

## SEARCH FOR AGGLUTINATING ANTIBODIES TO *Leptospira* AND *Leptonema* IN HORSES, SÃO PAULO, BRAZIL

ANTICORPOS AGLUTINANTES PARA *Leptospira* E *Leptonema* EM EQUÍNOS, SÃO PAULO, BRASIL

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### SUMMARY

Sera from 922 apparently health equines intended for therapeutic sera production in a farm of the Instituto Butantã in São Roque, State of São Paulo, Brazil, were examined for detection of antibodies to serovars of *Leptospira interrogans* and to *Leptonema illini* by the microscopic agglutination (MA) test. Significant antibody titres were found in 807 sera (87.5%) and 659 (81.7%) of those sera reacted to more than one serovar at titres ranging from 1:100 to 1:6,400 with predominance of low titres ( $\leq 1:400$ ). From the 23 screening serovars, only tarassovi did not react. The most common reactions among positive sera occurred to antigens from the *Icterohaemorrhagiae* serogroup (84%) and to *Leptonema illini* (79.2%).

UNITERMS: Leptospira; Leptospirosis; Equidae; Horses; Antibodies; Serology

### INTRODUCTION

Leptospirosis is a world-wide occurring zoonosis caused by members of the family Leptospiraceae. According to the antigenic classification, which is also based on pathogenicity and morphology, the family comprises the genera *Leptonema* and *Leptospira*<sup>7,9</sup>. The latter is subdivided in *Leptospira biflexa*, *Leptospira parva*<sup>8</sup> and *Leptospira interrogans*, which is composed of the pathogenic serovars<sup>9</sup>.

Many mammalian species and also cold-blooded animals are natural hosts of *Leptospira interrogans* serovars, spreading the infection among domestic and wild animals<sup>9</sup>. The economic and epidemiological importance of leptospirosis in domestic animals, including its close ecological relationship to man, has been reported in several countries<sup>2,3,10,19</sup>.

The infection in equines, although normally asymptomatic, has been associated with periodic ophthalmia, pyrexia and icterus<sup>6,11</sup>. Experimental proof that leptospiruria follows the acute phase of the disease for a period of up to 10 weeks in most horses<sup>13</sup> suggest that these animals may serve as important reservoirs for transmitting infection to either animals or men.

The present investigation was undertaken to determine the prevalence of leptospiral and leptonemal antibodies in sera from apparently healthy horses intended for therapeutic sera

production, maintained at the farm of the Instituto Butantã, São Roque, SP, Brazil.

### MATERIAL AND METHOD

The study involved all the 922 horses held at the farm of the Instituto Butantã, which were grouped according to the kind of antigen they received to produce hyperimmune sera. Each group was maintained in contiguous areas of 20,000 m<sup>2</sup> and shared the same water supply.

The herd consisted of animals of different origins and stay in the farm. The age varied from 1 to 20 years, with a mean of 9 years.

A total of 410 horses with at least 2.5 year-old had been submitted to hyperimmunization schedules with different snake venoms, 64 with *Clostridium tetani* and 35 with *Corynebacterium diphtheriae*. The other 413 horses had not been inoculated yet and among them 25 had been recently acquired.

Single blood samples were collected from each horse and the sera were submitted to the microscopic agglutination test (MA), as described by FAINE<sup>3</sup> (1982), using live bacterial strains of the following 23 serovars: *australis*, *autumnalis*, *bataviae*, *brasiliensis*, *butembo*, *canicola*, *castellonis*, *celledoni*, *copenhageni*, *cynopteri*, *djasiman*, *grippotyphosa*,

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*hebdomadis*, *icterohaemorrhagiae*, *javanica*, *panama*, *pomona*, *pyrogenes*, *ranarum*, *shermani*, *tarassovi*, *wolffi* and *illini*. Each serum was screened at a 1:100 dilution with each bacterial suspension. Those sera showing at least 50% agglutination with one or more serovars were further titrated in serial two-fold dilutions.

Data were analysed statistically for association between variables by the chi-square test and Yates correction with the level of significance set at 0.05 and 0.01.

## RESULTS

Significant antibody titres ( $\geq 1:100$ ) were found in 807 (87.5%) out of the 922 sera examined.

The titres to individual serovars in each serum are given in Tab.1. Reactions to serovar *icterohaemorrhagiae* and to *Leptonema illini* were the most frequent (73.3% and 69.3% respectively), followed by *autumnalis* (28.7%), *brasiliensis* (23%), *copenhageni* (19.8%) and *pyrogenes* (18.9%). A predominance of low titres ( $\leq 1:400$ ) and a large number of cross reactions were detected. The highest titre (1:6,400) was observed for *canicola* and *pyrogenes* in the same serum. Among the 23 serovars investigated no reaction was seen to the serovar *tarassovi*.

The positive sera were classified into 3 categories according to the reaction patterns observed: a) without coagglutination, i.e, reaction with only one serovar; b) with coagglutination and serovar predominance, that means, multiple titres within one serum but just one of the serovars reacting at the highest titre; c) with coagglutination and without serovar predominance, when more than one serovar reacted to the same and highest titre within one serum. In the latter case, no individual serovar was recorded as contributing to serological prevalences.

As shown in Tab. 2, reaction with a single serovar was detected in 148 sera (16%). The most frequent titres occurred to *icterohaemorrhagiae* and *illini* antigens (respectively 5.4% and 7.7%). High titres (1:800 to 1:3,200) were observed only with *Leptonema illini* in 19 sera.

Coagglutination was found in 659 (71.5%) sera and reactions with serovar predominance occurred in 525 of them. Prevalent titres were detected once more to *illini* and *icterohaemorrhagiae* (464 and 41 sera respectively). The most frequent titres to *Leptonema illini* were  $\geq 1:800$ .

The remaining 134 (14.5%) positive sera reacted against two or more antigens to the same titre.

The results of the MA test according to the groups of horses

(Tab. 3) revealed that the frequency of serologically-negative sera was significantly higher ( $p < 0.01$ ) among animals that had not been immunised (27.4%) compared with inoculated horses (0.4%). Significant differences in the prevalence of titres to different antigens were not observed between each group of animals.

## DISCUSSION

Previous serological surveys in São Paulo, Brazil, have shown the presence of leptospiral antibodies in horses with prevalence of titres ranging from 6.9% to 42%<sup>2,4,16,19</sup>. These prevalences are much lower than to that found in the present study (87.5%) and may reflect differences in exposure to risk factors as the presence of carrier animals and environment favourable to its maintenance and dissemination, besides the health conditions and the age of the animals.

In serological studies the causative serovars are speculative as the tests available are not serovar-specific, and cross reactions between antigens from different serogroups are a common occurrence in positive sera. However, if the positive reactions with antigens of the *Icterohaemorrhagiae* serogroup are considered, independently of titre predominance in positive sera (84%), the probability that leptospires from this group are being maintained at the site is high. Members of this serogroup have been the most commonly incriminated in human leptospirosis in the State of São Paulo either by serological<sup>1,14</sup> or isolation<sup>15</sup> findings.

According to HATHAWAY et al.<sup>6</sup> (1981) the equines do not appear to act as maintenance hosts for any specific serovar. The spectrum of leptospiral titres in horses is probably a reflection of exposure to serovars maintained by other domestic animals and wildlife in the same geographical region. Considering this interpretation and the high prevalence of *Icterohaemorrhagiae* serogroup among the horses examined, the probability of transmission from rodents within the area is very high. It should be stressed that different species of rodents could be found throughout the farm (in dams, swamps, boscages and fields). In spite of trials to decrease the infestation with brown rats they are abundant within the buildings as food and equipment warehouses. The isolation of 20 strains from *Rattus norvegicus* in Rio de Janeiro, all identified as members of the *Icterohaemorrhagiae* serogroup<sup>12</sup> provides evidence that rodents are the main reservoir of these leptospires in our environmental conditions.

The results presented in Tab. 1 and 2 indicate that infection with leptospires of serogroups other than *Icterohaemorrhagiae* may also occur. Nevertheless, the multiplicity of reactions with many antigens must also be attributed to the cross reactions frequently observed in positive sera.

TABLE 1

Prevalence of titres in sera from 922 horses to each serovar of the family *Leptospiraceae* examined by MA test. São Roque - SP, 1988.

Serogroup	Serovar	N <sup>o</sup> Positive Reactions	Preva- lence %	MAT Titres						
				100	200	400	800	1600	3200 6400	
<i>Australis</i>	<i>australis</i>	36	3.9	19	15	2	-	-	-	-
<i>Autumnalis</i>	<i>autumnalis</i>	265	28.7	92	122	44	7	-	-	-
	<i>butembo</i>	2	0.2	1	1	-	-	-	-	-
<i>Ballum</i>	<i>castellonis</i>	6	0.7	3	2	1	-	-	-	-
<i>Bataviae</i>	<i>bataviae</i>	16	1.7	8	7	1	-	-	-	-
	<i>brasiliensis</i>	212	23.0	92	98	17	5	-	-	-
<i>Canicola</i>	<i>canicola</i>	88	9.5	38	36	11	2	-	-	1
<i>Celledoni</i>	<i>celledoni</i>	2	0.2	2	-	-	-	-	-	-
<i>Cynopteri</i>	<i>cynopteri</i>	67	7.3	36	29	2	-	-	-	-
<i>Djasiman</i>	<i>djasiman</i>	34	3.7	23	9	1	1	-	-	-
<i>Grippotyphosa</i>	<i>grippotyphosa</i>	100	10.8	44	36	16	4	-	-	-
<i>Hebdomadis</i>	<i>hebdomadis</i>	3	0.3	2	1	-	-	-	-	-
<i>Icterohaemorrhagiae</i>	<i>copenhageni</i>	183	19.8	115	49	17	2	-	-	-
	<i>icterohaemorrhagiae</i>	676	73.3	173	312	144	41	5	1	-
<i>Javanica</i>	<i>javanica</i>	3	0.3	3	-	-	-	-	-	-
<i>Panama</i>	<i>panama</i>	14	1.5	9	5	-	-	-	-	-
<i>Pomona</i>	<i>pomona</i>	48	5.2	19	13	14	1	1	-	-
<i>Pyrogenes</i>	<i>pyrogenes</i>	174	18.9	55	75	33	8	2	-	1
<i>Ranarum</i>	<i>ranarum</i>	82	8.9	63	17	2	-	-	-	-
<i>Sejroe</i>	<i>wolffi</i>	15	1.6	10	4	-	1	-	-	-
<i>Shermani</i>	<i>shermani</i>	11	1.2	7	4	-	-	-	-	-
.....	<i>illini*</i>	639	69.3	23	67	149	233	130	37	-

\* of *Leptonema illini*; all other serovars of *Leptospira interrogans*

The serovar *ranarum* was introduced to the antigen battery due to its recovery from an aborted equine foetus in the State of São Paulo<sup>20</sup>. Titres to this serovar were detected in 8.9% of all sera and ranged from 1:100 to 1:400 (Tab. 1). Even admitting its circulation in the area, this serovar do not appear to have a major importance among the horses examined.

The high prevalence of titre (87.5%) in apparently healthy animals confirms the findings of other authors that the majority of leptospiral infections in equines are asymptomatic<sup>6,10,13</sup>.

Previous studies of *Leptonema illini* indicated that a large number of apparently healthy adult cattle and swine had antibodies to this species<sup>18</sup>. The high rate among the horses (69.3%) in the farm of the Instituto Butantã suggests a widespread circulation of *Leptonema illini* at that site. Titres to this species showed the highest mean (1:681), ranging from 1:100 to 1:3,200 (Tab.1) besides of being predominant in 535 (58%) sera (Tab.2). It is possible that as a non-pathogenic species it is adapted to and maintained by the horse popula-

tion in the farm. This study probably is the first serological survey of *Leptonema illini* in equines in Brazil.

Since *L.illini* was isolated, in 1965, from the urine of an asymptomatic bull whose serum was negative for *Leptospira*<sup>5</sup>, no clinical manifestations of disease were observed in different animals and the source of the antigen in nature is not yet known<sup>17</sup>.

The majority of negative sera (113 out of 115) were collected from animals that had not been inoculated with antigens for hyperimmune sera production (Tab. 3). It was not possible to analyse to what extent the stimulation of the immunologic system of the inoculated horses would interfere with their reactivity to leptospiral and leptonemal antigens. Titres of 1:3,200 should not be merely regarded as the result of cross reactions with antigens so diverse. We might attribute a relevant weight to the age of the animals, greater than 2.5 year-old among those inoculated horses. In studies of *Leptonema illini* involving cattle and swine, the majority of nonreactors were of young animals<sup>18</sup>. In leptospirosis it is

**TABLE 2**

Predominant titres in sera from 922 horses reacting in different patterns with serovars of the family *Leptospiraceae* by the MA test. São Roque-SP, 1988.

Reaction Pattern	Serovar	Posit. Sera		Reciprocal of Titres						
		Nº	%	100	200	400	800	1600	3200	6400
Without Coagglutination (148 sera; 16%)	<i>brasiliensis</i>	9	1.0	8	-	1	-	-	-	-
	<i>canicola</i>	1	0.1	1	-	-	-	-	-	-
	<i>cynopteri</i>	2	0.2	2	-	-	-	-	-	-
	<i>grippityphosa</i>	11	1.2	6	4	1	-	-	-	-
	<i>icterohaemorrhagiae</i>	50	5.4	30	19	1	-	-	-	-
	<i>pyrogenes</i>	3	0.3	3	-	-	-	-	-	-
	<i>ranarum</i>	1	0.1	-	-	1	-	-	-	-
Coagglutination and serovar predominance (525 sera; 57%)	<i>illini</i>	71	7.7	2	26	24	16	2	1	-
	<i>autumnalis</i>	4	0.4	-	2	2	-	-	-	-
	<i>brasiliensis</i>	7	0.8	-	7	-	-	-	-	-
	<i>copenhageni</i>	2	0.2	-	2	-	-	-	-	-
	<i>cynopteri</i>	1	0.1	-	1	-	-	-	-	-
	<i>grippityphosa</i>	5	0.6	-	2	3	-	-	-	-
	<i>icterohaemorrhagiae</i>	41	4.5	-	20	10	9	2	-	-
Coagglut. w/o predominance (134 sera; 14.5%)	<i>ranarum</i>	1	0.1	-	-	1	-	-	-	-
	<i>illini</i>	464	50.3	-	19	93	192	125	35	-
Total positive sera		807	87.5	85	137	173	242	132	37	1

**TABLE 3**

Results of the MA test with sera from horses inoculated and non-inoculated with different antigens in order to produce hyperimmune sera. São Roque-SP, 1988.

Group of equines	MA Test	
	Positive**	Negative
Inoculated	507/509* (99.6%)	2/509 (0.4%)
Non-inoculated	300/413 (72.6%)	113/413 (27.4%)
Total	807/922 (87.5%)	115/922 (12.5%)

$\chi^2=149.43$ ,  $p<0.01$

\* Nº examined/no.positive

\*\* titre  $\geq 1:100$

known that positivity increases as a function of age both for men and animals<sup>3,12</sup>.

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## RESUMO

Soros de 922 eqüinos aparentemente sadios, mantidos na Fazenda do Instituto Butantan (São Roque, SP) para produção de soros hiperimunes, foram analisados quanto à presença de anticorpos para sorovares de *Leptospira interrogans* e para *Leptonema illini*, através da reação de aglutinação microscópica (MA). Entre os 807 (87,5%) animais positivos, 659 (81,7%) reagiram com mais de um sorovar, com títulos entre 1:100 e 1:6.400, havendo predomínio de títulos baixos ( $\leq 1:400$ ); 84% dos soros positivos reagiram com representantes do sorogrupo *Icterohaemorrhagiae* e 79,2% com *Leptonema illini*. Dos 23 sorovares utilizados, apenas o *tarassovi* não reagiu.

UNITERMOS: Leptospirose; Anticorpos; Eqüinos; Sorologia; Leptospira

## REFERENCES

- 01-CORRÊA, M.O.A. Leptospiroses em São Paulo. *Revista do Instituto Adolfo Lutz*, v.29/30, p.29-37, 1969/70.
- 02-CORRÊA, M.O.A.; AMATO NETO, V.; VERONESI, R.; FABBRI, O.S. Leptospirose em eqüinos: inquérito sorológico. *Revista do Instituto Adolfo Lutz*, v.15, p.186-93, 1957.
- 03-FAINE, S. ed. *Guidelines for the control of leptospirosis*. Geneva, WHO, 1982. (WHO, publication n.67)
- 04-FREITAS, D.C.; SALLES GOMES, C.E.; LACERDA, J.P.G.; PEREIRA LIMA, F. Notas sobre leptospirose eqüina. *Arquivo do Instituto Biológico*, São Paulo, v.27, p.93-6, 1960.
- 05-HANSON, L.E.; TRIPATHY, D.N.; EVANS, L.B.; ALEXANDER, A.D. An unusual leptospira, serotype *illini* (a new serotype). *International Journal of System Bacteriology*, v.24, p.355-7, 1974.
- 06-HATHAWAY, S.C.; LITTLE, T.W.A.; FINCH, S.M.; STEVENS, A.E. Leptospiral infection in horses in England: a serological survey. *Veterinary Record*, v.108, p.396-8, 1981.
- 07-HOVIND-HOUGEN, K. *Leptospiraceae*, a new family to include *Leptospira noguchi* 1917 and *Leptonema* gen. nov. *International Journal of System Bacteriology*, v.29, p.245-51, 1979.
- 08-HOVIND-HOUGEN, K.; ELLIS, W.A.; BIRCH-ANDERSEN, A. *Leptospira parva* sp. nov.: some morphological and biological characters. *Zentralblatt für Bakteriologie Hygiene, I Abt. Orig. A*, v.250, p.343-54, 1981.
- 09-JOHNSON, R.C.; FAINE, S. Genus I. *Leptospira noguchi* 1917. In: KRIEG, N.R.; HOLT, J.G., eds. *Bergey's manual of systematic bacteriology*. 9.ed. Baltimore, Williams & Wilkins, 1984. v.1, p.62-7.
- 10-MYERS, D.M. Serological studies and isolations of serotype *hardjo* and *Leptospira biflexa* strains from horses of Argentina. *Journal of Clinical Microbiological*, v.3, p.548-55, 1976.
- 11-MORTER, R.L.; WILLIAMS, R.D.; BOLTE, H.; FREEMAN, M.J. Equine leptospirosis. *Journal of the American Veterinary Medical Association*, v.155, p.436-42, 1969.
- 12-PEREIRA, M.M.; ANDRADE, J. Epidemiological aspects of leptospirosis in a slum area in the city of Rio de Janeiro, Brazil. Search for Leptospire and specific antibodies in rodents. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, v.82, p.768-70, 1988.
- 13-ROBERTS, S.J. Comments on equine leptospirosis. *Journal of the American Veterinary Medical Association*, v.155, p.442-5, 1969.
- 14-SAKATA, E.E.; ROMERO, E.C.; YASUDA, P.H.; STILIANO, S.V. Leptospirose humana no Estado de São Paulo, triênio 1986-1988. *Revista de Microbiologia*, São Paulo, v.20, p.233, 1989. /Apresentado ao 15º Congresso Brasileiro de Microbiologia, Ribeirão Preto/
- 15-SAKATA, E.E.; YASUDA, P.H.; ROMERO, E.C.; SILVA, M.V.; LOMAR A.V. Sorovares de *Leptospira interrogans* isolados de casos de leptospirose humana em São Paulo, Brasil. *Revista do Instituto de Medicina Tropical de São Paulo*, v.34, p.217-21, 1992.
- 16-SANTA ROSA, C.A.; PESTANA DE CASTRO, A.F.; CAMPEDELLI FILHO, O.; MELLO, D. Leptospirose em eqüinos. *Arquivo do Instituto Biológico*, São Paulo, v.35, p.61-5, 1968.
- 17-TRIPATHY, D.N.; HANSON, L.E. Studies of *Leptospira illini*, strain 3055: pathogenicity for different animals. *American Journal of Veterinary Research*, v.34, p.557-62, 1973.
- 18-TRIPATHY, D.N.; HANSON, L.E. Studies of *Leptospira illini*, strain 3055: immunologic and serologic determinations. *American Journal of Veterinary Research*, v.34, p.563-5, 1973.
- 19-YANAGUITA, R.M.; SANTA ROSA, C.A.; ROSA, R.R.;

GIOMETTI, J. Prevalência de aglutininas anti-leptospiras em eqüinos mantidos para produção de soros terapêuticos. **Revista de Microbiologia**, São Paulo, v.13, p.22-5, 1982.

20-YASUDA, P.H.; SULZER, C.R.; GIORGI, W.; SOARES, M.E.G. *Leptospira biflexa* sorotipo *ranarum* isolado de feto abortado de eqüino. **Revista de Microbiologia**, São Paulo, v.17, p.25-7, 1986.

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