Prevalence of Tick-borne Hemolytic Microbes in Free-living Sika Deer (Cervus nippon) Captured in a Deer-overcrowded Area

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ABSTRACT

We collected 36 blood samples from free-living sika deer (Cervus nippon) that were captured in a deer-overcrowded area in Fukui prefecture, and examined the prevalence of tick-borne microbes, including hemoplasmas, Piroplasma, and Anaplasma. Of the 36 samples of deer, 35 (97.2%) were infected with at least one hemotropic microbes. Thirteen (36.1%) deer samples were positive for ‘Candidatus Mycoplasma erythrocervae’ and 13 (36.1%) deer were positive for ‘Ca. M. haemocervae’. Similarly, 25 (69.4%) deer samples were positive for Theileria sp. Thrivae. One (2.8%) was positive for Theileria sp. Sola, five (13.9%) were positive for Anaplasma phagocytophilum, eight (22.2%) were positive for A. bovis, and 28 (77.8%) were positive for Anaplasma sp. This showed a high prevalence of tick-borne blood microorganisms in the deer population. Phylogenetic analysis of the 18S rRNA gene showed that the Theileria sp. Thrivae that was detected in this study formed a monophyletic cluster with other Theileria species that previously were detected in sika deer. This suggests that a novel Theileria species is prevalent in sika deer. Phylogenetic analyses of 16S rRNA gene showed that Anaplasma sp. in this study formed monophyletic groups with other Anaplasma species that were previously detected in Japanese cattle and wild ruminants. This suggests that a novel Anaplasma species is prevalent in sika deer.

Key words: Anaplasma, deer, hemoplasma, sika, Theileria

INTRODUCTION

The sika deer (Cervus nippon) population has been increasing in Japan. This causes a serious problem in agriculture and forestry [1, 2]. In addition to the economic losses, the deer population can serve as a reservoir for livestock and human infectious agents [3-6]. The growth of the deer population increases the number of ticks in the deer habitat and, when combined with global warming in recent decades, this has increased and expanded the number and geographical distribution of ticks [6-9]. Thus, the prevalence of tick-borne diseases in sika deer should be of concern, especially in the areas where sika deer are overcrowded [6, 10].

Tick-borne haemotropic microbes in mammals include hemoplasmas, Piroplasma, and Anaplasma. Hemoplasma is a tribal name for a group of the hemotropic Mycoplasma species, and two hemoplasmas, ‘Candidatus Mycoplasma erythrocervae’ and ‘Ca. M. haemocervae’, which are known to currently exist in sika deer [11]. With regard to Piroplasma species, the presence of Theileria damad, Theileria sp., and Babesia sp. has been reported in sika deer [12-15]. In regard to the Anaplasma species, Anaplasma phagocytophilum, A. centrale, and A. bovis have been detected along with Ehrlichia sp. in free-living sika deer in the previous investigation conducted by Kawahara et al. [16]. They
identified their detected *Anaplasma* as *A. centrale*, based on 16S rRNA gene sequence homology with *A. centrale* Aomori strain (AF283007) detected in Japanese cattle. However, the low sequence identity of *A. centrale* Aomori strain (AF283007) to other erythrocytic *Anaplasma* species, including type strain *A. centrale* Israel vaccine (AF309869), implied that the species definition of the Aomori strain was unreliable [17, 18]. Thus, the *A. centrale* strains that were detected in sika deer in Japan have been considered to be unidentified *Anaplasma* sp. in this study. Consequently, *A. phagocytophilum*, *A. bovis* and unidentified *Anaplasma* sp. have been detected in wild sika deer in Japan.

There are several reports of a high incidence of hemoplasma and *Theileria* infections in captive deer herds [6, 10, 12, 19-21]. Further, a high prevalence of pathogens in ticks and a high incidence of human infection in areas where deer population is dense have been indicated [6, 10]. To date, the prevalence of hemoplasmas, *Piroplasma*, and *Anaplasma* in wild deer has been examined [13, 14, 16, 22]. Unlike previous studies of captive deer herds, the wild deer samples were collected from either unspecified area or various regions in Japan. Thus, how well those previous studies of wild deer reflect the actual prevalence of tick-borne disease in the local free-living deer herd is uncertain. The aim of the present study is to evaluate the prevalence of hemoplasmas, *Piroplasma*, and *Anaplasma* in wild deer in an area of overabundant deer population in Japan.

**MATERIALS AND METHODS**

The deer hunting spot was established in the Sagaki area in the Mihama district, Fukui prefecture where more than 32,000 sika deer live in 3,100km² woodland and more than 8,000 heads are hunted annually for population control [23]. Wire traps were set at the edge of the forest within a distance of 700-meters from the center of the Sagaki area (Fig. 1). This was done under the deer population control program in the Mihama district during the period of January 2014 to May 2015 (license numbers: 27, 38-6, 7-5, 19-5, 30-5, 40-5). Juvenile and adult individuals were divided by body weight together with antlers or mammary gland maturity [24].

Blood samples were collected in heparinized tubes and stored at -20 °C prior to analysis. Seventy-five µl of total DNA solution was extracted from 100 µl of the whole blood samples using FlexiGene DNA Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instruction, and stored at -20°C until examination by the PCR assay.

For hemoplasmas detection by PCR, two forward primers, CMec F (5’-GCAAGGGGTTCGCGTAAAA-3’) and CMhc F (5’-CCGCGAGTAGGATAGCAGCC-3’) are specific to ‘Ca. M. erythrocervae’ and ‘Ca. M. haemocervae’ respectively, and common reverse primer, T-R2 (5’-ACGCCCAATAAATCCGRATAAT-3’) was used to target the hemoplasma 16S rRNA gene as described previously [25]. Amplicon size of the PCR products using CMec F and T-R2, and CMhc F and T-R2 are 363 and 439 bp respectively. PCR amplification for ‘Ca. M. erythrocervae’ was carried out in 20µl reaction mixtures containing 1.0µl of DNA solution, 0.4µl of KOD FX Neo (Toyobo, Osaka, Japan), 10µl of 2×PCR Buffer for KOD FX Neo, 4µl of 2mM dNTPs, 0.4µl of CMec F and T-R2 (10 mM each), and sufficient water to provide a final volume of 20µl. The PCR reaction was carried out 40 cycles of denaturation at 98°C for 10 sec, annealing at 60°C for 30 sec, and extension at 68°C for 30 sec after an initial denaturation...
step at 94°C for 2 min. In the case of PCR amplification for ‘Ca. M. haemocervae’, 20µl reaction mixtures containing 1.0µl of DNA solution, 10µl of TaKaRa Taq HS Low DNA (Takara Bio Inc. Shiga, Japan), 0.2µl of CMhc F and T-R2 (10mM each), and 8.6µl of dH2O for Low DNA. Amplification was achieved with 40 cycles of denaturation at 94°C for 5 sec, annealing at 55°C for 1 sec, and extension at 68°C for 20 sec without initial denaturation step according to the manufacturer’s instruction of TaKaRa Taq HS Low DNA.

For *Piroplasma* screening by PCR, we used a pair of universal Apicomplexa primers to target the partial region of 18S rRNA gene (500bp); Babe-KF1 (5'-ATTAGGGTTCGATTCCGGAGAGGAGCC-3', equivalent to nucleotide numbers 314 to 344 of *B. gibsoni* [AF175300]) and Babe-MR (5'-GCTTTCGCAGTAGTTCGTCTTTAACAAATC-3', equivalent to nucleotide numbers 814 to 843 of *B. gibsoni* [AF175300]) as forward and reverse primers, respectively. The PCR reaction mixture was the same as ‘Ca. M. erythrocervae’ detection, and the reaction was carried out 40 times by a two-step cycle according to the manufacturer’s instruction of KOD FX Neo, denaturation at 98°C for 10sec and extension at 68°C for 30 sec after an initial denaturation step at 94°C for 2min.

For the preliminary screening of *Anaplasma* infection by PCR, universal primers (EH F1: 5'-AGAGTTTGATCCTGGCTCAGAACGAAC-3', equivalent to nucleotide numbers 4 to 30 of *A. phagocytophilum* [KC470064] and EH R1: 5'-TTGATTTTAGTCTTGCGACCGTAGTCC-3', equivalent to nucleotide numbers 844 to 871 of *A. phagocytophilum* [KC470064]) for the 16S rRNA gene (840bp) of *Anaplasma* were used. The PCR procedure was the same as the *Piroplasma* screening that described above, except for a 45 second extension at 68°C.

Ten-microliters of PCR products were mixed with 2µl of 6 × Loading Buffer (NIPPON GENE, Toyama, Japan) and subjected to agarose gel electrophoresis. After electrophoresis, the gels were stained in an ethidium bromide solution (0.5µg/ml) for 15 min.

Positive samples for *Anaplasma* screening PCR were further analyzed by PCR using species-specific primers for 16S rRNA of *A. phagocytophilum*, *Anaplasma* sp., and *A. bovis* to identify *Anaplasma* species as described previously [16]. A primer pair, which consisted of SSAP2f (5'-GCTGAATGTGGGGATAATTTAT-3') and SSAP2r (5'-ATGGCTGCTTCCTTTCGGTTA-3'), was used to amplify the partial region of 16S rRNA gene of *A. phagocytophilum*. AC1f (5'-CTGCTTTTAATACATGCAGACTA-3') and AC1r (5'-ATGCAGCACCTGTGTGAGGT-3') were used for *Anaplasma* sp., and AB1f (5'-CTTCGGACTCCAGTCTG-3') and AB1r (5'-TCTCCCCGACTCCAGTCTG-3') were used for *A. bovis*, respectively.

PCRs products that are positive for *Piroplasma* and *Anaplasma* using the primers F2 and R2 were then sequenced directly and compared to other relevant sequences in the GenBank DNA database by BlastN [26], and aligned with other closely related sequences using Clustal W [27]. An unrooted phylogenetic tree was generated by the neighbor-joining method [28] from a distance matrix and corrected for nucleotide substitutions by the Kimura two-parameter model [29].

After examining the prevalence of hemolytic microorganisms, the blood specimens were divided into four groups based on age and sex, and the prevalence of these hemotropic microbes was analyzed statistically. P-values were calculated using Pearson’s chi-square test. We defined the threshold for significance as P<0.05.

**RESULTS**

A total of thirty-six free-living sika deer were captured. They consisted of 18 males and 18 females, 17 adults and 19 juveniles (Table 1). All the deer seemed to be clinically and nutritionally healthy by a veterinarian’s macroscopical observation.

In the present study, 35 (97.2%) of 36 sika deer were found to be infected with at least one hemolytic microbe. Thirteen (36.1%) of the 36 blood samples that were examined were positive for ‘Ca. M. erythrocervae’, and 13 (36.1%) samples were positive for ‘Ca. M. haemocervae’. Of these, four samples were positive for both ‘Ca. M. erythrocervae’ and ‘Ca. M. haemocervae’ (Table 1). Consequently, 22 (61.1%) of the 36 sika deer were positive for hemoplasmas.

Twenty-six (72.2%) of the 36 blood samples were positive for *Piroplasma* using the PCR. BlastN [26] indicated that 25 of the 18S rRNA gene sequences were closely related to the *T. cervi* WU11 (HQ184411) that was detected in Chinese
sika deer [12], Theileria sp. Iwate (AB602888), and Theileria sp. CNY2A (AB012196) from Cervus nippon yesoensis inhabiting Hokkaido. Among these sequences, seven were 100% identical to T. cervi WU11 (HQ184411), 10 were 99.8%, four sequences were 99.6%, and the remaining five sequences showed homology of 99.4 to 96.4%. We excluded the five samples that showed homologies as low as 99.4 to 96.4% from the phyloanalysis due to the messy peak profiles observed in the chromatogram. Only ID # 27 appeared to be closely related to the Theileria sp. 3185/02 (DQ866842) that was detected in Cervidae and the chamois of Northern Spain and to the T. capreoli that was detected in the Mutiacus reevesi in China (KJ451473). Their sequence homologies were 97.9% and 97.8%, respectively. Of these obtained sequences, representative sequences that appeared to be closely related to T. cervi were submitted to GenBank with accession numbers AB981972, AB981975, AB981977, AB981978, AB981980 and AB981982 to AB981984. One sequence that was closely related to T. capreoli was submitted with the number LC060448. We tentatively named the predominant Theileria to Theileria sp. Thrivae, and the remaining ones Theileria sp. Sola. Thus, 25 (69.4%) of 36 were positive for Theileria sp. Thrivae and one (2.8%) of 36 was positive for Theileria sp. Sola (Table 1).

In the phylogenetic tree of Theileria species, Theileria sp. Thrivae formed an independent cluster with Theileria spp. (AB602888 and AB012196) and T. cervi WU11 (HQ184411), however, separated from the other Theileria species, such as T. cervi (U97056, U97054) that was isolated from white-tailed deer (Odocoileus virginianus) and elk (Cervus elaphus canadensis) (Fig.2). Theileria sp. Sola showed a close similarity to T. capreoli.

Thirty-three (91.7%) of 36 samples tested by PCR were positive for Anaplasma infection. Based on the results of the PCR, three samples were A. phagocytophilum, seven were A. bovis and 18 were Anaplasma sp. The rest of the 14 samples

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**Fig. 2** An un-rooted phylogenetic tree based on the 18S rRNA gene sequence comparison of Theileria species and sequence of three representative strains of Theileria sp. Thrivae (#7 {AB981977}, #8 {AB981978} and #16 {AB981984}) and Theileria sp. Sola (#27 {LC060448}) that were obtained in this study. Nucleotide sequences of Theileria sp. Thrivae and T. cervi detected from sika deer created independent taxa. Genetic distances were computed with CLUSTAL W [27]. Numbers in the relevant branches refer to the values of boot-strap probability of 1,000 replications. The scale bar indicates the estimated evolutionary distance.
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were screened as positive in PCR, but not identified by species-specific PCR, were subjected to another PCR procedure using the primer F2 and R2. Consequently, the 13 resulting PCR products were successfully sequenced. BlastN [26] search indicated that nine sequences had 98 to 99% homology with *A. centrale* detected in the deer and cattle (AB211164.

| ID  | Captured date | Sex | Age   | Hemoplasma CMe | Hemoplasma CMh | Piroplasma TsT | Piroplasma TsS | Anaplasma Ap | Anaplasma Ab | Anaplasma As |
|-----|---------------|-----|-------|----------------|----------------|---------------|--------------|-------------|-------------|-------------|
| 1   | 140121        | ♀   | Adult | +             |                | +             |              | +           | +           | +           |
| 2   | 140125        | ♀   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 3   | 140212        | ♀   | Adult | +             |                | +             |              | +           | +           | +           |
| 4   | 140304        | ♀   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 5   | 140311        | ♀   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 6   | 140312        | ♂   | Juvenile | +             |                |              |              | +           | +           | +           |
| 7   | 140313        | ♀   | Adult | +             |                | +             |              | +           | +           | +           |
| 8   | 140317        | ♂   | Juvenile | +             |                |              |              | +           | +           | +           |
| 9   | 140318        | ♀   | Adult | +             |                |              |              | +           | +           | +           |
| 10  | 140412        | ♀   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 11  | 140420        | ♀   | Adult | +             |                | +             |              | +           | +           | +           |
| 12  | 140426        | ♂   | Adult | +             |                | +             |              | +           | +           | +           |
| 13  | 140517        | ♂   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 14  | 140518        | ♂   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 15  | 140519        | ♀   | Adult | +             |                |              |              | +           | +           | +           |
| 16  | 140701        | ♀   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 17  | 140801        | ♀   | Adult | +             |                |              |              | +           | +           | +           |
| 18  | 140922        | ♀   | Juvenile | +             |                |              |              | +           | +           | +           |
| 19  | 140922        | ♂   | Adult | +             |                | +             |              | +           | +           | +           |
| 20  | 141008        | ♂   | Juvenile | +             |                |              |              | +           | +           | +           |
| 21  | 141026        | ♂   | Adult | +             |                | +             |              | +           | +           | +           |
| 22  | 141030        | ♂   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 23  | 141105        | ♂   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 24  | 141108        | ♀   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 25  | 141205        | ♂   | Adult | +             |                |              |              | +           | +           | +           |
| 26  | 141231        | ♂   | Juvenile | +             |                |              |              | +           | +           | +           |
| 27  | 150105        | ♂   | Juvenile | +             |                |              |              | +           | +           | +           |
| 28  | 150109        | ♂   | Adult | +             |                | +             |              | +           | +           | +           |
| 29  | 150119        | ♂   | Adult | +             |                |              |              | +           | +           | +           |
| 30  | 150120        | ♂   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 31  | 150219        | ♂   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 32  | 150222        | ♂   | Adult | +             |                | +             |              | +           | +           | +           |
| 33  | 150316        | ♂   | Adult | +             |                | +             |              | +           | +           | +           |
| 34  | 150316        | ♂   | Juvenile | +             |                | +             |              | +           | +           | +           |
| 35  | 150421        | ♂   | Adult | +             |                |              |              | +           | +           | +           |
| 36  | 150502        | ♂   | Adult | +             |                |              |              | +           | +           | +           |

| No. of infection | % of infection |
|------------------|---------------|
| 13               | 36.1%         |
| 13               | 36.1%         |
| 25               | 69.4%         |
| 1                | 2.8%          |
| 8                | 13.9%         |
| 28               | 22.2%         |
| 22               | 72.2%         |
| 26               | 88.9%         |

CMe='Candidatus M. erythrocervae'; CMh='Ca. M. haemocervae'; TsT='Theileria sp. Thrivae'; TsS='Theileria sp. Sola'; Ap='A. phagocytophilum'; Ab='A. bovis'; As='Anaplasma sp.'

*Juvenile and adult individuals were divided by body weight together with antlers or mammary grand maturity [24]*
AB588977 and AF283007) [16, 22, 30], Anaplasma sp. that are found in Japanese sika deer (AB454075) and Japanese serow (Capricornis crispus) (AB509223) in Japan [18]. Two sequences showed more than 99.6% homology with Anaplasma sp. NS108 (AB196721) and A. phagocytophilum SS33P-L (AB196721) detected in sika deer in Japan, and identified as A. phagocytophilum. One sequence showed 99.9% homology with A. bovis NR07 (AB196475) also detected in sika deer in Japan, and considered as A. bovis. Consequently, five (13.9%) of 36 deer samples were positive for A. phagocytophilum, eight (22.2%) were positive for A. bovis and 28 (77.8%) were positive for Anaplasma sp. (Table 1). In the phylogenetic tree of Anaplasma species, nine sequences formed an independent cluster with A. centrale (AB588977, AB211164, AF283007) and the other Anaplasma sp. (AB509223, AB454075). However, they were separated from other Anaplasma species (Fig. 3). Of all 16S rRNA gene sequences that were obtained, those closely related to A. centrale were submitted to GenBank with accession numbers LC002830 to LC002838. Two sequences of A. phagocytophilum were submitted under the accession number LC060986 and LC060987 and one sequence of A. bovis was submitted with the number LC060988.

Further, these blood specimens were grouped into four based on age and sex to examine the prevalence of these hemolytic microbes (Fig. 4). No suggestive trend was observed for the prevalence rate among these four groups of both haemoplasmas. Anaplasma sp. showed higher prevalence compared to other two Anaplasma species regardless of age and sex. In regard to Theileria sp. Thriva, juvenile deer had higher prevalence rate in contrast to adult ones. A statistical difference was indicated only in Theileria sp. Thriva infection between the juvenile and adult groups.
DISCUSSION

Our results show that the detection rate of hemoplasmas, *Piroplasma* and *Anaplasma* was generally higher than the rates reported previously [11, 13, 14, 16, 18, 22, 25]. In our last study, we identified ‘Ca. M. erythrocervae’ and ‘Ca. M. haemocervae’ from 13% of the free-living sika deer population [11], whereas Tagawa et al. [25] detected these hemoplasmas in 45.1% of the captive deer herd in Hokkaido. Thus, the prevalence of hemoplasmas in this study exceeded those of the previous studies including confined sika deer.

*Piroplasma*, *T. damad* and *Babesia* sp., which were found formerly in sika deer, were not identified in this research, although *Theileria* sp. Thrivae and *Theileria* sp. Sola were detected in 69.4% and 2.8% of the sika deer, respectively. Inokuma et al. [13] have reported that 72.7% of wild sika deer serve as a reservoir of *Theileria* sp. in Hokkaido. Our findings also demonstrate that *Theileria* sp. closely-related to the species Inokuma et al. [13] detected can be frequently found in sika deer inhabiting the main island of Honshu.

In using the BlastN [26] program search of the 18S rRNA gene of *Theileria* sp. Thrivae, all the sequences showed high homology with *T. cervi* WU11 detected in Chinese sika deer and *Theileria* spp. in Japanese sika deer. Furthermore, these sequences and the closely-related *Theileria* sequence formed an independent branch in the phylogenetic tree. Of those that relate to *Theileria* sp. Thrivae, *T. cervi* WU11 (HQ184411) was regarded as a novel *Theileria* species in the report published in 2012 based on the 18S rRNA gene and ITS sequence analyses [12]. Therefore, *T. cervi* WU11 (HQ184411) was considered to be a novel *Theileria* sp. in this study. *Theileria* sp. CNY2A (AB012196) detected from free-living sika deer in Hokkaido has been considered to be a novel *Theileria* species in ruminants [13]. Combined with this information, the phylogenetic analysis in this study may also support the conclusion that these *Theileria* sequences are of a novel *Theileria* sp. that is endemic to sika deer in Japan and China. With regard to *Theileria* sp. Sola, the sequence showed a close similarity to *T. capreoli* with 97.8% homology. This is the first report that the *Theileria* species related to *T. capreoli* is

Fig. 4 Comparison of infection rate of hemotropic microbes in each group divided by sex and age. AD=adult, JV=juvenile ♂ =male, ♀ =female. A statistical difference was indicated only in *Theileria* sp. Thrivae infection between adult and juvenile group (P< 0.05).
prevalent in sika deer in Japan. Further studies will be needed to identify the species of Theileria sp. Sola.

With respect to Anaplasma, Kawahara et al. [16] detected A. phagocytophilum, A. bovis, and Anaplasma sp. from sika deer in their earlier survey, with a rate of occurrence of 19%, 9%, and 12%, respectively. In addition, Sato et al. [18] identified Anaplasma sp. in Japanese Serows at an occurrence rate of 52.5%. Masuzawa et al. [22] detected A. phagocytophilum, A. bovis, and Anaplasma sp. in sika deer at an occurrence rate of 15.6%, 21.9%, and 37.5%, respectively. These results are consistent with our findings in which the detection rate for A. phagocytophilum was 13.9% and 22.2% for A. bovis, whereas the detection rate for Anaplasma sp. was significantly higher at 77.8%. Additionally, we examined the sequence homology between the primers used for the identification method [16] and the Anaplasma sp. DNA tested for phylogenetic analysis. The primer SSAP2f coincided with the A. phagocytophilum SS33P-L (AB196721), Anaplasma sp. NS108 (AB454076), #19 (LC060986) and #28 (LC060987). The primer SSAP2r corresponded to A. phagocytophilum SS33P-L (AB196721), Anaplasma sp. NS108 (AB454076), and #28 (LC060986), but varied from #19 (LC060986) by two bases. The primer AC1f coincided with all of the A. centrale, including the A. centrale Israel vaccine (AF309869), which is a type strain of A. centrale, and its relative species detected in Japan so far. In contrast, the primer AC1r corresponded to A. centrale and Anaplasma sp. that are closely related to A. centrale that has been identified in Japan, although they differ from A. centrale Israel vaccine (AF309869) and is closely related to two A. centrale strains (AF414868, AF414869) by two nucleotides. The primer AB1f showed a similarity to A. bovis Hiroshima-Z27 (HM131217) that was found in a dog in Hiroshima, although it was not homologous to A. bovis NR07 (AB196475) by five bases. The DNA region that was obtained in this study #35 (LC060988) did not include the primer AB1f. Consequently, we could not compare the homology of #35 (LC060988) to other sequences. The primer AB1r matched to A. bovis Hiroshima-Z27 (HM131217) that was detected in dogs in Hiroshima, but differed from #35 (LC060988) in this study and A. bovis NR07 (AB196475) by the single base.

On the whole, our results suggest the existence of A. bovis strain that cannot be detected by the PCR method with this primer set. Further examination also may be required for detection of the A. centrale type strain. In addition, the reason that we failed an Anaplasma characterization for the fourteen samples using primers of Kawahara et al. [16] in this study can be attributed to the lower sensitivity of our PCR than that of the nested-PCR method by Kawahara et al. [16]. A BlastN [26] program search of the obtained Anaplasma sp. 16S rRNA gene sequences showed high homology to Anaplasma sp. sequences that were detected in Japan. Furthermore, these obtained sequences and the closely related Anaplasma sp. sequences formed an independent branch in the phylogenetic tree apart from the type strain A. centrale Israel vaccine (AF309869) sequence. Although further analysis would be required, it seems logical to believe that there is an unidentified Anaplasma enzootic among wild ruminants and domestic cattle in Japan.

When comparing the infection rate of subgroups that are divided by age, statistically significant difference were recognized only for Theileria sp. Thrivae. All of the juvenile groups had a higher prevalence than the adult group, except for hemoplasmas. The causes of the difference among age groups needs to be investigated further. However, such age-dependent infection tendencies have been shown to be associated with Relapsing fever borreliae leading to persistent infection [31]. Similarly, hemoplasmas, Theileria and Anaplasma have been revealed to cause persistent infection in the host animals [32-34], which may be the reason that more infections were observed among young individuals.

Our study indicated that total hemolytic microorganisms infection of sika deer in a densely populated area was very high (97.2%) and that the infection rate varies widely by the microbes, from 2.8% for Theileria sp. Sola to 77.8% for Anaplasma sp.. The infection rates of Hemoplasmas, Theileria sp. Thrivae, and Anaplasma sp. were higher than those of previously reported. However, further study is warranted, since the pathogenicity of these microorganisms to sika deer and livestock as well as to human is not yet known. In contrast, A. phagocytophilum and A. bovis, pathogenic in cattle and humans [34] showed a similar rate to those of previous studies. Additionally, newly identified Theileria sp. Sola was found in only one case. Mixed infection of hemoplasmas were detected in four deer, and nine showed mixed infection of Anaplasma, suggesting the absence of cross protection, as mentioned in preceding literature [16].

Overall, in regard to Theileria and Anaplasma infections, it seems that the dominant parasitic species are transmitted...
much more easily than other infrequent species among free-ranging sika deer living in the same habitat. Although its mechanism is still unknown, further investigation, including determining the affinity of each microorganism to sika deer, or variations and the infection status of the ticks that are distributed in the studied area, might provide more detailed information on this issue.

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原著 細菌学

高密度生息地域で捕獲した野生ニホンジカ（Cervus nippon）におけるダニ媒介性病原体の保有状況

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要 約
福井県の高密度生息地域で捕獲した 36 頭の野生ニホンジカから血液を採取し、ダニ媒介性の住血液病原体であるヘモプラズマ、ピロプラズマ、アナプラズマの保有状況を調査した。36 頭中、13 頭（36.1%）に ‘Candidatus Mycoplasma erythocervae’、13 頭（36.1%）に ‘Ca. M. haemocervae’、25 頭（69.4%）に Theileria sp. Thrivae、1 頭（2.8%）に Theileria sp. Sola、5 頭（13.9%）に Anaplasma phagocytophilum、8 頭（22.2%）に A. bovis、28 頭（77.8%）に Anaplasma sp. の感染が認められ、高い感染状況にあることが確認された。本調査にて得られた Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Theileria sp. Thrivae の塩基配列の系統解析によると、過去にニホンジカから検出された Th

キーワード：アナプラズマ，タイレリア，ダニ，ニホンジカ，ヘモプラズマ

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