Short Communication

An Application of Duplex PCR for Detection of \textit{Leptospira} spp. and \textit{Orientia tsutsugamushi} from Wild Rodents

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(Received March 24, 2008. Accepted July 8, 2008)

SUMMARY: Duplex PCR is useful for detecting two different agents from the same specimen. Kidney specimens are the most suitable for detection of \textit{Leptospira} spp. For \textit{Orientia tsutsugamushi}, blood clots, spleen, and liver specimens are considered the most suitable. For this study, kidney tissues were the only specimens obtained for the PCR. Blood clots, spleen, and liver specimens were not available. However, by using the PCR for scrub typhus, \textit{O. tsutsugamushi} was detected in the kidney of one rodent. This result shows that kidney specimens can be used to detect \textit{O. tsutsugamushi} using PCR. Further studies will be necessary in order to be able to compare the detection ratio of \textit{O. tsutsugamushi} using kidney specimens and blood clots, spleen, and liver specimens.

Leptospirosis and scrub typhus, both of which are re-emerging diseases, are public health problems in Thailand. The clinical manifestations of leptospirosis and scrub typhus range from mild fever with few other symptoms to a fatal syndrome. These diseases are occurring among travelers returning from endemic areas associated with ecotourism (1,2). Both are zoonotic diseases, and epidemiologically wild rodents are involved in the prevalence of these infections in Thailand. Wild rodents are one of the most important reservoirs of leptospirosis. We studied the prevalence of leptospiral antibodies in these animals by the microscopic agglutination test (MAT), and revealed that 4.8\% (56/1,164) of them were serologically positive (3). On the other hand, wild rodents may not be the true reservoir of scrub typhus. These animals do, however, naturally maintain \textit{Orientia tsutsugamushi} by the cycle of mites and rodents, and the rodents often directly reflect the epidemic of disease. Surveillance of rat and infesting mites (vector of scrub typhus) in Chiang-Rai Province revealed that \textit{O. tsutsugamushi} was present in mites (\textit{Leptotrombidium} spp.) collected from approximately 31\% of \textit{Rattus rattus}, \textit{R. losea}, \textit{R. argentiventer}, and \textit{Bandicota indica} (4). The current methods for diagnosis of leptospirosis and scrub typhus usually depend upon detection of serum antibodies. As described previously (5-7), polymerase chain reaction (PCR) is a useful method for detection of leptospirosis and scrub typhus usually depend upon detection of serum antibodies. As described previously (5-7), polymerase chain reaction (PCR) is a useful method for detection of leptospirosis and scrub typhus when \textit{flaB} or \textit{groEL} genes are used as primers. The aim of the present study was to determine the applicability of duplex PCR for simultaneous detection of \textit{Leptospira} spp. and \textit{O. tsutsugamushi} from the same rodent specimen.

The 56 wild rodents examined were leftover from the Project of Survey of Ectoparasitic Fauna and Associated Diseases in Provinces along Thai-Myanmar, Thai-Cambodian borders (WHO Grant). These animals were captured during February - December 2004 and April and September 2005, and were anesthetized with ether. After the species of rodent was identified, the blood specimens were collected by heart puncture and kidney specimens were then collected for cultivation of leptospires. A piece of kidney tissue was minced with a scissors and then cultured for 26 days at 30°C with EMJH semi-solid medium containing 5-fluorouracil 200 µg/ml. All cultures were examined routinely by dark-field microscopy for 26 weeks before the specimens were regarded as negative, and they were identified by the authentic antisera kit provided by the Centers for Disease Control and Prevention (CDC), Atlanta, Ga., USA. MAT (8) was used for the detection of antibodies to \textit{Leptospira} spp. using a panel of 24 living leptospires of serovar authentic strains. The panel of 24 \textit{L. interrogans} serovars consisted of Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Manhao, Mini, Panama, Pomona, Pyrogenes, Ranarum, Samin, Sejroe, Shermani, Tarrassovi, and Semaranga. \textit{Leptospira} cultures for the antigen preparation grown in EMJH were adjusted to 1.5 × 10^5 cells/ml (0.5 McFarland standard) with PBS (pH 7.4). The end-point titer was defined as the highest serum dilution giving 50\% agglutination in comparison with the negative control. The positive serodiagnosis was considered to be a titer of 1:40 and for leptospira, a twofold serial dilution was used for a further diluted screening test for positive sera. A titer of 1:400 was diagnosed as positive. For detection of antigens by DNA, the DNA was extracted and purified from the kidney tissues by the NucleoSpin™-Machery-Nagel COM Ltd. kit purchased from Pacific Science Company, Thailand. By using pairs of primer constructed \textit{flaB} and \textit{groEL} genes were described by Kawabata et al. (5) and Park et al. (7). Duplex PCR was performed with PuReTag™ Ready-To-Go™ PCR (GE Healthcare, Buckinghamshire, UK). The amplification program involved 94°C denaturing for 20 s, 56°C annealing for 10 s, and 72°C extension for 60 s; 30 cycles. Amplified products of \textit{flaB} and \textit{groEL} were 790 bp and 360 bp, respectively.

To determine the sensitivity of the duplex PCR, purified...
leptospiral DNA and *O. tsutsugamushi* DNA adjusted from 1 ng/tl to 0.01 pg/tl were tested. The minimum concentrations of leptospiral DNA and *O. tsutsugamushi* DNA were both 1 pg/tl. We also tested the specificity of these primers to other bacteria (*Aeromonas sobria*, *A. hydrophila*, *Moraxella fava*, *Citobacter freundii*, *C. diversus*, *Staphylococcus aureus*, *S. epidermidis*, *Klebsiella pneumoniae*, *K. oxytoca*, *K. edwardsiella*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Salmonella typhi*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Streptococcus pneumoniae*, *Salmonella typhi*, *S. paratyphi* A, *Escherichia coli*, *Burkholderia pseudomallei*, *Enterobacter cloacae*). The *fts* was specific for leptospiral DNA, and *groEL* was specific for *O. tsutsugamushi*, and no product of them was detected to other bacteria.

Four techniques, the MAT/culture for leptospires, the IFA for scrub typhus, and the duplex PCR for both agents, were used to examine sera or kidney tissues from 56 rodents. By the MAT, all sera showed negative (antibody titers of sera used to examine sera or kidney tissues from 56 rodents. By for scrub typhus, and the duplex PCR for both agents, were other bacteria. Further studies are required to confirm the validity of the PCR for scrub typhus. However, the applicability of the PCR for scrub typhus remains unclear, though the method may be useful for small rodents such as *M. caroli*. Further studies are required to confirm the validity of the method for detection of *O. tsutsugamushi*.

REFERENCEs


ACKNOWLEDGMENTS

The authors wish to thank Assoc. Prof. Wijitr Fungladda, Faculty of Tropical Medicine, Mahidol University and Khun Amporn Imvithaya, NIH, Thailand for providing the rodent samples from project integrated studies of human and animal leptospirosis in endemic areas of Nakonratchasima, Thailand (Mahidol University grant) and survey of ectoparasitic fauna and associated diseases in provinces along Thai-Myanmar, Thai-Cambodian borders (WHO grant). We also thank Assist Prof. Thararat Kalambaheti for reviewing the manuscript.