ЭКСПЕРИМЕНТАЛЬНЫЕ ИССЛЕДОВАНИЯ В БИОЛОГИИ И МЕДИЦИНЕ

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COMPARATIVE STUDY OF ANAPLASMA IN JAPAN AND OTHER COUNTRIES

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To determine the reservoir animals and vector ticks for Anaplasma phagocytophilum in Far East Asia, which causes human granulocytic anaplasmosis, we analyzed tissue samples from deer and boars in Japan, rodents in Taiwan and Ixodes persulcatus in Russia by PCR-targeted to 16S rDNA. Anaplasma species including Anaplasma bovis and Anaplasma centrale-infected wild deer and boars were detected. The detection rates for A. phagocytophilum, A. bovis and A. centrale in deer were 15,6 %, 21,9 % and 37,5 %, respectively. These infection rates in wild boar were 3,6 %, 17,9 % and 3,6 %, respectively. Wild rodents captured in Taiwan were positive for A. phagocytophilum and A. bovis. Prevalence rate of A. phagocytophilum on I. persulcatus ticks in Irkutsk and in Khabarovsk were 6,3 % and 11,3 %, respectively. The 16S rDNA sequences detected from Russian ticks were identical to those of A. phagocytophilum detected in US and Europe, and from tick Ixodes ovatus and Ixodes persulcatus in Japan. However the sequence detected from deer and boars in Japan were identical to sequences previously detected from deer and cattle in Japan, and showed less similarity (98,6 %) with typical A. phagocytophilum. Sequences detected from wild rodents collected in Taiwan showed higher similarity (99,7 %) with typical A. phagocytophilum, but formed the branch from those of A. phagocytophilum detected in US and Europe. The finding suggests that the A. phagocytophilum-related sequence detected from deer and boars in Japan, and wild rodents in Taiwan were different from those of typical A. phagocytophilum found in Ixodid ticks.

Key words: *Anaplasma phagocytophilum, boar, deer, Rattus*

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ АНАПЛАЗМ В ЯПОНИИ И ДРУГИХ СТРАНАХ

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С целью установления животных-резервуаров и клещей-переносчиков бактерий Anaplasma phagocytophilum — возбудителя гранулоцитарного анаплазмоза человека — на дальнем востоке Азии мы проанализировали образцы тканей оленей и кабанов из Японии, грызунов из Тайваня и клещей Ixodes persulcatus в России с помощью ПЦР, направленной на амплификацию гена 16S rRNA. Дикие олени и кабаны были заражены Anaplasma bovis и Anaplasma centrale. Зараженность диких оленей A. phagocytophilum, A. bovis and A. centrale составила 15,6, 21,9 и 37,5 % соответственно. Среди диких кабанов зараженность этими микроорганизмами составила 3,6 %, 17,9 % и 3,6 % соответственно. Дикие грызуны, отловленные на Тайване, были заражены A. phagocytophilum и A. bovis. Зараженность клещей I. persulcatus в Иркутске и Хабаровске составила 6,3 и 11,3 % соответственно. Нуклеотидные последовательности гена 16S rRNA микроорганизмов, обнаруженных в клещах из России, были идентичны последовательностям A. phagocytophilum, обнаруженным в США и Европе, а также последовательностям A. phagocytophilum из клещей Ixodes ovatus и Ixodes persulcatus из Японии. Однако, последовательности из оленей и кабанов Японии были идентичны A. phagocytophilum, выделенным ранее от оленей и крупного рогатого скота в Японии и обладали меньшей схожестью (98,6 %) с типичными A. phagocytophilum. Последовательности, обнаруженные в грызунах на Тайване, были более сходны с типичными A. phagocytophilum (99,7 %), однако на филогенетическом древе формировали ветвь, отдельную от A. phagocytophilum из Европы и США. Данные результаты позволяют предположить, что A. phagocytophilum, инфицирующие диких оленей и кабанов в Японии и грызунов на Тайване, существенно отличаются от типичных A. phagocytophilum, инфицирующих иксодовых клещей.

Ключевые слова: *Anaplasma phagocytophilum, кабан, олень, Rattus*

Anaplasmosis is a tick-borne infectious disease of cattle, sheep, horse and other domestic ruminants caused by a gram-negative bacterium, *Anaplasma* species. Among *Anaplasma* species, *Anaplasma phagocytophilum* has been recognized as a tick-borne fever agent of cattle, sheep, goats and horses in the United States and Europe [4]. In 1990, it has been recognized that *A. phagocytophilum* potentially causes illness in humans (human granulocytic anaplasmosis, HGA) in the United States [1]. Roe and red deer and wild boar has been recognized for reservoir host for *A. phagocytophilum* in Europe. We previously demonstrated *A. phagocytophilum* infection in *Ixodes persulcatus* and *Ixodes ovatus* in Japan by PCR [3]. The 16S rDNA sequence detected from *I. ovatus* and *I. persulcatus* in Japan showed higher similarity with *A. phagocytophilum* isolated from US patient. The aim of this study was to determine reservoir host of *A. phagocytophilum* in Japan and Taiwan.

MATERIALS AND METHODS

Total DNA was extracted from the disrupted blood and/or spleen samples of 56 wild boars (*Sus scrofa*) and 32 Sika deer (*Cervus nippon*) captured in Japan, 138 wild small mammals (7 species) using QuickGene-800 Nucleic-acid Isolation System. To detect specific DNA of *Anaplasma* species, 16S rDNA was amplified with forward primer EC9 and reverse primer EC12A [2] in the first-step PCR. For the nested PCR, mixtures of forward primer Abpp (5′-TACTGCCAGACTAGAGTCCGGGA-3′) specific to sequences for *Anaplasma bovis, A. phagocytophilum,* and *Anaplasma platys,* and another forward primer Acom (5′- TACTGCAGGACTAGAGTCCGGAA-3′) specific to sequences for *Anaplasma centrale, Anaplasma ovis* and *Anaplasma marginale*, and reverse primer AP-R (5′- TTGCAACCTATTGTAGTC-3′) were used as inner primer sets, which amplified approximately 600 base pairs of DNA. For DNA sequencing analysis, the nested PCR amplicon purified with Microcon-PCR purification column (Millipore) was subjected to DNA cycle-sequencing analysis using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) with an ABI 3130-Avant Genetic Analyzer. A phylogenetic tree was constructed on the basis of the alignment of 16S rDNA sequences (600bp) and the previously published sequences by Clustal W algorithm using sequence analysis software, MegAlign (DNASTAR).

RESULTS AND DISCUSSION

Prevalence rate of *A. phagocytophilum* on *I. persulcatus* ticks in Irkutsk and in Khabarovsk were 6,3 % (28/445) and 11,3 % (23/204), respectively. A phylogenetic tree was constructed on the basis of 16S-rDNA sequence. The 16S rDNA sequences detected from Russian ticks were identical to those of *A. phagocytophilum* detected in US and Europe, and from tick *Ixodes ovatus* and *Ixodes persulcatus* in Japan.

Infection rates for *A. phagocytophilum*, *A. bovis* and *A. centrale* in Sika deer in Japan were 15,6 %, 21,9 % and 37,5 %, respectively. These rates in wild boar were 3,6 %, 17,9 % and 3,6 %, respectively. All positive results were obtained from whole blood samples, but not from spleen samples. *A. phagocytophilum* detected from deer and boars form cluster with these sequences previously found from deer (AB196720, AB196721, AB454076) and cattle (EU368727, EU368728) in Japan and that from dog in South Africa (AY570540). However, these branched from those detected from a patient in USA (AF093789), horse in Sweden (AY527214) and USA (AF172164), *I. ovatus* (AY969012) in Japan and *I. persulcatus* in Russia (k-7, 7-109, HM366590). Furthermore, these 16S rDNA-PCR positive samples gave negative result on *p44/msp2* PCR targeted to outer membrane protein gene. The finding suggests that the agents detected are genetically related, but different from the *A. phagocytophilum* that cause HGA in USA and European countries, and also those detected from ixodid ticks in Japan and Russia.

16S rDNA sequence related to *Anaplasma* species were detected from wild rodent, *Rattus losea* among wild small mammals (7 species, *R. losea, Apodemus agrarius, Mus fromosanus, Mus caroli, Bandicota indica, Suncus murinus, Crocidura suaveolens*). These sequences (99-73, 99-58) were identical to those detected from *R. losea* (HM439430) and *Niviventer coxingi* (DQ458808) in China and showed high similarity (99.7 %) with typical *A. phagocytophilum* detected from Ixodid ticks in US, Europe and Asia.

During the study, we detected *Anaplasma* species sequences identified as *A. centrale* and *A. bovis*, respectively. *A. centrale* was found to be the only bovine anaplasmosis pathogen in Japan and usually causes mild anemia. Ten and 2 sequences detected from deer and boars, respectively, were identical to that of *A. centrale* detected in cattle (AF283007), and Sika deer in in Japan (AB21116). The sequences detected in Japan blanched from these sequences detected in Europe EF520686, EF520690) and Africa (AF414869) (similarity value 98.5-98.6 %), indicating genetic differences between the pathogen in Japan and those in other countries. This classification remains as a problem that should be study. Furthermore, *A. bovis* sequences were detected from deer and also boars. *A. bovis* has been previously detected in Sika deer (AB21116), cattle (FJ169957), and vector ticks, *Haemaphysalis longicornis* (AB196475) and *Haemaphysalis megaspinosa*. *A. bovis* mostly infects cattle in Africa and is phylogenetically more closely related to *A. phagocytophilum* than *A. centrale*.

Further study is needed to confirm reservoir animals for *A. phagocytophilum*, and genetic characterization of *A. phagocytophilum* in Japan and Russia to understanding the status of diseases associated with these agents.

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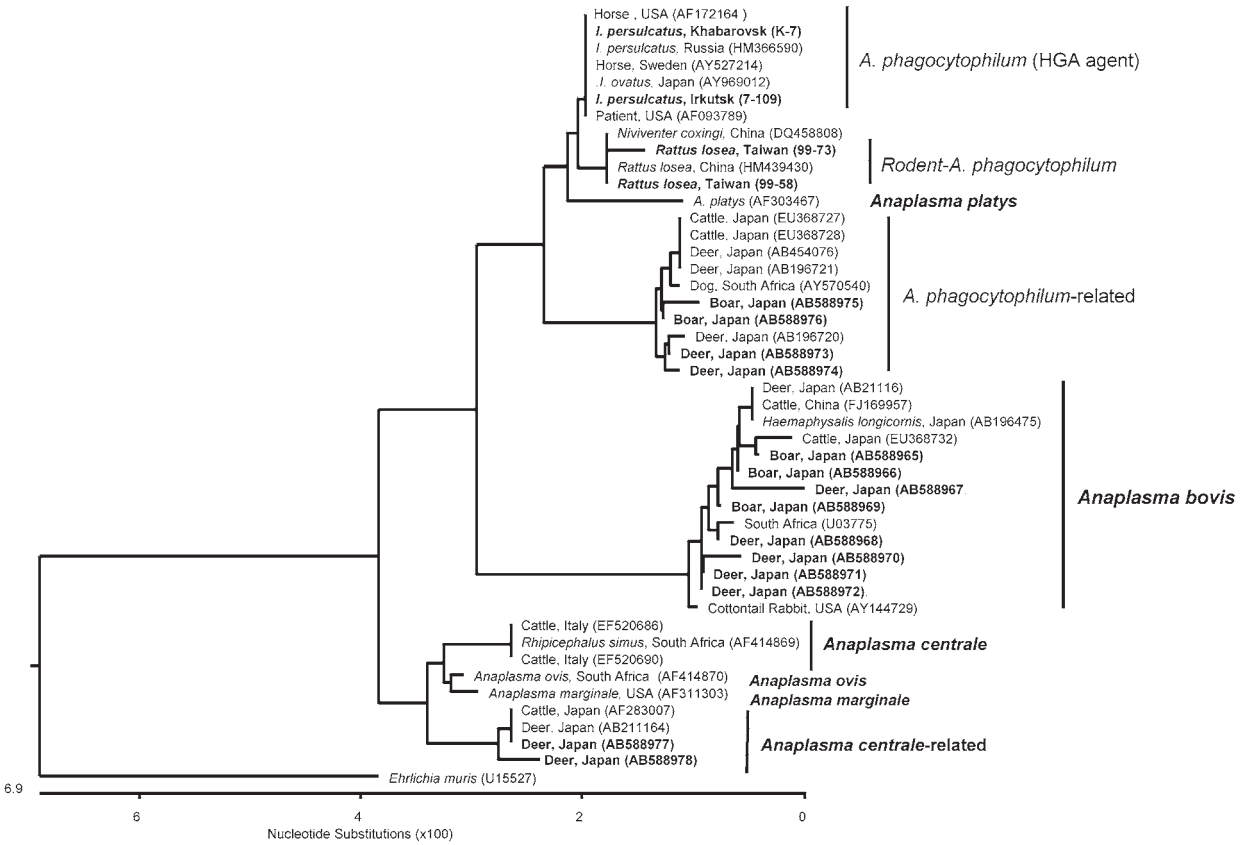

Fig. 1. Phylogenetic tree constructed on the basis of 16S rDNA sequences.

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The phylogenetic tree was constructed using the alignment of 16S rDNA sequences by Clustal W algorithm followed by the neighbor-joining method with 1000 bootstrap resamplings. Sequence accession numbers are in parentheses. Bold type indicates sequences determined by this study.

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