



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: www.elsevier.com/locate/apjtm

Document heading doi:

Investigation of anaplasmosis in Yiyuan County, Shandong Province, China

Lijuan Zhang^{1*}, Feng Cui², Lingling Wang², Lingling Zhang², Jingshan Zhang¹, Shiwen Wang¹, Shuxia Yang²

¹Department of Rickettsiology and Anaplasmosis, National Institute of Communicable Disease Control and Prevention, China CDC, Beijing 102206, China

²Zibo Center for Disease Control & Prevention, Zibo 255026, Shandong, China

ARTICLE INFO

Article history:

Received 11 February 2011

Received in revised form 11 April 2011

Accepted 15 June 2011

Available online 20 July 2011

Keywords:

Anaplasma phagocytophilum

Field investigation

Rickettsia

Prevalence

Yiyuan County

Shandong Province

China

ABSTRACT

Objective: To investigate the situation of anaplasmosis in Yiyuan county, Shandong Province. **Methods:** A total of 26 blood samples from febrile patients suspected of anaplasmosis, 48 blood samples from healthy farmers, 8 from dogs, and 10 from goats and 170 ticks were collected in the same area during 2005–2007, and detected by serological and molecular methods. **Results:** Eight confirmed cases and 6 probable cases were determined using serologic and molecular methods. The seroprevalence of *Anaplasma phagocytophilum* (*A. phagocytophilum*) was 26.7% in healthy cases. Nine out of 10 sheep samples and 7 out of 8 dog samples reacted positively to the *A. phagocytophilum* antigen. PCR amplification and sequencing of the 16SrRNA of *A. phagocytophilum* gene showed that some samples from patients, goats and ticks were 100% identical. The seroprevalence of *Rickettsia typhi* was 22.9%, *Orientia tsutsugamushi* 6.3%, *Rickettsia sibirica* 27.1%, *Coxiella burnetii* 18.8%, *Bartonella henselae* 31.3%, and *Borrelia burgdorferi* 41.6%. **Conclusions:** It is important to make differential diagnosis of febrile patients and to apply treatment with specific antibiotics. It is needed to enforce essential prevention and control measures including tick control and to improve sanitation conditions.

1. Introduction

Human granulocytic anaplasmosis (HGA) and human monocytic ehrlichiosis (HME) are acute and febrile tick-borne rickettsial diseases caused by the bacterium *Anaplasma phagocytophilum* (*A. phagocytophilum*) and *Ehrlichia chaffeensis* (*E. chaffeensis*), respectively[1,2]. As other rickettsioses, the prevalence of these diseases are found worldwide, but mostly in the USA and Europe[3–5]. Recently, serologic and molecular evidences of these diseases had been reported in some Asian countries,

including Korea, Japan and China[6–12]. In 2006, an unusual nosocomial human-to-human transmission was demonstrated by PCR and seroconversion in Anhui Province, China[13]. As a result of these events, a general retrospective laboratory survey of suspected HGA cases collected from 2004–2006 in Jiangsu, Zhejiang, Shandong and Hubei Provinces was undertaken by the Department of Rickettsiology of China and local health centers during 2006–2007. In this report, we outlined the findings from Yiyuan County of Shandong Province, China.

Yiyuan is one of the poorest counties in Shandong Province. It is reported that incidence of unknown endemic febrile (>38 °C) has increased from March to October every year[14]. Typical clinical characteristics of these cases include high fever, headaches, myalgias, hematological and biochemical abnormalities, leucopenia, thrombocytopenia, mild/moderately elevated hepatic transaminase levels, and (in some cases) high rates of urinary protein. Some of these patients ultimately die due to multi-organ failure. Laboratory tests of Dengue fever, scrub typhus, Lyme disease and epidemic hemorrhagic fever were performed

*Corresponding author: Lijuan Zhang, Department of Rickettsiology, National Institute of Communicable Disease Control and Prevention, China CDC, PO Box 5, Changping, Beijing 102206, China.

Tel: +86 23 58900780

Fax: 86 23 58900780

E-mail: zhanglijuan@icdc.cn

Foundation project: This study was supported by the National Basic Research Program of China (973 Program) 2010CB530200(2010CB530206); the National Natural Science Foundation of China (No. 30771854), National Key Science and Technology Projects of China (Project No. 2008ZX10004–008).

for each patient, but no positive result was obtained. Based on field epidemiology surveys, the occurrence of the disease was associated with poor sanitation of the patient's living room (*OR*: 12.000, 95%*CI*: 1.990–72.354, *P*<0.05), co-habitation with sheep and other domestic animals (*OR*: 6.400, 95%*CI*: 1.944–21.072, *P*<0.05), untreated open wounds (*OR*: 6.333, 95%*CI*: 1.501–26.732, *P*<0.05), wild grass around the patient's house (*OR*: 5.909, 95%*CI*: 1.690–20.660, *P*<0.05), and working on a field for more than one hour per day (*OR*: 5.250, 95%*CI*: 1.407–19.593, *P*<0.05)[11]. Local epidemiologists have suggested that the disease is a type of tick-related natural foci disease, because ticks are the most abundant disease vectors in domestic and wild animals in these areas. Hence, we first evaluated emerging tick-borne rickettsial diseases based on clinical features, particularly with *A. phagocytophilum* and *E. chaffeensis*. In this survey, we retrospectively investigated 26 blood samples from suspected cases collected from 2004–2006. We also evaluated blood samples from 48 healthy controls and predominant domestic animals (including 10 goats and 8 dogs) using serologic methods. In addition, tick samples from the same areas where the suspected patients lived were identified by molecular methods.

2. Materials and methods

2.1. Study area and populations

The study was conducted in 633 administrative hamlets, 33 administrative villages of 11 towns in Yiyuan County, China. These were all located in the mountainous areas of Yimeng (117°54′–118°31′E and 35°55′–36°23′N) and had a total population of 555 000, among which farmers accounted for 85%. Foothills with heavy wild grass, low, middle, and high upland were the most prevalent geographic features. The area is seasonal, with an average temperature of 11.9 °C, and average annual rainfall of 720.8 mm. Besides planting crops, most common human occupations are livestock-raising activities (predominantly by women) and active outdoor activities such as fishing and fruit tree farming (predominantly by men). Since many younger had moved to nearby urban areas, the major in families are older people (>50 years old). Goats (Boer sheep imported from America, and native black goats) farming plays an important role in each family, and pen-breeding in courtyards is the most common method. *Haemaphysalis longicornis* (*H. longicornis*) and *Haemaphysalis concinna* (*H. concinna*) ticks are predominant species in these areas[15] and actively grow from April to October. Higher density of these ticks are found in grass on hill areas. Ticks are common external parasites of goats and dogs from these areas.

2.2. Sample collection

We enrolled 26 un-confirmed febrile patients and 48 non-

infected healthy controls from the same area. Informed consent was obtained before data collection. Approval for the study was obtained from the Shandong provincial institutional review board. Patients' information was obtained, including name, age, gender, occupation, and history of tick bite/exposure. The mean age of the suspected cases was 59 years old, and all were farmers, including 10 male (mean age 60 years old) and 16 female (mean age 59 years old). They all had high fever (>38 °C), leucopenia, thrombocytopenia, and mild/moderately elevated hepatic transaminase levels; 65% had headache and myalgias, 20% had localized enlarged lymph nodes with lymphangitis and digestive system involvement, 5% had eye socket ache, and 5% presented a rash. Two patients died of multi-organ failure. The mean age of 48 was 50 years old, including 26 male (mean age = 52 years old) and 22 female (mean age =48 years old), and 82% were farmers.

Blood (5 mL) was collected from each suspected patient at the acute stage of illness; a second samples were collected from 14 patients at convalescent phase of illness (3 or 4 weeks after onset of illness). Separated serum was used for indirect immunofluorescence assays and DNA was extracted from red blood cells for PCR amplification. Blood (5 mL) from controls was collected to detect IgG-specific antibody titers of *A. phagocytophilum* and *E. chaffeensis*. All samples were temporally stored at –20 °C.

2.3. Diagnosis standard

A positive diagnosis was made if any one of the following conditions was met: 1) typical epidemiologic history of tick exposure/bite, or outdoor activity; 2) clinical manifestation of tick-borne rickettsioses, such as high fever (>38 °C), headache, myalgias, localized enlarged lymph nodes, or lymphangitis; 3) laboratory-based diagnosis of leucopenia, thrombocytopenia, or mild/moderately elevated hepatic transaminase levels.

2.4. Collection of ticks from goats and dogs

One hundred and fifty ticks from goats and 20 ticks from dogs were obtained in different farmer families from different villages. A total of 34 groups of ticks (5 ticks from the same animal as a group) were homogenized and their DNA was extracted for PCR amplification. Local slaughterhouses were selected for collecting animal blood, where 10 goat blood samples and 8 dog blood samples were obtained for serologic and molecular analyses.

2.5. Serologic testing

All serum samples were tested for IgM and IgG antibody against to *A. phagocytophilum* and *E. chaffeensis*, and detected using indirect immunofluorescence assays[16]. Antigen slices of *A. phagocytophilum* (Webster strain), *E.*

chaffeensis (Arkansas strain) and *Borrelia burgdorferi* (*B. burgdorferi*) were prepared at Johns Hopkins University School of Medicine (Baltimore, USA). Antigens for *Rickettsia typhi* (*R. typhi*), Karp, Kato and Gilliam *Orientia tsutsugamushi* (*O. tsutsugamushi*), *Rickettsia sibirica* (*R. sibirica*), *Coxiella burnetii* (*C. burnetii*) and *Bartonella henselae* (*B. henselae*) were prepared by our laboratory. Antibody titers of 1 : 80 (or 1 : 128 for *B. burgdorferi*) were used as IgG cutoff values and 1 : 40 as IgM cutoff values.

2.6. PCR amplification, sequencing, and phylogenetic tree analysis

DNA was extracted using the QIAamp Tissue Kit (Qiagen, Germany) according to manufacturer's manual. DNA extracted from the blood of healthy controls was used as a negative control, while DNA from *A. phagocytophilum* and *E. chaffeensis* were used as positive controls. These extracts were used as templates to amplify the 16SrRNA gene of genus-common *Anaplasma* and *Ehrlichia* by nested PCR^[11]. Primers were made by Shanghai Sangon Biological Engineering Technology Co, Ltd. In the first amplification round, the *Anaplasma* and *Ehrlichia* genus-common external primers out1 (5'-TTG AGA GTT TGA TCC TGG CTC AGA ACG-3') and out2 (5'-CAC CTC TAC ACT AGG AAT TCC GCT ATC-3') were used. This amplification yielded a 653-bp fragment. The second round amplification used three sets of inner primers respectively. The first general primers Eh-gs1 (5'-GTA ATA CT GTA TAATCC CTG-3') and Eh-gs2 (5'-GTA CCG TCA TTA TCT TCC CTA-3') were used to detect *A. phagocytophilum* and *E. chaffeensis*. This amplification yielded a 282-bp fragment. The second *A. phagocytophilum* species-specific rrs primers HGA1 (5'-GTC GAA CGG ATT ATT CTT TAT AGC TTG -3') and HGA2 (5'-TAT AGG TAC CGT CATTAT CTT CCC TAC-3') were used to amplify *A. phagocytophilum*. This amplification yielded a 389-bp fragment. The last set of primers - HME1 (5'-CAA TTG CTT ATA ACCTTT TGG TTA TAA AT-3') and HME2 (5'-TAT AGG TAC CGT CAT TAT CTT CCCTAT-3') - were used to amplify *E. chaffeensis*. This amplification yielded a 389-bp fragment. In addition, we performed another nested PCR incorporating the *groEL*-amplifying primer sets HS1-HS2 and HS43-HS45 for nested amplification of *A. phagocytophilum* and *E. chaffeensis*, as previously described^[4]. To identify and compare *A. phagocytophilum* and *E. chaffeensis* in human and animal specimens, nucleotide sequences of the nested PCR-amplified products were determined by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd and compared with sequences obtained from the GenBank database using the BLASTn program (National Center for Biotechnology Information, Bethesda, MD, USA). The phylogenetic tree analysis was made by software MEGA4.0.

3. Results

3.1. Serum analysis

A total of 14 patients had collection of blood during the convalescence phase of illness, and 6 of these showed seroconversion to *A. phagocytophilum*. Four patients had IgM-positive serologic results of *A. phagocytophilum*. Of the 6 patients with seroconversion, 1 had IgG-positive serologic results of *E. chaffeensis*. 26.7% of control cases had *A. phagocytophilum*-positive results and 1.8% had *E. chaffeensis*-positive results. Besides, 41.6% were positive to *B. burgdorferi*, 31.3% to *B. henselae*, 27.1% to *R. sibirica*, 22.9% to *R. typhi*, 18.8% to *C. burnetii*, and 6.3% to *O. tsutsugamushi*. Co-infection of *R. sibirica* and *B. burgdorferi* was 18.8%; while co-infection to *A. phagocytophilum* and *B. burgdorferi* was 16.6%.

Out of 10 blood samples collected from goats (8 male and 2 female at 1 to 2 years old), 9 reacted positively for *A. phagocytophilum*, but none reacted positively to *E. chaffeensis*. Seven out of 8 dog blood samples (6 male and 2 female at 1 to 5 years old,) tested positive to *A. phagocytophilum*, and all samples were tested positive to *E. chaffeensis*.

3.2. Identification of ticks

Classification of ticks from dogs and sheep showed that the dominant species were *H. longicornis* and *H. concinna*. The total positive rates of *A. phagocytophilum* were 17.6%, but no PCR-positive results of *E. chaffeensis* were observed.

3.3. PCR amplifying and sequencing

Nested PCR using general 16SrRNA primers identified the *A. phagocytophilum* DNA in 5 patients during the acute stage of illness. The sequencing results from 1 patient (251bp EU982709) showed 100% similarity to *A. phagocytophilum* identified from goat blood in the study (EU 982707), and 100% similarity to HGA nosocomial transmission patients in Anhui Province (China) (EF211110-EF211117, AY176588), in Europe (AY281788), and the USA (DQ088128). The remaining PCR products from the other four patients were not successfully sequenced. Of the 10 blood samples from goats, 7 showed expected bands of *A. phagocytophilum*, 5 of which were sequenced successfully and sequence analysis showed that there are 2 types of variation strains. One showed 100% similarity to *A. phagocytophilum*, detected from patients mentioned above (EU982709), and the other showed 100% similarity to *A. phagocytophilum* detected in South Korea, Japan and Chongqing, China (EU 409558, AB211163 and FJ389577). Comparisons between these 2 strains from goats in the study showed 96.8% similarity. For 8 dog blood samples, there were no positive results of *A. phagocytophilum*, but a faint band of *E. chaffeensis* was observed with PCR

amplification using species-specific 16SrRNA primers. Of the 34 groups tick samples (30 were collected from goats and 4 from dogs), 6 gave positive results, 2 (collected from goats) showed 100% similarity to *A. phagocytophilum* isolated from Chongqing (China), France, and Japan (FJ 389576, EU857675 and AB211164); the remaining 4 (3 collected from goats and 1 from dogs) showed 100% similarity to *A. phagocytophilum* isolated from goat strains (EU982704–EU982706, EU982708) in the study. For PCR amplification of *groEL* genes, only 1 tick sample with 16SrRNA gene-positive results showed 100% similarity to *A. phagocytophilum* isolated from patients in Anhui Province (China; EF47320108–EF473209) and those isolated from northern areas of the United States (DQ 680012) (Figure 1).

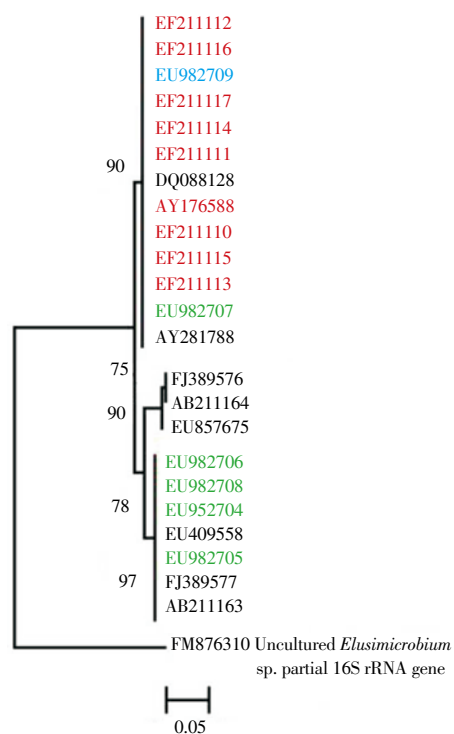


Figure 1. Phylogenetic tree resulting from analysis of nucleotide sequences of 16rRNA

Red: sequences of patients from Anhui Province 2006; Blue: sequences of patients in the study; Green: sequences of goats in the study; Black: other sequences from GenBank.

4. Discussion

Of the 26 febrile patients in this study, 8 were confirmed cases (six cases of seroconversion and 2 positive by PCR and IgG antibody) and 6 were probable patients (positive to single serum IgG antibody) according to the US CDC laboratory criteria for the diagnosis of HGA[17]. Although there was no laboratory evidence of HGA in the remaining 12 patients, we considered these as probable cases due to their typical clinical and epidemiologic features.

The average serological prevalence rates of HGA in local healthy control was 26.7%, which is substantially higher than that of farmers in Tianjin areas[18], but similar to

that reported from endemic areas in the USA[19,20]. High seroprevalence of *A. phagocytophilum* in goats (9/10) and dogs (7/9) was observed in this survey. All of 8 serum samples from dogs reacted positively to *E. chaffeensis*, while no positive results were obtained from goats samples. Based on the serologic and molecular evidence of this study, we suggest that domestic dogs and goats (Boer sheep imported from America, and native black goats) are commonly infected with *A. phagocytophilum*. Although a higher seroprevalence of *E. chaffeensis* exists in domestic dogs, no PCR-positive results of *E. chaffeensis* was found from patients' samples, tick samples, or goat samples—except one uncertain PCR product of *E. chaffeensis* from one dog sample. The main vectors for *A. phagocytophilum* are the worldwide distribution of *Ixodes* ticks, ie, *Ixodes scapularis*, *Ixodes pacificus*, *Ixodes ricinus* and *Ixodes persulcatus*. *H. longicornis* and *H. concinna*, which are found nationwide in China, were the predominant vectors in the study areas, with *A. phagocytophilum*-positive rates of 17.6% (6/34 tick samples). 16SrRNA sequences from tick samples showed 100% similarity to those from goats and febrile patients in the study. This suggests that *H. longicornis* and *H. concinna* might be the main vectors for *A. phagocytophilum* in these areas. Previous research indicated that *H. longicornis* and *H. concinna* are main vectors that are associated with some tick-borne diseases, including spotted fever and Lyme disease in China[21,22]. Moreover, *A. phagocytophilum* nucleic acids from *H. concinna* collected from northern areas in China were also identified[23]. Serologic and molecular evidence of HGA has been previously reported in many parts of China[24–26]. The serologic and molecular evidence from this study suggests that anaplasma infections in humans and domestic animals are common. However, the small sample size in this study makes it necessary for further investigation. The high seroprevalence of *A. phagocytophilum* implies that many cases were previously misdiagnosed. Serologic evidence also suggests that other tick-borne diseases co-existed in these regions. We suggest that further molecular investigations should be conducted in this area, including pathogen surveys and differential diagnosis.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We thank Dr Wu HX for assistance in tick classification. This study was supported by the National Basic Research Program of China (973 Program) 2010CB530200(2010CB530206); the National Natural Science Foundation of China (No. 30771854), National Key

Science and Technology Projects of China (Project No. 2008ZX10004–008).

References

- [1] Dumler JS, Choi KS, Garcia–Garcia JC, Barat NS, Scorpio DG, Garyu JW, et al. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. *Emerg Infect Dis* 2005; **11**: 1828–1834.
- [2] Paddock CD, Childs JE. *Ehrlichia chaffeensis*: a prototypical emerging pathogen. *Clin Microbiol Rev* 2003; **16**: 37–64.
- [3] Petrovec M, Lotric Furlan S, Zupanc TA, Strle F, Brouqui P, Roux V, et al. Human disease in Europe caused by a granulocytic *Ehrlichia* species. *J Clin Microbiol* 1997; **35**: 1556–1559.
- [4] Guero–Rosenfeld ME, Dumler JS. *Ehrlichia*, *Anaplasma*, *Neorickettsia*, *Aegyptianella*. In: Murray PR. *Manual of clinical microbiology*. Washington DC: ASM Press; 2003, p. 1015–1029.
- [5] Walker DH. *Ehrlichia* under our noses and no one notices. *Arch Virol* 2005; Suppl: 147–156.
- [6] Park JH, Heo EJ, Choi KS, Dumler JS, Chae JS. Detection of antibodies to *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* antigens in sera of Korean patients. *J Clin Microbiol* 2003; **40**: 3082–3085.
- [7] Ohashi N, Inayoshi M, Kitamura K, Kawamori F, Kawaguchi D, Nishimura Y, et al. *Anaplasma phagocytophilum*–infected ticks, Japan. *Emerg Infect Dis* 2005; **11**: 1780–1783.
- [8] Heo EJ, Park JH, Koo JR, Park MS, Park MY, Dumler JS, et al. Serologic and molecular detection of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* (human granulocytic ehrlichiosis agent) in Korean patients. *J Clin Microbiol* 2002; **40**: 3082–3085.
- [9] Cao WC, Zhan L, He J, Foley JE, SJ DEV, Wu XM, et al. Natural *Anaplasma phagocytophilum* infection of ticks and rodents from a forest area of Jilin Province, China. *Am J Trop Med Hyg* 2006; **75**: 664–668.
- [10] Wen B, Cao W, Pan H. *Ehrlichiae* and ehrlichial diseases in china. *Ann N Y Acad Sci* 2003; **990**: 45–53.
- [11] Wen B, Jian R, Zhang Y, Chen R. Simultaneous detection of *Anaplasma marginale* and a new *Ehrlichia* species closely related to *Ehrlichia chaffeensis* by sequence analyses of 16S ribosomal DNA in *Boophilus microplus* ticks from Tibet. *J Clin Microbiol* 2002; **40**: 3286–3290.
- [12] Cao WC, Zhao QM, Zhang PH, Yang H, Wu XM, Wen BH, et al. Prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in *Ixodes persulcatus* ticks from northeastern China. *Am J Trop Med Hyg* 2003; **68**: 547–550.
- [13] Zhang LJ, Liu Y, Ni D, Li Q, Yu Y, Yu XJ, et al. Nosocomial transmission of human granulocytic anaplasmosis in China. *JAMA* 2008; **300**: 2263–2270.
- [14] Cui F, Wang LL, Zhang LL, Yang SX, Zhang SF, Wang JK, et al. Field epidemiological survey of the human granulocytic anaplasmosis in Yiyuan county, Shandong Province. *Dis Surveill* 2009; **24**: 412–415.
- [15] Wang CX. *Pest management*. Beijing: Chemistry Industry Publishing House; 2005.
- [16] La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. *J Clin Microbiol* 1997; **35**: 2715–2727.
- [17] Chapman AS, Bakken JS, Folk SM, Paddock CD, Bloch KC, Krusell A, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis—United States: a practical guide for physicians and other health–care and public health professionals. *MMWR Recomm Rep* 2006; **55**: 1–27.
- [18] Zhang LJ, Shan A, Mathew B, Yin J, Fu XP, Zhang JS, Lu J, et al. Rickettsial seroepidemiology among farm workers, Tianjin, People’s Republic of China. *Emerg Infect Dis* 2008; **14**: 938–940.
- [19] Bakken JS, Goellner P, Van Etten M, Boyle DZ, Swonger OL, Mattson S, et al. Seroprevalence of human granulocytic ehrlichiosis among permanent residents of northwestern Wisconsin. *Clin Infect Dis* 1998; **27**: 1491–1496.
- [20] JW IJ, Meek JI, Cartter ML, Magnarelli LA, Wu C, Tenuta SW, et al. The emergence of another tickborne infection in the 12–town area around Lyme, Connecticut: human granulocytic ehrlichiosis. *J Infect Dis* 2000; **181**: 1388–1393.
- [21] BiDZ, Zhang JZ. Studies on isolation SFG rickettsiae using embryonated hen’s eggs. *Chin J Vector Biology & Control* 1997; **8**: 47–50.
- [22] Sun Y, Xu RM, Zhang PH. Experiment infection and trans–stadial transmission of Lyme spirochetes in *Haemaphysalis longicornis* and *H. concinna*. *Chin J Zoonoses* 2003; **19**: 73–77.
- [23] Gao DQ, Cao WC, Zhao QM. Detection of human granulocytic *Ehrlichia* 16SrRNA gene in ticks from north china. *Acta Parasitol Med Entomol Sin* 2007; **5**: 30–37.
- [24] Zhan L, He J, Wu XM. Investigation on the infection with *Anaplasma phagocytophilum* ticks from the forest area in Jilin province of China. *Chin J Zoonoses* 2007; **23**: 438–440.
- [25] Zhang QM, Wu XM, Zhang PH. Study on the coinfection of three tick–borne infectious diseases in China using polymerase chain reaction method. *Chin J Epidemiol* 2005; **26**: 9–13.
- [26] Zhang ZQ, Wu YM, Feng L. Detection and sequence analysis of human granulocytic *Ehrlichia* DNA in ticks collected in Jilin province. *Chin J Zoonoses* 2007; **23**: 266–268.