Most patients who came to the hospital with fever are usually diagnosed as "pyrexia of unknown origin" (PUO) when a definite diagnosis is not obtained. PUO may be caused by many diseases and leptospirosis is one. So this paper tried to study the incidence of leptospirosis in the patients which had been diagnosed as pyrexia of unknown origin.

MATERIALS AND METHODS

I. Antigens

Twelve Leptospira serotypes originally isolated from human beings and animals in Thailand were used for microscopic agglutination - lysis tests. (Table I.) Stock cultures of the **Leptospira** were obtained rfom The Bangkok Leptospirosis Reference Laboratory, Faculty of Tropical Medicine, Mahidol University.

Stock cultures were maintained in Fletcher's semisolid medium (Difco*) containing 10% rabbit serum, and were incubated at 28-30°C aerobically. Subcultures were performed at three weeks intervals.

Living leptospiral antigens were used in microscopic agglutination - lysis tests. They were grown in liquid Stuart's medium, (Difco*) containing 10% rabbit serum and incubated at 28-30°C for 4-6 days. After incubation, the cultures were examined by dark-field microscopy for density, autoagglutination, and contamination. Only active, smooth, non-clumping cultures were used. Optimal density was 100-200 organisms per high power field, and if they were more concentrated they were diluted with Stuart's liquid medium.

To obtain optimal growth it was necessary to seed with large amounts of materials. For subculturing, nearly 0.5 ml. of the old culture was inoculated into the fresh medium. Rabbit sera were checked for the presence of leptospiral antibodies by the microscopic agglutination-lysis tests. Only seronegative sera were used, because leptospiral antibody may be the cause of poor growth or autoagglutination.

II. Collection of Blood specimens

Blood specimens from the patients were collected and serum seperated from

the clot. All sera were stored at - 10 °C until used.

III. Serological Method

Sera were examined for the presence of leptospiral agglutinins by the microscopic agglutination-lysis tests of Schuffner Mochtar (17), in which living 5 days old culturs were used as antigen. Twelve serotypes were employed.

By this method the sera were diluted 1 : 50 and then three drops of antigens were added to three drops of diluted serum. The final dilution was thus 1:100. Negative controls consisted of three drops of normal saline and three drops of antigen. Serum - antigen mixtures were mixed by rotation and incubated at room temperature for three hours. A loopful of each mixture was examined by low dry dark - field. Illumination without the use of a cover glass. Per cent of agglutination or lysis or both was read by comparing with the negative control. Less than 50% agglutination or lysis was recorded negative; greater than 50% positive.

Reaction at 1:100 dilution or above were considered significant. Sera positive at 1 : 100 dilution were retested at higher dilutions and the extent of antibody titer determined. The titers were expressed as the reciprocal of the highest serum dilution showing at least 50% agglutination or lysis or both of leptospires.

When the titers were the same with two or more serotypes, all were reported. But if the titers were not the same, lower titers were considered as cross rections.

Results

Total 262 patients' sera were tested for leptospiral antibody and 70 or 26.7 % were positive. In these patients 196 cases were diagnosed as "pyrexia of unknown origin" and 47 cases or 23.9 % were positive (Table II). Most of the specimens were single collection; only in 10 cases was serum taken more than one time; and agglutination-lysis titers are shown in Table III.

Age distribution of 70 seropositive patients are shown in Table IV and most of them were in the 16 - 45 age group. Incidence of leptospiral serotypes among 70 seropositive patients are shown in Table V. The predominant serotype was **Leptospira wolffii,** 30% or 21 of 70, and secondarly was **Leptospira** icterohaemorrhagiae, 27.1% or 19 of 70.

Table I. Leptospiral Serotypes used for Agglutination-lysis Tests

GROUP	DEROTYPE	STRAIN
Icterohemorrhagiae	L. icterohemorrhagiae	M 20
Javanica	L. javanica	Veldrat Bataviae 46
Canicola	L. canicola	Hond Utrect IV
Pyrogenes	L. pyrogenes	Salinem
Autumnalis	L. autumnalis	Akiyami A
Australis	L. australis	Ballico
Pomona	L. pomona	Pomona
Grippotyphosa	L. grippotyphosa	Moskva V
Hebdomadis	L. hebdomadis	Hebdomadis
	L. wolffii	3705
Hyos	L. hyos	Mitis Johnson
Bataviae	L. bataviae	Swart

TABLE II

Provisional clinical diagnosis : Hospital patients *

Provisional diagnosis	No. of patients	No. of seropositives **
Pyrexia of unknown origin	196	47 (23.9%)
Leptospirosis	12	7
Jaundice	10	2
Hepatomegaly	2	1
Septicemia	4	2
Enteric fever	3	2
Pneumonia	7	2
Anemia	6	1
Weakness	1	–
Other	21	6
Total	262	70 (26.7%)

* From serological request form.

** Minimal titer 1 : 100

TABLE III

Result of agglutination-lysis titers for the ten cases on which a paired sera were submitted.

Patients	Agglutination - lysis titer at		
	1 st	2 nd	3 rd specimen
1	1000 H	1000 H	-
2	300 I	1000 I	-
3	1000 I	1000 I	-
4	Neg	Neg	300 I
5	1000 B	1000 B	-
6	100 I	100 I	-
7	100 G	30,000 G	-
8	3000 H	10,000 H	30,000 H
9	300 H	300 H	-
10	1000 A	3000 A	1000 A

H = Leptospira hebdomadis

I = Leptospira icterohemorrhagiae

B = Leptospira bataviae

G = Leptospira grippotyphosa

A = Leptospira akiyami A

TABLE IV

Age distribution in 70 seropositive * for leptospirosis from 262 hospital patients.

Age (year)	No.
under 15	1
16 – 20	13
21 – 25	7
26 – 30	11
31 – 35	10
36 – 40	11
41 – 45	7
46 – 50	2
51 – 55	2
57 – 60	3
over 60	3
Total	70

* Minimal titer 1 : 100

TABLE V

Serological distribution of Leptospira antigens giving the maximum titer in 70 seropositive* patients.

Serotype	No	Per cent
L. wolffii	21	30.0
L. icterohemorrhagiae	19	27.1
L. javanica	8	11.4
L. bataviae	7	10.0
L. grippotyphosa	7	10.0
L. hebdomadis	6	8.6
L. akiyami A	2	2.9
Total	70	100.0

* Minimal titer 1 : 100

DISCUSSION

The serologic procedures for study of leptospirosis have been done using various methods (17,18,19,20,21,22,23,24). They are based on the fact that antibody is regular produced after infection. Leptospires are excellent antigens. It has been previously shown that leptospiral cells contain two major antigenic components, P antigen or peripheral antigen and S antigen or somatic antigen (25). P antigen is type-specific antigen, functioning as an agglutinogen, complement fixing antigen, and precipitinogen. S antigen is genus-specific functioning as complement fixing antigen and precipitinogen.

After intection specific antibodies are developed and may be detectable for a long time depending upon the nature of the antibodies. So detection of leptospiral antibodies depends upon the serological method and relationship to the time of infection.

The microscopic agglutination - lysis test of Schuffner and Mochtar (17) is the generally accepted test in leptospiral serology, and employs living leptospires as antigen. It is type-specific and seem to be the most effective in serological studies of leptospirosis, especially in surveys for past infection. Specific agglutinins can be detected an average of 8-12 days after infection, and reach a high peak in a short time. They are then constant for a period of about five days to two weeks. The persistence of high levels of homologous antibodies differs with the animal species. In general the level of titer gradually decreases, but usually persists as high as 1 : 100 - 1: 300 for a year, and possibly for life.

The difficalty with using the agglutination-lysis test is that employing multiple antigens is laborious and involves the possibility of infection, as well as the necessity of maintaining a large number of antigenically stable stock cultures to provide antigens. The routine application of this test in a diagnostic laboratory is therefore limited.

The incidence of leptospiral infection in patients was investigated and it was found that 26.7 % of patients were seropositive. Most of these seropositive cases were in the age range of 16 - 45 years. Sex distribution of suspect cases serologically positive was not differenct. The predominant serotype was **Leptospira wolffii,** similar to the result of Charuchinda (26), but the incidence in our studies was higher than previous study by about 10%. This may be due to the rate of hospitalization of infected patients, collection of specimens and other clinical factors. such as the clinical suspicion of the doctor. Symptoms may be quite varied, so the doctor must suspect the possibity in order to make the diagnosis. We can see from Table II that the provisional diagnosis was varied although the clinical picture was apparently consistant with leptospirosis.

In our studies usually single specimens were obtained and the agglutination - lysis method of Schuffner and Muchtor was employed. Unfortunate the test is often not diagnostic before the eighth to tenth day (16). So agglutination studies have little value during the acute phase of illness and could not differentiate from past infection especially with single specimens.

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