Title of PhD project / theme	**The epidemiology and species diversity of spotted fever group rickettsial infection in India**
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Brief description of project / theme	**Background:** Spotted fever group rickettsioses (SFGR) are neglected infections in many parts of the world. SFGR are zoonotic and are transmitted by ticks, fleas, mites and possibly leeches[1] to accidental hosts, including humans in mainly forest and agricultural settings. In Asia, SFGR show a great species diversity. For example, studies have identified about 18 species in China, 11 in Laos, 10 in Korea, 3 in Japan, 8 in Taiwan and 7 in Thailand.[2] To date only a single species has been identified in India (*R. conorii*),[2] which remains a white spot on the SFGR map. A study at Vellore (the proposed study site) recently identified a previously unknown species (*Candidatus Rickettsia kellyi*).[3] There is evidence that SFGR may be a substantial public health problem in India, but is rarely tested for. A small case series of rickettsial infections at a CMC's community hospital where all suspected cases were tested for scrub typhus and spotted fever, found that 12 out of 53 confirmed rickettsial infections (23%) were due to SFGR. Unlike in Japan,[4] SFGR and scrub typhus infection clusters overlapped considerably. The clinical course was similar to scrub typhus with complications including ARDS, shock, renal failure and encephalitis. We are conducting a large population based cohort study on rickettsial infections in Vellore District from 2020 till 2022 (Funding approved by UK MRC, budget GBP 790,000). In parallel, we are conducting a multi-centre hospital-based surveillance study in 3 additional sites in India (Eastern Ghats, Western Ghats, North-East; funding: Indian Government-ICMR). The two studies will provide a

framework for the proposed PhD and, in particular, will provide human and vector samples the student can use.

Aim of the proposed PhD: To obtain a fundamental understanding of the epidemiology of SFGR in rural India, accounting for species diversity and vector parameters.

Specific Objectives:

1) Identify infection hot spots using population-based serological data.
2) Estimate the proportion of ticks and fleas infected with SFGR.
3) Isolate SFGR in ticks and human samples (blood, skin, eschar) and conduct genome sequencing to identify SFGR species diversity
4) Map species and vectors against human sero-prevalence data to identify SFGR species and vectors most closely associated with human infection

Methods: Objectives 1 and 4 will focus on the Vellore site. Objectives 2 and 3 will include human and vector samples from the 3 other sites. SFGR human sero-prevalence data and blood/eschar samples will be provided to the student from the two main studies. The student will conduct vector sampling at the Vellore site 1) by collecting ticks from life-stock (goats and cows), 2) flagging of vegetation assisted by local staff familiar with these methods. Fleas collected from rodents, as well as ticks from the other study sites will be provided to the student as these are already collected for other study purposes. Depending on the students' background and preferences, they can contribute to vector identification. Vector and human tissue samples will be tested for SFGR using nested PCR (*gltA*, *17 kDa*, *ompA*, and *ompB* genes).[5] Positive samples will undergo sequencing to identify SFGR species.[5] The student will use spatial analysis to explore SFGR infection clusters accounting for SFGR species, vector parameters, as well as geographic, behavioural (e.g. occupational) and socio-demographic characteristics of the study population.

Timeline: In the first year, the student will 1) use sero-prevalence data collected in the baseline survey of the Vellore site (completed by August 2020) to identify areas of high SFGR risk; 2) familiarise her/himself with vector sampling techniques and PCR/genome sequencing methods at CMC Vellore. In the second year the student will collate human and vector samples from all sites, collect vectors (Vellore site only) and conduct the laboratory analyses. The final year will mainly be used for mapping/spatial analysis and thesis write up.

Significance: The PhD is expected to substantially improve our understanding of SFGR ecology and species diversity in India, a country with hundreds of millions of rural people potentially at risk.

	References: 1. Sando E, Suzuki M, Katayama M, Taira M, Fujita H, Ariyoshi K. *Rickettsia japonica* **Infection after Land Leech Bite, Japan**. Emerg Infect Dis. 2019 Jun; 25(6): 1243–1245. 2. Satjanadumrong, J., Robinson, M.T., Hughes, T. et al. Distribution and Ecological Drivers of Spotted Fever Group Rickettsia in Asia EcoHealth (2019). **https://doi.org/10.1007/s10393-019-01409-3** 3. Rolain JM, Mathai E, Lepidi H, Somashekar HR, Mathew LG, Prakash JA, et al. '*Candidatus Rickettsia kellyi*', India. Emerg Infect Dis. 2006;12:483–5 4. **Sando E**, **Suzuki M**, **Katoh S**, **Fujita H**, **Taira M**, **Yaegashi M**, **Ariyoshi K**. Distinguishing Japanese Spotted Fever and Scrub Typhus, Central Japan, 2004- 2015. **Emerg Infect Dis.** 2018 Sep;24(9):1633-1641 5. **Prakash** JAJ, **Sohan Lal** T, **Varghese R**, et al. Molecular detection and analysis of spotted fever group *Rickettsia* in patients with fever and rash at a tertiary care centre in Tamil Nadu, India. **Pathog Glob Health**. 2012 Mar; 106(1): 40–45.
The role of LSHTM and NU in this collaborative project	NU has established expertise in the epidemiology and molecular diagnostics of rickettsial infections, including SFGR, and has a track record of publications in this field.[1,4] By contrast, LSHTM as the lead institution for the MRC grant on rickettsial infections is new to the field, but has expertise in conducting large cohort studies (WPS, NA) which will allow provision of large numbers of human tissue samples collected in a population based cohort. LSHMT also has expertise in vector sampling and identification (MC). This PhD will be a good opportunity to collaborate on the rapidly expanding field of rickettsial infections. The two institutions ideally complement each others' expertise, with future collaboration in this field being highly desirable.
Particular *prior* educational requirements for a student undertaking this project	This PhD is suitable for students with a background in microbiology, molecular biology, veterinary medicine, ecology or medicine. Strong interest or proven skills in molecular methods such as PCR or genome sequencing are essential.
Skills we expect a student to develop/acquire whilst pursuing this project	The student will be expected to further his/her skills in molecular methods, in particular pathogen detection in human tissue samples and disease vectors. The student will develop skills in genome sequencing and SFGR species identification. The students will have the opportunity to develop skills in vector sampling methods.