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Molecular survey of bovine vector-borne pathogens in Cebu, Philippines

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ABSTRACT

Vector-borne diseases (VBDs) continue to threaten the worldwide livestock industry, but comprehensive epidemiological surveys on such diseases have not been conducted in the Philippines. In the present study, we screened 408 bovine blood samples from 9 areas in Cebu, Philippines, for various VBD pathogens using specific PCR assays. The results revealed prevalences of 54.7, 15.4, 10.0, and 12.0% for *Anaplasma* spp., *Babesia bigemina*, *Babesia bovis*, and *Trypanosoma* (*Tr.*) *theileri*, respectively. In contrast, none of the samples were positive for *Trypanosoma* (*Tr.*) *evansi*, *Theileria* (*Th.*) *orientalis*, and *Theileria* (*Th.*) *annulata*. Mixed infections were observed in 24.2% of the samples tested. Phylogenetic analysis based on the 16S rRNA gene revealed that the *Anaplasma* spp. sequences from the present study were genetically close either to *Anaplasma marginale* or *Anaplasma phagocytophilum*. In addition, *B. bovis RAP-1* and *Babesia bigemina AMA-1* gene sequences were identical and monophyletic to other known *B. bovis* and *B. bigemina* sequences. On the other hand, *Tr. theileri* cathepsin-L like protein gene sequences shared 97.1–100% identities with those from the USA and Brazil and clustered within a single genotype in the phylogenetic tree. The molecular identification of several VBD pathogens in Cebu cattle calls for the implementation of control measures to prevent the spread of these pathogens to nearby localities or islands, and ultimately, economic losses to the Philippine economy.

1. Introduction

Vector-borne diseases (VBDs), which include anaplasmosis, babesiosis, theileriosis, and trypanosomiasis, continue to beset the livestock industry. Bovine anaplasmosis is caused by rickettsial Gram-negative pathogens, including *Anaplasma phagocytophilum* (Stuen, 2007) and

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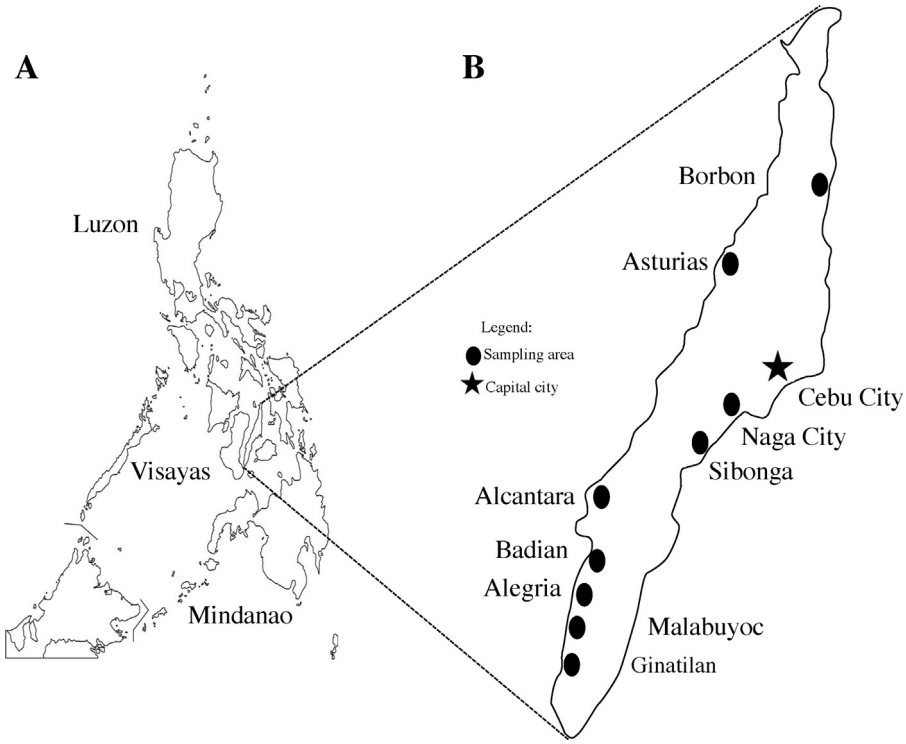

Fig. 1. Map of sampling area in Cebu, Philippines.

Anaplasma marginale (Ybañez et al., in press; Suarez and Noh, 2011). Meanwhile, bovine babesiosis is caused by intraerythrocytic apicomplexan protozoans *Babesia bovis* and *Babesia bigemina*, both of which are distributed worldwide and are often found in co-infection with *A. marginale* (Aboytes-Torres et al., 1994; Altay et al., 2008; Suarez and Noh, 2011).

Species of the genus *Theileria* (*Th.*) are intraerythrocytic apicomplexan protozoans that cause theileriosis (Aktas et al., 2006). Notable species include the highly pathogenic *Th. parva* (Norval et al., 1991) and *Th. annulata* (Kirvar et al., 2000), which infect cattle in Africa and tropical and subtropical countries, respectively. *Th. orientalis*, another important pathogen of cattle, causes anemia and occasionally death resulting in economic losses in Japan (Ota et al., 2009). A different type of VBD, trypanosomiasis, is caused by *Trypanosoma* (*Tr.*) spp., which are monophyletic kinetoplastids (Hamilton et al., 2004) that infect a wide range of hosts. *Trypanosoma* species capable of infecting livestock animals include *Trypanosoma congolense* (Boid et al., 1996), *Trypanosoma evansi* (Boid et al., 1996), and *Trypanosoma theileri* (Schlafer, 1979).

In the Philippines, reports on VBDs of veterinary importance have thus far been limited. Pathogens that have been reported to date include *A. marginale* in cattle (Ybañez et al., in press) and buffalo (Mingala et al., 2009), *Babesia* spp. in horses (Cruz-Flores et al., 2010), cattle (Dumag and Reyes, 1960; Molina and Montenegro, 1977; Mingala et al., 2009; Foronda et al., 2010), and dogs (Carlos et al., 1972; St. John et al., 1931; Cruz-Flores et al., 2008), and *Tr. evansi* in horses (Reid, 2002) and buffalo (Konnai et al., 2008). Trypanosomiasis caused by *Tr. evansi* is considered to be the second most important livestock disease after fasciolosis in the Philippines (Reid, 2002).

The Visayas, a region in the middle of the Philippines, is composed of several small islands compared to Luzon (upper region) and Mindanao (lower region) (Fig. 1). Human and animal movement between the Visayan Islands is usually done through sea travel. Situated within the Visayas is the second largest metropolitan city, Cebu City, which is located in the Cebu Island (or simply Cebu), the usual gateway of livestock trade in the area. To the author's knowledge, there have been no published epidemiological reports covering bovine VBDs in Cebu, with the exception of a recent report about bovine anaplasmosis (Ybañez et al., in press). Therefore, confirmation of the presence or absence of other VBDs in the area is highly desirable for the livestock sector, not only for the Visayas region, but also for the country as a whole. The aim of the present study was to determine if various bovine VBD pathogens, including *Anaplasma* spp., *B. bigemina*, *B. bovis*, *Th. orientalis*, *Th. annulata*, *Tr. evansi*, and *Tr. theileri* were present in Cebu. To determine the prevalence of such pathogens in the cattle studied, specific diagnostic PCR assays were used.

2. Materials and methods

2.1. Blood sample collection

Four hundred and eight bovine blood samples sourced from 9 areas (Borbon, Asturias, Naga, Sibonga, Alcantara, Badian, Alegria, Malabuyoc, and Ginatilan) in Cebu,