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RESEARCH ARTICLE

Rickettsiae reservoirs among small mammals (Rats, Mice And Shrews) and their Arthropod Vectors in Sri Lanka

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Abstract: Rickettsioses are a group of emerging diseases caused by closely related bacteria. In Sri Lanka, to date, studies have been focused mainly on human subjects. The present study aimed to identify small mammal reservoir hosts and vectors of *Rickettsia* spp. and *Orientia tsutsugamushi* in two districts of Sri Lanka. Quantitative-PCR was carried out to detect *Rickettsia* using citrate synthase gene and *Orientia* using 47-kD outer membrane protein antigen gene in blood of small rodents and their infested ectoparasites. In both districts ~7.5% blood samples were positive for *Rickettsia*. *Rattus rattus*, *Bandicota indica* and *Mus fernandoni* were carriers. Three individuals of *Suncus murinus*, *B. indica* and *Golunda ellioti* had only infected ectoparasites. Copies of *gltA*/100 µL ranged from 133-1.2 × 10^4 in blood and 197-1.9 × 10^7 in ectoparasites. Of small mammals with ectoparasites, 43% had *Rickettsia* positive ectoparasites. *Rhipicephalus haemaphysaloides, Ixodes ceylonensis*, *Haemaphysalis spinigera*, *Haemaphysalis* spp., *Stivalius aporus* and *Xenopsylla cheopis* were positive. All study sites except three had infected small mammals or ectoparasites. All samples were negative for *O. tsutsugamushi.* This is the first study to report *Rickettsia* spp. in small mammals and their ectoparasites in Sri Lanka. *Haemaphysalis spinigera*, *I. ceylonensis* and *S. aporus* are new records of vectors for *Rickettsia*. This is also the first report of endemic *M. fernandoni* as a carrier of *Rickettsia* and *G. ellioti* with *Rickettsia* infected ectoparasites. Though rickettsiosis is not life threatening in most cases, it can lead to severe or fatal disease in vertebrate animals and humans. Hence, the knowledge of the distribution of said pathogen in the reservoirs is essential to control the disease.

Keywords: murine rodents, shrews, rickettsioses, Kandy, Kurunegala.

INTRODUCTION

Rickettsiae are a group of obligate, intracellular, gram-negative bacteria, including two genera, *Rickettsia* and *Orientia*. Rickettsiae cause mild to severe diseases in human and animals collectively known as *Rickettsioses* (Azad and Beard, 1998). They are transmitted through arthropod vectors, such as ticks, fleas, lice and mites. Genus *Rickettsia* is classified into two groups, Spotted fever group (SFG) and Typhus group (TG). *Orientia tsutsugamushi* causes scrub typhus. Rickettsioses have been recognized as an emerging group of diseases in Sri Lanka (Premaratna, 2011; Kularatne *et al.,* 2013). Recent studies have reported predominance of SFG Rickettsioses from Central province and Scrub typhus from Western, North Western, Southern and Northern provinces (Kularatne *et al.,* 2013, Liyanapathirana and Thevanesam 2011; Predeepan *et al*., 2014). Reserch in Sri Lanka up to date have been reported mainly Rickettsioses in human subjects, forcusing on hospital based clinical, epidemiological and serological studies in selected areas (Kularatne *et al.,* 2013, Liyanapathirana and Thevanesam, 2011). Only few published data are available on reservoir hosts and vectors of Rickettsiae in Sri Lanka (Nanayakkara *et al.,* 2013; Liyanaarachchi *et al.,* 2012).

Number of vertebrates, such as dogs, cats, goats, sheep, horses, opossums and bats, have been identified as reservoir hosts for *Rickettsia* in different parts of the world (Wachter *et al.*, 2015; Tabuchi *et al.,* 2007; Ortuno *et al.,* 2012; Milagres *et al.,* 2013; D'Auria *et al.,* 2010). Many others have been identified as potential reservoir animals such as cattle, wild boars, domestic ruminants, sika deer, hedgehogs, wild rabbits and lizards (Parola et al., 2013). Among small mammals, rodents of genera *Rattus*, *Apodemus*, *Mus*, *Bandicota* and shrew *Suncus murinus* are reported as reservoirs. *Orientia tsutsugamushi* has been recorded from *Rattus* spp., *Bandicota* spp. and *Tupaia glis* (Okabayashi *et al.,* 1996; Chen *et al.,* 1998; Schex *et al.,* 2010; Chien Kuo *et al.,* 2015).

Although several arthropod parasites act as vectors for *Rickettsia*, ticks are the most important in transmission of *Rickettsia* of SFG. *Rhipicephalus sanguineus*, and members of the genera *Amblyomma* and *Dermacentor* are common vectors of Spotted fever *Rickettsia.* Murine typhus is transmitted mostly by *Xenopsylla cheopis,* and Scrub typhus by thromboculid mites (Merhej and Raoult, 2011). Many *Rickettsia* species are known to vertically transmit in invertebrates, and capable of amplifying within arthropod vectors suggesting that they act as reservoirs of *Rickettsia* in nature (Azad and Beard, 1998).

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In Sri Lanka, rickettsiosis was first recorded in 1937 with 6 Scrub typhus positive patients (Premaratna, 2011). The role of thromboculid mites in transmission of Scrub typhus was studied by Jayawickreme and Nilesin in 1946. First case of Murine typhus was reported in 1938 and its source was confirmed to be rat fleas (Wolf, 1939). At the time it was also reported that typhus-infested rats were transported to the country in Indian vessels (Premaratna, 2011). Later studies reported seroprevalence of *Rickettsia* among dogs in Kandy, Unawatuna and western slope of central hills (Nanayakkara *et al.,* 2013). SFG *Rickettsia* was also reported from *Amblyomma* ticks collected from three wild mammals, pangolin, wild boar, tortoise and *Rhipicephalus sanguineus* from a dog (Liyanaarachchi *et al*., 2012). More recently, the presence of *Rickettsia* was detected in *Amblyomma trimaculatum* ticks of snake, *Boiga forsteni* imported to Japan from Sri Lanka (Andoh *et al.,* 2015).

Small mammals, mainly rodent species, for example *R. rattus*, *R. norvegicus*, *Mus musculus* and some wild rodents have been identified as reservoirs or carriers of many *Rickettsiae* in the world. They also serve as hosts for immature stages of ticks and adult fleas, facilitating the transmission of vector borne diseases (Chen *et al.,* 1998; Hornok *et al.,* 2015). In Sri Lanka, prevalence and distribution of reservoirs of *Rickettsiae* and species of small mammals and their arthropod parasites involved in transmission of *Rickettsiae* is not adequately studied. Hence, the objectives of this study were to identify small mammal species and their ectoparasites involved in transmission of *Rickettsiae*, and their prevalence and distribution in two selected districts in Sri Lanka.

MATERIALS AND METHODS

Small mammals (murine rodents and shrews) were collected from two districts in Sri Lanka, Kurunegala and Kandy, from 2013 to 2014. They were identified using the descriptions given by Phillips (1980). Small mammals were captured using 40 mesh traps placed in each site for four consecutive days. Traps were placed in and around houses, paddy fields, tea and other cultivations. Eight localities were sampled in Kurunegala and 10 in Kandy (Table 1). A 100-300µl sample of blood from saphenous vein and ectoparasites were collected from anesthetized small mammals. Ticks and fleas were identified with the help of taxonomic keys and species descriptions (Kirwan, 1935; Kohls, 1950; Trapido *et al*., 1964; Seneviratna, 1965; Rajagopalan and Boshell, 1966; Walker *et al*., 2000; Hopkins and Rothschild, 1953; Mardon, 1981). Ethical clearance for the study was obtained from the Postgraduate Institute of Peradeniya, Sri Lanka.

Sample processing and DNA Extraction

Blood

Blood samples were centrifuged at 12,000 rpm for 10 minutes. DNA was extracted from the resulting blood cell pellet using Wizard® Genomic DNA Purification Kit, Promega, USA, according to the manufacturer's protocol for isolation of genomic DNA from blood, with few modifications. Volume of each blood pellet was adjusted to 300 µl by adding PBS. Following modifications were done to the original protocol; Step 3: After adding cell lysis solution centrifugation was done at 14,000 rpm for 1 min; Step 6: After adding Nuclei lysis solution samples were incubated at 80 ºC for 5 min; Step 15: After adding DNA Rehydration solution samples were kept at room temperature overnight to rehydrate the DNA; Step 16: DNA Samples were stored at -20 ºC.

DNA Extraction from Ectoparasites

DNA was extracted using the same extraction kit, according to the manufacturer's protocol for Genomic DNA isolation from animal tissues, with few modifications. Ectoparasites were cut into small pieces using a scalpel blade and transferred into EDTA and nuclei lysis solution. Following modifications were done to the original protocol; Step 3e: After adding Protenase K, tubes were incubated over night at 55 ºC; Step 13: For DNA Rehydration 50 µl of DNA rehydration solution was added and rehydrated overnight at room temperature; Step 14: DNA samples were stored at -20 ºC.

Quantitative PCR assay for *Rickettsia*

To detect *Rickettsia* in blood and ectoparasites, seventy-four base pair fragment of citrate synthase gene (*gltA*) was amplified. Primers used were: Forward-CS-F (5'TCG CAA ATG TTC ACG GTA CTT T 3'), Reverse-CS-R (5' TCG TGC ATT TCT TTC CAT TGT G3') with the probe-CSP (5'FAM-TGC AAT AGC AAG AAC CGT AGG CTG GAT G-TAMsp-3'). The assay specifically amplifies the members of spotted fever and typhus group of *Rickettsia*. This assay does not produce a positive reaction for the ancestral group *Rickettsia*, *R. bellii* nor other members of the order Rickettsiales or any non-Rickettsial bacteria (Stenos *et al.,* 2005). PCR reactions were prepared using 12.5 µl Go taq® Probe qPCR master mix (Promega, USA), 2.5 µl of 2 µM forward and reverse primer and probe, 1 µl of nuclease free water and 4 µl of template. Final volume of the PCR mixture was 25 µl. The thermal profile of the assay, adopted from Stenos *et al.* (2005) except for the number of cycles, composed of initial holding stage of 50 ºC for 3 min for Pre PCR read and 95 ºC for 5 min for heat activation, 45 cycles of amplification at 95 ºC for 20 sec and 60 ºC for 40 sec and final post PCR read stage of 50 ˚C for 3 min. The reactions were performed in Step one Real Time PCR System (Applied Biosystems, USA) and analyzed using Step One software version 2.2.2. Each sample was run in duplicate with a positive control and two negative controls. An additional third run was performed for samples that gave one positive and one negative result.

Quantitative PCR assay for *Orientia tsutsugamushi*

To detect *Orientia tsutsugamushi* in blood of small mammals 118 base pair fragment of 47-kD outer membrane protein antigen/high temperature requirement "A" gene was amplified. Primers used were; Forward – OtsuFP (5'AAC TGA TTT TAT TCA AAC TAA TGC TGC T 3'),

Reverse – OtsuRP (5' TAT GCC TGA GTA AGA TAC RTG AAT RGA ATT-3') with Probe 5' FAM- TGG GTA GCT TTG GTG GAC CGA TGT TTA ATC T-TAMsp-3'. The assay specifically amplifies *O. tsutsugamushi* and does not produce a positive reaction for *Rickettsia* spp., nor other non-Rickettsial bacteria (Jiang *et al*., 2005). PCR reactions were prepared using 12.5 µl Go taq® Probe qPCR master mix (Promega, USA), 2 µl of 1.25 µM forward and reverse primers, 2.5 µM probe, 2.5 µl of nuclease free water and 4 µl of template. Final volume of the PCR mixture was 25 µl. Thermal profile of the assay composed of initial holding stage of 60 ºC for 30 s for Pre PCR read and 94 ºC for 5 min for heat activation, 45 cycles of amplification at 94 ºC for 5 s and 60 ºC for 30s and final post PCR read stage of 60 ºC for 30 s. Samples were not run in duplicate since all were negative for *Orientia tsutsugamushi*. A positive control and two negative controls were used in each run.

Standard curve PCR efficiency and Quantification

To quantify *Rickettsia* spp. and *Orientia* spp., and to find out the PCR efficiency, a standard curve was generated using a synthetic double stranded gene fragment, gBlocks® (Integrated DNA Technologies) as standards (GATATGGGTAAC GGCATAGT**AACTGATTTTATTCAAA CTAATGCTGCTATTCATATGGGTAGCTTT GGTGGACCGATGTTTAA TCTTGAAGGAAAAATTATTGGAATTAATTCT ATTCATGTATCTTACTCAGGCAT**AA GTTTTGCTATTCCATCTAATTTTATAAAGCTA TGGGTATACCG**TCGCAAATGTTCA CGGTACTTTTTGCAATAGCAAGAACCG TAGGCTGGATGGC ACAATGGAAAGAAATGCACGA**AGACCC TGAACAAAAAATCA). It contains the target regions for both *Rickettsia* spp. and *Orientia* spp. with 20 additional bases on either side. A ten-fold dilution series of gene fragment was prepared from 3.533x 10^6 to 3.533x10^0 for the standard curve. The last point of the standard curve that had least number of copies (3.533×10^0) did not amplify. Hence, the standard curve was prepared with six points, with three replicates for each point, and three negative controls. The approximate quantity was determined using the least Ct values of the positive samples and reading the relevant quantity from the standard curve. For this a common threshold "one" was selected, that go through the linear phase of all the amplification plots of standard curve and the samples. According to the standard curve, the PCR efficiency for *Rickettsia* assay was 92.1 %, $R^2 = 0.994$, slope = -3.527, Y intercept = 40.572. According to the standard curve for *Orientia*, the assay had 87.2 % efficiency, $R^2 = 0.999$, slope = -3.672 and Y intercept = 43.177.

RESULTS

Overall, 7.56% (18/238) blood samples were positive for *Rickettsia*. *Rattus rattus*, *Bandicota indica* and *Mus fernandoni* were carriers, while three individuals of *Suncus murinus*, *B. indica* and *Golunda ellioti* had infected ectoparasites. Copies of *gltA*/100µL ranged from 133-1.2×10^4 in blood and 197-1.9×10^7in ectoparasites. Fourty-three percent of small mammals with ectoparasites had *Rickettsia* positive ectoparasites. Among the ectoparasites, *Rhipicephalus haemaphysaloides, Ixodes ceylonensis*, *Haemaphysalis spinigera*, *Haemaphysalis* sp., *Stivalius aporus* and *Xenopsylla cheopes* were positive. All study sites except three had infected small mammals or ectoparasites. All samples were negative for *O. tsutsugamushi.*

Kurunegala District

Out of the eight sites in Kurunegala, two sites were negative for *Rickettsia*; in three sites small mammals were negative but some individuals had positive ectoparasites (Table 1).

Out of the 131 small mammals trapped in Kurunegala, blood samples were obtained from 120 individuals. This included: *Rattus rattus* (92), *Bandicota indica* (9), *B. bengalensis* (7), *Mus cervicolor* (3) and *Suncus murinus* (9). Of these only *R. rattus* (9/92, 9.8%) were positive. All reproductive stages were among the infected. Copies of *gltA* in 100 µL of blood in them ranged from 162 - 1.2x10^4 (1.8x10^3 ± 3.9x10^3). Three localities had positive individuals (Table 1). One individual with 1070 *gltA* copies of *Rickettsia* also had a *Rickettsia* positive *R. haemaphysaloides* nymph with 889 *gltA* copies of *Rickettsia*. There was no relationship between the reproductive stage, locality or species with the number of *gltA* copies (Appendix 1).

Of the above 120 small mammals, 33 individuals of *R. rattus, B. indica* and *Suncus murinus* were infested with external parasites. Of these 33, 11 (33.33%) had *Rickettsia* positive ectoparasites, 21 were negative for both blood and parasites and one *R. rattus* was *Rickettsia* positive and had a negative *R. haemaphysaloides* larva. Of the 87 small mammals not infested with ectoparasites, 7 were positive for *Rickettsia*.

Among the ectoparasites carrying *Rickettsia* were *Rhipicephalus haemaphysaloides* ticks and *X. cheopes* fleas. The number of *gltA* copies in ectoparasites ranged from 197 to 9.1x10^5. *Rhipicephalus haemaphysaloides* nymph and a larval pool had high number of *Rickettsia*, 9.1x10^5 and 4.8x10^5, respectively. Flea pools of *X. cheopes* had relatively low number of *Rickettsia* ranging from 220 to 342 copies (Appendix 2). Most of the (8/11) *Rickettsia* positive ectoparasites were collected from *R. rattus*. However, *R. haemaphysaloides* ticks that had the highest quantity of *Rickettsia* were from two *S. murinus* and a *B. indica*.

Kandy District

All ten sites in Kandy, except one had infected ectoparasites. Five sites had infected small mammals and ectoparasites while four sites had infected ectoparasites though the host was free of the bacteria (Table 1).

Out of the 155 small mammals trapped, blood samples were obtained from 118 individuals; *Rattus rattus* (96), *Bandicota indica* (7), *B. bengalensis* (6), *Mus fernandoni* (5) and, *Golunda ellioti* (2), *S. murinus* (2). Seven point six percent (9/118) of the blood samples were positive for *Rickettsia*. *Rattus rattus, B. indica* and *Mus fernandoni*

Table 1: Site-wise distribution of *Rickettsia* infected small mammals and parasites in Kurunegala and Kandy Districts.

	Locations	Host species (Number infected)	Parasite species (Number of individuals or pools infected)
Kurunegala	Bogollagama (07˚٤٧'N, 80˚١٠'E, elevation 80m)	*R. rattus* (0)	*R. haemaphysaloides* (1) *X. cheopes* (3)
	Ipalawa (07˚34'N, 80˚27'E, elevation 145m)	(0)	(0)
	Herathgama (07˚52'N, 80˚25'E, elevation 155m)	*R. rattus* (7)	*R. haemaphysaloides* (2)
	Kiwlegedara (07˚23'N, 80˚12'E, elevation 75m)	*R. rattus* (0)	*R. haemaphysaloides* (1)
	Malliyagoda (07˚24'N, 80˚28'E, elevation 170m)	(0)	(0)
	Minhettiya (07˚35'N, 80˚18'E, elevation 100m)	*R. rattus* (1) *S. murinus* (0)	*R. haemaphysaloides* (1) *R. haemaphysaloides* (2)
	Polgahawela (07˚19'N, 80˚17'E, elevation 75m)	*B. indica* (0)	*R. haemaphysaloides* (1)
	Udawela (07˚33'N, 80˚02'E, elevation 40m)	*R. rattus* (1)	(0)
Kandy	Peradeniya University (site 1) (07˚15'N, 80˚35' E, elevation 485m)	*R. rattus* (3)	*R. haemaphysaloides* (5) *I. ceylonensis* (2) *H. spinigera* (1) *Haemaphysalis larva* (1)
		B. indica (0)	*H. spinigera* (2)
		G. ellitoi (0)	*R. haemaphysaloides* (1)
	Peradeniya University (site 2) (07˚15'N, 80˚36' E, elevation 530 m)	*R. rattus* (1)	D. *auratus* (0)
		B. indica (1)	(0)
		M. fernandoni (0)	*S. aporus* (1)
	Peradeniya University (site 3) (07˚15'N, 80˚36'E, elevation 565 m)	*R. rattus* (1)	*R. haemaphysaloides* (1)
		M. fernandoni (1)	*R. haemaphysaloides* (1) *S. aporus* (3)
	Rajawatta (07˚16'N, 80˚36' E, elevation 500 m)	R. *rattus* (0)	*X. cheopes* (1)
	Mahakanda (07˚13'N, 80˚36'E, elevation 650m)	*M. fernandoni* (0)	*S. aporusg* (3)
	Doluwa (07˚11'N, 80˚36'E, elevation575 m)	(0)	(0)
	Delthota (07˚10N', 80˚42'E, elevation 1000m)	*R. rattus* (1)	*R. haemaphysaloides* (1) *X. cheopes* (6)
	Gampola (07˚10'N, 80˚33'E, elevation 500m)	*R. rattus* (1)	*X. cheopes* (1)
	Nawalapitiya (07˚02'N, 80˚32'E elevation 620m)	R. *rattus* (0)	*R. haemaphysaloides* (1)
	Kadugannawa (07˚ 16'N, 80˚ 29'E, elevation 570 m)	R. *rattus* (0)	*H. spinigera* (1)

carried *Rickettsia* with a prevalence of, 7.3% (7/96), 14.3% (1/7) and 20% (1/5), respectively. All infected small mammals were female adults and subadults (Appendix 1). Copies of *gltA* in 100 µL of blood ranged from 133 - 1925 (614±716). Positive small mammals were collected from six sites. They were collected from scrubland, close to buildings or inside houses. A *R. rattus* and *M. fernandoni* captured from Peradeniya had the highest copies of the gene, but they were not as high as recorded from Kurunegala. Similar to Kurunagala, there was no relationship between the reproductive stage, locality or species with the number of *gltA* copies (Appendix 1).

Of the 118 small mammals, 41 individuals of *R. rattus*, *B. indica*, *M. fernandoni* and *Golunda ellioti* were infested with external parasites while *B. bengalensis* and *S. murinus* were not. Of the infested 41 small mammals, 20 (48.8%) had ectoparasites positive for *Rickettsia* spp., out of which, 3 were positive for host blood as well. Two *Rickettsia* positive small mammals had *Rickettsia* negative ectoparasites. Both hosts and ectoparasites were negative in the other 19 ectoparasite infested small mammals. Of the 77 non-infested small mammals 4 were positive for *Rickettsia* and 73 were negative. There were 37 small mammals without blood samples, of which 11 had parasites. Eight of them were positive for *Rickettsia*.

Of the ectoparasites, *R. haemaphysaloides, H. spinigera* and *I. ceylonensis* ticks and *X. cheopes* and *S. aporus* fleas were carrying *Rickettsia*. The number of *gltA* copies in them ranged from 50 to $1.9x10^7$. *Rhipicephalus haemaphysaloides* nymph individuals, a pool of two nymphs and pools of *R. haemaphysaloides* larvae, Nymphs of *H. spinigera* and *X. cheopes* fleas were carrying large quantities of *Rickettsia*. Three small mammals were positive for both host blood and parasites. *Rickettsia* positive ectoparasites were collected from *R. rattus*, *B. indica*, *M. fernandoni* and *G. ellioti*. Parasites with highest quantity of *Rickettsia* were from *R. rattus* and *B. indica* (Appendix 2).

DISCUSSION

This is the first extensive study to report small mammal species and their ectoparasites involved in transmission of *Rickettsia* in Sri Lanka. Only few small-scale studies have been carried out on reservoir and vector species of *Rickettsia* in Sri Lanka previously. During 1930s and 1940s prevalence of murine typhus among rats and the role of thromboculid mites in transmission of scrub typhus have been studied by Sri Lankan researches (Premaratna, 2011). Two latest studies reported seroprevalence of *Rickettsia* among two dog populations (Nanayakkara *et al*., 2013) and presence of SFG *Rickettsia* in *Amblyomma* ticks collected from Wild mammals (Liyanaarachchi *et al*., 2012).

Prevalence of *Rickettsia* among small mammals were similar in both districts studied here, but only *R. rattus* were carrying *Rickettsia* in Kurunegala while three species, *R. rattus, B. indica* and *M. fernandoni* were *Rickettsia* positive in Kandy. This difference however, could be accounted for the high small mammal and ectoparasite diversity in selected sites in Kandy. Sites in Peradeniya and Mahakanda were scrublands with minimum human interference. Endemic small mammal *Mus fernandoni*, *Goluna ellioti* and *I. ceylonensis*, *D. auratus* ticks and *S. aporus* fleas were found in these sites. Outside Sri Lanka, *Rickettsia* antibodies have been detected among *R. rattus* and *M. musculus* from Philipines (Camer *et al.,* 2012), *R. rattus* from Brazil (Milagres *et al.,* 2013) and *B. indica* from Thailand (Okabayashi *et al.,* 1996). Okabayashi reported high prevalence of antibodies among *B. indica* suggesting it to be a reservoir host for SFG *Rickettsia*. Studies from outside Sri Lanka also support the importance of *R. rattus* and *B. indica* as carriers of *Rickettsia* (Okabayashi, 1996; Camer *et al.,* 2000; Coleman *et al.,* 2003; Kim *et al,* 2006), however, this is the first report of endemic *Mus fernandoni* carrying *Rickettsia*.

Rhipicephalus haemaphysaloides and *X. cheopes* were the most abundant tick and flea species found from both districts. Both species were positive for *Rickettsia* in both districts. *Haemaphysalis spinigera*, *I. ceylonensis* ticks and *S. aporus* fleas were also positive in Kandy district. In other regions of the world *Rickettsia* has been detected from species of *Aponomma*, *Amblyomma*, *Dermacentor*, *Hyalomma* and *Rhipicephalus* (Merhej and Raoult, 2011). Sixty three percent of *Aponomma hydrosouri*, considered as a reservoir for *R. honei*, were reported as positive for *Rickettsia* in a study done in Australia (Stenos *et al.,* 2003). In northern Germany 33.3% of *I. ricinus*, a reservoir for *R. Helvetica* was reported positive (Schicht *et al.,* 2012), and 41.2% of *Amblyomma americanum* was carrying *Rickettsia* in USA (Mixson *et al.,* 2006). Of flea species, *X. cheopes* is considered a reservoir and the main vector for *R. typhi,* which has been isolated from *X. cheopes* in several studies (Laudisoit *et al.,* 2014).

When considering the *Rickettsia* quantity, *R. haemaphysaloides*, *H. spinigera* ticks and *X. cheopes* fleas are important as they carry large number of *Rickettsia*. Maximum quantities carried by them were 10^7, 10^5, and 10^5, respectively. Reports of *Rickettsia* quantity in reservoir animals and vectors are scarce, but similar quantities have been reported in other studies as well. One study has reported 10^6 to 10^7 copies of *R. rickettsia* in *Amblyomma* ticks (Eremeeva *et al.,* 2003). The first report of *Rickettsia* quantity from tick vectors was from northern Germany with a 33.3% prevalence of *Ixodes ricinus*, a reservoir for *R. helvetica* had maximum quantity of *Rickettsia* in larvae, nymphs and adults were $5x10^4$, $8.5x10^6$ and $2x10^7$, respectively (Schicht *et al.,* 2012). Present study, reports maximum of 10^7 *Rickettsia* from *R. haemaphysaloides* Nymphs and 10^5 from larvae of the same species..

Of the 18 sites sampled, only three sites were free of infected small mammals or ecoparasites. This shows that the distribution of the pathogen is very high. Though rickettsiosis is not a life threatening disease in most cases, it can lead to severe or fatal disease in vertebrate animals as well as in humans (Azad and Beard, 1998; Costa *et al.,* 2002; Nadchatram, 2008). The distribution of this pathogen in the reservoirs is useful in control of the disease and for taking precautionary measures in high rick localities.

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Appendix 1:

Small mammal individuals positive for *Rickettsia* with quantity of *Rickettsia* expressed as number of copies of *gltA* gene and locality of capture. *Hosts with *Rickettsia* positive parasites.

	Quantity of *Rickettsia*	Species (gender/reproductive stage)	Locality
Kurunegala	162	*Rattus rattus* (M/J)	Udawela
	276	*R.* rattus (F/A)	Herathgama
	319	*R. rattus* (F/SA)	Herathgama
	342	*R.* rattus (F/A)	Herathgama
	502	*R. rattus* (F/A)	Herathgama
	778	*R. rattus* (F/SA)	Herathgama
	889	*R.* rattus (F/A)	Minhettiya
	1070	*R.* rattus (F/A)*	Herathgama
	12394	*R. rattus* (M/J)	Herathgama
Kandy	133	*Bandicota* indica (F/A)	Peradeniya
	180	*R. rattus* (F/A)*	Peradeniya
	188	*R. rattus* (F/A)*	Delthota
	217	*R. rattus* (F/A)	Peradeniya
	230	*R. rattus* (F/A)*	Peradeniya
	367	*R. rattus* (F/A)	Peradeniya
	483	*R. rattus* (F/A)	Gampola
	1800	*Mus fernandoni* (F/A)	Peradeniya
	1925	*R. rattus* (F/SA)	Peradeniya

Appendix 2:

Parasites positive for *Rickettsia* with quantity of *Rickettsia* expressed as number of copies of *gltA* gene and their hosts (same host indicated by numbers 1-7).

	***Rickettsia* Quantity**	**Parasite, stage and number of parasite in the pool (N-Nymph, L-larvae, M-adult male, F-adult female)**	**Host (reproductive stage)**
Kurunegala	197	*Rhipicephalus haemaphysaloides* (N3)	[1]*Rattus rattus* (Adult Female)
	220	*Xenopsylla cheopes* (M1, F1)	*R. rattus* (Adult Female)
	225	*X.cheopes* (F2)	*R. rattus* (Juvenile male)
	238	*R. haemaphysaloides* (N2)	[1]*R. rattus* (Adult Female)
	280	*R. haemaphysaloides* (N1)	*R. rattus* (Adult Female)
	322	*R. haemaphysaloides* (N1)	[2]*R. rattus* (Sub adult male)
	342	*X.cheopes* (F2)	*R. rattus* (Adult male)
	583	*R. haemaphysaloides* (N1)	*R. rattus* (Adult Female)
	619	*R. haemaphysaloides* (N1)	[2]*R. rattus* (Sub adult male)
	895	*R. haemaphysaloides* (N1)	*R. rattus* (Adult Female)
	1.6 $x10^3$	*R. haemaphysaloides* (N1)	[1]*R. rattus* (Adult Female)
	1.7 $x10^3$	*R. haemaphysaloides* (N3)	[1]*R. rattus* (Adult Female)
	6.8$x10^3$	*R. haemaphysaloides* (N1)	*Suncus murinus* (Adult female)
	4.8 $x10^5$	*R. haemaphysaloides* (L16)	*Suncus murinus* (Adult male)
	9.1 $x10^5$	*R. haemaphysaloides* (N1)	*Bandicota indica* (Adult male)

	Rickettsia Quantity	Parasite, stage and number of parasite in the pool (N-Nymph, L-larvae, M-adult male, F- adult female)	Host (reproductive stage)
Kandy	50	*Xenopsylla cheopes* (F:3)	*Rattua rattus* (Adult male)
	100	*Stivalius aporus* (F:1)	*Mus fernandoni* (Sub adult female)
	100	*Xenopsylla cheopes* (F:2)	*R.rattus* (Adult female)
	138	*X. cheopes* (M:1, F:4)	*R.rattus* (Adult female)
	138	*X. cheopes* (M:2, F:1)	*R.rattus* (Adult female)
	150	*Rhipicephalus haemaphysaloides* (N:1)	*Golunda ellioti* (Adult female)
	150	*X. cheopes* (M:2, F:1)	*R.rattus* (Adult female)
	163	*X. cheopes* (F:2)	*R.rattus* (Sub adult male)
	188	*Ixodes ceylonensis* (A:1)	[3]*R.rattus* (Adult female)
	204	*X. cheopes* (M:3)	*R.rattus* (Sub adult male)
	225	*S. aporus* (F:1)	*M. fernandoni* (Sub adult Male)
	238	*S. aporus* (M:3, F:1)	*M. fernandoni* (Adult female)
	313	*S aporus* (M:2)	*M. fernandoni* (Adult female)
	388	*S. aporus* (M:1)	*M. fernandoni* (Adult female)
	438	*S. aporus* (M:3, F:1)	*M. fernandoni* (Adult female)
	480	*I. ceylonensis* (A:1)	[4]*R.rattus*(Adult male)
	$1.1x10^3$	*R. haemaphysaloides* (N:1)	[5]*R.rattus* μ(Adult male)
	$1.6x10^3$	*Haemaphysalis spinigera* (N:1)	*R.rattus* (Sub adult male)
	$2.2x10^3$	*S. aporus* (M:2, F:1)	*M. fernandoni* (Adult male)
	$6.6x10^3$	*H. spinigera* (N:1)	*R.rattus* (Adult male)
	$1.1x10^4$	*Rhipicephalus haemaphysaloides* (N:1)	*M. fernandoni* (Juvenile Male)
	$1.1x10^4$	*R. haemaphysaloides* (L2)	[6]*Rattus rattus*
	$1.1x10^4$	*H. spinigera* (N:1)	[7]*Bandicota indica* Δ(Adult male)
	$2.9x10^4$	*Haemaphysalis* (L1)	[4]*R.rattus* (Adult male)
	$5.1 x10^4$	*R. haemaphysaloides* (N:1)	[6]*Rattus rattus* (Adult male)
	$5.5 x10^4$	*R.haemaphysaloides* (L2)	[5]*R.rattus* μ(Adult male)
	$1.6 x10^5$	*X. cheopes* (F:3, M:3)	*R.rattus* (Adult male)
	$5.9x10^5$	*R. haemaphysaloides* (L8)	*R.rattus* (Juvenile Female)
	$7.7 x10^5$	*H. spinigera* (N:1)	[7]*B. indica* Δ(Adult male)
	$9.2 x10^5$	*R.haemaphysaloides* (N:1)	*R.rattus* (Adult female)
	$5.3 x10^6$	*R.haemaphysaloides* (N:1)	*R.rattus* (Adult male)
	$1.3 x10^7$	*R. haemaphysaloides* (N:2)	[3]*R.rattus* (Adult female)
	$1.9x10^7$	*R. haemaphysaloides* (N:1)	*R.rattus* (Adult male)