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Short communication

Epidemiological survey of hemoprotozoan parasites in cattle from low-country wet zone in Sri Lanka

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ABSTRACT

The diseases caused by hemoprotozoan parasites in cattle often result in economic losses. In Sri Lanka, previous studies found that the up-country wet zone, which is located in central Sri Lanka, was characterized by a high rate of *Theileria orientalis* and a low rate of *Theileria annulata* compared with the dry zone. In this study, DNA samples were prepared from the blood of 121 cattle in Galle, a coastal district located in low-country wet zone in Sri Lanka, and were PCR-screened for *Babesia bovis*, *Babesia bigemina*, *T. annulata*, *T. orientalis*, and *Trypanosoma theileri*. All the parasite species, except *B. bovis*, were detected among the surveyed cattle. The animals had a high rate of *T. orientalis* (100%) and a low rate of *T. annulata* (1.6%), as in the up-country wet zone. *Babesia bigemina* and *Tr. theileri* were detected in 19.0% and 20.6% of the animals, respectively, and their infection rates were higher in the animals reared in extensive management systems (32.8% and 27.9%, respectively) than in those managed in intensive/semi-intensive systems (5.0% and 13.3%, respectively). Genotypic analyses found that the *T. orientalis mpsp* type 5 was predominant similar to up-country wet zone, and that *Tr. theileri* consisted of seven *catl* genotypes, including two new genotypes (IL and IM) and four previously detected genotypes (IA, IB, II, and IK). These findings suggest that the hemoprotozoan infection profiles are largely conserved within the wet zone, despite differences in the geography, cattle breeds, and management practices between the up-country and low-country wet zones.

1. Introduction

Hemoprotozoan parasites, including species of *Babesia*, *Theileria*, and *Trypanosoma* parasites, infect cattle and cause clinical diseases, leading to economic losses in the cattle industry worldwide. Although various species of *Babesia* infect cattle, severe clinical babesiosis is caused by *B. bovis* and *B. bigemina* in the tropics and subtropics and by *B. divergens* in Europe [1,2]. On the other hand, bovine theileriosis is caused by several species of *Theileria*, including *T. parva*, *T. annulata*, and *T. orientalis*, which cause east coast fever in Eastern, Central, and Southern Africa, tropical theileriosis in North Africa, Southern Europe, and Asia, and oriental theileriosis worldwide, respectively [3–6]. Although *T. parva* and *T. annulata* are more virulent than *T. orientalis*, the latter sometimes causes severe disease, especially when newly introduced into an area [7,8]. The clinical signs of bovine theileriosis are similar to those of bovine babesiosis, except for the lack of hemoglobinuria and the presence of enlarged lymph nodes [9]. The virulence of *Trypanosoma* parasites differs among the species of the genus. *Trypanosoma congolense*, *Tr. vivax*, and *Tr. brucei*, which are only endemic to Africa, are more virulent than *Tr. evansi* and *Tr. theileri*, which have

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wide distributions. However, *Tr. evansi* often induces a chronic wasting disease in several host animals, including cattle [10], and *T. theileri* can also cause clinical disease, especially when it coinfects animals with other hemoparasites [11,12].

Once they infect their hosts, these hemoprotozoan parasites persist in the hosts' bodies for a long period [13–15]. Therefore, they can be acquired by their vectors and complete their life cycles. The detection of these carrier animals is vital in estimating the risks they pose, because the vectors can transmit the parasites from these animals to their next hosts, where the infection may result in clinical disease.

Sri Lanka is a tropical island in the Indian Ocean. The cattle farming systems in this country differ significantly among the climatic zones [16]. Throughout the dry zone, which is characterized by low annual rainfall, cattle breeds and management practice are similar, and predominantly local cattle breeds are managed with an extensive management system [17]. However, different cattle breeds and management systems are used in the wet zone [17]. In the up-country wet zone, which covers the high-altitude regions in central Sri Lanka, pure European breed cattle are reared with an intensive management system [17]. In contrast, in the low-country wet zone, which is situated around the low-elevation coastal areas in western and southern Sri Lanka, European and zebu hybrid cattle are managed with an intensive or semi-intensive system in urban areas and with an extensive system in rural areas [17].

In our previous studies in Sri Lanka, we surveyed cattle populations representing the dry zone (Jaffna, Polonnaruwa, and Amapara districts) and the up-country wet zone (Nuwara Eliya district) for infection with species of *Babesia*, *Theileria*, and *Trypanososma* parasites [15,18,19]. These studies indicated that the infection rates of some hemoprotozoan parasites, particularly species of *Theileria*, differed between the dry and up-country wet zones [15,18]. These differences were attributed to the variations in the species, densities, and activities of the tick vectors in the climatic zones [15]. However, the cattle in the low-country wet zone were not considered in these studies, although the geography, cattle breeds, and management practices vary between the up-country and low-country wet zones [17]. Therefore, in the present study, we surveyed the cattle in Galle, a coastal district located in the low-country wet zone, for infection with bovine *Babesia*, *Theileria*, and *Trypanosoma* parasites.

2. Materials and methods

2.1. Blood samples and DNA extraction

A total of 121 blood samples were collected from cattle in 16 farms in Akmeemana ($n = 60$) and 22 farms in Rathgama ($n = 61$) veterinary ranges in Galle in May 2017. Galle is a coastal district of southern Sri Lanka, located in the low-country wet zone. The animals sampled in both locations were cross-bred cattle. The cattle in Rathgama, a rural area, are usually reared in an extensive system, whereas those in Akmeemana are maintained in intensive or semi-intensive systems. In addition to cattle, seven buffaloes that were reared with cattle (five from Akmeemana and two from Rathgama) were also sampled. From each animal, approximately 2 ml of blood was collected from the jugular vein into a Vacutainer tube containing EDTA (Nipro, Osaka, Japan). The DNA samples were prepared from 200 µl of the whole blood from each animal with a commercial DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany), and then stored at −30 °C until analysis. All animal procedures were approved by the Committee on the Ethics of Animal Experiments, Obihiro University of Agriculture and Veterinary Medicine (Approval number 29–53). In addition, approval for the blood sampling was obtained from the Department of Animal Production and Health, Peradeniya, Sri Lanka.

2.2. PCR detection of Babesia, Theileria, and Trypanosoma parasites

All the DNA samples prepared from the cattle and buffaloes were screened for *B. bovis*, *B. bigemina*, *T. annulata*, *T. orientalis*, and *Tr. theileri*, with previously described PCR assays based on the rhoptry-associated protein 1 (*rap-1*) [20], apical membrane antigen 1 (*ama-1*) [21], merozoite-piroplasm surface antigen (*tams-1*) [22], major piroplasm surface protein (*mpsp*) [23], and cathepsin L-like protein (*catl*) [24] genes, respectively. The reaction mixtures and cycling conditions for the PCR assays have been described previously [18,19].

2.3. Type-specific PCR assays for T. orientalis

All the *T. orientalis*-positive DNA samples were screened with PCR assays specific for *mpsp* genotypes 1, 3, 5, and 7, which are known to be endemic to cattle in Sri Lanka, essentially as previously described [25].

2.4. Cloning, sequencing, and phylogenetic analyses

The PCR amplicons from selected samples of each of the parasite species were gel-extracted and then cloned into the PCR™2.1 plasmid vector (TOPO, Invitrogen, Carlsbad, CA). The inserts were sequenced with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Branchburg, NJ, USA). The *B. bovis rap-1* and *Tr. theileri catl* gene sequences obtained in the present study, together with those retrieved from GenBank, were used to construct maximum likelihood and neighbor-joining phylogenetic trees, respectively, based on the Tamura 3-parameter substitution model [26], using the MEGA version 6.0 software [27].

2.5. Statistical analyses

The confidence intervals for infection rates were calculated based on the Wilson score [28] using the OpenEpi software program (http://www.openepi.com/Proportion/Proportion.htm). The *P* values for the differences between the infection rates were calculated using an “N-1” χ^2 test (https://www.medcalc.org/calc/comparison_of_proportions.php) [29,30]. A P value $< .05$ was considered to indicate a statistically significant difference between the infection rates.

3. Results and discussion

The findings of the present study indicated that the cattle in the Galle district were infected with *B. bigemina*, *T. annulata*, *T. orientalis*, and *Tr. theileri*, whereas *B. bovis* was not detected among the cattle surveyed. The commonest parasite was *T. orientalis*, which was detected in all 121 animals (100%), followed by *Tr. theileri* (20.6%), *B. bigemina* (19.0%), and *T. annulata* (1.6%) (Table 1). Of 121 cattle, 46 (38.0%) had co-infections with two or three parasite species. Among these co-infected animals, four had co-infections with *T. orientalis*, *B. bigemina*, and *Tr. theileri*, while 21, 19, and two were co-infected with *T. orientalis* and *Tr. theileri*, *T. orientalis* and *B. bigemina*, and *T. orientalis* and *T. annulata*, respectively. These observations are in agreement with the results of previous studies, which clearly showed that the cattle in the up-country wet zone had high *T. orientalis* and low *T. annulata* infection rates, compared with the infection rates for both these parasite species in cattle in the dry zone [15,18]. Therefore, our present findings indicate that, despite the differences in the cattle-farming systems and geography of up-country and low-country wet zones, the rates of hemoprotozoan parasites displayed similar patterns in both regions, suggesting that the major factor influencing the hemoprotozoan parasitic epidemiology in Sri Lanka is climate, not geographic location.

The animals in the up-country wet zone are managed exclusively with an intensive management system [16]. However, the present study provided an opportunity to compare the infection rates between cattle managed with intensive/semi-intensive and extensive management

Table 1
PCR detection of *Babesia*, *Theileria*, and *Trypanosoma* in 121 cattle from Galle in Sri Lanka.

Parasite	Akmeemana (n = 60)		Rathgama (n = 61)		*P* value	Total	
	No. positive	% (CI)	No. positive	% (CI)		No. positive	% (CI)
B. bigemina	3	5.0 (1.6–12.7)	20	32.8 (22.3–45.2)	0.0001	23	19.0 (13.0–26.9)
T. annulata	0	0 (0.0–5.6)	2	3.3 (1.7–13.5)	0.1576	2	1.6 (0.45–5.82)
T. orientalis	60	100 (94.4–100)	61	100 (94.1–100)		121	100 (96.9–100)
Tr. theileri	11	13.3 (9.7–27.8)	17	27.9 (18.2–40.2)	0.0483	25	20.6 (14.4–28.7)

CI, 95% confidence interval.

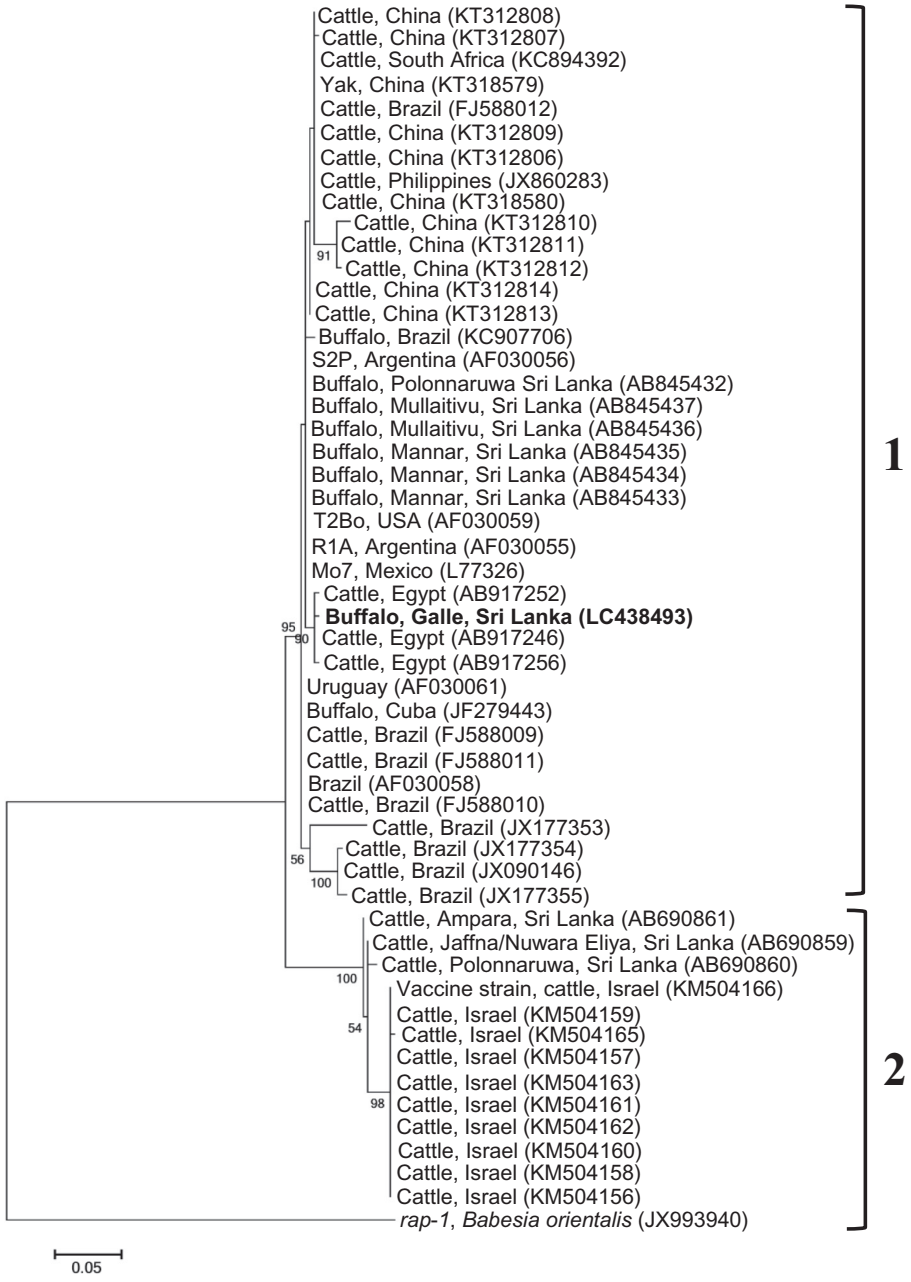

Fig. 1. Phylogeny of *Babesia bovis rap-1* gene. A buffalo-derived *B. bovis rap-1* sequence (indicated in boldface type) determined in this study and those retrieved from the GenBank database were used to construct a maximum likelihood phylogenetic tree. Note that the buffalo-derived sequence from Galle occurs in clade 1, together with the buffalo-derived sequences previously determined in Sri Lanka, and that the previously determined cattle-derived sequences from Sri Lanka occur in clade 2.

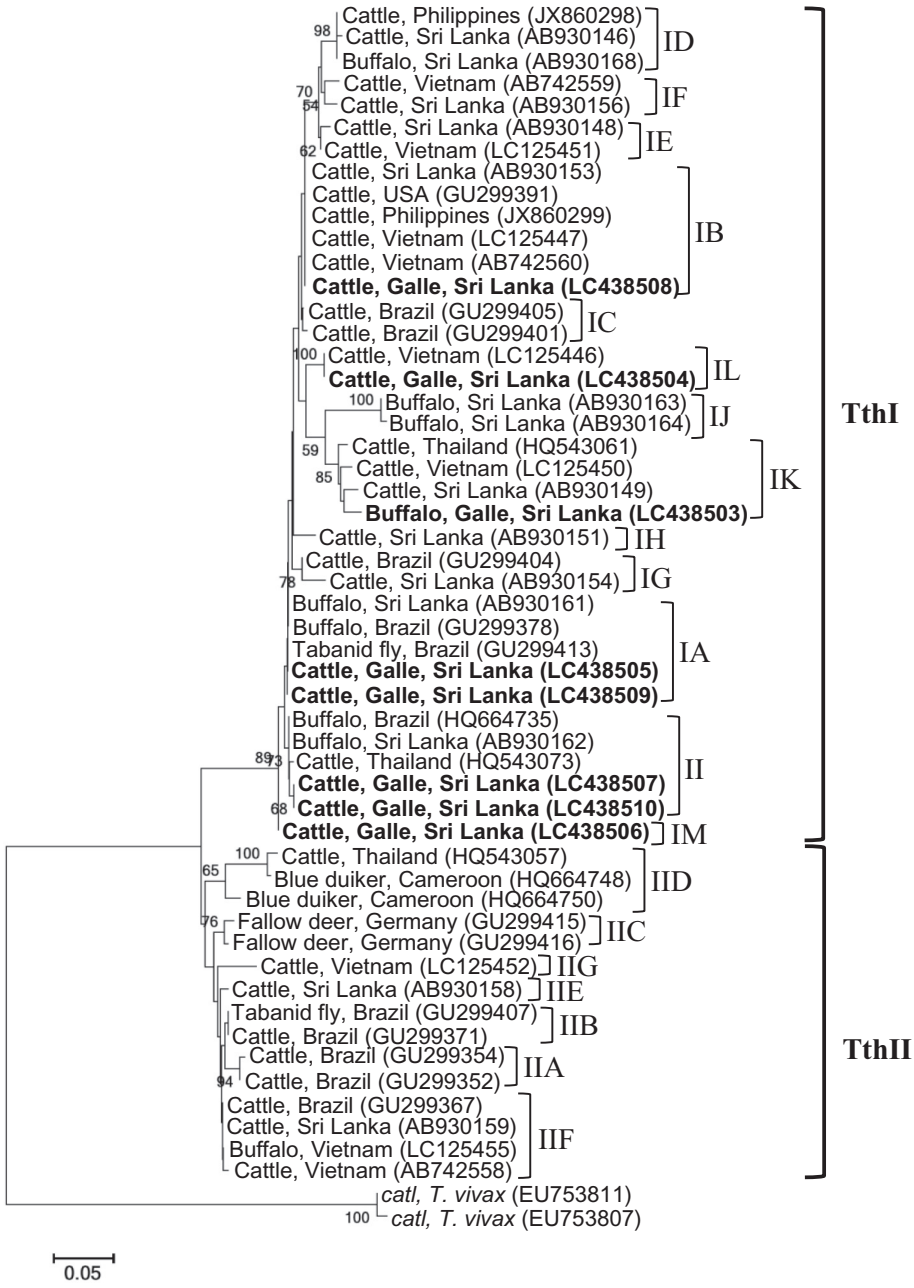

Fig. 2. Phylogeny of *Trypanosoma theileri catl* gene. The *catl* sequences from seven cattle and one buffalo from Galle (indicated in boldface type) and those previously reported in Sri Lanka and other countries were used to construct a neighbor-joining phylogenetic tree. Note that the newly determined sequences occur in six clades within the major TthI clade, including IA, IB, II, IK, IL, and IM, and that genotypes IL and IM are reported for the first time in Sri Lanka.

systems in the wet zone. We found that the infection rates for *Tr. theileri* and *B. bigemina* were higher in Rathgama (27.9% and 32.8%, respectively) than in Akmeemana (13.3% and 5.0%, respectively) (Table 1). The risk of exposure to vectors is higher for cattle in Rathgama than for those in Akmeemana, because the animals in these two areas are maintained with extensive and intensive/semi-intensive management systems, respectively, which explains why the *Tr. theileri*- and *B. bigemina*-positive rates were higher in Rathgama than in Akmeemana [31]. Thus, the prevalence of bovine hemoprotozoan parasites is influenced by climate, as well as management practices, in Sri Lanka. However, no comparison of the *T. annulata*-positive rates in the two sampling locations was possible because only two animals in Rathgama were positive for this parasite. All the animals sampled at both locations were positive for *T. orientalis*. A previous study conducted in Sri Lanka found that the animals had high *T. orientalis* infection rates, despite its low transmission rate, because the infection displays pronounced persistence [15]. This could explain the high infection rates for *T. orientalis* in both sampling locations in the present study, despite the differences in the management practices there.

Theileria orientalis consists of 11 *mpsp* genotypes, including types 1–8, N1, N2, and N3, and four of these (types 1, 3, 5, and 7) have been detected in Sri Lankan cattle [25]. Therefore, we screened all 121 cattle DNA samples, all of which were PCR-positive for *T. orientalis*, using previously established PCR assays specific for *mpsp* genotypes 1, 3, 5, and 7. The commonest genotype was type 5 (40.4%), followed by types 7 (30.5%), 1 (20.6%), and 3 (10.3%). These findings are also consistent with the previous observation that type 5 was predominant in the up-country wet zone [25]. However, of the 121 cattle DNA samples tested, only 52 were positive in at least one type-specific PCR assay, suggesting the presence of other genotypes. Therefore, we cloned and sequenced

the amplicons from the screening PCR assays of 28 (18 from Akmeemana and 10 from Rathgama) of the 59 samples that were negative in the type-specific PCR assays. The newly generated sequences represented either type 1 ($n = 5$; GenBank accession numbers LC438466–LC438470), type 5 ($n = 15$; LC438471–LC438485), or type 7 ($n = 7$; LC438486–LC438492). Therefore, the low DNA concentrations of individual genotypes in the DNA samples might explain the initial negative results for the type-specific PCR assays of the *T. orientalis*-positive DNA samples.

Although buffalo farming is uncommon in the up-country wet zone, buffaloes are sometimes reared together with cattle in the low-country wet zone. In the present study, DNA samples from seven buffaloes that were reared together with cattle at the sampling sites were also screened for hemoprotozoan parasites with PCR. *Babesia bovis*, *T. orientalis*, and *Tr. theileri* infections were detected among these animals. In common with the cattle, all seven buffaloes were positive for *T. orientalis*, and one and three animals were infected with *B. bovis* and *Tr. theileri*, respectively. One of the *Tr. theileri*-positive buffalo was co-infected with *T. orientalis* and *B. bovis*, and the remaining two were co-infected with *T. orientalis*.

To confirm the PCR findings, the PCR amplicons were cloned and sequenced. One resultant sequence of *B. bovis rap-1* (buffalo; GenBank accession number LC438493), seven sequences of *B. bigemina ama-1* (cattle; LC438494–LC438500), two sequences of *T. annulata tams-1* (cattle; LC438501 and LC438502), and eight sequences of *Tr. theileri catl* (one from buffalo and seven from cattle; LC438503–LC438510) shared high identity scores with those previously reported in Sri Lanka [18,19,32], confirming the PCR findings in this study. In a previous study, *B. bovis rap-1* variants were shown to differ between cattle and buffaloes in Sri Lanka [32]. The buffalo-derived *rap-1* sequence generated in the present study (LC438493) clustered together with previously reported buffalo-derived sequences from Sri Lanka (AB845432–AB845437) and those from GenBank in the phylogeny, whereas the previously determined cattle-derived sequences from Sri Lanka (AB690859–AB690861) occurred in a separate clade (Fig. 1). This finding confirms that the *B. bovis* populations differ between the cattle and buffaloes in this country. Therefore, the detection of *B. bovis* in a buffalo may not necessarily indicate that the cattle in Galle are infected with this parasite species. However, further studies with large number of samples are essential to rule out *B. bovis* infection in the cattle in Galle.

Trypanosoma theileri can be divided into several genotypes, based on the *catl* gene sequences [33,34]. In Sri Lanka, 12 *catl* genotypes have been reported, including IA, IB, and ID–IK within major phylogenetic clade TthI and IIE and IIF within major phylogenetic clade TthII [19]. In contrast, the *catl* sequences determined in the present study were classified into seven genotypes, including two new genotypes (IL and IM) and four genotypes (IA, IB, II, and IK) that were previously detected in Sri Lanka (Fig. 2). Investigations using large number of samples from different geographic regions are required to confirm whether the newly detected *catl* genotypes are unique to the low-country wet zone.

In conclusion, in this study, we analyzed infections of several hemoprotozoan parasite species among the cattle population in the low-country wet zone of Sri Lanka, and found that the infection profiles were similar to those observed in the up-country wet zone, despite the variations in cattle breeds, management practices, and geography between these two regions. Therefore, the major factor that influences the epidemiology of bovine hemoprotozoan parasites in Sri Lanka is not geography, but the local climatic zones.

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