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▣ BRIEF COMMUNICATION

Serological Detection of Antibodies against *Anaplasma* spp. in Cattle Reared in the Gyeongsangbuk-do, Korea

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Abstract: Anaplasmosis is a tick-borne, non-contagious, zoonotic disease caused by *Anaplasma* spp., which include *Anaplasma marginale, A. centrale, A. phagocytophilum, A. platys, A. ovis*, and *A. bovis*. Recently, in Korea, the prevalence of *Anaplasma* spp. has been investigated in some animals, such as dogs, horses, goats, cats, and Korean water deer. In cattle, *A. marginale* is the most virulent species and regarded as the typical type of species. However, data on the seroprevalence of *Anaplasma* spp. in cattle in Korea during the last decade is limited. This study was designed to investigate the seroprevalence of bovine anaplasmosis in Korea. From 2010 to 2013, blood samples were collected from 568 cattle. Forty animals (7.0%) tested seropositive for *Anaplasma* spp. by cELISA. Despite that current bovine anaplasmosis seropositivity rate in the Gyeongsangbuk-do is lower than those in tropical countries, anaplasmosis needs to be regarded as a concerning disease. The identification of the specific *Anaplasma* species infecting cattle in this province requires additional molecular studies. Moreover, further monitoring and control programs for bovine anaplasmosis is required, and the information from this study will be beneficial to develop these programs.

Key words: *Anaplasma*, bovine, ELISA, seroprevalence

Anaplasmosis is a tick-borne, infectious, non-contagious, zoonotic disease caused by *Anaplasma* spp. [1]. *Anaplasma* spp. are obligate, intracellular, gram-negative organisms with no cell wall. The genus *Anaplasma*, belonging to the family Anaplasmataceae and order Rickettsiales, comprises *Anaplasma marginale, A. centrale, A. phagocytophilum, A. platys, A. ovis*, and *A. bovis* [1]. For instance, *A. marginale* and *A. centrale* cause bovine anaplasmosis, and *A. ovis* infects other domestic and wild ruminant species. In cattle, *A. marginale* is the most virulent and regarded as the typical type of species; nevertheless, *A. marginale* does not cause clinical disease in humans [1].

The clinical signs of anaplasmosis are variable, from asymptomatic to fatal [1]. The symptoms of bovine anaplasmosis include anemia, pyrexia, jaundice, depression, anorexia, loss of weight, reduction of milk production, and death [1]. Anaplasmosis occurs worldwide but mainly in tropical and subtropical regions near the equator, and the distribution of *Anaplasma* spp. seems to be associated with that of ticks [2]. Nevertheless, global warming has driven the expansion of tick habitats, and, thus, of tick-borne diseases.

There are several studies on the detection of *A. phagocytophilum* in Korea in humans [3], dairy cattle, cats, and horses [4], birds [5], Korean water deer [6], shelter dogs [7], shelter cats [8], and horses [9]. Additionally, there are studies on *A. marginale*-infected cattle that have been performed by capillary tube agglutination test (CTAT) and ELISA [10] and on *A. centrale*-infected cattle performed by CTAT [11]. Nevertheless, no information exists on the last decade and on the current status of bovine anaplasmosis in Korea. Therefore, the purpose of this study was to investigate the prevalence of *Anaplasma* spp. in cattle in Korea, by ELISA.

In 2016, 3,121,169 cattle had been raised in 95,233 farms, 629,441 (20.2%) of which had been raised in 20,143 (21.2%) farms in the Gyeongbuk province, Korea [12]. The sample size to be used in the study was determined using the following formula, with an expected disease prevalence of 50%, an accepted absolute error of 5%, and a confidence level of 95%, with a simple random sampling design [13]:

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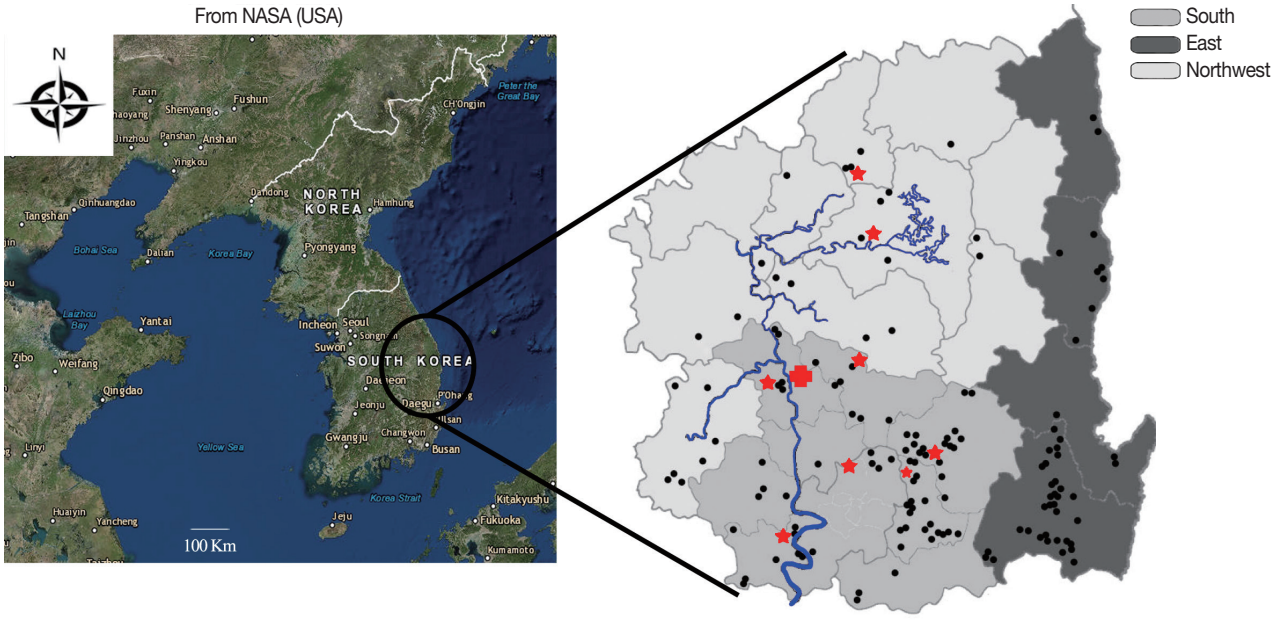

Fig. 1. Provincial map of Gyeongbuk, Korea, divided in 3 administrative districts. Samples collected from cattle farms are denoted by dots; the Haepyeong habitat for migratory birds is denoted by a red cross; local butcheries are denoted by red stars; and the Nakdong River is denoted by blue color.

$$n = \frac{1.96^2 p_{exp}(1-p_{exp})}{d^2}$$

where n = required sample size, p_{exp} = expected prevalence, and d = desired absolute precision.

According to the formula, a minimum of 384 samples were required. In this study, 568 animals were randomly selected in the Gyeongbuk province, in 2016 (Fig. 1).

The study area was divided into 3 regions by administrative districts, most of the areas being mountainous: the Northwestern region, which has mountains; the Eastern region, which has coast; and the Southern region, which is an urban and suburban area with lowland plains. The Nakdong River runs from the Northwestern to the Southern region, where a riverine marshland named Haepyeong, a habitat for migratory birds, is located (Fig. 1).

From 2010 to 2013, blood samples were collected from 568 cattle reared in the Gyeongbuk province. Sera were stored at -20°C until the analysis was performed. For the statistical analysis, the age and sex of the cattle and the location of the farm from which the samples had been collected were recorded (Table 1).

To detect antibodies against *Anaplasma* spp., a commercial *Anaplasma* antibody cELISA kit (VMRD, Pullman, Washington, USA), using recombinant major surface protein 5 to detect *A. marginale*, *A. ovis*, and *A. centrale* in bovine serum, was used, following the manufacturer's instruction. The percentage of inhibition was calculated for each sample as follows: Value (%) = 100 × [1-(OD_{sample}/$OD_{negative\ control}$)]. Samples with a percentage greater than 30% were considered positive; those with 30% or less were considered negative.

Table 1. Seroprevalence of *Anaplasma* spp. in 568 cattle in the Gyeongsangbuk-do as revealed by cELISA results, according to sex, age, and region

Group	No. tested	No. positive (%)	95% CI	*P*-value
Sex				
Male	22	3 (13.6)	0-28.0	<0.001
Female	74	15 (20.3)	11.1-29.4	
Castrated	472	22 (4.7)	2.8-6.6	
Age				
Young (≤1 y)	37	4 (10.8)	0.8-20.8	<0.001
Adult (2 y)	474	21 (4.4)	2.6-6.3	
Old (≥3 y)	57	15 (26.3)	14.9-37.7	
Region				
East	155	5 (3.2)	0.4-6.0	0.009
Northwest	124	7 (5.6)	1.6-9.7	
South	289	28 (9.7)	6.3-13.1	
Total	568	40 (7.0)	4.9-9.2	

CI, confidence interval.

Fisher's exact test and linear-by-linear association were conducted to analyze statistical differences among the groups, us-

ing SPSS 21.0 software (IBM, Armonk, New York, USA). Due to the difference in group sizes, the Bonferroni post hoc method was performed [14]. A *P*-value below 0.05 was regarded as statistically significant. The 95% confidence interval was also calculated.

Forty out of 568 (7.0%) animals tested seropositive for *Anaplasma* spp. (Table 1). According to the sex, 3 of 22 males (13.6%), 15 of 74 females (20.3%), and 22 of 472 castrated (4.7%) cattle were seropositive. By age, 4 samples of 37 (10.8%) from calves under 1 year old, 21 samples of 474 (4.4%) from 2-year-old animals, and 15 samples of 57 (26.3%) from 3-year-old or older animals were seropositive. Regarding the region, 5 of 155 (3.2%) from the East, 28 of 289 (9.7%) from the South, and 7 of 124 (5.6%) from the Northwest were seropositive. Statistical differences in seroprevalence were observed in age, sex, and region ($P < 0.05$).

In this study, cELISA was performed to identify antibodies against *Anaplasma* spp. in sera from cattle reared in the Gyeongbuk province. This method is a good choice for the serological survey of bovine anaplasmosis, including infection by *A. marginale*, because of its sensitivity (96.0%), specificity (95.2%), and low cost [15]. In this study, 7.0% of the animals tested were seropositive for *Anaplasma* spp. There were several studies in which the detection of *Anaplasma* spp. has been performed by ELISA, such as that in cattle in Iran (8/105, 7.6%) [16], cattle in Poland (23/1,326, 1.73%) [17], dairy cattle in Puerto Rico (743/2,414, 27.4%) [18], cattle and buffalo in North India (47/184, 31.7%) [19], cattle in USA (15.02%) [15], and goats in Korea (36/544, 6.6%) [20]. The overall seropositivity result obtained herein (7.0%) is quite lower than those in other studies but similar to that in Iran [16]. The countries showing high infection rates are located in tropical and subtropical regions; Iran is a region of highlands and savannas, and the Korean peninsula is not yet a subtropical climate. Therefore, the climate of the region seems to affect *Anaplasma* spp. infection rate.

Sorting the results by the sex of the animals, females presented the highest seropositivity (20.3%), followed by males (13.6%) and castrated cattle (4.7%), which showed the lowest value. According to previous gender-wise studies in tick-borne diseases, females are preferred hosts over males [21]. Regarding age, our study showed that 3-year-old or older animals presented a higher rate of seropositivity to anaplasmosis (26.3%) than calves (10.8%) and 2-year-old animals (4.4%). It seems that age affects the serum levels of antibodies against *Anaplasma* spp. This tendency in the age-related seroprevalence was similar to that observed in previous studies [1,16,19].

Regarding the results by region, the Southern area showed the highest seroprevalence (9.7%), followed by the Northwestern (5.6%) and the Eastern regions (3.2%). In Texas, a study performed on cattle on several local auction markets showed a close relationship between the region and the serological level of anaplasmosis, with the national and international herd movement increasing the potential exposure to ticks [15]. In the present study, 6 of the 8 butcheries were located in the Southern region, whereas the other 2 butcheries were located in the Northwestern region. They are in the center of the national cattle herd movement, which, similarly to the auction markets of Texas, may have effects on the serological results.

The riverside is a good habitat for ticks [22]. In this study, the riverine marshland located in the Southern region is a shelter for migratory birds travelling northward from Jeju Island to the mainland of Korea. Birds have a high potential for introducing infected immature ticks to distant areas [5]; therefore, this means that the movement of birds near the Haepyeong habitat can affect the seroprevalence of bovine anaplasmosis in this study area. Nowadays, the ticks' habitat area is expanding due to global temperature change [2], and tick-borne diseases, usually endemic to tropical regions, are gradually affecting areas of cold climates [23]. The number of reports of tick-borne diseases is increasing, and the areas of endemic regions in China are expanding [24]. These diseases can ultimately affect not only livestock but also humans; thus, more preventive efforts are required. The data obtained in this study would be useful for epidemic assays and prevention of sporadic infections in areas other than endemically affected regions.

Until now, it was difficult to access information on bovine anaplasmosis in Korea. We found that seroprevalence of *Anaplasma* spp. in cattle is relatively low in Korea. Nevertheless, the result shows that cattle is infected with *Anaplasma* spp. Identification of the specific species, *A. marginale*, A. ovis, *A. bovis*, *A. phagocytophilum*, and *A. centrale*, requires further tests, such as PCR and phylogenetic analysis. The current seropositivity rate of bovine anaplasmosis in the Gyeongsangbuk-do indicates that it is not yet a dominant tick-borne disease in cattle, unlike tropical countries; however, it needs to be regarded as a concerning disease. Therefore, continuous monitoring and control programs for bovine anaplasmosis are required, and the information from this study may be beneficial to establish such programs.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest related to this work.

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