STUDIES ON SHEEP BABESIOSIS IN KALUBYIA GOVERNORATE, EGYPT.

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A survey on haemoparasites of sheep in Kalubyia Governorate was carried out during the period extended from the beginning of September 1998 to the end of August 1999 . Haematological examination of sheep revealed that 23 out of 200 animals were infected with Babesia species .Babesia ovis was reported in 17 animals and six animals had both B.ovis and B.motasi .Seasonal dynamics of ovine babesiosis revealed that sheep examined during Summer showed high prevalence (22.22%) followed by those examined during Spring and Autumn (16.07 % and 4.4% respectively) .Sheep examined during Winter were free from infection . The present study revealed that sheep aged between 12 to 24 months were susceptable to infection with ovine babesiosis than other age groups . Tick Rhipcephalus bursa were collected from sheep ,dissected and developmental stages of Babesia were identified in their haemolymph .

INTRODUCTION

Babesia are intraerythrocytic protozoan parasites of domestic and wild animals .Sheep babesiosis caused by *Babesia ovis* (Babes 1892) and *Babesia motasi* (Wenyon 1926) are of considerable economic importance in the areas infested with *Rhipicephalus bursa* . *Rhipicephalus bursa* is widely distributed in the region between the 31 -45 parallets North including the Mediterranean basin (Hoffmann et al 1971 , Hoogstraal (1979) ,Iraq (Robson et al 1968) and Iran (Hoogstraal and Valdez 1980). Morbidity and mortality due to babesiosis in sheep corresponded closely to the seasonal activity of the vector *Rhipicephalus bursa* (Badescu et al 1968 ,Monov et al 1977 and Yeruham et al 1989,1995) .Studies on ovine babesiosis is comparatively rare compared with bovine babesiosis in Egypt . Nagaty (1974) reported the existance of both *B.ovis* and *B.sergenti* in Egyptian sheep and goats .Ezzat (1960) found sporadic cases of babesiosis in sheep and goats . Abo El -Khair (1980) stated that 23.86 % of the examined sheep at Dakahlia Governorate were infected with *B.ovis* .Recently Badawy (1998) indentified *B.ovis* from sheep in Moneeb slaughter house in Cairo .However , previous studies on sheep babsiosis in Kalubyia Governorate are lacking .

So the present work was planned to identify *Babesia* species in sheep , their seasonal incidence and age susceptabilty . Moreover , ticks were collected ,identified and dissected to study the developing stages of *Babesia* in their haemolymph.

MATERIAL AND METHODS

Animals:

blood samples were collected from 200 sheep of different ages during the period extending from September 1988 till August 1999 . the examined sheep were belong to private sheep flocks in Meet Kenana ,Shebeen El- Kanater and Moshtohor (Kalubyia Governorate) .

Blood smears:

Thin blood smears were prepared from a drop of blood collected from ear vein . The smears were air dried ,fixed in absolute methanol for 10 minutes and stained for 45 minutes in a 6% dilution of Giemsa stain in PBS PH 7.2 according to Pritchard and Kruse(1982). The stained films were examined under oil immersion lens.The detected piroplasms were identified according to (Soulsby1982) and Levine (1985)

Ticks:

Ticks in the present study were collected from the examined sheep and identified according to the keys of Hoogstraal (1956) and (Hoogstraal and Kaiser 1958) Haemolymph smears were made from 160 semi engorged *Rhipicephalus bursa* female ticks . The female tick was picked up with an entomological forceps and placed on a clean dry marked slide . The distal portion of the first coxae of tick were cut using clean ,dry microscissor. The slightly yellowish or colourless tansparent haemolymph was obtained on a marked glass slide . The smears were air dried ,fixed in absolute methanol for 5 minutes ,stained with Giemsa stain and examined under oil immersion lens .The detected parasites were identified accorging to Burgdorfer (1970) and Friedhooff (1981) .

RESULTS

Out of 200 examined sheep ,only 23 (11.5%) animals were infected with *Babesia* species . Two species of *Babesia* had been reported viz : *Babesia ovis* and *Babesia motasi* . *B.ovis* was smaller than *B.motasi* being 1 -2.4 um in length and 0.8-1.7 um in breadth. The majority of organisms were round occurring at the margin of the red blood cells (Fig 1). Pyriform organisms were comparatively rare and when they occur in pairs the angle between them was abtuse . *B.motasi* was a large pyriform in shape measuring 2.5 - 4 um x 2 um occupying the center of the red blood cells (Fig1). Table (1) displayed that 17 animals (8.5%) were infected with *Babesia ovis* and 6 ones (3%) were harbouring both *B.ovis* and *B.motasi* .None of the infected sheep was infected with *B.motasi* alone . The present data revealed that sheep examined during Summer and Spring showed highest percentage being (22.22 % and 16.07 %respectively) . In Autumn only 2 animals out of 45 were infected (4.4%). Non of the examined sheep during Winter were harboured *Babesia* in their blood.

Concerning the relationship between the age of the examined sheep and the rate of infection , (Table 2) displayed that the highest infection rate was among those aged 1 to 2 years .and the lowest rate was in sheep over three years (20% and 5% respectively)

The only collected and identified ticks from all investigated localities was *Rhipicephalus bursa* . Examination of 320 oil immersion feilds of haemolymph smears of the detected ticks revealed that 28.13 % were infected with developmental stages of Babesia spp..The obtained stages as shown in (Fig 2) were banana shaped merozoites with red stained nucleus, bluish cytoplasm and a vacule .They measured 1.6 -2.1 um (mean 1.8 um) in length and 0.60 to 0.79 um breadth (mean 0.68 um).Spherical stages ,fission bodies and stages with two long ends were also detected.

DISCUSSION

In the present work *B.ovis* and *B.motasi* were detected . *B .ovis* was found to be the dominant spp. and *B.motasi* was not seen alone . This agreed with Papadopoulos et al (1995) in Greece. *B.motasi* was reported in different countries of Western Europe Lewis and Herbert (1980) ,Uilenberg et al (1980) and Christensson and Thunegard (1981) . However, *B.motasi* were rare and in certain regions of Bulgaria and Iran unknown (Tomasevic 1962 and khalacheva and kyurtov 1981). Morphologically ,round and pear forms corresponded to *B .ovis* as described by Lestoquard (1925) in Algeria .As well as *B.motasi* reported here was identical to those described by Uilenberg et al (1980) and Soulsby (1982) . In the present study , the single rounded forms were abundant while in Algeria the annular forms were more frequent .The double pear forms were predominant in the illustration of *B.ovis* made by Cernaranu ,1958 (cited by Purnell 1981) .It is known that the proportion of the various forms of *Babesia* species changes during the infecion of sheep Lewis and Herbert (1980) . The behaviour of the parasite probably changes with breed ,age and the immunological response of the host .

In the present study, 11.5 % (23 out of 200) of examined sheep harboured *Babesia* spp. in their blood.This results agreed with that described by Yeruham et al (1995) who reported ovine babesiosis in 2-25% of examined sheep in Israel .On the other hand our results was lower than those of Abo -El -Khair (1980) ,Christensson and Thunegard (1981) ,Duzgun et al (1991),Singer et al (1997), Ferrer et al (1998) , Sayin et al (1998) and Yeruham et al (1998) who reported infection rates of 23.68 % ,60-80 %, 35 %, 32.6 %, 55.7-71.6 % and 88.9 % in sheep respectively.Those differences might be attributed to the change in husbandry , climatic conditions and the injudicious control of ticks .

With respect to the seasonal prevalence of *Babesia* spp. among sheep , the present study revealed that the peak was in Summer followed by Spring .That agreed with Yeruham et al (1995 ,1998) in Israel who found that the incidence of babesiosis reached a peak in May with a corresponding peak in the animal infestation rate of *R.bursa* ticks .Similar results have been also reported by Kohler et al (1967), Sherkov et al (1976),Liebisch and Zukari (1978) and Abo -Shehada et al (1988) .In our results the lowest rate of infection was recorded in Autumn . Similarly ,Sherkov et al (1976) reported a second season with a lower incidence of babesiosis in Autumn in Jordan .Abo El-Khair (1980) in Egypt found that the peak of natural infection with *B.ovis* was in June (42.6%) then the infection rate was decreased in July , May, April and August, being 41.3 % , 36.9 % ,32.6 % and 28.3 % respectively . The rate of infection varied between 17.4 % to 11.1 % at the period between September and December .

Concerning the relationship between the age of the examined sheep and the rate of infection , the present data revealed that the highest prevalence was among those between 12 to 24 months old . This result agreed with Habela et al (1990) and Yeruham et al (1995) who stated that morbidity in the sheep herds might be attributed to the fact that only 67.5% of colostrum samples of the ewes contained maternal immunity to *B.ovis* and the antibodies persisted for a period of only 3

months in the lambs . Thus it seemed that lambs must be exposed to *B .ovis* up to to the age of 4 months in order to maintain enzootic stability . However , Cleon (1988) reported that sheep aged between 6 to 12 months old have higher prevalence than animals of other age groups .

In the present study, *R. bursa* ticks were collected from infected sheep which morphologically identical to those described by Hoogstraal (1956) and Cleon (1988) .

The examination of haemolymph collected from *R .bursa* revealed that 28.13% positive cases of banana shaped merozoites , spherical stages ,fission bodies and stages with two long ends . These results was higher than that obtained by Ahmed (1980) who found that 7.14% of examined *Boophilus annulatus* ticks were infected with club or banana shaped merozoites of *Babesia spp.* being 1.5 -2 um x 0.50 -0.75 um .Such difference might be due to type of ticks , climatic condition and changes in control measure. Also developmental stages of *Babesia spp.* in ticks had been studied by Friedhoff (1981) and Castella et al (1995).

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Table (1) . The seasonal prevalence of ovine babesiosis

Parasite Season	No. of ex. sheep	No. of +ve	%	Babesia ovis No.	%	B. ovis + B. motasi Mixed infection No.	%
Autumn	45	2	4.4 %	2	4.4 %	0	0 %
Winter	45	0	0 %	0	0 %	0	0 %
Spring	56	9	16.07 %	7	12.5 %	2	3.57 %
Summer	54	12	22.22 %	8	14.81 %	4	7.47%
Total	200	23	11.5 %	17	8.5 %	6	3 %

Table (2). Relationship between age of the exemined sheep and rate of infection with Babesia species.

Age	Less than 6 months			6- 12 months			1-2 years			2- 3 years			Over 3 years		
	No of ex.	+ ve	%	No of ex.	+ ve	%	No of ex.	+ ve	%	No of ex.	+ ve	%	No of ex.	+ ve	%
Animals	40	0	0	80	11	13	40	8	20	20	3	15	20	1	5

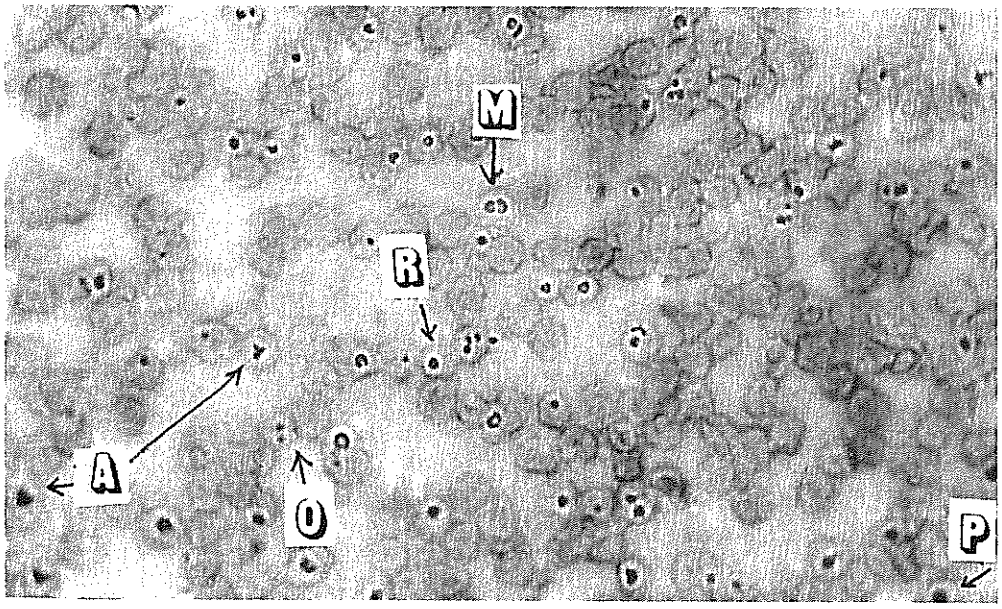

Fig (1) showing the different forms of *Babesia motasi* and *Babesia ovis* (X 1000)
M= B.motasi R = round form O = B .ovis P =pear shape of large *Babesia*
A = Amoebiod shape . DP=double ppear shaped .

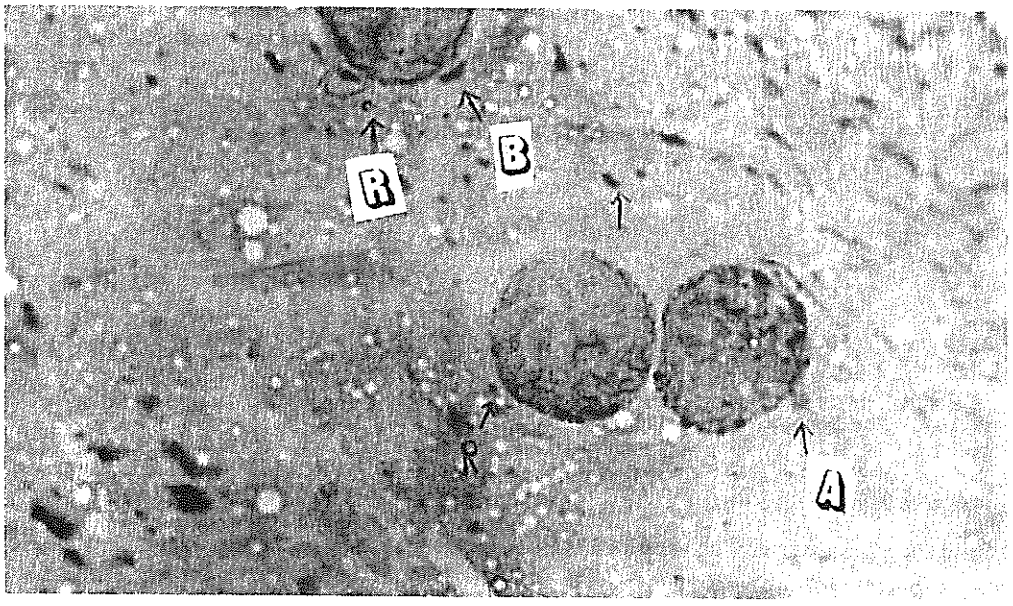

Fig (2) showing the different forms of *Babesia* in tick haemolymph (X 1000) .
A =Amoeboid form . R =Round form B = Banana shape .