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 bernandoni 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 × 10^6 in blood and 197-1.9 × 10^6 in ectoparasites. Of small mammals with ectoparasites, 43% had Rickettsia positive ectoparasites. Rhipecephalus haemaphysalooides, Ixodes ceylonensis, Haemaphysalis spinigera, Haemaphysalis spp., Stivallus 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. bernandoni 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, focusing 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 Tupai a 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 tromboculid 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 throbomculid mites in transmission of Scrub typhus was studied by Jayawickreme and Nileisin 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 *Rhipecephalus sanguineus* from a dog (Liyanarachchi 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 Protease K, tubes were incubated overnight 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 AGC GTA CTT T 3’), Reverse-CS-R (5’ TCG TGC ATT TCT TTC CAT G 3’) with the probe-CSP (5’FAM-TGC AAT AGC AAG AAC CGT AGG CTG GAT GTMasp-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 ºC 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 30 s 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 (GATATGGGGTAAC GGCATATGTAACCTTTATC AA A TCTATATGCTGCTATTTCAATAGGTTACCTTT GGTAGTACTTTTTGCAATAGCAAGAACCG GTTTTGCTATTCCATCTAATTTTATAAAGCTA ATTCATGTATCTTACTCAGGCAT TCTTGAAGGAAAAATTATTGGAATTAATTCT ATTCATGTATCTTACTCAGGCTAT AAGTTTGTATCTTCTAATTTTATAAGCTA TGGGTTGATCTGCAATAGCAGAATGC TGGTACATTTTTTGCAATAGCGACCAACGG TAAGCTGCTGGAGTGAGGC ACAAATGGAAAGAAAATGCAAGAAGACCC TGAACAAAAAATTCA). It contains the target regions for both Rickettsia spp. and Orientia spp. with 20 additional bases on either side. A ten-dilution series of a gene fragment was prepared from 3.533 x 10^6 to 3.533 x 10^9 for the standard curve. The last point of the standard curve that had least number of copies (3.533 x 10^9) 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 %, R² = 0.994, slope = -3.527, Y intercept = 40.572. According to the standard curve for Orientia, the assay had 87.2 % efficiency, R² = 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 x 10^4 in blood and 197-1.9 x 10^4 in ectoparasites. Forty-three percent of small mammals with ectoparasites had Rickettsia positive ectoparasites. Among the ectoparasites, Rhipicephalus haemaphysaloides, Ixodes ceylonensis, Haemaphysalis spinigera, Haemaphysalis sp., Stivalius aorpus and Xenopsylla cheopse 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, 9.9%) were positive. All reproductive stages were among the infected. Copies of gltA in 100 µL of blood in them ranged from 162 - 1.2 x 10^4 (1.8 x 10^3 ± 3.9 x 10^3). 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 nymph. 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. cheopse fleas. The number of gltA copies in ectoparasites ranged from 197 to 9.1 x 10^3. Rhipicephalus haemaphysaloides nymph and a larval pool had high number of Rickettsia, 9.1 x 10^3 and 4.8 x 10^5, respectively. Flea pools of X. cheopse 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) |
|-----------------------------------|-------------------------------|-----------------------------------------------------------|
| Bogollagama (07°47′N, 80°10′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) | *R. haemaphysaloides* (1) |
| Polgahawela (07°19′N, 80°17′E, elevation 75m) | *S. murinus* (0) | *R. haemaphysaloides* (2) |
| Udawela (07°33′N, 80°02′E, elevation 40m) | *B. indica* (0) | *R. haemaphysaloides* (1) |
| 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. aporus* (3) |
| Doluwa (07°11′N, 80°36′E, elevation 575 m) | (0) | (0) |
| Delthota (07°10′N, 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 1.9x10^6. *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 thomboctolitid 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 (Liyanarachchi 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*, *Golunda 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 Philippines (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* *hydroسورi*, 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. Helvatica* 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 (Laudisio 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 10^5, 10^6, and 10^7, 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. rickettsiae* 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 5x10^5, 8.5x10^5 and 2x10^6, respectively (Schicht et al., 2012). Present study, reports maximum of 10^7 *Rickettsia* from *R. haemaphysaloides* Nymphs and 10^4 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 rickettsioses 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.
ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from the Higher Education for the Twenty First Century (HETC) Quality and Innovation grant (QIG), Window 3. The field assistance given by Indika Senavirathna, Shivanthika Lakmali, Rajika Thilakaratana, Anushka Wanninayaka, Shashika Guruge, Nishadi Karunathilaka, P.P.C. Hemamala, Umesha Dissanyake and Ama Pothuhera is also acknowledged.

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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   |
|------------------------|-------------------------------------|------------|
| 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 |
| 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) |
|---------------------|-----------------------------------------------------------------------------------------------------|---------------------------|
| 197                 | *Rhipicephalus haemaphysaloides* (N3)                                                             | *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)                                                                       | *R. rattus* (Adult Female) |
| 280                 | *R. haemaphysaloides* (N1)                                                                       | *R. rattus* (Adult Female) |
| 322                 | *R. haemaphysaloides* (N1)                                                                       | *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)                                                                       | *R. rattus* (Sub adult male) |
| 895                 | *R. haemaphysaloides* (N1)                                                                       | *R. rattus* (Adult Female) |
| 1.6 x10^3           | *R. haemaphysaloides* (N1)                                                                       | *R. rattus* (Adult Female) |
| 1.7 x10^3           | *R. haemaphysaloides* (N3)                                                                       | *R. rattus* (Adult Female) |
| 6.8x10^3            | *R. haemaphysaloides* (N1)                                                                       | *Suncus murinus* (Adult female) |
| 4.8 x10^3           | *R. haemaphysaloides* (L16)                                                                      | *Suncus murinus* (Adult male) |
| 9.1 x10^3           | *R. haemaphysaloides* (N1)                                                                       | *Bandicota indica* (Adult male) |
| Quantity | Parasite, stage and number of parasite in the pool (N-Nymph, L-larvae, M-adult male, F- adult female) | Host (reproductive stage) |
|----------|--------------------------------------------------------------------------------------------------|--------------------------|
| 50       | Xenopsylla cheopes (F:3)                                                                         | Rattus 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 male) |
| 138      | *X. cheopes* (M:2, F:1)                                                                         | *R. rattus* (Adult female) |
| 150      | *Rhipicephalus haemaphysaloides* (N:1)                                                           | Golunda elliotti (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)                                                                         | *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)                                                                             | *R. rattus* (Adult male) |
| 1.1x10³ | *R. haemaphysaloides* (N:1)                                                                       | *R. rattus* μ(Adult male) |
| 1.6x10³ | Haemaphysalis spinigera (N:1)                                                                     | *R. rattus* (Sub adult male) |
| 2.2x10³ | *S. aporus* (M:2, F:1)                                                                           | *M. fernandoni* (Adult male) |
| 6.6x10³ | *H. spinigera* (N:1)                                                                             | *R. rattus* (Adult male) |
| 1.1x10⁴ | *Rhipicephalus haemaphysaloides* (N:1)                                                           | *M. fernandoni* (Juvenile Male) |
| 1.1x10⁴ | *R. haemaphysaloides* (L2)                                                                        | *Rattus rattus* |
| 1.1x10⁴ | *H. spinigera* (N:1)                                                                             | *Bandicota indica* Ą(Adult male) |
| 2.9x10⁴ | Haemaphysalis (L1)                                                                                | *R. rattus* (Adult male) |
| 5.1x10⁴ | *R. haemaphysaloides* (N:1)                                                                        | *Rattus rattus* (Adult male) |
| 5.5x10⁴ | *R. haemaphysaloides* (L2)                                                                        | *R. rattus* μ(Adult male) |
| 1.6x10⁵ | *X. cheopes* (F:3, M:3)                                                                           | *R. rattus* (Adult male) |
| 5.9x10⁵ | *R. haemaphysaloides* (L8)                                                                        | *R. rattus* (Juvenile Female) |
| 7.7x10⁵ | *H. spinigera* (N:1)                                                                              | *B. indica* Ą(Adult male) |
| 9.2x10⁵ | *R. haemaphysaloides* (N:1)                                                                        | *R. rattus* (Adult female) |
| 5.3x10⁵ | *R. haemaphysaloides* (N:1)                                                                        | *R. rattus* (Adult male) |
| 1.3x10⁶ | *R. haemaphysaloides* (N:2)                                                                        | *R. rattus* (Adult female) |
| 1.9x10⁷ | *R. haemaphysaloides* (N:1)                                                                        | *R. rattus* (Adult male) |