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Research Article

Seroprevalence and Risk Factors Associated with *Anaplasma phagocytophilum* Infection in Horses in Egypt

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Abstract

Anaplasma phagocytophilum is a zoonotic obligate intracellular pathogen, that infects horses and is transmitted by *Ixodes* ticks. The seropositivity for this pathogen does not necessarily associate with clinical signs. Limited data about the epidemiology of *A. phagocytophilum* in horses in Egypt are available. The purpose of the study was to determine the seroprevalence of *A. phagocytophilum* in Egyptian horses in three Egyptian governorates and to assess the associated risk factors for infection. A total of 395 serum samples from suspected horses raising in three Egyptian governorates at Northern Egypt were examined using SNAP®4DX® ELISA test. Overall, the seroprevalence of *A. phagocytophilum* was 11.1% (44/395), with highest rate found in Giza governorate 15.7%. The seroprevalence of A. *phagocytophilum* increased significantly in older animals, in thoroughbred horses and during summer. The multivariate logistic regression model revealed that age, thoroughbreds, summer season and presence of ectoparasites were identified as risk factors for *A. phagocytophilum* infection in horses. The results of this study confirmed the presence of antibodies against *A. phagocytophilum* in Egyptian horses. Thus, regular monitoring and genetic identification of pathogens are critical for implementing an effective control program and reducing public health risks.

Keywords: Equine granulocytic anaplasmosis, SNAP test, Risk factors, Horse, Egypt

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Introduction

Anaplasma phagocytophilum is obligate intracellular bacterium, causes granulocytic anaplasmosis, formerly known as granulocytic ehrlichiosis, in a variety of wild and domestic animal species, including horses [1,2]

This zoonotic pathogen is mostly spread throughout the world by *Ixodes* ticks. *A. phagocytophilum* is regarded as an emerging pathogen in humans and it causes a feverish state accompanied by headaches and myalgia [3].

Equine granulocytic anaplasmosis (EGA) varies according to the age of the horse and length of infection, ranging from subclinical to a noticeable condition. The clinical signs of the disease are vague and frequently manifest as ataxia, pyrexia, lethargy, distal limb oedema, and decreased appetite [4,5]. *A. phagocytophilum* may have a detrimental economic impact on the equine sector, because of its ability to inhibit the host defense system and hence cause decreased performance [6].

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EGA was initially documented in the United States in 1969 [7] and has since been recorded in Brazil, Israel and Europe including Germany [8-10]. EGA is endemic in some locations but not in others [11]. Seroprevalence investigations in horses have been conducted in numerous European countries such as Italy, Denmark, Sweden, France, Czech Republic and Spain, with seroprevalence rates ranging from 7% in Spain to 73% in Czech Republic [12-18].

The preliminary diagnosis of EGA is based on clinical symptoms or hematological abnormalities such as thrombocytopenia, anaemia and lymphopenia. Moreover, the inclusion bodies (morula) in granulocytes can frequently be identified cytologically to provide a conclusive diagnosis [19,20]. PCR is a sensitive and specific approach for detecting *A. phagocytophilum*-specific DNA, particularly in the early and late phases of the disease where microscopic detection of morulae is challenging [21-25]. EGA can be diagnosed using indirect detection approaches such as immunofluorescence test and ELISA [15]. For a precise diagnosis, four weeks with a four-fold increase in certain antibody titers for EGA is required [26].

In Egypt, *A. phagocytophilum* have been detected using serological and molecular techniques in human and ticks but no available data about the situation of *A. phagocytophilum* in equine.

Therefore, the purpose of this study was to determine the seroprevalence of *A. phagocytophilum* in horses in some localities at Northern Egypt and assess the associated risk factors for the infection.

Material and Methods

Ethical Statement

Benha University's ethical council approved all of the approaches and procedures conducted in this study (ethical number BUFVTM2-03-2023). All approaches were implemented in accordance with the standards and regulations of the Benha university committee. The entire study methodology followed the ARRIVE guidelines.

Study Area

The study was performed during the period between January to December 2023 in three governorates (Giza, Kafr ElSheikh and Qalyubia) located at Northern Egypt, *Fig. 1*.

The climate of Giza has a desert climate according to the Köppen-Geiger classification. During the year, there is virtually no rainfall, and the annual rainfall is 18 mm while the average annual temperature is 22°C. In addition, the climate of Kafr el-Sheikh and Qalyubia governorates has a subtropical desert climate (Classification: BWh), is situated at an elevation of 9 to 51 meters above sea level and average temperature of these areas is 23°C.

Sampling and Sample Size

The sample size was calculated using the following formula based on random sampling strategy with an expected disease prevalence of 50%, an acceptable absolute error of 5%, and a 95% confidence level.

$$N= (1.96)^{2*}P_{exp}\,(1\text{-}P_{exp})/d^2$$

Where n is the calculated sample, P_{exp} is the predicted prevalence rate and d is the absolute precision. A total of 395 serum samples were taken from horses raised in three governorates: Giza (140), Kafr ElSheikh (130), and Qalyubia (125).

All horses had no clinical signs for EGA at time of sampling. Blood (10 mL) was obtained from the jugular vein of presenting horses using a vacutainer tube without anticoagulants. The blood samples were centrifuged at 3000 rpm for 10 min to separate sera which kept at -20°C untill serological analysis.

A questionnaire was used to collect data on sex (male, female), age (1-4, 4-8, >8 years), breed (Arabian, Thoroughbred, mixed), season (summer, spring, winter, and autumn), and presence of ectoparasites (yes or no).

Serological Analysis

In accordance with the manufacturer's instructions, horses were screened using the SNAP® 4Dx test (IDEXX Laboratories, Westbrook, ME, USA), which uses the immunodominant p44 protein of the *A. phagocytophilum*. In brief, serum and conjugate were mixed and then added to the SNAP device's sample well. *A. phagocytophilum* test

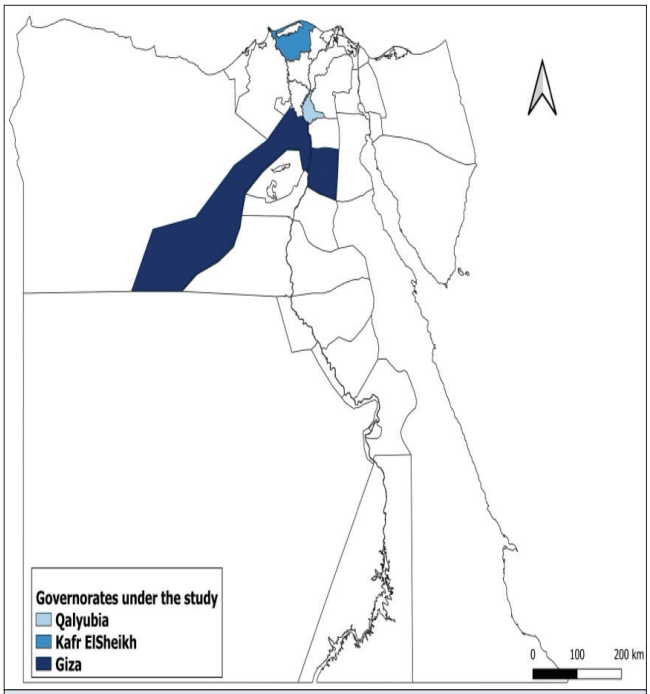

Fig 1. MAP showed location of studied governorates (MAP generated by QGIS software)

results were reported as positive or negative for each horse. The commercial SNAP test has been approved for use with horses despite being created initially for canine testing [27,28].

Statistical Analysis

Microsoft Excel was used to record and code the data from questionnaire surveys before being imported into IBM SPSS version 24 (USA). The relationship between the risk factors (sex, age, breed, season, and presence of ectoparasites) and the *A phagocytophilum* seropositivity was determined using univariate analysis. The significant variables from the univariate analysis were incorporated in a multivariate logistic regression model [29-32]. To determine the degree of association between different parameters and seropositivity, odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated.

Results

According to the results of the SNAP test, anti-*A. phagocytophilum* antibodies were found in 11.1% (44/395,

Table 1. *Seroprevalence of Anaplasma phagocytophilum in horses in relation to different factors*

Variable		No of Examined Horses	No of Positive	No of Negative	% of Positive	95% CI	Statistics
Locality	Giza	140	22	118	15.7	10.61-22.64	$\chi2$=4.683 df=2 P=0.096
	Kafr ElSheikh	130	12	118	9.2	5.36-15.44	
	Qalyubia	125	10	115	8.0	4.4-14.1	
Sex	Male	120	10	110	8.3	4.59-14.66	$\chi2$=1.371 df=1 P=0.242
	Female	275	34	241	12.4	8.98-16.78	
Age	1-4years	192	14	178	7.3	4.39-11.86	$\chi2$=10.664 df=2 P=0.005*
	4-8 years	95	9	86	9.5	5.06-17.03	
	>8 years	108	21	87	19.4	13.08-27.9	
Breed	Arabian	145	8	137	5.5	2.82-10.51	$\chi2$=10.523 df=2 P=0.005*
	Thoroughbred	115	21	94	18.3	12.26-26.31	
	Mixed	135	15	120	11.1	6.85-17.52	
Season	Summer	120	21	99	17.5	11.74-25.28	$\chi2$=8.631 df=3 P=0.035*
	Autumn	89	10	79	11.2	6.22-19.47	
	winter	93	8	85	8.6	4.42-16.06	
	Spring	93	5	88	5.4	2.32-11.98	
Presence of ectoparasites	Yes	295	40	255	13.6	10.12-17.94	$\chi2$=6.895 df=1 P=0.009*
	No	100	4	96	4.0	1.57-9.84	
Total		395	44	351	11.1	8.4-14.62	

The result is significant if P<0.05

Table 2. *Multivariate logistic regression analysis for factors associated with Anaplasma phagocytophilum infection in horses*

Variables		B	S.E.	OR	95% CI for OR		P Value
					Lower	Upper	
Age	4-8 years	0.253	0.464	1.3	1.2	3.2	0.029
	>8 years	1.388	0.396	4.0	1.8	8.7	<0.0001
Breed	Thoroughbred	1.449	0.461	4.3	1.7	10.5	0.002
	Mixed	0.789	0.475	2.2	1.4	5.6	0.027
Season	Summer	1.423	0.540	4.2	1.4	12.0	0.008
	Autumn	1.114	0.594	3.0	1.0	9.8	0.061
	Winter	0.419	0.609	1.5	1.2	5.0	0.019
Presence of ectoparasites	Yes	1.374	0.557	4.0	1.3	11.8	0.014

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

95% CI: 8.4-14.62) of the horses that were examined. The highest prevalence was found in the Giza governorate, where it was 15.7% (22/140), while the lowest prevalence was found at qalyubia with 8% (10/125), *(Table 1)*.

The seroprevalence of *A. phagocytophilum* in the horses under examination was not significant associated with both horse sex (P=0.242) and location (P=0.096). The seropositivity of *A. phagocytophilum* increased significantly in thoroughbred horses (18.3%), particularly those Particularly those over eight years old (19.4%). In addition, the highest positive results were highest in summer (21 out of 120; 17.5%), followed by autumn (10 out of 89; 11.2%), and winter (8 out of 93; 8.6%), while the prevalence increased significantly in presence of ectoparasites (40/295; 13.6%) *(Table 1)*.

The multivariate logistic regression model revealed that the likelihood of a positive for *A. phagocytophilum* increased by four times in in older horses over eight years (OR= 4, 95% CI: 1.8-8.7) in thoroughbreds (OR=4.3, 95% CI: 1.7-10.5). Horses were nearly four times more likely to test positive for SNAP in the summer (OR = 4.2) and in the presence of ectoparasites (OR = 4), *(Table 2)*.

Discussion

A. phagocytophilum is an obligate zoonotic intracellular bacterium that affects all mammals, including horses. It has a severe economic impact on the equine industry and reduces performance. To our knowledge, this is the first serological study to investigate presence of antibodies against *A. phagocytophilum* in Egyptian horses.

The study found a seroprevalence of 11.1% using the IDEXX SNAP 4DX Plus Test®, which was similar to the reported prevalences in southwest Virginia (8-11.2%) [33] and Brazil (11.3%) [34].

In addition, high prevalence rates of *A. phagocytophilum* was reported in Scandinavian countries. The prevalence ranged between 17-69% among Swedish horses using PCR [18,35], whereas 22% of horses in Denmark had positive results with ELISA testing [36]. In Europe, the prevalence rate was 5-73% using IFAT and 5% using PCR in the Czech Republic [16,37], 10% based on PCR in Netherlands and 4% using IFAT in Switzerland and ranged between20-26.9% in Germany based on IFAT and SNAP test [26,38],

The status of infection, time of sampling and size, vector distribution, presence of vectors, season, climatic condition, management practices and the tests used to assess infection can all be factors contributing to the fluctuations in global prevalence [39-41].

The current investigation found no significant effect of gender on the prevalence of *A. phagocytophilum*, which is consistent with Hinson et al.[33] and Nogueira et al.[34]. The high incidence was reported in females, which contrasts with the findings of Schäfer et al.[38]. This might be explained by the fact that most of the examined animals in the study were females or by the kind of care the animals received.

Interestingly, the presence of antibodies against *A. phagocytophilum* was significantly associated with the age of horse, with highest prevalence rate in old age. Other studies concluded similar findings [36]. On the contrary, Ribeiro et al.[42] reported no significant effect for age on prevalence of *A. phagocytophilum* in horses and observed high prevalence among young animals. This observation could be explained by the fact that young animals receive more care, while older animals are regularly exposed to illnesses throughout their careers [35,39].

The present finding are broadly in line with observation of Seo et al.[43], where the prevalence rate of *A. phagocytophilum* was higher in thoroughbreds and in presence of ectoparasites in comparison with others.

This could be attributed to thoroughbreds were more exposure for ticks infestation and absence of daily grooming [44]. In addition, the climatic characters of studied areas like relative humidity, temperature are favorable for development of ticks which are the main vectors of pathogen transmission [45].

In the present study, seasonality has a statistically significant effect on seropositivity for *A. phagocytophilum* (P=0.035). This could be due to the highest activity of the vector (*Ixodes* ticks) in late spring/early summer [46-50]. Conversely, Schäfer et al.[38] found that seasonality had no effect on the serological test results for horses which might be due to persist of antibodies titers for at least two years following pathogen exposure.

The results of this study showed that Egyptian horses frequently produce antibodies against *A. phagocytophilum*. The statistical analysis revealed that age, thoroughbreds, summer season and presence of ectoparasites were significantly associated with seroprevalence of *A. phagocytophilum*. Further studies are necessary for identification and genetic characterization of pathogens either in horses or ticks.

Declarations

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

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