ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

*Korean J Parasitol* Vol. 53, No. 6: 765-769, December 2015
http://dx.doi.org/10.3347/kjp.2015.53.6.765

▣ BRIEF COMMUNICATION

# Detection of *Anaplasma* sp. in Korean Native Goats (*Capra aegagrus hircus*) on Jeju Island, Korea

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**Abstract:** *Anaplasma* species are obligate intracellular pathogens that can cause tick-borne diseases in mammalian hosts. To date, very few studies of their occurrence in Korean native goats (*Capra aegagrus hircus*) have been reported. In the present study, we investigated *Anaplasma* infection of Korean native goats on Jeju Island, Republic of Korea, and performed phylogenetic analysis based on the 16S rRNA gene sequences. Our results showed that *Anaplasma* infection was found mostly in adult female goats. The phylogenetic tree revealed that the 7 sequences identified in Korean native goats could belong to *Anaplasma* sp. and were distinct from *A. marginale*, *A. centrale*, and *A. ovis*. The results indicated that the sequences identified to belong to *Anaplasma* were closely related to sequences isolated from goats in China and were clustered within the same group. To our knowledge, this is the first study to detect *Anaplasma* sp. infection in Korean native goats.

Key words: *Anaplasma*, Korean native goat, phylogenetic analysis, 16S rRNA gene

Anaplasmosis is a tick-borne disease caused by *Anaplasma* species, which are obligate intracellular pathogens that infect humans and animals [1,2]. Clinical manifestations of the disease include anemia, fever, weight loss, decreased milk production, abortion, and frequently, death. The genus *Anaplasma* is composed of 6 species that vary in host preference and cell tropism. *A. marginale*, *A. centrale*, and *A. ovis* infect the red blood cells of ruminants [3-5]. *A. bovis* infects the monocytes of ruminants and small mammals [6]. *A. phagocytophilum* is the causative agent of human granulocytic anaplasmosis and infects the neutrophils of humans, ruminants, dogs, and horses [7,8]. Finally, *A. platys* is a platelet pathogen that infects dogs, and can cause canine cyclic thrombocytopenia [9].

Of these *Anaplasma* species, *A. marginale*, *A. centrale*, and *A. ovis* are prominent pathogens in ruminants worldwide [3,10,11]. In particular, *A. marginale* and *A. ovis* have considerable economic importance in tropical and subtropical areas [4]. *A. marginale* is known to be highly pathogenic in cattle, while *A. centrale* is less pathogenic. *A. ovis* is moderately pathogenic in sheep, goats, and wild ruminants [1,12] and causes acute disease in animals exposed to stress or other predisposing factors such as hot weather, deworming, tick infestation, and animal movement [13].

The importance of anaplasmosis in small ruminants in the Republic of Korea (ROK) is not yet known. The infection in sheep and goats is usually asymptomatic; however, it can sporadically cause hemolytic anemia and hemoglobinuria. *Anaplasma* infection may likely be neglected because of its low economic importance in the goat production industry of the ROK. Although there have been previous reports of *Anaplasma* infection in Korean native goats (*Capra aegagrus hircus*) [14,15], epidemiological studies on *Anaplasma* infection in these animals have not been well reported. Therefore, the objective of this study was to investigate *Anaplasma* infection in Korean native goats pastured on Jeju Island where ticks are most widely distributed and to perform molecular characterization of *Anaplasma* species detected on Jeju Island.

Whole blood samples from 39 Korean native goats on Jeju

• Received 5 July 2015, revised 5 September 2015, accepted 30 September 2015.
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Island, ROK, were collected in April 2014. This herd typically grazed in the pasture for at least 1 season every year; that is, the animals were housed in stables in the cooler months (November-April), whereas in the warmer months (May-October), they grazed in the pasture. All goats were clinically healthy and no blood-sucking ticks were found on them. Blood samples were collected and immediately frozen at -80℃ until DNA extraction was performed.

Genomic DNA was extracted from whole blood samples using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions. For the detection of *Anaplasma* infection, PCR was performed using the *AccuPower*® Rickettsiales 3-Plex PCR Kit (Bioneer, Daejeon, Korea). Specific primer sets targeting the 16S rRNA were used to detect species belonging to *Anaplasma* (F, 5′-TACCTCTGTGTTGTAGCTAACGC-3′; R, 5′-CTTGCGACATTGCAACCTATTGT-3′), *Ehrlichia* (F, 5′-CGGAATTCCTAGTGTAGAGG-3′; R, 5′-AGGAGGGATACGACCTTC AT-3′), and *Rickettsia* (F, 5′-TAGGGGATGATGGAATTCCTA-3′; R, 5′-CCCCCGTCA ATTCCTTTGAG-3′). The predicted sizes of the amplified PCR products for *Anaplasma*, *Ehrlichia*, and *Rickettsia* were 429 bp, 340 bp, and 252 bp, respectively. The following cycling conditions were used: 95℃ for 15 min; 40 cycles of 95℃ for 10 sec, 58℃ for 30 sec, and 72℃ for 30 sec; and final extension at 72℃ for 5 min. PCR products were separated by gel electrophoresis on 1.5% agarose gels and visualized by staining with ethidium bromide.

The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen). The nucleotide sequences were determined by direct sequencing of the PCR products using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) and analyzed on ABI PRISM® DNA Analyzer (Applied Biosystems). The sequence data were aligned initially using the Clustal X program (version 1.8) [16]. Additional sequences from representative anaplasmosis isolates were obtained from the GenBank database and then integrated with each set of alignments. A phylogenetic tree based on the nucleotide alignment was constructed using the neighbor-joining method [17]. Bootstrap analysis was carried out with 1,000 replications and the tree was visualized using Treeview.

Statistical analyses were performed by one-way ANOVA with IBM SPSS Statistics (version 19.0) and GraphPad Prism (version 6.0). In all statistical tests, $P$-value of $<0.05$ were considered significant.

*Anaplasma* infection was analyzed in a population of 39 Korean native goats; blood samples from 7 animals (17.9%) tested positive for *Anaplasma* by 16S rRNA gene-based PCR. Infections with *Ehrlichia* and *Rickettsia* were not detected. None of the goats exhibited any clinical signs of illness, and no ticks were found on these animals. Additionally, no hematological abnormalities were observed. The incidence of *Anaplasma* infection in Korean native goats in all age groups and in male and female goats was investigated. *Anaplasma* infection was detected in female goats only (29.2%; Table 1), and the incidence was 38.9% in goats of 12 months of age (Table 2). *Anaplasma* infection was not detected in male goats or in other age groups.

Seven 16S rRNA gene sequences of *Anaplasma* sp. (GenBank Accession No. KR024571-KR024577) were obtained from the 7 *Anaplasma*-positive blood samples after direct sequencing. The sequence analysis of these 7 samples led to the identification of *Anaplasma* sp. The phylogenetic tree analysis revealed that there were 3 main clusters for the established *Anaplasma* spp.: *A. marginale*, *A. ovis*, and *A. centrale* clusters. The 7 sequences obtained from Korean native goats on Jeju Island formed a fourth, separate cluster, that is, *Anaplasma* sp., which diverged from *A. marginale*, *A. centrale*, and *A. ovis*, but was closely related to *A. centrale* (Fig. 1). These isolates had a 98% similarity with *A. centrale*, 96.4-97.5% similarity with *A. ovis*, and 96.9-97.8% similarity with *A. marginale*. The phylogenetic analyses showed that the 7 *Anaplasma* sp. identified in Korean native goats could potentially be new species, mainly because

**Table 1.** Comparison of the number of *Anaplasma*-positive Korean native goats (*Capra aegagrus hircus*) by sex

| Sex | No. of goats | No. of positive (%) |
|---|---|---|
| Male | 15 | 0 (0.0) |
| Female | 24 | 7 (29.2) |
| Total | 39 | 7 (29.2) |

**Table 2.** Comparison of the number of *Anaplasma*-positive Korean native goats (*Capra aegagrus hircus*) by age

| Age (months) | No. of goats | No. of positive (%) |
|---|---|---|
| 2 | 5 | 0 (0.0) |
| 3 | 5 | 0 (0.0) |
| 5 | 5 | 0 (0.0) |
| 10 | 5 | 0 (0.0) |
| 12 | 18 | 7 (38.9) |
| 24 | 1 | 0 (0.0) |
| Total | 39 | 7 (38.9) |

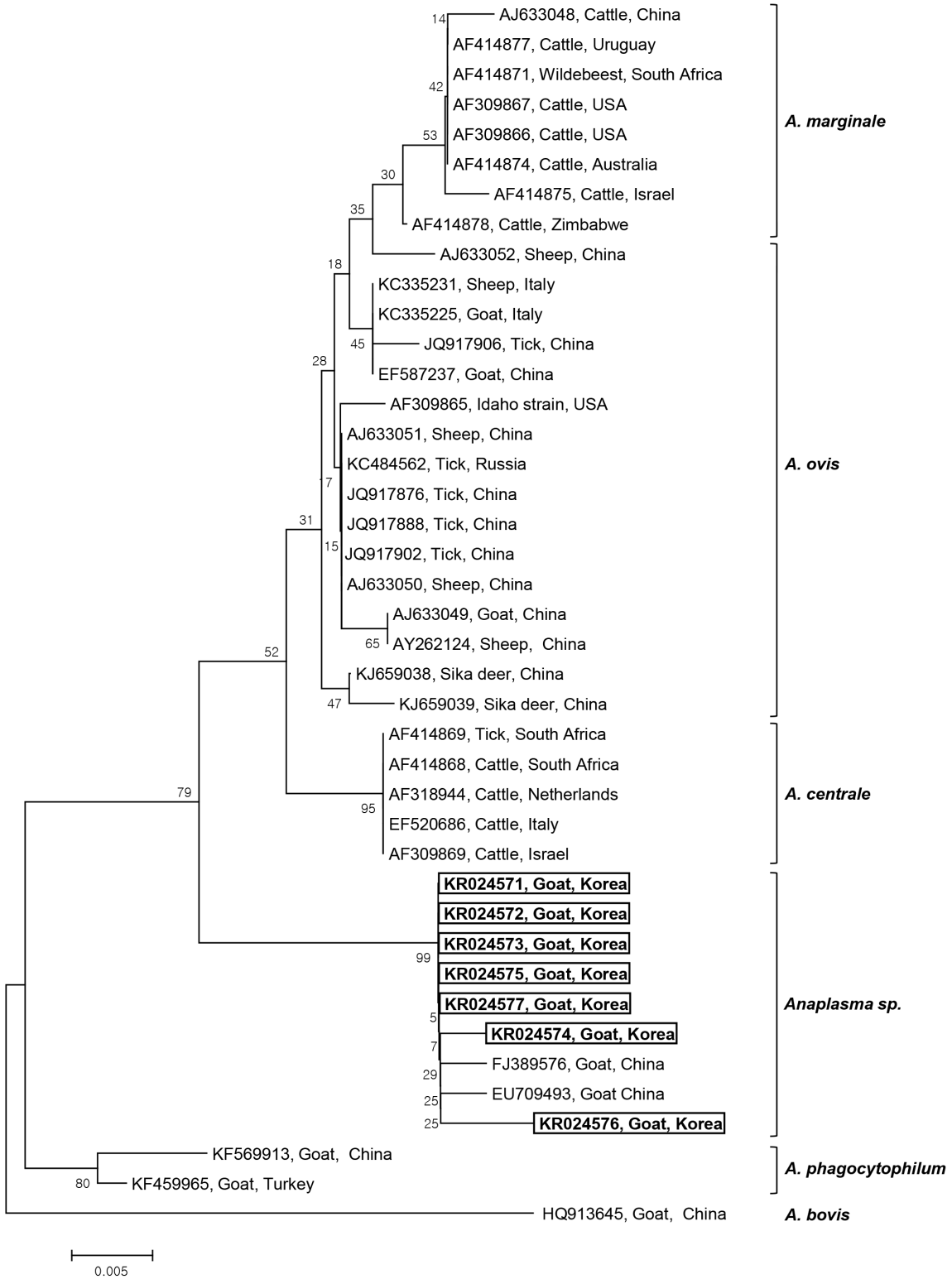


**Fig. 1.** Phylogenetic analysis of 16S rRNA gene sequences derived from 7 *Anaplasma*-positive blood samples obtained from Korean native goats (*Capra aegagrus hircus*) on Jeju Island, ROK, together with previously registered sequences from *Anaplasma* species. The 16S rRNA gene sequences identified in the present study are shown in the black-lined box. The unrooted phylogenetic tree was constructed using the neighbor-joining methods. Bootstrapping was carried out with 1,000 replications.

they clustered separately from the 3 established species, *A. marginale*, *A. centrale*, and *A. ovis*. Our 7 sequences are quite similar to the species isolated from goats in China (EU709493 and FJ389576), which also belong to *Anaplasma* sp., and are clustered in the same group. To our knowledge, this is the first study to identify *Anaplasma* sp. infection in Korean native goats by PCR.

Although anaplasmosis is one of the most widespread tick-borne diseases, very little is known about *Anaplasma* infection and its distribution in the ROK. Most studies concerning *Anaplasma* in the ROK have addressed ticks and humans [18,19], while the role of *Anaplasma* in goat pathology has not yet been described. In our study, a high prevalence of *Anaplasma* infection was observed in female and adult goats although it was not statistically significant. This result indicates that adult goats may have more opportunities for exposure to ticks carrying the pathogen than younger animals. Additionally, our findings suggest the presence of *Anaplasma* infection in Korean native goats on Jeju Island, which has a subtropical climate and a much higher distribution of ticks than other regions in the ROK. These conditions increase the possibility of tick-borne diseases spreading to livestock, wild animals, and humans.

In the present study, PCR and sequence analyses based on the 16S rRNA provided evidence of a new *Anaplasma* sp. infecting Korean native goats; these isolates were closely related to the previously reported Chinese isolates. The pathogenicity and role of *Anaplasma* sp. was not determined in Korean native goats; however, infection with this protozoan may have an impact on the health of these animals and consequently on their milk and meat production. Evidence of infection with a new *Anaplasma* sp. infection in Korean native goats would be very important, as the pathogens could considerably affect animal production, and outbreaks may occur under specific conditions. Accordingly, these findings indicate infection with a new species from the genus *Anaplasma* among Korean native goats on Jeju Island, ROK.

The current study demonstrates the presence of infection with *Anaplasma* sp. in Korean native goats, although none of the animals in our study exhibited clinical symptoms. Since the sample size was not sufficiently large to determine the incidence of *Anaplasma* infection, more studies are necessary to investigate epidemiological data and to elucidate the pathogenicity of *Anaplasma* sp. in these animals.

## ACKNOWLEDGMENT

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (project no. PJ010092)", Rural Development Administration, the Republic of Korea.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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