Prevalence of hard tick infesting cattle with a special reference to microscopic and molecular early diagnosis of tick born piroplasms

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A B S T R A C T

The current study aimed to conclude the prevalence of hard ticks infesting cattle in Qalyobia governorate and to investigate the developmental stages of cattle piroplasms in ticks hemolymph. A total of 600 cattle were haphazardly examined for tick infestation throughout the period from April 2013 to March 2014. Hemolymph samples of 1550 ticks were extracted, stained by Giemsa again and microscopically inspected under oil immersion lens. Moreover, PCR assay was applied to amplify 18SrRNA genes of *Babesia* and *Theileria* in 40 microscopically negative hemolymph samples. The study revealed that 6.17% of cattle were infested by ticks. *Rhipicephalus turanicus* and *Rhipicephalus praetextatus* (70.02% and 29.98% respectively) were the recorded tick species. Ticks showed a great tendency to attach to the udder and inguinal regions (34.46%), while the two sides were the least infested (7.69%). No recorded significant effect of sex on the rate of infestation ($P > 0.05$). Cattle at age of 3-5 years were significantly more infested (78.38%) than those of 2-3 years (16.22 %) and those of 8months -2 years (5.41%). Microscopic examination of tick hemolymph revealed the presence of piroplasm developmental stages in 39.23% and 49.4% of hemolymph samples obtained from *Rhipicephalus turanicus* and *Rhipicephalus praetextatus* respectively. PCR analysis further revealed that amongst 40 ticks whose hemolymph were microscopically negative, 25% and 12.5% were positive for *Babesia* and *Theileria* sp. respectively. The results concluded that *Rhipecephalus turanicus* and *Rhipicephalus praetextatus* are the common tick species infesting cattle in the study area. PCR assay is proved to be more efficient and sensitive than the microscopic assay for identification of the developmental stages of piroplasms in tick hemolymph. Thus, allowing the easy and rapid surveillance of the endemicity of piroplasm infection among tick sp. and consequently application of emphasized control programs

Keywords*:* Tick, *Babesia, Theileria*, Hemolymph, Sporokinetes.

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1. INTRODUCTION

In Egypt, cattle has a great socio- economic importance in people life because of their milk, meat, skin and hide production which are very important factors in generating export incomes. Ticks are haematophagous obligate ectoparasites live stock as well as humans. The genera of *Ixodidae* including *Dermacentor, Rhipicephalus*, *Haemaphysalis*, *Boophilus*, *Ambylomma*, *Hyalomma*, and *Aponomma* genera are of a great importance throughout the world (Wall and shearer 2001). Ticks act as a vectors for many haemoprotozoa (eg. *Babesia* and *Theileria*) which have a great hazard effect on livestock health resulting in economic losses for farmers (Eygelaar et al., 2015). Microscopic examination of hemolymph smears doesn't constitute a very sensitive diagnostic tool for detection of developmental stages of haemoprotozoa and doesn't enable differentiation between species involved (Guglielmone et al., 1996, 1997) as compared to PCR. In Egypt, there is scarcity of documented data dealt with PCR assay for investigation of the developmental stages of piroplasms in ticks hemolymph, consequently this surveillance help in early detection of infection and applying control program. Therefore, in this investigation, a survey was conducted aimed to recognize the abundance of ticks infesting cattle, assessing mean burden and distribution and determination the prevalence and intensity of *Babesia* and *Theileria* sp. sporokinetes in hemolymph of tick using microscopic and PCR assays.

2. MATERIALS AND METHODS

2.1. Animals

A total of 600 cattle of different ages and sexes were examined for tick infestation from April 2013 to March 2014 in three localities in Qalyobia governorate (Benha, Toukh and Shebin-El Qanater), Egypt.

2.2. Tick collection

Individual cattle were carefully inspected and tick were collected from half- body region, including head, neck, sides, abdomen, flank regions and under the tail. The collected ticks were transferred to the laboratory into capped tubes containing 70% ethanol (Walker et al., 2003). The total number of the collected tick species was duplicated to determine the tick load per animal. Morphological characterization of the collected ticks was done using dissecting microscope according to the identification keys given by Hoogstraal (1956) and walker et al. (2003). Total incidence, seasonal prevalence, mean burden and distribution of ticks on different body regions were recorded.

2.3. Hemolymph collection and examination

Hemolymph of 1550 engorged and semi-engorged female ticks were obtained by nipping the first legs just above the first Coxa. The released hemolymph was spread on a clean, dry glass slide, fixed in absolute methanol, stained by Giemsa and microscopically examined under oil immersion lens. The detected sporokinetes were measured by using of eyepiece micrometer and identified following Guglielmone *et al.* (1996). The intensity of infestation was defined as the number of sporokinetes counted in each hemolymph smear, divided by the number of fields microscopically examined per smear (Quintão-Silva and Ribeiro 2003).

2.4. PCR assay

2.4.1. DNA extraction

DNA was extracted from microscopically negative hemolymph samples obtained from 40 ticks (20 in winter and 20 in summer) using Promega kits (Promega BioSciences, LLC) according to manufacturer's instructions.

2.4.2. PCR amplification

The 18S rRNA gene was amplified using a forward primer: GTC TTG TAA TGG GAA TGA TGG and a reverse primer: CCA AAG ACT TTG ATT TCT CTC) which amplify 350 bp and 370 bp fragment of *Babesia* and *Theileria* species. PCR reaction was done in 25 ul solution containing 1.5 ul Emerlad AmpGTPCR mastermix (2x premix), 4.5 ul PCR grade water, 1ul Forward primer (20mol),1 ul reverse primer (20 mol), 5ul template DNA 10 mM Tris–HCl, 50 mM KCl, 1.5 mM MgCl2, 1.5 U TaqDNA-polymerase, 0.2 mM of each nucleotide, 10 pm of each primer and 5 ml of DNA samples. PCR protocol was done according to Adaszek and Winiarczyk (2008) as follow: Primary denaturation at 94^{o}c for 10 minutes, Secondary denaturation at 94^{o}c for 45 s, Annealing at 45^{o}c for 45 s, extension at 72^{o}c for 45 s and Final extension at 72^{o}c for 10 m. The amplified product was envisioned on agrose gel stained with ethidium bromide.

2.5. Statistical analysis

It was applied b by means of SPSS software program (ves. 16) using one way Anova test. (Steel et al., 1997).

3. RESULTS

3.1. Frequency of tick species

In the present work, a total of 37 out of 600 investigated cattle (6.17%) were infested by *Rhipicephalus* tick spp. The prevalence of tick infestation predominated in summer season (13.33%). The overall mean tick burden was 68.8 / cattle. The highest tick burden/ cattle was recorded in autumn (122.8/ cattle) and the lowest burden was in winter (43/ cattle) (Table 1). The identified tick species were *Rhipicephalus turanicus* (70.02%) and *Rhipicephalus praetextatus* (29.89%). Generally, there was a significant effect of season ($P< 0.05$) on the prevalence of the identified tick species (Table 2, Fig. 1). A total of 2584 ticks including 158 nymphs (6.20%), 1550 females (60.83%) and 840 males (32.96%) were collected from cattle during the study period. The highest number of the collected nymph, females and males was encountered in August, March/October and July (7.78, 66.67 and 40.25% respectively) (Table3). The mean tick burden/cattle of nymph, female and male were 4.27, 41.89 and 22.27 respectively. The highest nymph, female and male tick burdens were recorded in autumn (6.8, 80 and 36/cattle respectively), while the lowest burdens were found in winter (3, 25 and 15/cattle respectively). The mean of tick sex ratio of *Rhipicephalus* ticks was 1.8: 1. In autumn the sex ratio was 2.2 : 1, and it was 1.6 : 1 in both summer and winter (Table 4). The udder and inguinal regions of cattle showed the highest percentage of different developmental stages of ticks in summer (22.15% nymphs17.42% females and 22.62% males), whereas the two sides were the least infested (2.53% nymphs3.55% females 1.79% males) (Table 5). Bulls were frequently more

infested (6.80%) than cows (5.84%) (Table 6) without any significant effect (P> 0.05). Cattle of 3-5 years old showed a significantly (P< 0.05) higher infestation rate (78.38%) as compared with those of at age of 2-3 years (16.22%) and 8 months – 2 years (5.41%) (Table 7).

3.2. Developmental stages of piroplasms in tick hemolymph

Microscopic inspection of Giemsa stained hemolymph smears of *R. turanicus* revealed the existence of different developmemtal stages of piroplasms including: a. Sporokinete which were crescent shapes (9-16 µ long) with pointed or blunt ends and a centrally located nucleus. *Babesia* sp. sporokinete was 9-13 um long and *Theileria* sp. sporokinete was14-16 um long. b. Round, amoeboid shape developmental stages (2-9 um) were also noticed in tick hemolymph (Fig.2. A- D). In *R. turanicus*, piroplasm developmental stages were found in 39.24% of hemolymph samples. The sporokinetes were recorded in 294 samples (28%). *Babesia* sp. sporokinetes were found in 64.97% of tick, and *Theileria* sp. sporokinetes were registered in 36.05% of ticks. High percentages of *Babesia* and *Theileria* sporokinetes were recorded in summer (73.39% and 41.28% respectively). Mixed infection between *Babesia* and *Theileria* sp. sporokinetes was recorded at a percentage of 14.29%. Round and amoeboid shape developmental stages were also noticed in tick hemolymph at a percentage of 11.24% (Table 8). Whereas, the examination of 500 *Rhipicephalus praetextatus* female hemolymph, revealed that 49.4% were positive for piroplasm developmental stages. Sporokinetes were recorded in 40.2% of samples.The highest infection rate of tick by sporokinetes was in summer (45%) and the lowest was in winter (25%). Mixed *Babesia* and *Theileria* sporokinetes were found in 38.81% and 26.87 of hemolymph smears respectively. In the hemolymph of *R. praetextatus* female, *Babesia* and *Theileria* sporokinetes were most prevalent in winter season (60% and 40% respectively). Round and amoeboid forms of *Babesia* and *Theileria* were also seen in 9.2% of hemolymph smears (Table 9).

3.3. PCR evaluation

PCR assay of tick hemolymph succeeded to amplify 350 bp of *Babesia* in 25% of 40 microscopically negative hemolymphs (35% in summer and 15% in winter). Moreover, it amplified 370bp DNA fragments of *Theileria* sp. in 12.5% of the same samples (25% in summer and 0% in winter) (Table 10, Fig. 3).

4. DISCUSSION

In the present study, the lower vulnerability of cattle to tick infestation (6.17%) came in agreement with Gabaj et al., *(*1992) (9.6%) in Libya. Conversely, it was lower than that of Hassanain (1997) (80.44 %) in Behera, Egypt, Mangold *et al.*, (1989) (34%) in Argentina, Khan et al. (1993) (28.2%) in Pakistan. Such low incidence may be attributable to the method of cattle rearing and efforts given by veterinary authorities to control ectoparasite in the few last years. In accordance with our study, Aydn (2000) in Turkey, Rony et al. (2010) in Bangladesh and Kabir et al. (2011) in Ethiopia proved that the the seasonality of ticks elicit a significant increase in summer seasons.

Out of 2584 collected ticks, nymphs, females and males and were recognized in 6.20, 60.83 and 32.96% of the examined ticks respectively. In that matter, Nasibeh et al., (2010) in Northern Iran and Lorusso et al. (2013) in Nigeria collected a nearly similar percentage of both male and female ticks. The mean of sex ratio between females and males *Rhipicephalus* tick recorded in this current study (1.8: 1) was nearby that was found by Fanos et al. *(*2012) who recorded an average male to female sex ratio of 1.3:1.

The method of calculation of mean tick burden per infested cattle and/ or the different tick species examined might be from the factors which make our result (68.8 /cattle) differs from the other investigators (Tomassone et al., 2004) (247.55) in Guinea, Razmi et al. (2003) (6.35), Ica et al. (2007) (3.86), Belew and Mekonnen (2011) (5.9), Kalume et al. (2013) (6.5±0.22). Tick burden showed the highest prevalence in autumn (122.8/cattle) and this agreed with Guglielmone *et al.,* (1990). Whereas, Castellà et al. (2001) found that the peak of tick burden in the Spanish Mediterranean coast was in summer and spring (7.5 ticks/ cattle). The identified tick species (*R. turanicus* and *R. praetextatus*) in this work were previously recognized in Egypt by Shoukry et al. (1993), El-Kammah et al. (*2001)* and walker et al. (2003). Although these forementioned authors identified many other tick genera, our results point to the successful veterinary authorities efforts in last years for eradication of ticks, especially those of one host tick.

Ticks showed a great tendency to attach to cattle udder and inguinal region as previously reported by Opara et al. (2005) and Nady et al. (2014). This finding may be accredited to that the inguinal area and udder are thin skin and highly vascularized areas which facilitate tick penetration and allow easier feeding (Sajid, 2007). Alternatively, Tamiru

Table (1) Seasonal dynamics of *Rhipicephalus* species infesting cattle and tick burden.

Season	No. examined	No infected	%	No of ticks	%	Tick burden
Spring	150	10	6.67	719	28.21%	71.9
Summer	150	20	13.33	1129	44.31%	56.45
Autumn	150	5	3.33	614	25%	122.8
winter	150	2	1.33	86	3.38%	43
Total	600	37	6.17	2548	-	68.86

Table (2) The species of ticks infesting cattle during different seasons of the year

Season	Total ticks	*R. turanicus*		*R. Praetextatus*	
		No.	%	No	%
Spring	719	519	72.18^{aB}	200	27.82^{aA}
Summer	1129	709	62.80^{aA}	420	37.20^{aA}
Autumn	614	500	81.43^{aB}	114	18.57^{aA}
Winter	86	56	65.12^{aB}	30	34.89^{aA}
Total	2548	1784	70.02^{B}	764	29.98^{A}

Different superscripts letters (a, b, c) in the same column indicate significant differences at $P<0.05$. Different superscripts letters (A, B, C) in the same row indicate significant differences at $P<0.05$.

Table (3). Monthly prevalence of nymph, male and female of tick infesting cattle

Months	Collected tick No.	%	Nymph		Female		Male	
			No.	%	No.	%	No.	%
March	75	2.94	5	6.67^{aA}	50	66.67^{cC}	20	26.67^{bB}
April	320	12.56	20	6.25^{aA}	200	62.50^{bcC}	100	31.25^{bcB}
May	324	12.72	19	5.86^{aA}	200	61.72^{bcC}	105	32.41^{bcB}
June	310	12.17	15	4.84^{aA}	200	64.51^{bcC}	95	30.65^{bcB}
July	472	18.52	32	6.78^{aA}	250	52.97^{bC}	190	40.25^{cB}
August	347	13.62	27	7.78^{aA}	200	57.64^{bcC}	120	34.58^{bB}
September	305	11.97	15	4.92^{aA}	200	65.57^{bcC}	90	29.51^{bcB}
October	150	5.89	10	6.67^{aA}	100	66.67^{cC}	40	26.67^{bB}
November	159	6.24	9	5.66^{aA}	100	62.89^{bcC}	50	31.45^{bcB}
December	86	3.38	6	6.98^{aA}	50	58.13^{bcC}	30	34.88^{bcB}
January	0	0	0	0^{aA}	0	0^{aA}	0	0^{aA}
February	0	0	0	0^{aA}	0	0^{aA}	0	0^{aA}
Total	2548	-	158	6.20^{A}	1550	60.83^{C}	840	32.97^{A}

Table (4): Seasonal dynamics of nymph, female and male tick burden and sex ratio of Rhipicephalus species infesting cattle.

Season	No. ex.	No of infest. cattle	Infest. %	Total ticks	No of collected nymph	Nymph burden	No of collected female	Female burden	No of collected male	Male burden	Sex ratio
Spring	150	10	6.67	719	44	4.4	450	45	225	22.5	2: 1
%					6.12		62.59		31.29		
Summer	150	20	13.33	1129	74	3.7	650	32.5	405	20.25	1.6: 1
%					6.55		57.57		35.87		
Autumn	150	5	3.33	614	34	6.8	400	80	180	36	2.2: 1
%					5.54		65.15		29.32		
Winter	150	2	1.33	86	6	3	50	25	30	15	1.6: 1
%					6.98		58.14		34.88		
Total	600	37	6.17	2548	158	4.27	1550	41.89	840	22.7	1.8: 1
%					6.20		60.83		32.97		

Table (5): Percentage of seasonal distribution of ticks on different body regions of cattle

Seasons	Head and neck			Udder and inguinal regions			Abdomen			Sides			Under the tail		
	N	F	M	N	F	M	N	F	M	N	F	M	N	F	M
Spring	5.06^{aC}	4.52^{cBC}	2.86^{bA}	14.56^{cB}	9.68^{bA}	11.31^{bA}	3.16^{aA}	3.23^{aA}	5.00^{bA}	0.63^{aA}	2.58^{bB}	2.62^{bB}	4.43^{bA}	7.10^{bA}	5.00^{bA}
Summer	9.49^{bC}	5.16^{dA}	7.14^{cB}	22.15^{dB}	17.42^{cA}	22.62^{cB}	6.96^{bA}	9.68^{cB}	5.36^{bA}	2.53^{bAB}	3.55^{bB}	1.79^{bA}	3.80^{abA}	6.13^{bA}	10.12^{cB}
Autumn	3.16^{bA}	2.26^{bA}	2.98^{bA}	10.76^{bA}	10.65^{bA}	9.52^{bA}	2.53^{aA}	5.48^{bB}	3.21^{abAB}	0.63^{aA}	2.58^{bB}	1.43^{abAB}	4.43^{bA}	4.19^{abA}	4.29^{bA}
Winter	0.63^{aA}	0.32^{aA}	0.12^{aA}	1.90^{aA}	1.29^{aA}	2.38^{aA}	0.63^{aA}	0.65^{aA}	0.71^{aA}	0.00^{aA}	0.32^{aA}	0.12^{aA}	0.63^{aA}	0.65^{aA}	0.24^{aA}
Total (=2548)	352 (13.82)^{C}			878 (34.46)^{A}			336 (13.19)^{D}			196 (7.69)^{E}			466 (18.29)^{B}		

Different superscripts letters (a, b, c) in the same column indicate significant differences at $P<0.05$.
Different superscripts letters (A, B, C, …) in the same row indicate significant differences at $P<0.05$. Values in parentheses represent %. Abbreviations: N: Nymph, F: Female, M: Male

Table (6): Relationship between sex of cattle and tick infestation

Season	No. Examined	Examined bull	Examined Cows	Infested bulls		Infested cows	
				No	%	No	%
Spring	150	52	98	4	7.69^{bA}	6	6.12^{bcA}
Summer	150	40	110	7	17.50^{cB}	13	11.82^{cA}
Autumn	150	58	92	2	3.45^{abA}	3	3.26^{abA}
Winter	150	56	94	1	1.79^{aA}	1	1.06^{aA}
Total	600	206	394	14	6.80^{A}	23	5.84^{A}

Different superscripts letters (a, b, c) in the same column indicate significant differences at $P<0.05$.
Different superscripts letters (A, B, C) in the same row indicate significant differences at $P<0.05$.

Table (7): Relationship between age of cattle and tick infestation

Season	Total examined	Positive cases		8 m-2 y		2-3 y		3-5 y	
		No.	%	No.	%	No.	%	No.	%
Spring	150	10	6.67	1	10^{bA}	1	10^{bA}	8	80^{aB}
Summer	150	20	13.33	1	5^{abA}	4	20^{cB}	15	75^{aC}
Autumn	150	5	3.33	0	0^{aA}	1	20^{cB}	4	80^{aC}
Winter	150	2	1.33	0	0^{aA}	0	0^{aA}	2	100^{bb}
Total	600	37	6.17	2	5.41^{A}	6	$16.22^{B}B^{B}$	29	78.38^{C}

Table (8) Percentage of different forms of developmental stages of piroplasms encountered in *Rhipicephalus turinacus* hemolymph during different seasons of the year

Seasons	No of examined female ticks	Total Sporokinetes	Intermediate form of *Babesia* sp.	Large form of *Theileria* sp.	Mixed infection	Round and amoeboid shapes	Total
Spring	300	95	55	30	17	35	130
%		31.67%	57.89%	31.58%	17.89%	11.67%	43.33%
Summer	450	109	80	45	10	50	159
%		24.22%	73.39%	41.28%	9.17%	11.11%	35.33%
Autumn	270	80	50	28	14	30	110
%		29.63%	62.5%	35%	17.5%	11.11%	40.74%
Winter	30	10	6	3	1	3	13
%		33.33%	60%	30%	10%	10%	43.33%
Total	1050	294	191	106	42	118	412
%		28%	64.97%	36.05%	14.29%	11.24%	39.24%

Table (9) Percentage of different forms of developmental stages of piroplasms encountered in *Rhipicephalus praetextatus* hemolymph during different seasons of the year

Seasons	No of examined female ticks	Total Sporokinetes	Intermediate form of *Babesia* sp.	Large form of *Theileria* sp.	Mixed infection	Round and amoeboid shapes	Total
Spring	150	50	19	15	9	15	65
%		33.33%	38%	30%	18%	10%	43.33
Summer	200	90	33	22	9	15	105
%		%45	36.67%	24.44%	10%	7.5%	52.5
Autumn	130	56	23	43	6	14	70
%		%43.08	41.07%	76.79%	10.71%	10.77%	53.85
Winter	20	5	3	2	0	2	7
%		%25	60%	40%	0	10%	35
Total	500	201	78	54	24	46	247
%		%40.2	38.81%	26.87%	11.94%	9.2%	49.4

Table (10): Piroplasm species detected in tick hemolymph during different seasons using PCR assay

Seasons	No. examined	*Babesia* sp.	%	*Theileria* sp.	%
Summer	20	7	35%	5	25%
Winter	20	3	15%	0	0%
Total	40	10	25%	5	12.5%

Fig.1. Dissecting microscope (X10) showing *R. turanicus*: A. Male and B. Female, *R. praetextatus*: C. Female and D. male, E-F. *Rhipicephalus* species nymph dorsal and ventral view respectively.

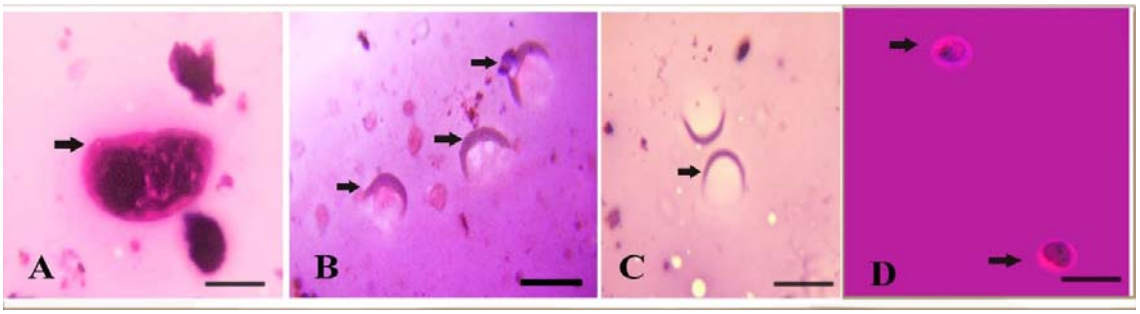

Fig.2. Giemsa stained developmental stages in tick hemolymph. A. Infected haemocyte by developmental stages of piroplasms B. Sporokinetes of *Babesia* sp., C. Sporokinetes of *Theileria* sp. D. rounded and amoeboid forms. Scale bar=10 µm

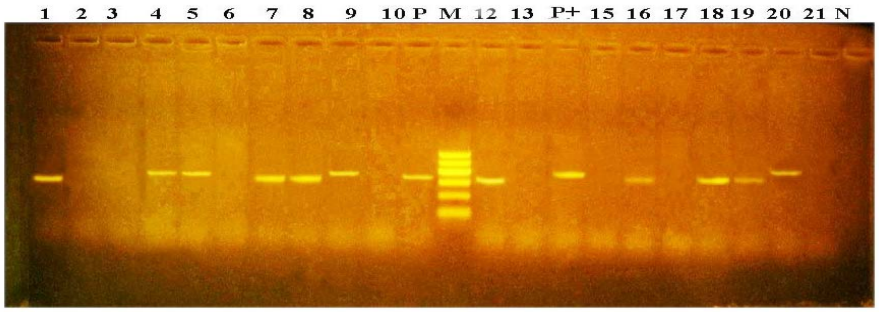

Fig.3. Amplified products of 18s rRNA gene of *Babesia* and *Theileria*. Lane, M: DNA ladder, Lane P: control positive for *Babesia*, Lane P+ control positive for *Theileria*, Lane 1,7,8,12,16,18,19 amplified 350 bp product of *Babesia sp.* Lane N: control negative lane 4.5.9..20 amplified 370 bp product of *Theileria* sp.

and Abebaw (2010) determined dewlap, head and back to be the prefered locations for Boophilus decolaratus Rhipicephalus evertsi-evertsi. Bossena and Abdu (2012) found most of Boophilus sp. attached to dewlap, back and hoof, while Rhipicephalus evertsi evertsi were mostly found under tail. The variation of tick species may be the cause associated with this matchlessness.

Eyo et al. (2014) was in agreement with our study proving the higher tick infestation rate in males than females. Though, the contrary observation was found by Kabir et al. (2011) and Chahardeh et al. (2015). This recorded variation may be ascribed to disparity of the ratio of examined females to males. Additionally, a significant effect of age on the rate of tick infestation was recorded in the present study, where older cattle (3-5 y) showed the peak of infestation (78.73%) than younger ones. This conclusion was consistent with that of Nady et al. (2014). Conversely, Kabir et al. (2011) recorded the peak of infestation in cattle of 1.5 years old (46.28%) than those >1.5 years of age (27.80%), and Eyo et al. (2014) proved a higher prevalence in cattle of 2 years of age (96.66%) than of cattle of >2 years of age (48.94%). Perhaps, the variability of the tick species infesting cattle or the host immune status explain the recorded differences.

The developmental stages of piroplasms were perceived in 39.24% and 49.4% of the screened hamolymph smears obtained from *Rhipicephalus turanicus* and *Rhipicephalus praetextatus* respectively. In Egypt, *Babesia* and *Theileria* developmental stages were observed in the 94.8% of *Hyalomma anatolicum*
(Mansour, 1996) and in 28.13% of *Rhipicephalus bursa* (Ramadan and El-Akabawy, 2000)

In the present study, there was a great sensitivity of PCR for the detection of Babesia and *Theileria* DNA fragments in 25% and 12.5% of microscopically negative hemolymph samples. In Egypt, Adham et al. (2009) found B. *bovis* and *B. bigemina* dual infections in 12% of Boophilus *annulatus* hemolymph samples, Zakkyeh et al., (2012) found *Theileria ovis in 55%* of Rhipicephalus *sanguineus* specimens and Oliveira-Sequeira et al. (2005) found *B. bigemina* and *B. bovis* in 56.2% and 4.7% of ticks and Quintão-Silva and Ribeiro (2003) found the same aforementioned species in 14.4% and 7.8% of ticks respectively.

5. CONCLUSION

R. turanicus and *R. praetextatus* were the identified tick species among the examined cattle with a total prevalence 6.17%. Both hemolymph microscopic examination and PCR were capable of performing the early diagnosis of piroplasm infection, with the superiority of PCR assay. The sensitive detection of *Babesia* and Theileria **18**SrRNA genes in microscopically negative hemolymph using PCR assay allows the easy rapid surveillance of the endemicity of piroplasm infection among tick species and consequently application of emphasized control programs.

6. ACKNOWLEDGMENT

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