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INCIDENCE OF THEILERIOSIS, BABESIOSIS AND ANAPLASMOSIS IN CATTLE IN TRIPOLI - LIBYA.

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SUMMARY

Bovine theileriosis, babesiosis and anaplasmosis are a haemoparasitic disease caused by protozoans of *Genus Theileria, Babesia* and *Anaplasma* respectively. These haemoparasites are responsible for considerable losses due to mortality, weight loss and a reduction in milk beside the cost of prophylactic measures. The results showed that 51 (11.7%) and 13(2.9%) out of 443 cattle from different localities in Tripoli-Libya by Giemsa stained blood and lymph node smears were infected only with Theileria species respectively. The incidence of *Theileria* species by Giemsa stained blood films examination in different localities in Tripoli was 0.0%, 22.7% and 35.4% in Engeela, Algyran and Fum-Mulgha respectively. While the total incidence of *T.mutans, B.bigemina* and *A.marginale* in the examined cattle by different ELISA kits were 50.6%, 12.9, and 3.4%, respectively. Also, the present work recorded mixed infection.

Keywords: *Theileria; Babesia*; *Anaplasma;* blood films; ELISA; Cattle.
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INTRODUCTION

Theileriosis, Babesiosis and Anaplasmosis are a haemoparasitic diseases caused by protozoans of genus *Theileria, Babesia* and *Anaplasma* respectively and are present in all tropical and subtropical areas were the vectors occur. They are responsible for considerable economic losses due to mortality, weight loss and reduction in milk production, and result in a state of premunity, which make the host asymptomatic carrier and serving as a source of infection for the tick or insect vec-

tor. Also they have been cited, as constraints to the health and improved production of cattle (Young et al., 1988) .The present study aims to clarify the incidence of blood parasites affecting cattle in some localities in Tripoli ñ Libya by blood films, and lymph node smears examination for detection of *Theileria* species schizonts and anaplasma morvlae serological examination by ELISA kits for diagnosis of *T.mutans.B.bigemina* and *A. marginalis.*

Anaplasma and other ehrlichiae are obligatory intracellular bacterial parasites that grow ineuokaryotic host cells form intracytoplasmic microcdonies (Dumler and Bakken,1998) Three genera are placed under family Anaplasmataceae namely genus Anaplasma, genus Ehrichia and genus Neorickettsia (Dumler et al.,2001).

MATERIALS AND METHODS

443 cattle (Friesian) 3 - 7 years old from farms located at Engeela (269), Elgyran (75) and Fum-Mulgha (99) districts in Tripoli, Libya were investigated in the present study for the presence of *Theileria, Babesia* and *Anaplasma* species by the use of the parasitological and serological methods.

2.1. Blood films and serum samples:

Thin and thick blood films were prepared from the ear vein of each of the 443 cattle, fixed and stained with Giemsa stain and examined microscopically. Serum samples were prepared from the 443 investigated cattle. The serum was collected and divided into labeled tubes and stored at - 20 C until used for ELISA.

2.2. Lymph node smears:

Lymph node smears(178) were prepared from each cattle by fixing the prescapular lymph node. The lymphatic juice was aspirated by sterile needle and smeared over a clean dry glass slide, air dried, fixed with methyl alcohol, stained with for detection of *Theileria* spp. schizont.

2.3. Serological examination:

Antibody ELISA kits for diagnosis of *T.mutans, B.bigemina* and *Anaplasma marginale* were used for detection of the specific antibodies against *T.mutans, B.bigemina* and *Anaplasma marginale* in the 178 cattle sera. The kits were obtained from Svanova Biotech AB Uppsala, Sweden. ELISA procedure was done according to the procedure of the manufacture manual.

2.4. Studying the cross reaction between T. mutans, B. bigemina and A. marginale infecting cattle by ELISA.

This study was conducted during run of ELISA test for serodiagnosis *T. mutans, B. bigemina* and *A. marginale* by adding 100 µl of positive control serum of each of *T. mutans, B.bigemina* and *A. marginale* in each selected well contains the above mentioned blood organisms antigens.

RESULTS

3.1. Results of blood and lymph node smears:

Examination of 443 Giemsa stained cattle blood smears revealed that 50 (11.3%) were infected only with Theileria species (Table1).The incidence of *Theileria* spp. according to the localities was 0.0% in Engeela, 17(22.7%) in Algyran and 35(35.4%) in Fum-Mulgha (Table 2).

Lymph node smears showed schizont in 13 (2.9%) out of 443 cattle lymph node smears (Table 1). Examination of the blood films did not reveal infection with *Babesia spp.* And *Anaplasma spp.*

Table (1): Incidence of Theileria species in cattle by examination of Giemsa stained blood and Lymph node smears.

No. of Examined samples	Blood smear		Lymph node smear	
	+ve	%	+ve	%
443	52	11.7	13	2.9

Table (2): Incidence of the *Theileria spp* by examination of Giemsa stained cattle blood films according to the localities.

Localities	No. of samples	Results	
		+ve	%
Engeela	269	0	0
Algyran	75	17	22.7
Fum-Mulgha	99	35	35.4
Total	443	52	11.7

3.2. Results of serological test

3.2.1. Incidence of T.mutans

The results of examination of 178 cattle serum samples by *T.mutans* antibody ELISA kit showed 90 (50.6%) of animals had antibodies against T.mutans. 50 out of the 90 seropositive samples were *Theileria* species blood film positive while the other 40 seropositive samples were blood film negative (Table 3).The distribution of the infection with T.mutans according to the localities were 31(38.8%) in Engeela, 22 (51.2%) in Algyran, and 37(67.3%) Fum-Mulgha (Table 4).

Table (3): Distribution of the 91 T.mutans seropositive samples.

Distribution / T.mutans	Theileria species blood film positive		Theileria species blood film negative	
	+ve	%	+ve	%
90	50	55.6	40	44.4

Table (4): Seroprevalence of *T.mutans* by ELISA kit according to the localities.

Localities	No.of samples	Results	
		+ve	%
Engeela	80	31	38.8
Algyran	43	22	51.2
Fum-Mulgha	55	37	67.3
Total	178	90	50.6

3.2.2. Incidence of B.bigemina:

The present study revealed that 23 (12.9%) out of the examined 178 cattle serum samples using *B.bigemina* antibody ELISA kit were positive for *B.bigemina*. All the 23 seropositive samples were negative for *B.bigemina* by blood film examination. In addition 6 out of the 23 seropositive were *T.mutans* seropositive (Table 5) The distribution of the infection with *B.bigemina* according to the localities were 11 (13.8%) in Engeela, 12 (21.8%) in Fum-Mulgha and 0.0% in other farm (Table 6).

Table (5): Distribution of 23 *B.bigemina* cattle serum samples.

Distribution / T.mutans seropositive	Theileria species blood film positive		Theileria species blood film negative	
	+ve	%	+ve	%
23	6	26.1	17	73.9

Table (6): Incidence of the seropositive *B.bigemina* among 178 cattle serum samples by *B.bigemina* antibody ELISA kit according to the localities.

Localities	No.of samples	Results	
		+ve	%
Engeela	80	11	13.8
Algyran	43	0	0
Fum-Mulgha	55	12	21.8
Total	178	23	12.9

3.2.3. Incidence of A.marginale:

The present investigation showed that examination of 178 cattle serum samples by *A.marginale* antibody ELISA kit showed that 6 (3.4%) had antibodies against *A.marginale*. All the 6 seropositive samples were negative for *A.marginale* by blood film examination. In addition, 3 out of 6 seropositive were *T.mutans* seropositive and the other 3 were *B.bigemina* seropositive and the 6 seropositive samples were Theileria species blood film negative (Table 7). The distribution of the infection with *A.marginale* according to the localities were found 2 (2.5%) in Engeela, 4 (7.3%) in Fum-Mulgha and 0.0% in other farm (Table 8).

Table (7): The result of serodiagnosis and blood examination for detection of *A.marginale*, *T.theileria*, *T.mutans* and *B.bigemina*.

A.marginale seropositive	Theileria species blood film positive		Theileria species blood film negative and seropositive		Theileria species blood film negative and *B.bigemina seropositive*	
	+ve	%	+ve	%	+ve	%
6	0	0	3	50.0	3	50.0

Table (8): Seropositive of the *A.marginale* in cattle serum samples by *A.marginale* antibody ELISA kit according to the localities.

Localities	No.of samples	Results	
		+ve	%
Engeela	80	2	2.5
Algyran	43	0	0
Fum-Mulgha	55	4	7.3
Total	178	6	3.4

3.2.4. Results of mixed infection:

The results of examination of 178 cattle serum samples by ELISA kits for diagnosing *T.mutans*, *B.bigemina* and *A.marginale* revealed that 6 (3.4%), 3 (1.7%) and 3 (1.7%) had mixed antibodies against *T.mutans* with B.*bigemina*, *T.mutans* with *A.marginale* and *B.bigemina* with *A.marginale* respectively (Table 9).

Table (9): Results of the mixed infection of the blood parasites in the cattle serum samples by ELISA kits.

No. of animals examined	Blood parasites					
	T.mutans with B.bigemina		*T.mutans with A.marginale*		*B.bigemina with A.marginale*	
	+ve	%	+ve	%	+ve	%
178	6	3.4	3	1.7	3	1.7

The results revealed no cross reaction between *T.mutans, B.bigemina* and *A.marginale* infecting by ELISA. The total incidence of blood parasites in the examined cattle in some localities in Tripoli Libya by Giemsa stained blood films was 11.3% Theileria species (Table 11). While examination of serum samples by different ELISA kits (*T.mutans, B.bigemina* and *A.marginale*) were 50.6% (T.mutans), 12.9% (B.bigemina) and 3.4% (*A.marginale*) (Table 10).

Table (10): Incidence of the blood parasites in the examined cattle by Giemsa stained blood smears and different ELISA kits according to the localities.

Parasites / Localities	*Theileria species* *			*T. Mutans***			*B. bigemina***			*A. marginale***		
	No of samples	(+)	%	No of samples	(+)	(%)	No of samples	(+)	(%)	No of samples	(+)	(%)
Engeeja	269	0	0	80	31	38.8	80	11	13.8	80	2	2.5
Algyran	75	17	22.7	43	22	51.2	43	0	0	43	0	0
Fum-Mulgha	99	35	35.4	55	37	67.3	55	12	21.8	55	4	7.3
Total	443	52	11.7	178	90	50.6	178	23	12.9	178	6	3.4

* As indicated by Giemsa stained blood smears.
** As indicated by ELISA kits.

DISCUSSION

The present study revealed that 52 (11.7%) out of 443 examined cattle by Geimsa stained blood smear were found infected with Theileria spp in Tripoli. This low incidence might be due to low parasitaemia and the cattle were chronically infected with Theileria species. The present results was nearly similar to that obtained by Mohammed, Mohammed, 1980 (11%) in Egypt. Low incidence of Theileria species was detected in different countries. In Jordan 4.5% - 6% (Sherkov et al., 1976) and in Bangladesh 8.47% (Samad et al., 1983) and in Kashmir vally, 1.4% (Shaw, 1989). On the other hand, high incidence was recorded 32.6% to 85% Saidu et al., 1984) and 44% Lawal et al., 1998) in Nigeria. 45.5% Schoepf et al., 1984) in Somalia, 20.75% (Darghouth et al., 1996) in Tunisia, 63% (Rouina, 1984) and 53.7% (Ziam and Benaouf, 2004) in Algeria. This in agreement might be due to the variation in the environmental conditions (Temperature and humidity) which affect the biology of both parasite and vector. Also, the examined cattle might be sampled from an endemic region. It was worthy to mention that this variation in the

incidence of Theileria infection among cattle from region to another might be regarded to presence of carrier population which serves as a reservoir of infection for ticks and variation of hygienic measures in the farms. In addition introduced susceptible livestock to farms and the potential for clinical relapse under serve nutritional and disease stress.

The results showed that the incidence of Theileria species by Giemsa stained blood smears in different localities in Tripoli were different (22.7% in Algyran, 35.4% in Fum-Mulgha, and 0.0% in Engeela). This variation in the incidence in different localities could be due to the difference in tick control and introducing susceptible chronically infected animals which can be infected easily.

The examination of 178 cattle serum samples for the detecting antibodies against *T.mutans* by *T.mutans* antibody ELISA kit showed that 90 (50.6%) were found positive. Low incidence was recorded in Kenya, 1.5% to 28% (Gitau et al., 1997). On the other hand high incidence was recorded in Ghana, 100% (Bell-Sakyi et al., 2004)
By examination of 178 cattle serum samples for the detecting antibodies against B.bigemina by B.bigemina antibody ELISA kit revealed that 23 (12.9%) were found positive. This finding agreed with Todorovic and Carson (1981) who mentioned that negative microscopic examination does not exlude the possibility of Babesia infection. So it is necessary to detect specific antibodies against Babesia species by serological tests (such as ELISA) rather than Babesia organisms. Nearly similar incidence was recorded in Morroco, 10 to 16% (EL Haj et al., 2002).The higher incidence were recorded in different countries. In Egypt, 27.5% and 22% at Giza and Ismailia (Nassar, 1992), and 64.3% to 100% (Ashmawy et al.,1998).In Tanzania, 88% (Woodford et al., 1990) and 27% (Swai et al., 2000); in Italy, 23.1% (Cringoli et al., 2002).

Also in the examination of 178 cattle serum samples for the detecting antibodies against A.marginale by A.marginale antibody ELISA kits 6 (3.4%) were found positive. Higher incidence was reported in many countries. In Egypt, 16.7% (Eid et al,.2001); in Uganda, 61.9% (Ssenyonga et al., 1991); in Italy, 55.6% (Ceci et al., 2002); in Brazil, 92.94% (Andrade et al., 2001).

The seropositivity of these blood parasites in cattle which were negative by blood smears examination indicated sub-clinical cases Sundar et al., 1993); (Soudarajan et al., 2000) . These incidence is higher than that obtained by the blood films examination, this indicates that ELISA are capable for detection of antibodies as early as seven days post infection and for more than three years after infection (Bary et al., 1982) . Also, ELISA had high sensitivity and specificity in the analysis of sera samples (Ashmawy et al., 1998) and (Morshedi et al., 2003) . Mtshali et al. (2004) demonstrated that blood smears were negative but were

positive for *Anaplasma* by ELISA. Kang et al. (1992) referred the negative results with direct light microscope but positive by ELISA to the maternal immunity. The high incidence recorded in different countries might be due to that the examined cattle were sampled from an endemic region.

Our study revealed that the incidence of *T.mutans* was 38.8%. 51.2% and 67.3% in Engeela, Algyran and Fum-Mulgha respectively. *B.bigemina* and *A.marginale* occurred only in two localities with incidence 13.8% and 2.5% in Engeela, 21.8% and 7.3% in Fum-Mulgha respectively. The variation in the incidence between the localities could be due to difference in the management, tick control and veterinary service.

The present study demonstrated mixed infection as detected by serological examination, 6 (3.4%), 3 (1.7%) and 3 (1.7%) out of 178 cattle serum samples showed mixed infection of *T.mutans* with *B.bigemina, T.mutans* with *A.marginale* and *B.bigemina* with *A.marginale* respectively. The previous studies recorded a mixed infection in Nigeria (0.75%) between Babesia and Theileria species ; Akinboade and Dipeolu, 1984) ; in Turkey 20% *T.annulata* and *T.mutans, T.mutans* and *B.bigemina* in one case and *T.annulata, T.mutans* and *B.bigemina* in another case Dumanli and Ozer, 1987) ; in Kashmir vally (0.69%) between anaplasmosis and babesiosis (Shaw, 1989) ; in Tanzania (45%) *B.bigemina* and *B.bovis* Woodford et al., 1990) ; in Turkey (13%) between B.bigemina with A.marginale or B.bovis (Acici,1995); in Italy (49.4%) between *B.bigemina* and A.marginale (Cringoli et al., 2002); in India 3 out of 10 calves had mixed infection with Anaplasma, Babesia and Theileria species (Julie et al., 2005) . These indicate that there is no cross reaction between the different genera of haemoparasites. This was also confirmed in the present study where their was no cross reaction was recorded between *T.parva, T.mutans, B.bigemina* and A.marginale. Madruga et al., 2001 reported that there is no cross reaction were verified with sera from calves inoculated three times with *B.bovis* for detection of B.bigemina by ELISA. Sundar et al. (1998) reported that their no cross reaction between *T.annulata* and *B.bigemina.*

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نسبة حدوث الإصابة بمرض التيليرياو البابيزيا والأنايلازما فى الأبقار بمنطقة طرابلس - ليبيا

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**كلية الطب البيطرى - جامعة القاهرة - مصر

اجرى هذا البحث على 443 مصل ابقار ووجد ان 11 و 2.9 % من هذه الأبقار مصابة بطفيل التيليريا وذلك بعد فحصها بواسطة استخدام صبغة الجمسا ومسحات الغدد الليمفاوية.

باستخدام اختبار الأيليزا وجد ان 50.6 و 12.9 و 3.4% من الأبقار التى فحصت مصابة بطفيل التيليريا ميوتانز و البابيزيا بيجيمنا و الأنا لازما مارجينال على التوالى . كما أوضح البحث وجود اصابات فى الأبقار باكثر من طفيل من الطفيليات المذكورة.