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Parasitology

Prevalence and Seasonal Dynamics of *Rhipicephalus annulatus* in Cattle and Buffaloes in Menoufia Governorate, Egypt.

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ABSTRACT

Ticks are the most significant ectoparasities of livestock in tropical and sub-tropical areas. Ticks are responsible for severe economic losses as it is a vector of various protozoal, rickettsial, bacterial and viral diseases. The purpose of the current study was to determine the prevalence of *Rhipicephalus annulatus* infestations in cattle and buffaloes in Menoufia governorate with their seasonal dynamics. A total of 1885 animals (973 cattle and 912 buffaloes) were randomly inspected for *Rhipicephalus annulatus* infestation from September 2020 and August 2021. In accordance with the ticks' preferred sites, the samples were obtained from cattle and buffaloes of various ages and sex in three different locations in Menoufia governorate (Tala, Shebien EL-Kom and EL-Shohadaa districts) during various seasons that subjected to parasitological examination. The results revealed that the prevalence of *Rhipicephalus annulatus* in cattle and buffaloes was 26% and 9.32%. The highest infestation in cattle was observed in summer season 38.5% and the lowest was in winter 11.92%. Meanwhile, in buffaloes spring and autumn recorded high infestation 10.84% and10.96% with lower prevalence in winter 8.58%. In relation to the effect of some risk factors in dynamic distribution of *Rhipicephalus annulatus* infestation, it was found that a significant effect of season in prevalence of *Rhipicephalus annulatus* in cattle, but not in buffaloes. Regarding to age, it was observed that 1-5 years old cattle and buffaloes recorded the highest infestations with 42.3% and 14.07 %. Although non- significant difference in relation to sex of examined cattle and buffaloes. Moreover, the effect of locality had a high significant effect in cattle recorded 31.43%, 25.38%, 20.88% in Tala, Shebien El-kom and Al-Shohadaa respectively and in buffaloes14.9%, 8.9%, 4.2% respectively. In conclusion, *Rhipicephalus annulatus* has endemic nature in cattle and buffaloes in Menoufia governorate and the adoption of prioritised control programmes should be directed.

Key words: Buffaloes, Cattle, Locality, Prevalence and *Rhipicephalus annulatus*.

INTRODUCTION

Hard ticks (Acari: Ixodidae) are a major pest with significant Medical and Veterinary significance (Guglielmone et al., 2014), they transmitted a variety of infections, such as bacteria (Parola and Raoult, 2001), rickettsiae (Donalisio et al., 2020), viruses (Labuda and Nuttall, 2004), protozoa (de la Fuente, 2008), and helminths. In many countries, including industrialised ones, ticks have become a serious public health illnesses problem (Gabriele-Rivet et al., 2015).

Ticks are the most significant carriers of pathogens that cause diseases such as theileriosis, babesiosis, anaplasmosis, and Crimean-Congo hemorrhagic fever in North Africa (Aouadi et al., 2017; Walker et al., 2003; Said et al., 2018 and Okely, 2020). These diseases have a substantial negative impact on the health of livestock .Although accurate identification of the specimens used in epidemiological and microbiological investigation of tick species is crucial, there is still a knowledge gap in identifying the most common tick species in the western Palearctic regions, particularly those belonging to the genera *Hyalomma* and *Rhipicephalus* (Guglielmone et al., 2014; Estrada-Pena et al., 2017). Morphological identification is a highly effective method for identifying ticks at species level (Estrada-Pena et al., 2017). *Amblyomma, Haemaphysalis, Hyalomma, and Rhipicephalus* are the common four genera that contain hard tick species that affected domestic animals in Egypt (Mazyad and Khalaf, 2002; Allam and Newman, 2018.).Hard tick taxonomy and morphology investigations were alternately conducted in Egyptian fauna (Hoogstraal and Kaiser, 1959; Abdullah et al., 2016).

As described by Murell and Barker (2003), the genera *Boophilus* and *Rhipicephalus* were joined, and the species *Annulatus* and *Decoloratus* were moved from one to the other. According to Abdel-Shafy et al. (2012), *Rhipicephalus annulatus* was the most important cow tick in terms of medicine. In Egypt, *Rhipicephalus annulatus* was an endemic pest that affected cattle, sheep, horses, donkeys, dromedary camels, buffalo, and sheep (Kaur et al., 2017). *Rickettsia africae, R. aeschlimannii, Anaplasma marginale, A. phagocytophilum, A. ovis, A. bovis, A. centrale, A. platys*, and other *Anaplasma* spp. are among diseases that *Rhipicephalus annulatus* plays an important part in their transmition (Loftis et al., 2006;Abd El-Baky and Allam, 2018), *Ehrlichia canis, Ehrlichia ovina* (Loftis et al., 2006), *Borrelia theileri* (Hassan et al., 2017), *Borrelia burgdorferi* (Adham et al., 2010), *Staphylococcus spp*. (ElKammah et al., 2007), *Babesia species* (Fahmy et al., 1983*), B. bovis* (Adham et al., 2009), and *B. bigemina* (El Kammah et al., 2007; Adham et al., 2009andHassan et al., 2017) .

In a accordance to (D'Amico et al., 2018) *Rhipicephalus annulatus* is a one-host tick that feeds on the same animal throughout all life stages and under ideal conditions, there can be two generations per year. The main morphological characters consisted of legs, abdomen, neck, and pectoral region that are favoured feeding areas (Walker et al. 2003). According to Kaur et al., 2017) described that the quick life cycle can cause extensive animal infestations that cause significant financial losses. Also, the larvae search for a host by scavenging on plants (Emmanuel et al. 2011). *Rhipicephalus annulatus* primary hosts are cattle, according to (D'Amico et al., 2018). Due to the endemic nature of *Rhipicephalus annulatus* in Egypt in livestock animals, this study aimed to determine the prevalence of *Rhipicephalus annulatus* infection in cattle and buffaloes in the Menoufia Governorate and associated risk factors affecting their dynamics and seasonal variation in the study area.

MATERIALS AND METHODS

Study area and sampling

From September 2020 to August 2021, at three distinct locations in the Menoufia Governorate (Tala, Shebien-Elkom, and El-Shohadaa districts), Egypt, a total of 1885 cattle and buffaloes (973 cattle, 9112 buffaloes) of different ages and sex were inspected for *Rhipicephalus annulatus* infestation. Individual cattle and buffalo were thoroughly examined, and ticks were collected from preferred locations of attachment, such as the ears,

around the anal and peri-anal, head, neck, sides, and under the tail.

Tick specimens

Using tiny forceps, tick specimens were extracted from the animals. Based on host and locations, the collected specimens were divided and encoded. Some of the engorged females were moved to the sterile, moist filter paper-lined, dark, and humid egg-laying containers, which were housed in an incubator until egg laying (2–7 days). The eggs were then photographed and preserved in 70% ethanol. Following the available standard dichotomous keys, all other specimens were identified based on morpho-taxonomic characteristics and then stored in vials containing a mixture of ethyl alcohol 70% and glycerol5%before being transported to the laboratory of the Parasitology Department at the Faculty of Veterinary Medicine, Sadat University at Sadat City. Each vial was tagged with a unique number, the collection site, the species of the host, age, sex, location, and the date of collection.

Ticks identification

The obtained specimens were identified in the lab utilising the local and regional taxonomic descriptions and keys (Robinson, 1926; Hoogstraal, 1956; Walker et al., 2003; Apanaskevich and Horak, 2008; Estrada-Pea et al., 2017 and Horak et al., 2018). Each tick was individually inspected with a dissecting microscope and shot with a Leica EC3 camera.

Statistical analysis

The previous study's formula was used to calculate the tick infestation rate as described by (Asmaa et al., 2014) and calculated the infestation rate as followed:

Infestation rate = Number of infested animals / Total number of animals × 100

The impact of location, age, sex, and seasonal fluctuations on the infestation was examined using the Chi-square test. $P< 0.05$ was regarded as a significant value. GraphPad Prism 5 (GraphPad Software, San Diego, CA) passed the test.

RESULTS

In the current study, a total of 253 out of 973 examined cattle (26%) and 85 out of 912 examined buffalo (9.32%) had *Rhipicephalus annulatus* infestations. The infestations of *Rhipicephalus annulatus* in cattle were more prevalent in the Summer and Autumn with 38.5% and 28.39% than in the Spring and Winter 21.14% and11.92%. The highest *Rhipicephalus annulatus* infestation in buffaloes was observed in the Autumn and Spring with 10.96 and 10.85% with low infestation in Winter and Summer with 8.58% and 7.29%. In general, the seasonal dynamics had a highly significant impact ($P<0.009$) on the frequency of *Rhipicephalus annulatus* in cattle. Although, non-discernible variance in the seasonal variation ($p<0.43$) in Buffaloes as showed in table 1.On the other hand, the ecological distribution had a high significant effect in infestation rate as showed in table 2, at which in cattle ($p<0.009$); in Tala 31.43%,Shebin-Elkom 25.38%, and El-Shohadaa district 20.88%.Furthermore,the locality in buffaloes had a high significant impact ($p< 0.000$); 14.9%, 8.9%, and 4.2% in Tala, Shebin-Elkom, and El-Shohadaa, districts respectively.

Concerning to the identification of that based on taxonomic descriptions and keys, it was founded that most Acarina have 4 pairs of legs. Ticks were visible by naked eye. The body covered by scutum which cover all the body in male and the anterior part only in female. The mouth part of composed of hypostomal teeth arranged in 4 + 4 columns, the first palpi have long concave internal margins without tuberance. In males the body length measured about 1.76 mm and yellow to brown color with no coadal appendage and genital opening while in females the body length is measured about 1.86–2.98 mm according to feeding status and the shape of body was parallel-side with posterior margin and round tip but no festoon. The scutum is covered the anterior part only as well as semicircular genital opening. The

nymph identification resemble the adult but genitally immature, and the scutum covered the anterior part only as illustrated in Fig. 1(A, B, C).

Regrading to the effect of some risk factors associated with the prevalence of *Rhipicephalus annulatus* in our study, the results demonstrated that the majority of infestation 42.3% was found in cattle between the ages 1-5 years. On the other hand, old aged cattle over 5 years had the least prevalence 13.14% while, animals less than 1 year old recorded prevalence rate17.3%.From these results, it was clear that the age difference showed high significant difference ($p<0.000$) as showed in Table 3. Similar findings were observed in buffaloes, the majority of *Rhipicephalus annulatus* infestation was found between 1-5 years 14.07% and the lowest prevalence 5.8% was detected in buffaloes over 5 years while, buffaloes under 1 year recorded prevalence rate7.3%. In addition to, the effect of age revealed a high significant effect ($P< 0.001$). Contrarily, the sex effect on the prevalence of *Rhipicephalus annulatus* infestation in cattle and buffaloes had non-significant effect with $P>0.712$, $P>0.743$ as illustrated in table 4.

Table (1) Seasonal prevalence of *Rhipicephalus annulatus* in cattle and buffaloes in Menoufia governorate, Egypt

	Cattle			Buffaloes		
Season	No. examined (N=973)	Frequency	%	No. examined (N=912)	Frequency	%
Autumn	243	69	28.39	228	25	10.96
Winter	218	26	11.92	198	17	8.58
Spring	227	48	21.14	212	23	10.84
Summer	285	110	38.5	274	20	7.29
	Chi square x^2 = 49.45 p<0.000 very high significant			Chi square x^2 = 2.77 p< 0.43 not significant		

Table (2) Infestation rate of *Rhipicephalus annulatus* in cattle and buffaloes in different districts of Menoufia governorate, Egypt

	Cattle			Buffaloes		
locality	No. examined (N=973)	Frequency	%	No. examined (N=912)	Frequency	%
Tala	334	105	31.43	301	45	14.9
Shebinelkom	323	82	25.38	303	27	8.9
Elshohadaa	316	66	20.88	308	13	4.2
	Chi square x^2 = 9.49 P<0.009 high significant			Chi square x^2 = 20.83 p<0.000 very high significant		

Table (3): The infestation rate of *Rhipicephalus annulatus* in different age groups of examined cattle and buffaloes in Menoufia governorate, Egypt

	Cattle			Buffaloes		
Age	No. examined (N=973)	Frequency	%	No. examined (N=912)	Frequency	%
Less than one year old	254	44	17.3	271	20	7.3
From one- to five year old	392	166	42.3	334	47	14.07
More than five year old	327	43	13.14	307	18	5.8
	Chi square x^2 = 92.44 p<0.000 very high significant			Chi square x^2 =14.47 p<0.001 very high significant		

Table (4): The infestation rate of *Rhipicephalus annulatus* in different sex of cattle and buffaloes in Menoufia governorate, Egypt

	Cattle			Buffaloes		
Sex	No. examined (N=973)	No. infected	%	No. examined (N=912)	No. infected	%
Male	253	68	26.8	695	66	9.4
Female	720	185	25.6	217	19	8.7
	Chi square x^2 = 0.14 p<0.712 Not significant			Chi square x^2 = 0.11 p<0.743 Not significant		

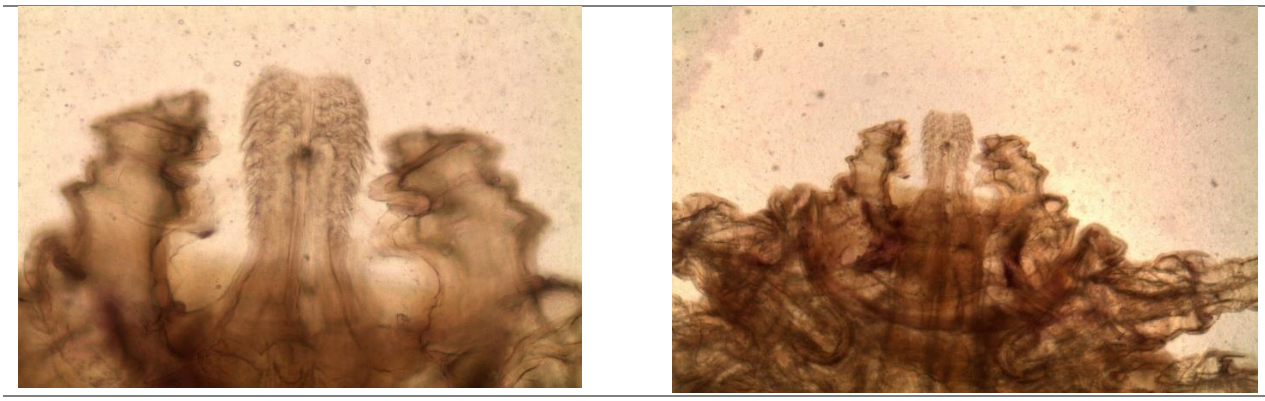

Fig. 1(A): 10 x &4 x showed mouth part of *Rhipicephalus annulatus* (hypostomal teeth arranged in 4and4 columns, the 1st palp has long concave internal margin without tuberance).



Fig. 1 (B): Nymph genitaly immature scutum cover anterior part of body | Male all body covered by scutum without caudal appendage | Unfeded female, yellow colour small size; while, feded female large size dark colour. scutum cover anterior part of body only


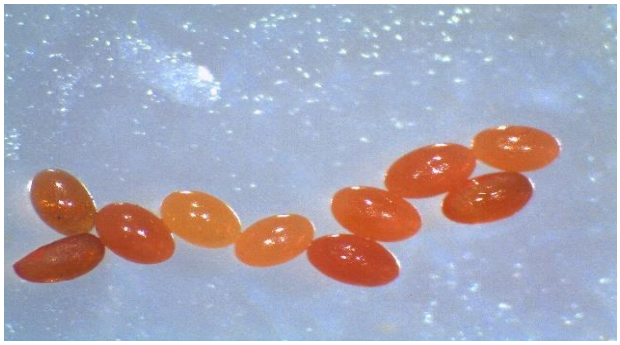

Fig. 1 (C): Un feded female, engorged female dark brawn bean shape reach 3mm size. | Egg batch

DISCUSSION

Rhipicephalus annulatus infestations in the current study in cattle (26%) and (9.32%) in buffaloes. Moreover, in cattle, the infestation was more prevalent in the Summer and Autumn with 38.5% and 28.39% than in the Spring and Winter 21.14% and 11.92%.While, in buffaloes Autumn and Spring with 10.96 and 10.85% with low infestation in Winter and Summer with 8.58% and 7.29%. In general, the seasonal dynamics had a highly significant impact (P<0.009) on the frequency of *Rhipicephalus annulatus* in cattle. Although, non-discernible variance in the seasonal variation (P<0.43) in Buffaloes. Furthermore, the locality in buffaloes had a high significant impact (P< 0.000); 14.9%, 8.9%, and 4.2% in Tala, Shebin-Elkom, and El-Shohadaa, respectively. These results were nearly similar to the observations of (Prakasan and Raman, 2007) as well as (Shyma, et al., 2013) illustrated that over prevalence rate of ticks infestation was 20.5% out of 342 examined animals as well as more prevalence of infestation in cattle 22.4% than buffaloes 12.8%. Also, they added that *Rhipicephalus annulatus* and *H. bispinosa* were the most prevalent ticks' species in three districts of northern Kerala, Iran. In addition, (Khan, 1993and Manan et al. 2007) documented that the overall prevalence rate of tick infestation in cattle ranged (20.4-33.7%) as compared to buffaloes (11.3-22.8%). This finding was in the same line of (Khan et al., 1993) who recorded the *Rhipicephalus annulatus* infestation rate was 28.2% in Pakistan. Meanwhile, lower prevalence rate was recorded by (Gabaj et al., 1992) who reported that *Rhipicephalus* infestations in Libya (9.6%). On the other hand, higher prevalence rate was reported in several studies in Egypt, Iran, Argentina and Algeria respectively with 80.44%, 51.3%, 34%, 31.26% as demonstrated by Hassanain (1997); Razmi et al. (2007); Mangold et al. (1989) and Bedouhene et al. (2022). The variation in the prevalence rate between our results and other studies

may attributed to the geographic location, methods of cattle rearing and Veterinary authorities cares and the applied control measures for ectoparasities in the study areas.

The identification of *Rhipicephalus annulatus* depend on the morphological and characteristic shapes as described previously by Graham and Price (1966) who identified *Rhipicephalus annulatus* based on the absence of a caudal appendage in males. Furthermore, Berry (2017) showed that *Rhipicephalus annulatus* contained internal margins of palp article I with long and slightly concave surface. However, these morphological characters may not considered appropriate for the identification of damaged specimens and engorged female ticks (Nava et al., 2008).

In our study some risk factors associated with the prevalence of *Rhipicephalus annulatus* were investigated and the results demonstrated that the majority of infestation 42.3% was found in cattle between the ages 1-5 years and old aged cattle had the least prevalence 13.14% and the age difference showed high significant difference ($P<0.000$) and similar findings were observed in buffaloes. In addition animal sex effect was non-significant effect within cattle and buffaloes with $P>0.712$ and $P>0.743$. This was previously was mentioned by Das (1994) who found that young calves had more susceptible to tick infestation than the adult and the high prevalence rate 48%, followed by growing animals 41.2%, heifers 39.8%and adult cattle 35.3%. Similar finding was also supported by Ramadan et al. (2016) who revealed that 3-5 years cattle were more significant age for *Rhipicephalus annulatus* infestation with prevalence rate 78.38% than those of 2-3 years 16.22 % and those of 8months -2 years with 5.41%.

In contrast to the sex effect on the prevalence of *Rhipicephalus annulatus* infestation in cattle and buffaloes that revealed non-significant effect. This was agreed with Ramadan et al. (2016) who revealed that non-significant influence of sex on the *Rhipicephalus annulatus* infestation. However, other studies were different as reported by Kabir et al. (2011); Eyo et al. (2014) and Chahardeh et al. (2015) they declared that high infestation in males more than females. This observed variation may be due to the ratio of examined females to males between different studies.

CONCLUSION

Rhipicephalus annulatus represented a serious vector for transmission of many diseases and economic impact in cattle and buffaloes. This study concluded that Summer season was the most associated season with high infestation with *Rhipicephalus annulatus* in cattle meanwhile, Spring and Autumn in buffaloes. Several risk associated factors were apparent with the dynamic distribution of *Rhipicephalus annulatus* infestation and significant effect of season in prevalence of *Rhipicephalus annulatus* in cattle, but not in buffaloes are recorded in our study as well as age of animal. Although, non- significant influence in relation to animal sex in cattle and buffaloes. Moreover, the effect of locality had a high significant effect in cattle and in buffaloes. Further studies are needed for investigation of more related determinants associated with *Rhipicephalus annulatus* infestation in cattle and buffaloes.

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