

Revista Latinoamericana de Microbiología

Volumen
Volume 44

Número
Number 3-4

Julio-Diciembre
July-December 2002

Artículo:

Evaluation of a rapid slide agglutination test for the diagnosis of acute canine leptospirosis

Derechos reservados, Copyright © 2002

:
Asociación Mexicana de Microbiología, AC

**Otras secciones de
este sitio:**

- 👉 [Índice de este número](#)
- 👉 [Más revistas](#)
- 👉 [Búsqueda](#)

***Others sections in
this web site:***

- 👉 [Contents of this number](#)
- 👉 [More journals](#)
- 👉 [Search](#)



Medigraphic.com

Evaluation of a rapid slide agglutination test for the diagnosis of acute canine leptospirosis

Walter Lilenbaum,* Paula Ristow,** Suzana Almeida Fráguas,** Emilson Domingos da Silva***

ABSTRACT. The standard method for the diagnostic of leptospirosis is the microscopic agglutination test (MAT). Nevertheless, it is time-consuming and presents risk of infection for laboratory personal. Several attempts to simplify and reduce the time, skill and expense required for serological testing were made. Rapid slide agglutination tests (RSAT) have been used nowadays as a reliable screening test for the detection of acute and recent infections by many laboratories. The purpose of that study was to evaluate a new antigen preparation for the rapid diagnoses of acute canine leptospirosis and to compare it with MAT results in clinically suspect serum samples. Two hundred and thirteen serum samples from dogs of both sexes and different ages were tested and 141 (66.2%) animals were considered as positive by MAT. The most frequent serovar was *icterohaemorrhagiae*, followed by *canicola*. RSAT results were very similar to those observed on MAT, with 139 (65.3%) reactive animals. Correlation between RSAT and MAT was positive (0.82) and significant ($p < 0.01$). Concordance of results was of 93.4% (199/213) of the animals correctly diagnosed by RSAT. Considering MAT as the standard test, sensitivity of RSAT was calculated on 94.3% (133/141) and specificity on 91.7% (66/72).

Key words: Leptospirosis, diagnosis, dogs.

RESUMEN. El método estándar para el diagnóstico de leptospirosis es la prueba microscópica de aglutinación (MAT). Sin embargo, ésta consume mucho tiempo y presenta riesgo de infección para el personal de laboratorio. Se han realizado varios intentos para simplificar y reducir el tiempo, la habilidad y los gastos requeridos por las pruebas serológicas. La prueba de aglutinación rápida en portaobjetos (RSAT) es usada actualmente por muchos laboratorios, como una prueba tamiz para la detección de infecciones agudas y recientes. El propósito de este estudio fue evaluar una nueva preparación antigénica para el diagnóstico rápido de la leptospirosis canina aguda y comparar este resultado con el de MAT en muestras de suero de animales clínicamente sospechosos. Doscintas trece (213) muestras de sueros caninos de ambos sexos y diferentes edades fueron estudiados y 141 (66.2%) animales considerados como positivos por MAT. El serovar más frecuente fue *L. i. ser icterohaemorrhagiae*, seguido por *canicola*. Los resultados obtenidos con RSAT fueron muy similares a aquellos observados usando MAT, con 139 (65.3%) de animales reactivos. La correlación entre RSAT y MAT fue positiva ($r = 0.82$) y significativa ($p < 0.01$). La concordancia en los resultados fue de 93,4% (199/213) de los animales correctamente diagnosticados por RSAT. Considerando a MAT como el método estándar, la sensibilidad calculada de RSAT fue de 94.3% (133/141) y la especificidad de 91.7% (66/72).

Palabras clave: Leptoespirosis, diagnóstico, perros.

INTRODUCTION

The current reference standard method for the diagnostic of leptospirosis in man and animals is the Microscopic Agglutination Test (MAT), using live organisms as antigens.⁷ Nevertheless, this requires good laboratory facilities for the maintenance of live cultures of different serovars, is time-consuming and presents an infection risk for laboratory personal.¹⁸ MAT is the reference method for serological diagnosis of leptospirosis, in spite of being a complex test to control, perform and interpret.¹² This way, few medical or veterinary diagnostic laboratories offer a service for the diagnostic of leptospirosis by the MAT.¹⁹

Several attempts have been made to simplify and reduce the time, skill and expense required for serological testing. Galton *et al.*⁹ developed in 1958 a rapid slide-agglutination

screening test (RSAT) for the diagnosis of leptospirosis. Ideal test should be safe, rapid and simple, stable and accessible even to little and distant laboratories. It should also present good sensitivity rates and be indicated to the diagnosis of the acute form of leptospirosis.

Stoenner & Davis²¹ have modified the preparation of plate antigens for leptospirosis diagnostic and concluded that this antigen could be used in rapid tests, obtaining similar sensitivities with the MAT in human, porcine and bovine sera. Wolff & Bohlander²² have tested plate antigens in human serum samples, and included *L. biflexa* antigens in the test. The conclusion was that the incorporation of *L. biflexa* antigens in the rapid macroscopic test has shown a sufficient agreement with the MAT. Nevertheless, the authors suggest that, although a good concordance occurred in human serum samples, results in animal serum samples are generally less favorable.

Animal serum samples, including ten dog samples, were tested in a macroscopic slide test using a single antigen, a thermo resistant antigenic fraction of *L. biflexa* serovar Patoc. Results were considered as satisfactory for the diagnostic of the disease in those samples.¹⁵ Nevertheless, Pa-

* MVD, PhD, Department of Microbiology and Parasitology, Universidade Federal Fluminense, R. Prof. Hernani Mello, 101, Niterói-RJ, CEP 24210-130, BRAZIL, E-mail: mipwalt@vm.uff.br

** Universidade Federal Fluminense, Rio de Janeiro, Brazil.

*** Bio-Manguinhos, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

toc slide tests were later re-evaluated and considered as insensitive.¹²

Nicolescu *et al.*¹⁶ have tested 571 sera of domestic animals suspected of leptospirosis, including 32 dogs, and reported the results of macroscopic agglutination test to be encouraging and valuable as screening test for veterinarian investigations. In Brazil Yanaguita *et al.*²³ have tested several *L. biflexa* strains for the rapid screening of canine serum samples and observed a sensitivity of 77.5% for the serovar *Buenos Aires*.

Also looking for a rapid and simple test, several serological tests have been developed, such as microcapsule agglutination tests,¹ ELISAs using different antigenic preparations¹⁹ or latex agglutination tests.¹⁸ Also molecular diagnosis has been studied for either human or veterinary clinical material.^{4,12} Although rapid slide agglutination tests have not been accepted universally and are still far from the desirable characteristics described by Galton *et al.*, they can be useful both for human and animal acute infections. It has been used nowadays as a reliable screening test for the detection of acute and recent infections by many laboratories.⁷

The purpose of that study was to evaluate a new antigen preparation for the rapid diagnosis of acute canine leptospirosis and to compare it with MAT results in clinically suspect serum samples.

MATERIAL AND METHODS

Samples - Two hundred and thirteen serum samples of dogs of both sexes and different ages were tested. Animals were examined by veterinarians and considered as clinically suspects for acute leptospirosis, following signs such as fever, jaundice or other signs of hepatic or renal disease. Epidemiological aspects as presence of rodents near the houses or contact with another confirmed animal case were also considered. Those animals were bled and serum samples were separated by centrifugation and stored in 2-ml aliquots on criotubes at -20°C, to be tested as batch. Positive (canine serum of confirmed leptospirosis, after isolation of the agent) and negative (canine serum of negative animals after various serological tests) controls were also used each time that the test was performed.

Antigen - The antigen for canine leptospirosis characterization is the form of antigenic suspension. It was prepared complying with technique described by Mazzonelli *et al.*,¹⁵ modified and standardized by Oswaldo Cruz Foundation, Brazil. Strains of *Leptospira interrogans* serovars *canicola* and *copenhageni* have been grown in 10 ml of EMJH medium tubes for seven days. After incubation, cultures were examined by dark-ground microscopy for density, auto agglutination and contamination. Later were inoculated on

bottles containing 100 ml of the same medium and grown for more seven days. After growing, cultures were formalized, centrifuged and once more examined by dark ground microscopy. Supernatant was poured and sediment was re-suspended in sodium chloride solution (0.85%), obtaining this way the antigenic preparation, which was maintained at the temperature of 4°C.

Rapid Slide Agglutination test (RSAT) - Tests were performed as previously described.³ Antigenic suspension was homogenized immediately after the use. Volumes of 15µl of each undiluted serum to be tested were added to 55µl of antigenic suspension on a glass slide divided into squares (2.5 by 2.5-cm) plate and mixed with sticks. Plates were left in an orbital shaker at 120 rpm for 6 minutes at room temperature. Reactions were immediately read under direct light (shaded from the eyes) against a dark background and classified into four categories - Non-reactive (absence of clots), Weak reaction (agglutination of approximately 25% of the leptospiras, with clots dispersed on opalescent bottom), Reactive (agglutination of approximately 50%), Strong reaction (75% of agglutination dispersed on a limpid supernatant fluid).

Microscopic Agglutination test (MAT) - To perform the microscopic agglutination test (MAT), samples were screened at a 1:100 dilution using a battery of live antigens strains of *Leptospira interrogans* serovars *australis* (Bailice), *autumnalis* (Akiyami A), *bratislava* (Jez bratislava), *bataviae* (Van Tienen), *canicola* (Hond Utrecht IV), *gripotyphosa* (Moskva V), *icterohaemorrhagiae* (RGA), *copenhageni* (M 20), *pomona* (Pomona) and *wolffi* (3705) grown in liquid medium -EMJH for about 7-10 days. All samples with agglutinating activity at the dilution of 1:100 were subsequently retested against reacting antigens using serial twofold dilutions of serum until the highest titer was obtained in order to determinate the infective serovar.

Statistics. The efficiency of the serological method using RSAT was evaluated by calculating the sensitivity and specificity.⁸ Correlation between RSAT and MAT was determined through Spearman's test.²⁰

RESULTS AND DISCUSSION

Laboratory tests are necessary to confirm the diagnosis of clinically suspected leptospirosis due to its varied symptoms. Moreover, leptospirosis must always be considered during the differential diagnosis of other illnesses, especially on those where jaundice is present. Laboratory analysis relies mainly on serological methods, and the most widely used reference standard method, MAT, has many disadvantages at least for screening purposes.³

From the 213 serum samples, 193 were reactive to the standard method, the microscopic agglutination test, which

represents 90.6% of the animals studied. This high percentage was not an unexpected finding, since leptospirosis is endemic in Brazil and many dogs have at least once been in contact with the agent. Besides it, animals were all clinically suspect and had been examined by veterinarians before the sampling.

From the 193 reactive samples, 52 (24.4%) presented titres of 100, which in Brazil are not enough to confirm diagnosis of acute leptospirosis, since background leptospiral antibodies might be present. For that study, we considered as necessary a minimum titre of 200. Therefore, the number of real positive animals, following the definitions described above, was of 141/213, which represents 66.2% of the studied dogs. One hundred twenty five (58.7%) animals presented titres ranging from 200 to 3200, and sixteen dogs (7.5%) titres from 6400 up to 102.400 (two animals).

In relation to the serovars observed at MAT, the great predominance was of serovar *icterohaemorrhagiae*, with 145 reactive samples (68.1%), followed by *canicola* (18.3%) and, less frequently, *pomona* (2.3%), *bratislava* (1.0%) and *grippityphosa* (1.0%). The distribution of the reactive serovars may be observed in Table 1.

In Brazil and many other parts of the world serovars *canicola* and *icterohaemorrhagiae* have been traditionally associated with canine leptospirosis. In this study, as expected,

those serovars were the most commonly observed, what agrees with other studies in Brazil^{5,6,14} and other countries.^{11,2} Serovars *pomona*, *bratislava*, and *grippityphosa* have emerged as significant causes of canine leptospirosis in North America and elsewhere.^{17,2,10} In the present study, a low incidence of those serovars was observed, especially because of the high spread of *icterohaemorrhagiae*, the most commonly serovar observed in Brazil. This is not an unexpected finding, since rodent control is not available in many regions of the country and 30% of the urban rats are infected with *icterohaemorrhagiae* strains of *Leptospira*.¹³

The Rapid Slide Agglutination test (RSAT) results were very similar to those observed on MAT. Twenty samples (9.4%) were Non-reactive to this test, and 54 (25.3%) were considered as Weakly reactive. Sixty-eight samples (31.9%) were classified as Reactive, and the most frequent result was Strong reaction, observed on 71 (33.4%) of the samples. Considering that, in order to confirm diagnosis of acute leptospirosis we determined as necessary a minimum Reactive result, than 139 (65.3%) animals were diagnosed as leptospirosis by the RSAT (Table 2).

Expected results were that Non-reactive results at RSAT should present titres zero at MAT, Weak samples should correspond to titres of 100, Reactive as 200 or 400 and Strong results at RSAT should correspond to titres > to 400 at MAT. Considering those definitions, the correlation of Non-reactive samples was of 95% (19/20), with one sample with titres of 200 and of 87% (47/54) to Weak results at RSAT, with seven serum samples presenting titres > than 200, even one sample with titre of 3200 and one of 6400 at MAT.

Correlation of Reactive results at RSAT was of 88.2% (60/68), with five samples presenting titres less than 200 and three samples presenting higher titres, up to 102.400 (one sample) at MAT. When the observed result at RSAT was Strong, correlation to MAT was of 91.5% (65/71), with one sample presenting titres of 100 and five samples with titres of 400 at MAT.

Table 1. Distribution of the reactive serovars of 213 canine serum samples by Microscopic Agglutination Test (MAT).

Serovar	Nº	%
<i>Icterohaemorrhagiae</i> RGA	145	68.1
<i>Canicola</i>	39	18.3
<i>Pomona</i>	5	2.3
<i>Bratislava</i>	2	1.0
<i>Grippityphosa</i>	2	1.0
Negative	20	9.3
Total	213	100.0

Table 2. Distribution of 213 canine serum samples by Microscopic Agglutination Test (MAT) and Rapid Slide Agglutination Test (RSAT)

RSAT	MAT titre					Total
	Negative		Positive			
	Zero	100	200	400	> 400	
Negative						74
Non-reactive	19	-	1	-	-	20
Weak	-	47	1	1	5	54
Positive						139
Reactive	1	4	28	32	3	68
Strong	-	1	-	5	65	71
Total	20	52	30	38	73	213

In order to simplify the interpretation of the results, we decided to rename Non reactive and Weak reactions as Negative and Reactive and Strong reactions as Positive at RSAT. At MAT samples with titres less than 200 were considered as Negative and more than that dilution as Positive. Therefore, the comparison of the two tests was facilitated, and the concordance was of 89.2% (66/74 samples) to Negative results, with eight false-negative samples to RSAT. One of those false-negative samples was reactive at MAT to serovar *pomona*, which was not included on the composition of the antigen, which could explain the false result. In relation to Positive samples at RSAT, concordance was of 95.7% (133/139) and six samples negative at MAT were positive at RSAT (Table 2). Considering all tested samples and using MAT as the standard test, the concordance was of 93.4% (199/213) of the animals correctly diagnosed by RSAT. Sensitivity of RSAT was calculated on 94.3% (133/141) and specificity on 91.7% (66/72). After statistical analysis through the Spearman's test, correlation between RSAT and MAT was positive (0.82) and significant ($p < 0.01$).

Those findings are very similar to those observed in Brazil using RSAT in human serum samples³ that reported high sensitivity (99%) and specificity (99%) to that test. When compared to other tests specificity of RSAT was very similar to ELISA, with 95.6% of specificity when compared to MAT,¹⁹ and superior to the majority of the molecular tests, considered being less sensitive than serology.¹² In the present study, we had not opportunity to retest the same animals after some months in order to evaluate the use of RSAT in the convalescent phase. Nevertheless, based on the findings of the acute phase and on the reports of other authors,^{7,3} we believe that RSAT may be specially indicated for the diagnosis of recent infections and of the acute phase of leptospirosis, when antibodies are more evident.

The main disadvantages of RSAT are that it is not suitable for epidemiological studies, identification of strains, assessment of the probable infecting serogroup, and confirmation of illness for public health surveillance. Interpretation may be doubtful and it shall be used only in acute cases of clinical leptospirosis, mainly in endemic areas. Infection by others serovars, such as *pomona*, *bratislava* and *grippotyphosa*, that have been increasing in some countries, are not well detected by this test. For those reasons, MAT, particularly when paired serology is possible, remains as the best diagnostic method available.

In summary, this study demonstrates that RSAT using formalized antigenic suspension of *L.i. ser. canicola* and *copenhageni* strains could be used as a screening test in the diagnostic of acute canine leptospirosis. It is more simple, faster and less expensive to perform than MAT and may be

used for the screening of large numbers of serum specimens. It is also comparable to ELISA or to Latex agglutination tests as an initial screening test for the detection of leptospiral antibodies in canine sera, with subsequent confirmation of results with the MAT, as suggested.^{18,19} In addition, it can be performed at the less specialized routine clinical laboratories, and, when compared to MAT, presented good sensitivity and specificity rates.

ACKNOWLEDGEMENTS

The authors would like to thank the assistance of Mr. A. Carmo, Dr. G.M. Oliveira, Dr. G.N. Souza and Dr. F. Palicchi for their collaboration.

REFERENCES

1. Arimitsu, Y., Kmety, E., Ananyina, Y., Baranton, G., Ferguson IR, Smythe, I. and Terpstra, W.J. 1994. Evaluation of the one-point microcapsule agglutination test for diagnosis of leptospirosis. Bull. WHO, 72:395-399.
2. Bolin, C.A. 1996. Diagnosis of Leptospirosis: A reemerging disease of companion animals. Sem. Vet. Med. Surg. 11:166-171.
3. Brandao, A.P., Camargo, E.D., Silva, E.D., Silva, M.V. and Abrao, R.V. 1998. Macroscopic agglutination test for rapid diagnosis of human leptospirosis. J. Clin. Microbiol. 36:3138-3142.
4. Cai HY, Hornby G, Key DW, Osuch MR, Maxie MG. 2002. Preliminary study on differentiation of *Leptospira grippotyphosa* and *Leptospira sejroe* from other common pathogenic leptospiral serovars in canine urine by polymerase chain reaction assay. Vet. Diagn. Invest. 14:164-168
5. Caldas, E.M. and Sampaio, M.B. 1979. Leptospirosis in the city of Salvador, Bahia, Brazil. Int. J. Zoon. 6:85-96.
6. Cordeiro, F. and Sulzer, C.R. 1983. *Leptospira interrogans*, serovar *copenhageni*, isolated from a dog in Belo Horizonte, Brazil. Rev. Microbiol. 14:38-41.
7. Faine, S., Adler, B., Bolin, C., Perolat, P. 2000. *Leptospira* and Leptospirosis. MedSci, Melbourne, Austrália, 2nd Ed.
8. Galen R.S. and Gambino, C.R. 1975. Beyond normality: the predictive value and efficiency of medical diagnosis. John Wiley and Sons, New York.
9. Galton, M.M., Powers, D.K., Hall, A.D. and Cornell, R.G. 1958. A rapid macroscopic-slide screening test for the serodiagnosis of leptospirosis. Am. J. Vet. Res. 72:505-512.
10. Harkin, K.R. and Gartrell, C.L. 1996. Canine leptospirosis in New Jersey and Michigan: 17 cases (1990-1995). J. Am. Anim. Hosp. Assoc. 32: 495-501.
11. Heat, S.E. and Jhonson, R. 1994. Leptospirosis. JAVMA, 205:1518-1523.
12. Levett, P. Leptospirosis. 2001. Clin. Microbiol. Rev. 14:296-326.
13. Lilenbaum, W., Ribeiro, V., Martin, E. and Bispo, V. 1993. Serological study for the detection of antibodies anti-leptospira in *Rattus norvegicus* from Duque de Caxias, Rio de Janeiro, Brazil. Rev. Latinoam. Microbiol. 35:357- 360.
14. Lilenbaum, W., Ribeiro, V.L.S. and Brunstein, R. 1994. Leptospirosis in dogs clinically suspect in Rio de Janeiro, Brazil. Hora Veterinária, 81:48-49.
15. Mazzonelli, J.D., Mazzonelli, M. and Mailloux, M. 1974. Possibilité de diagnostic sérologique macroscopique des leptospiroses à l'aide d'un antigène unique. Med. Mal. Infect. 4:253-254.

16. Nicolescu, M., Andreescu, N., Lupan, E. and Pop, A. 1982. The serological diagnosis of leptospirosis in domestic animals by the macroscopic agglutination test with patoc antigen. Arch. Roum. Path. Exp. Microbiol. 41:11-13.
17. Nielsen, J.N., Cochran, G.K., Cassells, J.A., Phil, B. and Hanson, L.E. 1991. *Leptospira interrogans* serovar *bratislava* infection in two dogs. JAVMA. 199:351-352.
18. Ramadass P, Samuel B and Nachimuthu K. 1999. A rapid agglutination test for detection of leptospiral antibodies. Vet Microbiol. 70:37-140
19. Ribotta, M.J., Higgins, R., Gottschalk, M. and Lallier, R. 2000. Development of an indirect enzyme-linked immunosorbent assay for the detection of leptospiral antibodies in dogs. Can. J. Vet. Res. 64:32-37
20. Sampaio, I.B.M. 1998. Statistics applied to animal experiments. First Edition. UFMG, Belo Horizonte, Brazil. Pp 221.
21. Stoenner, H.G. and Davis, E. 1967. Further observations on leptospiral plate antigens. Am. J. Vet. Res. 28:259-266.
22. Wolff, J.W. and Bohlander, J. 1967. Screening tests in human serum samples with *Leptospira biflexa* antigens incorporated in Galton's macroscopic slide test. Trop. Geogr. Med. 19:63-69.
23. Yanaguita, R.M. 1987. Diagnóstico rápido da leptospirose animal pela soroaglutinação macroscópica com antígenos de *Leptospira biflexa*. (Thesis). Universidade de São Paulo, Pp 68.

Correspondence to:

Dr. Walter Lilenbaum

Department of Microbiology and Parasitology
Universidade Federal Fluminense
R. Prof. Hernani Mello, 101,
Niterói-Rj, CEP 24210-130
Brazil
E-mail: mipwalt@vm.uff.br