Original Research

The Prevalence and Associated Infection Risk Factors of the Blood Protozoan Parasites among the Small Ruminants in the North Coast of Egypt

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ABSTRACT

Several factors are affecting the productivity of small ruminants, infectious diseases and their causative pathogens are one of the most detrimental factors. Blood protozoan parasites are pathogens affecting small ruminants worldwide, particularly Egypt. Thus, the current study was done for detecting the prevalence of the blood protozoan parasites among small ruminants in the North Coast of Egypt. This was done through collecting 366 blood samples from four districts at Matrouh governorate. Microscopic examination of the Giemsa-stained blood smears was done to reveal the overall infection rate. Results showed a total prevalence of 68.30% (Babesia ovis, Theileria ovis. and Anaplasma ovis.). Sheep recorded a higher prevalence of 84.8% than that of goat. Females recorded the highest infection rate with a highly significant association with infection in relation to the open breeding (X2=72.501, P. value=0.000). The same significant result was observed with the presence of ticks on the examined animals (X2 =46.711, P. value=0.000). When summed up, we can conclude that the lack of vector control in Matrouh governorate, associated with a bad housing of herd, ignorance of the proper animal management, and the open breeding system are the reasons for the widespread infection with blood protozoan parasites among small ruminants.

Keywords: : Anaplasma, Babesia, Goat, Sheep, Theileria

INTRODUCTION

Small ruminants are vital either present in large populations or as individual animals kept by farmers and herders particularly in the rural, suburban, and desert areas. They are a good and constant source of food and income for people in those areas. Thus, it was important to maintain animals' health by keeping them free from diseases, with balanced nutrition, and suitable housing (Al-Khalidi et al., 2013). Blood parasites of prokaryotic and eukaryotic origins that are transmitted through arthropod vectors are the most veterinary and medically important pathogens affecting the small ruminants. They are classified into two main categories: Anaplasma and Ehrlichia (Cowdria) are Rickettsia, while species of Theileria, Babesia, and Trypanosoma are protozoan parasites. These Blood parasitic diseases have great negative impacts on small ruminants, as they affect the health and productivity of these animals (Kasozi KI et al., 2019). The novelty of this study is that it is the first time to record the prevalence of blood protozoan parasites among sheep and goats in the Matrouh Governorate. In Qena, Hussein et al. (2017), reported that the total blood protozoan parasite was 38.60%. Moreover, the previous blood protozoan parasitic prevalence was done in Behera governorate, Egypt, which revealed that the overall prevalence of blood protozoan parasitic infection among sheep was 39 % with a precision of 5 and α of 5 % (Fadly, 2012). Generally, the diagnosis of blood protozoan parasites of small ruminants is based on the history, clinical signs, and the microscopic examination of blood smears from the suspected animals. Direct examination of Giemsa-stained blood smears by light microscope is very efficient in the diagnosis of acute cases, which consider a gold standard technique (Inciet al., 2010).

MATERIALS AND METHODS

Study setting

Small ruminants of sheep and goat were selected for blood sampling from Matrouh governorate (31.352778°N 27.236111°E). Animals were mainly distributed throughout four districts including EL-Hammam, EL-Alamein, EL-Dabaa, and Marsa-Matrouh districts. These districts were represented all Matrouh Governorate as they included herds with moderate to high animal densities, and they were located with geographically symmetrical distances from each other's.

Study design

Data for the current study were collected, analyzed, and presented in the format of the cross-sectional design.

Sample size

The minimum sample size needed for done current study was 366 small ruminants. The determination of this number is mainly based on the total small ruminants herd present among Marsa-Matrouh governorate, which is around 288,000 head.

Samples collection

About 2 ml blood sample was collected from the jugular vein of each selected animal for this study in EDTA vacutainer tubes as anti-coagulant. These blood samples were kept in an icebox and transported to the laboratory the Parasitological laboratory at the Faculty of Veterinary Medicine, Matrouh University, during the period from August 2020 to April 2021, then these samples were prepared for the microscopic examinations.

Data collection

Data of the present study was collected in questionnaire included: Demographic data, which considered as risk factors: Small ruminants location, species, sex, breeding, age; symptomatic data, which indicated possibly positive cases, general body condition, eye mucous membrane, animal temperature; and presence of ectoparasite.

Microscopic examination

Initially, blood smear was prepared from each blood sample on a glass slide, the blood smear was left till complete dryness. The dried blood films were fixed by methyl alcohol 5% for 5 min. The fixed blood films were left until complete evaporation of the methyl alcohol before being stained by Giemsa stain for about 30 min. Finally, the stained smears were washed gently with tap water to remove the excess stain and the stained smears were examined under the oil immersion lens (100 x magnification) of the light microscope (Kohli et al., 2014).

Statistical analysis

Data were statistically analyzed using a SPSS version 22, to analyze the data statistically and all the significance level was considered at $P<0.05$. The prevalence of infection was calculated for all data as the number of infected cases divided by the number of individuals and multiplied by 100. The tests determined the association between modified or non-modified risk factors of blood infection was Chi-square test to calculate the Pearson chi-square if had a cell with an expected frequency of less than 5.

RESULTS

Prevalence of the total blood parasites among Matrouh Governorate

Microscopic examination of 366 Giemsa stained blood smear (306 from sheep and from 60 goats) revealed that 68.3% (250/366) of sheep and goats were infected. The highest prevalence infection was 25.13% in El-hammam (Table 1).

Table 1: The total prevalence of blood protozoan parasites in relation to the area locality.

Overall infection	Districts				X^2	P
	El-hammam	El-dabaa	El-alamein	Matrouh		
68.30% (250/366)	25.13% (92/366)	21.03% (77/366)	4.09% (15/366)	18.03% (66/366)	8.434	0.038

Prevalence of the total blood parasites according to the blood protozoan parasite species among Matrouh governorate

The total prevalence of each blood protozoan parasite species was 59.6 (218/366), 16.9 (62/366), 27.9 (102/366), and 0.8 (3/366) for Anaplasma spp., Babesia spp., and Theileria spp., respectively **(Table 2).**

Table 2: Prevalence of the detected blood protozoan parasites.

Blood parasite species	Total infected	%
Overall blood protozoan parasites	250	68.3 (250/366)
Anaplasma species	218	59.6 (218/366)
Babesia species	62	16.9 (62/366)
Theileria species	102	27.9 (102/366)

Prevalence of the total blood parasites according to the mixed infection among Matrouh governorate

The single infection was 52.4% (131/250), while on other hand the mixed infection either double or triple was 47.6% (199/250) **(Table 3)**. The positive blood film of different blood protozoan parasite species shows mixed infection **(Figure 1A)** with Babesia ovis (the upper arrow) and Anaplasma ovis infection (the lower arrow). Meanwhile, **(Figure 1B)** show single infection with Theileria ovis

Table 3: the distribution of single and mixed infections with blood protozoan parasites among positive samples.

Single or multiple infection		Parasitic *spp*	Number	%
Singe infection n= 131(52.4%)		*Anaplasma*	103	41.2% (103/250)
		Babesia	26	10.4% (26/250)
		Theileria	2	0.8% (2/250)
Multiple n=119 (47.6%)	Double n=103 (41.2%)	*Anaplasma* with *Theileria*	81	32.4% (81/250)
		Anaplasma with *Babesia*	16	6.4% (16/250)
		Anaplasma with *Trypanosoma* like	2	0.8% (2/250)
		Babesia with *Theileria*	3.0	1.2% (3/250)
		Babesia with *Trypanosoma* like	1	0.4% (1/250)
	Triple n=16(6.4%)	*Babesia*+ *Theileria* +*Anaplasma*	16	6.4% (16/250)

The total prevalence of the positive cases associated with different risk factors Among the positive cases, sheep had recorded the highest prevalence compared with goats by infection rates of 84.8% and 15.2%, respectively. In relation to animal sex; the highest infection was detected in females 87.2% but according to breeding system the open breeding system was the highest infection rate of 64.8%. Based on the clinical symptoms, most of the positive cases were apparently with good health conditions with the majority of infected animals were normal eye mucous membrane. As regard to the presence of vector (hard ticks), there was significant association with blood protozoan infection **(Table 4).**

Table 4: The total prevalence of the positive cases associated with different risk factors (n= 250).

Demographic factors		Districts				Total	X2	P
		El-hammam	El-dabaa	El-alamein	Matrouh			
Animal species	Sheep	81 38.20%	68 32.10%	10 4.70%	53 25.00%	212	6.350a	0.096
	Goat	11 28.90%	9 23.70%	5 13.20%	13 34.20%	38		
Animal sex	Male	16 50.00%	6 18.80%	2 6.20%	8 25.00%	32	3.499a	0.321
	Female	76 34.90%	71 32.60%	13 6.00%	58 26.60%	218		
Breeding system	Open	77 47.50%	22 13.60%	7 4.30%	56 34.60%	162	72.501a	0.000
	Semi-closed	15 17.00%	55 62.50%	8 9.10%	10 11.40%	88		
General body condition	Good	65 33.50%	58 29.90%	10 5.20%	61 31.40%	194	12.160a	0.007
	Bad	27 48.20%	19 33.90%	5 8.90%	5 8.90%	56		
Eye mucous membrane	Normal	53 43.80%	33 27.30%	7 5.80%	28 23.10%	121	40.831a	0.000
	Pale	26 70.30%	5 13.50%	1 2.70%	5 13.50%	37		
	Congested	13 14.10%	39 42.40%	7 7.60%	33 35.90%	92		
Animal temperature	Normal	82 34.50%	76 31.90%	15 6.30%	65 27.30%	238	10.661	0.044b
	Feverish	9 81.80%	1 9.10%	0 0.00%	1 9.10%	11		
	Sub-normal	1 100.00%	0 0.00%	0 0.00%	0 0.00%	1		
Presence of ectoparasite	Yes	1 1.60%	24 39.30%	7 11.50%	29 47.50%	61	46.711a	0.000
	No	91 48.10%	53 28.00%	8 4.20%	37 19.60%	189		
Age of the animal	Year ≤1	20 40.00%	11 22.00%	4 8.00%	15 30.00%	50	10.942a	0.09
	1-3 years	25 48.10%	11 21.20%	5 9.60%	11 21.20%	52		
	Years ≥ 3	47 31.80%	55 37.20%	6 4.10%	40 27.00%	148		

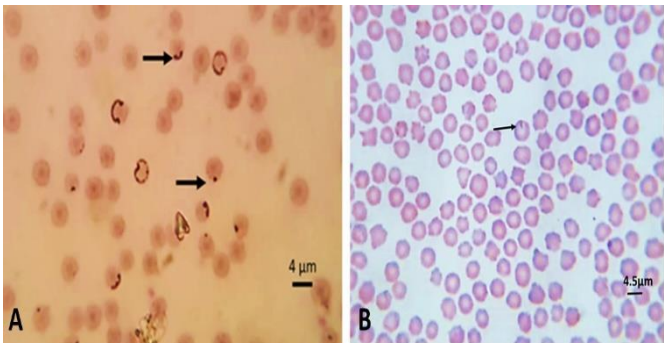

Fig. (1); (A): Mixed infection with Babesia ovis (the upper arrow) and Anaplasma ovis infection (the lower arrow), Giemsa stain, microscope power ˟1000. (B) Theileria ovis merozoite (arrow) comma shape, Giemsa stain, microscope power ˟1000.

DISCUSSION

Infections by the blood parasites in animals are known to impose substantial economic burdens on owners. The current study described the overall prevalence in Matrouh governorate of blood protozoan parasites by microscopic examination, which was recorded as 68.3%., Among positive cases, the highest infection rate was in El-hammam district at 25.13%. These results may be attributed to the fact that Matrouh Governorate is a more suitable region for hard ticks to survive due to the mean atmospheric temperature. Overall, the low temperature is 17.7 °C versus the high temperature is 25.3°C, an optimal weather condition for the propagation of vector hosts and their associated blood parasites, which may explain the relatively high prevalence rate that recorded in the current study. Gado et al. (2019) and Fieler (2019) reported that the highest activity is observed in most hard tick species near 30°C and the metabolic rate is the highest for most species around 40°C, where the activity and metabolic rates drop dramatically at temperatures below 10°C and above 50°C. In Behera province (north Egypt), Fadly (2012) reveals that the overall prevalence is 39% in sheep from April 2011 to March 2012, where the average weather temperature in summer is hot, humid, arid, and clear and the winters are cool, dry, and mostly clear. Over the course of the year in Behera province, the temperature typically varies from 6.7 to 35.5 °C. So, the activity of hard ticks is below normal, which directly reflects that the blood protozoan infection is lower than in Matrouh Governorate. On the other hand, Rosendale (2016) reported that the cold weather adaptations in hard ticks contributed to the success of cold tolerance because of reducing dehydration. Also, the capability of ticks to survive in unfavorable environmental conditions contributes to their success as vectors of disease. In the present study, with regard to types of infection, revealed that the single infection was nearly equal to either double or triple blood parasite infections, which contributed to the fact that the hard tick was not host specific for transmitting the blood parasites, and there are other sources of infection propagation such as contaminated needles during vaccination, where the blood was the main vehicle to convey the infection in the same direction. Approximately 73.24% of sheep are infected with at least one of the hard tick-born blood protozoans, with dual (24.41%), triple (9.03%), and quadruple (0.67%) co-infections were detected (Ceylan et al., 2021). In the current study, sheep were higher than goats, and the females were also come up with higher infection rates than male animals. This was due to the fact that in Matrouh governorate, the sheep herd was extremely higher than goats, which contributed 83.5% of the total small ruminants. So, the blood samples collected in this study were higher among sheep than among goats, and the total female heard was 87.8%. Shah (2017) agrees with this study as the prevalence of sheep is higher than in goats, but reverses the current study as the infection rate in females is lower than in male animals, which contributed to the same opinion as the current research. In recent studies, with regard to the area locality, the association with blood protozoan infection was significant between the different districts. Naz et al. (2012), on the other hand, reported that all the rest of risk factors, such as specie, age, and sex, are insignificant among sheep, nearly similar to the present findings in the current study. The present study found that the infestation by hard ticks was highly significant in association with all strains of blood protozoan parasite infection. This is due to the co-infection in the current study was 47.6%, in agreement with what was found by Hakimi (2019), who confirmed that the positive correlation may be a result of the simultaneous inoculation of these pathogens to the goats by ticks, or that both T. ovis and A. ovis are transmitted by the same tick species, R. sanguineus. Thus, the positive correlation could be due to ticks simultaneously inoculating these pathogens into goats or tick vectors are enhancing pathogen infectivity and transmission (Hakimi et al., 2019).

CONCLUSION

A relatively higher prevalence rate of infection by the blood protozoan parasites is reported in the current study than other similarly conducted studies. This is most likely contemplated by the infection enhancing factors such as bad management of animal herds such as unhygienic housing, the lack or insufficiency of programs to control ectoparasites in the open breeding system, a prevalent system of keeping the small ruminants herds in Matrouh governorate. Thus, in order to keep infection levels under controls, improved animal management should be practiced such as well-equipped housings, optimized rations, and continuous grazing rotations. This must be performed in parallel with an optimized vector control protocol and routine anti-parasitic protective therapies.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

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