

## USE OF SAPROPHYTIC LEPTOSPIRA STRAINS IN THE SERODIAGNOSIS OF EXPERIMENTAL LEPTOSPIROSIS IN GUINEA-PIGS (*Cavia* sp)(1)

Raul J. S. GIRIO(2) & Luis A. MATHIAS(2)

### SUMMARY

The efficiency of four *Leptospira biflexa* strains (Buenos Aires, Patoc 1, Rufino and São Paulo) as single antigen in the serodiagnosis in guinea-pigs experimentally infected with seven *Leptospira interrogans* serovars (*canicola*, *grippityphosa*, *hardjo*, *icterohaemorrhagiae*, *pomona*, *tarassovi* and *wolffi*) was evaluated by the microscopic agglutination test. The four saprophytic strains were not able to reveal antibody titres in sera of guinea-pigs experimentally infected with *Leptospira interrogans*. Serological cross-reactions were observed between strains Patoc 1 and São Paulo and between serovars *wolffi* and *hardjo*.

**KEY WORDS:** Leptospirosis; Serodiagnosis; Saprophytic strains.

### INTRODUCTION

The diagnosis of leptospirosis is usually performed by serological tests and the most widely utilized technique is the microscopic agglutination test. This technique is laborious because it requires the use of several leptospira serovars as antigens that demand constant maintenance, passages and other activities. Thus, the ideal situation would be a test easy to accomplish and using a few serovars as antigens.

In many studies using sera of naturally infected animals, several researchers have concluded that some saprophytic leptospira strains may be agglutinated by sera containing antibodies against pathogenic leptospira. Among saprophytic strains, serovar Patoc 1 has shown greater efficiency by agglutinating human sera containing antibodies against pathogenic serovars (1, 6).

Due to the high sensitivity shown, strain Rufino was recommended as screening antigen in seroagglutination tests to diagnose animal leptospirosis (3). On the other hand, some workers observed the inefficiency of this strain as polyvalent antigen in serological screening (7, 8, 9, 13).

The efficiency of strain Buenos Aires by agglutinating sera of animals naturally infected by *L. interrogans* was observed in bovine, swine, canine, water buffalo, caprine and ovine (5, 8, 13). This strain had shown good sensitivity to detect antibodies in the beginning of experimental infection period by serovars *canicola* and *icterohaemorrhagiae* in dogs (9). Conversely, this strain did not show sensitivity and specificity values that permit its recommendation as antigen to the diagnosis of swine leptospirosis experimentally induced by serovar *pomona* (14).

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(2) Departamento de Medicina Veterinária Preventiva, Faculdade de Ciências Agrárias e Veterinárias Campus de Jaboticabal — UNESP. CEP 14870 Jaboticabal, SP., Brasil.

By the microscopic agglutination test, standardized according to the World Health Organization recommendations, strain Buenos Aires did not show satisfactory results as screening single antigen to diagnose bovine, swine, equine, canine and buffalo leptospirosis (7).

Strains Patoc 1 and São Paulo revealed poor sensitivity to agglutinate sera of animals naturally (4, 7, 8, 11, 13) and experimentally (9) infected by *Leptospira interrogans*.

The purpose of this work, is research in sera of experimentally infected guinea-pigs, if pathogenic leptospires induce to forming antibodies crossly reacting against saprophytic *Leptospira* strains and to verify the possibility of the use of these strains as screening single antigen on leptospirosis serological diagnosis.

## MATERIAL AND METHODS

### *Leptospira* serovars

Each guinea-pig group was infected with one of the following *Leptospira interrogans* serovars: **canicola**, **grippotyphosa**, **hardjo**, **icterohaemorrhagiae**, **pomona**, **tarassovi** and **wolffi** or with one of the following *Leptospira biflexa* strains: Buenos Aires, Patoc 1, Rufino and São Paulo.

### Guinea-pigs

The animal groups were formed by 10 adult male guinea-pigs, weighing about 400 g, that before the first inoculation did not show antibody titres against any of the *Leptospira* serovars used in the experiment.

The animals were submitted to four subcutaneous inoculations of *Leptospira* grown in EMJH *Leptospira* medium (Difco). Each animal received 2 ml, 3 ml, 4 ml and 5 ml of the bacterial suspension and the interval between the inoculations was of one week.

Blood samples were taken, by cardiac puncture, 7, 14 and 21 days after the last inoculation.

### Serological test

Antibody titres against all the *Leptospira* serovars used in the experiment were searched in all guinea-pig sera, by the microscopic agglutination test (12).

## RESULTS

The sera of guinea-pigs infected with pathogenic leptospires did not agglutinate against saprophytic leptospira antigens. Sera of guinea-pigs infected with strains Buenos Aires, Patoc 1, Rufino and São Paulo also did not show agglutination against pathogenic leptospira antigens.

In sera of guinea-pigs infected with serovar **canicola**, the highest homologous antibody titre obtained was 1:800, on the 7<sup>th</sup> day after the last inoculation.

Guinea-pigs infected with serovar **grippotyphosa** and guinea-pigs infected with serovar **icterohaemorrhagiae** showed the highest homologous antibody titre, 1:400, on the 7<sup>th</sup> day after the last inoculation.

In the group of guinea-pigs infected with serovar **hardjo**, antibody titres against homologous antigen and against serovar **wolffi** were observed. The highest homologous titre, 1:800, was obtained on day 14<sup>th</sup> after the last inoculation. In the serum of one guinea-pig, the antibody titre against homologous antigen was slightly lesser than titre obtained against **wolffi** antigen.

Homologous antibody titres of up to 1:400 were found on day 14<sup>th</sup> after the last inoculation in sera of guinea-pigs infected with serovar **pomona** and also in sera of guinea-pigs infected with serovar **tarassovi**.

In sera of guinea-pigs inoculated with serovar **wolffi** antibody titres were also observed against serovar **hardjo**. The highest homologous antibody titre, 1:400, occurred on the 14<sup>th</sup> day after the last inoculation. On the three serological examinations the greater part of homologous antibody titres was higher than titres obtained against **hardjo** antigen.

The group of guinea-pigs infected with strain Buenos Aires showed the highest homologous antibody titre, 1:400, on the 14<sup>th</sup> day after the last inoculation. One guinea-pig showed a poor response to the inoculation since the highest titre was 1:25, observed on the 7<sup>th</sup> post-inoculation day.

In sera of guinea-pigs infected with strain Patoc 1, there were observed homologous antibody titres of until 1:1,600 on 14<sup>th</sup> day after the last inoculation. Those sera also agglutinated against the strain São Paulo, but with low titres, except one animal that showed titres against both antigens.

The sera of guinea-pigs infected with strain Rufino showed the highest homologous antibody titres, 1:400, on days 7<sup>th</sup> and 14<sup>th</sup> after the last inoculation.

In sera of guinea-pigs infected with strain São Paulo there were also observed antibody titres against strain Patoc 1. The highest homologous antibody titre was 1:400 whereas the highest heterologous titre was 1:200 and two animals showed the same titres on the three observations.

## DISCUSSION

The results showed that none of the four *Leptospira biflexa* antigens were able to agglutinate, by the microscopic agglutination test, against sera of guinea-pigs infected with any of the seven *Leptospira interrogans* serovars. The inefficiency of these saprophytic strains as screening antigen on leptospirosis serological diagnosis was also observed in sera of several domestic species naturally infected by pathogenic serovars (7).

The efficiency of strain Buenos Aires in agglutinating sera of bovine, swine and canine infected by serovars *wolffi*, *pomona*, *canicola* and *icterohaemorrhagiae* was verified (13). Other works (5, 8) concluded the viability of strain Buenos Aires as screening antigen in serological diagnosis of bovine and buffalo leptospirosis. In a experimental study in dogs, this strain was able to crossly react against serovars *canicola* and *icterohaemorrhagiae* during the acute period of the infection (9). However, these observations can not be confirmed in this work. In other experimental work (14) strain Buenos Aires did not show satisfactory results when used as antigen in the microscopic agglutination test to detect different antibody concentrations against serovar *pomona* in swine sera.

Strain Patoc 1 also showed a poor performance to reveal agglutinating titres in sera of guinea-pigs infected with pathogenic leptospiaras or with the another three saprophytic strains. The inefficiency of this strain as screening antigen to diagnose animal leptospirosis had already been demonstrated by Rumanian researchers (1). Identical results were obtained in another research that demonstrated the unviability of the use of strain Patoc 1 as single antigen (2). Conversely, this strain showed satisfactory results by revealing, in human sera, serological titres induced by pathogenic leptospiaras, mainly in infections caused by serovars *icterohaemorrhagiae*, *pomona*, *canicola* or *copenhageni* (6, 10).

The Rufino antigen was not able to diagnose, by the microscopic agglutination test, leptospirosis caused by pathogenic serovars. This confirms the results verified in human sera (4) and in sera of several animal species naturally (8, 13) or experimentally infected (9). However, Argentinean researchers verified a concordance of more than 93% between antibody titres obtained against strain Rufino and titres obtained against *Leptospira interrogans* in animal sera (3).

The infavourable results obtained with strain São Paulo in sera of guinea-pigs infected with pathogenic leptospira were in accordance with results obtained by several researchers, having had short diversification from the low sensitivity and specificity rates observed in sera of animals naturally infected by pathogenic serovars (1, 8, 11, 13).

During the experiment, it was verified that serological cross-reactions occurred only between serovars *hardjo* and *wolffi*, belonging to serogroup Sejroe and between strains Patoc 1 and São Paulo, belonging to serogroup Semarang.

Although several researchers have concluded by the usefulness of some saprophytic strains to be used as a single antigen in serological screening, it is worthy of notice that the greater part of these works was performed with sera of naturally infected animals and humans. Thus, these individuals were not submitted to

a control for the possibility of infection by more than one *Leptospira* serovars. This possibility is relatively great resulting from the frequency that saprophytic strains are found in free life, suggesting that titres eventually found may be attributed to mixed infections and not to cross-reactions.

## RESUMO

### Utilização de estirpes apatogênicas de *Leptospira* no diagnóstico sorológico de leptospirose em cobais (*Cavia sp.*) experimentalmente infectadas

No presente estudo foi avaliada, através da reação de soroaglutinação microscópica, a eficiência de quatro estirpes apatogênicas de *Leptospira biflexa* (Buenos Aires, Patoc 1, Rufino e São Paulo) como antígeno único para o diagnóstico sorológico em cobaias experimentalmente infectadas com sete diferentes sorotipos de *Leptospira interrogans* (*canicola*, *grippityphosa*, *hardjo*, *icterohaemorrhagiae*, *pomona*, *tarassovi* e *wolffi*). As estirpes Buenos Aires, Patoc 1, Rufino e São Paulo não foram eficientes em revelar títulos de anticorpos nos soros das cobaias infectadas com *Leptospira interrogans*. Durante o experimento, foi observada a ocorrência de reações sorológicas cruzadas entre as estirpes Patoc 1 e São Paulo e também entre os sorotipos *wolffi* e *hardjo*.

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