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Editor's Choice Article

Molecular detection of *Anaplasma phagocytophilum*-like *Anaplasma* spp. and pathogenic *A. Phagocytophilum* in cattle from South Korea

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ABSTRACT

Anaplasma phagocytophilum is the causative agent of human granulocytic anaplasmosis and tick-borne fever in domestic ruminants. Differential diagnosis of zoonotic and pathogenic tick-borne diseases like granulocytic anaplasmosis is important for the efficient implementation of control programs. Thus, the differentiation of pathogenic *A. phagocytophilum* from non-pathogenic *A. phagocytophilum*-like (APL) *Anaplasma* spp. is essential. Recent molecular analyses of APL revealed its distinct phylogenetic position from *A. phagocytophilum*. This study was conducted to detect *A. phagocytophilum* and genetically related strains in 764 cattle in South Korea using PCR and restriction fragment length polymorphism assays. APL clade A and *A. phagocytophilum* were identified in 20 (2.6%) and 16 (2.1%) cattle, respectively, with 16 cattle (2.1%) displaying co-infection. The 16S rRNA sequences of APL clade A were similar (98.3–99.9%) to those clustered in the APL clade A from eastern Asia. The *A. phagocytophilum* 16S rRNA sequence shared 98.6–100% identity to those of the *A. phagocytophilum* group. We used PCR to amplify the *groEL* and *msp2* genes from the 20 samples positive for the 16S rRNA gene and found that 16 were positive for the *groEL* sequences in the APL clade A, which showed identity (82.8–84.4%) to those clustered in the APL clade A from Japan. Amplification of *msp2* was unsuccessful. The co-infection results suggested sequence diversity in *Anaplasma* spp. Till date, both *A. phagocytophilum* and APL have been reported to be distributed separately in several animals throughout South Korea. This report is the first co-detection of *A. phagocytophilum* and APL in Korean cattle using molecular methods. Further studies are needed to provide additional molecular background and trace the evolutionary tree of *Anaplasma* species in animals and ticks.

1. Introduction

The genus *Anaplasma* contains obligate intracellular Gram-negative bacteria belonging to the order Rickettsiales and the family Anaplasmataceae (Rar and Golovljova, 2011). *Anaplasma phagocytophilum* is the causative pathogen of granulocytic anaplasmosis in many species, such as humans, dogs, horses, cattle, goat, sheep, and sometimes cats (Stuen et al., 2013). Many cases of tick-borne fever (TBF) have been described in domestic ruminants and have resulted in substantial economic losses (Woldehiwet, 2006). The most common symptoms of TBF include high fever, dullness, and anorexia (Stuen et al., 2013). Particularly, in cattle, TBF leads to reduced milk production, abortion, and immunosuppression, which in turn facilitates secondary infections (Woldehiwet, 2008).

The epidemiology of *A. phagocytophilum* is appreciably different between Europe and the USA (Dugat et al., 2014). In Europe, human granulocytic anaplasmosis (HGA) is rarely reported, whereas many TBF cases have been reported in domestic ruminants, resulting in major economic losses (Dugat et al., 2014). Conversely, HGA is an emerging public health problem in the USA, with 1761 cases and a 0.7% mortality rate in 2010 (CDC, 2016), whereas cases of TBF have not been reported to date (Dugat et al., 2014). These results suggest that *A. phagocytophilum* varies its host species between continents (Dugat et al., 2014).

The prompt and correct diagnosis of pathogenic and zoonotic diseases such as HGA is critical for risk estimation in tick-borne disease control programs (Ben Said et al., 2017). Thus, it is important to differentiate between pathogenic *A. phagocytophilum* and the closely related *A. phagocytophilum*-like (APL) species that do not cause clinical signs in infected animals, and that are currently considered non-

Abbreviations: APL, *Anaplasma phagocytophilum*-like *Anaplasma* spp.; *groEL*, heat shock protein; HGA, human granulocytic anaplasmosis; msp2, major surface protein 2; nPCR, nested PCR; RFLP, restriction fragment length polymorphism; TBF, tick-borne fever; CI, confidence interval

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pathogenic (Ben Said et al., 2017). For example, the phylogenetic clades of APL in Japan (APL clade A in the present study; Ybañez et al., 2012, 2013) and China (APL clade B in the present study; Dong et al., 2014; Kang et al., 2014) are different from those of *A. phagocytophilum*. In Tunisia, APL clades A and B were detected in cattle (1.9%, 7/367; 0.5%, 2/367), sheep (7%, 25/241; 5.4%, 19/241), and goats (13.3%, 32/355; 5%, 12/241) (Ben Said et al., 2017). In the latter study, the restriction enzyme fragment length polymorphism (RFLP) assay was utilized to rapidly distinguish among *A. phagocytophilum*, and APL clades A and B (Ben Said et al., 2017).

In South Korea, *Anaplasma* spp. have been detected by PCR in dogs, cats, water deer, and ticks (Doan et al., 2013; Kang et al., 2011, 2013a, 2013b, 2016a; Lee et al., 2016a, 2016b; Oh et al., 2009). *Anaplasma* spp. have also been detected by serological methods in cattle, cats, and horses (Chae et al., 2009; Jeon, 1978; Lee et al., 1997). However, no molecular information is available on *Anaplasma* spp. in ruminants. Therefore, this study is the first reported assessment of the prevalence, co-infection, and molecular characteristics of *A. phagocytophilum* and APL in cattle from South Korea using sequence analysis of several genes, and the RFLP assay.

2. Materials and methods

2.1. Ethics

This study, conducted in 2016, did not receive approval from the Institutional Animal Care and Use Committee at Kyungpook National University, as this committee evaluates laboratory animals maintained in indoor facilities and does not regulate research with outdoor animals. However, under the regulatory "Act on the Prevention of Contagious Animal Disease (Amendment Act 2016)", national and local veterinary institutes in South Korea conducted control measures in accordance with annual infectious animal disease control programs. Blood samples were collected from cattle by practicing veterinarians at the local government-run veterinary institutes during monitoring, surveillance, and treatment, or during regular check-ups after the receipt of verbal consent from the cattle owners. This blood collection at the government-run veterinary institutes was carried out according to the administrative rules of the Ministry of Agriculture, Food, and Rural Affairs, South Korea.

2.2. Sample size determination and sample collection

In 2016, a total of 3,121,169 cattle were raised on 95,233 farms in South Korea, including 629,441 (20.2%) cattle raised on 20,143 (21.2%) farms in Gyeongbuk province and 315,254 (10.1%) cattle raised on 12,740 (13.4%) farms in Gyeongnam province (KSIS, 2016). The sample size was determined using the following formula, with an expected disease prevalence of 25%, an accepted absolute error of 5%, and a confidence level of 99% with a simple random sampling design (Thrusfield, 2005):

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

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

As determined by the formula, a minimum of 289 samples were required. At first, 47 farms were randomly selected from Gyeongsang provinces using a simple random sampling method during monitoring or surveillance. Second, within each selected farm, the sample size needed for the detection of disease in a population was calculated according to epidemiological programs (Thrusfield et al., 2001). For example, assuming sensitivity and specificity rates of 100% for the diagnostic test, a total of 15 samples would be required depending on the formula-calculated desired level of confidence (e.g., 99%), population size (e.g., 100), and the number of expected diseased animals in the population (prevalence, e.g., 25%). We randomly selected 13–20 cattle per farm (depending on the total number of cattle in each farm) from 47 farms by a simple random sampling method. Thus, a total of 764 cattle were chosen from the Gyeongsang provinces in 2016 (Fig. 1). After blood collection from the jugular vein of each cow, the whole blood samples were stored at -20 °C. The data on age, sex, and collection region were recorded for analysis.

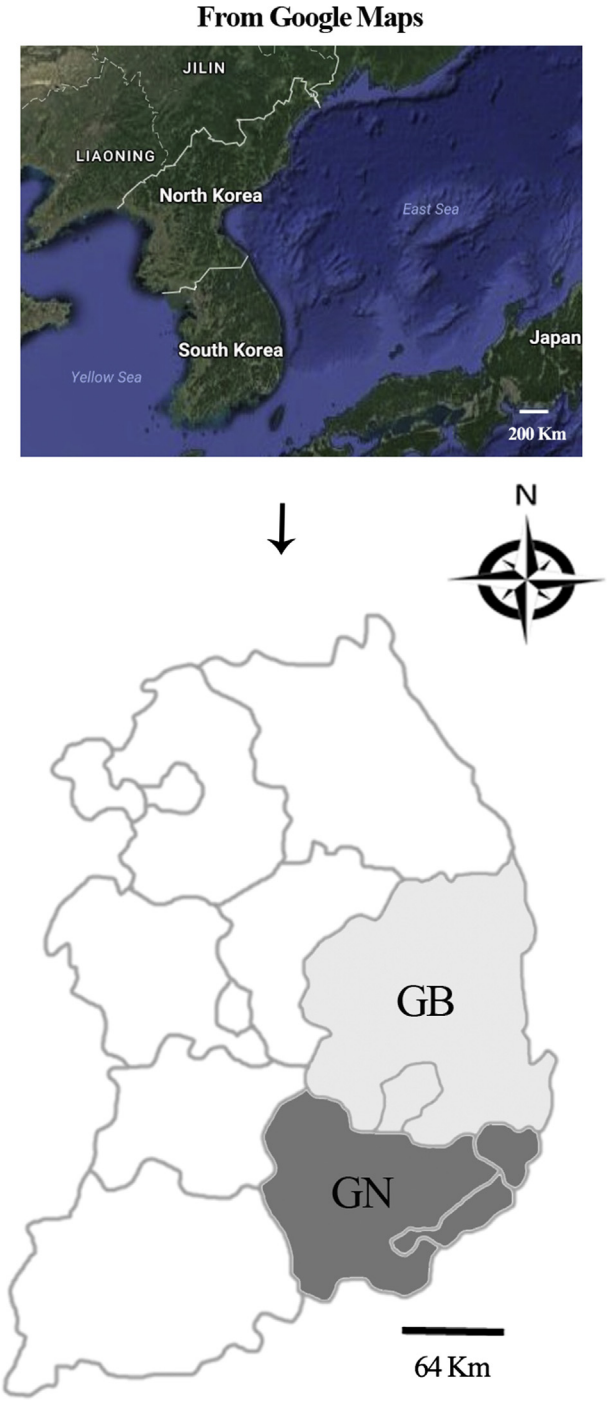

Fig. 1. Regions included in the study. A map of South Korea, indicating the regions in the Gyeongsang provinces from which cattle blood samples were collected to detect *Anaplasma* spp. GB, Gyeongbuk; GN, Gyeongnam.