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DOT ENZYME LINKED IMMUNOSORBENT ASSAY AS A QUICK RELIABLE DIAGNOSTIC AID FOR LEPTOSPIROSIS

(With One Table and One Fig)

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الدوت اليزا كوسيله تشخيصيه سريعه فى تشخيص مرض الليبتوسبيرا

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تم جمع عدد ٢٠٠ عينه سيرم أبقار من حالات تعانى عدم الخصوبه من مزارع خاصه فى مصر. وتم فحص هذه العينات سيرولوجيا باستخدام ثلاث تجارب وهى اختبار التلزن الميكروسكوبى - اختبار التلزن العيانى- الدوت اليزا وذلك للكشف عن الأجسام المناعية لميكروب الليبتوسبيرا. أظهرت النتائج وجود أجسام مناعية ضد العترات الأتيه الهاردجو- الجريبوتيفوزا- الأكتيروهيموراجيا بنسب (٦٪، ٥٪، ١٪) بالترتيب وذلك باستخدام كل من أختبار التلزن الميكروسكوبى والدوت اليزا بينما كانت النسب (٥و١٢٪، ٥و٧٪، ٥و٢٪) على الترتيب باستخدام أختبار التلزن العيانى. من هذه الدراسه يمكن أستنتاج أن الأختبارات الثلاثه صالحه لفرز الأبقار المصابة بالليبتوسبيرا وأن إختبارات الدوت اليزا كانت سهله وسريعه وحساسه جدا للكشف عن الأجسام المناعيه فى الأبقار الأيجابيه عند تخفيف ١: ٢٠٠ فأكثر.

SUMMARY

Two hundred cowserum samples were collected from infertile cases at private farms in Egypt. These samples were serologically screened by Microscopic Aglutination Test (MAT), Macroscopic Agglutination Test and Dot ELISA for detection of leptospiral antibodies. The obtained results

revealed that antibodies against *L.hardjo, L.grippotyphosa and L.icterohaemorrhagiae* were detected in a percentages of (6%), (5%) and (1%) respectively by using MAT and Dot ELISA, while (12.5%), 7.5%) and (2.5%) respectively by using Macroscopic Agglutination Test. It can be concluded that MAT, Dot ELISA and Macroscopic Agglutination Test can be used to screen leptospiral infection in cattle. However, Dot ELISA was easy,rapid and highly sensitive to detect leptospiral antibodies in cattle with positive titre at 1: 200 and more.

Key words: *Leptospirosis-Diagnosis-Dot ELISA.*

INTRODUCTION

Leptospirosis is a common zoonotic disease affecting most mammals. In livestock, the disease causes high economic losses due to abortion, stillbirth, decreased milk production, infertility and death of young ages. The diagnosis of bovine leptospirosis is usually based on serologic grounds, because leptospira are difficult to be isolated or detected in tissues or body fluids from infected animals (Fairbrother, 1985). The Microscopic Agglutination Test (MAT) is the standard technique for serological diagnosis of leptospirosis (Alexander et al., 1970) but its complexity limits its use to reference laboratories. Simpler techniques have been described, but information is lacking on their relative merits and adaptability to endemic areas where sophisticated laboratories may be absent. The Dot ELISA was recently shown to be comparable to the MAT in its ability to detect recent exposure to leptospirosis; it is also rapid and simpler to use (Pappas et al. 1985), therefore, the aim of the present study is to compare the efficiency and reliability of the Dot ELISA in the serology of cattle leptospirosis for the first time in Egypt.

MATERIALS and METHODS

Samples

blood samples were collected from 200 cows at private farms in Egypt and serum was harvested. These animals were non pregnant and suffering from infertility.

Leptospiral Strains

Leptospiral reference serovars: *hardjio, grippotyphosa, pomona, canicola* and *icterohaemorrhagiae with specific reference antisera (as control positive)* were kindly obtained from C. Sulzer, C.D.C, Atlanta, U.S.A.

Serological diagnosis

1) Microscopic Agglutination Test (MAT) (Alexander et al. 1970):

A serial double fold serum dilutions is done using Phosphate Buffer Saline (P.B.S.) begining with dilution 1/100. We considered positive titre at 1/200 and more.

2) Macroscopic Agglutination Test (Faine, 1982)

3) Dot Elisa (Pappas et al. 1983, 1985):

In brief, reference leptospiral strains were cultured for 7-10 days in E.M.J.H. media, then transefered to Nitrocellulose paper (NC). NC was treated with test sera and control positive sera (reference antisera) prepared in rabbit, and negative control sera, peroxidase conjugated antibovine and antirabbit immunoglobulins were added. 4-Chloro-1- naphthol and H_2O_2 as a substrate were added. Apurple colouration was developed in the presnece of specific reaction (control positive).

DISCUSSION

An accurate, rapid method for diagnosing leptospirosis has vital importance for Doctors and diseased animals. The results of this study are therefore encouraging. Concerning the results of serological examination of 200 cow sera using MAT we detected positive agglutinins at dilutions >1:200 against *L.hardjo, L.grippotyphosa* and *L.icterohaemorrhagiae* in percentages of (6%), 5%) and (1%) respectively. The same results of the same samples were obtained by using Dot ELISA, while, with Macroscopic Agglutination Test we detected agglutinins against *L.hardjo, L.grippotyphosa* and *L.ictero-haemorrhagiae* in percentages of (12.5%), 7.5%) and (2.5%) respectively.

The results of MAT were in agreement with those reported by Attia (1993), Turner (1988), Corboz et al. (1987), Wanyangue et al. (1987), Schonberg et al.(1985), Ellis et al. (1981) and Tawfik (1977).

Concerning the result of Macroscopic Agglutination Test, we detected agglutinins against *L.hardjo, L.grippotyphosa* and *L.icterohaemorrhagiae* in percentages of (12.5%), 7.5%) and (2.5%) respectively. The obtained results

agreed with Attia (1993) and with Stoenner and Davis (1967) who evaluated this test and recorded that it was sensitive for detection of leptospiral antibodies in human and animal sera, but they stated that the Macroscopic Agglutination Test was not as sensitive as the MAT when used for the serological survey. Also Trap and Gaumont (1980) reported that this test was simple and rapid and it can be employed as a screening test especially in herds with reproductive troubles. However, they stressed on the fact that the MAT must always be used in the epidemiological studies in herds with long-standing leptospiral infection.

Concerning the results obtained by Dot ELISA, it shows a harmony with the results of MAT where the specificity of Dot ELISA was excellent and there were no false positive results. The results were in agreement with that obtained by Watt et al. (1988), who reported that the Dot ELISA was more rapid than MAT and required no electrical equipments and only one serum dilution, wherease a Dark Field Microscope and several serum dilutions were needed for the MAT. Also, Kotani et al. (1987) detected positive reactions with antisera in order to 1: 1000 to 1: 30000 dilutions. Ahmed and Ibrahim (1996) reported that, an advantage of ELISA is its capability to detect specific Ig M. Ig A and Ig G in serum, which helps in preparing antibody-response profile of infected animals.

In conclusion, it was evident that MAT, Macroscpoic Agglutination Test and Dot ELISA can be used to screen leptospiral infection in cattle. However the Dot ELISA was easy, rapid and highly effective to detect antibodies in cattle with positive titre > 1: 200.

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Table (1) : Serological diagnosis of leptospiral antibodies in 200 cow sera

Serodiagnostic test	*L. hardjo*		*L. grippotyphosa*		*L. ictero - haemorrhagiae*	
	No.of Positive	%	No.of Positive	%	No.of Positive	%
Microscopic	12	6	10	5	2	1
Macroscopic	25	12.5	15	7.5	5	2.5
Dot ELISA	12	6	10	5	2	1

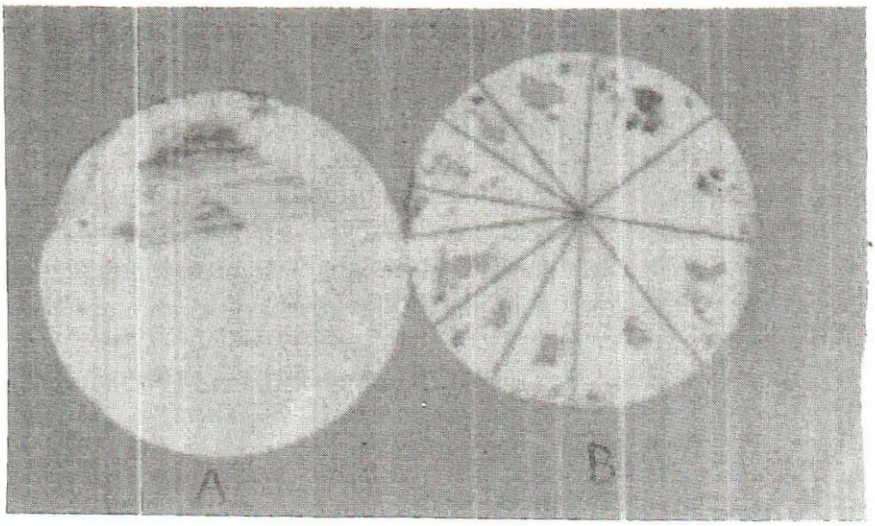

Fig. (1) A. Positive and negative control for leptospiral antibodies by Dot ELISA
B Positive sera for leptospiral antibodies by Dot ELISA