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Phylogenetic Analysis of the Chinese *Rickettsia* Isolate BJ-90

To the Editor: Five species of tick-associated rickettsiae have been identified in China; of these, three are human pathogens and two are of unknown pathogenicity (1). In 1990, one isolate, BJ-90, was first obtained from a *Dermacentor sinicus* tick, a newly recognized vector collected in a Beijing suburb, an atypical location for *Rickettsia sibirica* (2). Several taxonomic studies of the phenotype, antigenicity, and genotype of BJ-90 have been performed, with inconsistent results (2-6). Recently, phylogenetic analysis based on several gene comparisons has enabled the phylogenetic classification of this rickettsial species (7-11). To confirm the phylogenetic relationships between the BJ-90 strain and other rickettsiae, the 16S rRNA, *gltA*, and OmpA encoding genes were amplified and sequenced. Phylogenetic relationships between the BJ-90 strain and other rickettsia in the GenBank database were inferred by the parsimony and neighbor-joining methods (9). Bootstrap analyses were used to assess the reliability of the phylogenetic analysis.

Both methods showed a high degree of similarity between BJ-90, *R. sibirica* and "*R. mongolotimonae*," which were grouped in the same cluster in three inferred dendrograms. The data from the 16S rRNA and *gltA* sequences showed low statistical significance in the cluster (bootstrap values for the nodes 50% and 33%, respectively). However, data from the *rompA* gene sequence showed highly significant similarity in the cluster (bootstrap value 100%), confirming the reliability of the phylogenetic analysis. The results of this phylogenetic analysis are consistent with those of previous phenotypic, genotypic, and phylogenetic analyses (2,3,5-11), as well as taxonomy derived from direct antigenic comparison of the species (4). The sequences of 16S rRNA, *gltA*, and OmpA have been assigned the following GenBank accession numbers: AF178036 for 16S rRNA, AF178035 for *gltA*, AF179365 for the 611-bp sequence of ompA, and AF179367 for the 3174-bp sequence of ompA. According to previous genotypic and antigenic studies and our phylogenetic analysis, in which the BJ-90 strain is closer to *R. sibirica* than *R. mongolotimonae* in the dendrogram inferred from comparison of the ompA encoding gene sequences, the BJ-90 strain should be considered a variant of *R. sibirica*.

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Specimen Collection for Electron Microscopy

To the Editor: As virologists whose specialties include diagnostic electron microscopy (EM), we read with interest the discussion on bioterrorism scenarios (1,2) and the subsequent note by Marshall and Catton (3) on the rapid EM diagnostic process used for smallpox (1). EM diagnostics for viral agents offer an open, undirected view; a catch-all method; and speed. A negative stain preparation may be made and a result could be obtained within 5 minutes of the specimen's arrival in the EM laboratory. As suggested by Marshall and Catton, however, success depends as much on the quality of the sample collected as on the method of preparation and skill of the microscopist.

The Konsilarlaboratorium für die Elektronenmikroskopische Erregerdiagnostik in the Robert Koch Institut, Berlin, Germany, provides EM viral diagnostic services for up to 800 specimens per year and counsels other German diagnostic units. The Electron Microscope Unit for the Department of Medical Microbiology and Infectious Diseases, University of Manitoba, is used for EM viral diagnostics by both the major health-care facility in Manitoba, Canada, and the Manitoba Provincial Laboratories; it examines approximately 2,300 clinical specimens annually. Our two facilities examine 70 to 90 vesicular specimens of suspected viral origin annually. In our experiences, the most effective methods of specimen collection from virus-induced blisters (or ulcers) involve opening the vesicle with a 26-gauge needle. The exudate may then be collected and prepared for examination in one of three ways: 1) Draw lesion aspirates into the barrel of the needle with a tuberculin syringe and cap the needle (4); 2) touch a light microscope slide to the vesicle fluid; or 3) touch a 400-mesh, plastic-coated specimen grid directly to the base of the lesion (5). The samples may then be transported to an EM facility for preparation and examination. With the first two sample types, the sample is resuspended in approximately 20 µL of 0.2-µ pore-filtered, bidistilled water; this suspension is used to prepare a standard drop preparation on a 400-mesh, carbon-reinforced, plastic-coated grid. In all cases, the specimens are then negatively stained and examined.

Because of safety concerns about HIV infection, many health officials view transport of vesicle aspirates in capillary pipettes or needles as unacceptable. Glass slides are considered more acceptable, but still a risk. Since examination facilities or wards usually do not have the material to do direct touch preparations onto EM grids, many health officials advocate placing samples into transport medium. Alternatively, swabs may be used to prepare smears on glass slides for subsequent EM examination (6). Swabs in transport medium may be of value for culture or polymerase chain reaction procedures. However, in our experience these samples are not acceptable for EM diagnostics. Marshall and Catton suggest skin scrapings as an alternative to swabs (3). We find that these samples are preferable to swab specimens but not ideal. Our success rates in identifying herpesvirus and orthopoxvirus by drop method preparation (7-9) of vesicle aspirates are 62% to 80%, annually. The advent of sample transport as swabs has made additional procedures necessary to improve sensitivity and has delayed results. In Manitoba, direct centrifugation of samples to EM grids with the Beckmann Airfuge (Palo Alto, California, USA) is used as a nonspecific method of concentrating virus in sample preparations. This method increases the yield of viral particles by three or