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Genetic characterization of *Babesia* and *Theileria* parasites in water buffaloes in Sri Lanka

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ABSTRACT

Water buffaloes are thought to be the reservoir hosts for several hemoprotozoan parasites that infect cattle. In the present study, we surveyed Sri Lankan bred water buffaloes for infections with *Babesia bovis*, *Babesia bigemina*, *Theileria annulata*, and *Theileria orientalis* using parasite-specific PCR assays. When 320 blood-derived DNA samples from water buffaloes reared in three different districts (Polonnaruwa, Mannar, and Mullaitivu) of Sri Lanka were PCR screened, *B. bovis*, *B. bigemina*, and *T. orientalis* were detected. While *T. orientalis* was the predominant parasite (82.5%), low PCR-positive rates were observed for *B. bovis* (1.9%) and *B. bigemina* (1.6%). Amplicons of the gene sequences of the Rhoptry Associated Protein-1 (RAP-1) of *B. bovis*, the Apical Membrane Antigen-1 (AMA-1) of *B. bigemina*, and the Major Piroplasm Surface Protein (MPSP) of *T. orientalis* were compared with those characterized previously in Sri Lankan cattle. While the *B. bigemina* AMA-1 sequences from water buffaloes shared high identity values with those from cattle, *B. bovis* RAP-1 sequences from water buffaloes diverged genetically from those of cattle. For *T. orientalis*, none of the MPSP sequence types reported previously in Sri Lankan cattle (types 1, 3, 5, and 7) were detected in the water buffaloes, and the MPSP sequences analyzed in the present study belonged to types N1 or N2. In summary, in addition to reporting the first PCR-based survey of *Babesia* and *Theileria* parasites in water buffaloes in Sri Lanka, the present study found that the predominant variants of water buffalo-derived *B. bovis* RAP-1 and *T. orientalis* MPSP sequences were different from those previously described from cattle in this country.

1. Introduction

Livestock play a critical role in fulfilling the nutritional requirements of human beings. Therefore, maintaining healthy livestock is essential for the supply of livestock products. However, livestock are often at risk of becoming

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infected with harmful infectious agents. Among them, piroplasmosis of cattle, which is caused by various species of *Babesia* and *Theileria* parasites, has a worldwide distribution (Uilenberg, 1995). Bovine piroplasmosis imposes huge economic losses to cattle farming operations.

Babesia bovis, *Babesia bigemina*, and *B. divergens* are described as the parasite species potentially involved in severe clinical babesiosis in cattle (Bock et al., 2004). Among these species, *B. bovis* is the most virulent and causes neurological and respiratory disorders that lead to death in the cattle affected (Everitt et al., 1986). However, *B. bigemina* and *B. divergens* infections can also be fatal when animals are not cared properly (Bock et al., 2004). While *B. bovis* and *B. bigemina* are found mostly in tropical and subtropical regions of the globe, *B. divergens* is present mainly in Europe (Zintl et al., 2003). Similar to the virulent *Babesia* spp., *Theileria parva* and *Theileria annulata* cause severe clinical disease in cattle (McKeever, 2009). The geographical distribution of *T. parva* is limited to eastern, central, and southern Africa, whereas *T. annulata* is common in North Africa, southern Europe, and Asia (Lawrence et al., 1994). In contrast, a benign group of *Theileria* parasites including *Theileria orientalis*, *T. sergenti*, and *T. buffalo* has a wide host range and worldwide distribution (Sugimoto and Fujisaki, 2002). Although known to be benign, disease outbreaks in cattle caused by these species have been reported in several countries (Aparna et al., 2011; McFadden et al., 2011; Eamens et al., 2013).

Several studies have reported the presence of these *Babesia* and *Theileria* parasites in water buffaloes (Ferreri et al., 2008; Altangerel et al., 2011; Khukhuu et al., 2011; He et al., 2012). Although clinical disease in water buffaloes from these parasites is not common, these animals might act as reservoir hosts (Karbe et al., 1979; McKeever, 2009; Oura et al., 2010; Altangerel et al., 2011); hence, detection of *Babesia* and *Theileria* parasites in these animals is equally important to that in cattle. It makes sense, therefore, that the control strategies used for cattle should be expanded to water buffaloes to minimize the infection rates among these animals.

Recently, we demonstrated the presence of *B. bovis*, *B. bigemina*, *T. annulata* and *T. orientalis* in cattle populations reared in Sri Lanka (Sivakumar et al., 2012b). The predominant parasite detected among the cattle was *T. orientalis*, followed by *B. bigemina*, *B. bovis*, and *T. annulata*. When genetic diversity in the *T. orientalis* isolates from cattle was analyzed, four different genotypes (types 1, 3, 5, and 7) were defined based on the (homologous/allelic) Major Piroplasm Surface Antigen (MPSP) gene sequences (Sivakumar et al., 2013b). Type 7 found previously to be involved in several clinical theileriosis cases in southern India was the most common MPSP type in the Sri Lankan cattle populations. In addition, genetic diversity, as based on the Merozoite Surface Antigen (MSA) genes among the *B. bovis* isolates, was very high in Sri Lankan populations of the parasites (Sivakumar et al., 2013a). In Sri Lanka, water buffaloes are mainly bred in the dry zone and used to be in close contact with cattle, especially during grazing. However, the scientific literature is very sparse for hemoprotozoan parasites of Sri Lankan water buffaloes. Although *T. orientalis*-like parasites have been shown to be present in Giemsa-stained thin blood smears prepared from water buffaloes, the exact species was not determined (Weilgama et al., 1986).

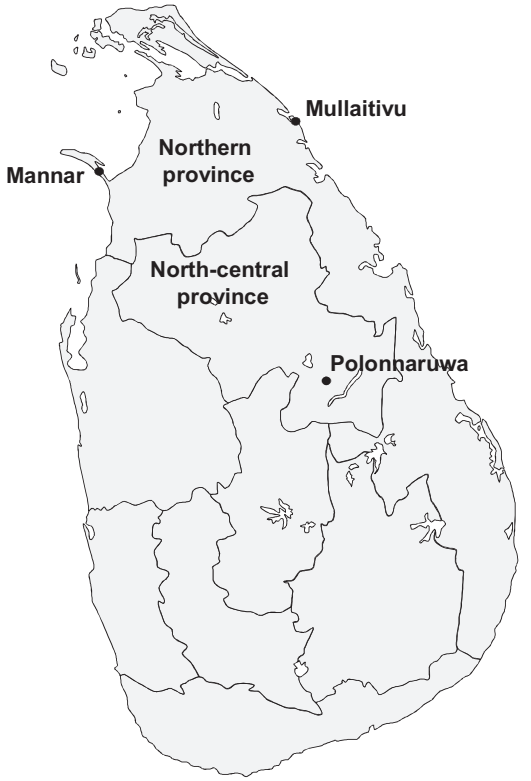

Fig. 1. Sampling locations in Sri Lanka. Blood samples were collected from water buffaloes in three different districts.

In the present study, we collected blood samples from water buffaloes reared in three different districts of Sri Lanka, and conducted a series of PCR-based surveys of *B. bovis*, *B. bigemina*, *T. annulata*, and *T. orientalis*. Subsequently, the PCR amplicons were sequenced, and the resultant gene sequences were compared with those generated from cattle in previous investigations (Sivakumar et al., 2012b, 2013b).

2. Materials and methods

2.1. Blood samples

Blood samples were collected from 320 water buffaloes reared in three different districts (Polonnaruwa, Mannar, and Mullaitivu, Fig. 1) of Sri Lanka in February 2013. All the animals surveyed in the present study were over 1 year of age and were apparently healthy during the sampling period. Approximately 2 mL of whole blood was drawn from each animal into a Vacutainer tube that contained EDTA (NIPRO, Osaka, Japan). The samples were labeled and then stored at −20 °C until they were used for DNA extraction.