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Molecular epidemiology of spotted fever group rickettsiae in Zambia and development of multiplex LAMP for simultaneous detection of the rickettsiae and malaria parasites

(ザンビアにおける紅斑熱群リケッチアの分子疫学とマルチプレックス LAMP を利用したマラリア原虫との同時検出法の開発)

Abstract

Spotted fever group (SFG) rickettsiae are obligate intracellular Gram-negative bacteria associated with blood-sucking arthropods. Several species within the SFG rickettsiae cause serious human diseases. Recently, flea-associated *Rickettsia felis* and its closely related *Rickettsia asembonensis* were identified from several arthropods and mammalian species, as well as humans with unknown pathogenicity. However, there is limited information on the ecology and epidemiology of SFG rickettsiae in Zambia. Hence, this thesis aimed at elucidating the molecular epidemiology of SFG rickettsiae in animals, arthropods, and humans in Zambia. Besides, the common clinical manifestation of rickettsioses is fever which could be undifferentiated from malaria. Therefore, a simple and rapid multiplex LAMP system for simultaneous detection of the rickettsiae and malaria parasites was developed.

In chapter 1, fleas and mosquitoes as well as dog blood and rodent organ DNA samples from Zambia were analyzed for *Rickettsia* genus by PCR and sequencing. As a result, *R. felis* was detected in 7/150 (4.7%) dog blood samples and in 12/77 (15.5%) *Mastomys* spp. organ samples. *Rickettsia felis* was also detected in 7/48 (14.5%) of cat fleas infesting dogs, which were all co-infected with *R. asembonensis*. Furthermore, 15/48 (31.3%) of cat flea samples were positive for only *R. asembonensis*. These observations suggest that *R. felis* is potentially circulating among domestic dogs and cat fleas as well as peridomestic rodents posing a potential public health threat in Zambia. As such, further studies of potential flea-borne rickettsioses in the Zambian population are warranted.

In chapter 2, the prevalence of SFG rickettsiae in humans from Zambia was assessed. The DNA samples from 1,153 residual human blood were tested for the presence of SFG *Rickettsia* spp. by PCR. As a result, *R. asembonensis* was detected in two individuals by PCR and sequencing of four partial genes. The *R. asembonensis* genotype detected in human blood

clustered with those previously detected in cat fleas in Chapter 1. These results signify the possible role of flea-borne *R. asembonensis* as a potential cause of human illness.

In chapter 3, a novel, simple, and rapid multiple pathogen detection LAMP method was developed for easy simultaneous detection of both SFG *Rickettsia* spp. and *Plasmodium* spp. Two primer sets that detect SFG *Rickettsia* spp. and *Plasmodium* spp. (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*) were mixed, and amplified products were visualized by hybridizing to dipstick DNA chromatography. The method was validated using spiked parasites in human blood mimicking infection. The developed diagnosis technique will contribute to the differential diagnosis of undifferentiated febrile illness caused by either spotted fever group *Rickettsia* spp. or *Plasmodium* spp. in resource-limited endemic areas.

In summary, this thesis highlights the circulation of *R. felis* in domestic dogs, peridomestic rodents, and cat fleas. *Rickettsia asembonensis* of identical genotype was detected in cat fleas and humans suggesting potential human infection. With the potential significance of *Rickettsia* infections, the novel developed multiplex LAMP technique could be useful for simultaneous detection of *Rickettsia* spp. and *Plasmodium* spp. which both presents with fever.