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Seroprevalence and risk factors associated with Equine piroplasmosis in North Egypt

Abdelfattah Selim [a,*], Hanem Khater [b]

[a] Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt
[b] Parasitology Department, Faculty of Veterinary Medicine, Benha University, Egypt

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ABSTRACT

Equine piroplasmosis (EP) is caused by *Theileria equi* and/or *Babesia caballi* and has economic importance particularly in equines reared in poor management systems. This study is based on cELISA test to study the seroprevalence of EP among 370 horses and 150 donkeys in four Governorates north Egypt. Additionally, its risk factors were studied for the first time. The seroprevalence rates 36.5 %, 20 %, and 5.6 % for *T. equi, B. caballi,* and mixed infections, respectively. The highest antibody levels against *T. equi* were detected in Kafr ElSheikh (40 %) and Giza (40.1 %) Governorates, whereas those of *B. caballi* were detected in Qalyubia (25 %) and Kafr ElSheikh (24.1 %) Governorates. Concerning *T. equi,* animals >10 years (OR = 2.06) were more likely to be infected with EP than those <5 years old. In addition, the seropositivity increased among grazing (OR = 5.7, 95 % CI: 1.73−19.27) males (OR = 1.8, 95 % CI: 1.23−2.61) infested with ticks (OR = 2.3, 95 % CI: 1.60−3.48) during summer (OR = 4.3, 95 %CI: 2.53−7.46); whereas the seropositivity of animals for *B. caballi* increased among grazing equines (OR = 7.8, 95 % CI: 1.05−58.25) over 10 years old (OR = 2.08, 95 % CI: 1.10−3.94) and infested with ticks (OR = 2.4, 95 % CI: 1.54−3.76) during summer (OR = 7.12, 95 % CI: 3.15–16.06). Therefore, EP is an important prevalent disease in Egypt and deserves further attention regarding the management system, treatment, and vector control.

1. Introduction

Equine piroplasmosis (EP) is a tick-borne disease affecting equids and caused by two distinct intra-erythrocytic protozoan parasites, *Theileria equi* and *Babesia caballi* [1]. The disease is distributed worldwide mainly in the tropical, subtropical, and temperate areas [2] and transmitted by ixodid ticks of the genera *Hyalomma, Dermacentor,* and *Rhipicephalus* [1,3,4]. Recently, microsatellite analysis for *T. equi,* Florida strain, revealed great genetic diversity and 48 SNP differences in the 18SRNA gene; after a whole-genome sequence, a novel species was described as *T. haneyi* [5] which detected also among horses in South Africa [6].

Clinical signs are not specific for EP and vary from no to severe symptoms, whereas signs in acute cases are characterized by fever, anemia, hemoglobinuria, jaundice, edema, and even death [7]. Furthermore, horses and donkeys may act as carriers for many years particularly after *T. equi* infection [8,9]. The monitoring of EP depends mainly on microscopic examination but it is unreliable during the latent phase of disease due to mild parasitemia [10,11]. Consequently, serological tests such as enzyme-linked immunoassay (ELISA) and an immunofluorescent antibody test (IFA) have been developed to detect mild infections and could be used in a large scale epidemiological studies [1,12,13,32].

The competitive ELISA (cELISA) using monoclonal antibodies (mAb 36/133.97) for *T. equi* merozoite antigen (EMA)-1 and rophtry associated protein (RAP-1 for *B. caballi* have been developed for successful detection of antibodies against both parasites in horses [2,14]. cELISA is the test of choice recommended by the World Organization for Animal Health [15]. The detection of DNA using PCR is very useful in the acute stage of the disease where the antibody levels are not yet detectable [16–19].

Despite the recent and sole epidemiological study made in Giza governorate, Egypt, reporting the occurrence of *T. equi* in horses [20] and *B. caballi* in a limited number of horses and donkeys [11], there is a lack of information about the full epidemiology of EP on a larger scale of animals and areas. Therefore, this study aimed to fill in the gap and determine the seroprevalence of EP caused by *T. equi* and *B. caballi* among equines in four Governorates north Egypt. Additionally, the risk

* Corresponding author at: Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Benha University, Egypt.
E-mail address: Abdelfattah.selim@fvtm.bu.edu.eg (A. Selim).

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Table 1
The characteristics of the examined equines.

Location	Number of Animals												Total
	Horse						Donkeys						
	<5 year		5–10 years		>10 year		<5 year		5–10 years		>10 year		
	M	F	M	F	M	F	M	F	M	F	M	F	
Kafr Elsheikh	12	9	33	29	11	4	20	3	28	9	7	5	170
Menofia	10	5	18	16	6	3	5	1	13	2	3	3	85
Qalyubia	15	8	23	11	8	6	11	2	11	3	7	3	108
Giza	23	28	26	54	6	6	2	1	6	2	2	1	157
Total	60	50	100	110	31	19	38	7	58	16	19	112	520

M: males.
F: females.

factors associated with EP were studied for the first time, as far as we know.

2. Material and methods

2.1. Ethical statement

Blood samples were collected from the examined animals after the consent of the owners and approval of the Internal Ethics Review Committee, Faculty of Veterinary Medicine, Benha University, Egypt.

2.2. Study area

The seroprevalence study for EP was conducted in four north Egyptian Governorates as Kafr El Sheikh, Menofia, Qalyubia, and Giza located at 38°18 N to 30°56 E; 30°25 N to 31°13 E; 30.41 °N to 31.21 °E; and 30°01′N to 31°13′E, respectively, Fig. 1. Such areas were selected according to equine populations in northern Egypt, the high density of ticks, and the cooperation of equine owners. The horses and donkeys are used mainly for tourism in Giza Governorate and for draught and transport in the other Governorates.

2.3. Sample collection

The sample size was calculated using Win Episcope 2.0 (www.winepi.net) for the estimation of the population's prevalence, 50 % expected prevalence, with 95 % confidence limits, and 5% expected error.

The study included 370 horses and 150 donkeys, 306 males and 214 females, and within the age group from <5 to >10 years old, Table 1. Serum samples were collected from equines from four Governorates during 2019. Blood samples (5 mL) were drawn from the jugular vein of each animal and collected in a sterile tube without anticoagulant and transported to the Laboratory in an icebox for further analysis.

A questionnaire to the owners about the characteristic of the examined animals was prepared including sex, breed, age, management, and tick infestation.

2.4. Serological examination

The serological examination was aimed to determine the seroprevalence of EP (*T. equi* and *B. caballi*) in equines based on cELISA test recommended by OIE [2]. Commercial cELISA kits (VMRD, Inc.,

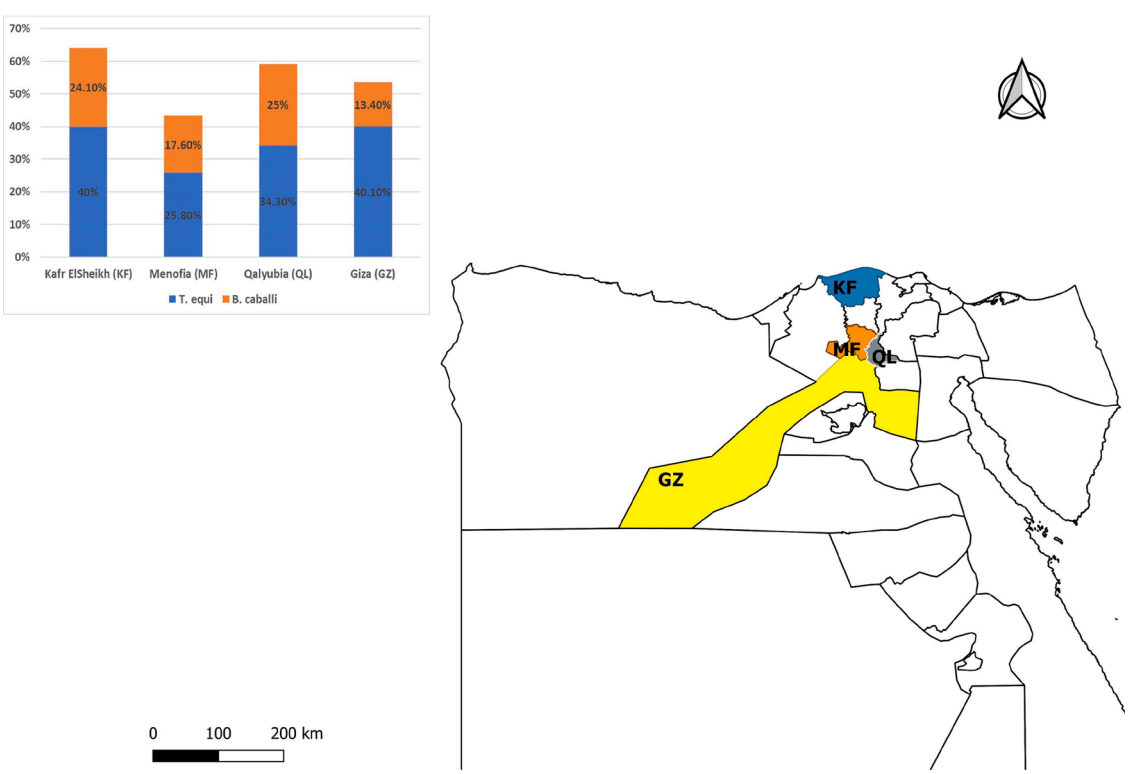

Fig. 1. Map of governorates targeted for sampling in the study.

Table 2
The seroprevalence and associated factors associated with equine piroplasmosis.

Factor		No of examined animals	*Theileria equi*				*Babesia caballi*				Mixed infection			
			No of positive	%	95 % CI	p value	No of positive	%	95 % CI	p value	No of positive	%	95 % CI	p value
Species	Horse	370	144	39	34.09–43.98	0.002#x20F0;	41	11	8.2–14.6	0.0001□	19	5.1	3.3–7.8	0.8
	Donkeys	150	46	30.6	23.85–38.46		63	42	34.4–50		10	6.6	3.6–11.8	
location	Kafr ElSheikh	170	68	40	32.6–47.8	0.12	41	24.1	18–31.4	0.03□	11	6.4	3.4–11.5	0.4
	Menofia	85	22	25.8	17.3–36.7		15	17.6	10.5–27.7		5	5.8	2.2–13.8	
	Qalyubia	108	37	34.3	25.6–44.1		27	25.0	17.4–34.4		8	7.4	3.5–14.5	
	Giza	157	63	40.1	32.5–48.3		21	13.4	8.6–19.9		5	3.2	1.2–7.6	
Age	<5 year	155	55	35.5	28.08–43.6	0.003*	26	16.7	11.7–23.4	0.02*	5	3.2	1.4–7.3	0.01*
	5–10 year	284	92	32.4	27.2–38.04		54	19.0	14.8–24		15	5.3	3.09–8.7	
	>10years	81	43	53.0	41.7–64.15		24	29.6	20.3–40.9		9	11.11	5.5–20.5	
Sex	Male	306	129	42.2	36.6–47.9	0.001*	68	22.22	17.7–27.4	0.13	21	6.8	4.4–10.5	0.12
	Female	214	61	28.5	22.6–35.1		36	16.8	12.2–22.6		8	3.7	1.7–7.5	
Season	Winter	110	21	19.1	12.4–28	0.0001*	7	6.3	2.8–13.1	0.0001*	4	3.6	1.2–9.6	0.12
	Spring	135	42	31.1	23.5–39.7		16	11.8	7.1–18.8		5	3.7	1.4–8.8	
	Summer	230	118	51.3	44.6–57.9		75	32.6	26.6–39.1		19	8.2	5.18–12.8	
	Autumn	45	9	20.0	10–35.05		6	13.3	5.5–27.4		1	2.2	0.12–13.2	
Grazing	No	31	3	9.7	2.5–27	0.001*	1	3.2	0.17–18.5	0.01*	0	0.0	0–13.7	0.16
	yes	489	187	38.2	34–42.7		103	21.1	17.6–25		29	6.0	4.08–8.5	
Tick infestation	Yes	153	78	51.1	42.8–59.1	0.0001*	47	30.7	23.6–38.7	0.0001*	21	13.7	8.9–20.4	0.8
	No	367	112	30.5	26–35.5		57	15.5	12.06–19.7		8	2.2	1.02–4.4	
Total		520	190	36.5	32.4–40.8		104	20	16.7–23.7		29	5.6	3.8–8.01	

* The results are significant at $P < 0.05$.

Table 3
Risk factors associated with seroprevalence of *Theileria equi* among Egyptian equines.

Risk factor	Comparative parameter	Odds ratio (OR)	95 % CI
Age	<5 year	ref[a]	
	5–10 year	0.87	0.58–1.32
	>10years	2.06	1.19–3.55
Sex	Female	ref[a]	
	Male	1.8	1.23–2.61
Season	Winter	ref[a]	
	Spring	1.9	1.05–3.48
	Summer	4.3	2.53–7.46
	Autumn	1.05	0.42–2.43
Grazing	No	ref[a]	
	Yes	5.7	1.73–19.27
Presence of ticks	No	ref[a]	
	Yes	2.3	1.60–3.48

[a]ref = reference category.

Pullman, USA) were used for detection of antibodies against recombinant *T. equi* merozoites antigen 1 (EMA-1), a surface protein on merozoites of *T. equi*, and recombinant rhoptry-associated protein (RAP-1) of *B. caballi* in examined equines, according to the manufacturer's instructions. Control positive and negative serums were run together on the same plate.

The optical density (O.D) was measured through an ELISA reader at 650 nm. The results were expressed based on the percentage of inhibition (I%), according to the following formula:

Percent Inhibition (I%) = 100 – (Sample O.D. × 100)/ (mean negative control O.D.)

Samples having a cut-off value ≥40 were considered positive; whereas samples with value <40 % were recorded as negative.

2.5. Statistical analysis

The obtained data were analyzed using SPSS (Ver16, USA). A Chi-square test was used to study the risk factors. The results were considered significant at a probability level of ≤0.05. The logistic regression method was used to analyze the effect of each variable of the seroprevalence of EP.

3. Results

3.1. Seroprevalence of EP

Out of 520 examined sera, the total seropositive cases against *T. equi*, *B. caballi,* and mixed infections were 190 (36.5 %), 104 (20 %), and 29 (5.6 %), respectively.

The seroprevalences of *T. equi* and *B. caballi* were significantly higher in horses (39 %, P = 0.002) and donkeys (42 %, P = 0.0001), respectively, when compared to those having mixed infections, Table 2.

The seroprevalence of *B. caballi* only showed a significant difference between different Governorates. The highest detectable antibody levels against *T. equi* were observed in Kafr ElSheikh (40 %) and Giza (40.1 %) Governorates; whereas those of *B. caballi* were detected in Qalyubia (25 %) and Kafr ElSheikh (24.1 %) Governorates, Table 2 and Fig. 1.

Table 4
Risk factors associated with seroprevalence of *Babesia caballi* among Egyptian equines.

Risk factor	Comparative parameter	Odds ratio (OR)	95 % CI
Age	<5 year	ref[a]	
	5–10 year	1.16	0.69–1.94
	>10years	2.08	1.10–3.94
Season	Winter	ref[a]	
	Spring	1.9	0.78–4.99
	Summer	7.12	3.15- 16.06
	Autumn	2.26	0.71–7.15
Grazing	No	ref[a]	
	Yes	7.8	1.05–58.25
Presence of ticks	No	ref[a]	
	Yes	2.4	1.54–3.76

[a]ref = reference category.

3.2. Risk factors associated with the prevalence of EP in equids

The logistic regression analyses of data indicated variations according to the studied factors. The seroprevalence rate of *T. equi* in equids showed significant differences between age groups and sex of examined animals, seasons, and management including grazing, and tick infestations.

The highest prevalence rate of *T. equi* was recorded among elder, >10 years old (53 %), grazing (38.2 %) males (42.2 %) highly infested with ticks (30.5 %), during the summer season (51.3 %). Concerning *B. caballi*, there was a significant difference (P < 0.05) among the previously mentioned factors, except for the gender. The detection of antibody levels was higher among elder, >10 years old (29.6 %), grazing (21.1 %) males (22.22 %), infested with ticks (30.7 %) during the summer season (33 %), Table 2.

Besides, few numbers of animals showed mixed infections with both piroplasms with a significant difference (P < 0.05) among age groups as the infections increased with aging, but there was no significantly detected antibody levels regarding the other factors, Table 2.

3.3. Multivariate logistic regression analysis

Five and four risk factors for *T. equi* and *B. caballi,* respectively, had been studied following a logistic regression model. Concerning *T. equi,* animals >10 years (OR = 2.06) were more likely to be infected with EP than those <5 years old. In addition, the seropositivity increased among grazing (OR = 5.7, 95 % CI: 1.73–19.27) males (OR = 1.8, 95 % CI: 1.23–2.61) infested with ticks (OR = 2.3, 95 % CI: 1.60–3.48) during summer (OR = 4.3, 95 %CI: 2.53–7.46), Table 3

The seropositive animals for *B. caballi* increased among grazing equines (OR = 7.8, 95 % CI: 1.05–58.25) over 10 years old (OR = 2.08, 95 % CI: 1.10–3.94) and infested with ticks (OR = 2.4, 95 % CI: 1.54–3.76) during summer (OR = 7.12, 95 % CI: 3.15–16.06), Table 4.

4. Discussion

Piroplasmosis is an important disease in equines leads to great economic impact. Recently, *T. haneyi* was reported in different areas in the US and Africa, inducing silent and persistent infections and superinfection to horses having anti-*T. equi* antibodies [21]. The epidemiological situation of EP is not fully estimated in Egypt, therefore, its seroprevalence based on cELISA test and its associated risk factors were investigated in the present study.

The seroprevalence rates of the present work revealed that *T. equi* and *B. caballi* were 39 % and 11 % in horses and 30.6 % and 42 % in donkeys, respectively, but few proportions of horses and donkeys, 5.1 % and 6.6 %, respectively, had mixed infections with both species. Such prevalence rate was higher than that previously reported in Giza, Egypt as the antibody levels against *T. equi* was 14.8 % and 23.5 % among horse and donkeys, respectively, but the *B. caballi* RAP-1-based cELISA failed to detect any positive cases [11].

On contrary, the prevalence rates in the present work are lower than that reported in Pakistan as the seroprevalence rates among horses and donkeys were 56.9 % and 37.3 % in the case of *T. equi*; 24.6 % and 20.2 % for *B. caballi;* and 12.3 % and 9.3 % for mixed infections, respectively [2].

This study indicated a higher prevalence of *T. equi* among equines than that of *B. caballi;* similar findings were observed [9,22] and

explained as the infection with *T. equi* remains life-long but that of *B. caballi* infection subsides within 5 years and responded well to treatment with imidocard [9].

About the locality of this study, the highest detectable antibody levels for both causative agents of EP were recorded for Kafr ElSheikh Governorate; whereas the lowest ones were noted for Menofia Governorate. Such variations could be contributed to climatic variation, tick control measures, or the presence of carrier equines in highly prevalent areas [23,24,33].

Concerning age in the present work, the seroprevalence rate of both parasite species was significantly increased with the age of infected animals. This finding is supported by previous studies [12,22,24]. In contrast, the seroprevalence rate of *T. equi* was more prevalent in young equines in Brazil [25]. This could be explained as different geographical areas, management systems, the dynamic of tick and parasitic populations, and their control measures [26].

Gender is another key factor for the prevalence of EP as males were more likely to be infected than females; similar observation was recorded [2,12]. This fact could be explained as most animals enrolled in this study were males and/or the level of care they received.

A strong relationship between seroprevalence of EP and season was observed where the infection rate was highly significant in the summer seasons which coincide with the presence of ticks. A similar finding was reported [16,22].

Our data demonstrated that the infection rate significantly increased in grazing equines than indoor reared ones, alike observation was previously recorded [3] because of the likelihood of exposure to tick during grazing outside and low awareness of the owner for tick infestation because ticks usually exist in areas which need careful examination [27,28].

The hard ticks especially *Dermacentor, Hyalomma*, and *Rhipicephalus* are more prevalent in the summer season in different areas in Egypt and play the main role in the transmission of haemoprotozoa in domestic animals [20]. Besides, the lack of control measures for external parasites, particularly ticks, was consistent with the high prevalence of EP among Egyptian equines [11,29].

5. Conclusion

EP has economic importance particularly in equines reared in poor management systems. This study revealed that EP was prevalent among 370 horses and 150 donkeys reared in four Governorates northern Egypt where equids in Kafr ElSheikh Governorate were highly infected with both *T. equi* and *B. caballi.* Moreover, the risk factors related to EP were revealed for the first time, according to our knowledge. The seroprevalence rate increased in summer among older animals, >10 years old, grazing outside, and infested with ticks for *T. equi* and *B. caballi.* The presence of ticks has a highly significant effect on the appearance of the disease among equines. As a consequence, tick control with safe materials [30,31] is an urgent need and EP deserves further attention in Egypt regarding the management system and treatment.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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