

Full Length Research Paper

Seroprevalence of human *Anaplasma phagocytophilum* in Bursa Province, Turkey

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The aim of this study was to determine the seroprevalence of *Anaplasma phagocytophilum* infections in rural areas of Bursa Province, where vector ticks and blood-sucking flies are commonly found. In total, 150 blood samples were collected from people living in four different locations where vector ticks are common, and 45 blood samples were collected from people living in non-tick-infested areas. Giemsa-stained blood smears were prepared from the blood samples and examined by light microscopy. The blood samples were also serologically examined using the indirect fluorescent antibody test (IFAT). One sample (0.7%) from a patient with a tick bite history was positive, as determined by the IFAT; however, the same sample was negative when its Giemsa-stained smear was examined. The co-seroprevalence of *A. phagocytophilum* and *Borrelia burgdorferi* was not observed.

Key words: Bursa, *Anaplasma phagocytophilum*, human, serology, prevalence.

INTRODUCTION

Human granulocytic anaplasmosis (HGA) is a zoonotic infection transmitted by ticks to vertebrate hosts. *Anaplasma phagocytophilum* is an obligate intracellular bacterium that is related to rickettsial organisms and that replicates within the hostile environment of neutrophils (Carlyon and Fikrig, 2003). Acute anaplasmosis do not have specific symptoms; its symptoms are flu-like, such as myalgia, headache and tiredness, and can occur similar to viral diseases. Acute anaplasmosis can cause acute renal failure, haemorrhage, and rhabdomyolysis and can cause death in some cases if treatment is delayed, depending on the host's immunological condition (Lotrič-Furlan et al., 2006). The HGA agent infects the cytoplasm of circulating leukocytes, causing intracellular clumps known as morulae, which are most likely to be

found during the first week of illness. The detection test has high specificity but low sensitivity. The presence of morulae in stained blood, bone marrow or cerebro-spinal fluid (CSF) leukocytes is an evidence of the presence of *Anaplasma* infection, but not for specific species, and supports the HGA diagnosis (Baken and Dumler, 2008). The first case of HGA was identified in the United States of America in 1994 from a patient with febrile illness (Chen et al., 1994). Since then the annual HGA incidence has been gradually increasing in the United States and in average, more than 600 cases were reported annually from 2004-2006 by the Centers for Disease Control and Prevention (CDC, Centers for Disease Control and Prevention, 2008). Local cases have also been reported in Europe (Koebel et al., 2012). *Ixodes* spp. ticks are

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primary vectors of this parasite. Previously, the *A. phagocytophilum* parasite was known as the species *Ehrlichia phagocytophila*, *Ehrlichia equi* and *Ehrlichia platys*. This parasite has recently been moved into the genus *Anaplasma* in the reorganised family Anaplasmataceae and unified into a single species (Dumler et al., 2001). Nearly half of the patients with severe HGA require hospitalization with 17% requiring admittance to intensive care (Baken and Dumler, 2008). *Ixodes ricinus* is a common tick, particularly in the northern regions of Turkey, which are rainy and covered with forest areas. In Bursa Province, *I. ricinus* (23.49%) is also the most common tick found on ruminants (Aydin, 2000). Therefore, detecting the seroprevalence of *Anaplasma* infections was the aim of this study.

MATERIALS AND METHODS

Study area

Bursa Province is along the coastline of the South Marmara region of Turkey (Coordinates: 40°11'N 29°03'E/40.183°N 29.067°E). This province has a moderate climate, with an average rainfall of 16.1-109.6 kg/m², the majority of which falls during the winter season. The average temperatures are 23.6°C in summer and 6.2°C in winter. Additionally, the temperature can drop below zero in winter. Mountains cover approximately 35% of the land and are generally in the form of mountain chains running from east to west. The annual temperature, rainfall distribution, average humidity levels and forest-covered areas are suitable for vector ticks (Çalışkan, 2012).

Sampling and parasitology examination

Four villages where *I. ricinus* ticks have been reported previously were selected for this study. These are Osmangazi-Gökçeören, Orhaneli-Koçuköy, Erenler and Keles-Alpagut villages. In total, 150 blood samples were taken from people living in these 4 villages (71 male, 79 female; ages: 15-87). Additionally, 45 blood samples were collected from people living in areas without the risk of tick infestation (39 male, 6 female; ages: 20-59). The participants were surveyed to identify risk factors before blood samples were collected. Age, gender and a history of tick bites were recorded for each individual. Serum samples were kept at -20°C in a freezer. The blood samples were collected in EDTA containing tubes and were used to prepare the blood smears. The blood smears were stained with Giemsa stain and examined under a light microscope (1000× magnification) for the presence of morulae. An indirect fluorescent-antibody (IFA) method was used for the detection of anti-*A. phagocytophilum* IgG antibodies employing a commercial IFA kit (Human *A. phagocytophila* IFA Antibody Kit; Fuller Laboratories, France). Seropositive samples were examined with *B. burgdorferi* IgG Antibody ELISA kit (Immuno laboratories *B. burgdorferi* IgG Antibody ELISA, USA) kit to determine the presence of *B. burgdorferi* IgG antibodies. The tests were performed in the usual manner according to the manufacturer's instructions.

Statistical analysis

Statistical analysis of the published data were performed using

Pearson's Chi-square and Fisher's exact tests. The necessary permissions were obtained from the establishment, and informed consent was obtained from each volunteer before participation in the study.

RESULTS

There were no significant differences between gender or age categories for rates of seroprevalance. As shown in Table 1, one sample (0.7%) (67-year-old male) in the IFAT analysis was positive for *A. phagocytophilum* IgG antibody. When the surveys were checked, this person had a tick bite history three years ago. *A. phagocytophilum* and *B. burgdorferi* co-seroprevalance was not observed (Table 1).

DISCUSSION

Around the world in recent years, the use of more effective surveillance programs, the development of new tests for diagnosis, increasing medical importance and tick-borne infections due to environmental changes have increasingly occurred. Additionally, an increase in the number of tick-borne infection cases has been observed. Ticks are one of the most important vectors that transmit many diseases to human and animals worldwide. Although many studies regarding anaplasmosis have been performed worldwide, the number of these studies are increasing since the 2000s (Stuen, 2003; Gokce et al., 2013; Altay et al., 2014; Aktas et al. 2010; Sen et al., 2011). In these studies, the presence of *Anaplasma* was determined from samples taken from both animals and ticks by serological and molecular methods. Depending on a region's geographic structure, climate and vegetation characteristics, the seroprevalance of tick bites among people varied between 8-25% (Gunes et al., 2011; Kilic et al., 2010; Ongut et al., 2006). In another study performed on people with a tick bite history, the seroprevalance of *A. phagocytophilum* was 8%, and the *I. ricinus* tick population was extremely high, at 80.4% (Ongut et al., 2006). In the Thrace region of Turkey, which is a neighbouring area that has similar environmental conditions to that of Bursa, *A. phagocytophilum* infection rate was 17.5% in *I. ricinus* adult ticks in 2008 (Sen et al., 2011). In another study, Göral et al. (1997) found that the seroprevalance of *A. phagocytophilum* and *B. burgdorferi* was 35.8% in the forest villages where the *I. ricinus* tick population was extremely high. In all geographical regions of Turkey, more than 30 tick species have been identified in six genera (*Rhipicephalus*, *Dermacentor*, *Hyalomma*, *Haemaphysalis*, *Argas* and *Ornithodoros*) in cattle, sheep and goats (Dumanli, 2012). Because the locations chosen for the study represented the province's meteorological aspect, most of the population makes a living from agriculture and forestry. The high seroprevalance of *B. burgdorferi*, which is transmitted by the same

Table 1. Demographics and seroprevalence of *A. phagocytophilum* in persons living in rural areas of Bursa province, Turkey.

Characteristics	Total
Seroprevalence, no. positive/no. tested (%)	
Total	1/ 150 (0.66)
Age, years	
Mean.±SD	54.8 ± 16.4
Seroprevalence by the age of 40, no. positive/no. tested (%)	
n≤40	0/30 (0.0)
n>40	1/120 (0.83)
P- value	1.000
Gender, no. (%)	
Male	71 (47.3)
Female	79 (52.7)
Seroprevalence by gender, no. positive/no. tested (%)	
Male	1/71 (1.4)
Female	0/79 (0.0)
P- value	0.475
Seroprevalence by tick bite, no. positive/no. tested (%)	
Yes	1/36 (2.8)
No	0/114 (0.0)
P- value	0.240
Co- seroprevalence. no. positive/no. tested (%)	
<i>A. phagocytophilum</i> and <i>B. burgdorferi</i>	0/150 (0.0)

vector ticks, and the rate of another tick-borne infection, tularaemia, which is found at the highest levels in this region, suggest that *Anaplasma* can affect people in the Bursa region (Helvacı et al., 2000). In this study, we aimed to determine the seropositivity of *Anaplasma* infections, not considering whether the participants have tick bite histories, and to determine the risk of *Anaplasma* infections. According to the 0.7% seropositivity, it was discovered that the inexistence of acute anaplasmosis reports in our region and country, lacked some levels of diagnosis in some cases. Only approximately 24% of the volunteers actually reported a tick bite; however, we noted that the serologically positive sample originated from a volunteer had a tick bite. The HGA infection level has risen since 1999 when HGA was added to the USA list of diseases that should be considered. In 2008, this level was 21% higher than in 2007 (Hall-Baker et al., 2010). The average HGA seroprevalence in Europe is 6.2%, and this level is as high as 28%, in some countries (Bakken and Dumler, 1994). *A. phagocytophilum* has an enzootic cycle that includes *Ixodes* ticks and mammalian

animals as reservoirs. HGA is transmitted by *Ixodes scapularis* in the eastern USA and by *Ixodes pacificus* in the northern USA. In Asia, the Ural Mountains, Siberia, the Far East and Baltic Russia, HGA is transmitted by *Ixodes persulcatus* (Woldehiwet, 2010). The vector in Europe is *I. ricinus* (Petrowec et al., 2002; Wittesjö et al., 2001). Christova et al. found ehrlichiosis agents in *Hyalomma*, *Rhipicephalus* and *Dermacentor* ticks collected from the cities of Antalya, Kayseri and Malatya, Turkey by using PCR and reverse line blot techniques. Although these authors did not provide the ratio for *A. phagocytophilum*, they reported 3% seropositivity in *I. ricinus* ticks and 22.2% seropositivity in *I. ricinus* nymphs (Christova et al., 2003). Ongut et al. (2006) found 8% seropositivity in Antalya, Sen et al. (2011) found 25% seropositivity in Thrace, and Gunes et al. (2011) found 10.62% seropositivity in Sinop and 5.77% seropositivity in Tokat. These results are sufficient evidence that diagnostic laboratories should have routine tests for diagnosing HGA (Kilic et al., 2010). The success in finding morulae varies in accordance with the experience

of the microscopist and with the duration of the illness (Bakken et al., 2002). Morulae have been detected in only three patients in Europe (Koebel et al., 2012). In this study, morulae were not visualised in the peripheral smear. Because they are transmitted by the same vector (*I. ricinus*) and have the same epidemiologic features, *A. phagocytophilum* and *B. burgdorferi* co-infections are often encountered in humans and animals (Koebel et al., 2012). Güneş and his colleagues reported that the co-prevalence rates were 3.3 and 0.55% with respect to *B. burgdorferi* in two different climatic regions, that is, Sinop and Tokat, respectively (Gunes et al., 2011). In our study, the co-prevalence rate was 0%. This could be attributed to unnoticed tick bites, e.g., bites from nymphs, which are small and feed for relatively shorter times than adults. Although infestations with ticks such as *Hyalomma spp.* and *Rhipicephalus spp.* are recorded as tick bites, these infestations can hardly be considered a source of HGE infection.

Conclusion

In Turkey, there are a limited number of human studies that have focused on this interaction. There is a need for a large-scale population studies especially on tickborne HGA, *Rickettsial* and Lyme diseases. Future studies on tick-borne diseases should focus more on aspects related to vector and this will be an important component in the study of epidemiology.

Conflict of interests

The authors did not declare any conflict of interest.

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