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Research Article

Seroprevalence and Assessment of Risk Factors Associated to *Borrelia burgdorferi* Infection in Egyptian Horses

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Abstract

Borrelia burgdorferi sensu lato can infect horses. However, the extent to which Egyptian horses get infected and seroconvert owing to this pathogen is unclear. Thus, this study aimed to determine the seroprevalence of *B. burgdorferi* and estimate its associated risk factors. A total of 385 serum samples collected from asymptomatic horses reared in three governorates in Egypt were screened using a commercial IDEXX SNAP 4Dx Plus kit. The results revealed that 58 (15.1%) out of 385 horses had antibodies against *B. burgdorferi* with Giza governorate showing the highest prevalence rate (30/130; 23.1%). According to univariate logistic regression analysis, locality, age, breed of examined horses and application of hygienic measures in house were significantly associated with *B. burgdorferi* infection. Furthermore, the multivariate logistic regression study revealed that thoroughbred horses older than four years old and raised in Giza governorate under unsanitary conditions, were more likely to be infected with *B. burgdorferi* than others. The serological evidence of *B. burgdorferi* in Egyptian horses and assessment of the risk factors associated with infection, necessitate wide range screening of this disease in other areas as well for efficient diagnosis and control.

Keywords: *Borrelia burgdorferi*; SNAP test; Risk factors; Horses; Egypt

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Introduction

Horse Lyme borreliosis (LB) was first reported in Wisconsin state at USA, an area where *Borrelia burgdorferi* is widespread [1]. *B. burgdorferi* is spread by the biting of hard ticks i.e. *Ixodes* spp., whereas mammals and birds act as reservoirs [2,3].

There is no typical symptom of Lyme disease among horses [4], but some may show meningitis, encephalitis [5], radiculoneuritis [6], arthritis, lameness [7], and panuveitis [1]. Human infection is usually associated with erythematous rash, neurological abnormalities, and arthritis, but some patients do not show any symptoms, as with horses [8].

The LB in horse is difficult to diagnose since specific antibodies against the *B. burgdorferi* sensu lato complex are not necessarily associated with clinical signs and symptoms [9]. Depending on the distribution of *Ixodes*

spp. ticks infected with *Borrelia burgdorferi*, equine LB seroprevalence varies across geographic regions. However, it is not clear whether high antibody levels correlate with clinical signs in infected hosts, or whether clinical signs are unrelated to antibody levels [10-12].

A number of previous studies on *B. burgdorferi* seroprevalence found 47.8% seropositive horses in Slovakia [13], 29.0% in Denmark [14], 25.6% in Poland [15], 6.8% in Sweden [16], 16.1% in Germany [17], 6.3% in Turkey [18], 34% in Mexico [19], 33% in France [20], 58.7% in Minnesota [12], 63% in Wisconsin [21], 60% in New Jersey [22] and 60-90% in Austria [23]. The available information concerning the seroprevalence of *B. burgdorferi* s.l. in horses in Egypt is rare.

Although both direct and indirect pathogen detection methods are available, direct detection of *Borrelia* spp. by culture is challenging and costly [24]. Although polymerase chain reaction (PCR) can detect *Borrelia* specific DNA during most stages of the disease, various in-house PCR tests have not been validated under standardized study conditions as methods for detecting *Borrelia* spp. from various sample materials. As a result, the use of PCR for detecting *Borrelia* spp. in routine diagnostics remains restricted [25,26]. Serological approaches for identifying specific antibodies against members of the *B. burgdorferi* complex remain a sensitive, low-cost, and quick laboratory diagnostic tool in both human and veterinary medicine [24].

Therefore, the purpose of this study was to determine the seroprevalence of *B. burgdorferi* s.l. among horses residing in three governorates in Egypt.

Material and Methods

Ethical Approval

The Benha University ethics committee for animal studies authorized all operations, including the collection and processing of blood samples. The animals' owners gave their informed consent for the collection of samples. The Benha ethics committee ensured that all operations followed all applicable laws and rules. The ARRIVE criteria were followed throughout the study process.

Study Area

The research was conducted in three Egyptian governorates: Giza, Kafr ElSheikh and Qalyubia which are located at 29.9870°N 31.2118°E, 31°06′42″N 30°56′45″E and 30.41°N 31.21°E, respectively *(Fig. 1)*.

Egypt has mostly hot and dry climate characteristics (Köppen climate classification BWh). Giza has a desert-like climate, which is characterized by warm summers with temperature range between 25-45°C and moderate winters with low rainfall (100 mm). Kafr ElSheikh and Qalyubia governorates situated at the Nile Delta, have hot desert climates (Köppen: BWh), like most of Egypt. In the Nile Delta, temperatures are relatively moderate, but they rarely reach 35°C during the summer. The delta region receives about 100-200 millimeters of rainfall on average per year, most of which falls in the winter. A variety of habitats, including lakes, marshes, grass pastures, and agricultural areas, may be found in the study area. These habitats together support many biological species and the proliferation of ticks.

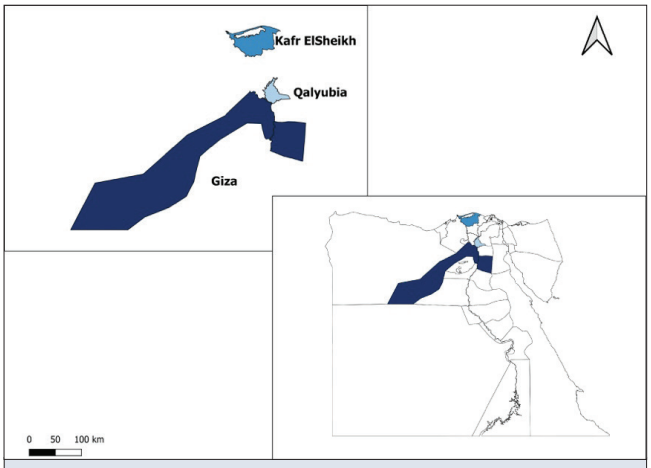

Fig 1. MAP showed the studied governorates (MAP generated by QGIS software)

Sample Size and Sampling

A cross-sectional study was carried out from January to December 2022. A sample size of 385 was calculated based on an estimated 50% prevalence. The samples were collected from asymptomatic horses distributed in three governorates (130 from Giza, 140 from Kafr El Sheikh and 115 from Qalyubia) under the study. These horses were raised by individual farmers and did not suffer from any disease or clinical signs.

Blood sample (10 mL) was collected from jugular vein of each horse, transferred immediately to Veterinary Diagnostic Laboratory, Benha university and centrifugated at 1500xg for 10 min to separate the sera. The sera samples were kept at -20°C till serological analysis.

Questionnaire

The examined horses included those kept for drafting and others being raised for breeding. These animals living in indoor house and some of them have outdoor yard. Each horse owner was asked to fill out a questionnaire that assessed each horse's farm management and demographics. Demographics included: sex (male or female), age (<2, 2-4 and >4 years) and breed (Arabian, Thoroughbred and mixed). Management related questions included: if owners apply regular cleaning of stable every week and application of acaricides (every month) or not.

Serological Analysis

The IDEXX SNAP 4Dx Plus (IDEXX Laboratories) was used in accordance with the manufacturer's instructions to test sera for the existence of antibodies against *B. burgdorferi*.

Three drops of serum and four drops of conjugate are added to the sample tube, the tube is inverted four times and then the entire contents of the tube are poured into the "sample well" of the SNAP device. Results are read 8 minutes after the test is activated. According to the published data by Chandrashekar, Daniluk [27], this test was validated for detection of antibodies to *B. burgdorferi* by 100% sensitivity and 95% specificity.

In addition, all examined samples with IDEXX SNAP 4Dx Plus were confirmed by by Viramed Biotech AG – Borrelia B31 IgG ViraStripe (Vuramed Biotech AG, Planegg, Germany).

Data Analysis

The IBM SPSS Statistics program (SPSS Inc., Chicago, USA, version 24) was used to conduct the statistical analysis. The variations in seroprevalences of variable groups were analyzed using the chi-square test. P-values under 0.05 were regarded as significant. The relationship between *B. burgdorferi* infection and the relevant risk variables was evaluated using univariate analysis.

All variables (locality, age, breed and hygienic measures) with *P* values less than 0.25 in the univariate analysis were subjected to multivariable logistic regression analysis [28-31]. To determine the strength of the correlation between the presence of *B. burgdorferi* and other factors, odds ratios (ORs) and 95% confidence intervals (CIs) were computed. The model's fit was assessed using the Hosmer and Lemeshow goodness-of-fit test.

Results

Using ELISA, 58 (15.1%) of 385 horse blood samples tested positive for *B. burgdorferi* with IDEXX SNAP 4Dx Plus and Borrelia B31 IgG ViraStripe®. The seroprevalence *B. burgdorferi* was varied significantly between different localities, Giza had the greatest prevalence (23.1%), followed by Kafr ElSheikh (12.1%) and Qalyubia (9.6%) *(Table 1)*.

There was no significant difference between sex and *B. burgdorferi* seroprevalence and females showed higher seroprevalence (16.7%) than males. In addition, the univariate analysis showed age, breed and hygienic measures had significant effect (P<0.05) on seroprevalence of *B. burgdorferi* in horses. Horses older than four years were more likely to be positive (24.5%) than horses of a middle age or younger than two years *(Table 1)*.

Regarding to breed, the seroprevalence of *B. burgdorferi* in thoroughbred horses (25.7%) was higher in comparison with Arabian horses (6.9%) especially in the absence of hygienic measures (17.5%) *(Table 1)*.

A multivariate logistic regression model was performed on the factors with a *P* value less than 0.25 in univariate analysis. The study found that horses raised in Giza, had

Table 1. *Seroprevalence of B. burgdorferi in horses in relation with different variables*

Variable		No of Examined Horses	Distribution (%)	No of Positive	No of Negative	% of Positive	95% CI	Statistic
Locality	Giza	130	33.8	30	100	23.1	16.67-31.03	χ2=10.175 df=2 P=0.006*
	Kafr ElSheikh	140	36.4	17	123	12.1	7.72-18.58	
	Qalyubia	115	29.9	11	104	9.6	5.43-16.32	
Sex	Male	115	29.9	13	102	11.3	6.72-18.38	χ2=1.812 df=1 P=0.178
	Female	270	70.1	45	225	16.7	12.7-21.58	
Age	<2 years	99	25.7	10	76	10.1	5.58-17.6	χ2=6.374 df=2 P=0.041*
	2-4 years	192	49.9	25	167	13.0	8.98-18.51	
	>4 years	94	24.4	23	84	24.5	16.9-34.05	
Breed	Arabian	145	37.6	10	135	6.9	3.79-12.23	χ2=16.893 df=2 P<0.0001*
	Thoroughbred	105	27.3	27	78	25.7	18.31-34.82	
	Mixed	135	35.1	21	114	15.6	10.41-22.62	
Hygienic measures	poor	286	74.3	50	236	17.5	13.52-22.3	χ2=3.910 df=1 P=0.048*
	good	99	25.7	8	91	8.1	4.15-15.14	
Total		385		58	327	15.1	11.83-18.98	

** The results with P value less than 0.05 considered significant*

Table 2. *Multivariate logistic regression analysis for the factors associated with B. burgdorferi in horses*

Variables		B	SE	OR	95% CI for OR		P Value
					Lower	Upper	
Locality	Giza	1.086	0.399	2.96	1.36	6.47	0.006
	Kafr ElSheikh	0.224	0.425	1.25	0.54	2.88	0.039
Age	2-4 years	0.359	0.412	1.43	0.64	3.21	0.038
	>4 years	1.230	0.436	3.42	1.46	8.03	0.005
Breed	Thoroughbred	1.647	0.415	5.19	2.30	11.72	0.000
	Mixed	0.992	0.420	2.70	1.18	6.14	0.018
Hygienic measures	Poor	0.744	0.427	2.10	0.91	4.86	0.081

B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval

a three-fold greater likelihood (OR=2.96, 95% CI: 1.36-6.47) of being infected with *B. burgdorferi* than horses in other areas *(Table 2)*. Moreover, Thoroughbreds were more likely to have *B. burgdorferi* infection (OR=5.19, 95% CI: 2.30-11.72), especially older examined horses more than four years (OR=3.42, 95% CI: 1.46-8.03), *Table 2*. Furthermore, absence of hygienic measures (OR=2.10, 95% CI: 0.91-4.86) was identified as a risk factor for *B. burgdorferi* infection in horses *(Table 2)*.

Discussion

Horses are frequently infested by ticks and might be directly infected by tick-borne diseases [32-35]. Lyme borreliosis is one of the most important tick-borne diseases in horses and is caused by *B. burgdorferi*. The serological detection of *B. burgdorferi* has been reported in dogs in Egypt but there is no available data about the disease in Egyptian horses.

Thus, the present study investigated the presence of antibodies against *B. burgdorferi* and the associated risk factor for infection.

The overall seroprevalence of antibodies against *B. burgdorferi* was 15.1% using IDEXX SNAP 4Dx Plus and the presence of IgG-specific antibodies against *B. burgdorferi* were confirmed by *Borrelia* B31 IgG ViraStripe.

The reported prevalence of the study lies in similar range (15-16%) reported by Tsachev et al.[36] among horses in Northern Bulgarian using method of rapid ELISA test (SNAP 4Dx Plus Test, IDEXX Laboratories Inc., USA) and in German horse [17] and in Northern Algeria using ELISA [37]. The seroprevalence rate in this study was higher than those reported in Sweden 6.8% [16] and Turkey 6% [18]. In addition, seroprevalence found in this study is lower than those reported in Mexico 34% [19], France 33% [20], Minnesota 58.7% [12], Wisconsin 63% [21], New Jersey 60% [22] and Austria 60-90% [23].

Various factors influencing seroprevalence between different countries that may explain the discrepancies in the values reported, include the number of surveyed horses, hygienic measures, environmental conditions, and the frequency of exposure to ticks [14,33,38].

In addition, the seroprevalence of *B. burgdorferi* was highest in Giza in comparison with other studied governorates which might be attributed to warmer weather of Giza which is significant for tick ecology [39-45].

There is no significant variation in seroprevalence between both sexes with a higher trend in females. This is consistent with previous findings which have been found by Lee et al.[46] and Laamari et al.[37].

The seroprevalence of *B. burgdorferi* increased significantly with age in horses, which concurs with a study conducted by Hansen et al.[14]. However, other studies found no significant difference between age groups and a higher presence of antibodies in young animals [37,46]. It may be attributed to horses being exposed to ticks frequently with age, increasing their chances of contracting Lyme disease [3,10,13,20].

Several studies have indicated that the breed is a significant risk factor for *B. burgdorferi* infection, and in the current study, the thoroughbred horses were found more likely to contract the infection than other breeds, consistent with previous results of Lee et al.[46]. However, Laamari et al.[37] found no significant correlation between breed and seroprevalence of *B. burgdorferi* and Arabian horses were found more frequently infected than others [41,47].

There is no clear explanation for the higher *B. burgdorferi* seroprevalence found in our study among thoroughbred horses compared to other breeds, but it might be attributed to a difference in management. Compared to Arabian breed horses that are kept in stables and are more protected from ticks than thoroughbreds, which are housed outdoors [48,49].

Additionally, the horses were not generally well cared or did not kept in clean houses, which made them more vulnerable to ticks and *B. burgdorferi* infection, as

previously reported by Neely et al.[50].

Limitation of the present study was sample selection bias and bias in timing of the sample.

To the best of our knowledge, this study is the first to reveal the countrywide seroprevalence of *B. burgdorferi* in Egyptian horses. This study concludes that *B. burgdorferi* exists in Egyptian horses and that the frequency is highest in the Giza governorate. Furthermore, the multivariate logistic regression analysis revealed that the locality, sex, breed and hygienic measures were identified as risk factors for *B. burgdorferi* infection in horses.

Declarations

Availability of Data and Materials:

The data used in this article will be provided by the corresponding author (MM and AS) upon request.

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