

The seroprevalence of *Anaplasma phagocytophilum* in humans from two different climatic regions of Turkey and its co-seroprevalence rate with *Borrelia burgdorferi**

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Aim: To investigate the seroprevalence of *Anaplasma phagocytophilum* in people living in 2 different climatic regions and to evaluate the co-seroprevalence rate of *A. phagocytophilum* with *Borrelia burgdorferi*. Sinop and Tokat provinces, both in the middle Black Sea region of Turkey, have distinct climatic features.

Materials and methods: In 2006-2007, serum samples were collected from people living in rural areas of Tokat and Sivas, and anti-*A. phagocytophilum* IgG antibodies were explored by the IFA method. Positive samples were further investigated for the possible presence of *B. burgdorferi* IgG antibodies.

Results: *A. phagocytophilum* seropositivity was found in 29 (10.62%) out of 273 serum samples in Sinop and 21 (5.77%) out of 364 serum samples in Tokat (P = 0.035). Co-seroprevalence for *A. phagocytophilum* and *B. burgdorferi* was found to be 3.30% in Sinop and 0.55% in Tokat (P = 0.012).

Conclusion: The current study suggests that *A. phagocytophilum* infections can be seen in humans from different climatic regions of Turkey. Both the seroprevalence of *A. phagocytophilum* and the possibility of mixed infections of *A. phagocytophilum* and *B. burgdorferi* are higher in places with more suitable habitats for *Ixodes ricinus* ticks.

Key words: *Anaplasma phagocytophilum*, human granulocytic ehrlichiosis, *Borrelia burgdorferi*, tick-borne disease, *Ixodes ricinus*, seroprevalence

Türkiye'nin iki farklı iklim bölgesinde yaşayan insanlarda *Anaplasma phagocytophilum* seroprevalansı ve *Borrelia burgdorferi* ile ko-seroprevalans oranı

Amaç: Bu çalışmanın amacı, iki farklı iklimsel bölgede yaşayan insanlarda *Anaplasma phagocytophilum* seroprevalansını araştırmak ve *A. phagocytophilum*'ün *Borrelia burgdorferi* ile ko-seroprevalans oranını ortaya koymaktır. Türkiye'nin Orta Karadeniz bölgesinde yer alan Sinop ve Tokat, farklı iklimsel özelliklere sahip yörelerdir.

Yöntem ve gereç: 2006-2007 yıllarında, Sinop ve Tokat'ın kırsal kesimde yaşayan insanlardan serum örnekleri elde edilmiş ve IFA yöntemiyle anti-*A. phagocytophilum* IgG antikorları araştırılmıştır. Pozitif örnekler, *B. burgdorferi* IgG antikorları yönünden tekrar incelemeye alınmıştır.

Bulgular: Sinop'ta 273 serum örneğinin 29'unda (% 10,62), Tokat'ta ise 364 serum örneğinin 21'inde (% 5,77) *A. phagocytophilum* seropozitifliği saptanmıştır (P = 0,035). *A. phagocytophilum* ve *B. burgdorferi* ko-seroprevalansının, Sinop'ta % 3,30 ve Tokat'ta % 0,55 olduğu saptanmıştır (P = 0,012).

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Sonuç: Çalışma, Türkiye'nin farklı iklim bölgelerinde *A. phagocytophilum* enfeksiyonlarının görülebileceğini ortaya koymaktadır. *I. ricinus* keneleri için daha uygun yaşam alanları olan yerlerde, hem *A. phagocytophilum* seroprevalansı, hem de *B. burgdorferi* ve *A. phagocytophilum* kaynaklı miks-enfeksiyon olasılığı daha yüksektir.

Anahtar sözcükler: *Anaplasma phagocytophilum*, insan granulositik ehrlihyozu, *Borrelia burgdorferi*, kene kökenli hastalıklar, *Ixodes ricinus*, seroprevalans

Introduction

Ehrlichiosis is caused by *Ehrlichia* species, which are tick-borne infectious agents. *Ehrlichia* species are obligate intracellular microorganisms that grow in the leukocytes of their vertebrate hosts. At least 5 *Ehrlichia* species, namely *E. chaffeensis*, *E. sennetsu*, *E. ewingi*, *E. canis*, and *E. phagocytophila* (*Anaplasma phagocytophilum*), are known to infect humans. *Ehrlichia chaffeensis* causes human monocytic ehrlichiosis (HME) and *A. phagocytophilum* causes human granulocytic ehrlichiosis (HGE). Both species of *Ehrlichia* can cause infection in animals such as dogs, sheep, and cattle (1-4).

Epidemiologic features of HGE are similar to those of Lyme disease, and *Ixodes* spp. are the main vectors for both of them (1-4). Co-infection of HGE and *Borrelia burgdorferi* can occur in *Ixodes ricinus* ticks (5-7). Furthermore, *B. burgdorferi* and *A. phagocytophilum* co-infection is seen in animals and humans living in regions in which *I. ricinus* is common (8-11).

HGE cases are reported from Africa, Asia, Europe, and America (1,2). Infection is acquired via bites of infected ticks and has an incubation period of 1-3 weeks. Although the appearance and symptoms of HGE and HME are similar, HGE can cause more severe illness than HME. The clinical appearance of HGE can also be asymptomatic (2-4).

Laboratory findings in patients with HME and HGE include leukopenia, thrombocytopenia, and elevated serum transaminase levels. HGE may be confirmed by culture, examinations of peripheral blood smears, serology, or PCR on an acute-phase blood sample. In Europe, verification of HGE has been based on PCR and the immunofluorescence antibody test (IFA), which is the most common diagnostic technique with a specificity and sensitivity of approximately 90% (1-3,12).

Ixodes ricinus is present in many regions of Turkey. It is especially abundant in the coastal areas of the Black Sea region, where humidity and dense vegetation provide good habitats for both the tick and its hosts (13,14). The goal of this study was to examine the prevalence of antibodies reactive with *A. phagocytophilum* in humans from the rural villages of 2 provinces with different climatic characteristics. In addition, some epidemiologic characteristics were investigated.

Materials and methods

Study area

Sinop Province is located along the coastline of the middle Black Sea region of Turkey. A temperate Mediterranean climate with an average rainfall of 679-1077 mm/m² prevails across the province. Average temperatures are 22 °C in summer and 7 °C in winter. Due to high humidity during all seasons, the province is covered with rich forests and vegetation. Tokat Province is located in the inner area of the middle Black Sea region. The average temperatures are 22 °C in summer and 3 °C in winter. It has a transitional climate, between the temperate Mediterranean and the steppe climate, with dominating continental features. Annual rainfall ranges between 385 and 485 mm/m². Due to the 2 provinces' distinct climatic characteristics, their tick fauna and abundances are different (Figure 1). While Sinop is mostly dominated by *I. ricinus* (13,14), Tokat Province has abundant fauna of ticks like *Hyalomma* spp. Meanwhile, very scattered and low numbers of *Ixodes* spp., *Rhipicephalus* spp., and *Dermacentor* spp. are common for both provinces (15).

Collection of blood samples

The statistical parameters used to calculate the sample size were at a 95% confidence level; error limits were ±5%. The prior estimated rate of

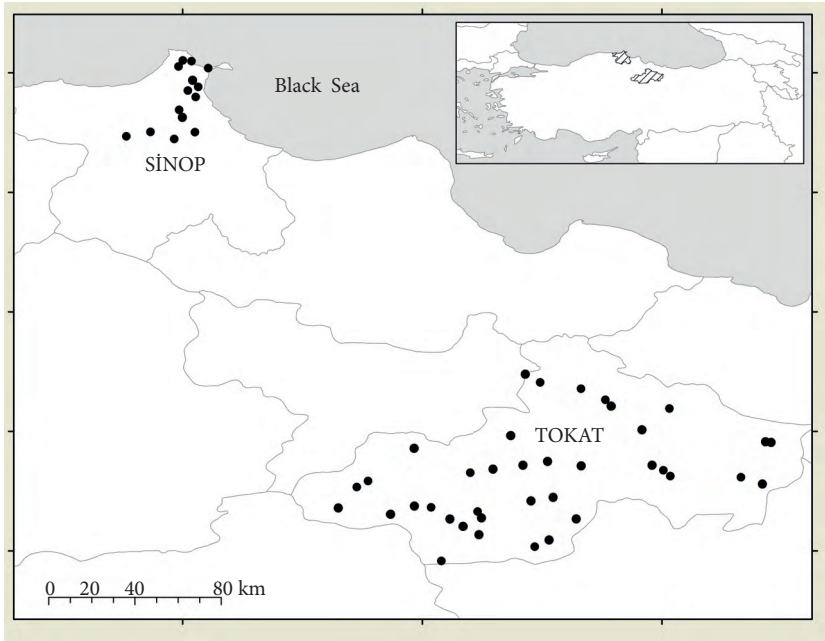


Figure 1. Villages of Sinop and Tokat provinces, Turkey, from which 637 persons were sampled.

seroprevalence for *A. phagocytophilum* was 20%, based on the seroprevalence data from previous studies in Europe (9,10,16-18). The estimated sample size required was calculated as 243 in Sinop and 246 in Tokat, respectively, for populations of 20,000 and 500,000 by using the free software openEpi (<http://www.openepi.com>, version 2.3). During the months of May and June, 2006 and 2007, a total of 637 blood samples were obtained from 273 individuals from 14 villages of Sinop and from 364 individuals from 38 villages of Tokat (Figure 1). Age, gender, and history of tick bites were recorded for each individual. The sera of these blood samples were then separated and kept frozen at -20°C until used.

Serological tests

A commercial IFA kit (Fuller Laboratories, USA) was used to demonstrate the presence of anti-*A. phagocytophilum* IgG antibodies in the collected sera. The test was performed according to the manufacturer's recommendations. At the end of the procedure, slides were covered with a mounting medium and examined under a fluorescence microscope at $400\times$ magnification

(Olympus BX50F, Olympus Optical Co., Japan). As suggested by the manufacturer, the appearance of immunofluorescence at 1/640 of positive controls was used as a cut-off level and titers greater than or equal to 1/80 of test sera, similar to the fluorescent appearance of the positive control, were considered positive for *A. phagocytophilum*-specific IgG (Figure 2).

In order to determine the co-seroprevalence rate of *A. phagocytophilum* with *Borrelia burgdorferi*, all *A. phagocytophilum* seropositive samples were also tested for *B. burgdorferi* IgG antibodies. For this purpose, a commercial ELISA kit (Zeus Scientific, Inc., Netherlands) was used. Considering false *B. burgdorferi* seropositivity, rapid plasma reagin (RPR), rheumatoid factor (RF), and antinuclear antibodies (ANA) were investigated in the positive sera.

Statistic

For categorical variables, P-values were determined by the chi-square test. Statistical analyses were performed with SPSS 17 software (SPSS, Inc., Chicago, IL, USA) and statistical significance was defined as $P \leq 0.05$, 2-tailed.

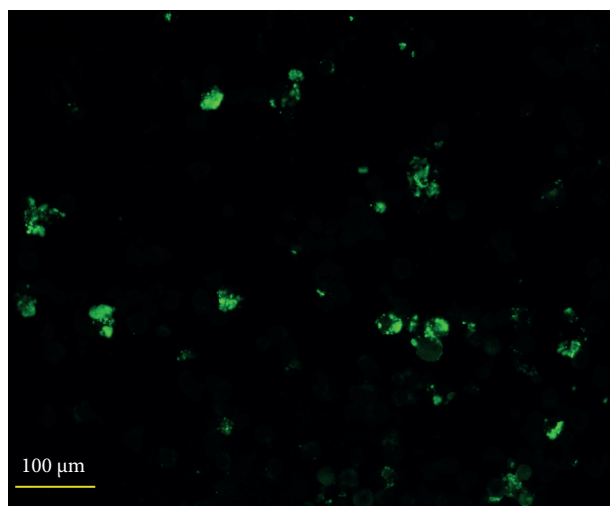


Figure 2. View of *Anaplasma phagocytophilum* antibody by indirect immunofluorescence test (IFA).

Results

The overall mean seroprevalence of *A. phagocytophilum* was found to be 7.85% (50/637) for the overall study population. As shown in the Table, 29 (10.62%) out of 273 individuals from Sinop and 21 (5.77%) out of 364 individuals from Tokat were positive for anti-*A. phagocytophilum* IgG. The seroprevalence difference between the provinces was significant ($P = 0.035$).

Antibodies against *A. phagocytophilum* were observed for females and males as 13.64% and 7.80% in Sinop and 6.22% and 5.35% in Tokat, respectively. For those under and above 40 years of age, seropositivity of *A. phagocytophilum* was found to be 7.14% and 13.61% in Sinop and 6.21% and 5.48% in Tokat, respectively. No statistical difference in seroprevalence was found between genders or between age groups under or above 40 in either region ($P > 0.05$).

In the last 4 years, in Sinop, 12.43% of people with a history of tick bites and 7.69% of people without a history of tick bites were positive for *A. phagocytophilum* IgG ($P = 0.303$). In Tokat, 6.70% of people with a history of tick bites and 4.29% of people without a history of tick bites were positive for *A. phagocytophilum* IgG ($P = 0.466$).

Meanwhile, 11 (22%) out of 50 *A. phagocytophilum* IgG-positive samples were also found to be positive for *B. burgdorferi* IgG antibodies. At the province

level, co-seroprevalence rates were significantly different ($P = 0.012$), being 3.3% (9/273) in Sinop and 0.55% (2/364) in Tokat.

Discussion

The most important factors that determine the prevalence of tick-borne infections in an area are climate and the density of vector ticks. *I. ricinus* ticks are especially abundant in coastal areas of Turkey like Sinop and are rarely encountered in transitional climate areas like Tokat (13,14,19).

The seroprevalence of HGE in humans has been reported from different countries in Europe ranging between 0% and 21% (2,9-11,16,17). Although there is very limited information on the clinical cases for human, in a limited study performed in Turkey, the prevalence of antibodies to *A. phagocytophilum* was found to be 8% in people exposed to tick bites in Antalya (18). In another study done by Kılıç et al. in the Trakya region, *A. phagocytophilum* seropositivity was detected in 25% of people with a history of tick bites (20). In the current study, the prevalence of antibodies reactive against *A. phagocytophilum* was 5.77% in Tokat and 10.62% in rural areas of Sinop, which may likely be caused by the different intensities of *I. ricinus* in the 2 provinces.

Although the findings are different among different countries, prevalence of antibodies to *A. phagocytophilum* in domesticated animals such as dogs, sheep, cattle, and horses are higher than those in people and can reach a level of 40% (8,21,22). In Turkey, the presence of *A. phagocytophilum* infection in domesticated animals has also been demonstrated by molecular and serological methods (23,24).

Depending on the geographic location, the prevalence of *A. phagocytophilum* in *I. ricinus* ticks ranges from 0.4% to 66.7% (2,5,7,25). Aktas et al. found *A. phagocytophilum* DNA in 14.86% of *I. ricinus* samples collected from humans in the Black Sea provinces (Giresun, Rize, and Trabzon) of Turkey in 2007 (26). All studies conducted on both ticks and animals in Turkey (23,24,26) indicate that *A. phagocytophilum* is circulating in the Black Sea region. Our study also supports these findings and highlights the risk of HGE infection by demonstrating the antibody prevalence in humans.

Table. Demographics and seroprevalence of *A. phagocytophilum* in persons living in rural areas of Sinop and Tokat provinces, Turkey.

Characteristic	Persons living in Tokat	Persons living in Sinop	P-value*	Total
Seroprevalence, no. positive/no. tested (%)				
Total	21/364 (5.77)	29/273 (10.62)	0.035	50/637 (7.85)
Age, years				
Mean \pm SD	44.92 \pm 17.48	43.50 \pm 18.28		44.32 \pm 17.83
Seroprevalence by the age of 40, no. positive/no. tested (%)				
n \leq 40	9/145 (6.21)	9/126 (7.14)	0.950	18/271 (6.64)
n > 40	12/219 (5.48)	20/147 (13.61)	0.012	32/366 (8.74)
P-value	0.951	0.126		0.409
Gender, no. (%)				
Female	177 (48.60)	132 (48.4)		309 (48.51)
Male	187 (51.4)	141 (51.6)		328 (51.49)
Seroprevalence by gender, no. positive/no. tested (%)				
Female	11/177 (6.22)	18/132 (13.64)	0.044	29/309 (9.39)
Male	10/187 (5.35)	11/141 (7.80)	0.502	21/328 (6.40)
P-value	0.896	0.172		0.211
Seroprevalence by tick bite, no. positive/no. tested (%)				
Yes	15/224 (6.70)	21/169 (12.43)	0.076	36/393 (9.16)
No	6/140 (4.29)	8/104 (7.69)	0.394	14/244 (5.74)
P-value	0.466	0.303		0.159
Co-seroprevalence, no. positive/no. tested (%)				
<i>A. phagocytophilum</i> and <i>B. burgdorferi</i>	2/364 (0.55)	9/273 (3.30)	0.012	11/637 (1.73)

*Tokat and Sivas were compared with each other.

Due to the fact that *I. ricinus* is the main vector for both *B. burgdorferi* and *A. phagocytophilum*, co-infections are commonly seen in humans and animals (9-11). Sinop is one of the endemic areas in terms of *B. burgdorferi* (14). In the current study, co-seroprevalence rates, which indicate the possibility of co-infections, were significantly different in Sinop (3.30%) and Tokat (0.55%), which can be explained by the different abundances of *I. ricinus* ticks.

In this study, no difference was found in gender and age groups in either Tokat or Sinop ($P > 0.05$). The main reason could be the fact that these groups share similar activities and are exposed to similar rates of tick bites. Although there was a higher level of seroprevalence in those with a history of tick bites, this difference was not statistically significant. The reason could be unnoticed tick bites, e.g. those from

nymphs, which are small and feed for relatively shorter times than adults. Another explanation would be the species of the tick involved. Although infestations with ticks like *Hyalomma* and *Rhipicephalus* are recorded as tick bites, they can hardly be considered as a source of HGE infection.

Our study demonstrates that, although it has been neglected for a long time, *A. phagocytophilum* is present in humans in Turkey and should be considered in clinical cases of unknown nature. The risk of infection in humans is high in coastline areas and becomes gradually lower from the coastline to inner areas of the country. Our study also indicates that, in the case of *A. phagocytophilum* infections, the possibility of co-infections with other tick-borne pathogens like *B. burgdorferi* should also be considered.

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