A COMPARATIVE STUDY USING MAT, ELISA AND ST FOR SERODIAGNOSIS OF LEPTOSPIROSIS

BY

Attia, E.R.H. and Ibrahim, I.G.A.
Animal Reproduction Research Institute, El-Haram EL Giza,

SUMMARY:

Two hundred and eighty nine serum samples were collected from cows suffering from reproductive disorders at private farms in Egypt. These samples were serologically examined by Microscopic Agglutination Test (MAT), Enzyme Linked Immuno Sorbent Assay (ELISA) and Macroscopic Agglutination Slide Test (ST) for detection of leptospiral antibodies. The obtained results revealed that antibodies against Leptosira (L) grippotyphosa, L. hardjo and L.pomona were detected in an incidence of (5.2 %), (3.8 %) and (1.4 %) respectively by using MAT. Similar results were obtained using ELISA with higher sensitivity and specificity, while (10.4 %), (6.9 %) and (2.8 %) respectively were detected by using ST. It could be concluded that MAT, ST and ELISA can be used to screen leptospiral infection in cattle, however the ELISA is highly sensitive, specific, rapid and highly effective to detect antibodies in cattle with positive titre of 1:200 and more.

INTRODUCTION:

Leptospirosis is a zoonotic disease with a worldwide distribution and causes high economic losses due to abortion, stillbirth, decreased milk production, infertility and death of young calves. The diagnosis of bovine Leptospirosis is usually based on serologic grounds because leptospirae are difficult to be isolated from tissues or fluids from infected animals (Fairbrother, 1985). A series of different methods in

2) Macroscopic Agglutination Slide Test (ST) (Faine, 1982 and Attia 1993):

The dead antigens were prepared from the abovementioned serovars, varying densities of antigens, determined by measuring the percentage of light transmission from 20 to 80 % on photoelectric colorimeter were tested both for sensitivity and ease of reading macroscopically. It was found that antigens adjusted 45 - 48 % light transmission at 550 nanometer read with less difficulty. The antigens were stored at 4 ^{0}C with pH 5.4 to 5.8, for serological studies 50 ul of each antigen was mixed with 50 ul of undiluted serum sample on a glass slide and rotation of the plate by hand. Then slide was placed on mechanical rotators, with speed regulator.

3) ELISA (Sting and Dura, 1994): -

The antigen was prepared from a dense culture in EMJH medium of the above mentioned reference leptospiral serovars were harvested by centrifugation at 10000 Xg of cultivated EMJH medium. The leptospirae were subsequently washed three times using 0.01 M PBS at pH 7.2.

Between each washing step the bacteria were spun down by centrifugation. After the washing procedures, the leptospirae were resuspended using 1 % sodium cholate and incubated in a water bath at 50 ^{0}C for 1 h. thereafter cell-free supernatants were obtained by centrifugation, the ELISA microtitre plates were coated with the extracted leptospiral antigens.

The antigen solutions were diluted 1:100 in coating buffer (0.2 M carbonate / bicarbonate buffer pH 9.6) and each well of the microtitre plates was filled with 0.1 ml. After incubation of the microtitre plates at 37 ^{0}C for 24 h, the plates were washed three times with washing buffer then dried at 37 ^{0}C and stored at 4 ^{0}C until use within 1 week, serum samples were diluted 1:100 in washing buffer. The diluted sera were pipetted in volumes of 0.1 ml in duplicate into each well of the microtitre plates coated previously with the extracted antigen serovar specific sera from immunized rabbits as a control positive and a serum obtained from a seronegative rabbit served as a negative control.

After incubation, the microtitre plates were washed three times and each well filled with 0.1 ml of antispecies immunoglobulins from cows with peroxidase diluted in the same buffer mentioned above. After 1 h of incubation at 37 ^{0}C, the plates were washed again three times, 0.1 ml of OPD – 0 – phenylenidiamine as a

substrate containing $H_2 O_2$ was pipetted into each well and optical densities were read at 405 nm. A positive reaction was indicated by a brown colour. The reaction exceeding two fold values of the negative control was considered as positive.

RESULTS:

A total of 289 serum samples were collected from cows, suffering from different reproductive disorders. The sera were subjected for three serologic tests as a comparative study.

Table (1) Summarizes the results obtained by MAT, it was noticed that leptospiral agglutinins against three leptospiral serovars were detected in 30 out of 289 (10.4%); meanwhile, *L.grippotyphosa* antibodies was the most predominant in cow sera with (5.2%) of the total number of the examined sera followed by *L.hardjo* (3.8%) and then *L. pomona* (1.4%).

Table (2) Summarizes the results obtained by ELISA it was noticed that these results were similar to that obtained by MAT.

Table (3) Summarizes the results obtained by ST, it was noticed that the results were higher than that obtained by MAT and ELISA because serum samples were undiluted and this test was used as screening test.

Table (4) Summarizes the comparison between different serologic tests using MAT, ELISA and ST on examined cow sera and it was noticed that similar results were obtained using MAT and ELISA at serum dilutions 1 ? 200.

DISCUSSION:

Serological tests for leptospirosis are used not only for diagnosis of infection in humans but even more frequently used for serodiagnosis in domestic animals. Tests are also applied for epidemiological studies, which frequently entail retrospective determination of antibodies which may have been provoked many months previously.

In the present study, the results of serological examination of 289 undiluted cow sera using ST agglutinins were detected against *L.grippotyphosa, L.hardjo* and *L.pomona* in percentages of (10.4 %), (6.9 %) and (2.8 %) respectively as shown in (table 3). The obtained results agreed with Attia et al (1996), and with Stoenner and

Davis (1967) who evaluated this test and recorded that it was specific and sensitive for detection of leptospiral antibodies in human and animal sera, but they stated that the ST was not as sensitive as the MAT when used for the serological survey. Also Trap and Gaumont (1980) reported that this test is simple and rapid and it can be employed as a screening test especially in herds with reproductive disorders. However they stressed on the fact that the MAT must always be used in the epidemiological studies in herds with long standing leptospiral infection.

On the other hand, the results of serological examination of 289 cow sera using both MAT and ELISA as shown in tables (1&2),
positive agglutinins at dilution ≥ 1:200 was detected against *L.grippotyphosa, L.hardjo and L.pomona* in percentages of (5.2 %), (3.8 %) and (1.4 %) respectively for MAT. Similar results were obtained using ELISA, the comparison of the various serologic tests for the detection of leptospiral antibodies in infected cattle indicates a harmony between ELISA and MAT where the ELISA has excellent specificity and sensitivity in correlation to MAT.

The results obtained by MAT were in agreement with those reported by Schonberg et al (1985), Corboz et al (1987), Wanyangue et al (1987), Turner (1988), Attia (1993), Attia et al (1996), Collares-Pereira and Rocha (1991) and Woodward et al (1997).

The results obtained using ELISA in comparison with that obtained by MAT meet with that obtained by Thiermann and Garrett (1983) who reported that the ELISA antiglobulin system using the homologous antigen, is the most sensitive test. Although its sensitivity was considerably higher than the MAT and the ELISA anti Ig G system, no false-positive results were obtained when testing known negative samples.
They also added that the MAT may be detected mostly Ig G levels, when using ELISA with pomona antigen in samples of hardjo-infected animals, some antibody levels were detected; this occurs in those samples showing high homologous titers. Some somatic antigenic components have a role in this test, whereas the MAT reaction is mostly based on agglutination of surface antigen components present on the live organisms.

Sting and Dura (1994) reported that the ELISA proved to be somewhat more sensitive than the MAT. Cho et al. (1989) and Mendoza and Prescott (1992) also reported high sensitivity of ELISA system when testing sera from cattle. The obtained results were in agreement with that obtained by Woodward et al (1997), Ramadass et al (1999), Ribotta et al (2000) and Surujballi and Mallory (2001). They reported that the ELISA is easily standardized technically more advantageous than MAT and uses antigenic preparation that can be routinely prepared in large amounts.

Therefore it can be concluded that the ELISA is a sensitive and reproducible test, also offers the advantage of using purified dead antigen, which are readily stored and transported. It does not require the costly and tedious maintenance of a constant supply of fresh leptospiral cultures of numerous serovars and avoiding the hazard of personel infection during dealing with the live strains.

REFERENCES:

Alexander, A.D.; Gochenour, W.S.; Reinhard, K.R.; Ward, M.K and yager, R.H. (1970): Leptospirosis. Am. Pub. Hlth. Assoc. Inc., New York pp. 382 – 421.

Attia, E.R.H. (1993); Microbiological Assay of Animal leptospirosis in Egypt. Ph.D. Thesis, Fac of Vet. Med., Micorbiol Dep., Alexandria university.

Attia, E.R.H.; Ibrahim I.G.A. and.Tawfik M.S. (1996): Dot Enzyme Linked Immunosorbent Assay as a Quick Reliable diagnostic aid for Leptospirosis. Assiut Vet Med. J. vol. 36 No 71, 1-5

Cho, H.J., Gale, S.P.,.Masri, S.A and Malkin K.L. (1989): Diagnostic specificity, sensitivity and Cross reactivity of an Enzyme Linked Immuno Sorbent Assay for the detection of antibody against Leptospira interrogans serovars pomona, sejroe and hardjo in Cattle. Can. J. Vet. Res. 53, 285 – 289.

Collares–Pereira M. and Rocha, T. (1991): Leptospira infection in cattle serologically negative for brucellosis in Portugal, Revista Portuguesa deciencias veterinarias 86, 90 – 94.

Corboz, L.; Brieger, C.; Brack, A. and Bertschinger, H. (1987): Epidemiology of leptospirosis in swiss cattle: Prevalence of L.hardjo in carriers and its role in abortion. Trop. And Georg. Med., 39 (1) 57 – 65.

Faine, S (1982): Guide lines for the control of leptospirosis, Offset Publication no. 67. WHO, Geneva.

Fairbrother,J.M.(1985): Antibody response to genus and serovar specific leptospiral antigens in leptospira infected cows. Am. J.vet. Res. Vol. 46, (7), July.

Mendoza, L. and Prescott, J.F. (1992) Serodiagnosis of leptospirosis in pigs using an axial filament Enzyme Linked Immuno Sorbent Assay. Vet. Microbiol. 31, 55 – 70.

Ramadass P, Samuel B and Nachimuthu K. (1999) A rapid latex agglutination test for detection of leptospiral antibodies. Vet. Microbiol; 70 (1 – 2), 137 – 140.

Ribotta, MJ; Higgins R; Gottschalk M; Lallier R (2000) Development of an indirect Enzyme Linked Immuno Sorbent Assay for the detection of leptospiral antibodies in dogs. Can. J. Vet. Res.; 64 (1) 32 – 37.

Schonberg, A.; Staak, C. and Kamp, U. (1985): leptospirosis in the Federal Republic of Germany. Results of an investigation programme in animals carried-out in 1984.5th meeting of European leptospira workers, Amsterdam 10 - 11 October1985. Published in Tropical and Geographical Medicine 39 (1), 55 – 63.

Surujballi O and Mallory M. (2001) Competitive Enzyme Linked Immunosorbent Assay for detection of leptospira interrogans serovar pomona antibodies in bovine sera. Clin. Diagn. Lab. Immunol.; 8 (1): 40 – 43.

Sting, R. and Dura, U. (1994): Isolation of serovar-specific leptospiral antigens for use in an Enzyme Linked Immuno Sorbent Assay (ELISA) compared with the Microscopic Agglutination Test and Immunofluorescence. J.Vet.Med. B. 41, 166 – 175.

Stoenner, H.G. and Davis, E. (1967): Further observation of leptospiral plate antigens. Am. J. Res, 28; 259 – 266.

Thiermann A.B., and Garrett L.A. (1983) Enzyme Linked Immuno Sorbent Assay for the detection of antibodies to Leptospira interrogans serovars hardjo and pomona in cattle. Am.J.Res. 44, (5) 40 – 48.

Trap, D. and Gaumont, R. (1980): Serological diagnosis of leptospirosis: Comparison of results obtained by macroscopic slide agglutination test and an agglutination lysis test. Ann. Res. Vet., 11 (3); 241 – 244.

Turner, G.V. (1988): The seroepidemiology of bovine leptospirosis in a densely populated cattle area: preliminary findings Acta Vet. Scand 84, 274 – 276.

Voller, A., Bartlett A., and Bidwell D.E. (1978): Enzyme immunoassay with special refernce to ELISA techniques. J. Clin. Pathol. 31, 507 – 520.

Wanyangue, S.W., Olubayo,.O.R Ositter, P.B. and Waitkins, S.A. (1987), The study of the ecology and prevalence of leptospirosis in large wild ruminants and domesticated bovines found in Kenya. Israel, J. Vet. Med., 43 (4) 340 – 341.

Woodward M.J, Swallow C, Kitching A, Dalley C, and A.R. Sayers (1997): Leptospira hardjo serodiagnosis a comparison of MAT, ELISA and Immunocomb Vet. Rec. 6., 603 – 604.

Table (1) MAT results on 289 cow sera for detection of leptospiral antibodies

serovar	End titres					1 ? 200	
	1/ 200	1 / 400	1/800	1/1600	1/3200	No	%
L.grippotyphosa	2	6	4	3	--	15	5.2
L.hardjo	1	3	3	4	--	11	3.8
L.pomona	1	3	--	--	--	4	1.4
Total	4	12	7	7	--	30	10.4

Table (2) ELISA results on 289 cow sera for detection of leptospiral antibodies

serovar	End titre					1 ? 200	
	1/200	1 / 400	1/800	1/1600	1/3200	No	%
L.grippotyphosa	2	6	4	2	1	15	5.2
L.hardjo	1	3	3	5	--	12	4.2
L.pomona	1	2	--	--	--	3	1.0
Total	4	11	7	7	1	30	10.4

Table (3) ST results on 289 undiluted cow sera for detection of leptospiral antibodies

Dead Antigen	Positive	
	No	%
L.grippotyphosa	30	10.4
L.hardjo	20	6.9
L.pomona	8	2.8
Total	58	20.1

Table (4): Comparison between different serologic tests on 289 cow sera for detection of leptospiral antibodies:

Serovar	Serological test					
	MAT		ELISA		ST	
	No positive	%	No positive	%	No positive	%
L.grippotyphosa	15	5.2	15	5.2	30	10.4
L.hardjo	11	3.8	12	4.2	20	6.9
L.pomona	4	1.4	3	1.0	8	2.8
Total	30	10.4	30	10.4	58	20.1

الملخص العربى

دراسة مقارنة باستخدام اختبار التلزن الميكروسكوبى والأليزا واختبار التلزن العيانى للتشخيص السيرولوجى لليبتوسبيرا

. السيد رضوان حسن عطية و إبراهيم جاد عبد الله

تم فحص عدد ٢٨٩ عينة سيرم من أبقار تعانى من مشاكل تناسلية من مزارع خاصة فى مصر وتم فحص هذه العينات سيرولوجيا باستخدام ثلاث إختبارات مختلفة وهم إختبار التلزن الميكروسكوبى وإختبار التلزن العيانى والأليزا ، وذلك للكشف عن الأجسام المناعية لميكروب الليبتوسبيرا ، وأظهرت النتائج وجود أجسام مناعية ضد العترات الأتية : الجريبوتيفوزا والهاردجو والبومونا بنسب (٥,٢ %) ، (٣,٨ %) ، (١,٤ %) على التوالى مستخدما إختبار التلزن الميكروسكوبى ، وأظهرت الأليزا نتائج مشابهة مع نتائج إختبار التلزن الميكروسكوبى بينما اختلفت نتائج إختبار التلزن العيانى وظهرت بالنسب الأتية (١٠,٤ %) ، (٦,٩ %) ، (٢,٨ %) على التوالى .

من هذه الدراسة يمكن إستنتاج أن الإختبارات السيرولوجية الثلاثة صالحة لفحص الأبقار المصابة بالليبتوسبيرا وأن الأليزا كان اكثر حساسية وسريع وفعال جدا فى الكشف عن الأجسام المناعية فى الأبقار الإيجابية عند تخفيف ١ : ٢٠٠ فأكثر .