

## Evidence of *Anaplasma* spp. exposure in native Korean goats (*Capra hircus coreanae*)

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**ABSTRACT:** Anaplasmosis in animals is caused by *Anaplasma* spp. including *A. phagocytophilum*, *A. marginale*, *A. centrale*, *A. ovis*, and *A. bovis*, which are obligate intracellular rickettsial pathogens transmitted by ticks. Infection in animals is considered an important constraint on livestock production. In Korea, the prevalence of *Anaplasma* spp. has been investigated in several species, including cattle, dogs, and rodents, but there are no available data on anaplasmosis in goats. The purpose of this study was to investigate the presence of *Anaplasma* spp. in native Korean goats (*Capra hircus coreanae*) using a commercial competitive ELISA which specifically detects antibodies against *A. marginale*, *A. centrale*, and *A. ovis*. A total of 36 (6.6%) of 544 goat serum samples tested seropositive for *Anaplasma* spp. With regard to age, 4.9% (7/144), 9.5% (27/283), and 1.7% (2/117) of samples tested seropositive in the young (< 1 year), adult (≥ 1 year), and unknown age groups, respectively, with significant differences among groups ( $P < 0.05$ ). The seroprevalence by region was 1.7% (2/121), 2.6% (2/77), and 9.2% (32/346) in the northern, central, and southern regions, respectively, with significant differences among regions ( $P < 0.05$ ). With regard to the season of sample collection, 3.3% (4/122) and 7.6% (32/422) samples tested seropositive during the cold and warm seasons, respectively. To the best of our knowledge, this is the first known study reporting the seroprevalence of *Anaplasma* spp. in native Korean goats. Despite the relatively low prevalence of *Anaplasma* spp. in native Korean goats compared with that in animals from other countries, these results should not be disregarded because infection with *Anaplasma* spp. in animals has long been recognised, and the potential for horizontal transmission cannot be excluded.

**Keywords:** anaplasmosis; seroprevalence; ELISA; caprine

Anaplasmosis in animals is caused by *Anaplasma* spp., which are small, Gram-negative, obligate intracellular bacteria that reside within the endothelial cells of blood vessels or the cytoplasm of blood cells such as neutrophils, monocytes, macrophages and erythrocytes (Rar and Golovljova 2011). The genus *Anaplasma* includes *A. marginale*, *A. centrale*, *A. ovis*, and *A. bovis*, which infect ruminants, including goats and sheep; *A. platys*, which infects dogs; and *A. phagocytophilum*, which infects several mammalian species (de la Fuente et al. 2007; Rar and Golovljova 2011).

Clinical manifestations of anaplasmosis vary according to the infected host species (Rar and Golovljova 2011). Clinical manifestations including weight loss, icterus, and fever are described in cattle infected with *A. marginale* (Kocan et al. 2003), and anaemia, icterus, depression, anorexia, and possibly, death are reported upon *A. ovis* infection (Tibbitts et al. 1992). *Dermacentor*, *Rhipicephalus*, and *Hyalomma* ticks are known as vector ticks of *A. ovis* (Derdakova et al. 2011), and *A. marginale* is transmitted mechanically by biting flies and biologically by various tick species (Kocan et al. 2003).

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Native Korean goats (*Capra hircus coreanae*) are primarily raised for meat and for use as an invigorant in traditional Korean medicine (Jung et al. 2014). In Korea, *Anaplasma* spp. have been isolated from various animal species, including *A. phagocytophilum* and *A. platys* from ticks and rodents (Chae et al. 2008), *A. phagocytophilum* from dogs (Jung et al. 2012), and *A. bovis* from cattle (Kang et al. 2013). However, there are no available data on the prevalence of *Anaplasma* spp. in native Korean goats. The objective of this study was to investigate the prevalence of *A. marginale*, *A. centrale*, and *A. ovis* in native Korean goats using ELISA.

## MATERIAL AND METHODS

**Study areas and blood sampling.** Blood samples ( $n = 544$ ) were collected from the jugular vein of goats from November 2009 to August 2011. Sera were separated from the whole blood samples by centrifugation at 1500 rpm for 10 min and stored at  $-20^{\circ}\text{C}$  until analysis. Age, geographical regions, and the season of collection were recorded for statistical analysis. The regions of sample collection included the northern, central, and southern regions of Korea according to the administrative boundaries (Figure 1). The temperature in Korea during the study period ranged from  $8.2^{\circ}\text{C}$  to  $17.9^{\circ}\text{C}$ , and the mean annual temperature was  $12.7^{\circ}\text{C}$  with a mean annual precipitation of 1499 mm (Jung et al. 2014).

**Serological examination.** To detect antibodies against *Anaplasma* spp., a commercial *Anaplasma* antibody test cELISA kit (VMRD, USA) was used, which detects serum antibodies against major surface protein 5 (MSP5) in *A. marginale*, *A. centrale*,

and *A. ovis*. Although this kit is approved for use in bovines by the U.S. Department of Agriculture, it has also been used to detect antibodies against *Anaplasma* spp. in goats and experimentally infected sheep because the MSP5 epitope is conserved among the three species of *Anaplasma* (Ndung'u et al. 1995; Hornok et al. 2007; Torina et al. 2008; Goda et al. 2009). To determine the seroprevalence, the optical density (OD) of each sample was measured using a NanoQuant Plate (Tecan, Switzerland), and the percentage inhibition was calculated according to the manufacturer's instructions as follows: percentage inhibition =  $100 \times [1 - (\text{sample OD} / \text{negative control OD})]$ . Samples with an inhibition of  $< 30\%$  and  $\geq 30\%$  were considered negative and positive, respectively.

**Statistical analysis.** The Chi-square test and Fisher's exact test were used to analyse significant differences between variables using SPSS 21.0 (IBM Co., USA).  $P$ -values of  $< 0.05$  were considered statistically significant. The 95% confidence intervals (CI) were also calculated.

## RESULTS

In total, 36 (6.6%) of 544 goat serum samples tested seropositive for *A. marginale*, *A. centrale* and *A. ovis* (Table 1). Among the seropositive goats, 4.9% (7/144) and 9.5% (27/283) were aged  $< 1$  year and  $\geq 1$  year, respectively, while the age of 1.7% (2/117) goats was unknown. With regard to region, the seroprevalence was 1.7% (2/121), 2.6% (2/77), and 9.2% (32/346) in the northern, central, and southern regions, respectively. With regard to the season of collection, 3.3% (4/122) samples

Table 1. Seroprevalence of *Anaplasma* spp. in 544 native Korean goats according to age, region, and season of collection

Group		Positive (%)	Negative	Total	95% CI
Age*	young ( $< 1$ year)	7 (4.9)	137	144	1.4–8.4
	adult ( $\geq 1$ year)	27 (9.5)	256	283	6.1–13.0
	unknown	2 (1.7)	115	117	0–4.1
Region*	northern	2 (1.7)	119	121	0–3.9
	central	2 (2.6)	75	77	0–6.2
	southern	32 (9.2)	314	346	6.2–11.8
Season	cold (October–March)	4 (3.3)	118	122	0.1–6.4
	warm (April–September)	32 (7.6)	390	422	5.1–10.1
Total		36 (6.6)	508	544	4.5–8.7

CI = confidence interval, \* $P < 0.05$

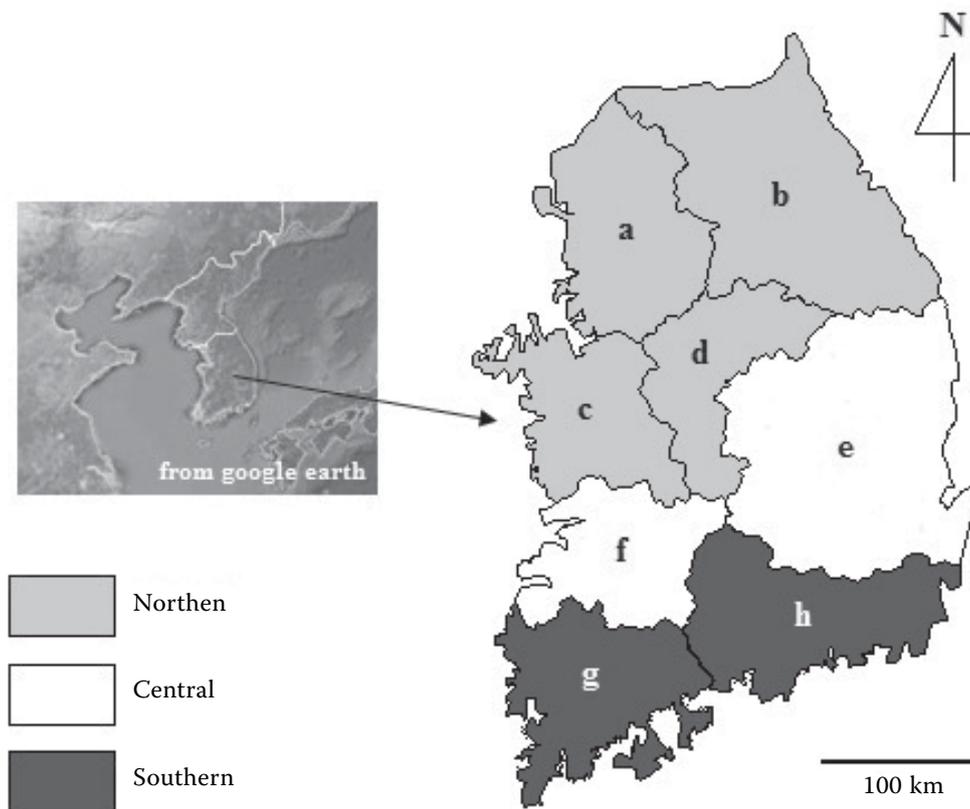


Figure 1. The regions in Korea from where goat serum samples were collected for the detection of anti-*Anaplasma* spp. antibodies. The regions of sample collection were divided according to the administrative boundaries of Korea: northern [Gyeonggi-do (a), Gangwon-do (b), Chungcheongnam-do (c), and Chungcheongbuk-do (d)], central [Gyeongsangbuk-do (e) and Jeollabuk-do (f)], and southern [Jeollanam-do (g) and Gyeongsangnam-do (h)] regions

were seropositive during the cold season (October–March) and 7.6% (32/422) were seropositive during the warm season (April–September). Statistically significant differences in seroprevalence were observed with regard to age and region ( $P < 0.05$ ).

## DISCUSSION

In the present study, 36 (6.6%) of 544 goat serum samples tested seropositive for *A. marginale*, *A. centrale*, and *A. ovis*. These results were then analysed after stratification of the goats by age, region, and season of collection. Adult goats showed a higher seroprevalence (9.5%; 27/283) compared with the young (4.9%; 7/144) goats and those of an unknown age (1.7%; 2/117), with significant differences among groups ( $P < 0.05$ ). The higher seroprevalence in the adult goats may reflect the increased opportunities for exposure to ticks carrying *Anaplasma* spp. In case of calves, maternal antibodies against *A. marginale* could be detected

for 21 weeks (Toye et al. 2013). Similarly, young goats in this study might have harboured maternal antibodies. Although exploring the possibility of maternal antibody transfer to kid goats is beyond the scope of this study, it would be interesting to assess the possible involvement of maternal antibodies in seropositive kid goats.

The highest seroprevalence was observed in the southern region (9.2%; 32/346), followed by the central (2.6%; 2/77) and northern regions (1.7%; 2/121), with significant differences among regions ( $P < 0.05$ ); this was potentially caused by variations in climate. The distribution and abundance of vector ticks are affected by climatic conditions (Leger et al. 2013). In Korea, the southern region is located at a lower latitude and is warmer, receives more precipitation and has a higher humidity than the northern and central regions. These climatic conditions are favourable for the survival of vector ticks that transmit diseases, including *Anaplasma* spp., thus resulting in a higher seroprevalence as observed in this study.

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With regard to the season of collection, the seroprevalence was higher in samples collected during the warm seasons (7.6%; 32/422) than in those collected during the cold seasons (3.3%; 4/122). However, there was no significant difference between seasons ( $P > 0.05$ ). As noted previously, the distribution and abundance of vector ticks is affected by climatic conditions, which are closely related to the season (Leger et al. 2013). Antibodies can last for several months within the host; therefore, analysis based on season may have limited ability to reveal any seasonality in anaplasmosis occurrence on the basis of seroprevalence only.

Validation of the ELISA kit in goats requires additional studies with true positives and negatives to determine appropriate cut-off values. However, data has clearly shown that the MSP5 from all the recognised species of *Anaplasma*, including *A. marginale*, *A. centrale*, and *A. ovis*, possesses a common epitope recognised by the monoclonal antibody ANAF16C1 (Ndung'u et al. 1995; Goda et al. 2009). A couple of studies have used the commercial ELISA kit with the same cut-off values in goats as well as cattle, and the following prevalence rates were described in goats: a 45.5% seropositivity (10/22) for *Anaplasma* spp. among goats in Italy (Torina et al. 2008) and a 48.0% (144/300) seropositivity for *A. ovis* among goats in Egypt (Goda et al. 2009). The positive rate (6.6%; 36/544) for native Korean goats in this study was lower than that obtained from the other countries, possibly because of differences in the detection method, climate, rearing system and the presence of vector ticks.

Regarding the cross-reactivity of the ELISA kit used in this study, sera from humans or canines that were experimentally infected with *A. phagocytophilum* showed negative results when the kit was used as recommended (Strik et al. 2007). This indicates that this ELISA kit was able to distinguish the infection of *A. marginale*, *A. centrale*, and *A. ovis* from that of *A. phagocytophilum*. However, identification of a specific species among *A. marginale*, *A. centrale*, and *A. ovis* requires further tests such as PCR.

In conclusion, we found that *Anaplasma* spp. are prevalent in native Korean goats. Although this prevalence is relatively low, these results should not be disregarded for several reasons. Most importantly, infection with *Anaplasma* spp. in animals has long been recognised; therefore, the potential for horizontal transmission should not be excluded. Furthermore, the influence of anaplasmosis on eco-

nomical losses in the goat production industry should be considered. Therefore, a consistent monitoring system for goat anaplasmosis should be established.

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