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

Erandi Gunasekara[a,b], Thillaiampalam Sivakumar[a], Hemal Kothalawala[b], Thuduwege Sanath Abeysekera[c], Amitha Sampath Weerasingha[d], Singarayar Caniciyas Vimalakumar[e], Ratnam Kanagaratnam[f], Palitha Rohana Yapa[g], Atambekova Zhyldyz[a], Ikuo Igarashi[a], Seekkuge Susil Priyantha Silva[b], and Naoaki Yokoyama[a,*]

[a] *National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan*

[b] *Veterinary Research Institute, Peradeniya, Sri Lanka*

[c] *Government Veterinary Surgeon's Office, Akmeemana, Galle, Sri Lanka*

[d] *Government Veterinary Surgeon's Office, Ambalangoda, Galle, Sri Lanka*

[e] *Deputy Director's Office, Department of Animal Production and Health, Mannar, Sri Lanka*

[f] *Government Veterinary Surgeon's Office, Kandavalai, Kilinochchi, Sri Lanka*

[g] *Government Veterinary Surgeon's Office, Haputale, Badulla, Sri Lanka*

*** Corresponding author at:** N. Yokoyama, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro,

20 Hokkaido 080-8555, Japan. Tel.: +81 155 49 5649; Fax: +81 155 49 5643

21 E-mail: yokoyama@obihiro.ac.jp

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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 *T. orientalis* and a low rate of *T. 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 *B. bovis*, *B. bigemina*, *T. annulata*, *T. orientalis*, and *Tr. 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.

*Keywords:* Cattle, Epidemiology, Hemoprotozoa, Low-country wet zone, Sri Lanka

## 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 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,

84 particularly species of *Theileria*, differed between the dry and up-country wet zones [15, 18].
85 These differences were attributed to the variations in the species, densities, and activities of
86 the tick vectors in the climatic zones [15]. However, the cattle in the low-country wet zone
87 were not considered in these studies, although the geography, cattle breeds, and management
88 practices vary between the up-country and low-country wet zones [17]. Therefore, in the
89 present study, we surveyed the cattle in Galle, a coastal district located in the low-country wet
90 zone, for infection with bovine *Babesia*, *Theileria*, and *Trypanosoma* parasites.
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## 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" $\chi^2$ test (https://www.medcalc.org/calc/comparison_of_proportions.php) [29, 30]. A *P* value < 0.05 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

164 management system [16]. However, the present study provided an opportunity to compare the
165 infection rates between cattle managed with intensive/semi-intensive and extensive
166 management systems in the wet zone. We found that the infection rates for *Tr*. *theileri* and *B*.
167 *bigemina* were higher in Rathgama (27.9% and 32.8%, respectively) than in Akmeemana
168 (13.3% and 5.0%, respectively) (Table 1). The risk of exposure to vectors is higher for cattle
169 in Rathgama than for those in Akmeemana, because the animals in these two areas are
170 maintained with extensive and intensive/semi-intensive management systems, respectively,
171 which explains why the *Tr*. *theileri*- and *B*. *bigemina*-positive rates were higher in Rathgama
172 than in Akmeemana [31]. Thus, the prevalence of bovine hemoprotozoan parasites is
173 influenced by climate, as well as management practices, in Sri Lanka. However, no
174 comparison of the *T. annulata*-positive rates in the two sampling locations was possible
175 because only two animals in Rathgama were positive for this parasite. All the animals
176 sampled at both locations were positive for *T. orientalis*. A previous study conducted in Sri
177 Lanka found that the animals had high *T. orientalis* infection rates, despite its low
178 transmission rate, because the infection displays pronounced persistence [15]. This could
179 explain the high infection rates for *T. orientalis* in both sampling locations in the present
180 study, despite the differences in the management practices there.

181 *Theileria orientalis* consists of 11 *mpsp* genotypes, including types 1–8, N1, N2, and
182 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.*

202 *bovis* and *Tr*. *theileri*, respectively. One of the *Tr*. *theileri*-positive buffalo was co-infected
203 with *T*. *orientalis* and *B*. *bovis*, and the remaining two were co-infected with *T*. *orientalis*.
204 To confirm the PCR findings, the PCR amplicons were cloned and sequenced. One
205 resultant sequence of *B*. *bovis rap-1* (buffalo; GenBank accession number LC438493), seven
206 sequences of *B*. *bigemina ama-1* (cattle; LC438494–LC438500), two sequences of *T*.
207 *annulata tams-1* (cattle; LC438501 and LC438502), and eight sequences of *Tr*. *theileri catl*
208 (one from buffalo and seven from cattle; LC438503–LC438510) shared high identity scores
209 with those previously reported in Sri Lanka [18, 19, 32], confirming the PCR findings in this
210 study. In a previous study, *B*. *bovis rap-1* variants were shown to differ between cattle and
211 buffaloes in Sri Lanka [32]. The buffalo-derived *rap-1* sequence generated in the present
212 study (LC438493) clustered together with previously reported buffalo-derived sequences
213 from Sri Lanka (AB845432–AB845437) and those from GenBank in the phylogeny, whereas
214 the previously determined cattle-derived sequences from Sri Lanka (AB690859–AB690861)
215 occurred in a separate clade (Fig. 1). This finding confirms that the *B*. *bovis* populations differ
216 between the cattle and buffaloes in this country. Therefore, the detection of *B*. *bovis* in a
217 buffalo may not necessarily indicate that the cattle in Galle are infected with this parasite
218 species. However, further studies with large number of samples are essential to rule out *B*.
219 *bovis* infection in the cattle in Galle.
220 *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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250 **References**


251 1. R. Bock, L. Jackson, A. de Vos, W. Jorgensen, Babesiosis of cattle, Parasitology 129
252 (2004) S247–S269.

253 2. A. Zintl, G. Mulcahy, H.E. Skerrett, S.M. Taylor, J.S. Gray, *Babesia divergens*, a bovine
254 blood parasite of veterinary and zoonotic importance, Clin. Microbiol. Rev. 16 (2003)
255 622–636.

256 3. A.D. Irvin, Characterization of species and strains of *Theileria*, Adv. Parasitol. 26 (1987)
257 145–97.

258 4. D.J. McKeever, Bovine immunity – a driver for diversity in *Theileria* parasites?, Trends
259 Parasitol. 25 (2009) 269–276.

260 5. T. Sivakumar, K. Hayashida, C. Sugimoto, N. Yokoyama, Evolution and genetic
261 diversity of *Theileria*, Infect. Genet. Evol. 27 (2014) 250–263.

262 6. W. Weir, T. Karagenc, M. Baird, A. Tait, B. R. Shiels, Evolution and diversity of
263 secretome genes in the apicomplexan parasite *Theileria annulata*, BMC Genomics 11
264 (2010) 42.

265 7. M.M. Izzo, I. Poe, N. Horadagoda, A.J. De Vos, J.K. House, Haemolytic anaemia in
266 cattle in NSW associated with *Theileria infections*, Aust. Vet. J. 88 (2010) 45–51.

267 8. A.M.J. McFadden, T.G. Rawdon, J. Meyer, J. Makin, C.M. Morley, R.R. Clough, K.
268 Tham, P. Mullner, D. Geysen, An outbreak of haemolytic anaemia associated with

infection of *Theileria orientalis* in naive cattle, N. Z. Vet. J. 59 (2011) 79–85.

9. B.S. Gill, Y. Bhattacharyulu, D. Kaur, Symptoms and pathology of experimental bovine tropical theileriosis (*Theileria annulata* infection), Ann Parasitol Hum Comp. 52 (1977) 597–608.

10. R. Brun, H. Hecker, Z. Lun, *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review), Vet. Parasitol. 79 (1998) 95–107.

11. J.M. Mansfield, Nonpathogenic trypanosomes of mammals, in: J.P. Kreier (Ed.), Parasitic Protozoa, Academic Press, London, 1977, pp. 297–327.

12. E.A. Wells, Subgenus Megatrypanum, in: W.H.R. Lumsden, D.A. Evans (Eds.), Biology of the kinetoplastida, Academic Press, London, 1976, pp. 257–275.

13. D.R. Allred, Babesiosis: persistence in the face of adversity, Trends Parasitol. 19 (2003) 51–55.

14. M. Desquesnes, P. Holzmuller, D.H. Lai, A. Dargantes, Z.R. Lun, S. Jittaplapong, *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects, Biomed. Res. Int. 2013 (2013) 194176.

15. T. Sivakumar, H. Kothalawala, G. Weerasooriya, S.S.P. Silva, S. Puvanendiran, T. Munkhjargal, I. Igarashi, N. Yokoyama, A longitudinal study of *Babesia* and *Theileria*

infections in cattle in Sri Lanka, Vet. Parasitol. Reg. Stud. Rep. 6 (2016) 20–27.

16. H. Abeygunawardena, D. Rathnayaka, W.M.A.P. Jayathilaka, Characteristics of cattle farming systems in Sri Lanka, J. Natn. Sci. Found. 25 (1997) 25–38.

17. M.N.M. Ibrahim, S.J. Staal, S.L.A. Daniel, W. Thorpe, Appraisal of the Sri Lanka dairy sector. Colombo, Sri Lanka: Ministry Livestock Development and Estate Infrastructure (1999).

18. T. Sivakumar, H. Kothalawala, A.S. Abeyratne, S.C. Vimalakumar, A.S. Meewawe, D.T. Hadirampela, T. Puvirajan, S. Sukumar, K. Kuleswarakumar, A.D.N. Chandrasiri, I. Igarashi, N. Yokoyama, A PCR-based survey of selected *Babesia* and *Theileria* parasites in cattle in Sri Lanka, Vet. Parasitol. 190 (2012) 263–267.

19. N. Yokoyama, T. Sivakumar, S. Fukushi, M. Tattiyapong, B. Tuvshintulga, H. Kothalawala, S.S.P. Silva, I. Igarashi, N. Inoue, Genetic diversity in *Trypanosoma theileri* from Sri Lankan cattle and water buffaloes, Vet. Parasitol. 207 (2015) 335–341.

20. J.V. Figueroa, L.P. Chieves, G.S. Johnson, G.M. Buening, Multiplex polymerase chain reaction based assay for the detection of *Babesia bigemina*, *Babesia bovis* and *Anaplasma marginale* DNA in bovine blood, Vet. Parasitol. 50 (1993) 69–81.

21. T. Sivakumar, K. Altangerel, B. Battsetseg, B. Battur, M. AbouLaila, T. Munkhjargal, T. Yoshinari, N. Yokoyama, I. Igarashi, Genetic detection of *Babesia bigemina* from Mongolian cattle using *apical membrane antigen-1* gene based PCR technique, Vet

307 Parasitol 187 (2012) 17–22.

308 22. E. Kirvar, T. Ilhan, F. Katzer, P. Hooshmand-Rad, E. Zweygarth, C. Gerstenberg, P.
309 Phipps, C.G.D. Brown, 2000. Detection of *Theileria annulata* in cattle and vector ticks by
310 PCR using the Tams1 gene sequences, Parasitology 120 (2000) 245–254.

311 23. N. Ota, D. Mizuno, N. Kuboki, I. Igarashi, Y. Nakamura, H. Yamashina, T. Hanzaike, K.
312 Fujii, S. Onoe, H. Hata, S. Kondo, S. Matsui, M. Koga, K. Matsumoto, H. Inokuma, N.
313 Yokoyama, Epidemiological survey of *Theileria orientalis* infection in grazing cattle in
314 the eastern part of Hokkaido, Japan, J. Vet. Med. Sci. 71 (2009) 937–944.

315 24. A.C. Rodrigues, H.A. Garcia, P.A. Ortiz, A.P. Cortez, F. Martinkovic, F. Paiva, J.S.
316 Batista, A.H. Minervino, M. Campaner, E.M. Pral, S.C. Alfieri, M.M. Teixeira, Cysteine
317 proteases of *Trypanosoma* (*Megatrypanum*) *theileri*: cathepsin L-like gene sequences as
318 targets for phylogenetic analysis, genotyping diagnosis, Parasitol. Int. 59 (2010) 318–325.

319 25. T. Sivakumar, T. Yoshinari, I. Igarashi, H. Kothalawala, A.S. Abeyratne, S.C.
320 Vimalakumar, A.S. Meewawe, K. Kuleswarakumar, A.D.N. Chandrasiri, N. Yokoyama,
321 Genetic diversity within *Theileria orientalis* parasites detected in Sri Lankan cattle, Ticks
322 Tick Borne. Dis. 4 (2013) 235–241.

323 26. K. Tamura, 1992. Estimation of the number of nucleotide substitutions when there are
324 strong transition-transversion and G+Ccontent biases. Mol. Biol. Evol. 9, 678–687.

325 27. Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular

evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (1992) 2725–2729.

28. E.B. Wilson, Probable inference, the law of succession, and statistical inference, J. Am. Stat. Assoc. 22 (1927) 209–212.

29. I. Campbell, Chi-squared and Fisher-Irwin tests of two-by-two tables with small sample recommendations, Stat. Med. 26 (2007) 3661–3675.

30. J.T. Richardson, The analysis of 2 × 2 contingency tables—yet again, Stat. Med. 30 (2011) 890.

31. A. Rehman, A.M. Nijhof, C. Sauter-Louis, B. Schauer, C. Staubach, F.J. Conraths, Distribution of ticks infesting ruminants and risk factors associated with high tick prevalence in livestock farms in the semi-arid and arid agro-ecological zones of Pakistan, Parasit. Vectors 10 (2017) 190.

32. T. Sivakumar, M. Tattiyapong, S. Fukushi, K. Hayashida, H. Kothalawala, S.S. Silva, S.C. Vimalakumar, R. Kanagaratnam, A.S. Meewewa, K. Suthaharan, T. Puvirajan, W.K. de Silva, I. Igarashi, N. Yokoyama, Genetic characterization of *Babesia* and *Theileria* parasites in water buffaloes in Sri Lanka, Vet. Parasitol. 200 (2014) 24–30.

33. H.A. Garcia, K. Kamyingkird, A.C. Rodrigues, S. Jittapalapong, M.M. Teixeira, M. Desquesnes, High genetic diversity in field isolates of *Trypanosoma theileri* assessed by analysis of cathepsin Llike sequences disclosed multiple and new genotypes infecting cattle in Thailand, Vet. Parasitol. 180 (2011) 363–367.

345 34. H.A. Garcia, A.C. Rodrigues, F. Martinkovic, A.H. Minervino, M. Campaner, V.L.
346 Nunes, F. Paiva, P.B. Hamilton, M.M. Teixeira, Multilocus phylogeographical analysis of
347 *Trypanosoma* (Megatrypanum) genotypes from sympatric cattle and water buffalo
348 populations supports evolutionary host constraint and close phylogenetic relationships
349 with genotypes found in other ruminants, Int. J. Parasitol. 41 (2011) 1385–1396.
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353 **Figure legends**

354

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

361

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

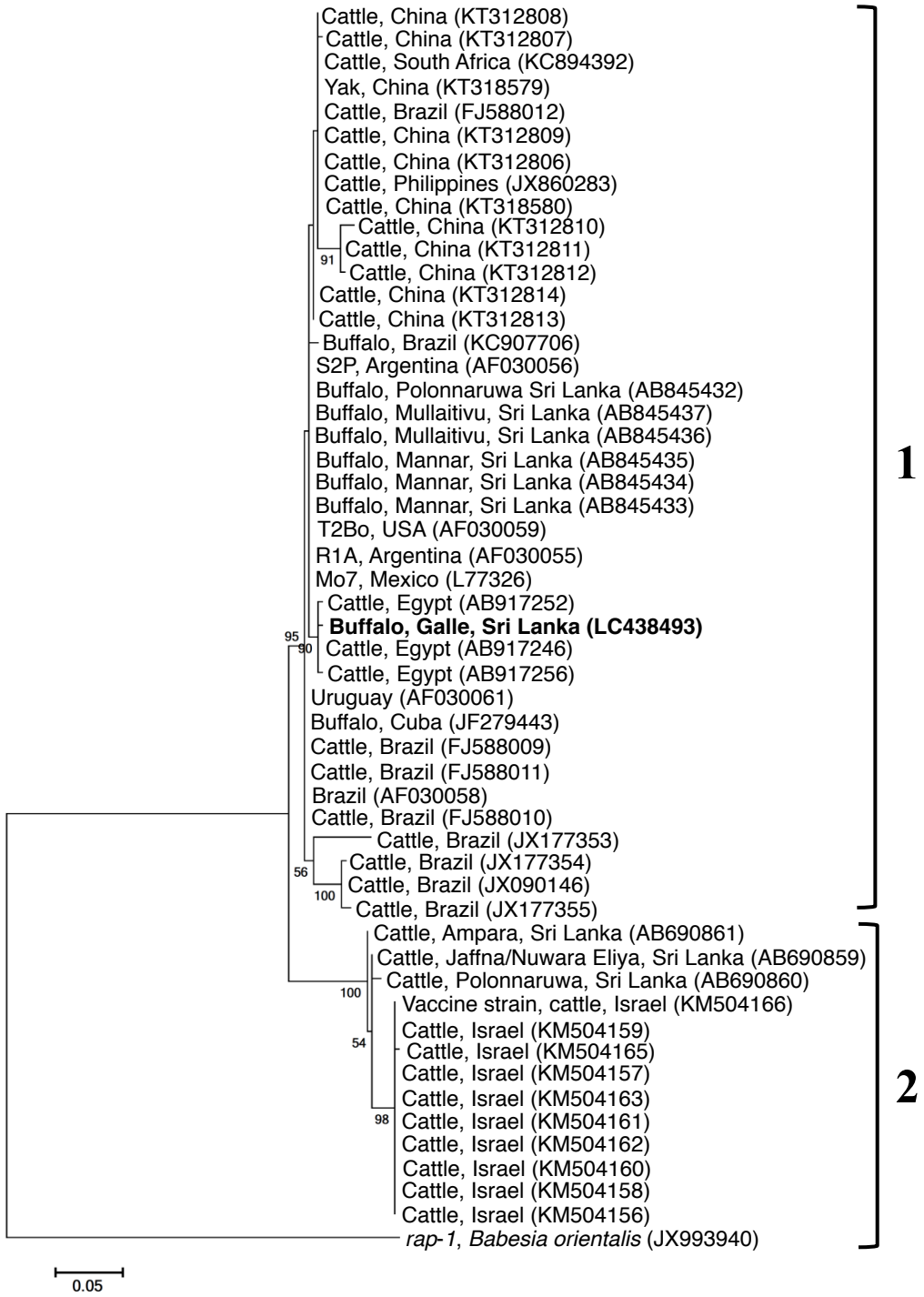
Cattle, China (KT312808)
Cattle, China (KT312807)
Cattle, South Africa (KC894392)
Yak, China (KT318579)
Cattle, Brazil (FJ588012)
Cattle, China (KT312809)
Cattle, China (KT312806)
Cattle, Philippines (JX860283)
Cattle, China (KT318580)
Cattle, China (KT312810)
Cattle, China (KT312811)
Cattle, China (KT312812)
Cattle, China (KT312814)
Cattle, China (KT312813)
Buffalo, Brazil (KC907706)
S2P, Argentina (AF030056)
Buffalo, Polonnaruwa Sri Lanka (AB845432)
Buffalo, Mullaitivu, Sri Lanka (AB845437)
Buffalo, Mullaitivu, Sri Lanka (AB845436)
Buffalo, Mannar, Sri Lanka (AB845435)
Buffalo, Mannar, Sri Lanka (AB845434)
Buffalo, Mannar, Sri Lanka (AB845433)
T2Bo, USA (AF030059)
R1A, Argentina (AF030055)
Mo7, Mexico (L77326)
Cattle, Egypt (AB917252)
Buffalo, Galle, Sri Lanka (LC438493)
Cattle, Egypt (AB917246)
Cattle, Egypt (AB917256)
Uruguay (AF030061)
Buffalo, Cuba (JF279443)
Cattle, Brazil (FJ588009)
Cattle, Brazil (FJ588011)
Brazil (AF030058)
Cattle, Brazil (FJ588010)
Cattle, Brazil (JX177353)
Cattle, Brazil (JX177354)
Cattle, Brazil (JX090146)
Cattle, Brazil (JX177355)
Cattle, Ampara, Sri Lanka (AB690861)
Cattle, Jaffna/Nuwara Eliya, Sri Lanka (AB690859)
Cattle, Polonnaruwa, Sri Lanka (AB690860)
Vaccine strain, cattle, Israel (KM504166)
Cattle, Israel (KM504159)
Cattle, Israel (KM504165)
Cattle, Israel (KM504157)
Cattle, Israel (KM504163)
Cattle, Israel (KM504161)
Cattle, Israel (KM504162)
Cattle, Israel (KM504160)
Cattle, Israel (KM504158)
Cattle, Israel (KM504156)
rap-1, Babesia orientalis (JX993940)
91
95
90
56
100
100
54
98
1
2
0.05

**Fig. 1**

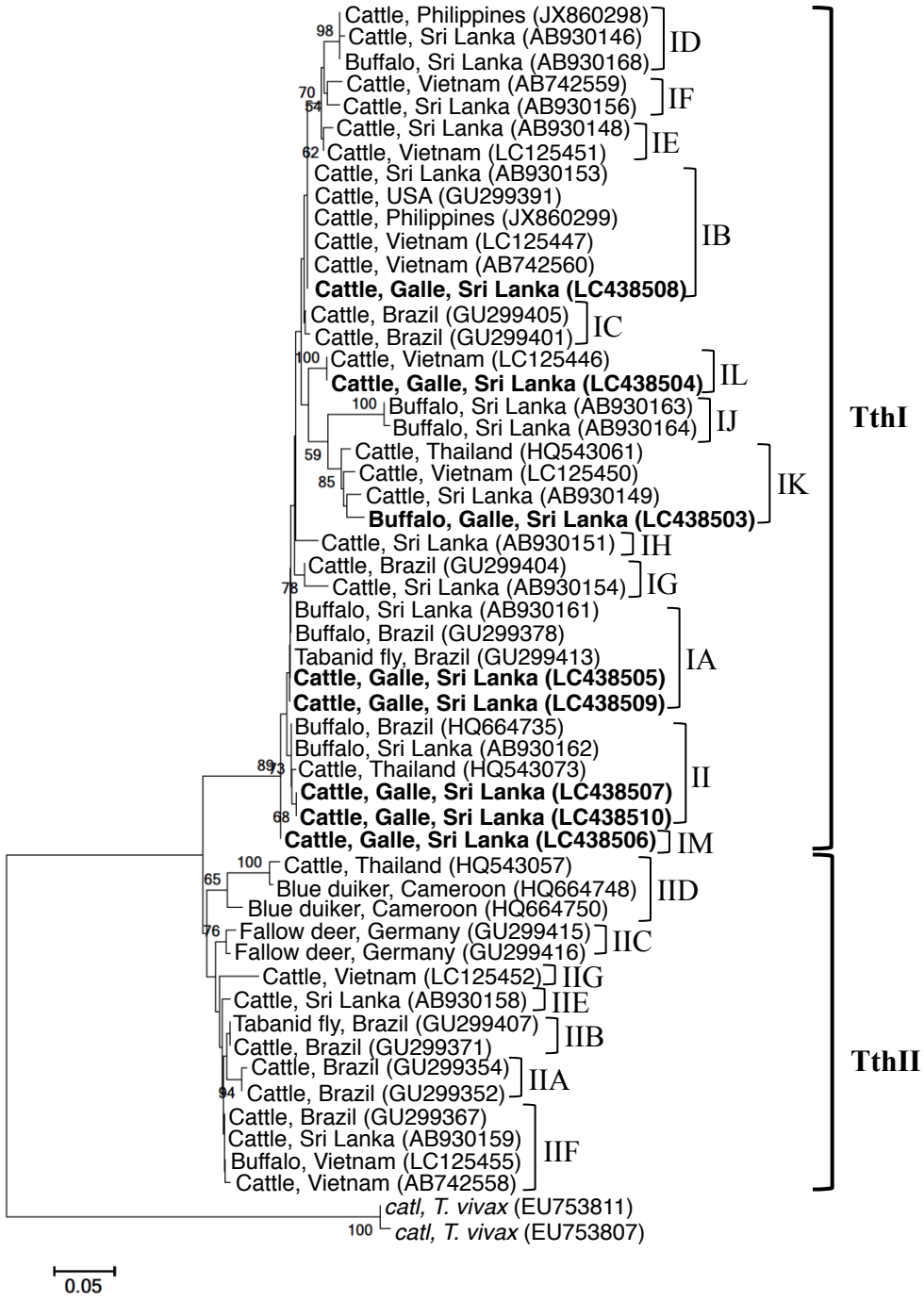
Cattle, Philippines (JX860298)
Cattle, Sri Lanka (AB930146)
Buffalo, Sri Lanka (AB930168)
ID
Cattle, Vietnam (AB742559)
Cattle, Sri Lanka (AB930156)
IF
Cattle, Sri Lanka (AB930148)
Cattle, Vietnam (LC125451)
IE
Cattle, Sri Lanka (AB930153)
Cattle, USA (GU299391)
Cattle, Philippines (JX860299)
Cattle, Vietnam (LC125447)
Cattle, Vietnam (AB742560)
Cattle, Galle, Sri Lanka (LC438508)
IB
Cattle, Brazil (GU299405)
Cattle, Brazil (GU299401)
IC
Cattle, Vietnam (LC125446)
Cattle, Galle, Sri Lanka (LC438504)
IL
Buffalo, Sri Lanka (AB930163)
Buffalo, Sri Lanka (AB930164)
IJ
Cattle, Thailand (HQ543061)
Cattle, Vietnam (LC125450)
Cattle, Sri Lanka (AB930149)
Buffalo, Galle, Sri Lanka (LC438503)
IK
Cattle, Sri Lanka (AB930151)
IH
Cattle, Brazil (GU299404)
Cattle, Sri Lanka (AB930154)
IG
Buffalo, Sri Lanka (AB930161)
Buffalo, Brazil (GU299378)
Tabanid fly, Brazil (GU299413)
Cattle, Galle, Sri Lanka (LC438505)
Cattle, Galle, Sri Lanka (LC438509)
IA
Buffalo, Brazil (HQ664735)
Buffalo, Sri Lanka (AB930162)
Cattle, Thailand (HQ543073)
Cattle, Galle, Sri Lanka (LC438507)
Cattle, Galle, Sri Lanka (LC438510)
II
Cattle, Galle, Sri Lanka (LC438506)
IM
TthI
Cattle, Thailand (HQ543057)
Blue duiker, Cameroon (HQ664748)
Blue duiker, Cameroon (HQ664750)
IID
Fallow deer, Germany (GU299415)
Fallow deer, Germany (GU299416)
IIC
Cattle, Vietnam (LC125452)
IIG
Cattle, Sri Lanka (AB930158)
IIE
Tabanid fly, Brazil (GU299407)
Cattle, Brazil (GU299371)
IIB
Cattle, Brazil (GU299354)
Cattle, Brazil (GU299352)
IIA
Cattle, Brazil (GU299367)
Cattle, Sri Lanka (AB930159)
Buffalo, Vietnam (LC125455)
Cattle, Vietnam (AB742558)
IIF
TthII
catl, T. vivax (EU753811)
catl, T. vivax (EU753807)
0.05

**Fig. 2**

**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) |
| *T. 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