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COMPARATIVE STUDY BETWEEN COMPLEMENT FIXATION AND MICROSCOPIC AGGLUTINATION TESTS FOR LEPTOSPIRAL DIAGNOSIS

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SUMMARY

The results of a comparative study between complement fixation and microscopic agglutination tests, for leptospiral diagnosis are reported. An agreement of 88.8% was found, with highly significant titer in human sera. In animals sera the concordance was 77.7% for dog, 57% in mule and 19.5% in horse.

INTRODUCTION

The microscopic agglutination test with live antigens is the accepted procedure in most laboratories, as a routine for the detection of leptospiral antibodies in human and animal sera. Although several Authors have applied the complement fixation test to detect antibodies in humans and also in many animal species, using as antigens a non-pathogenic strain of Leptospira (*Leptospira biflexa, Patoc I*).

This strain, in view of its antigenic composition, is capable of agglutinating in presence of the most specific sero-agglutinins for most of the pathogenic Leptospirae.

The first observations to demonstrate that *L. biflexa Patoc I* is agglutinated by human positive sera, for different types of Leptospirae were carried out by COMBIESCU et al. [3]

STURDZA et al. [8] and STURDZA & EILIAN [7], using antigens prepared from *L. biflexa Patoc I*, in both complement fixation and microscopic agglutination tests, obtained better results in complement fixation test. They recommended this type of antigens for screening, as a routine, in suspected cases of leptospirosis.

ADDAMIANO & BABUDIERI [1] ascertained that antigens prepared from *Patoc I* strain are able to be agglutinated by human positive sera to pathogenic Leptospirae. The same Authors, carrying out this type of research on animals, observed that sera with a negative microagglutination reaction, were positive only when the test was done with samples of pathogenic Leptospirae.

Recently, FUCHS [5] compared the results obtained with antigens prepared from Patoc I and São Paulo strains, and found that Patoc I was antigenically superior.

In Brazil, CORREA et al. [4] using serotype *patoc* as a polyvalent screening antigens, in microscopic agglutination test, on patients sera suspected of leptospirosis, compared the results with other found when tested with pathogenic leptospirae. In 5,942 samples of sera they showed 98,7% of agreement in the results, what confirmed the considerable practical value of serotype *patoc* in the serological diagnosis of human leptospirosis.

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In this paper, a comparative study was done between the microscopic agglutination and complement fixation tests for the evaluation of this test in human and animal leptospirosis.

MATERIAL AND METHODS

Antigens — For the complement fixation tests, the antigen was prepared according to the technique used by STURDZA et al. [8]. It was used a non-pathogenic strain of Leptospira (Patoc I). Cultures of 12 days old, in 500 ml Korthoff medium, incubated at 28°C were centrifuged at 4,000 rpm for one and a half hour. After centrifuging, the sediment was retaken in merthiolated physiological saline 1/10,000, (1/20 of the initial volume). This antigens was stored at + 4°C, for the time it was used. During the experiment the same lot of antigen was used. The titration of the antigen was done against homologous serum and the fixing titer choosed was 1/20 with 4 units of complement 50% of hemolysis.

For the microscopic agglutination test the antigen consisted in cultures of leptospirae, in Korthoff medium, from 4 to 14 days old according to GALTON et al. [6]. The following serotypes were used: *copenhageni, icterohaemorrhagiae, canicola, pomona, grippotyphosa, tarassovi, szwajizak, wolffi, australis, bataviae, brasiliensis, castellonis, panama, pyrogenes, javanica, autumnalis, butembo, patoc, hebdomadis, sejroe, saxkoebing, djasiman, sentot, cynopteri* and *andamana.*

Sera — 326 samples of sera were studied distributed as follows: 192 human sera, 143 of which from the Adolfo Lutz Institute and 52 from Biological Institute of São Paulo, most of them positive for leptospirosis; 114 horse sera, 13 from dog and 7 from mule.

For the complement fixation test all sera were inactivated at 56°C for 30 minutes and then followed by twofold dilutions, starting with the dilution 1/20 for human and 1/5 for animal sera.

Complement fixation test — The complement fixation test were carried out by the 50% haemolysis method. For reading of the reaction (degrees of haemolysis) a Coleman Jr. Spectrophotometer, Model 6 A was employed, at a wave lenght of 545 mu. The components of the haemolytic system were standardized according to the technique described by CAMARGO et al. [2].

The reactions were performed in haemolysis tubes; 0.2 ml of serum plus equal volume of antigen and complement (4 UC 50%) were used. After one hour of incubation at 37°C, the haemolytic system, 0.4 was added followed by another incubation for one hour. Then, all tubes were centrifuged at 1,500 rpm for 5 minutes. The final reading was done as mentioned above. The titers for the sera were obtained by determining the inverse of their dilutions that fixes 50% of the complement.

Microscopic agglutination test — The microscopic agglutination test was carried out in tubes. The sera was initially diluted at 1/100 in physiological saline. From this dilution, 0.2 ml was delivered in 13 x 100 tubes as many as there were the antigens to be used. An equal amount of each antigen was dispensed, one for each tube. The tubes were incubated at 28°C for 3 hours and examined. A drop of each tube was examined by dark field microscopy using low power objective and 10 x ou 15 x oculars. The degree of agglutination was interpreted according GALTON et al. [6].

RESULTS

The results are summarized in Table I. As expressed, the titers were higher in microscopic agglutination test, for serotype *icterohaemorrhagiae.* They varied from 1/200 to 1/51,200 in human sera, 1/400 to 1/25,600 in horse sera, 1/200 to 1/1,600 in dog whereas only 1/800 in mule. Lower titers were also found for other serotypes.

The best agreement, 88.8% between the two methods, with highly significant titers was found in human sera. For animals the concordance was 77.7% for dog, 57% in mule and 19.5% in horse.

TABLE I

Results of the microscopic agglutination and complement fixation tests according serotypes and titers — São Paulo 1972

Serotype	Titers in MA			
	Human	Horse	Mule	Dog
icterohaemorrhagiae	1/200 to 1/51.200	1/400 to 1/25.600	1/800	1/200 to 1/1.600
canicola	1/200 to 1/1.600	1/200 to 1/1.600	1/200	1/200 to 1/400
grippotyphosa	1/200 to 1/12.800			1/200
mini	1/400			
pomona		1/200 to 1/3.200	1/200 to 1/3.200	
tarassovi		1/200 to 1/800		
wolffi		1/200 to 1/1.600	1/200	
australis		1/200		
Titers in CFT with strain Patoc I as antigen	1/30 to 1/2.560	1/5 to 1/670	1/50 to 1/220	1/30 to 1/400

MA = Microscopic Agglutination
CFT = Complement Fixation Test

DISCUSSION

By the analysis of the results, the conclusion can be drawn that *L. Semaranga Patoc I* is efficient for the laboratory diagnosis of leptospirosis. When human sera were used 88.8% of concordance were found between the microscopic agglutination and complement fixation tests.

These data are supported by the studies carried out by STURDZA et al. [8]; using different method they found a diagnostical agreement of 79.23% of the total examinations. According the same Authors, the use of microscopic agglutination test alone would allow the diagnosis of 80.32% of the sera, whereas through the complement fixation test 86.88% of the samples would give positive results.

CORREA et al. [4] using the strain Patoc I in microscopic agglutination test found concordance of 98.7%, that confirms the practical value of this strain as antigen for serodiagnosis in human leptospirosis. The total of sera tested was 5,942 from leptospiral suspected patients.

On the other hand, it was not possible to obtain the same concordance in animal sera, probably due to the time when the animal sera were collected.

STURDZA et al. [8] showed that the complement fixation test is more precocious and at least as sensitive as the microscopic agglutination test, possessing the advantage of not registering old cases, which is very important from the clinical and epidemiological point of view.

In our experience some sera from horses, mules and dogs showed antibodies that reacted only against homologous antigen. This results of this research will be subject of another paper. In relation to animal sera our results agree with those of ADDAMIANO & BABUDIERE [1] who had a high percentage of positive sera in animal with leptospirosis, using specific antigens. When the same sera were used against Patoc antigen the results

were negative. For this reason, Patoc I antigen is not recommended for routine diagnosis when using animal sera.

RESUMO

Estudo comparativo entre as reações de fixação do complemento e soro-aglutinação microscópica para o diagnóstico de leptospirose

Em 192 amostras de soros humanos e 134 de animais (eqüinos, muares e caninos) foi feita uma comparação entre as reações de soro-aglutinação microscópica e fixação do complemento para o diagnóstico de leptospirose. Na soro-aglutinação microscópica foram usadas 25 extirpes de leptospira como antígenos e na de fixação do complemento apenas uma, apatogênica, a *patoc*, cepa Patoc I.

A reação de fixação do complemento se fez pelo método 50% de hemólise, com leitura em espectrofotômetro e o sistema hemolítico padronizado segundo CAMARGO & col. [2].

Nos soros humanos encontrou-se uma concordância de 88,8% entre as duas reações, com títulos altamente significativos. Nos soros de animais a maior concordância se verificou em soros de cães, 77,7% com títulos fixadores do complemento variando de 1/30 a 1/400. Em muares foi verificada uma concordância de 57% enquanto que em eqüinos ela foi de 19,5%.

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