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INCIDENCE OF *BABESIA BIGEMINA* INFECTION IN NATIVE BREED CATTLE, BEHERA PROVINCE, EGYPT USING DIFFERENT METHODS OF DIAGNOSIS

(With 3 Tables)

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دراسات على البابيزيا بايجيمينا في الأبقار المرباه محلياً بمحافظة البحيرة - مصر

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أجريت هذه الدراسة بمحافظة البحيره على عدد ٣٢٨ من الأبقار المحلية ذات أعمار مختلفه ومُمثلة لأربعة مناطق مختلفه وهي إيتاى البارود (عدد ٥٦ حيوان) - شبراخيت (١١٦ حيوان) - حوش عيسى (٥٦ حيوان) ودمنهور (١٠٠ حيوان) وقد تم التشخيص بإستخدام الفحص الروتيني للمسحات الدموية المصبوغة بصبغة الجيمسا والتي أظهرت نسبة إصابة ٨, ٣٤٪. وكذلك تم إستخدام طريقتين من التشخيص السيرولوجي وهي :

أولاً : إستخدام إختبار التلازن الأنبوبي الشعري (C.A.T.) على عدد ١٣٠ حيوان من الحيوانات التي تم فحصها بالطريقة السابقة ، وقد أظهر هذا الإختبار نسبة إصابة ٥, ٦١٪.

ثانيا: إستخدام إختبار الإنزيم المرتبط (ELISA) وأجرى هذا الإختبار على عدد ٢١٥ حيوان وأظهر نسبة إصابة ٤, ٨١٪.

وقد أظهرت الدراسة إختلاف نسبة الإصابة بين المناطق الأربعة التي أجريت فيها الدراسة ، كما أثبتت الدراسة كفاءة وحساسية إختبار الإنزيم المرتبط (ELISA) بالمقارنة بالإختبارات الأخرى التي تم إستخدامها.

SUMMARY

Local breed cattle in Behera province are investigated for *Babesia bigemina* infection. The incidence is found higher in Hoosh-Easa and Shobra- kheet followed by Damanhour and Etay El-Baroud respectively. Examination of

Giemsa stained blood films together with two serological tests [Capillary tube agglutination test (CAT) and Enzyme linked immunosorbent assay (ELISA)] are used in the diagnosis. In comparison, ELISA technique offers a number of advantages overall other tests; being more sensitive, requires a simple apparatus for large number of samples, can be automated (less subjected to operator error) and can also be adopted as a simple field screening test especially in intensive breeding programms and dairy farms.

Key words: *Babesia bigemina - Native cattle-Diagnosis - Egypt.*

INTRODUCTION

Babesiosis, a world wide tick borne haemoparasitic disease of domestic and wild animals, continues to be a major deterrent to livestock production. It is being enzootic in countries with tropical and subtropical climates leading to significant loss in meat and milk production. Besides, it prevents the opportunity to upgrade the local herds through the importation of genetically superior animals to and from the enzootic areas (McElwain et al., 1987).

It is necessary to emphasize that identification of *Babesia* protozoans in either thin or thick blood films is a true evidence of infection. However, negative microscopic examination does`nt exclude the possibility of infection. On the other hand, carrier animals in endemic regions have a very low parasitaemia and the infection can`t be easily diagnosed by light microscopy. Also, in the very early or chronic stages of infection, the detection of *Babesia* parasites in the stained blood films being uncommon. So, it was necessary to develop serological methods to detect specific antibodies of *Babesia species* rather than *Babesia* organisms (Todorovic and Carson, 1981 and Weiland and Reiter, 1988).

In Behera province, Babesiosis had little attention of the concerned workers. Therefore, this study was conducted for the incidence and diagnosis of *Babesia bigemina* using two serological tests; the capillary tube agglutination test (CAT) and the enzyme linked immunosorbent assay (ELISA) in comparison to the routine parasitological examination of Giemsa stained blood films (light microscopy).

MATERIAL and METHODS

1. Animals:

328 local breed cattle of different ages and a history of Babesia infection without treatment from different regions in Behera province including Hoosh-Easa (56), Shobra-Kheet (116), Damanhour (100) and Etay El-Baroud (56) are investigated by the light microscopy. According to the available materials, sera of 215 cases are subjected to ELISA while sera of 130 cases are tested by CAT.

2. Blood and serum samples :

2.1. Blood smears: Blood smears were made from the peripheral blood of the investigated animals, dried in air, fixed with methanol, stained with Giemsa stain and examined microscopically under the oil immersion lens. Identification of *Babesia species* was made according to the key given by Levine (1971).

2.2. Serum samples: 10 ml of jugular blood were taken from each animal into a clean, sterile Mckartiney bottle , allowed to clot at room temperature. Then, cooled overnight at 4°C for clot separation. Samples were then centrifuged at 5000 r.p.m. for 15 minutes for serum separation. The sera were collected in labelled sterile vials and kept froozen at -20^{0}C for serological examination.

3. Serological tests:

From 328 samples, previousely examined by the light microscopy, sera of 215 cases are subjected to ELISA while 130 were tested by CAT. This was controled by the available chemicals and reagents used in the concerned serological tests.

Preparation of antigens was carried out in Serum and Vaccine Research Institute, Cairo. The crude antigen was prepared according to that described by Schaffler (1963). The purified antigen was prepared from the crude antigen using the technique of Ristic (1962) .

Control positive sera obtained from *Babesia bigemina* naturally infected cattle (with ticks) 2, 4 and 6 weeks post infection according to the suggestion of Dwivedi and Gautam (1978). Control negative sera are obtained from clinically normal cattle.

Capillary tube agglutination test was prepared and carried out according to Ristic (1962). ELISA technique was adopted according to Kemney and Challacombe (1988) and determination of it`s optimal conditions were applied according to Voller et al. (1976 b).

RESULTS

Examination of Giemsa stained blood films from 328 cattle revealed that 114 (34.8 %) were infected with *B.bigemina*. The incidence is found higher in Hoosh-Easa (67.8 %), followed by Damanhour (37 %) and Shobra-Kheet (25 %) while the lowest incidence is recorded in Etay El-Baroud (17.8 %) [Table 1].

Out of 130 sera samples, tested by the capillary tube agglutination test, 80 samples (61.5 %) are found positive to *B.bigemina* infection. The incidence is found higher in Hoosh-Easa (68%) followed by Shobra -Kheet (58 %) and Damanhour (56.6%) [Table 1].

On using ELISA , the brown colour produced by the action of the enzyme on it`s substrate was determined spectrophotometrically at 492 nm.

Screening of sera from 215 animals revealed that 175 (81.4%) are *B.bigemina* positive.The incidence is higher in Hoosh-Easa (100 %) followed by Shobra-kheet (83.3 %) and Damanhour (76.7%) while the lowest incidence is recorded in Etay El-Baroud (64.3 %) [Table 1].

Various *B.bigemina* specific antibody levels are also recorded where 18.6% of the tested sera were negative; 3.7% week positive; 2.3% moderate positive; 27.9% strong positive; 24.2 % very strong positive and 23.3% showed severe strong positive reaction (Table 2).

DISCUSSION.

This study on *Babesia bigemina* is considered to be one of the first records in Behera province. Examination of stained blood films prepared from 328 local breed cattle revealed that 114 (34.8 %) were infected with *B.bigemina* .

In Egypt, *B. bigemina* infection had been investigated by different authors in different provinces (Table, 3). Our above mentioned results of light microscopy is found to be in agreement with that obtained by El-Bahi (1986) and El-Seify (1989) in Fayoum and Beni-Suef provinces respectively. On the other hand, lower incidences have been recorded by El-Allawy (1973); Sakla (1975); Ahmed (1980); Gattas (1983 and 1990); Chafick (1987); Abo El-Kheir (1989) and Abd El-Gawad (1993). These different incidences may attributed to the climatic variations, different control and hygienic measures applied by the owners and/or the different immunological status of animals in different localities.

To much attention has been paid to serology of *B.bigemina* in cattle. Each serological test had it`s own advantages and disadvantages depending on it`s sensitivity, specificity, simplicity and cost effectiveness as well as it`s field application. At present, most specialists propose the combination of at least two different assays to increase the diagnostic reliability of serodiagnosis of *Babesia* infection (Weiland and Reiter, 1988). Therefore, it was planned to apply ELISA and CAT tests on conducting this study.

Using the capillary tube agglutination test, 61.5% of the tested animals proved positive to *B.bigemina* infection (Table 1). This incidence is much higher than that obtained by the blood film examination. This indicates that the CAT has more ability to determine latent and chronic infection than light microscopy which able only to detect the parasite during the acute stage which lasts for few days (Popovic and Ristic, 1970). This result coincided with Dwivedi and Gautam (1982) who stated that CAT able to detect antibodies in 33.33 % of cases on the day 14 and in 100 % on the day 35 post infection in experimentally infected calves.

Animal sera tested with ELISA revealed that 81.4 % were positive to *B.bigemina* infection. This result being much higher than that obtained by light microscopy and CAT. These results confirm those previousely acheived by O` Donoghue et al. (1985) who stated that ELISA capable for detection of antibodies of *B.bigemina* as early as seven days post infection and Barry et al. (1986) who said that ELISA can detect antibodies for more than three years after infesction.

Concerning the incidence in different localities , the same incidences arrangement is obtained on using both ELISA and CAT . The highest incidence is recorded in Hoosh-Easa [CAT (68 %) and ELISA (100%)]. The followed incidence is recorded in Shobra-Kheet [CAT (58%) and ELISA (83.3%)] followed by Damanhour and Etay El-Baroud respectively. On the other hand, a different arrangement of incidences is recorded on using the blood films examination (Table 1).

These results explain the reliability of serological tests in comparison to the blood films examination agreeing with Todorovic (1975) who stated that diagnosis of Babesiosis by light microscopy is time consuming, tedious and not always successful particularly in endemic areas. Also, Popovic and Ristic (1970) stated that blood film examination can only detects the circulating parasites in acute stage which lasts for few days .

From this investigation, we can conclude that *B.bigemina* infection is common among local breed cattle in Behera province. It is wisely to

recommend the use of serological tests for it`s perfect diagnosis especially in intensive breeding programms and dairy farms.

Overall, ELISA technique is found to be the pereferable test as it is proved to be more sensitive, less subjected to operator error and/or stress and can also be adopted as a simple field screening procedures involving large number of samples. Moreover, ELISA capable of determining the various specific antibody levels which should be considered during the vaccine application programms.

REFERENCES

Abd El-Gawad, M. (1993): Some studies on *Babesia species* in cattle in Beni-suef Governorate. Ph.D.thesis, Fac. Vet. Med., Cairo University.

Abo El-Kheir, E.A. (1989): Epidemiological aspect on the distribution of haemotropic parasites among cattle in upper Egypt confirmed by the identification of encountered organisms. M.V.Sc. thesis, Fac. Vet. Med., Cairo University.

Ahmed, A.B. (1980): Some biological studies on blood parasites of farm animals. Ph.D. thesis, Fac. Vet. Med., Zagazig University.

Barry, D.N.; parker, R.J.; Devos, A.J.; Dunster, P.and Rodwell, B.J. (1986): A microplate ELISA for measuring antibodies to Anaplasma marginale in cattle serum. Aust.Vet.J.,63(3):76 - 79.

Chafick, M.L. (1987): serological tests for the field diagnosis of Babesia infection in calves. M.V.Sc.thesis, Fac.Vet.Med.,Cairo Univ.

Dwivedi, S.K. and Gautam, O.P. (1978): A note on indirect haemag-glutination test for the evaluation of experimental Babesiasis in calves. Ind.J.Anim.Sci., 48(2): 148 - 149.

Dwivedi, S.K. and Gautam, O.P. (1982): A note on comparative efficacy of indirect haemagglutination test , capillary tube agglutination and indirect fluorscent antibody tests in the diagnosis of experimental Babesiosis in calves. Ind.Vet.J.,59:404 - 406.

El-Allawy, T.A. (1973): Some studies on bovine piroplasmosis in Assiut. M.V.Sc. Thesis, Fac.Vet. Med., Assiut Univ.

El-Bahi, N.M. (1986): Some studies on tick borne disease among ruminants in Fayoum Governorate. M.V.Sc.thesis ,Fac.Vet.Med.,Cairo Univ.

El-Ghaysh, A.A. (1993): Studies on *Babesia species* infecting cattle and water buffaloes in Egypt. Ph.D.thesis,Fac.Vet.Med.,Cairo Univ.

El-Sawalhy, A.A. (1987): Some studies on cattle blood parasite in Kaluopia province.M.V.Sc.thesis, Fac.Vet.Med.,Zagazig University.

El-Seify, M.A. (1989): Incidence and geographical distribution of some blood parasites among cattle and buffaloes in Beni-Suef Governorate.Vet. Med. J. Giza, 37(3): 611 - 622.

Gattas, M.W. (1983): Some studies on cattle Babesiosis in Suez-Canal zone. M.V.Sc.thesis ,Fac.Vet.Med.,Cairo University.

Gattas, M.W. (1990): Further studies on the biological aspect of *Babesia species* in Suez-Canal zone with special reference to their control. Ph.D. thesis, Fac. Vet. Med., Cairo University.

Kemney, D.M. and Challacombe. S.J. (1988): ELISA and other solid phase immuno assays. United Medical and Dental schools of Guy`s and St.Thomas`s Hospitals, London SEL, 9 RT (UK) PP.: 345 - 352.

Levine, N. D. (1971): Taxonomy of the piroplasms. Trans. Am. Microsc. Soc., 90: 2 -33.

McElwain,T.F.; Pyerrymanl, E.; Davis, W.C. and McGuire, T.C. (1987): Antibodies define multiple proteins with epitopes exposed on the surface of live *B.bigemina* merozoites. J.Immunol.,138(7):2298 - 2304.

Mohan, R.N. (1968): Diseases and parasites of buffaloes (Review article). Vet. Bull. Abst., 4405 - 4852.

Nassar, A.M. (1992): Serological diagnosis of *Babesia bigemina* by Dot enzyme linked immunosorbent assay and indirect fluorscent antibody test. Proc 2nd. Cong., Fac. Vet. Med., Cairo Univ. 31-33.

O`Donoghue, P.J.; Friedhoff, K.T.; Vizcaino, O.G. and Weyreter, H. (1985): The detection of IgM and IgG antibodies against *Babesia bigemina* in bovine sera using semifined antigen in enzyme immuno assay. Vet.Parasitol. 18: 1 -12.

Popovic, N.A. and Ristic, M. (1970): Diagnosis of canine Babesiosis by gel precipitation test. Am. J. Vet. Res., 31: 2201 - 2204.

Ristic, M. (1962): A capillary tube agglutination test for Anaplasmosis. A preliminary report. J. Am.Vet. Med. Ass., 141(5): 588 - 594.

Sakla, A.A. (1975): Studies on ticks in Assiut Governorate,with special reference to their role in transmission of parasitic diseases. M.V.Sc. Thesis, Fac. Vet. Med., Assiut University.

Schaffler, W.F. (1963): Serological tests for *Theileria cervi* in white tailed deer and for other species of Theileria in cattle and sheep. Am.J.Vet.Res.,24(101): 784 - 791.

Todorovic, R.A. (1975): Serological diagnosis of Babesiosis: A review Trop. Anim. H. Prod., 7: 1 - 14.

Todorovic,R.A. and Carson,C.A. (1981): Methods for measuring the immunological response to Babesia in: Babesiosis. (ed. M.Ristic and J.P. Kreier): 381 - 410.

Voller, A.; Bidwell, D.E. and Bartlett, A. (1976 b): Enzyme immuno assay in diagnostic medicine theory and practice. Bull.World Health Organiz.,53: 56 - 63

Weiland, G. and Reiter, I. (1988): Methods for the measurement of the serological response to Babesia in (Babesiosis of domestic animals and man). C.R.C. Press: 143 - 162.

Table (1) : Incidence of *B. bigemina* in Behera province using three different methods of diagnosis.

Locality	light microscopy			CAT *			ELISA **		
	NO. ex .	NO. + ve	%	NO. ex .	NO. + ve	%	NO. ex .	NO. + ve	%
Hoosh. Easa	56	38	67.8	50	34	68	56	56	100
Shobra - Kheet	116	29	25	50	29	58	60	50	83.3
Damanhour	100	37	37	30	17	56.6	43	33	76.7
Etay El-Baroud	56	10	17.8	---	---	---	56	36	64.3
Total	328	114	34.8	130	80	61.5	215	175	81.4

* CAT = Capillary tube agglutination test .

** ELISA = Enzyme linked immunosorbent assay .

Table (2) : Various *B. bigemina* specific antibodies recorded by ELISA in Behera province .

Locality	NO.	- ve	%	+ ve	%	++ ve	%	+++ve	%	++++ve	%	+++++ve	%
Hoosh. Easa	54	0	0	0	0	1	1.9	6	11.1	18	33.3	29	53.7
Shobra - Kheet	61	10	16.4	1	1.6	1	1.6	25	41	23	37.7	1	1.6
Damanhour	44	10	22.7	1	2.3	1	2.3	4	9	10	22.7	18	41
Etay El-Baroud	56	20	35.7	6	10.7	2	3.6	25	44.6	1	1.8	2	3.6
Total	215	40	18.6	8	3.7	5	2.3	60	27.9	52	24.2	50	23.3

- ve	negative	(OD = 0.176 - 0.500)	+++ ve	strong positive	(OD = 1.00 - 1.228)
± ve	doubtfull	(OD = 0.500 - 0.600)	++++ ve	very strong positive	(OD = 1.228 - 1.428)
+ ve	week positive	(OD = 0.600 - 0.800)	+++++ ve	severe strong positive	(OD > 1.428)
++ ve	moderate positive	(OD = 0.800 - 1.000)	OD = optic denisty		

Table (3) :Prevalence of *Babesia Species* infecting cattle in Egypt .

Author	Year	Locality	Animal spp	Babesia spp	Method	Result
1-Mohan	1968	Egypt	Cows	*B. bigemina*	Direct	20%
2- El Allawy	1973	Assiut	Cows	*B. bigemina*	Direct	7.05 - 10.6%
3- Sakla	1975	Assiut	Cows	*B. bigemina*	Direct	8.7%
4- Ahmed	1980	Sharkia	Cows	*B. bigemina*	Direct	8.56%
5- Gattas	1983	Port Said	Cattle	*B. bigemina*	Direct	25.5%
		Ismalia	Cattle	*B. bigemina*	Direct	27.77%
6- El Bahi	1986	Fayoum	Cattle	*B. bigemina*	Direct	38.25%
7- Chafick	1987	Beni Suef	Cattle-Calves	*B. bigemina*	Direct CAT HAT	8.87% 10.19% 20.94%
8- El Sawalhy	1987	Kalubia	N. Cattle F. Cattle	*B. species* *B. species*	Direct Direct	4.65% 8.7%
9-Abo Elkheir	1989	Giza	Cattle	*B. bigemina*	Direct IFA	2.98% 23.5%
		Beni Suef	Cattle	*B. bigemina*	Direct IFA	4.46% 28.5%
		Minia	Cattle	*B. bigemina*	Direct IFA	6.27% 35.6%
		Fayoum	Cattle	*B. bigemina*	Direct IFA	10.17% 40.5%
10- El Seify	1989	Beni Suef	Cattle	*B. bigemina*	Direct IHAT	31.1% 44%
11- Gattas	1990	Suez Canal	Cattle	*B. bigemina*	Direct IHAT ELISA	7.62% 19.72% 71.56%
12- Nassar	1992	Giza Ismailia	Cattle Cattle	*B. bigemina* *B. bigemina*	Dot ELISA Dot ELISA	27.5% 22%
13-Abd ElGawad	1993	Beni Suef	Cattle	*B. bigemina* *B. bovis* *B. bigemina*	Direct Direct CAT IHAT IFAT Dot ELISA	8.26% 1.65% 13.94% 23.86% 36.17% 32.13%
14- El Ghaysh	1993	Cairo	Cattle	*B. bigemina* *B. bovis*	IFA	12.5% 13.5%
		Delta	Cattle	*B. bigemina* *B. bovis*	IFA	4% 8%
		Upper-Egypt	Cattle	*B. bigemina* *B. bovis*	IFA	5.7% 8.6%
			Cattle	*B bigemina*	ELISA	42%
Present Study	**1996**	**Behera**	**Cattle**	**B. bigemina**	**Direct** **CAT** **ELISA**	**34.8%** **61.5%** **81.4%**