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SEROLOGICAL AND BIOCHEMICAL STUDIES ON BOVINE LEPTOSPIROSIS

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ABSTRACT

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In the present study 286 cows with reproductive disorders were serologically tested for natural infection with the most prevalent leptospiraserovars using MAT and ELISA. Estimation of the corresponding serum biochemical changes were carried out also. Serological survey on cows sera showed that leptospiral seropositivity using MAT and ELISA were (45.81%) and (52.10%) respectively. The most prevalent antibodies were detected against *L*.grippotyphosa*, L*. Canicola, *L*.Pomona, *L*.icterhaemorrhagiae*, L*.wolfi using MAT (11.89 %, 10.49 %, 10.49 %, 8.55 % and 4.55 %) respectively. And using ELISA (12.94 %, 12.59 %, 11.54 %, 10.14 % and 4.89 %), respectively. The biochemical analysis of cow sera revealed that cows suffered from leptospirosis showed increase in blood urea, creatinine, ALT, AST and GGT.

Key words: *Bovine leptospirosis, Serological; biochemical studies.*

INTRODUCTION

Leptospirosis is an anthropozoonosis caused by a spirochete of the genus Leptospira that lives mainly among rodents. Transmission to humans primarily occurs through contact with environments contaminated by the urine of infected animals causing serious problems including hepato-nephritis and clinical diagnosis is usually difficult because of the clinical polymorphism (Levett, 2001). Early diagnosis of leptospirosis is urgent for effective medical care, improving patient outcomes (Assez *et al.,* 2013).

The genus Leptospirais classified serologically into two species, the pathogenic species *L. Interrogans* and the saprophytic species *L. biflexa*. There are more than 200 serovars of *L. interrogans* and more than 60 serovars of *L. biflexa*. Most of these serovars can infect different animal species, but there is a primary host reservoir for each serovar, which ensures the survival and dissemination of the organisms (Birnbaum *et al.,* 1998). Leptospirosis causes high economic losses in live-stock due to abortion, still-birth, decreased milk production, and death of young ages. The present work was adopted to fulfill two tasks: a) diagnosis of leptospirosis in dairy herds by using two serological techniques (Microscopic Agglutination Test (MAT) and ELISA, b) estimation of some biochemical parameters to study the effect of leptospirosis on animal health.

MATERIALS and METHODS

1. Animals:-
Two hundred and eighty six (286) cows were investigated in this study. These animals were distributed in private farms in five governorates (Behera, Alexandria, Kalubia, Sharkia and Assiut) in Egypt. Some of them suffered from repeat breeders (36), some suffered from abortion (13), some had abnormal milk (100) and 137 cows were apparently healthy. All cows were non vaccinated to Leptospira.

2. Samples:
Blood sera samples of the investigated cows were collected and stored at -20°C till serological examination except sera that used for estimation of liver enzymes were immediately tested.

3. Leptospiraserovars:
Five leptospiraserovars were obtained from Animal Reproduction Research Institute, the source of these reference strains was leptospirosis reference laboratory in Center for Diseases Control (C.D.C.), Atlanta, Ga.30333, USA. Leptospiraserovarsare *L.int*. icterohaemorrhagiae, *L.int*.canicola, *L.int*.pomona, *L.int*.grippotyphosa and *L.int*.wolffi. These serovars were used for MAT, ELISA.

4. Leptospiral media:
It is a serum free medium which has been used in the continuous subculture of leptospiral strains both for their maintenance and propagation (EMJH (Ellinghausen, McCullough, Johnson and Harris) base medium (Difco) USA - EMJH Enrichment (Difco) USA).

5. The Microscopic Agglutination Test (MAT):
The MAT was employed in this study to determine the presence of leptospiral antibodies and their titers in the sera of adult dairy cows against 5 leptospiralserovars (*L.int*.icterohaemorrhagiae, *L.int*. canicola, *L.int*.pomona, *L.int*.grippotyphosa and *L.int*.

wolffi. It was carried out according to Faine *et al.* (1999). The MAT was performed with living reference leptospira strains cultivated for 7 days in EMJH medium at 30 ^{O}C. For serological studies a serial double fold serum dilution is done using Phosphate Buffer Saline (PBS) beginning with dilution 1:100.

6. ELISA Test was carried out according to Hajkova and Jurmanova (1986)

7. Biochemical examinations:-

1- **Estimation of urea** was done based on Batton and Crouch (1979).

2- Estimation of creatinine: was carried out according to the method described by Bowers and Wong (1980).

3- Estimation of aspartate aminotransferase (AST): using kinetic method which carried out adopting the method of Breuer (1996).

4- Estimation of alanine aminotransferase (ALT): using kinetic method which carried out adopting the method of Breuer (1996).

5- Estimation of γ-glutamyltransferase (GGT):- Using kinetic method which is carried out adopting the method of Kaplan (1992).

8. Statistical analysis:

Collected data were analyzed for the mean and standard error of mean. Significance of the results was evaluated by F-test, (analysis of variance) according to Petrie and Watson (1999).

RESULTS

Table 1: Leptospira seropositive cases among examined cows with reproductive disorders:

	MAT		ELISA	
	No	**%**	**No**	**%**
Positive	**131**	**45.80**	**149**	**52.10**
Negative	**155**	**54.20**	**137**	**47.90**
Total	**286**	**100**	**286**	**100**

Table 2: Results of sero-diagnosis of leptospirosis using MAT among cows with reproductive disorders:

Leptospiral Serovars	**Total cases, n=286**	
	No	**%**
L. int. Grippotyphosa	**34**	**11.89**
L. int. canicola	**30**	**10.49**
L. int. pomona	**30**	**10.49**
*L. int.*icterohaemorrhagiae	**24**	**8.39**
*L. int.*wolfi	**13**	**4.55**
Total	**131**	**45.81**

Table 3: Results of sero-diagnosis of leptospirosis using ELISA among cows with reproductive disorders:

Leptospiral Serovars	**Total cases, n=286**	
	No	**%**
L. int. grippotyphosa	**37**	**12.94**
L. int. canicola	**36**	**12.59**
L. int. pomona	**33**	**11.54**
*L. int.*icterohaemorrhagiae	**29**	**10.14**
*L. int.*wolfi	**14**	**4.89**
Total	**149**	**52.10**

Table 4: Distribution of positive titers against different leptospiralserovars among cows:

Leptospiral Serovars		Titers							
		1:200		1:400		1:800		1:1600	
	No ≥1:200	No	%	No	%	No	%	No	%
L. int. Grippotyphosa	34	9	26.48	12	35.29	12	35.29	1	2.94
L. int. Canicola	30	4	13.33	15	50.00	8	26.67	3	10.00
L. int. pomona	30	20	66.67	7	23.33	2	6.67	1	3.33
L. int.icterohaemorrhagiae	24	4	16.67	15	62.50	4	16.67	1	4.16
L. int. wolfi	13	8	61.54	3	23.08	2	15.38	0	0.00

Table 5: Values of ALT, AST and GGT associated with leptospiral infection in cows (u/l):

Parameter	Control	Can	Ict	Pom	Grip	Wol	Ict +Grip	Can +Grip	Can +Pom	Grip +Pom	Ict +Pom	Wol +Grip	Grip +Can +Ict	Grip +Can +Ict +Pom	Grip +Can +Wol +Ict +Pom
	C	* B	* AB	* AB	* AB	* AB	* AB	* AB	* AB	* AB	* AB	* AB	* AB	* A	* A
ALT	16.96 ±0.23	23.21 ±0.93	25.14 ±1.67	26.37 ±1.50	27.13 ±1.77	26.50 ±1.77	23.25 ±1.39	27.30 ±1.61	26.38 ±1.29	24.75 ±1.18	26.63 ±1.25	25.25 ±1.00	27.59 ±1.23	30.25 ±1.41	31.00 ±1.16
	C	* B	* B	* B	* B	* B	* B	* B	* B	* B	* B	* B	* AB	* B	* A
AST	30.50 ±0.32	43.68 ±1.87	43.21 ±2.48	46.84 ±1.84	48.00 ±3.35	47.30 ±3.01	45.88 ±2.68	50.30 ±2.19	49.00 ±2.59	42.83 ±3.16	47.69 ±2.23	44.75 ±2.22	55.22 ±1.23	54.00 ±3.27	64.40 ±1.46
	B	AB	AB	AB	AB	* A	AB	AB	AB	AB	* A	AB	* A	* A	* A
GGT	12.15 ±0.20	15.26 ±1.01	17.93 ±1.11	16.74 ±0.77	19.80 ±0.44	18.00 ±0.66	16.75 ±0.23	18.10 ±0.99	12.50 ±0.11	16.92 ±0.54	20.50 ±1.03	14.00 ±1.11	20.77 ±0.95	21.88 ±0.44	21.60 ±0.23

* Significant at P<0.05 Means that have different subscripts were significantly different at P<0.05.
Can= *L.int*.canicolaIct= *L.int*.icterohaemorrhagiae Pom= *L.int*.pomona Grip= *L.int*.gripotyphosa Wol= *L.int*.wolfi

Table 6: Kidney function parameters associated with leptospiral infection in cows (mg/dl):

Parameter	Control	Can	Ict	Pom	Grip	Wol	Ict +Grip	Can +Grip	Can +Pom	Grip +Pom	Ict +Pom	Wol +Grip	Grip +Can +Ict	Grip +Can +Ict +Pom	Grip +Can +Wol +Ict +Pom
	B	* A	* A	* A	* A	* A	* A	* A	* A	* A	* A	* A	* A	* A	* A
Urea	26.16 ±0.22	32.84 ±0.34	37.79 ±0.36	37.23 ±0.29	34.93 ±1.02	36.50 ±1.00	35.38 ±0.88	35.00 ±0.67	35.13 ±1.03	36.08 ±1.00	36.25 ±0.86	36.00 ±0.22	37.41 ±0.88	38.13 ±1.74	41.20 ±1.88
	C	* ABC	* ABC	* ABC	* ABC	* BC	* ABC	* ABC	* ABC	* ABC	* ABC	* ABC	* AB	* AB	* A
Creatinine	0.96 ±0.01	1.18 ±0.03	1.20 ±0.02	1.15 ±0.02	1.17 ±0.04	1.09 ±0.02	1.15 ±0.03	1.19 ±0.01	1.12 ±0.02	1.17 ±0.03	1.12 ±0.02	1.21 ±0.01	1.27 ±0.03	1.28 ±0.03	1.38 ±0.03

* Significant at P<0.05
Means that have different subscripts were significantly different at P<0.05.
Can= L.int.canicolaIct= *L.int*.icterohaemorrhagiae Pom= *L.int*.pomona Grip= *L.int*.gripotyphosa Wol= *L.int*.wolfi

DISCUSSION

In the present study, titers of 1:200 or greater were recorded as positive. According to the report of (WHO 1982) titers of 1:800 or greater were considered as indicative for an active leptospiralinfection. The results of serological tested cows sera indicated that leptospiral seropositive cases using MAT and ELISA were 45.81% and 52.10% respectively. Vanasco *et al.* (2001) suggested that ELISA could constitute a very useful indicator for epidemiological purposes of past or present leptospiral infection in rodents and agreement between ELISA and MAT would be much higher if ELISA cut-off points were lowered, being 1:20. It could be concluded that MAT and ELISA could be used to screen leptospiral infection in cattle. However the ELISA was more sensitive, specific and rapid to detect antibodies in cattle with positive titer of 1:200 and more Attia and Ibrahim (2002). The obtained

results revealed that agglutinins against *L.int.*grippotyphosa were predominant (11.89%) and (12.94%) by using MAT and ELISA respectively. The distribution of its positive titers at 1:200, 1:400, 1:800 and 1:1600 was in the following percentages 26.48%, 35.29%, 35.29% and 2.94% respectively. These results were approximately similar to that obtained by Berovich (1987), Wanyangu *et al.* (1987) and El-Sukhon *et al.* (1992). They detected agglutinins against *L.int.*grippotyphosa in 14%, 10.3% and 15.1% respectively in infertile cattle sera.

In Egypt, Attia (1993) detected agglutinins against *L.int.*grippotyphosa in 2% in cattle at Dakahlia Governorate, while 5.5% in Baladi cows sera at Giza abattoir. The author also detected antibodies in 5% in cow sera from infertile cases in private farms in Egypt. The obtained results are lower than that obtained by Eman S. Ibrahim (2007) who detected agglutinins against *L.int.*grippotyphosa in 17.07% in infertile cows, and higher than that obtained by Hatem (1979), Kilany (1988), Prescott *et al.* (1988), Truner(1988), Sebek *et al.* (1989) and Espi *et al.* (2000) who detected agglutinins against *L.int.*grippotyphosa in 1%, 6.5%, 2%, 7.1%, 2.9% and 2.37% respectively. The current study revealed that agglutinins against *L.int.*canicola were (10.49%) and (12.59%) by using MAT and ELISA respectively. The distribution of its positive titers at 1:200, 1:400, 1:800 and 1:1600 was in the following percentages 13.33%, 50.00%, 26.67% and 10.00% respectively. The obtained results were higher than results of Attia, (1993) at Giza abattoir, who detected agglutinins against *L.int.*canicola in 9.5% and 1% in cow sera respectively. On the other hand Ibrahim (2007) recorded agglutinins against *L.int.*canicola in 21.34% in cows suffering from infertility.

Concerning some biochemical studies associated with leptospiral infection in cows suffered from reproductive disorders, it was found that ALT and AST were significantly high in cows suffered from leptospirosis compared with control group. This elevation behaved rather similar in different leptospiraserovars. Moreover GGT was increased slightly in cows suffered from leptospirosis compared with control group. Blood urea and creatinine were significantly elevated compared with control group. This elevation was markedly seen in cows affected with *L.int.*icterohaemorrhagiae mixed with *L.int.*grippotyphosa, *L.int.*canicola, *L.int.*pomona and *L. int.* wolfi. The obtained results were similar to Levett (2001) who reported that in severe leptospirosis, the rise in AST and ALT activities were due to release of bacterial toxins or immunological reactions. Renal function impairment is indicated by raised plasma creatinine levels. The degree of azotemia varies with severity of illness. The outer membrane of leptospires contains lipopolysaccharide (LPS) and outer membrane proteins (OMPs). The LPS is highly immunogenic and is responsible for serovar specificity. An inverse relationship between expression of transmembrane OMPs and virulence was demonstrated in serovar *L.int.*grippotyphosa, outer membrane components may be important in the pathogenesis of interstitial nephritis (Barocchi *et al.,* 2002).

REFERENCES

Assez, N.; *Mauriaucourt, P.*; *Cuny, J.*; *Goldstein, P. and Wiel, E.* (2013): [Fever and jaundice... and if it was a leptospirosis. About a case of *L. interrogans* icterohaemorrhagiae in Northern France] Ann FrAnesth Reanim. 2013 Jun; 32(6): 439-43.

Attia, E.R.H. (1993): "Microbiolgical assay of animal leptospirosis in Egypt". Ph.D. thesis (Microbiology), Faculty of Veterinary Medicine, Alexandria University.

Attia, E.R.H.; Ibrahim, I.G.A. and Tawfik, M.S. (1996): "Dot ELISA as a quick reliable diagnostic aid for leptospirosis". Assiut Veterinary Medical Journal, 36 (71) 167-172.

Attia, E.R.H. and Ibrahim, I.G.A. (2002): "A comparative study using MAT and ELISA for serodiagnosis of leptospirosis". Minufyia. Vet. J., 2 (1): 137–146.

Barocchi, M.A.; Ko, A.I. and Reis, M.G. (2002): "Rapid translocation of polarized MDCK cell monolayers by Leptospirainterrogans, an invasive but nonintracellular pathogen". Infect. Immun.; 70: 6926-6932.

Batton, C.J. and Crouch, S.R. (1979): "Estimation of Urea". Anal. Chem., 1977, 49: 464-469.

Bercovich, Z.; Taaijke, R. and Bokhout, B.A. (1990): "Evaluation of an ELISA for the diagnosis of experimentally induced and naturally occurring *Leptospira* Hardjo infections in cattle". Vet. Microbiol. 21: 255–262.

Birnbaum, N.; Barr, C.; Center, S.A.; Schererhorn, J.F.; Randolph, D.K. and SIMPSON, W. (1998): "Naturally acquired leptospirosis in 36 dogs: serological and clinicopathological feature". Journal of Small Animal Practice 39. 231-236.

Bowers, L.D. and Wong, E.T. (1980): "Kinetic serum creatinine assays. II. A critical evaluation and review". Clin. Chem., 526-555.

Breuer, J. (1996): "Report on the symposium "drug effects in clinical chemistry methods"". Eur. J. Clin. Chem. Clin. Biochem., 34: 385-386.

Brem, S. and Schreyer, K. (1989): "Occurrence of Leptospira antibodies in serum samples from cattle in Bavaria Barl. Und Munch". Teir. woch., 101 (12): 416-419.

El-Sukhon, S.; Abo-Shehada, M.; Abuharfil, N. and Atmh, R. (1992): "Prevalemce of leptospiral antibodies in cattle in Northern Jordan". Trop. Anim. Hlth. prod.,24 (2): 127-128.

Espi, A.; Prieto, J.M.; Fernandez, M. and Alvarez, M. (2000): "Serological prevalence to six leptospiralserovars in cattle in Asturias (Northern Spain)". Epidemiology and Infection, (124): 599-602.

Faine, S.; Adler, B.; Bolin, C. and Perolat, P. (1999): "Leptospira and leptospirosis".2nd ed. Melborne: MediSci. The C. V. Mosby Combany, St. Louis. Baltimore. Philadelphia. Toronto.

Hajkova, M. and Jurmanova, K. (1986): "Detecting mycoplasma antibodies by enzyme linked immunosorbent assay". Veterinarni Medicina. 31: 8, 487-496.

Hatem, M.E. (1979): "Further studies on Leptospirosis in animals and man in Egypt with special reference to isolation and identification". PhD. Thesis (Microbiology), Faculty of Veterinary Medicine. Cairo University.

Hassan, W.H. (2007): "Serodiagnostic studies on bovine leptospirosis in Beni-Suef Goverorate". Beni-Suef, Vet. Med. J.(18) 22-25

Ibrahim (2007): "Leptospirosis in bovin with special reference to reproductive disorder and mastitis". M.V.Sc. thesis (Microbiology), Faculty of Veterinary medicine, Cairo University.

Kaplan, L.A. (1992): "Estimation of γ-Glutamyl Transferase (GGT)". A.J. Clinical Chemistry, 2nd Ed., St. Louis, C.V. Mosby Company.

Kilany, S. (1988): "Incidence of leptospirosis in cattle at Behiragovernorate". M.V.Sc. Thesis (Microbiology), Faculty of Veterinary Medicine, Cairo University.

Levett, PN. (2001): (Leptospirosis). ClinMicrobiol Rev.; 14: 296–326.

OIE (2004): "Manual of diagnostic tests and vaccines for terrestrial animals". 5th edition part 2 section 2.2 chapter 2.2.4.

Petrie, A. and Watson, P. (1999): Statistics for Veterinary and Animal Science" 1st ed., the Black Well Science LTD, United Kingdom, 90-99.

Prescott, J.F.; Miller, R.B.; Nicholoson, V.M.; Marttin, S.W. and Lesnick, T. (1988): "Seroprevalence and association with abortion of leptospirosis in cattle in Ontario". Can. J. Vet., (2): 210-215.

Sebek, E.; Sixl, W.; Reinthaier, F. and Nabi, O. (1989): "Leptospirosis in the |Melat district-upper-Nile province (south Sudan)". Geogr. Med. Suppl., 5: 161-178.

Turner, L.H. (l988): "Leptospirosis II. Serology". Trop. Med. Hyg., 62 (6): 880-899.

Vanasco, N.B.; Lottersberger, J.; Sequeira, M.D. and Tarabla, H. (2001): "Development and validation of an ELISA for the detection of leptospires–specific antibodies in rodents". Vet. Microbiol. 82: 321–330.

Wanyangu, S.M.; Olubay, R.O.; Rositter, P.B. and Witkins, S.A. (1987): Leptospirosis in Kenya, low serological prevalence in cattle, sheep and goat on Rusinga Island in Kenya". Bull. of anim. Health prod. In Afr., 36(2): 188-189.

WHO (1982): "Guidelines for the control of leptospirosis" q.Geneva, Switzerland: WHO offset publication No. 67.

دراسات سيرولوجية وبيوكيميائية على مرض الليبتوسبيرا فى الآبقار

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اجريت هذه الدراسة على عدد ٢٨٦ حالة من ابقار تعانى اضطرابات تناسلية وقد تم أخذ عينات سيرم لتلك الحالات وتم فحصهم سيرولوجيا للكشف عن الاجسام المناعية لمعظم عترات الليبتوسبيرا المنتشرة فى مصر باستخدام اختبارى التلزن الميكروسكوبى والاليزا وكذلك تقدير التغيرات الكيميائية لتلك العينات أيضا. وأظهرت نتائج الفحص السيرولوجى عن وجود حالات ايجابية للليبتوسبيرا باستخدام التلزن الميكروسكوبى والاليزا بنسب (٤٥.٨١ %) ، (٥٢.١٠ %) على الترتيب.وكانت غالبية الاجسام المناعية التى تم الكشف عنها ضد العترات الاتية: ليبتوسبيرا جريبوتيفوسا، ليبتوسبيرا كانيكولا، ليبتوسبيرا بومونا، ليبتوسبيرا أكتيروهيموراجيا، ليبتوسبيرا ولفاى باستخدام اختبار التلزن اليكروسكوبى بنسب: (١١.٨٩ %)، (١٠.٤٩ %)، (١٠.٤٩ %)، (٨.٥٥ %)، (٤.٥٥ %) على الترتيب. وأظهرت نتائج الآختبارات الكيميائية لسيرم الابقار المصابة بالليبتوسبيرا عن زيادة فى البولينا والكرياتينين عن المعدلات الطبيعية، وكذلك زيادة فى أيه.أل.تى، أيه. أس. تى، جاما. جى. تى عن المعدلات الطبيعية لخلل فى وظائف الكبد.