

## PREVALENCE OF BLOOD PARASITES OF SOME FARM ANIMALS AT BEHERA PROVINCE

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### ABSTRACT

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Farm animals especially (cattle, buffaloes and sheep) are the main source for meat and milk production in Egypt. blood parasites are one of the most important and serious pathogenic protozoal disease, caused by *Babesia* and *Theileria* species which infest these animals. The aim of present work is to investigate the prevalence of blood parasites in some animals (cattle, buffaloes and sheep) by blood film examination. we examined 800 animals (300 cattle, 200 buffaloes and 300 sheep) aged from < one year till > 3 years in period of April 2011 to March 2012. At Behera province. Using thin blood smears, stained with Giemsa stain and examined under microscope. It was found that 130 out of 300 (43.3%), 40 out of 200 (20 %) and 117 out of 300 (39%) cattle, buffaloes and sheep were positive by microscopic examination. Then the incidence of *Babesia* spp. in cattle, buffaloes and sheep by blood film examination were 19.33 %, 9 % and 17%. while the incidence of *Theileria* Spp. in cattle, buffaloes and sheep by blood examination were 23 %, 10 % and 20 %. Mixed infection by *Babesia* spp and *Theileria* spp were 1 %, 1 % and 2 % in cattle, buffaloes and sheep. Regarding the prevalence rate of infection in relation to age, high incidence of both parasites *Babesia* spp. and *Theileria* spp. at (2 -3 years) were 28 %, 16 % and 25% in cattle, buffaloes and sheep positive for *Babesia* spp. and were 30 %, 18 % and 28 % in cattle, buffaloes and sheep positive for *Theileria* spp. by Giemsa stained blood smears examination. The peak of incidence of the blood parasites was noticed in Summer and Spring months.

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**Key words:** Blood parasites, *Babesia* & *Theileria*, Cattle.

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### INTRODUCTION

Blood parasites and their vectors are the most destructive diseases of our animal health and the biggest hindrance for successful production.

Piroplasmosis is highly fatal and has serious economic impact on livestock. This disease is caused by protozoan parasites belonging to family *Babesiidae* and family *Theileriidae* of suborder piroplasmida.

Babesiosis and theileriosis are of the most important and serious blood parasitic disease affecting animals in Egypt (Ashmawy, *et al.*, 1998; Radwan and El Kelesh 2009; Mervat *et al.*, 2010) Theilerial parasites affect a wide range of mammals mainly, ruminants and are particularly important as pathogens of domestic cattle, buffaloes, sheep and goats in the tropical and sub tropical regions.

Tropical theileriosis is severe, often fatal disease caused by *Theileria annulata*. The parasite is transmitted by the bite of tick from *Hyalomma* spp.

Diagnosis *Theileria* spp. infection was based on the light microscopic detection of parasite in thin blood smears and presence of schizonts in stained lymph node biopsy smears (Uilenberg, 1981).

Babesiosis is a tick born disease of domestic wild animals and human being. *Babesia* is a small protozoan found in vertebrates red blood cells.

Perfect diagnosis is essential for successful control measures, Giemsa stained blood smears was the method of choice to detect *Babesia* in the blood of infected animals especially in acute cases (Salem *et al.*, 1999).

The aim of the present work was planned to identify *Theileria* and *Babesia* Species in cattle, buffaloes and sheep and its seasonal incidence.

### MATERIALS and METHODS

#### Animals:

A total 800 animals from private farms located in Behera Province cattle, buffaloes and sheep aged between (< 1 - > 3 years) were examined for the existence of blood parasites. Samples were collected from animals showed clinical signs of the disease.

#### Samples:

Two blood samples were collected from the jugular vein by using steril sharp needle with wide pore used for blood smear and were collected in clean and dry test tube containing EDTA as anticoagulant.

#### Blood smear:

Some precautions should be taken into account like using very clean, dry glass slides, clean, dry regular edges speeder slides, sharp sterile needle, and absolute methyle alcohol.

**Giemsa stain stock solution:**

Giemsa powder (0.5 g) dissolved in glycerin absolute (33ml), the powder was dissolved in glycerin with vigorous shaking. Then the mixture was added to one liter methyle alcohol. The stain was transferred to a tightly stopper brown bottle and stored in dark place for two months the stain was filtered through filter paper prior to use.

**Blood film:**

Thin blood films were prepared and left in air to dry and fixed in absolute methyle alcohol for 1 – 2 min.

Staining with freshly filtered and diluted 10%Giemsa stain for 30 – 45 minutes then washed with distal water to remove excess of stain.

The Slides were left to dry, then put one drop of cider oil examined under oil immersion lens according to (Levine, 1985).

**Examination of blood film for blood parasites:**

1/4 – 1/ 2 inch from the end of the film and transferred from one side of film to other (cross – sectional method) to give constant and representative examination. Animal can be considered negative if the three slides were negative.

**RESULTS**

Table (1) Incidence of theileriosis and babesiosis among cattle, buffaloes and sheep ,by microscopic examination of blood smears of 800 animals (300 cattle, 200 buffaloes and 300 sheep) aged from <1 years until > 3 years at the period from May 2011 until April 2012 at Behera province. Out of 300 cattle 130 (43.3 %) were positive for blood parasites 58 (19.33%) positive for *Babesia* spp. and 69(23%) positive for *Theileria* spp. and mixed infection with *Theileria* spp. and *Babesia* spp. was 3(1 %), Out of 200 buffaloes 40 (20%) were positive for blood parasites 18 (9%) positive for *Babesia* spp., 20 (10%) positive for *Theileria* spp. and mixed infection with *Theileria* spp. and *Babesia* spp. was 2(1 %). Out of 300 sheep 117(39%) were positive for blood parasites which 51(17%) positive for *Babesia* spp.and 60 (20%)

positive for *Theileria* spp. and mixed infection with *Theileria* spp. and *Babesia* spp. was 6( 2%) by Giemsa stained blood smears examination.

Table (2) &Table (3) showed that the infection rate increased with age in cattle, buffaloes and sheep. The highest infection rate at age group (2-3years) were 28%, 16% and 25% in cattle, buffaloes and sheep positive for *Babesia* spp. and were 30%,18% and 28% in cattle, buffaloes and sheep positive for *Theileria* spp. by Giemsa stained blood smears examination.

Table (4) & Table (5) revealed that the maximal infection rate was recorded during summer and spring. The infection rates of *Babesia* spp. were 28 % , 23 % , 12 % and 8 % in summer, spring, Autumn and winter in cattle, 16 % , 14 % , 6 % and 4 % in buffaloes while it was 24 % , 22 % , 12 % and 10 % in sheep. The same relation between seasonal incidence and infection rate of *Theileria* spp. among cattle, buffaloes and sheep, were recorded high incidence rate of infection in summer and spring followed by Autumn and winter 30 % , 28% , 16% and 12 % in cattle, 18 % , 16 % , 6% and 4 % in buffaloes while in sheep it was 28% , 26% , 16 % and 8 %.

The positive blood smears for *Babesia* spp. revealed that the majority of the parasites were rounded and tend to occur peripherally in erythrocytes, while other forms (dot, ring, ovoid, single and double pear shape) were also found. The morphometric data showed the 2.8u is average for the round forms single pyriform was 2.8 - 3.8 X 1.8 - 2.2 u and double pyriform measured was 2.8 – 3.8 X 1.8 – 2.2 u.

Positive blood smears for *Theileria* spp. showed that, the parasites was found in different morphological forms, Ring forms measured 1.3 u was found in infected ertthrocytes with high percentage. The schizonts (koch's blue bodies) were detected macroschizonts and microschizonts. Macroschizonts measured 8.5 X 3.5 u while microschizonts measured 9.75 X 3.75 u. Figs(1) (2), (3) (4) Show morphological chreacter's of blood parasites.

**Table 1:** Prevalence of blood parasites in cattle, buffaloes and sheep by blood film examination at Behera province

Animals	Examined animals			Parasite spp.					
	Total	+ve	%	<i>Babesia</i> spp.		<i>Theileria</i> spp.		Mixed infection	
				+ve	%	+ve	%	+ve	%
Cattle	300	130	43.3	58	19.33	69	23	3	1
Buffaloes	200	40	20	18	9	20	10	2	1
Sheep	300	117	39	51	17	60	20	6	2

**Table 2:** Prevalence of *Babesia*. Spp. infection among cattle, buffaloes and sheep of different age groups using blood film examination

Age	< year			1 year - 2 years			2 years - 3 years			> 3 y		
	No .	+ve	%	No .	+ve	%	No.	+ve	%	No.	+ve	%
Animals												
Cattle	50	1	2	100	22	22	100	28	28	50	10	20
Buffaloes	50	0	0	50	7	14	50	8	16	50	5	10
Sheep	50	0	0	100	23	23	100	25	25	50	9	18

**Table 3:** Prevalence of *Theileria* spp. infection among cattle, buffaloes and sheep of different age groups using blood film examination

Age	< year			1 year - 2 years			2 years - 3 years			> 3 y		
	No .	+ve	%	No .	+ve	%	No.	+ve	%	No.	+ve	%
Animals												
Cattle	50	2	4	100	26	26	100	30	30	50	14	28
Buffaloes	50	0	0	50	8	16	50	9	18	50	5	10
Sheep	50	3	6	100	24	24	100	28	28	50	11	22

**Table 4:** Seasonal prevalence of *Babesia* Spp. in cattle, buffaloes and sheep by blood film examination at Behera province

Animals	Cattle			Buffaloes			Sheep		
	No.	+ve	%	No.	+ve	%	No.	+ve	%
season									
Summer	100	28	28	50	8	16	100	24	24
Autumn	50	6	12	50	3	6	50	6	12
Winter	50	4	8	50	2	4	50	5	10
Spring	100	23	23	50	7	14	100	22	22

**Table 5:** Seasonal prevalence of *Theileria* Spp. in cattle, buffaloes and sheep by blood film examination at Behera province.

Animals	Cattle			Buffaloes			Sheep		
	No .	+ve	%	No .	+ve	%	No.	+ve	%
season									
Summer	100	30	30	50	9	18	100	28	28
Autumn	50	8	16	50	3	6	50	8	16
Winter	50	6	12	50	2	4	50	4	8
Spring	100	28	28	50	8	16	100	26	26

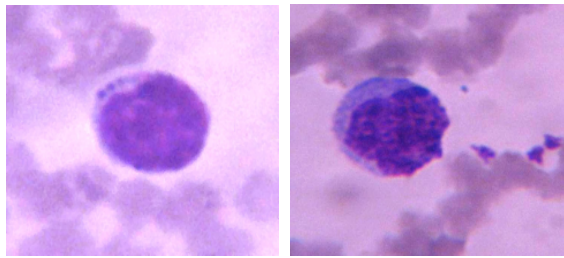


Fig (1): Macroscopic stage in lymphocytes      Fig (2): Microscopic stage in lymphocytes

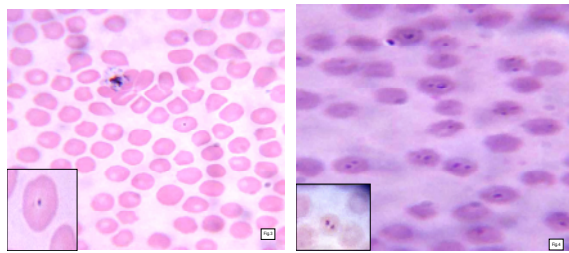


Fig (3): Erythrocytic stage of *Theileria* spp.      Fig (4): Erythrocytic stage of *Babesia* spp.

## DISCUSSION

Babesiosis and Theileriosis are a tick protozoal disease that imposes large economic losses such as, drop in meat and milk production reproductive disorders, losses from deaths, costes of treatment and prevention programs (Abosakaya, 2009 and chowdhury *et al.*, 2006) During the last 20 years human cases of Babesiosis have been reported *B. bovis* transmitted from animals to human and a human case was recorded in Egypt by (El Bahnasawy and Morsy 2008; Mazyad *et al.*, 2010).

(Table 1) revealed that ( 43.3 % , 20% and 39 % ) from cattle , buffaloes and sheep were positive for blood parasites. Cattle were the highest susceptible of blood parasites, followed by sheep and buffaloes, the same result recorded by (Abdul Manan *et al.*, 2007 and ziapour *et al.*, 2011) This explain that tick infestation was high in cattle and sheep than buffaloes.

Prevalence of *Babesia* spp. revealed 19.33 % , 9 % and 17 % in cattle buffaloes and sheep by blood smears.

In Egypt many researchers carry out a comparsion between cattle and buffaloes babesiosis and they found higher prevalence of *Babesia* spp. in cattle than buffaloes. Our results were 19.33 % and 9 % in cattle and buffaloes with regard to Egyptian studies , the results agree with, El Sawalhy (1987) in Kalubia governorate who recorded 8.7 % of cattle and 1.2 % of buffaloes; Gattas (1990) in Suez canal who recorded 7.26 % and 5.91% incidence of *Babesia* spp. in cattle and buffaloes. While our results disagree with, El Ghaysh (1993) who recorded 24 % , 5.4 % in buffaloes and 12.5 % and 4% in cattle in Cairo and Delta while El Badr *et al.* (2009) who recorded 1.9 % *Babesia* spp . in buffaloes and 1.3 % in cattle. In other

counteries, Zulfiqar *et al.* (2012) in Pakistan reported higher incidence of *Babesia* in buffaloes than cattle.

The higher prevalence of *Babesia* spp. in cattle than buffaloes might be attributed to that cattle has longer and thick hair which allow higher tick infestation which act as avector of blood parasites (Abdul Manan *et al.*, 2007) while the difference between our results and perivous authors may be due to climatic variation, immunological status of animals.

(Table 1) revealed, 17% ( 51 out of 300 ) of examined sheep harboured *Babesia* spp. in their blood , it was to be in agreement with Mahmoud (1992) in Beni Suef, Kafir Elsheikh, El-Fayoum and El-Shorkia governorates (23.7 % , 23.1 % , 21.2 % and 20.6 % respectively). On the other hand, high incidence recorded by Ibrahim *et al.* (2000) in Egypt was 50.7% while low incidence recorded by Ramadan and El A Kabawy (2000) in Kalubya governorate (8.5 %).

In other countries there were different rates, Ziapour *et al.* (2011) in Iran found similar rate (16.03%) while low incidence recorded by yeruham *et al.* (1992) In Israel, (5.56 and 7.04 %) of *Babesia ovis* in ewes and lambs and Savini *et al.* (1999) in Italy (10% ) and higher incidence recorded by Fakhar *et al.* (2012) in Iran found in sheep (51.4%).

The Variation in incidence rates of infection could be explained by the climatic condition of the area of the study that enhances the life cycle of ticks and gives higher chance to ticks to infest animals and subsequently increasing the prevalence of blood parasites. Also, Variation may be due to the immunological status of animals and different ages, different breeds and sexes.

(Table 2) The age susceptibility of *Babesia* spp. in present study showed that highest rate of infection at age group 2 – 3 years were (28%, 16% and 25%) in (cattle, buffaloes and sheep) these results agreed with that obtained by Ibrahim *et al.* (2000) and El Bader *et al.* (2009) while our results disagree with Cleon (1988) who reported that sheep aged between 6 to 12 month old have higher prevalence than animals of other age group.

The infection rate was low among young animals may be due to young calves posses innate resistance enhanced by maternal antibodies, these resistance declined gradually leaving the animal with high susceptibility to the disease.

The seasonal incidence of *Babesia* spp. of our study revealed that maximal infection rate was recorded during Summer months followed by Spring. These results agreed with that obtained by Gattas (1990) and Garcia *et al.* (2006) in large animals Ramadan and El Akabawy (2000) and Hosein *et al.* (2007) Fakhar *et al.* (2012) in sheep.

The variation on infection rates in different seasons may be attributed to the effect of climatic condition

on the tick activity which increased in summer and spring.

(Fig 4) blood smears showed presence of *Babesia* spp. inside R. B .CS . Our results agree with, Reda (2001), Ali (2005) and Guerrero *et al.* (2007).

Typical and a typical forms of *Babesia* might be resulted from multiple infection and / or multiple division severe infection with babesiosis which result in multiple invasion of erythrocytes.

Mixed infection with blood parasites include *Babesia* spp. and *Theileria* spp. which recorded in 3 cases in cattle , 2 cases in buffaloes and 6 cases in sheep (1 % , 1 % and 2 %) cattle, buffaloes and sheep. Our results agree with. Nasir *et al.* (2000) (3.1%) and Garcia *et al.* (2006) (3.9 %), Mixed infection may attribute to exposure of animals to more than one genus of ticks or the ability of tick to transmit more than one parasite.

Mediterranean coast fever or Tropical theileriosis is a Tick born disease of (cattle, buffaloes and sheep) caused by *Theileria* spp. infection, extending From Morocco to china and consider one of the Major health problems.

In (Table 1), *Theileria* spp. was 23 % in cattle by Giemsa stain blood films this result was relatively similar to Abd El Raof *et al.* (2000); Hoda and Osman (2009); Hosny *et al.* (2010) whose recorded that the incidence of theileriosis among cattle were 33.66 % 31.16 and 31.58 %. However higher prevalence rates were reported by Gamal El Dien (1993) in Behera province and El Metenawy (2000) Saudi Arabia , who recorded that theileriosis among cattle were 65.4 % , 76.5 % ,and 50.4% respectively and lower prevalence rates reported by El Bader *et al.* (2009) in South vally was 10.3 % .

*Theileria* spp. was 10 % in buffaloes by Giemsa stained blood film , this percentage is relatively similar to that obtained by El Bader *et al.* (2009) who recorded that *Theileria* spp. in buffaloes was 12 . 5 %.

*Theileria* spp. was 20 % in sheep by Giemsa stained blood film this percentage is approximately lower to those recorded in Egypt by Harfoush (2001); Kafrelsheikh, Ibrahim *et al.* (2000); Hala and El Kelesh (2006); Radwan and El Kelesh (2009) were 58.2 % , 50.7 % , 87.5 % and 33.75 % respectively.

These difference in prevalence may be attributed to differences in locality, sex, age, breed and climatic condition.

In (Figs 1-3) blood films revealed the presence of different forms of the *Theileria* spp in addatiton to koch's blue bodies. This finding is agree with perviously described by Salah and Mahran (2003); Radwan and El Kelesh (2009); Hosny *et al.* (2010).

In (Table 3) The age susceptibility of *Theileria* spp. showed that highest rate of infection at age group 2 – 3 years were (30%, 18% and 28 %) in (cattle, buffaloes and sheep). These results a greed with that obtained by Abd ElKader (1991) who reported that the

susceptibility of clinical theileriosis was low among the calves of less than 1 years old then increased in age of (1 – 3 years), Anada, *et al.* (2009); Ziapour *et al.* (2011) whose recorded similar results.

The infection rate was low among young animals due to young animals posses innat resistance enhanced by maternal antibodies than This resistance declined gradually leaving the animal with high suscptibility to disease.

In (Table 5) The seasonal incidence of *Theileria* Spp. of our study revealed that, the maximal infection rate was recorded during Summer and Spring months followed by Autumn and winter the results agreed with that obtained by Abd ElKader (1991) The varitation of infection rates in different seasons may attributed to effect of climatic condition on tick activity which increased in Summer as well as period of the vector activity.

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### انتشار طفيليات الدم في بعض حيوانات المزرعة بمحافظة البحيرة

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حيوانات المزرعة وبالأخص (الأبقار والجاموس والأغنام) من المصادر الرئيسية للحوم والألبان في مصر وتعتبر طفيليات الدم واحدة من الأوليات الخطيرة التي تسببها كل من أجناس البابيزيا والتيليريا. الهدف من هذه الدراسة التعرف على مدى تواجد طفيليات الدم في بعض حيوانات المزرعة (الأبقار والجاموس والأغنام) عن طريق فحص مسحات دموية. تم فحص ٨٠٠ حيوان (٣٠٠ أبقار و٢٠٠ جاموس و٣٠٠ أغنام) تتراوح أعمارهم من أقل من عام حتى أكبر من ثلاثة أعوام في الفترة من إبريل ٢٠١١ حتى مارس ٢٠١٢ بمحافظة البحيرة تم عمل مسحات دموية رفيعة صبغت بصبغة الجمسا وتم فحصها عن طريق الميكروسكوب الضوئي وقد أظهرت النتائج إن نسبة الإصابة بطفيليات الدم بصفة عامة كانت ٤٣.٣% و ٢٠% و ٣٩% في كل من الأبقار والجاموس والأغنام على التوالي وكانت نسبة الإصابة بأجناس البابيزيا في (الأبقار / جاموس / أغنام) عن طريق المسحات الدموية المصبوغة بصبغة الجمسا (١٩.٣٣% ، ٩% ، ١٧%) على التوالي بينما كانت نسبة الإصابة بأجناس التيليريا في (الأبقار / جاموس / أغنام) عن طريق المسحات الدموية المصبوغة بصبغة الجمسا (٢٣% و ١٠% و ٢٠%) و كنت نسبة الإصابة المشتركة بكل من البابيزيا والتيليريا معاً في كل من (الأبقار / الجاموس / أغنام) (١% / ١% / ٢%) على التوالي و تحققت أعلى نسبة إصابة بطفيليات الدم (البابيزيا والتيليريا) في الفئة العمرية من (٢-٣) أعوام حيث كانت نسبة الإصابة بأجناس البابيزيا في (الأبقار / جاموس / أغنام) عن طريق المسحات الدموية المصبوغة بصبغة الجمسا (٢٨% ، ١٦% ، ٢٥%) على التوالي بينما كانت نسبة الإصابة بأجناس التيليريا في (الأبقار / جاموس / أغنام) عن طريق المسحات الدموية المصبوغة بصبغة الجمسا (٣٠% و ١٨% و ٢٨%) وكانت أعلى نسبة إصابة بطفيليات الدم في فصلي الصيف والربيع على التوالي في حيوانات المزرعة .