Attachment 2:4(a)18

infections become activated and cause medical problems later? These questions will be discussed.

O40 Assessment of a quantitative multiplex 5 nuclease PCR for Orientia tsutsugamushi, spotted fever, or typhus group rickettsioses

JAJ Prakash[1], MF. Reller[2], N Barat[2], JS Dumler[2]
E-mail: mreller1@jhmi.edu

[1]Dept. Microbiology, Christian Medical College, Vellore, India and [2]Dept. Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Introduction: Scrub typhus and spotted fever group rickettsioses are re-emerging causes of acute undifferentiated febrile illness in South Asia.

Objective: To design a quantitative multiplex 5?nuclease real-time PCR assay for scrub typhus, spotted fever and typhus diagnosis.

Methods: A consensus ompA spotted fever group Rickettsia spp. (SFGR) sequence, the R typhi (and R. prowazekii [TGR]) 17-kDa lipoprotein antigen gene, and the O. tsutsugamushi (OT) Kato strain 56 kDa antigen gene sequence were examined using AlleleID 6 (PREMIER Biosoft, Palo Alto, CA) to develop minor groove binding primers and probes. A BioRad iQ5 PCR Detection System was used. After optimization, analytical sensitivity was determined with plasmid clones (R. conorii, R. akari, R. felis, R. rickettsii, R. parkeri, R. typhi, R. prowazekii, and O. tsutsugamushi Kato, Karp and Gilliam). Analytical specificity was determined using genomic DNA from Anaplasma phagocytophilum, Ehrlichia canis, Ehrlichia chaffeensis, Neorickettsia helminthoeca, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Borrelia burgdorferi, Plasmodium falciparum, and from humans. Multiplex validation was performed using all 3 primer/probe sets and DNA from human blood spiked with genomic rickettsial DNA.

Results: Multiplex analytical sensitivity for all cloned targets was linear over 105 to 100 copies, and >10 target copies/IL of each were detected. Genomic DNA from all SFGR and TGR species, and OT strains was detected. Multiplex analytical specificity showed no cross-reactions among genomic DNA from SFGR, TGR, and OT, nor detected genomic DNA from other pathogens in human blood.

Conclusion: This multiplex quantitative PCR assay detects 1-5 O. tsutsugamushi, SFGR or TGR gene copies in blood DNA and can be conducted in <3 hours. The assay holds great promise for early rapid rickettsiosis diagnosis when treatment decisions are being made. Clinical sensitivity and specificity of this assay using samples from individuals with and without disease needs to be determined to assess its diagnostic efficacy.

O41 Predicted cost benefits of establishing rickettsial disease diagnostics in Sri Lanka

Premaratna R.[1], Dissanayaka T.G.I.[1], Chandrasena T.G.A.N.[2], Attanayake N.[3], de Silva H.J.[1]
E-mail: ranjan_premaratna@lycos.com

Departments of Medicine[1], Parasitology[2], Faculty of Medicine, University of Kelaniya Department of Economics[3], University of Colombo, Sri Lanka.

Objectives: Rickettsial diseases are re-emerging in Sri Lanka. Presence of an eschar or a vasculitic rash helps in the early clinical diagnosis of probable rickettsial infection (PRI). However, absence of specific symptoms leads to longer hospitalization as pyrexia of unknown origin (PUO), in settings where facilities for diagnosis of rickettsial diseases are not available. We aim to calculate the treatment cost differences between these two categories of patients (PRI vs PUO) as a quantitative method of predicting the cost benefits accruing from establishment of rickettsial disease diagnostics in Sri Lanka.

Methods: Twenty nine PRI patients and 33 PUO patients, who were confirmed to have rickettsial infections by serological testing were selected for the study. Institutional costs (costs for accommodation, staff, electricity, water, telephone, meals and consumables), costs of laboratory investigations and medications were calculated for each patient to obtain mean per-patient costs for each category. The mean per-patient cost difference between PRI and PUO was then calculated to measure the cost benefit of establishing rickettsial disease diagnostics in Sri Lanka.

Results: The mean and standarddeviation (SD) of hospital stays for PRI patients was 3.2 (0.5) days compared to 12.3(2.5) days for PUO patients. The mean (SD) costs (in US $) of medication per-patient for PRI was $0.12 (0.03) compared to $88.8 (21.2)for PUO. The mean (SD) costs for laboratory testing per-patient for PRI was $2.2 (0.02) compared to $25.5 (9.7) for PUO, and the mean institutional cost per-patient for PRI was $0.1 compared to $0.5 for PUO. Therefore the average cost difference per-patent between the two groups was $112.38.

Conclusions: Considerable cost benefits could be achieved by establishing rickettsial disease diagnostics in Sri Lanka This direct cost benefit must be balanced by general economic arguments considering the indirect income losses by patients and the added costs for establishing and maintaining new diagnostic facilities.

042 An case of eschar-associated spotted fever rickettsiosis in Brazil

[1]Márcio A. M. Galvão. [2]Simone B. Calic. [3]Paulo Sérgio G. Costa. [4]Marcelo 6. Labruna. [5]Cláudio L. Mafra. [6]Dárlen C. H. Pena. [6]Bruno S. Milagres. [7]David H Walker
E-mail: galvaomarcio@oi.com.br

[1]Universidade Federal de Ouro Preto (UFOP), Brasil; World Health Organization Collaborating Center for Tropical Diseases of University of Texas Medical Branch. USA.
[2]Fundação Ezequiel Dias (FUNED), Brasil.
[3]Hospital Monte Sinai , Faculdade de Ciências Médicas da Saúde, Juiz de Fora - MG, Brad.
[4]Faculdade de Medicina Veterinária e Zootecnia (FMVZ) - Universidade de São Paulo (USP), Brasil.
[5]Universidade Federal de Viçosa (UFV) - MG. Brasil.
[6]Universidade Federal de Ouro Preto - MG,Brasil. 7University of Texas Medical Branch at Galveston - USA ; World Health Organization Collaborating Center for Tropical Diseases of University of Texas Medical Branch, USA.

Herein we describe a supposed Brazilian spotted fever (BSF) 3 year-old boy's case. He became ill an July 1,2003 with fever, myalgia. headache and was admitted in Juiz de Fora Hospital University, Minas Gerais State,Brazil in July 8. He eventually developed rash, nausea, vomiting and coma, and an eschar was identified on the patient's leg. He had contact with ticks near his residence several days prior to the onset of his symptoms. The serum samples of this boy were tested by an indirect imunofluorescence Assay (IFA) for the presence of antibodies to Rickettsiarickettsii, Rickettsia parkeri and Rickettsia felis showing high titers to R. rickettsii and R. parkeri in the first and second samples. No skin biopsies or blood samples were obtained to attempt molecular or culture-based diagnostic assays, and the serology results did not allow us to distinguish between an infection caused by R. rickettsii or R. parkeri. In the Brazilian scientific literature the only relate of an eschar in a supposed case of BSF was made by Piza in 1932.The severity of this patient's illness is greater than previously reported for R. parkeri. Nonetheless, it is possible that R. parkeri may cause severe disease in some patients, reflecting confusion that may exist between diagnoses of BSF and other rickettsial diseases from spotted fever group.

043 A meta-analysis to determine the accuracy of scrub typhus diagnostic assays

Stuart D. Blacksell, Daniel H. Paris, Nicholas P.J. Day
E-mail: stuart@tropmedres.ac

Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Centre for Tropical Medicine, University of Oxford, United Kingdom

Objectives: Scrub typhus a an acute tropical illness caused by the mite-borne rickettsia, Orienna tsutsugamushi. The early diagnosis of acute scrub typhus can greatly reduce the chance of complications and guide optimal therapy The objective of thrs study was perform a meta-analysis of scrub typhus diagnostics from published literature to determine the accuracy of the different assays.

Methods: Relevant studies were identified using Internet search engines and hand searching of reference lists. Data was extracted and a 2 x 2 table was constructed where a reference comparator result was compared to the index test to calculate diagnostic accuracy indices of sensitivity and specificity. Pooling the individual study results was used to generate an overall estimate of diagnostic accuracy for each test.