Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 56 (1): 137-144 Jan. 2018
DOI: 10.5455/ajvs.284484

Dynamics of *Boophilus* Ticks and its Role in Transmission of Piroplasms at Behaira District

Soad M. Menshawy, Eman K. Bazh, Abdelrahman A. Abou-Rawash

Pathology and Parasitology Department, Faculty of Veterinary Medicine, Damanhour University.

Key words:
Tick, Hemolymph, salivary gland, ookinetes, sporozoites, Boophilus, Behaira

Correspondence to:
soadelmenshawy@vetmed.dmu.edu.eg

ABSTRACT

Dynamics of Boophilus tick was investigated at Behaira district. A total number of 86 out of 839 cattle (10.25%) were infected with Boophilus spp. The prevalence of Boophilus spp infection predominated in summer season (21.33%) followed by autumn (10.78%) and last spring (6.34%) and the lowest burden was in winter. Concerning the locality, El-Dalangat was representing the highest locality infected with tick vector (17.39%) and according to age, the animals less than one year was the lowest one infected with tick (5.17%) then animals which were aged 1-3 years (9.8%) and animals more than 3 years (10.75%).The neck and dewlap representing the highest predilection seats of Boophilus spp. (81.39%) in cattle and the lowest one was udder (2.94%). Hemolymph and salivary gland samples of 56 Boophilus spp were extracted, stained by Giemsa and microscopically inspected under oil immersion lens. the total infection of Hemolymph with ookinete was 28.57% that increased in summer season (32.14%). The total infection rate was 21.43% and increased in summer (25%) and no infection was recorded in winter. Electron microscopic examination of salivary gland of Boophilus spp. showed a presence of electron-dense, irregular-shaped sporozoites in some infected cells

1-INTRODUCTION

Cattle were raised for their great economic importance as they providing the human with milk, meat and skin production. They are very important factors in generating export incomes. Ticks are blood-sucking arthropods of animals and humans. They occur worldwide and are highly distributed in Egypt especially in villages (farms and pastures) (Adham et al., 2009). Tick infection in cattle is one of the major constraints to the livestock industry in developing countries which adversely affects economic performance, mainly by transmission of serious pathogens of animals (Das et al., 2005). Ticks act as vectors for many haemoprotozoa (eg. *Babesia* and *Theileria*) which have a great hazard effect on livestock health resulting in economic losses for farmers. *Boophilus annulatus* was well known in Egypt and found that cattle and horses were often heavily infected (Hoogstraal and Kaiser 1958). The detection of parasitic stages in the vector becomes essential, as this forms a component for assessing the infection rate in vectors and also helps to curtail the risk of babesiosis and theileriosis in enzootic areas. Examination of tick tissues and fluid (hemolymph) was found as effective for detecting the clinical and subclinical forms of babesiosis and theileriosis in cattle and found as a suitable epidemiological tool. Vermicule of the parasite consumed times for development inside vector tissue. However, vector tissue may be infected, but without transmitting infection. So, cytological studies of blood parasites in vector tissue will be helpful in this study on the epidemiology of bovine piroplasmids. This study aimed to detect the prevalence of hard ticks infecting cattle in Behaira district and to investigate the developmental stages of cattle piroplasms in ticks hemolymph and salivary gland.

2-MATERIALS AND METHODS

2.1- Animals and study area

A total of 839 cattle of different ages and sexes were examined for tick infection in five localities in Behaira district which represent the Behaira governorate. Whereas Itay- Elbaroud was located in the South of the District, Shobrakheit from the East, El-Dalangat and Abo-Elmatameir from the West, and Abouhomos from the North.

2.2-**Collection and identification of tick samples**

The whole body of each animal was inspected for the presence of tick's infection after visual examination. The ticks were manually captured from the host and orientated anticlockwise until the capitulum detaches from the host, kept alive in glass tubes and labeled with collection points noted. For identification, the ticks were examined under stereo-microscope. Then mounted on glass slides according to (Chhillar 2014 with modification). Adult ticks were identified according to general key Hoogstraal (1956), Arthur (1960) and Walker et al., (2003).

2.3- Collection and examination Hemolymph

Hemolymph was collected by severing the forelegs at the coxal–trochanteral joint, applying gentle pressure on the tick’s body and drawing the clear liquid expressed into a slide according to (Patton et al., 2012). The collected hemolymph was left to dry on a clean and dry slide and fixed with methanol for 5 minutes then stained with Giemsa stain, finally examined under oil immersion.

2.4- Harvesting and examination of salivary gland

The isolated ticks were washed with normal saline (0.9% NaCl) and dissected according to Edwards et al; (2009) was put part of salivary gland on the slide and crushed with edge of other slide or between two slides, let to dry and fixd with methanol then stained with Giemsa stain for detection of sporozoites according to Kruse and Pritchard (1982) and examined under oil immersion.

2-5- Electron microscope of salivary gland according to Yano et al; (2005)

Tick tissues including the salivary gland were fixed in cold 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 6 hr, and washed gently in the buffer. They were post- fixed with 1% OsO4 in the buffer for 3.5 hr, dehydrated with acetone and embedded in epoxy resin. Observations with an electron microscope (Hitachi H-600, 75 kV) were made of the salivary gland from ticks which were positive for *Babesia spp*. Processing of samples and examination occurred in electron microscope unit in Faculty of Science, Alexandria University.

3-RESULTS

3.1- Prevalence of *Boophilus* species.

In the present work, the total number of 86 out of 839 (10.25%) investigated cattle were infected by tick species and the identified tick was *Boophilus spp*. The prevalence of *Boophilus* infection predominated in summer season (21.33%) and the lowest burden was in winter (1.57%), table (1) and monthly infection rate of tick vector showed the highest rate in July (25%) and not recorded in January (0.00%), table (2). Concerning the locality, El- Dalangat represented the highest locality infected with tick vector (17.39%) and the lowest one was Abouhomos (5.78%), table (3). Cattle of more than 3 years old showed higher infection rate (10.75%) as compared with those at age of 1-3 years (9.8%) and less than one year (5.17%), table (4). The neck and dewlap regions of cattle showed the highest percentage of ticks (81.39%) and the lowest one was the udder (2.94%), table (5).

3.2- Detection of developmental stages of piroplasms in tick hemolymph

Microscopic inspection of Giemsa stained hemolymph smears of *Boophilus spp*. revealed that the total infection rate was 28.57% that increased in the summer season (32.14%) spring (30%) lowest in autumn (26.67%) and no infection was recorded in winter table (6).The existence of different developmental stages of piroplasms including ookinete were rod shapes, banana and club- shaped developmental stages (Figure 1).

3.3- Detection of developmental stages of piroplasms in tick salivary gland

Microscopic examination of Giemsa stained salivary gland smears of *Boophilus spp*. showed the presence of different developmental stages of piroplasms including sporozoites which were crescent and rod shapes developmental stages (Figure 2).The total infection rate was 21.43% and increased in summer (25%) also winter had no infection, table (6).

Concerning locality, El-Dalangat was the highest locality in hemolymph and salivary gland infection (44.44% and 38.89%) followed by Abo-Elmatameir (26.67% and 20%), Itay-Elbaroud (25% and 12.5%) then Shubrakheit (16.67% and 00.00%) and Abouhomos (11.11% and 11.11%), table (7).

3.4- Ultrastructure examination of salivary gland.

Semithin section of salivary gland of *Boophilus spp*. tick showed different shapes of sporozoites, Fig. (3). Electron microscopic examination of the salivary gland of *Boophilus spp*. Revealed presence of a pale-staining spheroidal structure formed of a cluster of undifferentiated branching parasites, the developmental stages called sporoblast.

The sporoblast existed freely in the host cytoplasm and had a single cytoplasmic membrane. There was no surrounding host membrane or vacuole. It was also difficult to distinguish between the nucleoplasm and cytoplasm, because both had almost the same density.

The infected cells were filled with numerous developing sporozoites. They were of the same ovoid shape and surrounded by a single cytoplasmic membrane and single rhoptry, which is a characteristic organelles for sporozoites. Some of the mitochondria showed enlargement and vacuolation. The examination also revealed detachment of ribosomes which were free in the cytoplasm. More electron-dense and more irregular shaped sporozoites were also detected in some infected cells. (Figures 4,5,6)

Table (1): Seasonal dynamics of *Boophilus spp*. infected cattle in Behaira district.

Season	Total no. of exam. Animals	No. of infected cattle (%)
Spring	205	13 (6.34%)
Summer	211	45 (21.33%)
Autumn	232	25 (10.78%)
Winter	191	3 (1.57%)
Total	839	86 (10.25%)

Table (2): Monthly prevalence of *Boophilus spp*. infected cattle in Behaira district.

Months	No. of exam. Animals	No. of infected Animals (%)
January	62	0 (0%)
February	61	2 (3.28%)
March	51	6 (11.76%)
April	73	3 (4.11%)
May	71	4 (5.63%)
June	78	14 (17.95%)
July	72	18 (25%)
August	87	13 (14.94%)
September	76	10 (13.16%)
October	77	5 (6.49%)
November	63	10 (15.87%)
December	68	1 (1.47%)
Total	839	86 (10.25%)

Table (3): Infection rate of *Boophilus spp*. in different locality in Behaira district.

Location	No. of exam. Animals	No. of infected Animals (%)
Abouhomos	173	10 (5.78%)
El-Dalangat	161	28 (17.39%)
Itay-Elbaroud	166	14 (8.43%)
Abo-Elmatameir	170	21 (12.35%)
Shubrakheit	169	13 (7.69%)
Total	839	86 (10.25%)

Table (4): Infection rate of *Boophilus spp*. concerning to animal age in Behaira district.

Age	No. of exam. Animals	No. of infected Animals (%)
< than one year	58	3 (5.17%)
1-3 years	102	10 (9.8%)
> 3 years	679	73 (10.75%)
Total	839	86 (10.25%)

Table (5): Predilection seats of ticks among cattle in Behaira district.

Predilection seats	Frequency of infection	%
Head	7	8.14%
Neck and dewlap	70	81.39%
Root of tail	4	4.65%
Udder	2	2.94%
Back	8	9.3%
Abdomen	10	11.63%
All body	7	8.14%
Total	86	100.00%

Table (6): Seasonal distribution of different developmental stages of piroplasmides in *Boophilus spp.* hemolymph and salivary gland.

Season	No. of examined tick	Hemolymph		Salivary gland	
		+ve No.	%	+ve No.	%
Spring	10	3	30%	2	20%
Summer	28	9	32.14%	7	25%
Autumn	15	4	26.67%	3	20%
Winter	3	0	00.00%	0	0%
Total	56	16	28.57%	12	21.43%

Table (7): Infection rate of developmental stages of piroplasmides in tick hemolymph and salivary gland concerning to locality.

Location	No. of exam. Tick	Hemolymph		Salivary gland	
		+ve No.	%	+ve No.	%
Abouhomos	9	1	11.11%	1	11.11%
El-Dalangat	18	8	44.44%	7	38.89%
Itay-Elbaroud	8	2	25%	1	12.5%
Abo-Elmatameir	15	4	26.67%	3	20%
Shubrakheit	6	1	16.67%	0	0%
Total	56	16	28.57%	12	21.43%

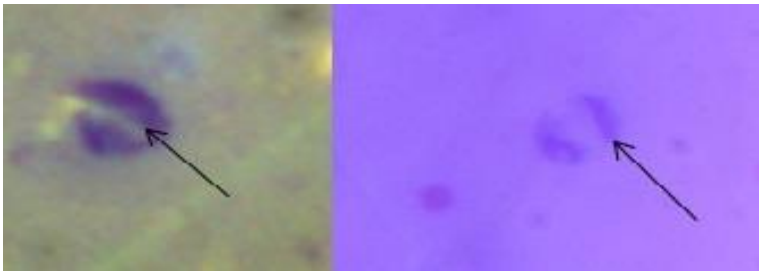

Fig. (1): Boophilus spp. hemolymph smear stained with Giemsia showing different developmental stages of blood parasites (banana form) as indicated by arrow.

Fig. (2): Giemsa stained Boophilus spp. salivary gland smear showing different developmental stages, crescent-shaped (arrow).

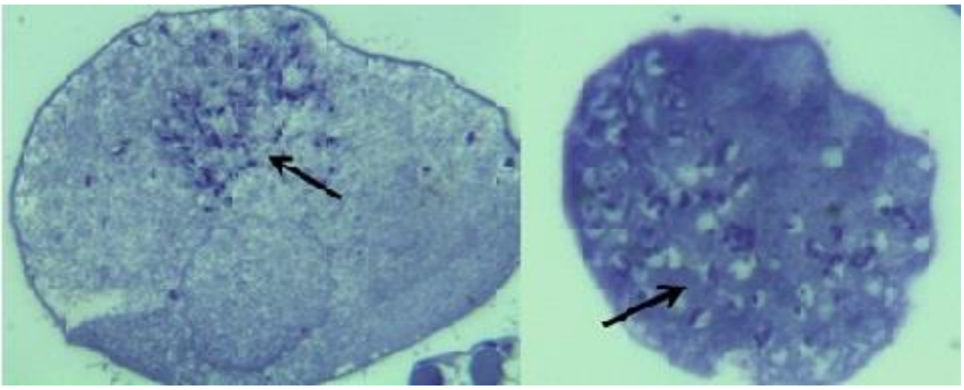

Fig. (3): A semithin section of acini of *Boophilus spp*. tick salivary gland which arrows referred to different shapes of Sporozoites.

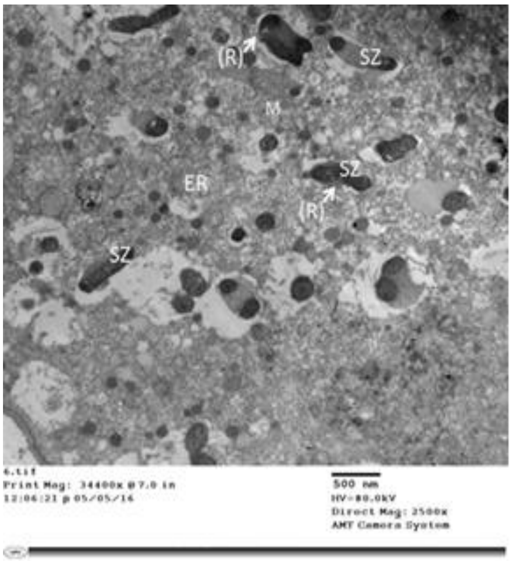

Fig. (4): Electron micrograph showing sporozoites (sz) in a salivary gland cell tick. The sporozoites are more electron-dense and more irregular in shape. (rhoptry =R), mitochondria=M.

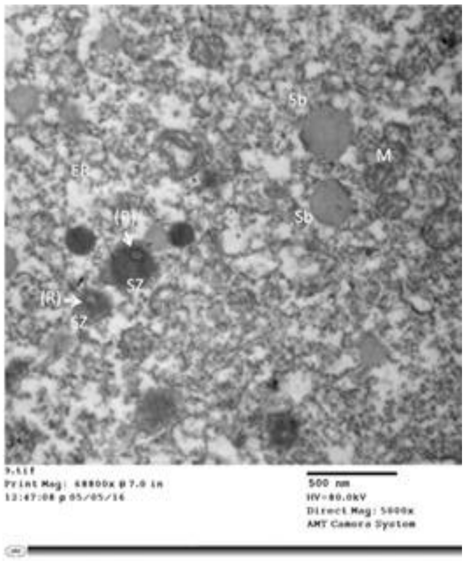

Fig. (5): Electron micrographs showing. Undifferentiated masses of sporozoites (sporoblast) (sb) of piroplasm spp. in a salivary gland cell of Boophilus ticks., Sporozoites (sz) have rhoptery (R). mitochondria (M), Endoplasmic Reticulium (ER).

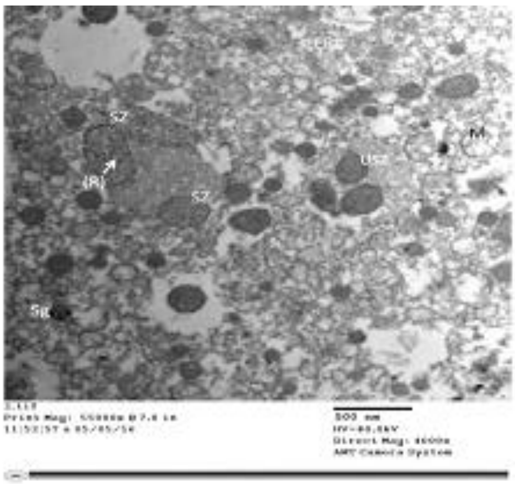

Fig. (6): Electron micrographs showing. undifferentiated masses of sporozoites (sporoblast) (USZ) of piroplasm spp. in a salivary gland cell of Boophilus ticks., Sporozoites (SZ) have rhoptery (R). mitochondria (M), Endoplasmic Reticulium (ER), Secretory granules (Sg).

4- DISCUSSION

In current study, the obtained results showed that *Boophilus* tick was the only tick species infect cattle, these results were in agreement with Said and Atif (1958) in Shebin El-Kanatir district, Salem (1986) at Sidi Salem district, Darweesh (1999) in Elbehaira Province, El-Kammah et al., (2001) in Giza, Sharkia, Ismailia, El Beheira and Sinai who recorded *Boophilus annulatus* was found only on cows (100%), Patel et al., (2013) in India and Ramadan et al., (2016) in Qalyobia governorate.The more prevalence of *Rhipicephalus* (*Boophilus*) species on cattle might be attributed to their being brevirostrate thus preferring thin- skinned and hairier hosts.

Low prevalence of *Boophilus* spp. (10.25%) came partially in agreement with Darweesh (1999) in Elbehaira, Ndhllovu et al., (2009) in Zimbabwe and Ramadan et al., (2016) in Qalyobia governorate and disagree with El-Housary (1981) at Kafr El-Sheikh and Ibrahim (1981) in El-Minyia, Rony et al., (2010) in Bangladesh. Mossie et al., (2016) in Ethiopia and Ali et al., (2016) in Pakistan.The difference may be due to use of acaricides and hygienic measures in different localities as well as the immune status of animals. Also low, incidence may be attributed to the method of cattle rearing and effort given by veterinary authorities to control ectoparasites in the few last years.

The prevalence of *Boophilus* infection was predominated in summer season (21.33%) followed by autumn (10.78%) then spring (6.34%) and the lowest burden was in winter because of at low temperature. In winter ticks try to protect themselves by entering in diapauses leading to delayed morphogenesis and reduced behavioral activities while in summer season increase activity of tick due to increase temperature which enhances the vector life cycle. The results are in agreement with the results of Gattase (1983) in Suez Canal Zone, Darweash (1999) in Elbehaira , Abbas et al., (2006) in Giza and Ismailia, Salim abadi et al., (2010) in Iran and Moges et al., (2012) in Chilga district, northwestern Ethiopia. On the other hand, disagreement with Hassan et al., (1958) in Egypt, El-Housary (1981) in Kafr El-Sheikh governorate and Singh and Rath (2013) in India, who recorded the activity of tick was increasing in the rainy season (autumn and spring) Thus rainfall (humidity) seemed to be an important macroclimatic factor influencing seasonal variation in tick infection.

Concerning the locality, El- Dalangat was representing the highest locality infected with *Boophilus* tick vector (17.39%) followed by Abo-Elmatameir (12.35%), Itai-Elbaroud (8.43%) then Shubrakheit (7.69%) and the lowest one was Abouhomos (5.78%), thus it may be due to use of acaricides and hygienic measures of different localities.

According to age, the results of the present study showed that the older ages were susceptible to tick infection than young ages; these findings were in agreement with Darweesh (1999) in Behaira and Alemu et al., (2014) in Ethiopia, The differences could be attributed partially to the less exposure to grazing field with other animals and partially to host immune status explained the recorded differences.

The most favorable predilection seats of *Boophilus spp.* in cattle were the neck and dewlap which in agreement with Tessema and Gashaw (2010) in Ethiopia, Ikpeze et al., (2011) in Nigeria, Patel et al., (2013) in India Wasihun and Doda (2013) and Gedilu et al., (2014) in Ethiopia While disagree with Hasson (2012) in Aldoura region south Baghdad, Ali et al., (2016) in Pakistan.The variation of tick species may be the cause associated with this matchlessness, as *Boophilus spp*. has short mouth part so prefer sites of thin- skinned and long hair.

Examination of Giemsa stained hemolymph smears of *Boophilus spp*. revealed that the total infection rate was (28.57%) that increased in summer season (32.14%) followed by spring (30%) then in autumn (26.67%) and winter had no infection. These findings are with agreement with Ramadan et al. (2016) in Qalyobia governorate and disagree with El-Bahy (1986) in Fayoum governorate and Quintao-Silva and Ribeiro (2003) in Brazil who found 10% infected hemolymph smears.

Microscopic examination of Giemsa stained salivary gland smears of *Boophilus spp*. showed that the total infection rate was (21.43%) and increased in summer (25%) also winter had no infection. The results are in agreement with El- Bahy (1986) who found the percent of infected salivary gland was less than the percent of infected hemolymph of *Boophilus spp*.

The difference in percent of infection may be due to the life cycle in salivary gland took a long time than in hemolymph also its asexual reproduction leading to the formation of small infective stages in salivary gland.

The findings of electron microscopic examination of salivary gland of *Boophilus spp*. are in agreement with Potgieter and Els (1976) and Yano et al., (2005) in Japan.

We can conclude that the prevalence of *Boophilus spp* was predominated in the summer season. El-

Dalangat was representing the highest locality. Animals more than 3 years were highest one .The neck and dewlap representing the highest predilection seats of *Boophilus spp.* The infected hemolymph and salivary gland increased in summer season and ultrastructure revealed that electron-dense and more irregular shaped of sporozoites were detected in some infected cells, from this study; we recommend to make periodical control for ticks especially in summer season.

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