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Research Article

# The First Detection of anti-*Anaplasma phagocytophilum* Antibodies in Horses in Turkey

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## Abstract

*Anaplasma phagocytophilum*, the causative agent of equine granulocytic anaplasmosis, affects several species of wild and domesticated mammals, including horse, besides human. In Turkey, there were many reports on *A. phagocytophilum* circulation among cattles, sheep, dogs, mice, humans, except horses. In this study, we aimed to inquiry whether *A. phagocytophilum* were circulating among the horse population or not. For this purpose, 105 mare horse blood sera were examined for the presence of Anti-*Anaplasma phagocytophilum* IgG antibodies by IFAT. The seroprevalance rate of 8.57% horse sera were found to be positive. This was the first report about the presence of anti-*A. phagocytophilum* antibodies in horses in Turkey..

***Keywords:*** *Anaplasma phagocytophilum, Horse, IFAT*

# Türkiye'de Atlarda anti-*Anaplasma phagocytophilum* Antikorlarının İlk Tespiti

## Öz

Equine granulocytic anaplasmosis'in etkeni olan *Anaplasma phagocytophilum*, atların dahil olduğu pek çok vahşi ve evcil memeliyi, yanı sıra insanları etkilemektedir. Türkiye'de *A. phagocytophilum*'un atlar hariç, sığırlar, koyunlar, köpekler, fareler, insanlar arasında sirküle olduğuna dair birçok çalışma mevcuttur. Bu çalışmada, *A. phagocytophilum*'un at populasyonu arasında mevcut olup olmadığını araştırmayı amaçladık. Bu amaçla, 105 dişi at kan seumu Anti- *A. phagocytophilum* IgG antikorlarının varlığını tespit etmek için IFAT ile incelendi. At kan serumlarının %8.57'si pozitif bulundu. Bu çalışma, atlarda anti-*A. phagocytophilum* antikorlarının varlığı ile ilgili ilk rapordur..

***Anahtar sözcükler:*** *Anaplasma phagocytophilum, At, IFAT*

## INTRODUCTION

*Anaplasma phagocytophilum (A. phagocytophilum),* a member of Anaplasmataceae in the order of Rickettsiales is a causative agent of Equine Granulocytic Anaplasmosis (EGA) was defined in 2001 [1]. The agent is an obligate, intracellular, gram negative tick-borne, zoonotic rickettsiale bacterium of human and animals [2,3].

The family Anaplasmataceae contains arthropod-borne α-proteobacteria which causes important economic and health losts both in veterinary and human medicine relevant to endemic and emerging infectious diseases. Particularly, *Anaplasma* genera infects peripheral blood cells, *A. phagocytophilum* infects myeloid cells of bone marrow, especially neutrophills and sometimes eosinophils [3,4]. *A. phagocytophilum* is transmitted by ticks of Ixodes genera

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during seasons of tick activity [5]. Agent replication takes place within the vacuoles of phagocytes [3,6].

The first reported case of Equine Granulocytic Anaplasmosis (EGA) was in California, USA [7,8] in 1969, *A. phagocytophilum* infection in domesticated animals, ticks and people has a worldwide geographic distribution such as Europea [9-11], Great Britain [12], Asia [13,14] and Africa [15,16]. Also, infections of *A. phagocytophilum* have been reported in neighbouring countries of Turkey such as Greece [17], Bulgaria [18] and Iran [19]. Many available studies conducted on *A. phagocytophilum* infection on horses were present [20-25]. In Turkey, although studies conducted on *A. phagocytophilum* including a wide variety of animal species such as cattle, sheep, dog, mice, besides human [26-32] were exist, but there is no report about equine anaplasmosis. The aim of this study was to detect anti-*A. phagocytophilum* antibodies in horses in Nevşehir province of Turkey.

# MATERIAL and METHODS

### *Ethical Approval*

The ethics committee of Veterinary Control Central Research Institute (Date 27.11.2015, Report no: 2015/07) approved the protocol used in this study.

### *Samples*

The material of this study was consist of 105 mares from different races in Nevsehir province of Turkey. Ages of animals were between 3 and 24. Horses were rising for touristic purposes. All animals were clinically healthy. Blood samples were collected between February-April in 2016. Blood samples were collected by jugular vein puncture into vacutainer tubes without anticoagulant and store at 4ºC until arrival at the laboratory. After arriving to the laboratory, blood samples were centrifuged at 5.000 rpm 10 min, subsequently sera were seperated. Serum samples were stored at -20ºC until analysis performed.

### *Serologic Analysis*

Samples were screened for IgG against *A. phagocytophilum* according to the instructions of commercially available IFAT Kit *(Anaplasma phagocytophilum* IFA Equine Antibody kit; Fullerton, California, USA; Cat no: EEE-120) based on *A. phagocytophilum* HGE-1 isolate antigens derived from HL-60 cells. Slide examination was performed using fluorescence microscope at 400-fold magnification.

### *Interpretation of the Results*

All samples tested at a 1:80 as starting dilution in phosphate buffer saline solution (PBS) pH 7.2 according to the manufacturer's protocol. IgG titers 1:80 and greater were considered as positive. Samples were considered positive when bright green flurescence of *A. phagocytophilum* morulae observed at 1:80 and greater IgG titers. Samples were considered negative if no flurescence was seen at 1:80 titer.

### *Statistical Analysis*

The animals were divided into two age groups: animals aged equal and up to 7 years, and more than 7 years. Association between the presence of *A. phagocytophilum* and age of animals is evaluated by 2x2 contingency table and analyzed by using Fisher's Exact test. For statistical analysis, Statistical package IBM SPSS is used.

# RESULTS

Anti- *A. phagocytophilum* IgG antibodies were detected in nine out of 105 (8.57%) horse sera.

The statistical analysis was shown in *Table 1*. N represented the number of animals from two age categories. Presence of *A. phagocytophilum* (in terms of proportion) was given in parenthesis. The results were showed that approximately 9% of all the blood samples of all animals aged equal and up to 7 years were positive for *A. phagocytophilum*. Additionally, this rate seemed similar for the animals aged more than 7 years, i.e., approximately 8%. For $\alpha$=0.05, the result of the Fisher's Exact test *P*-value=0.545 indicated that the difference between the presence of *A. phagocytophilum* infection in respect to horse ages could not reach a statistical significance. In statistical sense, analysis of data revealed that there was not a significant relationship between the age of the horses and the infection of *A. phagocytophilum*.

# DISCUSSION

As previously emphasized before, many studies published for the presence of *A. phagocytophilum* in various animals in Turkey [26,27,29,30]. Best of our knowledge, there is no study about the presence of *A. phagocytophilum* in horses and this will be the first study about this subject in Turkey.

In this study we determined the seroprevalence rate as

***Table 1.*** *Results of the statistical analysis*

| Age | N | Positive n (%) | Negative n (%) | Fisher's Exact Test *P-value* |
|---|---|---|---|---|
| Equal and up to 7 years | 43 | 4 (9.30%) | 39 (90.70%) | 0.545* |
| More than 7 years | 62 | 5 (8.06%) | 57 (91.94%) | |

** P>0.05 = Not significant*

8.57% which is lower than France (11.3%) [33], Guatemela (13%) [34], Sweden (16.6%) [35], Italy (17.03%) [36], Denmark (22.3%) [37], Tunus (16.3-67%) [15,38], USA (17-29%) [39], and Czech Republic (73%) [40], and higher than Korea (2.9%) [41], Sub-Saharan Africa (0%) [42], Taiwan (2.5%) [43], and Japan (3.4%) [44]. Our result was found to be approximately equal with the studies conducted in Italy (8.9-9%) [45,46].

Equine Granulositic Anaplasmosis is usually diagnosed by interpreting the combination clinical signs, results of laboratory tests and epizootic history. Various diagnostic methods can be used to determine the disease according to the course of infection [47]. Since having no argument about the existence of the disease in horses in Turkey, we first had to decide which diagnostic tests provided usefullness by revealing the advantages and disadvantages of them. In solely acute stage of the infection, appearing morula in granulocytes usually 2-4 days after infection can be seen from blood smears taken from the infected animals stained using Wright, Giemsa methods which is highly specific, but have a limited sensitivity was not preffered to inquire *A. phagocytophilum* due to the sampling group of healthy horses in the study [48,49]. In addition to this, lack of sensitivity observed in healthy carrier hosts which have low parasitaemia was also reported [50]. Another diagnostic tool, PCR is an excellent diagnostic tool for the detection of early stage of the infection fastly. PCR becomes positive between 1-21 days post infection, sporadic PCR positivenes can be observed after 21 days, but not for long time [50]. Due to short course of bacteriemia, while IgG antibodies were detectable, *A. phagocytophilum* DNA cannot. This situation correspond to past infection. Both serology and PCR positive results correspond to early infection [50]. Due to having no information about EGA in horses in Turkey, purposeful diagnostic tool for our study in our sampling group consisting of randomly selected healthy horses with no apparent clinical signs was seeking IgG against *A. phagocytophilum* by IFAT. Since it provides an excellent screening method to explore whether the circulation of bacteria exist or not among the horses.

Early production of specific IgG titers occurred during cell mediaed and humoral immun-response can be observed 19 days after the infection date with a peak being reached approximately 8 weeks after the infection [51]. In naturally infected horses, immunity persists for at least 2 years and does not appear to depend on latent infection and carrier status [52]. Nine out of 105 seropositivness with no clinical manifestation determined in the study showed that horses were somehow exposed to tick infestation and *A. phagocytophilum* circulated among horse population. Anaplosmosis is usually seen in Aegean, Black-Sea, Marmara region of Turkey, where humidity and dense vegetation provide good habitats for *Ixodes ricinus (I. ricinus)* [53,54]. On the other hand, the presence of *I. ricinus* was reported on sheep in all regions of Turkey [55,56]. There was also a report from the neighbouring province, Kayseri whom had the similiar geographic conditions to Nevşehir [57]. The authors reported 8% *A. phagocytophilum* rate in a study conducted on dogs, in Kayseri [57]. The Anatolian Plateau (Central Anatolia) is much more subject to extremes than are the coastal areas. Winters on the plateau are especially severe. Because of central Anatolia's geographical conditions, one cannot speak about a general overall climate. Hence, it was thougt that presence of *I. ricinus* was also exist in the Central Anatolia [53].

In this study seropositivness was attributed to sub-clinical EGA where the clinical signs of the disease mild and absent [58]. Persistent subclinical EGA was also hypothesized by Chang et al.[59] in experimental infections.

The severity of the disease varies according to the age of the horse. Horses less than 1 year old exhibit limited clinical signs, those younger than 4 years old and 4 years show mild clinical signs, horses older than 4 years develop characteristic symptoms of disease [60]. However, according to the statistical analysis, no significant relationship between the age of the horses and the infection of *A. phagocytophilum* in the study.

The outcome of the present study provided to obtain a knowledge about the presence of *A. phagocytophilum* circulating on horses, in Nevşehir province of Turkey. Asymptomatic animals may be reservoirs for humans and other animals. From this point of view, we think that; further studies in domestic and wild animals will help us for a better understanding the epidemiology and effective control strategies of the disease.

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