INTRODUCTION

Ticks, a group of specialized obligate hemophagous ecto-parasites, parasitize abundant host species and are the vectors of a wide range of pathogens of veterinary and public health importance [1-6]. Recently, they are considered to occupy the second place after mosquitoes as vectors of human infectious diseases in the world. As of May 31 2015, there were at least 5,568 cases of human tick-borne diseases reported around China, including large number of patients with Lyme diseases and newly emerging severe fever with thrombocytopenia syndrome [1].

China has the complex distributions and the great diversity of tick species because of its diverse ecological habitats. Ticks in China were reported to be carriers of various human pathogens including protozoans and bacteria like *Borrelia* spp. and *Rickettsia* spp. [1,7,8]. Poyang Lake region, belonging to Jiangxi (a province of southeastern China), has already recorded sporadic human tick-borne diseases and at least 13 tick species. Our previous work detected some tick-borne pathogens in a few kinds of hosts, such as rodents and dogs in Poyang Lake region [4-6]. However, knowledge on tick-borne pathogens in tick vectors in this region is limited. Therefore, in this study we showed evidence to illustrate the distribution of pathogens comprising *Borrelia* spp., *Rickettsia* spp., and protozoa in tick vectors from Poyang Lake region in Jiangxi, and elucidated its relation with tick species, developmental stage, host and vegetation. The results will be a basis for future epidemiological studies and risk assessment of human tick-borne pathogens in Poyang Lake region.
MATERIAL AND METHODS

Study area
The study had been conducted for 3 years (2013-2015) in Poyang Lake region of Jiangxi Province, southeastern China, which has altitudes higher than 35 m and lower than 190 m above sea level. This area experiences a subtropical climate with over 1,000 mm of annual rainfall, -10˚C of maximum low temperature and 40˚C of maximum high temperature. Temperatures usually vary from 10 to 37˚C between May and October when tick populations are active. Types of vegetation cover include mixed broadleaf and coniferous woodland and grassland (Table 1). We selected 12 counties in Poyang lake region as investigation sites (Table 1).

Tick collection and identification
Ticks in vegetation covers were collected by flagging or dragging both at ground level and over and through the vegetation with a cotton cloth (100×60 cm). Each site was visited at least 3 times to cover all of 3 categories of habitats (grassland, woodland, and shrubs). Each habitat category was selected to cover a 900-m² area with many animal trails and tracks. Ticks were removed from the cotton cloth every 2 minutes. Ticks parasitizing hosts were collected from 24 villages and 12 wild animal markets. In villages, domestic animals and fowls were restricted by owners for sampling. In markets, wild animal bodies were employed for tick collection. Rodentia around villages were trapped using peanut baited rodent traps for tick examination. All the procedures were carried out according to ethical guidelines for the use of animal samples permitted by Obihiro University of Agriculture and Veterinary Medicine (Animal experiment access num: 28-100). The information regarding all of the collected specimens, including their location, vegetation type, host, number of ticks collected from the body of each animal and the date of collection, were recorded. Ticks were collected from the entire body of each host into separate sample bottle containing 70% ethanol. Standard taxonomic keys were used to morphologically identify adults [9]. Larvae and nymphs were identified individually based on molecular methods [10]. The specimens were kept in 70% ethanol and used for further molecular identification and detection of tick-borne pathogens.

DNA isolation
Tick specimens immersed in 70% ethanol were air dried, and then rinsed in sterile water for 3 times. After rinsed in sterile phosphate-buffered saline, ticks were dried on sterile filter paper in a biosafety hood, and individually ground in sterile tubes. DNA was extracted using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. The genomic DNA was stored at 4˚C until used as a template in PCR assays.

Pathogen identification
A total of 3 groups of pathogens were assayed: *Borrelia* spp., *Rickettsia* spp. and protozoa. A conventional PCR was performed with a set of primers (forward: 5'-ACATATTCAGATGCAGACAGAGGT-3', reverse: 5'-GCAATCATAGCCATTGCAGATTGT-3') designed to amplify the 665-bp flagellin gene of *Borrelia* spp. For citrate synthase encoding gene (*glt*A), a primer set of primer 1 (5'-GCAAGTATCGGTGAGGATGTAAT-3') and primer 2 (5'-GCTTCCTTAAAATTCAATAAATCAGGAT-3') was used and expected to yield a 401-bp fragment depending on the *Rickettsia* spp. For amplification of 209-214 bp fragment of 18S ribosomal RNA (rRNA) in the protozoa, a set of primers (forward: 5'-GCAAGTATCGGTGAGGATGTAAT-3') and primer 2 (5'-GCTTCCTTAAAATTCAATAAATCAGGAT-3') was used and expected to yield a 401-bp fragment depending on the *Rickettsia* spp. For amplification of 209-214 bp fragment of 18S ribosomal RNA (rRNA) in the protozoa, a set of primers (forward: 5'-GCAAGTATCGGTGAGGATGTAAT-3').

Table 1. Location and vegetation type of 12 plots sampled in this study

| Location | Geographic coordinates | Vegetation type | Year surveyed |
|----------|------------------------|----------------|--------------|
| Anyi     | N 28.6173°, W 115.5423° | G, S, W       | 2014, 2015   |
| Wanli    | N 28.8400°, W 115.7589° | W             | 2014         |
| Xinjian  | N 28.9800°, W 115.9154° | G             | 2014         |
| Qingyunpu| N 28.6389°, W 115.9127° | G             | 2013, 2014   |
| Duchang  | N 29.2542°, W 116.1946° | W             | 2015         |
| Hukou    | N 29.7469°, W 116.2330° | W             | 2015         |
| Wuning   | N 29.2574°, W 115.0986° | G, S          | 2015         |
| Poyang   | N 29.0000°, W 116.6730° | G             | 2015         |
| Wannian  | N 28.6899°, W 116.9728° | W             | 2015         |
| Wuyuan   | N 29.2709°, W 117.75793° | G, S, W      | 2015         |
| Yichun city | N 27.5914°, W 114.3252° | G, S, W      | 2015         |
| Xingan   | N 27.7327°, W 115.3791° | G, S, W      | 2015         |

W, woodland; S, shrubs; G, grassland.
same conditions as flagellin gene except the extension at 72˚C for 45 sec for the proteozoa and the annealing at 50˚C for 30 sec. Positive samples were sequenced to identify potential microbial species with a resemblance to known species based on an online software (http://www.bioinformatics.org/sms2/ident_sim.html).

Sequence analysis
All obtained sequences were assembled and edited by using SeqMan software. We compared them with sequences in the GenBank database. We performed multiple sequence alignments by using the ClustalX program. Phylogenetic trees were constructed by using the Neighbor-Joining (NJ) algorithm in the MEGA v.7.0.26 software. Support for the tree nodes was calculated with 1,000 bootstrap replicates.

Data analyses
All the raw data were collated in Excel spreadsheets. The differences in infection rates of ticks at species levels, at developmental stages, on hosts, in habitat categories, and the difference in infection rates of ticks collected in vegetation covers and on hosts were evaluated using Chi square (http://quantpsy.org). In the $2 \times 2$ case of the chi-square test of independence, if expected frequencies is less than 5, Yates’ correction is employed [11].

RESULTS

Tick samples
A total of 311 ticks belonging to 5 genera and 11 species were collected from 5 species of domestic animals (Canis familiaris, Capra aegagrus hircus, Bos spp., Bubalus bubalis, and Equus ferus), 5 species of wild animals (Lepus sinensis, Erinaceidae, Apodemus agrarius, Rattus norvegicus, Rattus rattoides), a species of bird (Phasianus colchicus) and a species of chicken (Gallus gallus domesticus), in 2 kinds of vegetation types from 12 locations in Poyang Lake region (Table 1). L. sinensis harbored abundant

---

**Table 2.** Summary of species and number of ticks collected from hosts and by flagging over vegetation cover

| Vegetation covers/Animal hosts | Tick species                          | No. of ticks collected | Density* |
|-------------------------------|---------------------------------------|------------------------|----------|
|                               |                                       | L | N | A |                           |           |
| Woodland (a= 800 m$^2$)       | Haemaphysalis longicornis              | 0 | 0 | 2M | 0.0038                  |
|                               | Dermacentor auratus                   | 0 | 0 | 1M |                       |
| Grassland (a= 800 m$^2$)      | H. longicornis                        | 3 | 34 | 5M6F | 0.06                  |
| Subtotal (a= 1,600 m$^2$)     |                                       | 3 | 34 | 8M6F | 0.032                |
| Canis familiaris (n= 24)      | H. longicornis                        | 0 | 5 | 4M25F | 1.79                  |
|                               | R. sanguineus                         | 0 | 0 | 1M8F |                       |
| Capra aegagrus hircus (n= 44) | H. longicornis                        | 0 | 2 | 0 | 0.80                  |
|                               | Haemaphysalis flavicola               | 2 | 2 | 3M1F |                       |
|                               | Rhipicephalus microplus               | 0 | 8 | 6M1F |                       |
| Bos spp. (n= 13)              | H. longicornis                        | 1 | 8 | 0 | 4.31                  |
|                               | R. microplus                          | 0 | 20 | 8M19F |                       |
| Bubalus bubalis (n= 7)        | H. longicornis                        | 0 | 0 | 1F | 1                      |
|                               | R. microplus                          | 0 | 0 | 5F |                       |
|                               | Amblyomma testudinarium               | 0 | 0 | 1F |                       |
| Phasianus colchicus (n= 5)    | Haemaphysalis phasiana                | 0 | 5 | 0 | 1                      |
| Lepus sinensis (n= 22)        | H. longicornis                        | 57 | 12 | 5M1F | 3.77                  |
|                               | Ixodes acuminatus                     | 0 | 0 | 1F |                       |
|                               | Ixodes sinensis                      | 0 | 0 | 2M2F |                       |
|                               | Rhipicephalus haemaphysaloides        | 3 | 0 | 0 |                       |
| Erinaceidae (n= 3)            | H. flavacaudata                       | 0 | 1 | 3M9F | 4.33                  |
| Apodemus agrarius (n= 206)    | Ixodes granulatus                     | 0 | 0 | 1M4F | 0.02                  |
| Rattus norvegicus (n= 95)     | I. granulatus                        | 0 | 0 | 3M1F | 0.04                  |
| Rattus rattoides (n= 8)       | I. granulatus                        | 0 | 5 | 2M | 0.88                  |
| Equus ferus (n= 6)            | H. longicornis                        | 0 | 0 | 1F | 0.17                  |
| Gallus gallus domesticus (n= 30) | H. longicornis           | 1 | 0 | 0 | 0.03                  |
| Subtotal (n= 463)             |                                       | 64 | 68 | 38M90F | 0.56               |
| Total (n= 483; a= 1,600 m$^2$)|                                       | 67 | 102 | 46M96F |                |

L, larvae; N, nymph; A, adult; M, male; F, female.

*Tick population density is denoted as ticks/hosts for ticks on hosts, ticks/m$^2$ for ticks collected from vegetation covers.
ticks such as *Haemaphysalis longicornis*, *Ixodes acuminatus*, *Ixodes sinensis* and *Rhipicephalus haemaphysaloides*, with the third highest tick population density of 3.77 ticks per a host. Hosts with the first highest and second highest tick loads were Erinaceidae (4.33 ticks per host) and Bos spp. (4.31 ticks per host), respectively. Other hosts with higher tick abundance were *B. bubalis* and *C. aegagrus hircus*, harboring 3 tick species. Sixty-seven female (14.79%) and 102 male (30.87%) adult ticks were obtained. Sixty-seven larvae and 102 nymphs accounted for 21.54% and 32.80% of the total number of ticks collected respectively. Of the 11 tick species collected, 3 species belonged to the genus *Haemaphysalis*, 3 species belonged to *Rhipicephalus*, 3 species belonged to *Ixodes*, 1 species belonged to *Dermacentor*, and 1 other species belonged to the genus *Amblyomma*. The most abundant species was *H. longicornis* (55.63%), found in a kind of vegetation cover and infesting the most diverse host species (7 species). Three other common species included *H. flava*, *R. microplus* and *I. granulatus* (Tables 2, 3).

**Pathogen infections in ticks**

Protozoa, *Borrelia* spp. and *Rickettsia* spp. were detected in 4 tick species. Overall, 7.07% of ticks were tested positive for at least 1 pathogen. In detail, 2.31% of *H. longicornis* were detected positive for *Rickettsia* spp., or/and *Protozoa*, 18.75% of *I. granulatus* for *Borrelia* spp., 52.38% of *H. flava* for *protozoa* or/and *Rickettsia* spp. and 5.19% of *R. microplus* for protozoa. Infection rate in *H. flava* was significantly greater than that in *H. longicornis* ($\chi^2 = 61.24, P < 0.001$). Coinfection with protozoa and *Rickettsia* were found in *H. longicornis* and *H. flava*, with coinfection rate of 0.58% and 47.62%, respectively. There was no positive samples found in 7 tick species (*H. phasiana*, *I. acuminatus*, *R. sanguineus*, *R. haemaphysaloides*, *I. sinensis*, *A. testudinarium* and *D. auratus*) (Table 3).

The effect of risk factors on the pathogen distribution

The overall prevalence of pathogens in larvae, nymphs, male, and female ticks were 1.49%, 2.94%, 10.87%, and 13.54%, respectively. There was major difference in the prevalence of these pathogens between immatures (larvae and nymphs) and matures (males and females) ($\chi^2 = 10.12, P = 0.018$). However, there was no significant difference in prevalence of these pathogens in ticks among host species and vegetation, although the positive rate of pathogens in ticks collected from hosts was approximately 2 times more than that collected by flagging over vegetation ($\chi^2 = 0.44, P = 0.51$). Prevalence of these pathogens in ticks collected from *Canis familiaris*, *C. aegagrus hircus*, Muridae and Erinaceidae were 4.65%, 11.43%, 18.75%, and 84.62%, respectively, and ticks on Erinaceidae were at significantly higher risk for pathogen infection compared to ticks on other hosts ($\chi^2 = 108.44, P < 0.001$). There was no positive ticks found on other host species. Prevalence of these pathogens in ticks in grasslands and woodland were 2.08% and 0, respectively, and there was on significant difference ($\chi^2 = 1.37, P = 0.24$) (Table 4).

**Pathogen identification and sequence analyses**

Further sequencing and sequence alignment showed that 1 *Borrelia* species (*Borrelia yangtzensis*), 2 protozoan species (*Babesia vogeli* and *Hepatozoon canis* or *Hepatozoon felis* related geospecies), and 1 *Rickettsia* species (*Rickettsia slovaca* or *Rickettsia raoulitii* related genospecies) were successfully sequenced from 4 tick species. The 665-base pair sequence of *Borrelia* spp. flagellin gene (MG717513) yielded in the study was 99.21-99.37% identical to other 2 sequences of MG717514 and MG717515 pro-

Table 3. Pathogen infection rates in ticks collected in Poyang Lake region

| Tick species (number collected) | Borrelia | Rickettsia | Protozoa | Protozoa+ Rickettsia | Infection rate (%) | $\chi^2$ | $P$-value |
|--------------------------------|----------|------------|----------|----------------------|--------------------|--------|----------|
| *Haemaphysalis longicornis* (n=173) | 0        | 4 (2.31)   | 1 (0.58) | 1 (0.58)             | 4 (2.31)            | 61.24  | <0.001   |
| *Ixodes granulatus* (n=16)        | 3 (18.75)| 0          | 0        | 0                    | 3 (18.75)           |        |          |
| *Haemaphysalis flava* (n=21)      | 0        | 11 (52.38) | 10 (47.62)| 10 (47.62)           | 11 (52.38)          |        |          |
| *Rhipicephalus microplus* (n=77)  | 0        | 0          | 4 (5.19) | 0                    | 4 (5.19)            |        |          |
| *Haemaphysalis phasiana* (n=5)    | 0        | 0          | 0        | 0                    | 0                  |        |          |
| *Ixodes acuminatus* (n=1)         | 0        | 0          | 0        | 0                    | 0                  |        |          |
| *Rhipicephalus sanguineus* (n=9)  | 0        | 0          | 0        | 0                    | 0                  |        |          |
| *Rhipicephalus haemaphysaloides* (n=3) | 0        | 0          | 0        | 0                    | 0                  |        |          |
| *Ixodes sinensis* (n=4)           | 0        | 0          | 0        | 0                    | 0                  |        |          |
| *Amblyomma testudinarium* (n=1)   | 0        | 0          | 0        | 0                    | 0                  |        |          |
| *Dermacentor auratus* (n=1)       | 0        | 0          | 0        | 0                    | 0                  |        |          |
duced in the study. When compared to other fragments deposited in GenBank, MG717513 showed 98.73-98.89% identity to *B. yangtzensis* (EU135599, EU135601, and EU135602), 98.57-98.73% identity to *Borrelia valaisiana* (AB022134 and AB022135), and 95.25% identity to *Borrelia burgdorferi* sensu lato (X75202, X63413, and D63364). Therefore, 3 individuals of *Borrelia* spp. in the study were identified as *B. yangtzensis* or *B. yangtzensis*-related species. In *Rickettsia* spp., the 401 base-pair sequence of *gltA* gene (MG717516) obtained in a *H. longicornis* tick collected in grassland was 100% identical to the sequences of *gltA* gene isolated from 2 *H. longicornis* ticks (MG717517 and MG717523) on *C. familiaris* and 10 *H. flava* ticks on Erinaceidae (MG717518-MG717522, MG717524-MG717528), and 96.26% identical to the sequence in a *H. flava* tick on Erinaceidae (MG717529) (Table 5; Fig. 1). Our 13 sequences (MG717516-MG717528) showed 99.75% identity to the sequences of *R. raoultii* (MF002517) and *R. slovaca* (MF002529) deposited in GenBank, in addition, 1 remaining sequence (MG717529) presented 96.01% identity to *R. raoultii* and *R. slovaca*. The *Rickettsia* spp. pathogens in the study were identified as *R. raoultii* or *R. slovaca* related genospecies. Of 15 protozoa-positive specimens for amplification of 209-214 base-pair 18S ribosomal RNA by means of PCR method, 2 specimens were successfully sequenced (Table 5). The closest matches of 209 base-pair 18S ribosomal RNA of protozoa in our study were *B. vogeli* isolated in dogs from Jiangsu, China (MG586235, 100%), Serbia (KY747491, 100%), and Argentina (KY290978, 99%), and in *R. sanguineus* from India (MG050159, 100%) and from Australia (MG758132, 100%), in *Haemaphysalis concinna* from Czech Republic (KX857477, 100%).

### Table 4. Comparison of difference of collected ticks and positive rates of pathogens among ticks by life stage, host species and vegetation type

| Group             | Sampled ticks | Positive ticks | χ²    | P-value |
|-------------------|---------------|----------------|-------|---------|
| **Life stage**     |               |                |       |         |
| Larvae            | 67            | 1              | 1.49  | 10.12   | 0.018   |
| Nymph             | 102           | 3              | 2.94  | 9.25    | 0.067   |
| Male              | 46            | 5              | 10.87 | 13.54   |         |
| Female            | 96            | 13             | 13.54 |         |         |
| **Vegetation type** |              |                |       |         |
| Grassland         | 48            | 2              | 4.17  | 1.37    | 0.24    |
| Woodland          | 3             | 0              | 0.00  |         |         |
| **Host**          |               |                |       |         |
| Muridae           | 16            | 3              | 18.75 | 108.44  | <0.001  |
| Canis familiaris  | 43            | 2              | 4.65  |         |         |
| Capra aegagrus hircus | 35       | 4              | 11.43 |         |         |
| Lepus sinensis    | 83            | 0              | 0.00  |         |         |
| Erinaceidae       | 13            | 11             | 84.62 |         |         |
| Babalculus bubalis| 7             | 0              | 0.00  |         |         |
| Bos spp.          | 56            | 0              | 0.00  |         |         |
| Phasianus colchicus| 5          | 0              | 0.00  |         |         |
| **Vegetation vs host** |       |                |       |         |
| Vegetation        | 51            | 2              | 3.92  | 0.44    | 0.51    |
| Host              | 260           | 20             | 7.69  |         |         |

### Table 5. Pathogens in ticks collected from different hosts in different locations

| Pathogens                          | Ticks species (No. positive) | Host species | Sampling site | GenBank accession No. |
|------------------------------------|-----------------------------|--------------|---------------|-----------------------|
| Borrelia *B. yangtzensis*          | *i. granulatus* (1♂1♀1♀)    | *R. norvegicus* | Anyi          | MG717514-MG717515    |
| *B. yangtzensis*                   | *i. granulatus* (1♀)        | *R. rattiodes* | Anyi          | MG717513              |
| Rickettsia *R. raoultii* or *R. slovaca* related genospecies | *H. longicornis* (2♂) | *Canis familiaris* | Grassland | MG717517, MG717523 |
| *R. raoultii* or *R. slovaca* related genospecies | *H. longicornis* (1♀) | *Canis familiaris* | Grassland | MG717517, MG717523 |
| *Rickettsia* sp.                   | *H. flava* (1♀)             | *Canis familiaris* | Grassland | MG717517, MG717523 |
| Protozoa *Babesia vogeli*          | *H. flava* (1♀)             | *Canis familiaris* | Grassland | MG717517, MG717523 |
| *Babesia sp.*                      | *H. flava* (9♂)             | *Canis familiaris* | Grassland | MG717517, MG717523 |
| *Hepatozoon canis* or *Hepatozoon felis* related genospecies | *R. microplus* (1♀) | *Canis familiaris* | Grassland | MG717517, MG717523 |
| *Hepatozoon canis* or *Hepatozoon felis* related genospecies | *H. longicornis* (1♀) | *Canis familiaris* | Grassland | MG717517, MG717523 |

Zheng et al.: Pathogens in ticks from southeastern China 593
ed in *H. longicornis* from grassland in the study showed 94.86% to *H. canis* (MG917719 and MG209594) and *H. felis* (KUJ232308), 92.99% identity to *Hepatozoon ursi* (KUJ232308), hence we proposed the protozoan as *H. canis* or *H. felis* related genospecies. *R. slovaca* or *R. raoultii* related genospecies was most frequently identified (14 times, 3 from the tick *H. longicornis*, 11 times from the tick *H. flava*), followed by *B. yangtzensis* (triple from *I. granulatus*). The other 2 protozoan species were detected only once. Twenty one of the 33 detections of pathogens were on *H. flava* collected from Erinaceidae (Table 5).

For phylogenetic analyses, 3 sequences of *B. yangtzensis* flagellin gene obtained from *I. granulatus* belonged to the same cluster where they shared with the strain QLZSP, QSYSP, and QTMP2 of *B. yangtzensis* and strain CKA3a and CMN1b of *B. valaisiana* (Table 5; Fig. 2). The sequences of *R. raoultii* or *R. slovaca* related *Rickettsia* spp. (MG717516-MG7175129) were clustered with those of *R. raoultii* (MF002517) and *R. slovaca* (MF002529) (Fig. 2).

**DISCUSSION**

In Poyang Lake region, the common animals and birds with potential for tick parasitism and easy to contact human were *C. familiaris*, *C. aegagrus hircus*, *A. agrarius*, *R. norvegicus*, *G. gallus domesticus*, and *L. sinensis*, accounting for over 90% of hosts captured. Rodents like *A. agrarius* and *R. norvegicus* were trapped with large number, but a few ticks were found, where as *B. yangtzensis* was occasionally detected in ticks removed from the rodents. *B. yangtzensis*, a *Borrelia* species in the *B. burg-
The complex was originally discovered in Chinese Yangtze River Valley region in 2015, and it was reported in _H. longicornis_ and _I. granulatus_ ticks from small mammals in China and isolated in rodents or shrews in Japan [12]. However, _B. yangtzensis_ was not detected in _H. longicornis_ albeit greater than 50% ticks collected in the study were _H. longicornis_. The reason, we guessed, might be that _H. longicornis_ was not an efficient vector of _B. yangtzensis_, hence the pathogen was rarely presented in the ticks. Poyang Lake region belongs to part of Yangtze River Valley region, and has similar distribution pattern of ticks and tick related small mammals to other parts of Yangtze River Valley region, therefore _B. yangtzensis_ can also be found in _I. granulatus_ collected in rodents in our study. The sequences of flagellin gene in _B. yangtzensis_ in the study showed higher identity to _B. valaisiana_ than to other known Lyme Borreliosis group spirochaete species, which was in agreement with the previous study [12].

Despite some _L. sinensis_ were majorly tick infested, pathogens were not found in those ticks. We had 3 Erinaceidae hosts, and found diverse pathogens like _R. slovaca_ or _R. raoultii_ like genospecies and _Babesia_ spp. in attached ticks with high infection rate. Ticks on Erinaceidae might serve as vectors within Erinaceidae populations in this region, thus readily leading to high infection rate. This increases the chance that ticks transport pathogens from a natural hedgehog cycle to other hosts, including humans [13]. _C. familiaris_, usually functioning as a guard dog and a pet in investigated sites, were closely related to human, furthermore, some ticks on dogs in our study were positive for _R. slovaca_ or _R. raoultii_ related genospecies which is likely considered as human pathogen. Dogs, incidental hosts for the agent of spotted fever group, can become infected by a bite of ixodid ticks, and then transmit the pathogens to human [14]. Therefore, people should avoid contact with such dogs and ticks.

In this study, 11 tick species were collected, with _H. longicornis_ acting as the predominant species, and other common ticks included _H. flava_, _R. microplus_ and _I. granulatus_. These common tick species were also reported in other subtropical regions of China like Zhejiang and Hubei [15]. Our findings indicated that _H. flava_ and _H. longicornis_ were the ticks frequently detected positive for presence of _R. raoultii_ or _R. slovaca_ related genospecies. _R. raoultii_ and _R. slovaca_ were reported as human pathogenic agents [3,16,17]. Previous researches showed that _R. raoultii_ had been reported in northern regions of China [3,7], and _R. slovaca_ recorded in Europe and Xinjiang, China [7,16,17]. Although natural infection with tick-borne pathogens occurs [1], other tick species like _R. sanguineus_, _R. haemaphysaloides_, _I. sinensis_, and _A. testudinarium_ were tested negative for _Borrelia_ spp., _Rickettsia_ spp. and protozoa infection in our study. The possible reason might be because of a few numbers of ticks collected and thus decreasing the probability of pathogen detection.

Compared to immature ticks, mature ticks tended to pathogen infection, furthermore, we found that females had comparable positivity rate with males. In contrast, a study conducted in Europe showed higher pathogen infection rate in immatures than matures [18]. Our study demonstrated that relatively high infection rate were determined in adult ticks collected from hedgehogs. The result suggests hedgehogs functioning as important pathogen reservoirs, and corresponds with a previous indication that several species of birds played a role as Lyme disease spirochetal reservoirs infective to ticks [18]. Therefore, in some cases, positivity rate is not depended by tick developmental stage but by which reservoir hosts that ticks attach to. For vegetation types, grassland sheltered more ticks than woodland and shrubs, and there were some ticks infected with _R. slovaca_ or _R. raoultii_ related genospecies in grassland. However, non-infected ticks were found in woodland, in contrast to more than 6 tick-borne pathogens infection in ticks from French suburban woodland [19]. Workers and visitors for travelling in the field should pay more attention to questing ticks in grassland in prevention of occurrence of tick-borne diseases. In addition, people in this region should keep a distance from hosts with tick infestation, especially the hosts with high risk for human tick-borne pathogens including hedgehogs, dogs and rodents.

**ACKNOWLEDGMENTS**

This study was supported financially by Jiangxi Provincial Department of Science and Technology (grant number 2016 BBG70005); Nanchang Science and Technology Bureau (Hong Scientific Research Program [2016] No. 96 Item 77); and the Health and Family Planning Commission of Jiangxi Province (grant number 20162007). The sponsors have no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. The authors thank Rongman Xu, Yi Sun, and Ze Chen for morphological identification of tick species, and also wish to acknowledge the contribution of Yuanping...
Deng (the director of Anyi Center for Disease Control and Prevention), for technical assistance during the collection of samples. Weiqing Zheng was partially supported by Sasakawa Medical Fellowship through Japan-China Medical Association.

**CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest.

**REFERENCES**

1. Fang LQ, Liu K, Li XL, Liang S, Yang Y, Yao HW, Sun RX, Sun Y, Chen WJ, Zuo SQ, Ma MJ, Li H, Jiang JF, Liu W, Yang YE, Gray GC, Krause PJ, Cao WC. Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect Dis 2015; 15: 1467-1479.

2. Saito K, Ito T, Asashima N, Ohno M, Nagai R, Fujita H, Koizumi N, Takano A, Watanabe H, Kawabata H. Borrelia valaisiana infection in a Japanese man associated with traveling to foreign countries. Am J Trop Med Hyg 2007; 77: 1124-1127.

3. Li H, Zhang PH, Huang Y, Du J, Cui N, Yang ZD, Fan YD, Li XM, Cui XM, Fan FY, Li XM, Sun RX, Sun Y, Chen WJ, Zuo SQ, Ma MJ, Li H, Jiang JF, Liu W, Yang YE, Gray GC, Krause PJ, Cao WC. Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect Dis 2015; 15: 1467-1479.

4. Zheng WQ, Chen HY, Liu MM, Adjou Moumouni PF, Efstratiou A, Liu ZB, Xuan XN. First evidence of Mycoplasma haemocanis in China. Trop Biomed 2018; 6: 401-411.

5. Zheng WQ, Liu Y, Zhao H, Li Z, Xuan X, Liu X, Adjou Moumouni PF, Wu Y, Liu W, Chen H. First molecular evidence of Anaplasmaphagocytophilum in rodent population of Nan chang, China. Jpn J Infect Dis 2018; 71: 129-133.

6. Zheng W, Liu M, Adjou Moumouni PF, Liu X, Efstratiou A, Liu Z, Liu Y, Tao H, Guo H, Wang G, Gao Y, Li Z, Ringo AE, Jirapathasarate C, Chen H, Xuan X. First molecular detection of tick-borne pathogens in dogs from Jiangxi, China. J Vet Med Sci 2017; 79: 248-254.

7. Han R, Yang J, Niu Q, Liu Z, Chen Z, Kan W, Hu G, Liu G, Luo J, Yin H. Molecular prevalence of spotted fever group rickettsiae in ticks from Qinghai Province, northwestern China. Infect Genet Evol 2018; 57: 1-7.

8. Takada N, Masuzawa T, Ishiguro F, Fujita H, Kudeken M, Mitani H, Fukunaga M, Tsuchiya K, Yano Y, Ma XH. Lyme disease Borrelia spp. in ticks and rodents from northwestern China. Appl Environ Microbiol 2001; 67: 5161-5165.

9. Teng GF. Chinese Economic Insect Archive, Ixodidae. Beijing, China. Science Press. 1978, pp 1-174.

10. Lv J, Wu S, Zhang Y, Chen Y, Feng C, Yuan X, Jia G, Deng J, Wang C, Wang Q, Mei L, Lin X. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acaria: Ixodida). Parasit Vectors 2014; 7: 53.

11. Preacher KJ. Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence [Computer software]; Available from: http://quantpsy.org/. 2001.

12. Margos G, Chu CY, Takano A, Jiang BG, Liu W, Kurtenbach K, Masuzawa T, Fingerle V, Cao WC, Kawabata H. Borrelia yangtzeensis sp. nov., a rodent-associated species in Asia, is related to Borrelia valaisiana. Int J Syst Evol Microbiol 2015; 65: 3836-3840.

13. Skuballa J, Petney T, Pfaffle M, Taraschewski H. Molecular detection of Anaplasma phagocytophilum in the European hedgehog (Erinaceus europaeus) and its ticks. Vector Borne Zoonotic Dis 2010; 10: 1055-1057.

14. Nicholson WL, Allen KE, McQuiston JH, Breitnuser EB, Little SE. The increasing recognition of rickettsial pathogens in dogs and people. Trends Parasitol 2010; 26: 205-212.

15. Lu X, Lin XD, Wang JB, Qiu XC, Tian JH, Guo WP, Fan FN, Shao R, Xu J, Zhang YZ. Molecular survey of hard ticks in endemic areas of tick-borne diseases in China. Ticks Tick Borne Dis 2013; 4: 288-296.

16. Gouriet F, Rolain JM, Raoult D. Rickettsia slovaca Infection, France. Emerg Infect Dis 2006; 12: 521-523.

17. de Sousa R, Pereira BI, Nazareth C, Cabral S, Ventura C, Crespo P, Marques N, da Cunha S. Rickettsia slovaca infection in humans, Portugal. Emerg Infect Dis 2013; 19: 1627-1629.

18. Olsén B, Jaenson TG, Bergström S. Prevalence of Borrelia burgdorferi sensu lato-infected ticks on migrating birds. Appl Environ Microbiol 1995; 61: 3082-3087.

19. Reis C, Cote M, Paul RE, Bonnet S. Questing ticks in suburban forest are infected by at least six tick-borne pathogens. Vector Borne Zoonotic Dis 2011; 11: 907-916.