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Los desafíos en el diagnóstico, la investigación y la concientización sobre las rickettsiosis en América Latina

(The challenges of rickettsial diagnosis, research, and awareness in Latin America)

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Resumen

Los retos para establecer el diagnóstico de una enfermedad rickettsial específica, desarrollar un programa de investigación clínica o científica que se base en métodos eficaces de laboratorio, y promulgar la conciencia y el conocimiento de las rickettsiosis entre los médicos de atención primaria y los organismos de salud pública son hechos sustanciales. El logro de estas metas es una misión mancomunada. En esta mini revisión se presentan los principales desafíos en estos aspectos y se proponen algunos métodos para superarlos.

Descriptores: Infecciones por Rickettsiaceae, *Rickettsia*, América Latina, zoonosis

Abstract

The challenges to establish the diagnosis of a specific rickettsial disease, to develop a clinical or scientific research program that relies upon effective laboratory methods, and to promulgate awareness and knowledge of rickettsial diseases among primary care physicians and public health agencies are substantial. Achieving these goals is our mission. This minireview delineates the challenges and proposes some approaches to surmount them.

Keywords: Rickettsiaceae infections, *Rickettsia,* Latin America, zoonoses

Challenges of Diagnosis

Establishing a laboratory-confirmed diagnosis is the cornerstone of the foundation upon which the study of rickettsial diseases depends. The standard diagnostic tool, serologic demonstration of antibodies to rickettsiae, remains the major approach to document the diagnosis of rickettsial diseases. The deficiencies of serologic diagnosis include frequent absence of diagnostic antibodies early in the clinical course when critical therapeutic decisions are needed, inability to distinguish among the etiologic agents within the spotted fever (SFG) or typhus group (TG) owing to shared antigens, and presence of preexisting antibodies to the test antigens during the acute phase of illness owing to prior stimulation by crossreactive antigens. Currently in the U.S., historically high numbers of cases of SFG rickettsiosis are being reported on the basis of the presence of antibodies in a single serum collected in the acute phase of illness. These diagnoses are reported as "probable", not as "confirmed cases". It is quite possible that these antibodies had been stimulated by exposure to the highly prevalent *Rickettsia amblyommii* in *Amblyomma americanum*, the predominant tick in the southeastern and south central U.S. and spreading northward.[1-5] A study of military personnel heavily exposed to these infected ticks in field exercises could be interpreted as revealing seroconversion with subclinical infection in the majority of seroconverters and a self-limited symptomatic illness in a significant number of persons. *Rickettsia amblyommii* is widely distributed in Latin America. In a study in a village in Veracruz state of Mexico, the majority of the

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healthy inhabitants had antibodies reactive with *R. amblyommii* and reported a history of frequent tick bites without serious illness suggestive of SFG rickettsiosis. Eleven isolates of *R. amblyommii* were obtained from local *A. cajennense* ticks. A single serum sample from any of these persons who had a non-rickettsial illness could be interpreted as indicating a probable diagnosis of SFG rickettsiosis on the basis of the preexisting antibodies as has occurred in the U.S. in patients with human monocytotropic ehrlichiosis.[6]

A major challenge to the performance of confirmatory serologic diagnosis of rickettsial infection on the basis of seroconversion between acute and convalescent sera is the availability of reagents, namely rickettsial antigens. Commercially available antigens are expensive and generally would be imported. Few laboratories in the world cultivate *Rickettsia*, *Ehrlichia*, or *Orientia*. The methods for cultivation require antibiotic-free cell culture or propagation in yolk sacs of embryonated eggs of chickens from flocks maintained on antibiotic-free feed. This approach demands skilled expertise and is threatened by contamination with bacteria and fungi. Motivated scientists can become proficient if trained by an experienced rickettsiologist. There are laboratories in Latin America and the U.S. that although not numerous are available where the methods can be mastered by scientists with basic microbiologic and cell culture skills.

Another challenge is the broad classification of *Rickettsia* as requiring biosafety level-3 (BSL-3) biocontainment by U.S. public health authorities based on the significant mortality of scientists working with *R. rickettsii* and *R. prowazekii*, largely in the preantibiotic era. These restrictions have loosened slightly as scientists have begun to work with *R. bellii*, *R. montanensis*, *R. parkeri*, and probably a few other non-lifethreatening rickettsiae under BSL-2 conditions. If I lived in another country where there were no official restrictions, I would cultivate many SFG rickettsiae that do not cause lifethreatening illness and other attenuated pathogenic rickettsiae in a BSL-2 laboratory using an effective biosafety hood, N-95 mask, gown, and gloves in space where other personnel were not present during the procedures of inoculation and harvesting of rickettsiae. Latin American diagnostic laboratories need plentifully available sources of affordable rickettsial serologic reagents if appropriate awareness of rickettsial and ehrlichial diseases is going to occur. Ehrlichiae are classified as requiring BSL-2 biocontainment, but their cultivation for use as serologic antigens requires different cell lines than *Rickettsia*, but the technical skills for growing the organisms and manufacturing glass slides for indirect fluorescent antibody assay are similar.

It has been arbitrarily considered that reactivity of a serum sample at four-fold or greater titer with one antigen than the rest indicates that species is the causative agent. Frequently the antibody titers do not differ by four-fold dilutions, and etiologically proven infections have occasionally stimulated antibodies reactive at a higher titer with antigens of another *Rickettsia* species than the etiologic agent. A serologic assay that has detected species-specific antibodies is a method developed by Jorge Zavala-Castro that demonstrates antibodies to a fragment of outer membrane protein A of *R. felis*.[7] This achievement suggests that further research could identify species-specific peptides that might serve as effective serologic antigens. Another promising approach that could be pursued is the whole genome protein array developed by Felgner.[8,9] The goal would be to identify which antigens are recognized early in the course and most strongly by a high proportion of patients and to determine whether any of the antigens detect reactivity to only the causative *Rickettsia* species[8,9] so that serological assays could be manufactured using the ideal combination of antigens capable of yielding highly sensitive and specific results.

Molecular diagnosis by polymerase chain reaction (PCR) seems deceptively easy after one has obtained a thermal cycler and a source of primers. However, positive results that are not supported strongly by clinical, epidemiologic, and other laboratory data are viewed skeptically. Contamination of PCR by target DNA, particularly amplicons generated in previous PCR runs, can occur despite extensive precautions. Amplification and sequencing of multiple gene targets increases the strength of support for a PCR diagnosis, but not as much as seroconversion and an appropriate clinicoepidemiologic history would support the PCR result. Real time PCR and isothermal amplification methods based on transcription-mediated techniques are much less likely to suffer target DNA contamination.

Blood is not the ideal sample for diagnosis of rickettsial diseases by PCR because of the low concentration of circulating organisms. Rickettsiae are located predominantly in endothelial cells in the tissues. The eschar scab and a swab from its base are excellent samples for patients who have this lesion at the tick feeding site and should be examined with suspected infection by *R. parkeri*, *R. massiliae*, and *R. akari*[10].For patients such as those infected by *R. rickettsii*, *R.* typhi, and *R. prowazekii* in whom most of the bacteria are located in the lesions rather than in peripheral blood, approaches such as needle aspiration of the rash could be evaluated. Low cost multiplex instrument-free point-of-care nucleic acid amplification devices with built-in lyophilized reagents and microfluidic processing have been developed that could be applied to the diagnosis of rickettsioses and ehrlichioses.

Challenges of Rickettsial Research

The definitive and most convincing evidence for the presence of an infectious disease is isolation of the pathogen from a patient with compatible clinical manifestations. This goal has been achieved in very few Latin American laboratories. The obstacles are similar to those related to the production of antigens for serologic diagnosis, namely cultivation of rickettsiae in antibiotic-free cell culture without bacterial or fungal contamination and manipulating potentially highly pathogenic organisms without accidental infection of personnel in the laboratory or its nearby environment. Appropriate use of a biosafety cabinet, laboratory safety precautions, and personal protective equipment in a facility engineered or arranged to prevent escape of aerosolized bacteria is the ideal situation. Under any circumstances it is necessary to institute surveillance of febrile disease in laboratory personnel and to treat illness suspected to possibly represent laboratory-acquired infection early in the course. Many rickettsiae such as *R. parkeri* and all

Ehrlichia can be cultivated in a BSL-2 laboratory. Cultivation of *R. felis*, *R. massiliae*, *E. chaffeensis*, *Orientia tsutsugamushi*, and any novel member of the order Rickettsiales from patients in Latin America would constitute a major research achievement.

Other topics related to rickettsial diseases that would be major research achievements include active prospective clinical studies of acute undifferentiated febrile illness that determined the actual incidence of rickettsioses and ehrlichioses in a defined population, development of effective species-specific serologic methods, and extensive characterization of human immune responses to rickettsiae. Determination of the likely mechanism(s) of greater virulence could include comparison of the differences between the more severe Latin American and less severe North American infections with *R. rickettsii* in terms of rickettsial genome comparisons, differential rickettsial gene expression during infection, rickettsial growth rates, and host immune responses as could be revealed by network analysis of several cytokines, chemokines and growth factors. Recognizing the superior achievements of Marcelo Labruna and his colleagues in the elucidation of the natural ecologic cycles of *R. rickettsii* in vertebrate reservoirs and tick vectors in Brazil, they could be challenged to use their experience in transmission by ticks to animals to approach the vector biology of identification of the initial target cells of vertebrate infection and the effects of tick saliva on experimental infections. This foundation of knowledge could be a prelude to elucidating the mechanisms of immune modulation by tick saliva and identification of the tick salivary effector molecules.

Challenges of Increasing Awareness of Rickettsial Diseases

The challenges of achieving increased awareness of rickettsial diseases in Latin America differ little from this unmet goal worldwide. The aim would be to educate all family medicine, emergency medicine, and primary care internal medicine physicians to consider the diagnosis of rickettsial diseases in all tick- and flea-exposed patients with acute undifferentiated febrile illnesses during the appropriate season. This cannot be accomplished by publication of our research articles alone. We must publish review articles and perspectives in journals read by primary care physicians and organize educational sessions at national and regional medical meetings. We need to influence the inclusion of rickettsial diseases in medical school curricula as an important differential diagnosis for the patient with acute undifferentiated febrile illness. We should engage the lay press with topics of potential interest to the general public. Performance of the results of longitudinal active prospective surveillance of cases of acute febrile illnesses for rickettsial infection would fuel credence in their importance. Ultimately collaborative interactions among academicians, physicians, veterinarians, vector biologists, and public health officers could be the most productive challenge to establish.

References

1. Walker DH, Paddock CD, Dumler JS. Emerging and re-emerging tick-transmitted rickettsial and ehrlichial infections. Med Clin North Am 2008; 92:1345-61
2. Yevich SJ, Sanchez JL, DeFraites RF, Rives CC, Dawson JE, Uhaa IJ, *et al.* Seroepidemiology of infections due to spotted fever group rickettsiae and *Ehrlichia* species in military personnel exposed in areas of the United States where such infections are endemic. J Infect Dis 1995; 171:1266-1273.
3. Sanchez JL, Candler WH, Fishbein DB, Greene CR, Coté TR, Kelly DJ, *et al.* A cluster of tick-borne infections: association with military training and asymptomatic infections due to *Rickettsia rickettsii.* Trans R Soc Trop Med Hyg 1992; 86:321-325.
4. McCall CL, Curns AT, Rotz LD, Singleton JA Jr, Treadwell TA, Comer JA, *et al.* Fort Chaffee revisited: The epidemiology of tick-borne rickettsial and ehrlichial diseases at a natural focus. Vector Borne Zoonotic Dis 2001; 1:119-127.
5. Kardatzke JT, Neidhardt K, Dzuban DP, Sanchez JL, Azad AF. Cluster of tick-borne infections at Fort Chaffee, Arkansas: *Rickettsiae* and *Borrelia burgdorferi* in Ixodid ticks. J Med Entomol 1992; 29:669-672.
6. Apperson CS, Engber B, Nicholson WL, Mead DG, Engel J, Yabsley MJ, *et al.* Tick-borne diseases in North Carolina: Is "*Rickettsia amblyommii*" a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? Vector Borne Zoonotic Dis 2008; 8:597-606.
7. Zavala-Castro JE, Dzul-Rosado KR, Leon JJA, Walker DH, Zavala-Velázquez JE. Short report: *Rickettsia felis* outer membrane protein A: A potential tool for diagnosis of patients with flea-borne spotted fever. Am J Trop Med Hyg 2008; 79:903-906.
8. Felgner PL, Kayala MA, Vigil A, Burk C, Nakajima-Sasaki R, Pablo J, *et al.* A *Burkholderia pseudomallei* protein microarray reveals serodiagnostic and cross-reactive antigens. Proc Natl Acad Sci USA 2009; 106:13499-504.
9. Vigil A, Ortega R, Nakajima-Sasaki R, Pablo J, Molina DM Chao CC, *et al.* Genome-wide profiling of humoral immune response to *Coxiella burnetii* infection by protein microarray. Proteomics 2010; 10:2259-2269.
10. Myers T, Lalani T, Dent M, Jiang J, Daly PL, Maguire JD, *et al.* Detecting *Rickettsia parkeri* infection from eschar swab specimens. Emerg Infect Dis 2013; 19:778-780.