Asian Journal of Epidemiology

ISSN 1992-1462

science alert
http://www.scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Epidemiology

ISSN 1992-1462
DOI: 10.3923/aje.2017.123.127

Research Article
Molecular Characterization of *Orientia tsutsugamushi* in Domestic Rodents In Kolar Region, Karnataka

Gangannagari Vijay Kumar and Parvangada Madappa Beena

Department of Microbiology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India

Abstract

Background and Objective: *Orientia tsutsugamushi* (*O. tsutsugamushi*) is the causative agent of scrub typhus, which is an acute febrile illness seen in human beings. Rodents were implicated as the natural reservoirs for *Orientia tsutsugamushi* and they served as hosts for chigger mite. This study was undertaken to investigate the molecular prevalence and characterization of *Orientia tsutsugamushi* in domestic rodents in Kolar region. **Methodology:** Blood samples were collected from 177 rodents (138 *Bandicota bengalensis*, 16 *Rattus rattus*, 9 *Bandicota indica*, *Rattus norvegicus* and 2 *Mus musculus*). Conventional PCR, targeting the 56 kDa type specific gene was performed and DNA sequenced. **Results:** *O. tsutsugamushi* DNA was detected by conventional PCR in 10 (5.6 %) rodents. Two PCR purified samples were sequenced, phylogenetic analyses showed that one strain was closely related to *Orientia chuto* Dubai strain and another was closely related to Boryang strain. **Conclusion:** This suggests a high incidence of scrub typhus in this region. Further research on the characterization of *O. tsutsugamushi* from rodents as well as vectors throughout this district is needed to study the prevalence of *O. tsutsugamushi* in reservoir hosts.

Key words: *Orientia tsutsugamushi*, scrub typhus, 56 kDa type specific antigen, rodents, PCR, DNA sequencing

Citation: Gangannagari Vijay Kumar and Parvangada Madappa Beena, 2017. Molecular characterization of *Orientia tsutsugamushi* in domestic rodents In Kolar region, Karnataka. Asian J. Epidemiol., 10: 123-127.

Corresponding Author: Parvangada Madappa Beena, Department of Microbiology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India
Tel: +91 9980164277

Copyright: © 2017 Gangannagari Vijay Kumar and Parvangada Madappa Beena. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Orientia tsutsugamushi is the causative agent of scrub typhus, which is an acute febrile illness seen in human beings. Rodents were implicated as the natural reservoirs for *O. tsutsugamushi* and they serve as hosts for chigger mite which in turn transmits the bacteria to man following the bite[1]. Scrub typhus is endemic in the Asia-Pacific region and report an annual incidence of 1 million cases/year[2]. Mortality rates in untreated cases range from 0-30%[3]. Scrub typhus was also known to occur all over India and have been reported in states like Haryana[4], Himachal pradesh[5], Jammu and Kashmir[6], Karnataka[7], Assam[8], Tamil Nadu[3], Andhra Pradesh[9], Kerala[10] and Utharakand[11]. Antigenic variations among the strains of *O. tsutsugamushi* were reported and also interstrain variability associated with the virulence of the disease resulting in mild disease or fatal disease among humans as well as rodents[12]. Till the date more than 20 antigenic variants of *O. tsutsugamushi* were identified by immunological and molecular methods, which included previously identified prototype strains Karp, Gilliam and Kato[13]. 56 kDa type specific antigen is the one of the major immunogen of *O. tsutsugamushi*[14] and used as a target gene in methods like genetic sequence analysis[15]. However, there were no studies regarding molecular characterization of *O. tsutsugamushi* in the rodents in India. This study was undertaken to investigate the molecular prevalence and characterization of *Orientia tsutsugamushi* in domestic rodents in Kolar region.

MATERIALS AND METHODS

The study was done in the Department of Microbiology, Sri Devaraj Urs Medical College attached to R.L Jalappa Hospital and Research Centre, Kolar. From December, 2014-November, 2015, 177 domestic rodents were trapped from the residences of farmers in rural areas, chicken market and the tomato market in the Kolar region (Table 1). Rodents included in this study were not an endangered or protected species. Captured rodents were euthanized and species identified[16]. Two milliliter of whole blood was collected by cardiac puncture and placed in tubes containing EDTA[17] and stored at -80°C before testing. This study was approved by the Institutional Ethical Committee.

DNA amplification: The DNA was extracted from the Blood sample of 177 rodents using the QIAamp Blood DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. Conventional PCR, targeting the 56 kDa type specific gene was standardized and performed with slight modifications; initial denaturation at 94°C for 5 min, followed by 40 cycles, each consisting of denaturation at 94°C for 30 sec, 58°C for 30 sec, 72°C for 1 min and the final extension at 72°C for 10 min using the primers of 56 kDa F: 5-AATTGCTAGTGCAATGTCTG-3' and of 56 kDa R: 5-GGCATTATAGTAGGCTGAG-3' (Sigma Aldrich, Bangalore, India)[18]. This particular region encompasses 410 bp and contains the VD I-III hyper variable regions[19]. The PCR products were visualized by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and were purified by using the Gene JET Gel Extraction Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Purified products were subjected to a sequencing reaction using the BigDye Terminator Mix (Applied Biosystems, Foster City, CA, USA) and subsequent automatic sequencing using the ABI 3500 Genetic Analyzer (Applied Biosystems).The sequence obtained was submitted to GenBank (accession nos. KY497019, MF140631). A phylogenetic and molecular evolutionary analysis was conducted by using Mega 7 software. A phylogenetic tree with 1,000 bootstrap replications was constructed by using the neighbour-joining methods with distances calculated by the maximum composite likelihood[20].

RESULTS AND DISCUSSION

Rodents were recognized as reservoirs of various zoonotic diseases. Scrub typhus is a zoonotic disease caused by *O. tsutsugamushi*, where rodents act as reservoir hosts. The present study helped to understand the prevalence of

Table 1: Domestic rodents trapped in Kolar region

Species	Arh	Bmn	Ckm	Elm	Hrl	K k	Khl	Mbr	Prh	Rhn	Shn	Tmk	San
Bandicota bengalensis	35	16	2	2	12	-	15	14	18	5	4	6	9
Bandicota indica	-	-	1	-	2	-	-	1	1	-	1	3	-
Rattus rattus	4	-	3	-	-	7	-	1	-	1	-	-	-
Rattus norvegicus	-	1	2	-	-	2	3	2	1	1	-	-	-
Mus musculus	1	-	-	-	-	-	-	-	1	-	-	-	-
Total	40	17	8	2	14	9	18	18	21	7	5	9	9

Arh: Arahalli, Bmn: Bommidi nagar, Ckm: Chicken Market, Elm: Eelam, Hrl: Horhalli, Kk: Karanji Katte, Khl: Kuppahalli, Mbr: Mulbagal road, Prh: Prasanth nagar, Rhn: Rahmath nagar, Shn: Saheed nagar, Tmk: Tomato market, San: Sangondahalli

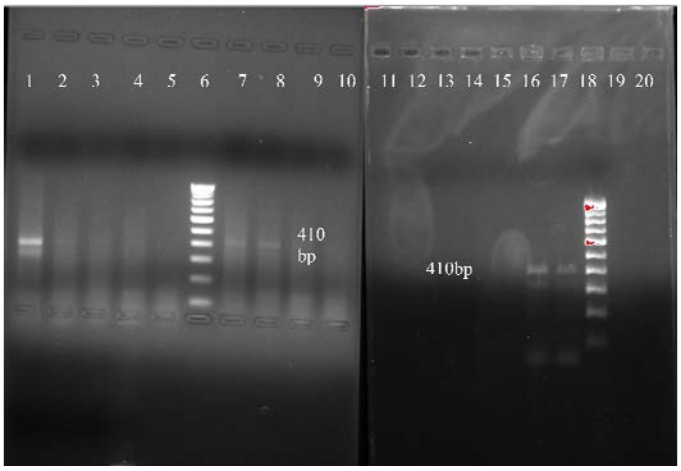

Fig. 1: Agarose-gel electrophoresis of domestic rodents PCR products
Lane 1 and 16: PC, Lane 6 and 18: 100 bp DNA marker, Lane 7, 8 and 17: PCR positives

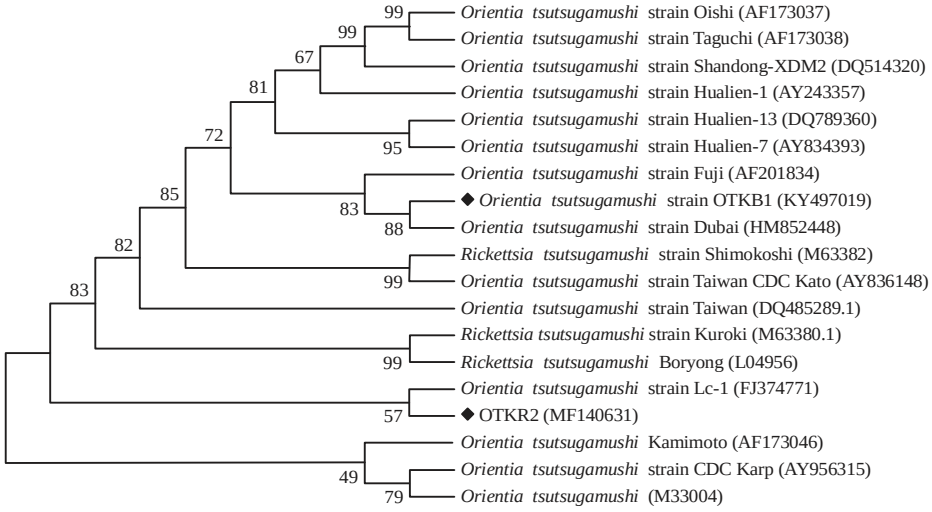

Fig. 2: Phylogenetic relationships of *Orientia tsutsugamushi* detected in domestic rodents in Kolar region, from December, 2014-November, 2015
Relationships were determined on the basis of the partial 56 kDa type-specific antigen gene of *O. tsutsugamushi* by the minimum-evolution method with the maximum composite likelihood. Bootstrap values >50% were showed at the branches. Location and GenBank accession numbers are indicated for each sequence. Solid diamonds indicate sequences determined in this study

O. tsutsugamushi among domestic rodents in Kolar region. Among 177 rodents trapped, 138 (78.4%) were identified as *Bandicota bengalensis*, 16 (9.09%) *Rattus rattus*, 9 (5.11%) *Bandicota indica*, 12 (6.7%) *Rattus norvegicus* and 2 (1.12%) *Mus musculus* (Table 1), which were captured during the months of December, February and March. *O. tsutsugamushi* DNA was detected by conventional PCR in 10 (5.6%) rodents (Fig. 1), 7 from *Bandicota bengalensis*, 2 from *Rattus rattus* and 1 from *Rattus norvergicus*; *O. tsutsugamushi* DNA was detected in the rodents captured from Arahalli (4), Bommidi nagar (3), Karanji katte (2) and Sangondahalli (1) villages situated in Kolar. This indicates high risk of acquiring scrub typhus infection in this area. Zhang *et al*.[21], reported 2.6% positivity for *O. tsutsugamushi* DNA by semi nested PCR among 385 domestic rodents from Northern China, which was slightly lower than the results reported in this study. The predominant rodent species in this study was *Bandicota bengalensis* as the reservoir host and the study by Zhang *et al*.[21], reported *Mus musculus* as the predominant species.

Among 10 purified DNA recovered from the rodents blood sample, 2 were sequenced and analyzed. One strain was phylogeneticaly closely related to novel *Orientia* species *O. chuto* Dubai strain, which was isolated from Humans[22] in Dubai and another strain was closely related to Boryang strain which was reported in wild rodents from South Korea[23] (Fig. 2).

Studies from different parts of China, reported multiple genotypes of *O. tsutsugamushi* in rodents which were phylogeneticaly related to Kawasaki, Fuji, SDM related types, Karp, Gilliam, Kato, TA763 strains and Sdu-1, similar to Japan Kawasaki strain[21,24,25] indicating different genotypes of *Orientia tsutsugamushi* exists in the environmental ecology.

CONCLUSION

Scrub typhus is one of the important cause of acute febrile illness in Karnataka. This present study was done in the Kolar region in Karnataka state from where a large number of clinical cases of scrub typhus were reported in recent years in humans. To the best of researchers knowledge this is the first molecular characterization of *O. tsutsugamushi* in domestic rodents in India. The strain characterized in this study was phylogeneticaly closely related to novel *Orientia chuto* Dubai strain isolated from human and Boryang strain from wild rodents. These strains were circulating in reservoir rodents suggesting a high incidence of scrub typhus in this region. Further research on the characterization of *O. tsutsugamushi* from rodents as well as vectors throughout this district are needed to study the prevalence of *O. tsutsugamushi* in reservoir hosts.

SIGNIFICANCE STATEMENT

This study highlights the prevalence and molecular characterization of *Orientia tsutsugamushi* in domestic rodents in the Kolar region, Karnataka, India. This study will helps the researchers to uncover the circulating strains of *Orientia tsutsugamushi* in domestic rodents and increases the knowledge in the community about epidemiological role of rodents in scrub typhus in this region.

ACKNOWLEDGMENT

The authors acknowledge Mr. vaigundan, Research Assistant and Mrs. Divya Bose, Research Assistant in Cell Biology and Molecular Genetics, Sri Devraj Urs Academic Higher Education and Research, Kolar, India for the assistance in DNA sequencing. Researchers used the equipment and materials provided by the institution for research work.

REFERENCES

1. Traub, R. and C.L. Jr. Wisseman, 1968. Ecological considerations in scrub typhus. 1. Emerging concepts. Bull. World Health Organ., 39: 209-218.
2. Watt, G. and P. Parola, 2003. Scrub typhus and tropical rickettsioses. Curr. Opin. Infect. Dis., 16: 429-436.
3. Mathai, E., J.M. Rolain, G.M. Verghese, O.C. Abraham, D. Mathai, M. Mathai and D. Raoult, 2003. Outbreak of scrub typhus in Southern India during the cooler months. Ann. N. Y. Acad. Sci., 990: 359-364.
4. Chaudhry, D., A. Garg, I. Singh, C. Tandon and R. Saini, 2009. Rickettsial diseases in Haryana: Not an uncommon entity. J. Assoc. Physicians India, 57: 334-337.
5. Sharma, A., S. Mahajan, M.L. Gupta, A. Kanga and V. Sharma, 2005. Investigation of an outbreak of scrub typhus in the Himalayan region of India. Jpn. J. Infect. Dis., 58: 208-210.
6. Mahajan, A., D.S. Jasrotia, R.S. Charak, T. Kumar, P.L. Bhagat, N. Sharma and B.B. Gupta, 2010. Scrub typhus: Jammu outbreak-2009. JK Sci., 12: 98-101.
7. Koraluru, M., I. Bairy, R. Singh, M. Varma and J. Stenos, 2016. Molecular confirmation of scrub typhus infection and characterization of *Orientia tsutsugamushi* genotype from Karnataka, India. J. Vector Borne Dis., 53: 185-187.
8. Baruah, M.K. and J. Sharma, 2015. First report of scrub typhus cases in Dhemaji, Assam, India. South Indian J. Biol. Sci., 1: 156-157.
9. Boorugu, H., M. Dinaker, N.D. Roy and J.A. Jude, 2010. Reporting a case of scrub typhus from Andhra Pradesh. J. Assoc. Physicians India, 58: 520-520.
10. Ittyachen, A.M., 2009. Emerging infections in Kerala: A case of scrub typhus. Natl. Med. J. India, 22: 333-334.
11. Singh, P., 2004. Scrub typhus, a case report: Military and regional significance. Med. J. Armed Forces India, 60: 89-90.
12. Kelly, D.J., P.A. Fuerst, W.M. Ching and A.L. Richards, 2009. Scrub typhus: the geographic distribution of phenotypic and genotypic variants of *Orientia tsutsugamushi*. Clin. Infect. Dis., 48: S203-S230.
13. Kelly, D.J., A.L. Richards, J. Temenak, D. Strickman and G.A. Dasch, 2002. The past and present threat of rickettsial diseases to military medicine and international public health. Clin. Infect. Dis., 34: S145-169.
14. Hanson, B., 1985. Identification and partial characterization of *Rickettsia tsutsugamushi* major protein immunogens. Infect. Immun., 50: 603-609.
15. Ohashi, N., H. Nashimoto, H. Ikeda and A. Tamura, 1992. Diversity of immunodominant 56-kDa Type-Specific Antigen (TSA) of *Rickettsia tsutsugamushi*. Sequence and comparative analyses of the genes encoding TSA homologues from four antigenic variants. J. Biol. Chem., 267: 12728-12735.
16. Hoff, J., 2000. Methods of blood collection in the mouse. Lab. Anim., 29: 47-53.
17. Lunde, D.P. and T.S. Nguyen, 2001. An Identification Guide to the Rodents of Vietnam. Center for Biodiversity and Conservation, American Museum of Natural History, New York.

18. Mahajan, S.K., J.M. Rolain, R. Kashyap, D. Bakshi and V. Sharma *et al*., 2006. Scrub typhus in Himalayas. Emerg. Infect. Dis., 12: 1590-1592.
19. Fournier, P.E., S. Siritantikorn, J.M. Rolain, Y. Suputtamongkol and S. Hoontrakul *et al*., 2008. Detection of new genotypes of *Orientia tsutsugamushi* infecting humans in Thailand. Clin. Microbiol. Infect., 14: 168-173.
20. Kumar, S., G. Stecher and K. Tamura, 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evolut., 33: 1870-1874.
21. Zhang, M., Z.T. Zhao, X.J. Wang, Z. Li, L. Ding, S.J. Ding and H.L. Yang, 2013. Genetic variants of *Orientia tsutsugamushi* in domestic rodents, Northern China. Emerg. Infect. Dis., 19: 1135-1137.
22. Izzard, L., A. Fuller, S.D. Blacksell, D.H. Paris and A.L. Richards *et al*., 2010. Isolation of a novel *Orientia* species (*O. chuto* sp. nov.) from a patient infected in Dubai. J. Clin. Microbiol., 48: 4404-4409.
23. Shim, S.K., E.N. Choi, K.O. Yu, H.J. Park and C.M. Kim *et al*., 2009. Characterisation of *Orientia tsutsugamushi* genotypes from wild rodents and chigger mites in Korea. Clin. Microbiol. Infect., 15: 311-312.
24. De, W., K. Jing, Z. Huan, Z.H. Qiong and C. Monagin *et al*., 2015. Scrub typhus, a disease with increasing threat in Guangdong, China. PLoS ONE, Vol. 10, No. 2. 10.1371/journal. pone.0113968.
25. Yang, L.P., Z.T. Zhao, Z. Li, X.J. Wang, Y.X. Liu and P. Bi, 2008. Comparative analysis of nucleotide sequences of *Orientia tsutsugamushi* in different epidemic areas of scrub typhus in Shandong, China. Am. J. Trop. Med. Hyg., 78: 968-972.