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Short Report: Seroprevalence of Human Granulocytotropic Anaplasmosis in Central and Southeastern China

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Abstract. Human granulocytic anaplasmosis (HGA) is an emerging tick-borne infectious disease. To determine the prevalence of HGA in central and southeastern China, a total of 323 human sera were collected from individuals at high risk for exposure to ticks and animals. The IgG antibody against the etiologic agent of HGA, *Anaplasma phagocytophilum* was detected with indirect immunofluorescence assay. The results showed that 20% of the tested individuals (64/323) were positive to *A. phagocytophilum* and that the incidence was higher in male (22%) than female (16%). We concluded that *A. phagocytophilum* infection was prevalent in central and southeastern China.

INTRODUCTION

Human granulocytic anaplasmosis (HGA) is a tick-borne disease caused by an obligatory intracellular bacterium *Anaplasma phagocytophilum*. It was previously known as human granulocytic ehrlichiosis (HGE). The HGA was first identified in the United States of America in 1994 from a patient with febrile illness.[1] Since then the annual incidence of the disease gradually increased in the United States and in average more than 600 cases were reported annually from 2004 to 2006 by the Centers for Disease Control and Prevention (CDC).[2] The disease has also been reported in Europe.[3] *Anaplasma phagocytophilum* DNA has been detected from ticks in Israel, Japan, and China.[4–6] The HGA was first reported in China in 2008 and the first HGA outbreak in China was noteworthy because it caused nosocomial infection.[7] Because HGA only emerged recently in China, the pathogen of HGA has not yet been isolated and the epidemic data is incomplete. To determine the epidemiology of HGA in China, we studied the seroprevalence of HGA in risk populations from two areas in China, one forestry mountain area in southeastern China and one hilly area in central China. These areas were chosen because they are endemic for Lyme disease, and the pathogens of Lyme and HGA share the same tick vectors in the United States and Europe.[8,9]

MATERIALS AND METHODS

Geographic characteristics of sample collection sites. Two areas that had been reported for Lyme disease were selected: Wuyi Mountain area including Guangzhe and Jianyang counties that are located in Fujian Province of southeastern China; and a hilly area including Jiaocheng, Yicheng, Quwo, and Yuncheng counties that are located in Shanxi Province of central China (Figure 1). Both areas have a mild climate where several species of tick have been identified. Tick species identified in Shanxi including *Ixodes persulcatus, Haemaphysalis concinna, H. japonica, H. campanulata, H. longicornis, H. verticalis, Dermacentor reticulatus, D. silvarum, D. sinicus, Hyalomma ditritum, Rhipicephalus sanguineus,* and *Boophilus microplus* and tick species identified in Fujian including *Ornithodoros papillipes, Haemaphysalis yeni, H. formosensis, H. longicornis, Ixodes granulatus,* and *B. microplus*.[10–13]

Sample collection. Three hundred three human sera were collected from May to July in 2007 from forestry workers, shepherds, or hunters that were at high risk for exposure to ticks. The other 20 serum samples collected from March to August in 2006 were combined with the 2007 samples for testing of HGA antibody. A 5 mL sample of blood was collected from each individual by venipuncture. The serum samples were obtained after centrifugation of whole blood and were stored at –20°C until use. Epidemiologic data were collected for each sampled individual including age, gender, job type, living place, medical history, and history of animal contact and tick bite.

Indirect immunofluorescence assay (IFA). An IFA kit from Focus Diagnostics (Cypress, CA) was used to detect human serum IgG antibodies against *A. phagocytophilum* in collected samples following the protocols of the manufacturer. A serum titer of ≥ 1:64 was considered as positive to *A. phagocytophilum* according to the definition of the manufacturer.

RESULTS AND DISCUSSION

To evaluate the prevalence of *A. phagocytophilum* infection among population in regions where cases of Lyme disease had been reported,[14,15] we performed IFA tests on sera from individuals at high risk for exposure to ticks in the Fujian

TABLE 1

Results of *Anaplasma phagocytophilum* IgG antibody in human serum samples from Shanxi and Fujian

Location	Sera tested	Serum antibody titers				Seroprevalence
		1:64	1:128	1:256	1:512	
Fujian Province						
Guangzhe County	105	8	9	3	0	19%
Jianyang County	116	7	7	4	1	16%
Shanxi Province						
Yuncheng County	27	0	4	2	1	26%
Yuncheng County	20	4	1	1	0	30%
Yicheng County	31	4	2	1	0	23%
Quwo County	24	3	1	1	0	21%
Total	323	26	24	12	2	20%

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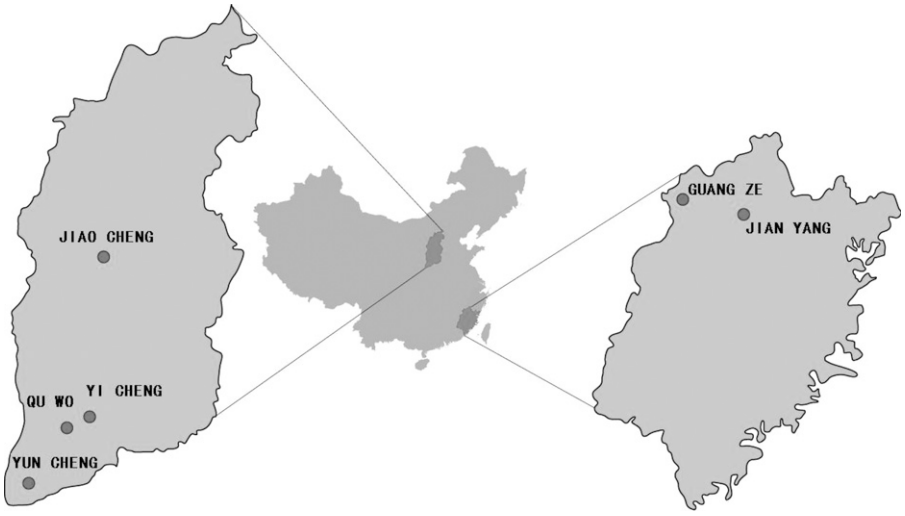

FIGURE 1. The areas where the serum samples were collected.

and Shanxi provinces. Sixty-four of 323 sera (20%) were found positive to *A. phagocytophilum*. The *A. phagocytophilum* seroprevalence rate in samples from Fujian and Shanxi were 18% (39 out of 221) and 25% (25 out of 102), respectively (Table 1). Comparison of *A. phagocytophilum* seroprevalence by age group revealed the highest incidence of *A. phagocytophilum* infection in the age group of 41 to 60 years (21%), followed by the age group of 22 to 40 years (17%), and the age group of over 60 years (18%, Table 2). *Anaplasma phagocytophilum* seroprevalence rate was higher in male (22%) than in female (16%, Table 3).

Little is known about the epidemiology of human infection caused by *A. phagocytophilum* in China. Polymerase chain reaction (PCR) detection of 16S rRNA gene showed that *A. phagocytophilum* existed in ticks and rodents from northern China.[6,16] Very recently a death case was confirmed as a result of *A. phagocytophilum* infection. This case also caused a cluster of 9 cases of nosocomial infection among health care workers and family members after exposure to the index patient.[7] Our serologic survey revealed a seroprevalence rate of 20% for *A. phagocytophilum*, indicating the existence of infection by *A. phagocytophilum* with a relatively high frequency among population at high risk for exposure to ticks in central and southeastern China. The seroprevalence rates in our study were higher than those reported in a recent study from North China.[17] This may be explained by the fact that we studied serum samples from high-risk population, which may not reflect that of the general population of the same country. Nevertheless, these studies indicated the urgency and necessity for large-scale serologic survey of high-risk population for *A. phagocytophilum* infection in China. Study of HGA was initiated only a few years ago and HGA is still not recognized by most doctors in China. The first HGA outbreak in China was mistakenly diagnosed as hemorrhagic fever. It is most likely that in the past and even right now the HGA cases in China were mistaken as hemorrhagic fever or other viral infections because clinical manifestations and laboratory test results of HGA are similar to viral infections. However, the treatment of HGA is very different from viral infection because HGA is sensitive to tetracycline. Thus, serologic studies of *A. phagocytophilum* infection among high-risk populations would provide valuable insights for developing public health strategies to prevent and control HGA. We hope this study can serve as an indicator to our clinicians that *A. phagocytophilum* infection was present among the population in the area studied. Patients in the endemic areas who present with nonspecific febrile illness after exposure to ticks should be evaluated for HGA.

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TABLE 2
Distribution of *Anaplasma phagocytophilum* IgG antibody in serum samples among different age groups

Age group	Sera tested	Serum antibody titers 1:64	1:128	1:256	1:512	Seroprevalence
22–40	64	4	4	2	1	17%
41–60	183	19	13	7	0	21%
> 60	76	3	7	3	1	18%
Total	323	26	24	12	2	20%

TABLE 3
Distribution of *Anaplasma phagocytophilum* IgG antibody in serum samples among different sexes

Sex	Sera tested	Serum antibody titers 1:64	1:128	1:256	1:512	Seroprevalence
Male	222	18	19	10	1	22%
Female	101	8	5	2	1	16%
Total	323	26	24	12	2	20%

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