NOTE  Bacteriology

Mycoplasma ovis Detected in Free-Living Japanese Serows, Capricornis crispus

Yoshihiro OHTAKE1), Ikuo NISHIZAWA1), Makoto SATO1), Yusaku WATANABE1), Takashi NISHIMURA2), Kazuei MATSUBARA2), Kazuya NAGAI3) and Ryô HARASAWA1)*

1)Departments of Veterinary Microbiology, 2)Animal Science and 3)Cryobiofrontier Research Center, Faculty of Agriculture, Iwate University, Morioka 020–8550, Japan

(Received 5 September 2010/Accepted 2 October 2010/Published online in J-STAGE 15 October 2010)

ABSTRACT. Nineteen blood samples collected from free-ranging wild Japanese serows, Capricornis crispus, between 2006 and 2008 in Iwate prefecture were examined for the hemoplasma infection by real-time PCR targeting the 16S rRNA gene. Five (26.3%) out of the 19 samples were positive in real-time PCR with an average melting temperature at 85.18°C. The positive samples in the real-time PCR were reconfirmed by conventional PCR, and one of them was successful for direct DNA sequencing. The nucleotide sequence of the 16S rRNA gene of the representative strain was identical to that of Mycoplasma ovis. This was the first demonstration of hemotropic mycoplasma infections among the free-living Japanese serows in Japan.

KEY WORDS: hemoplasma, Japanese serow.


Hemoplasmas are epierythrocytic mycoplasmas, but have never been cultured in vitro [8]. Hemoplasmas infections have been reported in wild ungulates such as reindeer (Rangifer tarandus) raised on a farm in Michigan [14] and splenectomized deer captured from a wild population in Texas [7] in the United States. However prevalence of hemoplasma infections in free-living ungulates has not well been understood. Recently, we have newly detected hemoplasma infections in wild sika deer in Iwate Prefecture [17]. In the present study, we subsequently examined for hemoplasma infections in free-living Japanese serows (Capricornis crispus).

Whole blood samples collected from 19 free-living Japanese serows during three years from 2006 to 2008 in the Iwate prefecture were examined for the presence of hemoplasmas by the real-time PCR procedure using the hemoplasma specific primers to amplify the 16S rRNA gene region. Heparinized-blood or clots of blood samples were stored at –20°C prior to analysis. Smear preparations were not available because of frozen blood samples. Total DNA was extracted from 200 μl of the whole blood samples by using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions, eluting into 200 μl of buffer AE, and stored at –20°C until examination in the PCR assays.

Real-time PCR was performed in a SmartCycler instrument (Cepheid, Sunnyvale, Calif., U.S.A.) with SYBR Premix Ex Taq (Code #RR041A, TaKaRa Bio., Shiga). Hemoplasma specific PCR primers (forward primer: 5'-ATATTCCTACGGGAAGCAGC-3' and reverse primer: 5'-ACCGCAGCTGCTGGCACATA-3') for the 16S rRNA gene of hemoplasmas were used as described previously [12]. After real-time PCR, melting experiment was performed from 60 to 95°C at 0.2°C/sec with smooth curve setting averaging one point, by using a nonspecific fluorescent dye, SYBR Green I. Melting peaks were visualized by plotting the first derivative against the melting temperature (Tm) as described previously [3]. By Preliminary screening by real-time PCR detected five (26.3%) out of the 19 samples were positive for hemoplasma infections, and an average Tm was 85.18°C (Fig. 1). High-resolution of melting curve experiments allow discrimination of variations in nucleotide sequences, which indicate specificity of the real-time PCR [11].

The positive samples were re-examined by conventional PCR targeting the 16S rRNA gene for nucleotide sequencing. The conventional PCR was carried out with 50-μl reaction mixtures containing 1 μl of DNA solution, 0.5 μl of TaKaRa LA Taq™ (5 units/μl), 5 μl of 10X LA PCR™ Buffer, 8 μl of 25 mM MgCl₂ (final 4.0 mM), 8 μl of dNTP mixture (2.5 mM each), 0.2 μl of forward primer (5'-ATATTCTACGGGAAGCAGC-3', equivalent to nucleotide numbers 328 to 347 of M. wenyonii), reverse primer (5'-TACCTTGGTACGACTTA-3', equivalent to nucleotide numbers 328 to 347 of M. wenyonii, Hilden, Germany) according to the manufacturer’s instructions, eluting into 200 μl of buffer AE, and stored at –20°C until examination in the PCR assays.

Fig. 1. Melting curve analysis of the PCR product depicted by using SYBR Green I. A representative Tm curve for Kamosika3 strain detected from a Japanese serows, showing a characteristic peak at 85.17°C in the melting experiments.

a) Correspondence to: Prof. Harasawa, R., Department of Veterinary Microbiology, Faculty of Agriculture, Iwate University, Morioka 020–8550, Japan.
e-mail: harasawa-tyk@umin.ac.jp
nucleotide numbers 1446 to 1465 of *M. wenyonii* (50 pmol/μl each) and water to a final volume of 50 μl. After the mixture was overlaid with 20 μl of mineral oil, the reaction cycle was carried out 35 times with denaturation at 94°C for 30 sec, annealing at 60°C for 120 sec and extension at 72°C for 60 sec in a thermal cycler.

The PCR products were fractionated on horizontal, submerged 1.0% SeaKem GTG agarose gels (FMC Bioproducts, Rockland, Me., U.S.A.) in TAE (40 mM Tris, pH8.0, 5 mM sodium acetate, 1 mM disodium ethylenediaminetetraacetate) buffer at 50 volts for 60 min. After electrophoresis, the gels were stained in ethidium bromide solution (0.4 μg/ml) for 15 min. DNA was extracted by using NucleoSpin Extract II kit (Macherey-Nagel, Düren, Germany) and was subjected to direct sequencing in a 3500 Genetic Analyzer (Applied Biosystems, Foster City, Calif., U.S.A.). Only one sample developed a clear nucleotide sequence. This suggests that the other samples were of mixed-infection. The nucleotide sequence of the partial 16S rRNA gene has been deposited in the DDBJ, EMBL, GSDB and NCBI nucleotide sequence databases under the accession number AB571119.

In the present study, nucleotide sequences of the 16S rRNA gene from the Kamosika3 strain along with the 13 established hemoplasma species were aligned using CLUSTAL W (version 1.83; DDBJ) with further adjustment made manually by eyes as necessary [16]. An unrooted phylogenetic tree constructed by the algorithms implemented in the PHYLIP program (DDBJ) using the neighbor-
joining method [13] indicated that the hemoplasma strain detected in the Japanese serows was most closely related to *Mycoplasma ovis* (Fig. 2) which was also close to ‘Candidatus *M. haemovis’ [4]. Similarity of the 16S RNA gene sequences between Kamosika3 strain and *M. ovis* ranged from 99.5 to 99.8%, while 96.3 to 96.6% between Kamosika3 and ‘Candidatus *M. haemovis’.

The Japanese serows, one of Japan’s official national treasures, usually live on steep, thickly wooded hillsides on the islands of Honshu, Kyushu and Shikoku in Japan. All the Japanese serows examined were found in Morioka and Rikuzentakata or their vicinal areas. They have been problematic for taxonomists due to their unique characteristics. Although the serows were tentatively given the generic name *Nemorhaedus* [2], recent study indicates that the Japanese serows are distinct from gorals, the genus *Nemorhaedus*, and should be allocated to the genus *Capricornis* [9]. The serows and sheep (*Ovis aries*) are both the members in the subfamily *Caprinae* of the family *Bovidae*.

*Mycoplasma ovis* is prevailed world-widely and causative of hemolytic anemia in sheep and goats of all ages from 4 weeks upwards [1, 10], resulting in economic losses. Young sheep, particularly weaners, are most severely affected but older sheep can also be noticeably affected. The effect of *M. ovis* infection is more severe if sheep are stressed by other conditions such as internal parasites or malnutrition. Deaths may occur in severely affected young sheep, if they are stressed by yarding. Role of *M. ovis* in wild ungulates is largely unknown, though there is some evidence that variants of this particular hemoplasma species have also been isolated from humans [5, 15], suggesting a zoonotic pathogen. We first demonstrated *M. ovis* infections in free-living Japanese serows. Therefore, it will be necessary to further examine that the wildlife could serve as a reservoir for hemoplasma infections in not only domestic animals, but also humans, though there has never been reported on *M. ovis* infections in sheep or goats in this country.

REFERENCES


