

Full Length Research Paper

Prevalence of *Rickettsia typhi* in rodent fleas from areas with and without previous history of plague in Mbulu district, Tanzania

Claus A. Thomas^{1*}, Abdul A. S. katakweba², Apia W. Massawe², Rhodes H. Makundi², Robert S. Machang'u¹ and Stella T. Kessy²

¹Department of Veterinary Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, Morogoro, Tanzania.

²Pest Management Centre, Sokoine University of Agriculture, Morogoro, Tanzania.

Received 21 August, 2019; Accepted 16 December 2019

Murine (endemic) typhus is a flea-borne infectious disease caused by *Rickettsia typhi*. The disease transmission cycle has similarities to that of *Yersinia pestis* causing plague. It is hypothesized that murine typhus is prevalent in areas with plague transmission. This study aims at detection of *R. typhi* in rodent fleas by conventional polymerase chain reaction (PCR). A cross sectional study was carried out in Mbulu district in villages with, and without previous history of plague from November 2018 to February 2019. Sherman[®] traps were set in forest and agricultural habitats while box traps were set inside houses. Captured rodents were anaesthetized using halothane and fleas were removed from the fur using a hard brush and preserved in 70% ethanol. PCR amplification of the targeted citrate synthase (*gltA*) gene of *R. typhi* was done using primers RpCS.877p and RpCS.1258n. 12 (24%) of the DNA from rodent fleas was positive for *R. typhi*. Of these, 5 (10%) and 2 (4%) were from farms and forests with previous plague history respectively, while 3 (6%) and 2 (4%) were from houses and farms with no previous plague history, respectively. This suggests the prevalence of murine typhus is independent of plague infections.

Key words: Polymerase chain reaction (PCR), plague, prevalence, *Rickettsia typhi*, rodents.

INTRODUCTION

Rickettsial diseases are worldwide emerging arthropod borne zoonoses that are caused by an obligate intracellular Gram-negative bacterium often found in vector fleas (Abdad et al., 2019; Noh et al., 2017). Rickettsioses are traditionally divided into the spotted fever, typhus, and the scrub typhus groups (McLeod et al., 2004; Giulieri et al., 2012).

Murine typhus, also called endemic typhus or flea-

borne typhus is caused by *Rickettsia typhi* (Civen and Ngo, 2008). The disease is transmitted by fleas (*Xenopsylla cheopis*) found on rodents (Eremeeva et al., 2008). People get murine typhus when rodent flea faeces containing the rickettsial agents contaminate the bite sites or other skin openings during feeding (La Scola et al., 2000). The conjunctiva can also be the port of entry for *R. typhi* (Noden et al., 2017). Similar to plague,

*Corresponding author. E-mail: clauthomas2017@gmail.com. Tel: +255712819789/+255757808512.

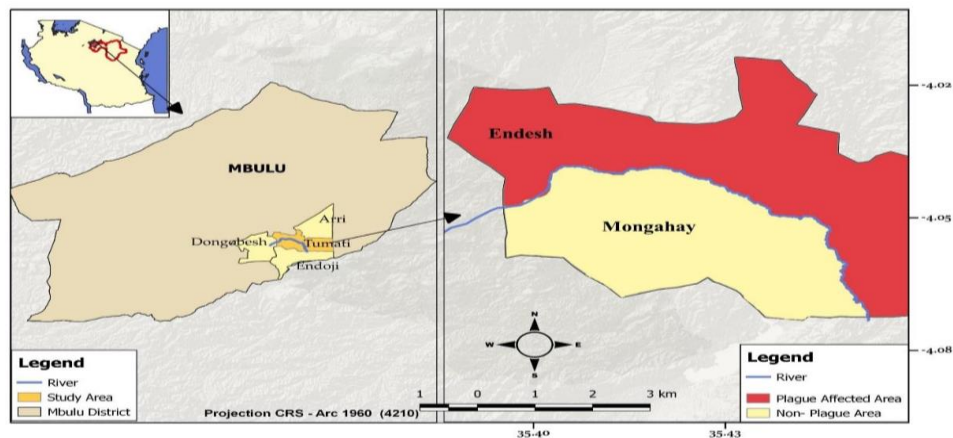


Figure 1. Map of Mbulu district showing villages with and without previous history of plague.

murine typhus would occur in areas where rodents and the fleas are abundant mostly in farms, forests and residential houses (Laudisoit et al., 2014).

Limited studies on murine typhus have been done in Tanzania, specifically in Mbeya and Moshi districts by Dill et al. (2013) and Prabhu et al. (2011). However, more extensive studies have been done in other countries, such as the serological evidence of exposure to *R. felis* and *R. typhi* in Australian veterinarians by Teoh et al. (2017) and the short report on murine typhus in Caldas Colombia (Hidalgo et al., 2008).

Apart from the limited information on murine typhus in Tanzania, little is known on its prevalence in the areas with previous history of *Yersinia pestis* plague, such as Mbulu district. Since murine typhus transmission cycle is similar to that of plague, there is the likelihood that murine typhus is prevalent in Tanzania in areas with previous history of plague. Mbulu District is known as one of the hotspots of plague in Tanzania (Makundi et al., 2008). The outbreak was revealed in some of villages including Tumati and Arri in the Division of Dongobesh in Mbulu, where 35 cases of plague were initially reported, with six deaths (Makundi et al., 2008). The victims had clinical symptoms of plague, including buboes, high fever, chills, headache, weakness, vomiting, nausea and prostration (Ben-Ari et al., 2011; Leirs et al., 2010). This study aimed at detecting *R. typhi* in rodent fleas by conventional polymerase chain reaction (PCR) from areas with and without previous history of plague in Mbulu District. This information will contribute to the design of disease control strategies of murine typhus and other rickettsial diseases in the future.

MATERIALS AND METHODS

Study area and design

A cross sectional study was carried out in Mbulu district

(3°45'00.0"S 35°20'00.0"E) with the altitude ranging from 1930 to 2250 m above sea level (Ziwa et al., 2013). This involved two villages with and without previous history of plague, namely Endesh and Mongahay respectively (Figure 1). Samples were collected in three habitats, agricultural land, forest and inside houses in both villages.

Rodent trapping and sample collection

During rodent trapping four transect lines were set in agricultural and forest habitats in previously plague and non-plague affected villages. Each habitat had 20 Sherman® traps (Standard medium size LFA: 7.6 X 8.9 X 23 CM) in each line placed 10m apart from line to line and from trap to trap. Ten percent of the houses in the village were randomly selected making a total of 43 houses in both villages. Each of five Sherman® traps and box traps were randomly set inside each house. Trapping was conducted once per month from November 2018 to February 2019 for three consecutive nights, left overnight and inspected every morning. A total of 50 rodents were captured and anaesthetized using halothane. The following information was recorded rodent species, by looking their morphology, site of collection, trap used (whether box or Sherman® traps), sexual maturity and number of fleas collected. Total of 100 fleas, were removed from the fur of rodents using a hard brush and collected in a clean basin covered with a white paper. The fleas were then preserved in Eppendorf tubes with 70% ethanol before being transported to the laboratory for the molecular studies. Only 50 fleas were tested by PCR.

DNA extraction and storage

Fleas were rinsed twice in distilled water for 10 min and then dried on sterile filter paper. Handling was performed in a laminar flow biosafety cabinet. Fleas were individually crushed by sterile mortar and pestle then preserved in sterile Eppendorf tubes, as described by Leulmi et al. (2014). DNA was extracted from each flea using the kit (QUICK-gDNA™ Min prep USA) according to the manufacturer's instructions. The genomic DNA was stored at -20°C under sterile conditions until used in PCR assays.

PCR amplification of *R. typhi* DNA

The DNA extracted from each flea was tested by conventional PCR

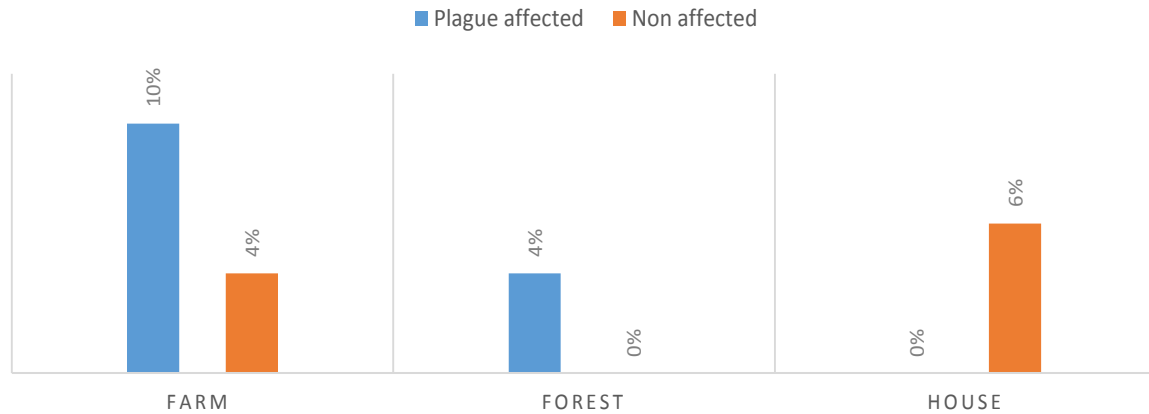


Figure 2. PCR results showing prevalence of *R. typhi* in rodent flea.

using forward and reverse primers RpCS.877p: GGGGGCCTGCTCACGGCGG RpCS.1258n: ATTGCAA AAAGTACAGTGAACA respectively (MacroGen Humanizing Genomics, Republic of Korea). This amplifies a 381 bp fragment of the citrate synthase encoding gene (*gltA*) for *R. typhi* (Regnery et al., 1991; Webb et al., 1990; Portillo et al., 2017). DNA amplification was done in a total volume of 17 μ l using Taq polymerase (Cycler (Perkin-Elmer Cetus, Norwalk, Conn). Mixed samples were amplified for 40 repeated cycles, denaturation at 94°C for 30 s, annealed at 58°C for 30 s, and subjected to the sequence extension at 68°C for 1 min and 30 s for 50 samples as described by Webb et al. (1990).

Agarose gel electrophoresis of PCR product

A total of 1.5 g of agarose powder was weighed and dissolved into 1X dissolving buffer in a conical flask to make 1.5% agarose gel. Before loading the agarose gel was pre-stained with Gel Red (Biotium, Hayward, CA). A total of 17 μ l of the PCR product was loaded onto the 1.5% agarose gels and electrophoresed at 100V for 40 min (Sousa et al., 2017). The amplified products were visualized against a ladder marker followed by appropriate examination of the band using UV trans illuminator. *R. typhi* targeted amplified DNA sequence by PCR in (*gltA*) is at 381 bp band on agarose gels upon electrophoresis (De Sousa et al., 2006). The positive control was kindly provided by the Molecular Biology Laboratory, University of Dar es Salaam (UDSM).

RESULTS

A total of 50 fleas were sampled from 50 rodents in villages with previous history of plague (Endesh) and villages without previous history of plague (Mongahay). DNA from each flea was tested by conventional PCR. A 381 bp fragment of the citrate synthase encoding gene (*gltA*) for *Rickettsia* spp was amplified then visualized on agarose gels after electrophoresis using UV trans illuminator. Altogether 12 (24%) of the DNA from rodent fleas was positive for *R. typhi* (*gltA*). Of these 5 (10%) and 2 (4%) were from farms and forests of Endesh respectively, while 3 (6%) and 2 (4%) were from houses

and farms in Mongahay (Figure 2). Chi square test showed that prevalence of *R. typhi* was not statistically different between villages with or without plague history ($\chi^2 = 50.62$ df = 49 p = 0.084). This entails that prevalence of *R. typhi* is not associated with plague outbreak in an area. Suggesting that *R. typhi* can be equally prevalent in plague affected as well as non-affected areas. Amplification of the gene (*gltA*) encoding for *Rickettsia* was done in six fleas from plague affected and non-plague affected villages of Mbulu district followed by agarose gel electrophoresis. For negative control PBS was used. 381 bp fragment was observed in samples 1, 23, 24, 25, 26 and 27 (Figure 3). The size of band was determined by the DNA ladder Marker (LD). For rodent species, total of seven species of rodents were identified to be positive for *R. typhi* in both plague and non plague affected areas as shown in (Figure 4).

DISCUSSION

The aim of this study was to determine the prevalence of *R. typhi* in rodent fleas from areas with and without previous history of plague in Mbulu district, Tanzania. Demonstration of *R. typhi* was done by detecting *R. typhi* DNA of rodent fleas by PCR. Results from rodent fleas showed the prevalence of *R. typhi* not to be significantly higher in a village with previous history of plague than the one without previous history of plague (p>0.05). This suggests that prevalence of *R. typhi* in fleas in Mbulu district may not be necessarily associated with plague infection in the studied areas.

Murine typhus and plague have similarities in their epidemiology (Drancourt and Raoult, 2016) however, they do not necessarily infect the host concomitantly (Fenollar and Mediannikov, 2018). The rather faint bands in the electrophoresis of some of the flea PCR products (Figure 3) was possibly due to reduced amount of DNA available. Therefore, it is recommended to pool more

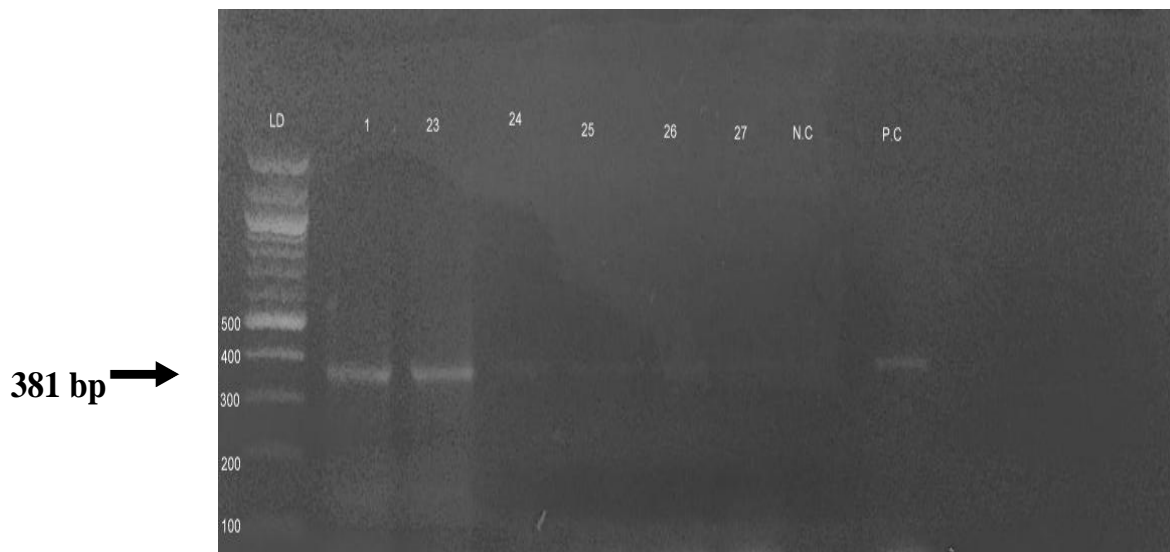


Figure 3. Demonstration of citrate synthase gene (*gltA*) of 381 bp in rodent flea by agarose gel electrophoresis. LD= Ladder Marker; 1, 23, 24, 25, 26 and 27= Positive Samples. PC= Positive Control: NC= Negative Control: bp = Base Pairs.

fleas in order to get a clearer gel electrophoresis band (Phan et al., 2011). Also, instead of extracting the DNA from fleas one can use DNA from rodent serum and get similar results with a clear *gltA* band when the right primers are used (Giulieri et al., 2012).

Other diagnostic tools namely ELISA and IFA have been used to detect *R. typhi* infection (Portillo et al., 2017). However, long-term reliance on serological tests and microscopy has led to underdiagnosis, inappropriate therapy, and undocumented morbidity and mortality. Recent approaches therefore integrate molecular approaches in the diagnosis of murine typhus to enable early detection and appropriate treatment (Paris and Dumler, 2016). The PCR was used as a highly sensitive and specific test in studies carried out in Tanzania among pregnant women (Scola and Raoult, 1997). These studies found a prevalence of 28% in Dar es Salaam and 0.5 to 9.3% in the towns of Kilimanjaro and Mbeya respectively (Leulmi et al., 2014).

Generally, murine typhus is more prevalent in areas where rodents and fleas are abundant (Conlon, 2007). Similar to plague, murine typhus occurs in agricultural land, forests and in residential houses (Laudisoit et al., 2014). Some domestic animals are also known to harbor the plague pathogen (Nyirenda et al., 2018). Murine typhus has no dramatic clinical effects and it is often neglected however, it can damage body organs, lead to coma, and even death. Hence intervention has to be done as soon as possible in an affected or suspected area.

The rodent species *Mastomys natalensis* showed overall more positive sera followed by *Rattus rattus*. The other rodent species namely *Arvicantis* spp, *Lophuromys*

spp, *Lemniscomys* spp, *Grammomys* spp gave lower numbers of positive sera possibly due to high abundance of *Mastomys natalensis* in both farms, forest and domestic house though more studies have to be conducted in the future to scientifically prove this assumption (Figure 4).

In conclusions the prevalence of *R. typhi* may not necessarily be associated with previous plague infection in the studied areas. Nevertheless, murine typhus is a potential health threat to communities in Mbulu and possibly other part of Tanzania. Further research and longitudinal surveillance of fleas, their rodent hosts and disease carrier status is needed to determine the geographical distribution, habitats, and prevalence of the pathogens. Isolation and characterization of the Rickettsia is essential in order to identify locally circulating strains and their potential threat to humans. The prevalence data reported herein provides a basis for the development of species-specific assays that will give a clearer picture of rickettsial diseases in Tanzanian communities in the future.

Study limitation

One among the shortcomings is that the number of villages were limited and therefore number of specimen collected were few.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

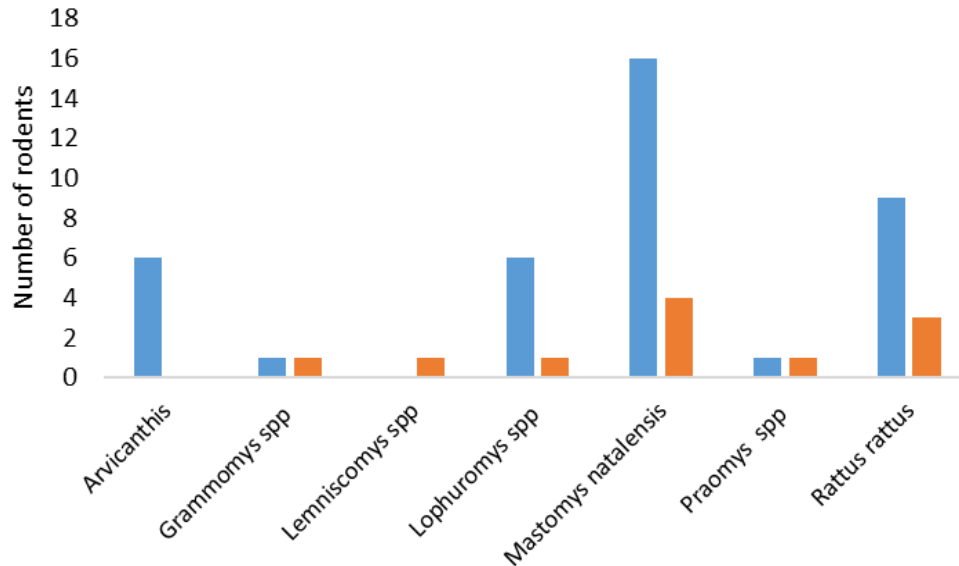


Figure 4. Rodent species carrying fleas positive for *R. typhi* in plague affected and non-affected villages.

ACKNOWLEDGEMENTS

The authors are pleased to appreciate ACE-IRPM & BTM for supporting research work for Mr. Claus Augustino Thomas (main author). Special thanks to Miss. Stella Kessy, Dr. Christopher Sabuni, Dr Ladislaus Mnyone, Mr. Hashim Matambwa and Mr. Ginethon Mhamphi for their technical inputs, and Mr. Salim, Saddick and Rama for logistical and field support.

REFERENCES

- Abdad MY, Abdallah RA, Karkouri K E, Beye M, Stenos J, Owen H, Raoult D (2019). *Rickettsia gravesii* spp. a novel spotted fever group rickettsia in Western Australian Amblyomma triguttatum triguttatum ticks. International Journal of Systematic Evolutionary Microbiology 67:3156-3161.
- Ben-Ari T, Neerincx S, Gage KL, Kreppel K, Laidisoit, A (2011). Plague and climate scales matter. Plos Pathology 7(9):e1002160.
- Civen R, Ngo V (2008). Murine Typhus, an unrecognized suburban vector borne Disease. Clinical Infectious Diseases 46(6):913-918.
- Conlon JM (2007). The historical impact of epidemic typhus. Insects, Disease and History P 19.
- De Sousa R, Edouard P, Santos M, Amaro F, Bacellar F, Raoult D (2006). Molecular detection of *Rickettsia felis*, *Rickettsia typhi* and two genotypes closely related to *Bartonella elizabethae*. American Journal of Tropical Medicine and Hygiene 75(4):727-731.
- Dill T, Dobler G, Saathoff E, Clowes P, Kroidl I, Ntinginya E, Heinrich N (2013). High seroprevalence for typhus group rickettsiae, southwestern Tanzania. Emerging Infectious Diseases 19(2):317-320.
- Drancourt M, Raoult D (2016). Molecular history of plague. Clinical Microbiology and Infection 08.031.
- Eremeeva ME, Warashina WR, Sturgeon MM, Buchholz AE, Olmsted GK, Park SY, Karpathy SE (2008). *Rickettsia typhi* and *Rickettsia felis* in rat fleas (*Xenopsylla cheopis*), Oahu, Hawaii. Emerging Infectious Diseases 14(10):1613-1615.
- Fenollar F, Mediannikov O (2018). Emerging infectious diseases in

Africa in the 21st Century. New Microbes and New Infections 26:S10-S18.

- Giulieri S, Jatou K, Cometta A, Trelu LT, Greub G (2012). Development of a duplex real-time PCR for the detection of *Rickettsia* spp and typhus group rickettsia in clinical samples. FEMS Immunology and Medical Microbiology 64(1):92-97.
- Hidalgo M, Salguero E, De La Ossa A, Sánchez R, Vesga JF, Orejuela L, Valbuena G (2008). Short report: Murine typhus in Caldas, Colombia. American Journal of Tropical Medicine and Hygiene 78(2):321-322.
- La Scola B, Rydkina L, Ndiokubwayo JB, Vene S, Raoult D (2000). Serological differentiation of murine typhus and epidemic typhus using cross-adsorption and western blotting. Clinical and Diagnostic Laboratory Immunology 7(4):612-616.
- Laidisoit A, Falay D, Amundala N, Akaibe D, De Belloq JG, Van Houtte N, Socolovschi C (2014). High prevalence of *Rickettsia typhi* and *Bartonella* species in rats and fleas, Kisangani, Democratic Republic of the Congo. American Journal of Tropical Medicine and Hygiene 90(3):463-468.
- Leirs H, Neerincx S, Laidisoit A, Makundi RH (2010). Emergence and growth of plague foci in Africa. Vector-Borne and Zoonotic Diseases 10(97).
- Leulmi H, Socolovschi C, Laidisoit A, Houemenou G, Davoust B, Bitam I, Parola, P(2014). Detection of *Rickettsia felis*, *Rickettsia typhi*, *Bartonella* species and *Yersinia pestis* in Fleas (*Siphonaptera*) from Africa. PLoS Neglected Tropical Diseases 8(10):4-11.
- Makundi RH, Massawe AW, Mulungu LS, Katakweba A, Mbise TJ, Mgode G (2008). Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, northern Tanzania, in 2007. Mammalia 72(3):253-257.
- Mcleod MP, Qin X, Karpathy SE, Gioia J, Highlander SK, Fox, GE, Weinstock GM (2004). Complete Genome Sequence of *Rickettsia typhi* and Comparison with Sequences of Other Rickettsiae 186(17):5842-5855.
- Nyirenda SS, Hang BM, Simulundu E, Mulenga E, Moonga L, Machang'u RS, Kilonzo BS (2018). Molecular epidemiological investigations of plague in Eastern Province of Zambia. BMC Microbiology pp. 1-7.
- Noden BH, Davidson S, Smith JL, Williams F (2017). First detection of *rickettsia typhi* and *rickettsia felis* in fleas collected from client-owned companion animals in the Southern Great Plains. Journal of Medical Entomology 54(4):1093-1097.

- Noh Y, Lee YS, Kim H, Chong S, Klein TA, Jiang J, Kim SY (2017). Molecular detection of *Rickettsia* spp in ticks collected from the southwestern provinces of the Republic of Korea. *Parasites and Vectors*, pp. 1-10.
- Paris DH, Dumler JS (2016). State of the art of diagnosis of rickettsial diseases, the use of blood specimens for diagnosis of scrub typhus, spotted fever group rickettsiosis and murine typhus pp. 433-439.
- Phan JN, Lu CR, Bender WG, Marion R.S, Zhong J (2011). Molecular detection and identification of *Rickettsia* spp in *Ixodes pacificus* in California 11(7).
- Portillo A, De Sousa R, Santibáñez S, Duarte A, Edouard S, Fonseca IP, Oteo JA (2017). Guidelines for the detection of *Rickettsia* spp. *Vector-Borne and Zoonotic Diseases* 17(1).
- Prabhu M, Nicholson W L, Roche AJ, Kersh GJ, Fitzpatrick KA, Oliver LD, Crump JA (2011). Q fever, spotted fever group, and typhus group rickettsioses among hospitalized febrile patients in Northern Tanzania. *Clinical Infectious Diseases* 53(4).
- Regnery RL, Spruill CL, Plikaytis BD (1991). Genotypic identification of rickettsiae and Estimation of intraspecies sequence divergence for portions of two rickettsial genes. *Journal of Bacteriology* 173(5):1576-1589.
- Scola BL, Raoult D (1997). Laboratory Diagnosis of Rickettsioses, current approaches to diagnosis of old and new rickettsial diseases. *Journal of Clinical Microbiology* 35(11):2715-2727.
- Sousa R, De Santiba S, Duarte A, Edouard S, Novakova M, Palomar AM, Zu S (2017). Guidelines for the Detection of *Rickettsia* spp. *Vector-Borne and Zoonotic Diseases* 17(1):7831.
- Teoh YT, Hii SF, Stevenson MA, Graves S, Rees R, Stenos J, Traub RJ (2017). Serological evidence of exposure to *Rickettsia felis* and *Rickettsia typhi* in Australian veterinarians. *Parasites and Vectors* 10(1):4-11.
- Webb L, Carl M, Malloy DC, Dasch GA (1990). Detection of Murine typhus infection in Fleas by using the Polymerase Chain Reaction. *Journal of Clinical Microbiology* 28(3):530-534.