ISSN 0001-6012

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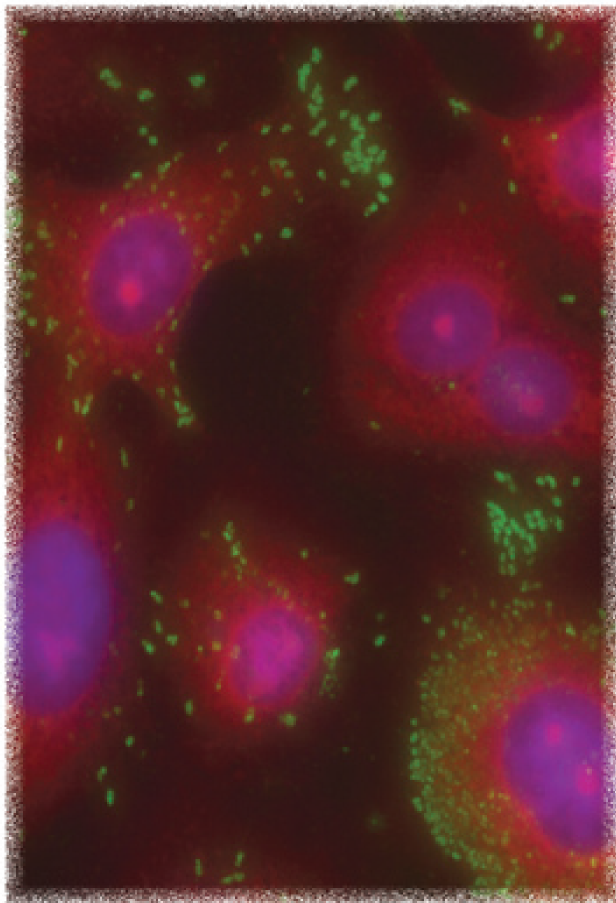
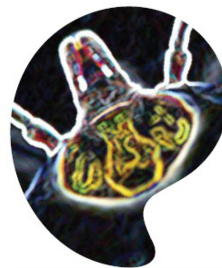
Acta Médica Costarricense

Publicación Científica Oficial del Colegio de Médicos y Cirujanos de Costa Rica





IV Congreso Latinoamericano de Enfermedades Rickettsiales

San José, Costa Rica 2013

22 al 24 julio 2013

Ciudad de la Investigación, Universidad de Costa Rica

Volumen 55 / Suplemento 1 / Julio 2013

Acta Médica Costarricense
Fundada en 1957 por el Dr. Rodolfo Céspedes Fonseca

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Tiraje total 6000 ejemplares. Revisión y editorialización Comité Editorial Acta Médica Costarricense. Acta Médica Costarricense

AMC
Vol. 55 No. 3
2013

Presentación

Para Costa Rica es un honor haber sido escogida para organizar el IV Congreso Latinoamericano de Enfermedades Rickettsiales, ya que representa un reconocimiento a los trabajos del Dr. L.G. Fuentes, pionero de la investigación en Rickettsiales en nuestro país y al grupo de investigadores del CIET, UCR que continúan hasta el presente aportando nuevos conocimientos en este campo.

La sola participación de distinguidos conferencistas de E.E.U.U., Brasil, Argentina, Uruguay, México y Costa Rica nos asegura la calidad y diversidad de enfoques basados en la experiencia de tan distinguidos especialistas en un campo tan complejo como el de las enfermedades Rickettsiales, considerando el actual mundo globalizado en el campo médico.

Bástenos destacar las recientes investigaciones de Parola P, Paddock CD, Raoult D, Walker D, Oteo JA, Galvao M, Labruna MB, Dumler J, McBride J, Nava S, Venzal JM, Zavala-Castro J, Valbuena G y Pinter A, entre otros. Tales investigadores permiten estimar la complejidad del problema de las enfermedades Rickettsiales tanto a nivel mundial como Latinoamericano.

Debe preocuparnos y llamar la atención de todos los especialistas de nuestro continente, el lograr coordinar esfuerzos para así poder enfrentar el problema que nos atañe.

Mario Vargas Vargas, PhD

“Debemos aprender a vivir con las Rickettsias, esos microorganismos causantes de la enfermedad conocida como fiebre manchada de las montañas Rocosas”

Luis Guillermo Fuentes Leiva, PhD
(1935-2005)

ISSN 0001-6012/2013/55/3/4-6
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Conferencias Magistrales

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)

David H. Walker

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

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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

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.

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ISSN 0001-6012/2013/55/3/7-10
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Conferencias Magistrales

Enfermedades rickettsiales: ¿Un problema de salud pública en América Latina?

(Rickettsial diseases: a public health problem in Latin America?)

Márcio Antônio Moreira Galvão, Amanda de Freitas Padilha

Resumen

Para que una enfermedad sea considerada un problema de salud pública, los parámetros epidemiológicos son magnitud, vulnerabilidad y trascendencia. Si se considera sólo magnitud y vulnerabilidad basadas en la incidencia de las enfermedades rickettsiales en América Latina y en la ausencia de una vacuna efectiva contra esta clase de enfermedades, se puede decir que las enfermedades rickettsiales no son una prioridad ni un problema de salud pública. Sin embargo, si se investigan otros parámetros epidemiológicos se puede tener otra visión acerca de estas enfermedades. El objetivo de este trabajo es analizar mediante un estudio histórico retrospectivo, los factores de riego epidemiológicos en las enfermedades rickettsiales, para confirmar nuestra hipótesis sobre su relevancia en salud pública.

Descriptores: salud pública, rickettsiosis, epidemiología.

Abstract

The epidemiologic parameters for a disease to be considered a public health problem are magnitude, vulnerability and transcendence. If you consider only magnitude and vulnerability based on incidence of rickettsial diseases in Latin America and in the absence of an effective vaccine against these kind of diseases, you can say that rickettsial diseases are not priority and are not a public health problem. If you investigate other epidemiologic parameters we can have another vision about the rickettsial diseases. The objective of this paper is to analyze the epidemiologic risk factors in rickettsial diseases by an historic retrospective in Latin America, to confirm our hypothesis of the public health relevance of rickettsial diseases.

Keywords: Public Health; rickettsioses; epidemiology.

In Latin America, human cases of infection by the genus *Rickettsia* have been described in several countries in the last twenty years. The increase of notification of this kind of rickettsial diseases is the result of implementation of epidemiologic surveillance and too due to the modifications in the environment changing the spatial distribution of these diseases. The introduction of molecular biologic techniques in the investigation of rickettsial diseases has permitted the detection of new species of the genus *Rickettsia* in vectors, humans and animals. The sequence of conserved genes from amplified regions by PCR permitted the identification of Rickettsiae not cultivated yet. With this strategy was possible to identify new species as the *Rickettsia felis*. The description of *R. felis* human cases in Latin America by 2000s opened new perspectives for comprehension of the role performed by new species of *Rickettsia* in the epidemiology of rickettsioses. In 2000 only three species of *Rickettsia* were known in South America. In the beginning of this century more seven species were related in this continent, confirming the importance of the introduction of these techniques in rickettsiology area.[1] The use of molecular biology helps too to understand better about the geographic distribution of rickettsioses and about the association vector-rickettsia.[2]

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Some countries, such Brazil, have a long history of rickettsioses, while others such as Argentina, have only recently detected these diseases. In Brazil, *Rickettsia rickettsii* has been described as the agent of Brazilian spotted fever (BSF) transmitted by the tick *Amblyomma cajennense* since the 1920s.[3] In the last 20 years, cases of BSF have been confirmed by immunofluorescence serology and more recently by molecular techniques in the states of Minas Gerais, São Paulo, Rio de Janeiro and Espírito Santo.[4] Between 1990 and 2011, Brazil registered 269 deaths due to BSF. During this period the number of confirmed cases in southeast region reached 747 cases, representing 77.2 % of the total of cases of this country. São Paulo was responsible for 45.7 % of these cases and Minas Gerais for 20.2%.[5] Cases of cat flea-associated rickettsiosis caused by *Rickettsia felis* were reported too in Minas Gerais state, [6] with the detection of this bacterium in *Ctenocephalides* genus fleas by PCR.[7]

Spotted fever caused by *R. rickettsii* were identify too in Mexico, Panama, Costa Rica, Colombia and Argentina.[2,8] The Argentine cases were described in Jujuy Province in 1999, when six children with fever, rash and a history of recent tick bite were evaluated for rickettsial infections and immunohistochemistry staining of tissues obtained by \ autopsy from one fatal case.[9] In Panama the description of *R. rickettsii* re emergence occurred in 2007 after more than fifty years without the description of spotted fever cases.[10] In Mexico the first case of spotted fever caused by *R. rickettsii* was described in 1930. From 1930 to 1950 many cases of this disease were reported in many states.[11] The same phenomenon that was seen in Panama was observed too in Mexico with an epidemiologic silence occurred from 1950 till 2005, when new cases of this re emergent disease initiated to happen again.[10] Costa Rica registered the first outbreak of *rickettsii* spotted fever in 1975. After this date several cases were described in this country.[12] In Colombia nothing was known or published about rickettsioses since 1937 when a report of an epidemic caused by *R. rickettsii* was related and named as "Fiebre de Tobia". In 2007 two fatal cases of *R. rickettsii* spotted fever were related near the same locality were the disease was reported in the 1930s.[13, 14] Peru has a long history of epidemic typhus caused by *Rickettsia prowazekii* (transmitted by body lice), [15] isolated cases of murine typhus, and the detection of *R. felis* in *Ctenocephalides canis* fleas, indicating the possibility of human cases of this rickettsiose.

The principal vectors of rickettsioses found in Latin America as in other parts of the world are arthropods like ticks, fleas, lices and mites. The transmission of *Rickettsia* by the vector is influenced by different factors, including physiologic and ecologic process. These last factors influence the intensity of *Rickettsia* transmission for humans and animals. Intrinsic and extrinsic factors can affect significantly the competency of these arthropods as agents of rickettsioses. The risk of occurrence of these diseases due to extrinsic factors includes the population level of the arthropods, the susceptibility of the preferential hosts to *Rickettsia*, the immunity of the host, the genetic variation determined by infectivity of the agent and the environmental relationships. The intrinsic factors related to the vectors are food, physiologic and compartment questions, transovarial and transstadial transformation, the presence of other organisms or pharmacology actives substances, and the time of permanence of the vector in the host.

The rates of vector infection can variety depending of pathogen virulence, *Rickettsia* susceptibility, the presence of co-infections and modulation of immune response at the host. The *R. rickettsii* maintains in general levels of infection lower than 1%.[1,16] Although some authors don´t consider difference between endemic and non endemic areas, asserting that the presence of infected ticks cannot be sufficient to produce human disease, the high infestations by ticks in the environment and in the animals can modify this relation.

Conclusions

To judge if rickettsial diseases are an important public health problem or not in Latin America we need to evaluate all these factors related. Summarizing all them by epidemiologic parameters they can be represented by magnitude, spreading potential, vulnerability and transcendence (measured by fatality, severity, social and economic relevance/spatial distribution of the diseases).

If you consider only magnitude and vulnerability based on incidence of rickettsial diseases in Latin America and the absence of an effective vaccine against this kind of disease, you can say that rickettsial diseases are not priority and are not a public health problem. But if you consider the other epidemiologic parameters we can have another vision about the importance of rickettsial diseases. We can try to do it discussing the situation of each rickettsiose of the genus *Rickettsia* in Latin America.

The spotted fever caused by *R. rickettsii* is the more severe rickettsiose among the rickettsial diseases in Latin America with a high case-fatality ratio.[1] The primary vectors for *R. rickettsii* in Latin American are ticks from *Amblyomma cajennense* specie (Mexico, Panama, Costa Rica, Colombia, Argentina e Brazil), *Amblyomma aureolatum* (Brazil), *Amblyomma imitator* (Mexico) and *Riphicephalus sanguineus* (Mexico).[8]

Rickettsia parkeri is considered an emergent pathogen in Latin America with suspected human cases related in Uruguay[17] and *Amblyomma triste* as vector.[18] Brazil also have suspected cases of this rickettsiose described in the south of the country.

The riquetsiose felis caused by *Rickettsia felis* has the fleas of *Ctenocephalides* genus as the principal vector. Although the symptoms of this disease are common in other rickettsioses (fever, myalgia, rash and abdominal pain) and can be synonym of mild cases, neurologic symptoms occurred in Brazilian and Mexican cases can traduce the possibility of severity of this disease in some cases.[19]

The occurrence of epidemic typhus in Latin America caused by *Rickettsia prowazekii* can be observed in Peru and Mexico.[15, 20] This disease is transmitted by the feces of *Pediculus humanus corporis*. The outbreaks of epidemic typhus are related to social

problems as absence of hygiene and extreme poverty. The case fatality ratio of cases without treatment can reach 30%, with a tax of 60% in elderly people.

Rickettsia typhi the agent of endemic typhus occurs in all Latin America having *Xenopsylla cheopis* as primary vector and *Rattus norvegicus* as host. The case fatality ratio is lower than 5%. The elimination of infected fleas with the use of insecticides has diminished the number of human cases and the importance of murine typhus as a public health problem.

The analysis presented of the epidemiologic situation of each rickettsiose help us to conclude that rickettsial diseases are an important public health problem in Latin America due their great social and economic impact. At the time the rickettsioses reach poor regions, the association of agglomeration of people to the potential dissemination of the vector originates an increase in the number of cases in a short period of time. The exemplification of this thesis can be demonstrated in Brazil with *R. rickettsii* and in Peru and Mexico with *R. prowazekii*. The phenomenon of familial clusters of *R. rickettsii* spotted fever rickettsiosis has been noted numerous times in Latin America.[21] In fact, the simultaneous occurrence of severe febrile illness in more than one patient generally suggests person-to-person or a point-source transmission of infection. Few physicians in USA may be aware that 4.4% of cases of Rocky Mountain spotted fever occur in the household of another case-patient with the disease, a situation that often lends further diagnostic confusion for this illness that can mimic other febrile exanthems, such as dengue, as well as gastrointestinal infection, other abdominal conditions, pneumonia, and meningoencephalitis.

We conclude with the affirmation that the analysis of the spatial distribution of rickettsial diseases besides social/ economic impact is fundamental to understand the occurrence and the spread of these diseases in the present moment in Latin America. We can exemplify it affirmation by the re emergence of cases of Brazilian spotted fever in Minas Gerais state, Brazil since 1980 in urban areas. This occurrence suggests the invasion of natural focus of the disease by the man and an expansion of rickettsioses due to a new ecologic reorganization associated to the way of life of a population excluded in the social economic context.[22, 23, 24] The presence of ricketsioses focus in areas of urban expansion was first related in São Paulo by 1920, [25] but continues to be a reality now in Latin America as related in Brazil by 1980's and recently in Costa Rica.[26]

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ISSN 0001-6012/2013/55/3/11-12
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Rickettsia felis: un patógeno emergente en Latinoamérica

(*Rickettsia felis*: an emerging pathogen in Latin America)

Jorge E. Zavala Castro

Resumen

El primer caso humano infectado con *Rickettsia felis* fue descrito en un paciente diagnosticado con tifus murino en Estados Unidos en 1994. Los reportes acerca de la presencia de *R. felis* se han incrementado en los últimos años y ya se ha reportado en la mayoría de los países de América Latina. Los síntomas y signos de la enfermedad causada por *R. felis* son inespecíficos y es importante que cada país lo incluya en el diagnóstico diferencial con respecto a otras enfermedades que tienen las mismas manifestaciones clínicas.

Descriptores: *Rickettsia felis*, enfermedad, América Latina

Abstract

The first human case of *Rickettsia felis* was described in 1994, in a patient diagnosed with murine typhus in the United States. Reports about the presence of *R. felis* have increased in the last years, and it has been reported in most countries of Latin America. Signs and symptoms of disease caused by *R. felis* are unspecific, and it is important for each country to include it in the differential diagnosis with other diseases that show the same clinical manifestations.

Keywords: *Rickettsia felis*, disease, Latin America

En 1992 se reportó un hallazgo inesperado en una colonia comercial de la pulga *Ctenocephalides felis*. El organismo detectado fue nombrado como agente ELB porque fue descubierto en pulgas mantenidas en condiciones *in vitro* en los laboratorios de El, Soquel, CA.[1] El primer caso humano infectado con el agente ELB fue descrito en un paciente diagnosticado con tifus murino en Estados Unidos en 1994.[2]

En 1996, el agente ELB se propuso como nueva especie de *Rickettsia* perteneciente al grupo tifus y fue sugerido el nombre de *Rickettsia felis*.[3] Sin embargo, una aparente contaminación del aislado descrito y una mayor caracterización molecular de *R. felis*, muestran que pertenece a la familia de grupo de la fiebre manchada.[4] Posiblemente por esta discrepancia, la infección humana por *R. felis* no fue totalmente aceptada en un inicio.

Con un nuevo enfoque molecular y evidencias serológicas, fue descrito por primera vez la presencia de casos humanos infectados con *R. felis* en México, tras la identificación de *C. felis* como el vector y la presencia de la infección en perros.[5,6] En los años siguientes se documentaron más casos humanos en Perú, Brasil, Estados Unidos y México.[7,8,9,10]

Los reportes acerca de la presencia de *R. felis* en América se han incrementado en los últimos 8 años, probablemente debido a que se ha aceptado su presencia en nuestro continente como un agente patógeno que amenaza potencialmente la salud humana. Es así como se ha reportado en Guatemala, Costa Rica, Panamá, Argentina, Uruguay, Chile; y una búsqueda de información revela que se ha reportado prácticamente en todos los países de América Latina en alguna ocasión.[11-17]

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Los síntomas y signos de la enfermedad causada por *R. felis* son inespecíficos y se confunden con los causados por muchas otras bacterias e infecciones virales. Así mismo, pueden presentar cierta heterogeneidad entre pacientes de diferentes países, e incluso en pacientes de un mismo país. Por ejemplo, en México fueron diagnosticados 9 casos de 2000 a 2006, y ninguno de los signos se observaron en el 100% de los pacientes. El más común era la fiebre (95%) y la otra manifestación clínica varía de mialgia (75%) a la implicación respiratoria superior (25%). Otros signos y síntomas fueron dolor de cabeza, sarpullido, diarrea, participación CNS, lesiones cutáneas, náuseas y vómito y dolor abdominal. Cada paciente presenta diferentes manifestaciones clínicas sin un evidente patrón común. Cuando comparamos las manifestaciones clínicas entre los pacientes de Brasil y México encontramos diferencias en la tasa de cada signo y síntoma, y algunos de los signos están ausentes.[18] Tras el análisis de las manifestaciones clínicas, consideramos la importancia que cada país en América Latina haga su propio esquema de los signos y síntomas de la enfermedad causada por *R. felis* y determine sus propios parámetros para la infección en seres humanos, y lo incluyan en el diagnóstico diferencial con respecto a otras enfermedades que sin duda tienen las mismas manifestaciones clínicas.

Recientemente, hemos reportado casos atípicos causados por la infección de *R. felis*, teniendo manifestaciones clínicas severas, que asemejan a las causadas por el virus de la hepatitis, y hemorragias pulmonares que pueden presentarse en otro tipo de infecciones bacterianas o virales.[19] Es importante considerar éstas variaciones clínicas para evitar muertes en la población causadas por ésta infección que apareció para quedarse en América Latina.

Agradecimientos: Este trabajo fue facilitado por el Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo- CYTED, y gracias a las aportaciones de los miembros de la Red Iberoamericana para la Investigación y Control de las Enfermedades Rickettsiales-RIICER.

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ISSN 0001-6012/2013/55/3/13-24
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Perspectivas sobre el diagnóstico de laboratorio de enfermedades rickettsiales en el siglo 21

(Perspectives on the laboratory diagnosis of rickettsial diseases in the 21st century)

Christopher D. Paddock

Resumen

Es necesario contar con métodos de laboratorio muy eficientes y específicos para poder distinguir las rickettiosis de otras enfermedades infecciosas y diferenciar con exactitud unas rickettsias de otras. Las categorías más importantes de métodos utilizados para diagnóstico de rickettsias son: Visualización directa por histología o tinciones inmunohistoquímicas, serología, cultivo y métodos moleculares, cada una de ellas posee sus fortalezas y debilidades. La presente revisión discute algunas de estas ventajas y desventajas inherentes a cada método y cómo estas técnicas pueden evolucionar colectivamente en el futuro. Históricamente, muchas enfermedades rickettsiales han sido pobremente estudiadas, generalmente porque estos patógenos se consideran difíciles de cultivar, difíciles de teñir y peligrosos de propagar en el laboratorio. Progresando hacia el siglo 21, un mayor uso de estos métodos de diagnóstico, especialmente en países tropicales y en vías de desarrollo podría beneficiar un reconocimiento global de las rickettsiosis y el impacto que estas tienen en un enorme segmento de la población mundial. Es cada vez más importante para los ricketsiólogos contemporáneos abstenerse de utilizar los métodos clásicos tales como cultivo, serología y hasta la visualización directa con la evolución de métodos moleculares más rápidos y sofisticados. Se pueden obtener resultados extraordinarios cuando se utilizan múltiples técnicas de manera concomitante.

Descriptores: *Rickettsia*, *Ehrlichia*, *Orientia*, *Coxiella*, diagnóstico

Abstract

Robust laboratory methods are necessary to distinguish rickettsioses from other infectious diseases and to accurately distinguish one rickettsiosis from another. The major categories of diagnostic techniques used for rickettsioses, i.e., direct visualization by histological or immunohistochemical stains, serology, culture, and molecular techniques, each have unique strengths and weaknesses. This review discusses some of the advantages and disadvantages inherent to each method and how these techniques might evolve collectively in the future. Many rickettsial diseases have been historically understudied, often because the pathogens were considered difficult to grow, difficult to stain, and dangerous to propagate. As we progress into the 21st century, the broader use of diagnostic assays, particularly in many tropical and developing countries, will better leverage the global recognition of rickettsioses, and the impact that these infections have on enormous segments of the world population. It will be increasingly important for contemporary rickettsiologists to refrain from replacing entirely classical methods such as culture, serology, or even direct visualization with more rapidly evolving and increasingly sophisticated molecular techniques. Extraordinary results can be achieved when multiple techniques are used in tandem.

Keywords: *Rickettsia*, *Ehrlichia*, *Orientia*, *Coxiella*, diagnosis

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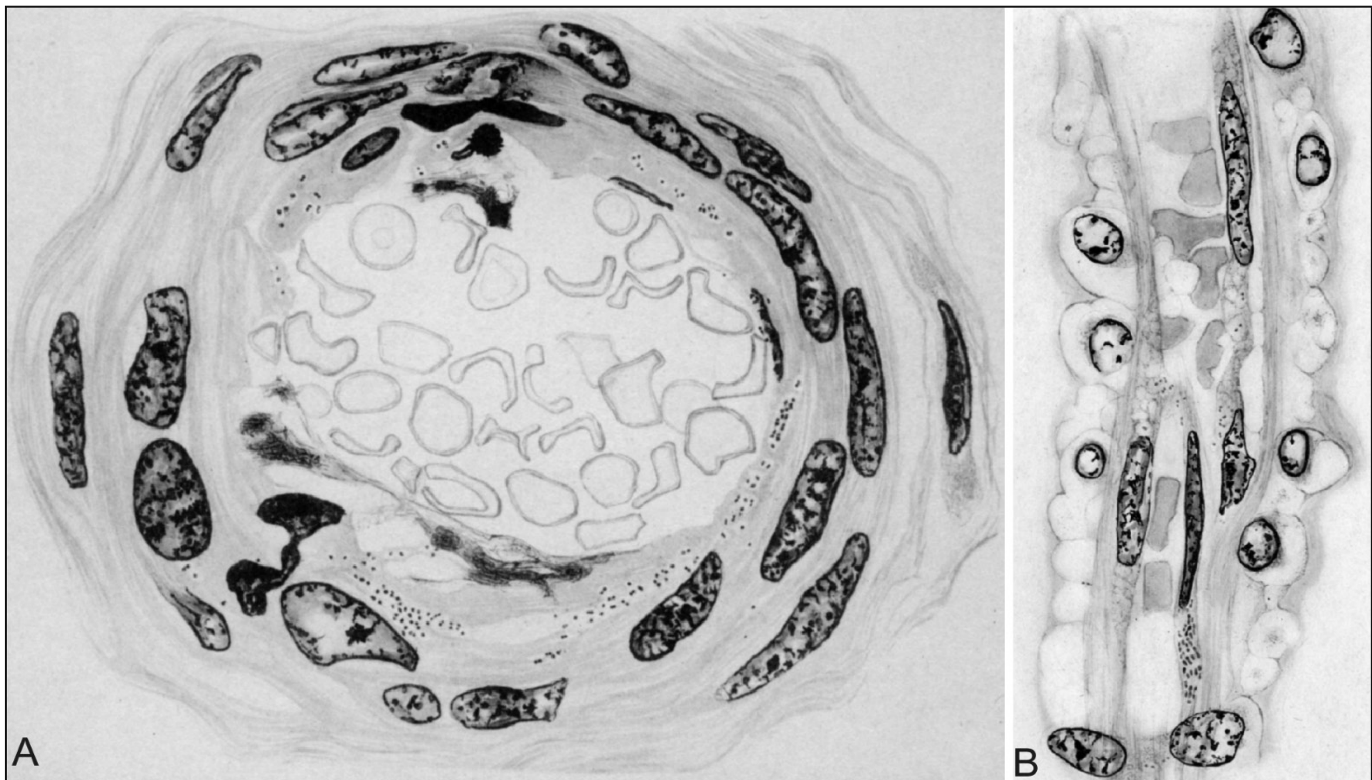

Figure 1. Early drawings of histologic appearance rickettsiae in the tissues of patients with fatal rickettsioses identified by using Giemsa stain. (A) *Rickettsia rickettsii* bacteria in an arteriole from the skin of a man with from the Bitterroot Valley, Montana, who died from Rocky Mountain spotted fever, showing the distribution of rickettsiae in endothelial cells.[11] (B) *Rickettsia prowazekii* rickettsiae in an arteriole of a woman who died from epidemic typhus.[12]

An early presumptive diagnosis, based on careful consideration of the clinical and epidemiological features associated with the illness, represents the foundation for successful treatment of patients with rickettsial diseases. For more than 50 years, rickettsiologists and public health professionals have emphasized this dictum because so many of the rickettsioses, including Rocky Mountain spotted fever (RMSF), Mediterranean spotted fever, scrub typhus, louse-borne typhus, and *Ehrlichia chaffeensis* ehrlichiosis can be life-threatening diseases that characteristically offer a relatively narrow window of time during when appropriate antibiotic therapy is most effective at minimizing morbidity and mortality. The term 'acute doxycycline deficiency' is used occasionally by clinicians in the United States during the spring and summer months to describe an acute febrile illness, presumably rickettsial in origin, that responds favorably to therapy with this antibiotic. The choice of appropriate therapy for these diseases is crucial, but must also be coupled with laboratory efforts that validate clinical suspicion. A response to doxycycline is not necessarily confirmatory evidence of a rickettsiosis, and not all febrile, rash-associated illnesses will necessarily respond to treatment with doxycycline. In this context, robust laboratory methods are needed to distinguish rickettsioses from other infectious diseases, and to accurately distinguish one rickettsiosis from another.

Laboratory diagnostics provide physicians with vital data that validate the accuracy of their clinical diagnoses and help define the true clinical spectrum of individual infections. These tools also occupy a fundamental role in the science of rickettsiology as drivers of pathogen discovery and by providing information necessary to uncover nuanced epidemiological and ecological features unique to each rickettsiosis. During the last decade, several publications have provided detailed descriptions of the various techniques used to diagnose the rickettsiosis,[1-5] and the enormous downstream influence these assays have on epidemiological statistics for these diseases.[6,7] The following discussion is not intended to comprehensively evaluate each method, but rather to emphasize some general strengths and weaknesses of the 4 major classes of these techniques, and how these might evolve collectively in the future.

Direct visualization

The small size, relatively sparse distribution and specific tinctorial properties of rickettsiae in tissues, blood, and other body fluids have historically posed challenges to clinicians and scientists attempting to visualize these pathogens in clinical samples. Howard Ricketts was the first to microscopically identify rickettsiae in animals and humans when he described "diplococcoid bodies, sometimes short bacillary forms" in Giemsa-stained smears of blood from patients with RMSF and guinea pigs and monkeys infected experimentally with *Rickettsia rickettsii.* [8,9] Ricketts was hesitant to state definitively that these structures were the etiologic agent of RMSF because he could not cultivate these bacteria by use of axenic media; nonetheless, his observations were soon corroborated by Simeon Burt Wolbach who used the same staining method to describe the characteristic appearance and distribution of rickettsiae in human tissues: …

Table 1. Comparison of selected signs and symptoms reported for patients with Brazilian spotted fever in the states of São Paulo and Santa Catarina, Brazil, during 2003-2006.[37]

Sign or symptom	São Paulo (n = 126)	Santa Catarina (n = 61)	p-value
Fever	112 (89%)	58 (95%)	0.16
Rash	44 (35%)	30 (49%)	0.06
Nausea or vomiting	40 (32%)	24 (39%)	0.3
Adenopathy	5 (4%)	30 (49%)	< 0.01
Petechiae	46 (36%)	5 (8%)	< 0.01
Hemorrhage	33 (26%)	1 (2%)	< 0.01
Hypotension	30 (24%)	2 (3%)	< 0.01
Coma	24 (19%)	0	< 0.01
Convulsion	18 (14%)	0	< 0.01
Death	46 (37%)	0	< 0.01

"a short rod in pairs, joined end to end…found in apparently uninjured endothelium of normal vessels, in areas of proliferated endothelium of the intima of vessels, in hyaline necrosed intima in more advanced lesions, in apparently normal and necrosed smooth muscle fibers of vessels with lesions, and in endothelial cells in the perivascular zones of proliferation."[10]

Traditional staining methods thus facilitated identification and description of the fundamental lesions of RMSF and louse-borne typhus (Figure 1), and provided a foundation for the pathophysiology of all vasculotropic rickettsiosis;[11,12] nonetheless, the use of traditional histological stains as a diagnostic technique to detect rickettsiae in clinical samples is vanishingly rare in contemporary scientific and medical literature.[13-15]

In 1976, investigators first coupled hyperimmune animal serum with immunofluorescence techniques to detect spotted fever group (SFG) rickettsiae in tissues of ill patients;[16] during the last 25 years, investigators have developed immunohistochemical (IHC) assays using monoclonal or polyclonal antibodies to identify various genera of rickettsial pathogens in formalin-fixed, paraffin-embedded biopsy or autopsy tissue specimens, including multiple SFG and typhus group (TG) *Rickettsia* species,[17-23] *E. chaffeensis,*[24-26] *Anaplasma phagocytophilum*[24] and *Orientia tsutsugamushi.*[27] Advantages of these methods include: (1) direct evidence of infection; (2) high sensitivity during the early stages of infection when other methods, particularly serology, are non-diagnostic; and, (3) excellent positive predictive value. The advantage provided by direct observation of the pathogen in the appropriate histopathological context is considerable and provides a second level of confirmation inherent only to this class of diagnostics.

Another useful and somewhat unique feature of IHC techniques is its application to specimens obtained years or even decades earlier. The cause of death of a Maryland patient from 1901 was confirmed ninety years later as RMSF by use of an IHC stain applied to archival paraffin-embedded tissues, predating retrospectively the first descriptions of this disease in the eastern United States by thirty years.[28] Application of immunohistochemical techniques identified *O. tsutsugamushi* in archival tissues >50 years old.[27] Finally, by identifying additional cellular targets of these pathogens, and by dissecting the specific inflammatory response of the vertebrate host, IHC techniques build on the foundation of rickettsial pathobiology established almost 100 years ago by conventional histological staining methods.[19, 21, 26, 27] Disadvantages of IHC staining methods include: (1) the acquisition of the analyte, i.e., tissue, is typically more complex than collection of blood or serum; (2) the requirements of specimen processing and evaluation that often limit diagnostic capacity to specialized regional or national centers; and, (3) the use of immunologic reagents that are generally group-specific rather than species-specific.

Careful microscopical examination peripheral blood smears stained with eosin-azure type dyes will detect morulae in the cytoplasm of infected leukocytes in as many as 20%-30% patients infected with *E. chaffeensis* and approximately 20%-80% of those infected with *Anaplasma phagocytophilum.*[29-31] The accuracy of these techniques is biased by the number of examined cells or blood smears, the level of immune compromise of the host, and the relative experience of the microscopist, who must distinguish morulae from other structures such as Döhle bodies, toxic granulations, or phagocytosed bacteria or fungi that may occur in the cytoplasm of white cells in other infectious conditions.[29] This technique is relatively insensitive and inconsistent; however, its simplicity and ubiquity provided the initial discoveries of human monocytic ehrlichiosis and human anaplasmosis in the United States during the late 1980s and early 1990s.[29, 31]

Serology

Serological methods represent the most widely recognized, broadly available and frequently used tools for the diagnosis

of rickettsial diseases; however, these techniques are also deeply ingrained in the minds of clinicians, often to the exclusion of any other category of confirmatory test. Despite the ease of use and accessibility of serological assays, these are distinguished from all other diagnostic methods by providing indirect evidence of infection or exposure, i.e., detecting host antibodies reactive with rickettsiae, rather than identifying directly antigens, nucleic acids, or live rickettsiae. The indirect immunofluorescence antibody (IFA) assay, generally considered the serological reference method for rickettsioses, was first applied for the diagnosis of murine and louse-borne typhus in 1959 and subsequent uses of IFA to diagnose other rickettsial diseases increased dramatically during the next several decades.[32,33]

The principal advantages of IFA, enzyme immunoassay (EIA), and other antibody detection methods include: (1) commercial availability; (2) relative simplicity; and, (3) opportunities for retrospective diagnosis weeks after the patient has recovered from the acute illness. Serologic methods can be used effectively to screen individuals and populations for rickettsial infections in regions where these diseases were previously unrecognized. By example, the recent identification of an unexpected and novel *Orientia* infection in a traveler to Dubai, hundreds of miles beyond the recognized range of classical scrub typhus, was initiated by a serologic result that indicated recent infection with a pathogen antigenically similar to *O. tsustugamushi.*[34]

Serological assays, particularly IFA methods, can be very effective at confirming rickettsial infections when 2 or more serum specimens are collected at appropriately timed intervals that generally range between 2 to 6 weeks, depending on the relative severity of the particular infection;[35] however, when this protocol is not followed, the opportunities for misinterpreting data generated by serological assays are considerable, particularly when no other laboratory methods are included in the diagnostic evaluation of the patient. Paradoxically, serological methods are particularly insensitive during the acute stage of rickettsial infections, when most patients seek medical attention and when the majority of specimens are collected for subsequent evaluation by these techniques. As an example, initial serum samples collected from 11 of 22 patients with laboratory-confirmed rickettsialpox failed to demonstrate IgG antibody levels reactive with *Rickettsia akari* antigens at or above the conventionally recognized cutoff dilution when tested by using an IFA assay; confirmatory evidence of infection with *R. akari* in these 11 patients was obtained only because diligent clinicians collected additional specimens, including convalescent-phase serums and skin biopsy specimens for IHC, culture, and PCR.[20]Approximately 50% of patients with RMSF lack a diagnostically relevant IFA titer (i.e., > 64) during the first week of illness; however, at least half of all deaths attributed to *R. rickettsii* occur within 7-9 days after illness onset, explaining the large percentage of persons who die of RMSF without serological confirmation.[18] In these circumstances, IFA methods have an exceptionally low negative predictive value and molecular methods (see below) can greatly enhance a diagnosis in cases of fatal disease, particularly if a serum sample collected during the acute illness is the only diagnostic specimen obtained by clinicians.[36]

Patients infected with or exposed to *Rickettsia, Ehrlichia, Anaplasma, Coxiella* or *Orientia* species characteristically generate antibodies that are genus- or group-specific rather than species-specific. An important limitation of serological methods occurs when antibody titers are over-interpreted to ascribe a species-specific etiology based entirely on reactivity to a particular rickettsial antigen. In 2003, cases of Brazilian spotted fever (BSF) were reported for the first time in the state of Santa Catarina in southern Brazil. Because serological testing revealed antibodies reactive with *R. rickettsii* in these patients, their illnesses were categorized officially as BSF. However, observant clinicians recognized that the clinical composite of "BSF" in Santa Catarina differed significantly from that of historically recognized disease in the state of São Paulo, particularly with respect to life-threatening manifestations and death (Table 1).[37] Indeed, no deaths were reported among the 139 cases of "BSF" identified in Santa Catarina during 2003-2009; in contrast, the case-fatality rate of BSF in São Paulo during this same interval was approximately 30% (www.cve.saude.sp.gov.br/htm/zoo/fm_i8503.htm). Because many of the severe cases in São Paulo were confirmed infections with *R. rickettsii* [38] investigators suggested that a *Rickettsia* species other than *R. rickettsii* was responsible for the mild cases of "BSF" in Santa Catarina.[36] Indeed, subsequent entomological surveys for SFG rickettsiae in ticks in Santa Catarina detected a strain of *Rickettsia parkeri,*[39] recognized previously in other parts of Brazil as the cause of a rickettsiosis far milder than BSF.[40,41]

Serological diagnoses of rickettsioses are often confounded by the occurrence of preexisting levels of antibodies in the population at-risk that are reactive with a particular pathogen that may be different than the actual disease under investigation. In the United States, antibodies reactive with antigens of *R. rickettsii* occur at "diagnostic" levels in as many as 5% to 10% of the general population.[42-46] There are several possible explanations for the serological noise created by diagnostically relevant antibody titers to pathogenic rickettsiae among otherwise healthy persons. These include serum reactivity with antigenically related but minimally or non-pathogenic species, and persistence of antibody levels among persons previously infected with or exposed to rickettsial antigens weeks, months, or perhaps years earlier (Figure 2).[47]

In some areas of the world the background seroprevalence to rickettsial agents is considerably higher. From separate studies in Colombia, approximately 40% of 392 randomly selected healthy adults in Cundinamarca Province demonstrated antibodies reactive with *R. rickettsii* at titers $\geq$ 64 by using a well-validated IFA assay,[48] while in Caldas Province, a staggering 490 (72%) of 682 sampled volunteers demonstrated IgG antibody titers $\geq$ 64 to antigens of *Rickettsia felis*, *Rickettsi typhi* or both rickettsiae.[49] In the Department of Loreto in the Amazon basin of Peru, a serosurvey of 1,195 persons during 2006 revealed antibodies reactive with SFG and TG *Rickettsia* species in 521 (43.6%) and 123 (10.3%) participants, respectively.[50] Diagnostically relevant titers (i.e., $\geq$ 64) to antigens of *E. chaffeensis* were identified among 15 (14%) of 105 healthy persons in a rural area of Jujuy Province, Argentina, despite the absence of any recently reported illnesses compatible with ehrlichiosis among the persons sampled.[51]

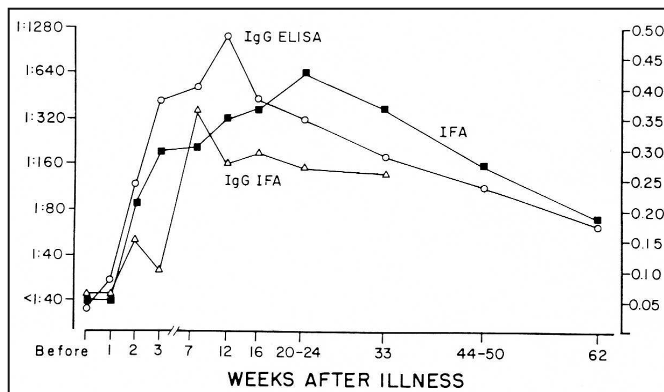

Figure 2. Kinetics of immunoglobulin G (Δ) and total immunoglobulin (■) reactive with *Rickettsia rickettsii* antigens, as determined by indirect immunofluorescence antibody (IFA) assay and enzyme immunosorbant assay (ELISA) (○), in volunteers infected experimentally with *R. rickettsii*. [47]

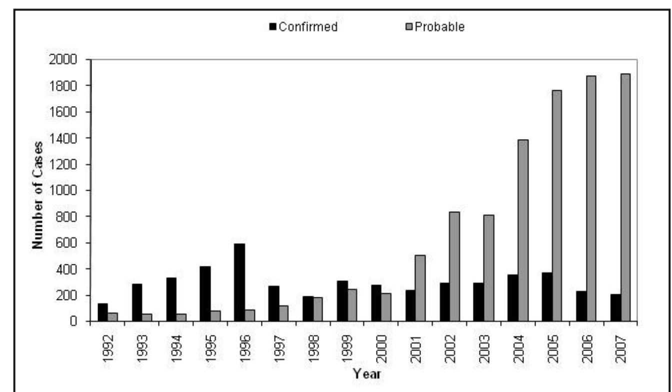

Figure 3. Reported cases of Rocky Mountain spotted fever in the United States, by case classification status, 1992-2007.[6]

Serological methods, particularly IFA, are also subject to a lack of standardization among laboratories and inter-observer variability. A recent analysis of 109 published studies where IFA was used for the diagnosis of acute scrub typhus or to determine seroprevalance in a particular region found broad variation among studies with respect to the specific strains of *O. tsutsugamushi* used as antigen, the targeted antibody isotype, and the selection of cutoff titers that defined a positive result. In the majority of these studies no clear justification for the cutoff titer was provided. These authors concluded that no single antibody titer can reliably be considered diagnostic unless prior studies have been performed to determine the seroprevalence levels in the normal population of that locality.[52] Important serological discrepancies occur even when identical specimens are evaluated by different laboratories. When a panel of serum specimens, collected from 52 persons 6 years after a point source outbreak of Q fever in Australia, were evaluated by IFA methods at 3 different international reference centers for serological evidence infection with *Coxiella burnetii*, the concordance status of IgG and IgM titers used to determine acute and chronic infections, past exposures, and serologically negative persons was only 35% among these highly respected laboratories.[53]

Nonetheless, serological methods are used with increasing frequency, often to the exclusion of other diagnostic tests. Results from these assays, most commonly represented as a single IFA titer or EIA absorbance value, are then used to generate epidemiological statistics; as a result, fewer cases are confirmed and a far greater percentage of cases are considered probable (Figure 3). The impact of diagnostic inaccuracy upon epidemiologic observations may be considerable. During 2000-2007, the reported case-fatality rate for RMSF in the United States was 0.5%, based on CRF denominator data comprising 7,796 cases, or approximately 1000 cases each year.[6] One explanation for this estimate lies in the composition of the denominator, which is likely populated with patients with milder infections, caused by SFG *Rickettsia* species other than *R. rickettsii*.[7]

In some cases, western blotting and cross-adsorption techniques offer greater resolution with respect to the specific rickettsial pathogen; however, these assays are generally more expensive and technically difficult to perform, and are characteristically limited in availability to only a few reference centers around the world.[3] In theory, cross-adsorption is the best serological method of identifying the specific rickettsial agent responsible for the infection and is based on the principle that homologous and heterologous antibodies will be removed entirely when the patient serum is incubated with the antigens of the specific pathogen responsible for the disease. When incubated with a heterologous antigen, only the group-specific antibodies responsible for the cross-reaction will be removed, whereas species-specific antibodies are retained. This technique has been used successfully to discriminate serologic responses to *R. typhi* from those to *R. prowazekii*.[54] Nonetheless, the rationale for this technique can unravel when applied to SFG rickettsioses because: (1) the inherent pretest bias, i.e., an assumption that one of the antigens used in the assay is the pathogen responsible for the infection; and (2) the absence of appropriate positive and negative controls to validate the technique. In practice, most applications of the cross-adsorption technique assume only 2 possible pathogens, and the panel consists of 4 reactions; however, if correctly applied, adsorption panels must become considerably larger and more complex as additional SFG *Rickettsia* species are discovered and considered as potential pathogens. For example, if 3 sympatric SFG rickettsioses exist in a region, a panel should require 9 separate cross-adsorptions. If there are 4 possible agents, a correctly designed panel becomes prohibitively large with 16 separate reactions, which in practice is never observed. In this context, the utility of cross-adsorption is diminished in regions where there are multiple co-circulating and antigenically similar pathogens that cause similar clinical syndromes.

Culture

Culture represents the reference standard for microbiological diagnosis and is the least biased of all diagnostic techniques. Most pathogenic *Rickettsia* species will proliferate in many commonly used cell lines such as Vero E6 cells and human embryonic lung fibroblasts. The recent discovery of an axenic media for *C. burnetii* suggests that culture of other rickettsiae might also be achieved in carefully defined, cell-free mediums.[55]

Historically, rickettsiologists have been adept at culture techniques and these skills have resulted serendipitously in the isolation of several other important and novel non-rickettsial pathogens, including *Legionella pneumophila*, *Tropheryma whipplei*, and Heartland virus.[56-58] Nonetheless, diagnostic laboratories are often reluctant to attempt isolation of rickettsial agents, founded largely on the perception of the dangers associated with this task. For much of the 20th century, cultivation of rickettsiae was inextricably linked with the unfortunate and surprisingly frequent occurrence of laboratory-acquired rickettsioses, often resulting in the death of the investigator. Rickettsioses accounted for approximately 15% of 3921 laboratory-associated infections and 14% of the infection-related deaths tabulated through 1974.[59]

Rickettsia typhi, R. prowazekii, O. tsutsugamushi, R. akari, R. africae, R. australis, and especially *C. burnetii*, have been associated with laboratory-acquired infections;[59-67] however, it is the notoriety of *R. rickettsii* that instills the greatest fear among clinical microbiologists. During 1912-1942, 12 investigators in the United States, Brazil, Colombia, and Japan who worked with *R. rickettsii* died from laboratory-associated RMSF.[68] The deaths of a custodian and glassware worker at the Centers for Disease Control and Prevention in 1977 represent the only laboratory-associated fatal illnesses in the history of this agency. Although neither employee worked directly with *R. rickettsii*, both worked in the building and had access to laboratories where infected embryonated chicken eggs were processed and where discard pans containing rickettsiae were autoclaved.[69] During 1955-1965, 5 cases of laboratory-acquired RMSF occurred at Ft. Detrick, Maryland via tick bite, needle inoculation and respiratory transmission,[70] and 9 laboratorians engaged in research into the pathogenesis and immunology of rickettsioses at the United States Army Medical Research Institute of Infectious Diseases developed RMSF during 1971-1976, following inhalation of infectious aerosols containing *R. rickettsii*.[71]

Nonetheless, it important to place these laboratory accidents in perspective: 20 (80%) of 25 fatal rickettsial infections occurred prior to 1945,[59] before the availability of effective antibiotic therapy for these infections, and typically in circumstances lacking biological safety cabinets. Many of these deaths resulted from failure to use simple barrier precautions, such as disposable latex gloves, eye protection, or filtering facepieces. Finally, almost all laboratory-acquired rickettsioses have occurred in research facilities where: (1) large numbers of infected arthropods were housed for study or vaccine development;[68] (2) massively infected cell cultures, embryonated chicken eggs, or animal tissues were manipulated by using unsafe techniques that generated infectious aerosols,[60-62,64,65,70,71] or; (3) routine safety precautions and reporting of laboratory accidents were not followed.[63,66,69] These facilities and circumstances are far different than those reasonably expected in a routine diagnostic laboratory. Finally, it is should also be noted that through 1974, there were 439 cases and 40 deaths attributable to laboratory-associated typhoid, leptospirosis, and psittacosis,[59] to underscore the fact that cultivation of any pathogenic agent posed far greater risks to microbiologists before the implementation of the safeguards and guidelines that are now used in diagnostic laboratories around the world.

Biosafety level-3 practices and facilities are currently recommended for diagnostic activities involving the manipulation of known or potentially infectious materials and for inoculation and incubation of cell cultures with any recognized pathogenic *Rickettsia*, *Orientia*, or *Coxiella* species.[72] For exquisitely infectious agents such as *C. burnetii,* and highly virulent rickettsiae such as *R. prowazekii* and *R. rickettsii*, this level of containment seems justified. However, different species of *Rickettsia* vary greatly in pathogenicity, and a rational application of species-specific rather than genus-wide recommendations for cultivating rickettsial agents deserves some consideration in the future, particularly if more clinical laboratories are ever expected to pursue culture isolation as a contemporary diagnostic technique. Microbiologists are confronted continuously with pathogens that pose some level of occupational risk. As an example, *Neisseria meningitidis* is classified as a biosafety level 2 pathogen;[72] nonetheless, 18 cases of laboratory-acquired meningococcal disease, including 8 deaths, were identified in laboratories around the world during 1985-2002, predominantly among laboratorians who worked with isolates on an open benchtop.[73] An argument might be made that the hazards associated with some rickettsial pathogens are no greater, and quite possibly less, than those associated with *N. meningitidis*, particularly if all work is performed using a biological safety cabinet. One additional level of complexity was alleviated in December 2012, when *R. rickettsii* was removed from the list of select agents, so that possession, storage, or transfer of cultures infected with this pathogen no longer require registration with and oversight by the Centers for Disease Control and Prevention.[74]

Molecular methods

> *"In the years ahead, as we compare the nucleotide sequences of various rickettsiae, it should be possible to develop group-specific, species-specific, and perhaps even strain-specific probes that can be used both for diagnosing diseases and for conducting precise epidemiologic investigations."*[75]

It is perhaps difficult to imagine a time when molecular tools were not routinely available to rickettsiologists; yet as recently as 1991, as suggested by Joseph McDade, these resources could only be imagined. In 2013, the genetic codes unique to each species, subspecies, and strain of rickettsiae provide the foundation for confirmatory diagnosis and molecular epidemiology of the rickettsioses, and represent the cornerstone of pathogen discovery in this discipline. Largely because of the revolution in molecular biology and its direct application to rickettsiology, the number of distinct *Rickettsia*, *Orientia*, *Ehrlichia*, and *Anaplasma* spp. recognized as pathogens of humans has more than doubled since 1985.

Molecular techniques have been instrumental in epidemiological assessments of the origins of outbreaks and distributions of subtypes or strains. A devastating outbreak of RMSF that occurred during 2008-2009 in Mexicali, Mexico,[76] shared several epidemiological and entomological features with a large cluster of cases that occurred several years earlier in a rural community in Navajo County in eastern Arizona.[77] Both events were triggered by an abundance of free-roaming

Table 2. Molecular typing of DNA of *Ehrlichia chaffeensis* from 2 episodes of ehrlichiosis in a liver transplant recipient from rural Missouri, 1997 and 1999.[81]

Gene, variable	Episode 1 (June 1997)	Episode 2 (May 1999)
Variable-length PCR target (VLPT)		
Number of repeat units	5	5
Repeat unit profile	1, 2, 3, 4, 5	1, 2, 3, 3, 4
Single nucleotide substitutions		
Position -69	G	A
Position 6	G	A
Position 27	A	G
Position 487	G	G
Aspartic acid codon deletion	Yes	No
Gap of 9 base pairs	No	No
120-kDa protein gene		
Number of repeat units	3	4

and stray dogs and massive populations of *Rhipicephalus sanguineus* ticks in the peridomestic environments. Because of the similarities between these outbreaks, recognized 5 years apart and separated by a distance of approximately 450 km, molecular typing assays were used to evaluate DNA samples of *R. rickettsii* obtained from ticks and ill patients at both locations. A detailed analysis of 3 variable intergenic regions, used previously to characterize all known isolates of *R. rickettsii,*[78] provided investigators the data needed to determine that the outbreaks of RMSF in Arizona and Mexicali were independent events involving genetically different strains of *R. rickettsii.*[79]

The granularity of molecular methods allows careful assessment of clinical features of specific rickettsioses that were previously obscured by less specific assays. In a recent study, investigators used molecular methods to accurately diagnose 140 consecutive Portuguese patients with Mediterranean spotted fever. The case-fatality rate in this series, determined by using specific diagnostic assays, was 21%, more than 8 times greater than the previously recognized lethality of this disease. In addition, they identified a particular strain of *R. conorii* that was more frequently associated with severe disease in this patient population.[80]Molecular methods can be used to examine the natural history and disease dynamics of rickettsioses. The first description of confirmed reinfection of a human host with *E. chaffeensis* was identified by using molecular techniques.[81] A 56-year-old liver transplant reciepient from a rural area of Missouri developed laboratory confirmed ehrlichiosis in 1997 and 1999. Whole blood specimens collected during each episode were evaluated separately by using PCR and sequence analysis to determine the nucleotide pattern of the variable-length PCR target (VLPT) and 120-kDa genes of *E. chaffeensis* present in each sample. Because the VLPT and 120-kDa antigen genes demonstrate a variety of strain-specific patterns, it could be determined conclusively that the 2 episodes resulted from infections with separate and distinct strains of *E. chaffeensis*, rather than recrudescence of a persistent infection (Table 2).[81]

The sensitivity and specificity of molecular methods have been enhanced considerably by continuously evolving technologies, including real-time and loop-mediated isothermal PCR techniques.[82-86] It has been proposed that these types of diagnostic tests can be implemented easily in laboratories with basic molecular capacity and developed as acute point-of-care assays.[84,85] Highly specific molecular assays have great utility in regions where particular rickettsial pathogens are known to be endemic; however, broad-range molecular methods can be extremely useful in some circumstances, particularly when multiple, genetically distinct agents occur sympatrically, or in regions where the existence of a novel rickettsiosis is unrecognized. In Missouri, investigators used molecular methods to discriminate infections caused by *Ehrlichia ewingii* from those caused by *E. chaffeensis*. Their discovery unveiled a second, clinically and ecologically similar illness, the identity of which was previously obscured because of sufficient overlap of disease manifestations and a shared tick vector.[87] The initial report of a scrub typhus-like illness in Chile in 2011, caused by a pathogen with highest molecular similarity to *O. tsutsugamushi,* represents the first identification of an *Orientia* species infection in the Western Hemisphere and resulted from the use of a broad-range PCR assay designed to amplify a large segment of the prokaryotic 16S rRNA gene.[88]

The acquisition and evaluation of clinical samples previously not considered, or believed suboptimal for a particular molecular method, are recognized increasingly as important sources of diagnostic information. Concentration of rickettsiae by centrifugation of serums of infected animals was first described by Ricketts in 1909;[9] today, cellular sediments obtained from serum represent just one of many non-conventional specimens used increasingly as successful sources of template for molecular assays. This technique has been used effectively to provide diagnostic material for PCR-confirmation of various rickettsioses, including RMSF,[36,77,79] Israeli spotted fever,[89] Thai tick typhus,[90,91] *Rickettsia felis* rickettsiosis,[92,93]

Table 3. Frequency of rickettsial diseases among patients with acute febrile illnesses in selected case series from tropical and developing countries, 2001-2011.					
Location	**Year(s)**	**No. of patients evaluated (% with rickettsiosis)**	**Pathogen(s) identified**	**Assay(s) used for diagnosis**	**Reference**
Vientiane and Xieng Khang Provinces, Laos	2001-2003	427 (26.9)	*Orientia tsutsugamushi* *Rickettsia typhi* *SFG Rickettsia spp.*	IFA WB	110
Uda Walawb State, Sri Lanka	2007	883 (17.7)	*SFG Rickettsia sp* *TG Rickettsia sp.* *O. tsutsugamushi*	IFA EIA	111
Sine-Saloum region, Senegal	2008-2009	134 (6.0)	*Rickettsia felis* *Rickettsia conorii*	PCR	92
Nyanza Province, Kenya	2008-2010	699 (7.2)	*R. felis*	PCR	112
Caldas Province, Colombia	2010-2011	26 (34.6)	*R. typhi* *R. felis* *SFG Rickettsia sp.*	IFA	49
Piura, Junin and Cusco Departments, Peru	NS	170 (17.6)	*SFG Rickettsia sp.*	IFA EIA	113

IFA = Indirect immunofluorescence antibody
WB = Western blot
EIA = Enzyme immunoassay
NS = Not specified

and scrub typhus.[83] Improvements in nucleic acid extraction technology permit better recovery of rickettsial DNA from formalin-fixed, paraffin-embedded skin biopsy or autopsy tissues to allow species-specific PCR assays. Included among infections confirmed recently by using this technique are those caused by *R. rickettsii*,[94,95] *R. parkeri*[96] and *Rickettsia* 364D.[97]

The use of swabs to collect rickettsial nucleic acids from eschars and vesicular rash lesions for PCR analysis was first described in 2009.[98] Subsequent reports described successful application of this simple and minimally invasive method to confirm cases of Queensland tick typhus,[67] African tick bite fever,[67,99] Mediterranean spotted fever,[100] *R. parkeri* rickettsiosis,[101] and *Rickettsia* 364D rickettsiosis.[102] Eschar swabs represent highly versatile specimens that can provide confirmatory results retrospectively from healing lesions, in some cases as long as 2 weeks after initiation of antibiotic therapy.[98,101] Because the technique is more rapid, easier to perform, and generally less painful than biopsy techniques, there is greater patient and physician acceptance, particularly in areas with limited medical resources;[67,100] Scab material from the eschar surface may also provide a source of rickettsial DNA suitable for molecular confirmation.[103] Despite these important advantages, eschar swabs should not be considered categorically as a replacement for cutaneous biopsy methods, for unlike swab material, tissue specimens provide histological context that may assist in an alternate diagnosis if the skin lesion is caused by something other than a *Rickettsia* species.[104] Skin biopsy techniques also allow for cultivation of rickettsiae from the clinical specimen and are more likely than swabs to provide confirmatory results by molecular evaluation.[22,67,104]

Prospectus

Improving access to and awareness of species-specific diagnostic methods remain important challenges for 21st century rickettsiologists. Broader use of these techniques around the world might change considerably existing notions about the ecology, epidemiology, and clinical presentations of many rickettsioses. During the last 30 years, adherence to this principle has leveraged the discovery of >20 newly recognized rickettsioses and has clarified several long-standing epidemiologic questions about the clinical heterogeneity and unusual geographical distributions of many historically recognized rickettsial diseases.

In regions of the world where malaria, typhoid, leptospirosis, or dengue are endemic, there is accumulating evidence that rickettsial diseases also circulate with considerable frequency, embedded as misdiagnosed cases among statistics for these other, more classically recognized tropical diseases. By example, 10% of Cameroonian patients with undifferentiated febrile illnesses for whom malaria and typhoid were excluded had molecular evidence of infection with *E. chaffeensis*.[105] During a serological study in the states of Jalisco and Yucatan in Mexico, an astounding 20 (40%) of 50 of patients with a clinical diagnosis of dengue had robust antibody titers reactive with SFG *Rickettsia* species, yet no demonstrable antibodies reactive with dengue virus.[106] In a similar manner, serological evidence of recent infections with SFG *Rickettsia* species was identified in 22% of 96 serum samples collected during 2000-2001 as part of regional surveillance for dengue in Cundinamarca, Colombia. [107] Two confirmed and 7 probable cases of *R. parkeri* rickettsiosis in Argentina were

identified by careful laboratory evaluation of patients presenting to one hospital with fever, myalgias and rash, several of whom were diagnosed initially with leptospirosis or dengue.[108]

The scope and magnitude of rickettsial infections in many densely populated and geographically diverse areas of the world including India, Africa, Southeast Asia, and Central and South America remain poorly understood, yet during the last decade, diagnostic tools have uncovered a plethora of agents and diseases that impact the health of populations in these regions.[109] The rigorous and routine use of rickettsial diagnostics in several prospective studies of febrile patients in these understudied regions has unveiled a surprisingly high prevalence of rickettsioses in these areas and signals an interesting and important period of discovery (Table 3). The frequency of undetected rickettsioses may be even greater when diseases associated with fever and cutaneous eruptions are investigated by these methods. During a prospective study at 2 hospitals in eastern Algeria, 108 adults who presented with fever and rash during 2000-2006 were evaluated for laboratory evidence of a rickettsiosis; acute infection with a SFG or TG *Rickettsia* species was confirmed in 14 (13%) of 108 enrolled patients during this interval.[114] Confirmatory evidence of SFG rickettsioses was obtained from PCR analysis of skin biopsy specimens obtained from 24 (58.6%) of 58 consecutive patients with fever and rash who presented for care at a hospital in Tamil Nadu State, India, during 2006-2008.[115]

Many rickettsial diseases have been historically understudied, often because the agents were considered difficult to grow, difficult to stain, and dangerous to propagate. As we progress into the 21st century, the diagnostic techniques outlined above will leverage the global recognition of rickettsial diseases and the impact that these have infections on enormous segments of the world population. Each method has unique strengths, and it is important for rickettsiologists to refrain from replacing entirely classical methods with rapidly evolving and increasingly sophisticated molecular techniques. The pitfall of routinely relying on a single diagnostic method was recognized by Pijper and Crockett in 1938,[116] when they stated,

> *"Different authors stress different methods of approach, and few use all of them…In Rickettsioses [sic] research, few methods are conclusive when used by themselves. There are so many approaches, and it is unfortunate that different workers value them differently…Identical methods are not always applicable to the various diseases. Only then can a Rickettsiosis [sic] be said to have been completely studied when all possible approaches have been explored."*

Pursuits in rickettsiology are most successful when traditional and contemporary methods are used in a complementary approach. Despite some specific and defined limitations inherent to each of the classes of rickettsial diagnostic methods, extraordinary results are achieved when multiple techniques are used in tandem. As an example, when high titers of antibodies reactive with *E. chaffeensis* were detected among a cohort of ill patients in Wisconsin and Minnesota, who presented with classical signs and symptoms of ehrlichiosis but who resided hundreds of miles beyond the established range of the recognized tick vector of *E. chaffeensis,* the application of cell culture and molecular techniques subsequently revealed a newly recognized ehrlichial pathogen in the United States.[117] Laboratories that develop expertise in and routinely apply multiple combinations of these methods provide the highest level of diagnostic accuracy and are positioned characteristically at the leading edge of discovery.

Disclaimer: The findings and conclusions are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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ISSN 0001-6012/2013/55/3/25-28
Acta Médica Costarricense, © 2013
Colegio de Médicos y Cirujanos
de Costa Rica

Conferencias Magistrales

Rickettsiosis en Costa Rica

(Rickettsiosis in Costa Rica)

Laya Hun

Resumen

La Fiebre de las Montañas Rocosas causada por *Rickettsia rickettsii* es una enfermedad reportada en Costa Rica desde 1977 con casos en humanos, originalmente provenientes de zonas endémicas del bosque tropical húmedo; sin embargo, recientemente se han confirmado casos en habitantes de la meseta central, de zonas urbanas en San José. Por otra parte, se han detectado anticuerpos contra *Rickettsia sp.* en animales silvestres y en perros domésticos, además se demostró presencia de la bacteria en sus ectoparásitos. Todos estos estudios confirman la importancia de esta zoonosis en el país y demuestran la necesidad de alertar a profesionales y autoridades nacionales en salud para que estén atentos a posibles casos de esta enfermedad que puede ser letal sino se trata a tiempo.

Descriptores: Rickettsiosis, Costa Rica, vectores, zoonosis

Abstract

Rocky Mountain spotted fever caused by *Rickettsia rickettsii* was first reported in Costa Rica in human cases since 1977, the first cases from endemic areas of humid tropical forests and recently reported human cases in the urban areas of San José. Also, positive serology as well as presence of the bacteria in ectoparasites of wild and domestic animals has been demonstrated. All these scientific results confirm the importance of this zoonosis in the country and demonstrate the urgency to alert public health professionals and political authorities to be aware of possible cases of this disease which can be lethal if not treated on time.

Keywords: Rickettsiosis, Costa Rica, vectors, zoonoses

Rickettsiosis en humanos

En 1952 se reporta por primera vez posibles casos de rickettsiosis a partir de unos sueros colectados en el país y procesados en los Estados Unidos.[1] En 1971 Peacock,M, *et al.*[2] reportan serología positiva en sueros procedentes de Costa Rica y en 1974 Campbell et al. [3] reportan un aparente brote de una enfermedad letal similar a rickettiosis en la zona este de Costa Rica.

Un año después (1975) se documentan los primeros casos en dos norteamericanos que habitaban en Cedral de San Carlos en una casa carro (camper) quienes se enferman y son trasladados en estado grave al Hospital México. Uno de ellos falleció y el otro recibió tratamiento adecuado, lo que permitió su recuperación parcial dado que quedó con secuelas importantes de parálisis flácida de la pierna izquierda y amnesia parcial.[4] El Dr. Luis Guillermo Fuentes, Jefe de la Sección de Virología de la Facultad de Microbiología de la Universidad de Costa Rica sospechó de posibles casos de rickettsiosis, por lo que se tomaron muestras de sangre de ambos pacientes y los coágulos se inocularon intraperitonialmente en cobayos machos, los cuales enfermaron y presentaron las reacciones características de la enfermedad, fiebre alta, baja de peso (Reacción de Neil Mooser) y posteriormente edema y necrosis testicular.

Una vez aislado el agente en cobayos, se realizaron varios pasajes inoculando saco vitelino de huevos embrionados y se detectó la presencia del agente por medio de la tinción de Giménez. Con el suero del paciente y utilizando la prueba de Fijación de Complemento se demostró por

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seroconversión que se trataba de una infección por una bacteria del grupo de las fiebres manchadas y se descartó que fuera causada por agentes del grupo tifo. En vista de no contar en aquel momento con los reactivos apropiados, las muestras se enviaron al Laboratorio de las Montañas Rocosas en Montana, Estados Unidos donde se confirma por medio de inmunofluorescencia la identidad del agente infecciosos como *Rickettsia rickettsii,* indistinguible de la cepa prototipo Sheila Smith.[4]

El siguiente caso fatal ocurre en junio de 1980, un niño de 14 años proveniente de Puerto Limón. El diagnóstico del agente causal, *Rickettsia rickettssii* se realizó mediante el aislamiento e identificación del agente, así como por inmunoflourescencia con sueros pareados en la Sección de Virología de la UCR. En 1985 se reportan los resultados de un estudio seroepidemiológico realizado con los sueros de los familiares del niño fallecido,[5] así como de muestras tomadas de residentes de localidades cercanas a Puerto Limón tales como Cedral y Jiménez. En tres de los familiares se detecta que tuvieron infección con la rickettsia, ya que presentaron títulos de anticuerpos muy altos (1/2048) sin haber presentado sintomatología alguna; dicho de otra manera, se demuestra que puede haber infección asintomática con esta peligrosa bacteria.[5] También es importante recalcar que en la localidad de Cedral 22 sueros de 31 (70 %) presentaron títulos positivos y en Jiménez 16 de 16 sueros (100 %) sin embargo, nunca se han reportado caso humanos en estas localidades.

En 1982 fallecieron dos personas en la localidad de Guápiles, en enero de 1987 dos casos letales provenientes de la urbanización El Bosque, Puerto Limón y en Marzo del mismo año un caso letal proveniente de La Virgen de Sarapiquí, Heredia. En todos se logró aislar el agente etiológico el cual se identifica como *R. rickettsii* indistinguible de la cepa prototipo Sheila Smith.[6]

Vale la pena mencionar que el supuesto brote reportado por Campell et al. en 1974,[3] ocurrió en la misma zona de El Bosque en los Altos de Herediana de la Provincia de Limón; lo que epidemiológicamente indica que la zona endémica en Costa Rica es el bosque tropical húmedo al Norte de la Cordillera Volcánica Central para terminar en Puerto Limón.

En 1994 la autoridades de salud notifican de un brote con cinco personas fallecidas en Turrialba, en la Provincia de Cartago; 108 Km al oeste de la Provincia de Limón. En un inicio se sospechó de otros agentes etiológicos, sin embargo en otros pacientes hospitalizados de la misma zona se demostró por serología que se trataba de un brote por *R. rickettsii,* lo cual fue posteriormente corroborado por el Centro de Control de Enfermedades (CDC) de Atlanta por medio de la técnica de PCR (Hun L. datos no publicados).

En el 2003, 5 estudiantes que estaban realizando una gira por San Ramón, Provincia de Alajuela fueron hospitalizados y gracias a la intervención inmediata del Jefe de Laboratorio del Hospital de San Ramón, las muestras fueron enviadas a nuestro laboratorio y se logró aislar e identificar una *R. rickettsii*, lo que además fue corroborado por la seroconversión en los 5 casos. Estas muestras también fueron enviadas y confirmadas por el CDC. Interesantemente, esta zona tampoco se consideraba endémica para esta zoonosis. (Hun L. datos no publicados)

Finalmente en el 2010 se presentó un caso fatal, una niña de 8 años residente de la Provincia de San José, es el único caso en el que se demuestra una escara en la parte abdominal; las muestras de la autopsia revelaron una infección por *R. rickettsii.*[7] En el 2011 se presentaron dos casos, uno en San José y otro en Cartago con sintomatología que sugería una posible rickettsiosis, lo cual fue confirmado por seroconversión. Es importante recalcar que estos tres pacientes son residentes de zonas urbanas del país.

Estudio en ectoparásitos

A raíz de los primeros casos humanos en 1975, se iniciaron los estudios epidemiológicos en la zona considerada endémica del país, la zona atlántica. El primer estudio se realizó entre 1979 y 1980 capturando 37 conejos silvestres (*Sylvilagus braziliensis)* de los cuales se recuperaron 127 garrapatas, todas de la especie *Haemaphysalis leporispalustris.*[5] En este estudio se infectaron cobayos con pooles de 10 garrapatas cada uno. Dos de estos pooles produjeron enfermedad severa en los cobayos con orquitis severa y necrosis testicular y se logró el aislamiento del agente el cual fue identificado como *R. rickettsii.* La importancia de este trabajo radica en que la *R. rickettsii* aislada de esta especie de garrapata en Estados Unidos no es patógena para cobayos, mientras que la costarricense demostró ser muy virulenta. Aunque se aísla por primera vez infección en una especie de garrapata en Costa Rica, esta no tiene avidez por los humanos, por lo que no podría considerarse un vector importante en la transmisión de esta enfermedad al ser humano.

Otras garrapatas identificadas en ese estudio fueron *Dermacentor nitens* y *Amblyomma cajennense* en caballos de las zonas de Jiménez, Cedral y Matina y otras *Amblyomma* sp. en perros y en la vegetación de las mismas zonas las cuales fueron procesadas e inoculadas en cobayos, sin embargo los resultados fueron negativos para *Rickettsia* sp.[8]

En el año 2008 se reporta un estudio molecular comparativo entre los genomas de las *R. rickettsii* aisladas de humanos de diferentes zonas del país y la de la garrapata *Haemaphysalis leporispalustris* y se demuestra que no hay diferencia entre estas rickettsias y la prototipo Sheila Smith, así como con la cepa Iowa, considerada avirulenta.[9]

En los últimos 5 años se han realizado varios estudios con el fin de caracterizar posibles vectores de las Fiebres Manchadas en Costa Rica; para lo cual se han efectuado múltiples giras a las zonas endémicas del país, cercanas a los sitios donde se originaron históricamente los casos humanos: Turrialba, la Virgen, Limón, Cahuita y Guápiles.

Se obtuvieron más de 3000 ectoparásitos de diferentes grupos de animales, bovinos, equinos, caninos, felinos, tortugas, didelfos, cabras y roedores. Se demostró que un porcentaje importante, aproximadamente el 60 %, de garrapatas del genero *Amblyomma* contienen bacterias del genero *Rickettsia; Amblyomma cajennense*, observada con frecuencia en caballos y *Amblyomma ovale* en perros domésticos. La garrapata *Rhipicephalus sanguineus* presentó muy baja positividad,

apenas un 2 % para *Rickettsia* sp. (Troyo A. y col. datos no publicados)

De las muestras positivas para el género *Rickettsia* sp. se logró identificar por PCR: *R felis* RF 2125 y *R. felis* URRWXcal2 de perros y gatos,[10] *R amblyommii,* de equinos, bovinos, perros y de la vegetación, *R. rickettsii* de hospedero desconocido*, R. belli* de tortugas (Troyo A. y col. datos no publicados).

Es importante mencionar que durante el año 2010, 15 garrapatas adultas identificadas como *Ammblyommma cajennense* obtenidas de caballos en Cahuita, Limón y 7 pulgas identificadas como *Ctenocephalis felis* obtenidas de un perro de San José fueron procesadas con el fin de aislar rickettsias en cultivos celulares.[11] Se aislaron por primera vez rickettsias de 3 de las garrapatas que fueron identificadas como *Rickettsia ammblyommii* y de las pulgas se logró aislar una rickettsia identificada como *Rickettsia felis.*[11]

Por otra parte, en un estudio que se está llevando a cabo actualmente en colaboración con la Universidad Nacional (UNA) se demostró la presencia de *Rickettsia sp.* en 30 % tanto en pulgas (principalmente *C. felis*) como en garrapatas (principalmente *R. sanguineus*) obtenidas de perros en parques recreativos de todas las provincias del país. (Pacheco y col. datos no publicados)

Estudio en animales

Del estudio realizado con el suero de los 37 conejos en 1979-1980, 5 de los conejos mostraron un título muy alto por inmunoflourescencia contra la cepa prototipo Sheila Smith. [5]

En otro estudio ecológico realizado alrededor de la casa de los primeros casos humanos reportados de Cedral de San Carlos y del primer caso de Limón, se capturaron roedores pequeños, conejos silvestres y perros de las zonas de Cedral, Jiménez, Matina, Limón y Herediana con el fin de tomarles una muestra de sangre para realizar inmunofluorescencia contra rickettsias. Un 55 % de los sueros de los conejos y un 62 % de los sueros de los perros fueron positivos contra la cepa Sheila Smith.[8]

A partir del 2010 se realizaron estudios con sangre de perros ubicados en sitios urbanos donde se reportaron los tres casos humanos confirmados en el área metropolitana de San José. Los sueros se estudiaron contra antígenos de *Rickettsia rickettsii, Rickettsia ammblyomii, Rickettsia felis, Rickettsia bellii, Rickettsia riphicephali y Rickettsia parkeri* por inmunofluorescencia. Un total de 36 perros de 168 (21.4 %) mostraron positividad más frecuentemente contra *R. rickettsii* y *R. amblyommii* (Moreira-Soto A y col. Datos no publicados). Se encontraron algunos sueros positivos para *R. felis* y para *R. parkeri* aunque con títulos más bajos, la *R. felis* fue hallada predominantemente en pulgas. (Moreira-Soto A y col. Datos no publicados).

Otro estudio realizado con sueros de perros obtenidos en refugios de la zona metropolitana del país, demostró 27.5 % de seropositividad para una o más de las mismas especies de rickettsias del grupo de las fiebres manchadas evaluadas en el estudio anterior. (Carranza y col. datos no publicados).

En el estudio con la UNA, se encontró de 0 a 26 % (promedio 14 %) de positividad contra *Rickettsia sp.* en los perros de los parques recreativos de Costa Rica. (Pacheco y col. datos no publicados)

En resumen podemos indicar que la presencia de *R. rickettsii* en Costa Rica está distribuida en relación con la presencia de su vector, los cuales infestan animales de diversa índole como perros domésticos, ganado vacuno, caballos y otros. Lastimosamente no se ha podido demostrar en ningún caso el vector responsable de la infección en humanos, a pesar de su detección en animales y garrapatas cercanas a la residencia de la persona; pero nunca asociadas a ellas.

Todos estos estudios demuestran que la rickettsiosis es una zoonosis presente de forma importante en el país y que debe alertarse y educarse a los profesionales en salud, autoridades y población en general sobre el potencial riesgo de contraer esta infección, que tal como se demuestra, si no es diagnosticada y tratada a tiempo puede ser fatal.

Agradecimientos y colaboradores: En la investigación de rickettsiosis en Costa Rica han colaborado de manera significativa un gran grupo de investigadores, asistentes y estudiantes. Destacan: Dr. Luis Guillermo Fuentes q.p.d, Dr. Mario Vargas, Dra. Libia Herrero, MSc. Laya Hun, Dra. Adriana Troyo, Dra. Lizeth Taylor, MSc. Olger Calderon, MSc Adrian Avendaño, Lic. Andrés Moreira, Dra. Gaby Dolz, MSc Gilberth Alvarado, Lic. Jussara Ortíz, Lic. Carlos Mata, Marco Carranza, Juan José Rivas, Katherine Pacheco, Lic. Alexander Barrantes, Bach Luis E. Vargas Castro, Edwin Carrera, Iván Coronado, Francisco Vega, Carlos Vargas.

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ISSN 0001-6012/2013/55/3/29-33
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Colegio de Médicos y Cirujanos
de Costa Rica

Conferencias Magistrales

Ehrlichiosis y anaplasmosis humanas en América

(Human Ehrlichiosis and Anaplasmosis in America)

J. Stephen Dumler

Resumen

Se realiza una descripción de los agentes de *Ehrlichia* y *Anaplasma* que han sido vinculados con la generación de enfermedad los seres humanos, dando especial énfasis *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum* y *Ehrlichia canis*. Se describe además, el cuadro clínico relacionado con cada agente, su correspondiente diagnóstico y tratamiento.

Descriptores: Ehrlichiosis, anaplasmosis, humanos, América

Abstract

A description of *Ehrlichia* and *Anaplasma* agents that have been linked to human disease is presented. A particular emphasis is given to *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, and *Ehrlichia canis*. The clinical features associated with each agent, as well as the corresponding diagnosis and treatment are also described.

Keywords: Ehrlichiosis, anaplasmosis, humans, America

Ehrlichia and *Anaplasma* are two major genera in the family Anaplasmataceae, order Rickettsiales. While infections by these genera have been well known in animals for many years, infections by Anaplasmataceae species have only been documented in humans since the mid-1980s. Thus, the focus of this mini-review is on ehrlichiosis and anaplasmosis in humans.

Since the first descriptions, human infections caused by new species, including *Ehrlichia chaffeensis* (cause of HME or human monocytic ehrlichiosis),[1] *Ehrlichia ewingii*,[2] an *Ehrlichia muris*-like agent (EMLA),[3] the Panola Mountain Ehrlichia (PME) which has similarities to *Ehrlichia ruminantium*,[4] and *Anaplasma phagocytophilum* (cause of human granulocytic anaplasmosis-HGA) have been identified in the Americas.[5,6] The only evidence of human infections by any of these species in Central or South America is limited to the cultivation of *E. canis* from an asymptomatic person in Venezuela,[7] several cases of *E. canis* infection in symptomatic patients in Venezuela,[8] a single case of *E. chaffeensis* infection in a Venezuelan child,[9] or to limited serologic suspicion based on high antibody titers in seroprevalence studies or seroconversions in individual patients. All species in these genera are transmitted to their vertebrate hosts by tick bites, including *Amblyomma americanum* in the US for *E. chaffeensis*, *E. ewingii* and the PME; EMLA and *A. phagocytophilum* are transmitted by *Ixodes scapularis* in the US. It is speculated that *E. canis* is transmitted to humans in Venezuela by *Rhipicephalus sanguineus* ticks,[10] the known vector for dogs.

Since first recognized and data was collected in the US, the CDC has recorded 8,404 cases of HME and 10,181 cases of HGA. In the US, the geographic location for HME and *E. ewingii* infection largely correspond to areas of *A. americanum* abundance and to areas of *I. scapularis* abundance for HGA and EMLA infection.[11] Whereas *E. canis* and *R. sanguineus* ticks are widely distributed throughout all of North, South and Central America, human infection has only been recognized among 7 individuals so far, and only in a single location in Lara State, Venezuela.[7,8] Although *A. phagocytophilum* has been identified

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Table. Serologic, culture, blood smear, and PCR evidence of ehrlichiosis or anaplasmosis in Latin America										
Country	**Year**	**N**	**Population or patient presentation**	***Serological tests***			**Final report**	**Dx based on**	**Notes**	**PMID**
				E. chaffeensis	***E. canis***	**VHE5**				
Argentina	1999	105	healthy subjects	15	nd6	nd	*E. chaffeensis*[1]	serology		10463693
Brazil	2004	?	suspected Brazilian spotted fever	2	nd	nd	*E. chaffeensis*[2]	serology		15476059
Brazil	2005	437	healthy subjects	46	nd	nd	*E. chaffeensis*[1]	serology		16444416
Brazil	2006	771	fever	9	nd	nd	*E. chaffeensis*[3]	serology		16767308
Chile	2003	19	healthy subjects exposure to dogs with ehrlichiosis	2	nd	nd	*E. chaffeensis*[4]	serology	2 samples 128 titer for *E. chaffeensis* and *A. phagocytophilum*	12643221
Mexico	1999	1	fever, rash	1	nd	nd	*E. chaffeensis*[1]	serology	*E. chaffeensis* titer 128; blood smear negative	10341193
Peru	2009	160	healthy subjects	21	nd	nd	*E. chaffeensis*[2]	serology		19190221
Venezuela	1996	49	43 healthy adults; 6 children with clinical signs	2	1	2	VHE/*E. canis*[1]	culture	isolate from asymptomatic person with 2,560 VHE titer and 320 *E. chaffeensis* titer; 2nd person with titers VHE 640, *E. chaffeensis* 2560 and *E. canis* 1280	8862572
Venezuela	1996	1	"viral" illness	1	nd	nd	*E. chaffeensis*[1]	serology	blood smear; platelet inclusions; 128 *E. chaffeensis* titer	8920030
Venezuela	2008	1	fever; ? Dengue	1	nd	nd	*E. chaffeensis*[1]	PCR	*E. chaffeensis* DNA in blood by nested PCR, blood smear +, 256 *E. chaffeensis* titer with seroconversion; dengue virus seroconversion	18325283
Venezuela	2010	6	fever	nd	1	nd	VHE/*E. canis*[1]	PCR	all VHE PCR+; 5/6 *E. canis* seronegative	17114689
TOTAL		**1550**		**100**	**2**	**2**				

[1,2,3,4] *A. phagocytophilum* serology was not tested ([1]), negative in all tested ([2]), negative in 5 tested ([3]), or positive at 128 titer in all ([4])
VHE = Venezuelan human *Ehrlichia*
Dx = diagnosis
PMID = PubMed identification number
nd = not done

as an infectious agent of dogs, horses, cattle, and wildlife in South America, human infection by this bacterium has not yet been reported either there or in Central America. In a search of PubMed using the phrases "ehrlichiosis", "anaplasmosis", "chaffeensis", "phagocytophilum", and country names in Central and South America, a total of 100 individuals had antibodies reactive with *E. chaffeensis, E. canis* and/or Venezuelan human *Ehrlichia* strain (VHE) of *E. canis* antigens in serologic tests

(Table 1). In addition, 3 symptomatic persons (2 from Venezuela and 1 from Mexico) had blood smear, seroconversion, or PCR evidence of infection by *E. chaffeensis*,[9,12,13] 4 Brazilian patients had clear *E. chaffeensis* seroconversions,[14] while 6 symptomatic patients had PCR evidence of *E. canis* infection (although 5 were seronegative),[8] and 1 asymptomatic seropositive individual was the source of a blood isolate similar to *E. canis*, the Venezuelan Human Ehrlichia (VHE) agent.[7] At least serologic evidence of infection has been detected in Argentina (1 case),[15] Brazil (57 cases),[14,16,17] Chile (2 cases),[18] Mexico (1),[13] Peru (21 cases),[19] and Venezuela (11 cases)[7-9,12] so far.

The median age of those diagnosed with HME and HGA is 47 to 52 years,[11] and for EMLA infection, 60 years,[3] yet all infection has been reported in all age groups.[11] Men are affected more often than women by a ratio of 1.4:1. Infection is often reported in those with HIV infection, where the course can be fulminant.[20] Other immune compromising conditions such as cancer, diabetes, arthritis, or organ transplantation are reported in up to 12% of HME patients.[11] For HGA, increased incidence or severity of infection with HIV infection has not been well documented, and fewer (6.5%) of patients reported pre-existing immune compromising conditions, including asplenia.[11]

The clinical features of infection have been best delineated in patients from the US. Both are generally characterized as undifferentiated fever, and many have a recent history of tick exposure or tick bite within 10 days.[21,22] Patients often present with sudden fever (92-100%), headache (62-93%), myalgia (63-90%), malaise (73-98%), and nausea or vomiting (35-59%). Rash is more frequent in HME (median 26%) than in HGA (median 6%) where coinfection with *Borrelia burgdorferi* and the occurrence of erythema migrans can confound the presentation. Confusion or changes in mental status are reported in 19-22% of HME patients and in 16-17% of HGA patients. The laboratory features especially include thrombocytopenia (61-91%) and leukopenia (44-73%). Increased serum activities of alanine and aspartate aminotransferases, reflective of mild to moderate hepatic lobular inflammation, are frequent in both HME and HGA (69-100%). Both *E. ewingii* and EMLA infection present similarly, but with less morbidity and no deaths have yet been reported.[2,3] The average age is lower and severity of infection worse for a small group of Brazilian patients with HME.[14]

Nearly 50% of HME and 36% of HGA patients require hospitalization.[11] Complications of infection can occur, including a septic- or toxic-shock syndrome, acute respiratory distress syndrome, acute abdominal syndromes, cardiac failure, renal failure, cranial nerve palsies, brachial plexopathy, demyelinating polyneuropathy, meningoencephalitis (for HME), and opportunistic infections.[23] There is very limited evidence that even with recovery from active infection, patients with HGA do not report feeling entirely well up to one year later.[24]

Diagnosis is suspected with undifferentiated fever or an influenza-like illness after exposures to ticks or reported tick-bites, especially given thrombocytopenia with leukopenia and mild to moderate increases in serum AST or ALT. The diagnosis can be confirmed rapidly by review of a peripheral blood or buffy coat smear stained by Giemsa, Wright or similar Romanowsky methods that demonstrate inclusions (morulae) in monocytes in up to 10% of HME patients, or in neutrophils in up to 75% of HGA patients.[25] A specific diagnosis can be made by identification of *Ehrlichia* spp. or *Anaplasma* DNA in blood, CSF or tissues using methods such as PCR. The most frequent method for diagnosis is the demonstration of a seroconversion or four-fold increase in specific antibody titer, which is highly sensitive when comparing acute and convalescent sera, but has not been rigorously tested for specificity. Diagnostic serological tests usually use indirect immunofluorescent methods, where the sensitivity and specificity are highest for IgG antibodies. A role for IgM testing has not been clearly established.

All forms of ehrlichiosis and anaplasmosis appear to respond to tetracycline antibiotics, especially doxycycline, although no randomized clinical trials have been conducted. All isolates so far tested are susceptible to these drugs in vitro at easily achieved MICs.[26-29] Chloramphenicol should not be used owing to lack of in vitro susceptibility and frequent empirical clinical failures. Although *A. phagocytophilum* is sensitive to fluoroquinolones *in vitro*, treatment failures with levofloxacin that required subsequent retreatment with doxycycline are reported.[30] Rifampin has low MICs in vitro and has been successfully used in children in empiric studies.[31,32]

Other forms of human infections by Anaplasmataceae species are reported outside of the Americas, but present potential risks to people and animals residing in the western hemisphere. This includes *Neoehrlichia mikurensis* infection of humans that is reported as ranging from mild febrile illness to a sepsis-like severe infection.[33-36] It is transmitted by *Ixodes ricinus* ticks in Europe and perhaps by *Haemaphysalis ticks* in China, and a related species, *Neoehrlichia lotoris* has been readily found in wild animals in North America.[37] Likewise, *Neorickettsia* spp. related to the human pathogen, *Neorickettsia sennetsu*,[38] are abundantly present in aquatic environments throughout North and South America.[39] Unlike other Anaplasmataceae prokaryotes, *Neorickettsia* are largely vectored by trematodes that require part of their life cycle to pass through fresh water snail species. The greatest risk to humans so far seems to relate to the consumption of uncooked or raw fish products, although no sushi or sashimi-related outbreaks have been reported.

Acknowledgements: This work was supported in part by funding from the US National Institutes of Allergy and Infectious Diseases including grants R01 AI044102, R21 AI096062, and grant 011DUM2013 from the Fisher Center Discovery Program at The Johns Hopkins University.

Conflict of interest: JSD receives royalty payments for patent on the method to grow *Anaplasma phagocytophilum* to prepare serological reagents.

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ISSN 0001-6012/2013/55/3/34-40
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Colegio de Médicos y Cirujanos
de Costa Rica

Conferencias Magistrales

Ehrlichiosis y anaplasmosis en Costa Rica

(Ehrlichiosis and anaplasmosis in Costa Rica)

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Resumen

La ehrlichiosis y la anaplasmosis son enfermedades infecciosas producidas por bacterias de la familia *Anaplasmataceae* y transmitidas por garrapatas. Ambas afectan, entre otras especies, al hombre, ocasionando sintomatología que puede ser asociada a un resfriado común o con signos clínicos compatibles con el dengue hemorrágico, patología que se presenta frecuentemente en Costa Rica. Tanto la ehrlichiosis como la anaplasmosis son consideradas también enfermedades de importancia en Medicina Veterinaria. A continuación se brinda una revisión sobre los hallazgos obtenidos en investigaciones realizadas en el país para determinar la presencia y distribución de *Ehrlichia* y *Anaplasma* en Costa Rica. *Ehrlichia canis* se encuentra ampliamente distribuida en el país y es la especie predominante en perros y garrapatas (*Rhipicephalus sanguineus*). Adicionalmente, se ha detectado, aunque en menor porcentaje, la presencia de *Anaplasma platys* y *Anaplsma phagocytophilum* en perros y sus garrapatas. También se ha determinado la presencia de *A. phagocytophilum* en un venado cola blanca, y de *E. canis* en humanos donadores de bancos de sangre mediante técnica serológica y molecular.

Descriptores: *Ehrlichia*, *Anaplasma*, humanos, perros, venados cola blanca, garrapatas

Abstract

Ehrlichiosis and anaplasmosis are tick borne infectious diseases caused by bacteria from the family Anaplasmataceae. They both can infect humans, amongst other species, and their clinical presentation can be associated to a common cold or compatible to dengue hemorrhagic fever, a common disease in Costa Rica. Ehrlichiosis and anaplasmosis are also considered diseases with veterinary importance. Following is a review of the findings from research conducted in the country to determine the presence and distribution of *Ehrlichia* and *Anaplasma* in Costa Rica. *Ehrlichia canis* is widely distributed in the country, prevailing in dogs and their ticks (*Rhipicephalus sanguineus*). Also, the presence of *Anaplasma platys* and *Anaplasma phagocytophilum* has been detected in dogs and their ticks, although less frequently. We also detected the presence of *A. phagocytophilum* in white-tailed deer, and *E. canis* in humans blood bank donors by serological and molecular techniques.

Keywords: *Ehrlichia*, *Anaplasma*, humans, dogs, white tail deer, ticks

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La ehrlichiosis y la anaplasmosis son producidas por organismos pertenecientes al subgrupo α-*Proteobacteria,* orden *Rickettsiales*, familia *Anaplasmataceae*, géneros *Ehrlichia* y *Anaplasma*, respectivamente. Se caracterizan por ser bacterias intracelulares obligadas pequeñas (0.4 a 1.5μm), Gram-negativas, generalmente redondas, pero algunas veces altamente pleomórficas, que se replican dentro de una vacuola derivada de la membrana de la célula eucariota del hospedero, vertebrado o invertebrado.[1,2]

Los géneros *Ehrlichia* y *Anaplasma*, infectan diferentes células hematopoyéticas, nombrándose a las enfermedades, según las células sanguíneas infectadas. *Ehrlichia canis* y *Ehrlichia chaffeensis* afectan monocitos y linfocitos, por el contrario *Ehrlichia ewingii* y *Anaplasma phagocytophilum* tienen especial

tropismo por granulocitos, y *Anaplasma platys* predilección por las plaquetas. Las bacterias ingresan a las células sanguíneas por fagocitosis, y se alojan en vacuolas citoplasmáticas, en donde se dividen hasta formar colonias de bacterias conocidas como mórulas, característica distintiva de este grupo de patógenos.[1, 3] Típicamente aparecen en las extensiones sanguíneas, como inclusiones de 2 a 7 mm de diámetro, redondeadas o elongadas, detectadas mediante diversas tinciones como Romanowsky, Wright y Giemsa.[4, 5]

El tamaño del genoma de las especies de *Ehrlichia* y *Anaplasma* es relativamente pequeño (0.8 a 2.5Mb).[2] Los progresos en tecnología molecular han permitido avances en el desarrollo de análisis genéticos, indicando la verdadera posición filogenética de la mayoría de organismos del orden *Rickettsiales,* de tal manera que *E. canis*, *E. chaffeensis*, *E. ewingii*, *Ehrlichia ruminantium* y *Ehrlichia muris* han sido ubicadas dentro de un mismo género, por presentar una similitud de al menos 97.7% en su secuencia genética del segmento 16S de ARNr, mientras que *A. platys*, *A. phagocytophilum* y *Anaplasma bovis,* anteriormente reconocidos como miembros del género *Ehrlichia* (*E. platys, E. phagocytophila y E. bovis*), pasaron a formar parte del género *Anaplasma* por presentar similitudes de al menos 96.1% en sus secuencias genéticas del gen 16S de ARNr.[1]

En humanos se han reportado infecciones emergentes con *E. chaffeensis*, *A. phagocytophilum, E. ewingii*, y *E. canis.*[2] En perros han sido reportados *E. canis*, *E. ewingii*, *A. platys*, *E. chaffeensis*, y *A. phagocytophilum.*[6, 7,8]

E. canis fue la primera especie del género *Ehrlichia* reconocida y desde su descubrimiento en Argelia en 1935 por Donatien y Lestoquard ha sido identificada en regiones tropicales y subtropicales de todo el mundo ocasionando la ehrlichiosis monocítica canina (CME, por sus siglas en inglés). Inicialmente fue denominada como *Rickettsia canis*, y cobró mucha importancia durante la guerra de Vietnam por causar la muerte de cientos de perros militares.[9] Aunque originalmente se consideró como agente exclusivo de caninos, recientemente se discute su importancia como agente con potencial zoonótico, debido a la detección de infección en humanos de Venezuela con y sin asociación de signos clínicos, [10,11] además de evidencia serológica en humanos de Argentina[12] y de Brasil.[13, 14]

E. chaffeensis fue descrita por primera vez en humanos de Estados Unidos por Maeda y colaboradores en 1987 quienes la caracterizaron equivocadamente como una nueva cepa de *E. canis.* Estudios posteriores revelaron su verdadera etiología, al ser aislada de un recluta de la Armada de Estados Unidos en Fort Chaffee, Arkansas, de donde adquirió su nombre.[15] Desde entonces, ha sido reconocida por causar la ehrlichiosis monocítica humana o HME (por sus siglas en inglés) y años más tarde se descubrió que también era capaz de infectar naturalmente a perros[16] y coyotes en Estados Unidos;[17] además, se reporta su presencia en cabras[18] y venados de cola blanca, confirmando a éstos últimos como reservorios naturales del agente, aunque muchas especies de vertebrados pueden servir como reservorios.[19] La mayoría de los casos de HME se han reportado en Estados Unidos.[20] Hallazgos serológicos de *E. chaffeensis* en seres humanos en diferentes partes del mundo[21,22] deben de interpretarse con cuidado, ya que se reportan reacciones cruzadas entre *E. canis* y *E. chaffeensis*, y por otro lado, la garrapata vector está restringida a Norte América.[23]

E. ewingii es el agente etiológico de la ehrlichiosis granulocítica canina (EGC) o ehrlichiosis ewingii humana. Fue descubierta como causante de enfermedad en perros de Estados Unidos en 1971, pero no fue hasta 1985 que se le consideró como una especie distinta, ya que inicialmente se pensó que era una cepa de *E. canis* menos virulenta.[24, 25] En 1999 se encontró de forma accidental en humanos de Estados Unidos, que presentaban síntomas de ehrlichiosis. Desde entonces se reportan casos en perros y humanos de Estados Unidos, en donde incluso estudios epidemiológicos la han descrito como la especie más prevalente en perros del estado de Missouri.[26, 27] Fuera de América, existe un sólo reporte del agente en Camerún, donde se encontró en 2 perros naturalmente infectados.[28] Infecciones naturales han sido confirmadas en ciervos (*Odocoileus virginianus*), los cuales pueden ser importantes reservorios de este agente.[29]

A. *platys*, causante de la trombocitopenia cíclica infecciosa canina (ICCT, por sus siglas en inglés) fue reportado en perros por primera vez en Estados Unidos.[30] La enfermedad afecta solamente a caninos. A la fecha, no se ha encontrado en otras especies, incluyendo a los humanos.[8]

A. phagocytophilum es el agente causante de la anaplasmosis granulocítica humana (HGA, por sus siglas en inglés), y comprende los anteriores géneros de *Ehrlichia equi* y *Ehrlichia phagocytophila.*[1] Fue reportada por primera vez como patógeno en ovejas de Escocia en 1932, luego en caballos de Estados Unidos en 1969, y finalmente en perros de ese país en 1971 y 1982.[7] No fue hasta en 1990 que se describió por primera vez la enfermedad en seres humanos.[4] Afecta tanto a humanos, como a numerosas especies de animales domésticos y silvestres, entre las que se reportan perros, caballos, cabras, ovejas, gatos, rumiantes y animales silvestres, incluyendo aves, que podrían desempeñar un papel en la diseminación de la enfermedad.[31,32] Los reservorios de *A. phagocytophilum* se consideran sobre todo los roedores y los cérvidos. El agente se mantiene en la naturaleza en un ciclo biológico garrapata-mamífero-garrapata y los humanos son hospederos accidentales al ser infectados ocasionalmente por garrapatas portadoras de la bacteria.[33] Las diferencias en la preferencia de diversos hospedadores, en la variedad de manifestaciones clínicas y en la distribución geográfica del agente se atribuyen a la existencia de múltiples cepas de *A. phagocytophilum.*[1] En ciertos animales reservorios han sido evidenciadas recientemente cepas apatógenas para humanos, lo que hace reconsiderar el rol definitivo del venado cola blanca en el mantenimiento de cepas infectivas de *A. phagocytophilum.*[34]

Transmisión y manifestaciones clínicas

Las garrapatas actúan como vectores biológicos de *la ehrlichiosis y anaplasmosis,* siendo *Rhipicephalus sanguineus*

el vector biológico *de E. canis.*[35] Experimentalmente, también se ha logrado la transmisión por *Dermacentor variabilis.*[36] La transmisión de este agente en la garrapata *R. sanguineus* ocurre transestadialmente.[37] La garrapata *Amblyomma americanum* sirve como vector biológico de *E. chaffensis* y *E. ewingii*[38,39] y se sospecha de *D. variabilis*, debido a que el ADN de los dos agentes han sido detectados en dicha garrapata,[40] *E. ewingii* además ha sido encontrado *en R. sanguineus*; sin embargo, son necesarios más estudios para determinar el posible rol de estas garrapatas como transmisores. Se sospecha que *A. platys* es transmitida por la garrapata *R. sanguineus*; sin embargo, el papel de esta garrapata, como vector biológico, no ha sido confirmado.[30] Como vectores de *A. phagocytophilum* se consideran sobre todo las garrapatas de los géneros *Ixodes* y *Amblyomma* .[33]

La garrapata adquiere el agente al alimentarse de animales que se encuentran en la fase aguda de la enfermedad y aún quizá en la fase subclínica.[41,42] Dentro del vector, la bacteria pasa del intestino a las glándulas salivales transmitiéndose a un nuevo hospedador cuando la garrapata se alimenta nuevamente. Estudios han comprobado que una garrapata adulta puede sobrevivir desde 155 hasta 568 días sin alimentarse y que posee capacidad de transmitir el agente hasta 155 días post infección.[42]

La CME se manifiesta en perros con síntomas como fiebre, depresión, anorexia y pérdida de peso en la fase aguda, encontrándose en el laboratorio trombocitopenia, leucopenia, anemia ligera e hipergamaglobulinemia. La fase crónica se caracteriza por hemorragia, epistaxis y edema.[43] La CME cursa en humanos con síntomas similares a los reportados para la HME.[2, 11]

E. chaffeensis produce en humanos una enfermedad aguda caracterizada por manifestaciones clínicas no específicas, principalmente presentando fiebre, dolor de cabeza, escalofrío, anorexia, vómitos y en algunos casos leucopenia, trombocitopenia y un elevado nivel de transaminasas hepáticas.[15,23] En perros naturalmente infectados no se han podido determinar signos clínicos significantes; sin embargo, un reporte de tres perros naturalmente infectados con *E. chaffeensis* documentó serios signos incluyendo vómitos, epistaxis, linfadenomegalia y uveítis.[44]

En el primer reporte de *E. ewingii* en humanos se reportó fiebre, dolor de cabeza, trombocitopenia y leucopenia, parece afectar sobre todo a personas inmunosuprimidas.[26, 33] Los perros infectados con este agente presentan principalmente fiebre y trombocitopenia, pero también pueden mostrar ataxia y paresis, además la fase crónica ha sido asociada con poliartritis.[27]

La infección aguda con A. platys se caracteriza por una parasitemia en las plaquetas, seguida por episodios de trombocitopenia que ocurren en intervalos de 7 a 14 días, [30] rara vez ocurre hemorragia; sin embargo, trabajos realizados en Estados Unidos[45] y en Venezuela, [46] han reportado signos clínicos de mayor severidad, similares a aquellos asociados a infecciones con E. canis: disfunción de plaquetas, anemia, leucopenia, hipoalbuminemia e hipergamaglobulinemia.

El cuadro clínico de la angranulocítica humana es semejante al ocasionado por *E. chaffeensis* y *E. ewingii.* Usualmente, la enfermedad cursa con sintomatología febril, frecuentemente acompañada de anormalidades hematológicas (leucopenia y trombocitopenia), y elevación de las transaminas hepáticas.[34] En todos los casos, la severidad de la infección dependerá de varios factores, entre los que se incluyen edad, estado del sistema inmune y variante de *A. phagocytophilum* involucrada.[47,48] En caninos y equinos los principales síntomas de la anaplasmosis granulocítica son fiebre, letargia, inapetencia, vómitos y diarrea. Menos frecuente se presenta cojera, polidipsia y hemorragias, edema de los miembros, ataxia, ictericia y los mismos hallazgos de laboratorio arriba mencionados.[49, 50] La alteración en la función neutrofílica y la leucopenia producida por la infección por *A. phagocytophilum* puede predisponer al desarrollo de infecciones oportunistas secundarias, que potencialmente pueden ser causa de mortalidad.[50]

Diagnóstico

Para el diagnóstico de la ehrlichiosis y anaplasmosis se emplean diversas técnicas laboratoriales que incluyen: la identificación de mórulas o cuerpos de inclusión en frotis sanguíneos[51] y la detección de anticuerpos mediante inmunofluorescencia indirecta (IFI).[22] Recientemente se ha incrementado el uso de técnicas moleculares tales como la técnica de reacción en cadena de la polimerasa (PCR, por sus siglas en inglés). La secuenciación y el aislamiento primario en cultivo celular son otras técnicas empleadas en casos importantes y generalmente para fines de investigación.[11]

El método más simple, rápido y económico para detectar la bacteria, es la visualización de la mórula en células de sangre periférica; sin embargo, es también la técnica menos sensible e inespecífica, debido a que no se detectarán bajas cantidades circulantes de bacterias en sangre, y en ocasiones, es posible encontrar inclusiones no relacionadas a *Ehrlichia y Anaplasma* que pueden causar confusión en el diagnóstico.[52] Finalmente, esta técnica no permite la diferenciación entre las diferentes especies de la familia *Anaplasmataceae*, por presentar una morfología idéntica y poseer tropismo por las mismas células.[1]

Actualmente hay disponibles pruebas serológicas inmunoenzimáticas e inmunofluorescentes de excelente sensibilidad, especificidad y confiabilidad, pero los anticuerpos generalmente están ausentes durante las dos primeras semanas de aparición de los síntomas de la enfermedad, y pueden persistir hasta ocho meses después de la eliminación del agente del organismo. También es posible que se presenten reacciones cruzadas entre los miembros de la familia *Anaplasmataceae,* y con otros agentes rickettsiales, ocasionando asi diagnósticos poco precisos. Solamente un incremento o una disminución de 4 veces en el título de anticuerpos en sueros pareados tomados con 4 semanas de diferencia se considera como diagnóstico confirmatorio para casos clínicos de ehrlichiosis y anaplasmosis.[33]

En cuanto el aislamiento de estas bacterias mediante cultivo celular, ésta sigue siendo la prueba de oro para confirmar el diagnóstico; sin embargo, implica un proceso de incubación de

varias semanas, requiere de laboratorios de seguridad grado 3 y personal especializado, resultando además ser una técnica muy laboriosa y poca sensible.[33, 34] Además, en el caso de E. ewingii no se ha logrado su cultivo celular.[16]

En los últimos años, las técnicas moleculares han ganado aceptación como un método importante para la detección de agentes rickettsiales, implementándose muchos protocolos de investigación, esto debido a que son métodos altamente sensibles, específicos, rápidos y confiables. El PCR permite detectar el ADN de la bacteria en la muestra de sangre del paciente, en animales reservorios y en garrapatas, mientras que la secuenciación confirma o determina la especie infectante. Debido a su alta sensibilidad, permite el diagnóstico temprano del agente, antes de que se desarrollen los anticuerpos, permite determinar el estado de portador y diferenciar entre las especies de *Ehrlichia* y *Anaplasma*.[6,40,41,44] Además, debido a la imposibilidad de aislar *E. ewingii* en cultivo celular, el diagnóstico de la infección con este agente puede ser determinado solamente con el uso de técnicas moleculares.[26]

La técnica de PCR convencional basada en la amplificación de regiones del gen *16S ARNr* de *Ehrlichia* y *Anaplasma* ha demostrado efectividad en muestras de sangre de humanos y animales, reportándose un límite de detección de 20pg para *Ehrlichia,*[16,53,33] y se han obtenido aún mejores resultados utilizando técnicas de PCR de tipo anidado, que han incrementado la sensibilidad de la prueba hasta un límite de detección de 0.2 pg de ADN.[54,55,56] Para *Ehrlichia* spp. también se emplea una técnica de PCR basada en la amplificación del gen *dsb* (gen Thio-Disulfido Oxireductasa). El producto de las reacciones puede utilizarse para fines de secuenciación.[28,55]

Tratamiento y profilaxis

Se cree que un posible mecanismo mediante el cual *Ehrlichia* sobrevive dentro de la célula, es inhibiendo la fusión fagosoma-lisosoma, efecto que es restaurado tras la administración de doxiciclina. También existen otras drogas con comprobada eficacia como tetraciclina, oxitetraciclina, y cloranfenicol.[42] Se recomienda administrar doxiciclina como tratamiento de elección en seres humanos cada 12 horas durante 14 días.[33] Para perros el protocolo recomendado es de 10mg/Kg una vez al día por 28 días;[57] sin embargo, Harrus *et al.*[44] encontraron que este tratamiento elimina el agente en 16 días. En la mayoría de los casos, los perros en fase aguda de la enfermedad responden al tratamiento con doxiciclina dentro de 24 a 72 horas posteriores a la primera administración. Por otro lado, el tratamiento para animales que se encuentran en la fase subclínica y crónica de la enfermedad debe ser aún evaluado, ya que perros subclínicamente infectados pueden permanecer portadores aún después de 6 semanas de tratamiento con doxiciclina[41] y solamente existe un reporte de tratamiento exitoso con recuperación de la fase crónica de la enfermedad.[42] El único método profiláctico existente es el control del vector.

Ehrlichiosis en Costa Rica

El agente causal de la CME se reportó por primera vez en Costa Rica en 1995, por medio del hallazgo de cuerpos de inclusión y mórulas en leucocitos de caninos infectados, confirmándose estos hallazgos por medio de IFI en muestras de suero de los caninos previamente diagnosticados.[58] Durante muchos años la CME fue detectada rutinariamente en caninos mediante frotis sanguíneos y serología, considerándose una enfermedad endémica en el país, con casos constantes y aumentos durante ciertos periodos del año (Marzo – Abril, Agosto – Setiembre), cuando las condiciones climáticas, generaban un ambiente propicio para el desarrollo y supervivencia del vector (Dra. Ana Meneses Guevara, comunicación personal).

Fue hasta en el año 2005 que se diagnosticó en el Sistema de Salud de Costa Rica el primer caso de ehrlichiosis en una niña de Santa Ana; San José, que presentaba fiebre, manifestaciones cutáneas y neurológicas, y cuya muestra, así como la de los perros que convivían con ella, se enviaron al Centro de Control de Enfermedades (CDC, por sus siglas en inglés) en Atlanta, determinándose la presencia de *Ehrlichia* spp. en la sangre de los caninos. A la niña se le trató exitosamente con antibióticos (Dra. Gaby Dolz, comunicación personal).

Un estudio realizado en 2006, encontró 70% de casos seropositivos en 30 perros sospechosos de sufrir ehrlichiosis utilizando un inmunoensayo enzimático comercial (Tesis de Grado, UNA. Prevalencia serológica y citológica de la ehrlichiosis canina en Costa Rica. Mónica Rímolo, 2006).

En el 2007 se reportaron en Costa Rica dos casos clínicos de ehrlichiosis granulocitotrópica humana en dos hospitales diferentes del Valle Central de Costa Rica, basados en el cuadro clínico y la presencia de mórulas granulocíticas en sangre periférica.[59,60] Estos hechos ocasionaron que la Caja Costarricense de Seguro Social (CCSS) incorporara esta patología en el diagnóstico diferencial de las enfermedades febriles.[60]

El diagnóstico molecular, la caracterización y el aislamiento celular de *E. canis* en perros de Costa Rica se logró por primera vez en el 2010[61]. Romero y colaboradores lograron amplificar mediante PCR *16S ARNr* anidado, ocho muestras sanguíneas de caninos con sintomatología compatible a ehrlichiosis, resultando positivas para *E. canis*. La secuenciación de parte del gen *dsb* de esas ocho muestras y su análisis a través del BLAST mostró 100% de identidad con todos los aislamientos de *E. canis* depositados en el GenBank, además se logró aislar cuatro de estas muestras mediante cultivo celular.[61] Se logró detectar además ADN de *E. canis* en un 47.7% (148/310) de muestras sanguíneas provenientes de perros con sintomatología sugestiva de CME de diferentes provincias de Costa Rica (San José 17%, Alajuela 38%, Heredia 15%, sin datos geográficos 30%) utilizando el PCR *16S ARNr* anidado. En ninguna de las muestras procesadas se detectó ADN de *E. chaffeensis* o *E. ewingii.*[61]

Al comparar los resultados del diagnóstico de *E. canis* obtenidos mediante frotis sanguíneo y PCR en sangre de perros del Valle Central de Costa Rica con sintomatología sospechosa de CME, solamente en 103 (57,9%) de los casos positivos en frotis sanguíneo se logró el diagnóstico molecular de *E. canis,* mientras que de las 122 muestras reportadas negativas (sin inclusiones) un 36,0% (44) de los casos resultaron positivas

en la técnica de PCR. Para la prueba de frotis sanguíneo se determinó una sensibilidad del 70,1%, especificidad del 51,0%, valor predictivo positivo de 57,9% y valor predictivo negativo de 63,9%, comparado con la técnica de PCR. El análisis de regresión logística con la información recolectada de los animales reveló asociación positiva (p<0,05) con la presencia de inclusiones, pero no con la edad o el sexo del animal; mientras que la aplicación de la prueba t-student detectó diferencias altamente significativas en los promedios de las variables de hematocrito y hemoglobina entre perros PCR positivos y perros PCR negativos a *E. canis*.[62] De 30 muestras evaluadas previamente mediante serología, solamente un 25,0% de los casos seropositivos y un 36,4% de los casos seronegativos resultaron positivos en el diagnóstico molecular de *E. canis*. El trabajo concluye, que el hallazgo de valores bajos de hematocrito y hemoglobina en combinación con signos clínicos y la detección de inclusiones en muestras sanguíneas representan un fuerte indicio de infección con ehrlichiosis; sin embargo, es necesaria la aplicación de una técnica molecular como método diagnóstico confirmativo.[62]

En el trabajo realizado por Ábrego *et al.*[63] se analizó mediante PCR la presencia de *A. platys* en 300 muestras sanguíneas provenientes de perros atendidos en clínicas veterinarias del Valle Central de Costa Rica. Un total de 19 (6.33%) muestras de diferentes áreas geográficas (San José, Heredia, Alajuela, Cartago y Guanacaste) resultaron positivas a *A. platys*. En 7 muestras caninas se encontró además infección mixta (*A. platys* y *E. canis*). Una muestra sanguínea positiva a *A. platys* fue secuenciada mostrando una similitud del 100%.[63] En tres de estas muestras se detectó además ADN de *A. phagocytophilum*, una muestra provenía de un perro de Jacó, Puntarenas, mientras que de las otras dos muestras no se pudo obtener su procedencia.[64] *A. phagocytophilum* fue sin embargo, reportado por primera vez en Costa Rica por Soto (Tesis de Posgrado UNA. Detección molecular de *Ehrlichia canis, Ehrlichia chaffensis, Ehrlichia ewingii*, y *Anaplasma phagocytophilum* en garrapatas y venados de Costa Rica. José Luis Soto Rivas. 2010). En este estudio se analizaron 34 muestras de sangre de venados cola blanca, por medio de PCR, encontrándose el agente en un venado de San Carlos. La secuenciación de la muestra confirmó los hallazgos.

Por otro lado, estudios realizados con 160 garrapatas recolectadas de perros, detectaron por PCR anidado *E. canis*, *A. platys* y *A. phagocytophilum* en 43 (26.87%), 5 (3.12%) y 2 (1.25%) garrapatas *R. sanguineus*, respectivamente, siendo éste el primer reporte de la presencia de estos agentes en garrapatas de Costa Rica. Dos garrapatas presentaron además infecciones mixtas con *E. canis* y *A. platys*. Las garrapatas positivas a *E. canis* provenían de San José, Heredia, Alajuela y Cartago, las positivas a *A. platys* de Heredia y Alajuela, y las positivas a *A. phagocytophilum* de San José y Heredia.[64,65]

En el trabajo realizado por Bouza-Mora (Tesis de Posgrado UCR. Uso de reacción en cadena de la polimerasa (PCR) e inmunofluorescencia indirecta (IFA) como técnicas de diagnóstico confirmatorio para *Ehrlichia* spp en donadores de Bancos de Sangre. Laura Bouza-Mora. En preparación) en donadores de Bancos de Sangre de Costa Rica, se determinó anticuerpos contra *E. canis* en 35 de 100 sueros analizados utilizando IFI; de ellos, 30 (85,7%) evidenciaron títulos bajos (1:64 a 1:256), mientras que en 5 (14,3%) se determinaron títulos altos (1:1024 a 1:8192). El 68.6% (24/35) de las muestras positivas correspondió al sexo femenino. En ambos sexos las edades coincidieron entre los 18 y los 35 años. Mediante PCR se detectaron 15 (5.3%) muestras de un total de 280 analizadas mediante *dsb* PCR como positivas. La secuenciación determinó en 10 muestras la presencia de *E. canis*.

En el 2011 se vuelven a reportar dos casos clínicos de ehrlichiosis humana en el Sistema de Salud de Costa Rica, basados en el cuadro clínico y la presencia de mórulas monocíticas en sangre periférica, los pacientes responden adecuadamente al tratamiento con doxiciclina.[66] Hasta la fecha, el diagnóstico de anaplasmosis y erhlichiosis en el Sistema de Salud incluye solamente la observación de inclusiones en las células sanguíneas utilizando las tinciones de Giemsa y Wright, ya que se carece de técnicas diagnósticas sensibles y específicas como IFI, cultivo celular o técnicas moleculares.[59,60] Los estudios realizados hasta la fecha demuestran la presencia de *E. canis, A. platys* y *A. phagocytophilum* en hospedadores, reservorios y vectores de Costa Rica, además de que se evidencia una amplia distribución en el país. Se recomienda realizar más estudios y establecer el diagnóstico molecular de estos agentes en los Sistemas de Salud de Costa Rica.

Agradecimientos y colaboradores: Al Consejo Nacional de Rectores (CONARE), por aportar el financiamiento de la presente investigación (Fondos del Sistema), al Servicio Alemán de Intercambio Académico (DAAD) por la beca de estudio otorgada a Leyda Ábrego y Luis Romero, y al Programa de Ciencia y Tecnología para el Desarrollo (CYTED) por el apoyo brindado a la Red Iberoamericana para la Investigación y Control de las Enfermedades Rickettsiales (RIICER-CYTED). Un especial agradecimiento al Dr. Ganta, Dr. Labruna, Dr. Aguiar, y a todos los veterinarios que participaron en esta investigación.

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ISSN 0001-6012/2013/55/3/41-44
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de Costa Rica

Conferencias Magistrales

Ehrlichia: Avances en vacunas, diagnóstico y patobiología

(*Ehrlichia:* Advances in vaccines, diagnostics and pathobiology)

Jere W. McBride

Resumen

Ehrlichia spp. es responsable de una zoonosis humana emergente y una enfermedad veterinaria importante en las Américas. *Ehrlichia chaffeensis* emerge en Norte América en 1986 y nuevas erlichias asociadas con enfermedad humana continúan emergiendo junto con la identificación reciente de una *E. muris*-like en Minnesota y Wisconsin en el 2011. *E. canis* es prevalente en toda América en los perros y ha sido asociada con enfermedad humana en América del Sur. La erlichiosis humana causada por todas estas erlichias junto con *E. ewingii*, es un importante problema de salud pública. En años recientes se han descrito importantes avances en el desarrollo de la vacuna, inmuno-diagnóstico y la pato biología de estas enfermedades. Sin embargo es necesario entender por completo el mecanismo de la enfermedad producida por estos patógenos emergentes trasmitidos por garrapatas, tanto en humanos como a nivel de medicina veterinaria.

Descriptores: *Ehrlichia,* vacunas, diagnóstico, patobiología

Abstract

Ehrlichia spp. is responsible emerging human zoonoses and diseases of veterinary importance in the Americas. *Ehrlichia chaffeensis* emerged in North America in 1986 and new *Ehrlichia* spp. associated with human disease continue to emerge with the recent identification of an *E. muris*-like agent in Minnesota and Wisconsin in 2011. *E. canis* is prevalent throughout the Americas in dogs and has been associated with human disease in South America. The human ehrlichioses caused by all these erlichias together with *E. ewingii*, is an important public health problem. In the last years have been important advanced in vaccine development, immunodiagnostic and pathobiology of these diseases. However, is necessary the understanding of disease mechanisms of these emerging tick-transmitted pathogens in human and veterinary medicine.

Keywords: *Ehrlichia,* vaccine, diagnostic, pathobiology

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Ehrlichia spp. is responsible emerging human zoonoses and diseases of veterinary importance in the Americas. *Ehrlichia chaffeensis* emerged in North America in 1986[1] and new *Ehrlichia* spp. associated with human disease continue to emerge with the recent identification of an *E. muris*-like agent in Minnesota and Wisconsin in 2011.[2] *E. canis* is prevalent throughout the Americas in dogs and has been associated with human disease in South America.[3] The emergence of human ehrlichioses caused by multiple agents including *E. chaffeensis, E. ewingii*, *E. canis* and most recently the *E. muris*-like agent (**EMLA**) is an important public health problem. The need for broadly effective countermeasures, diagnostics and understanding of disease mechanisms of these emerging tick-transmitted pathogens in human and veterinary medicine is needed.

Vaccine development

Recent progress in the immunomolecular characterization of *Ehrlichia* spp. has substantially improved understanding of the molecular basis of antigenicity of *Ehrlichia.* Effective immunity

to ehrlichiae involves humoral and cell-mediated immunity against antigens that have recently been molecularly identified in *E. chaffeensis* including a group of tandem repeat protein (TRP) effectors,[4,5,6,7] and a paralogous outer membrane protein family (OMP-1).[8,9] Several TRPs in *Ehrlichia* have been completely molecularly characterized, and major continuous species-specific antibody epitope(s) have been mapped to the acidic serine-rich TRs of *E. chaffeensis* and *E. canis* TRPs, [5,6,7] and passive transfer of antibodies against *E. chaffeensis* TRPs provides protection against challenge in mice.[10]

Transcriptional analysis of ehrlichial gene expression in mammalian and tick cells has also revealed that TRPs are highly upregulated in the mammalian host.[11] In addition, many genes were found to be differentially expressed, most of which have unknown function,[11] suggesting that proteins encoded by these genes should be considered as vaccine candidates. The development of animal models that faithfully reproduce human disease,[12,13] delineating protective and pathologic innate and adaptive immune mechanisms,[10,14,15] and defining the ehrlichial phenotypic profile in both mammalian hosts and arthropod vectors[11] collectively offers new opportunities for rational development of an effective multivalent subunit vaccine for this group of tick-transmitted pathogens.

Immunodiagnostics

Clinical diagnosis of human ehrlichosis is usually confirmed retrospectively by detection of *Ehrlichia*-specific antibodies in sera using an indirect fluorescent-antibody assay (IFA).[16] IFA is the current gold standard, but has limitations that include lack of standardization between laboratories, false positive interpretations due to autoantibodies or antibodies directed at conserved bacterial proteins, and cross-reactive antibodies produced by related organisms that can make identification of the specific etiologic agent difficult.[17] Furthermore, IFA requires expensive microscopy equipment and highly skilled technicians to produce the antigen and interpret results. Molecular diagnostic methods such as PCR are useful for specific and sensitive detection of *E. chaffeensis* prior to development of reactive antibodies,[18] but PCR is not useful after antibiotic therapy is initiated, and the clinical sensitivity of PCR in the primary care setting has not been unequivocally determined. Therefore, PCR is currently considered a valuable adjunct to IFA for diagnosis.

Advances in the immunomolecular characterization of *E. chaffeensis* have provided new opportunities to dramatically improve the sensitivity, specificity and standardization of immunodiagnostics for the ehrlichioses. Species-specific continuous epitopes have been identified in the tandem repeats (TRs) of *E. chaffeensis* TRP32, TRP47, and TRP120[5,6,7] The *E. chaffeensis* TRP32 has two to six nonidentical 30-amino acid TRs, and two major species-specific antibody epitopes (continuous and discontinuous) have been identified in the tandem repeats.[7] Single major molecularly distinct continuous antibody epitopes (18 to 22 amino acids) have also been identified in the TRP47 and TRP120, and corresponding orthologs of *E. canis*.[6] These peptide epitopes can be used for development of solid phase high throughput assays. Synthetic peptides can be produced consistently in highly pure forms and can be produced quickly and efficiently without costly and laborious purification procedures and need for defined expression vectors and hosts. The development of standardized and commercially available assays will be advanced by a molecularly defined polypeptide epitopes that provide comparable or better sensitivity than IFA and analytical and clinical specificity that is much higher than IFA. Hence, the future of immunodiagnostics for the ehrlichioses can be substantially improved through the development of peptide-based immunodiagnostics.

Pathobiology

Substantial progress in defining *Ehrlichia*-host interactions and effector proteins involved has been advanced by recent studies on the role of ehrlichial TRPs as effector proteins in reprogramming host cell through interactions with host cell targets and DNA. Early immunoelectron microscopy studies identified TRP120 extracellularly, associated with the morular fibrillar matrix and the morula membrane.[19] It is now known that *E. chaffeensis* TRPs are secreted by the type 1 secretion systems and are related to the repeats-in-toxin exoprotein family.[20] Generally, TRPs in pathogenic bacteria have been associated with host-pathogen interactions such as adhesion and internalization,[19,21] actin nucleation[22] and immune evasion.[23] In *Ehrlichia* spp., long period tandem repeats are distributed in intergenic and coding regions of *Ehrlichia* and appear to have evolved after divergence of the species, through active locally occurring independent events that create and delete TRs through a mechanism compatible with DNA slippage.[24]

Studies have determined that three *E. chaffeensis* TRPs (TRP47, 120, & 32) are involved in a diverse array of interactions with the host cell.[25,26,27] Host cell proteins that are targeted by TRPs include proteins involved in signaling, vesicle trafficking, and transcriptional regulation. The interactions between TRPs and host targets cause the redistribution of some host proteins to ehrlichial morula or cytoplasm adjacent to the morulae in *E. chaffeensis*-infected cells, further indicating the profound effects of TRPs on host cell protein recruitment. Experiments using RNA interference to knockdown genes encoding host proteins such as CD63, DAZAP2 and FTL that interact with *E. chaffeensis* TRP32 resulted in decreased bacterial load in infected cells demonstrating an important functional significance for intracellular survival of *Ehrlichia*.[27] The reduction of a single target protein could not abolish the ehrlichial growth completely; therefore, further study is needed to understand the importance of each specific TRP-host protein interaction in ehrlichial pathobiology.

Nuclear effector proteins (nucleomodulins) have been identified in several intracellular human bacterial pathogens including *Ehrlichia, Anaplasma, Shigella* and *Yersinia*. It is well documented that *E. chaffeensis* significantly alters the transcriptional levels of approximately 5% of host genes within 24 hr of infection.[28] Genes that are modulated include those coding for apoptosis inhibitors, regulation of cell cycle and differentiation, signal transduction, proinflammatory cytokines, biosynthetic and metabolic proteins, and membrane trafficking

proteins. How *Ehrlichia* regulate host cell gene transcription is not known, but two nucleomodulins have recently been described including *E. chaffeensis* TRP120 and Ank200. These nucleomodulins interact with the host cell chromatin through distinct DNA motifs, targeting many host cell genes.[29,30] We have determined the *E. chaffeensis* TRP120 directly binds a defined host cell DNA motif through a novel tandem repeat DNA binding domain that has never been described previously in a human pathogen.[30] Using a direct DNA sequencing approach, we found that TRP120 has a large number of binding sites throughout the genome, but binds strongly to a defined group of host cell process genes involved in transcriptional regulation, protein modification, signaling, and apoptosis. TRP120 directly activated host cell target genes, supporting the conclusion that *E. chaffeensis* TRP120 acts as transcriptional activator of eukaryotic host genes.[30] These new findings have broad implications related to prokaryotic-eukaryotic interactions that include mechanisms of transcriptional modulation of the host cell, the characteristics of bacterial effector proteins, structure and function of prokaryote DNA binding proteins, functional and non functional eukaryote *cis*-regulatory code, and alternative mechanisms for immune escape through direct transcriptional regulation of host defense genes by obligately intracellular pathogens.

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ISSN 0001-6012/2013/55/3/45-47
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Colegio de Médicos y Cirujanos
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Conferencias Magistrales

Epidemiología de rickettsiosis por *Rickettsia parkeri* y otras especies emergentes o re-emergentes asociadas a la antropización en Latinoamérica

(Epidemiology of rickettsioses by *Rickettsia parkeri* and other emerging and reemerging species associated with anthropization in Latin America)

José M. Venzal

Resumen

Se describe la importancia regional de *Rickettsia parkeri* y sus respectivos vectores. Se hace énfasis en los factores de antropización que favorecen la aparición de hospedadores alternativos para las garrapatas en los entornos domésticos y peridomésticos, generando modificaciones en la epidemiología del agente etiológico. También se menciona las modificaciones ecológicas que pueden favorecen el incremento de poblaciones de reservorios para las garrapatas incrementando el riesgo para el ser humano de sufrir enfermedades rickettsiales.

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

Abstract

A description of the regional importance of *Rickettsia parkeri* and their vectors is presented. There is emphasis on the factors of anthropization that favor the development of alternative hosts for ticks in domestic and peridomestic environments, generating changes in the epidemiology of the etiological agent. The environmental changes that can promote the increase in populations of tick reservoirs, increasing the risk for humans for rickettsial diseases, is also mentioned.

Key words: Rickettsiaceae Infections , *Rickettsia* Latin America, zoonoses

Los estudios sobre patógenos transmitidos por garrapatas han crecido en las últimas décadas gracias a la aparición de técnicas basadas principalmente en la biología molecular. De esta variedad de patógenos varios corresponden a rickettsias del género *Rickettsia*. Antes de estos nuevos estudios solo unas pocas especies de *Rickettsia* fueron determinadas como patógenas para humanos Dentro de estas figuran *Rickettsia rickettsii* en las Américas, *Rickettsia conorii* en Europa y África, *Rickettsia sibirica* en Asia, y *Rickettsia australis* en Australia.[1]

Para Sudamérica las rickettsiosis referían solamente a *R. rickettsii* (grupo de las fiebres manchadas) y dos del grupo de las fiebres tíficas (*R. prowazekii* y *R. typhi*).[2]

Este avance en los estudios también llegó a Latinoamérica, y al menos 10 especies, cepas o "Candidatus" pertenecientes al grupo fiebres machadas o grupos asociados se han determinado sobre garrapatas, de las cuales algunas han sido confirmadas como patógenas para el humano.[3] Aunque, es probable que este número de rickettsias aumente ya que por ejemplo de lo que actualmente se considera como *Rickettsia parkeri*, recientemente se han descubierto varias cepas diferentes del mismo grupo.[3]

Actualmente, desde el punto de vista de la salud pública, del grupo de las fiebres machadas las especies de rickettsias más relevantes en Latinoamérica y el Caribe son *R. rickettsii*, *R. parkeri, R. massiliae* y *R. africae*, aunque

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recientemente una *Rickettsia* denominada cepa Mata Atlántica relacionada *R. parkeri* ha sido diagnosticada como patógena en Brasil.[4,5]

Un caso interesante es el de *Rickettsia parkeri*, la cual fue inicialmente denominada como "maculatum agent" al ser aislada sobre la garrapata *Amblyomma maculatum* halladas en bovinos. La cual luego fue denominada en honor a R.R. Parker y resultó ser patógena en cobayos.[6,7,8] Varias décadas después, *R. parkeri* fue confirmada como patógena para humanos y actualmente es considerada como una rickettsiosis emergente en las Américas.[9,10,11]

Los diagnósticos de casos de rickettsiosis humana por *R. parkeri* provienen de Estados Unidos, Uruguay y Argentina, en el primer país el vector es *A. maculatum* y en los dos restantes *Amblyomma triste.* [10, 11, 12] En Sudamérica, *R. parkeri* ha sido detectada infectando a *A. triste* en Argentina,[13] Brasil,[14] Uruguay[15,16] y en *Amblyomma tigrinum* de Bolivia.[17] Si bien *R. parkeri* fue determinada en *A. triste* de Brasil, no se han reportado casos de rickettsiosis asociados a este vector, aunque, las cepas de *Rickettsia* denominadas Mata Atlántica y Bahia se consideran pertenecientes a cepas de *R. parkeri* y causantes de rickettsiosis, poseen una epidemiología totalmente diferente.[5,18]

Un caso notable sobre como la alteración ecológica ambiental por parte del hombre se relaciona con casos de rickettsiosis humana por *R. parkeri* es en Uruguay. Si bien inicialmente los casos de rickettsiosis en Uruguay fueron atribuidos a *Rickettsia conorii* (Fiebre Botonosa o del Mediterráneo)[19,20] posteriormente fueron confirmados como causados por *R. parkeri.*[21]

La distribución de los casos de rickettsiosis humana en Uruguay en los departamentos del sur del país coincide con el área ocupada por el vector, *A. triste.*[11,22] Además, la actividad estacional de los adultos de *A. triste* también coincide en general con la mayoría de los casos de rickettsiosis por fiebre manchada reportados tanto en Uruguay como en Argentina.[20,23]

Estos casos en Uruguay se producen principalmente en áreas rurales y suburbanas donde *A. triste*, la cual parasita principalmente a perros y otros vertebrados domésticos de mayor porte como equinos, bovinos, etc., y los inmaduros a pequeños roedores y marsupiales.[24] El problema radica que en Uruguay en las zonas donde la rickettsiosis es endémica, el principal hospedador natural primario de esta garrapata en Sudamérica que es el "Ciervo de los pantanos" *Blastocerus dichotomus* y el otro que bajo condiciones experimentales el "Carpincho" *Hydrochoerus hydrochaeris* también demostró ser un hospedador competente,[25, 26] son escasos o está ausente por la presión de caza ejercida y el Ciervo de los pantanos está extinto desde hace ya muchos años en el país.[27] En estas áreas, la urbanización ha sido muy importante, principalmente en forma de asentamientos, muchos de ellos ilegales. En estos asentamientos hay una fuerte presencia de *A. triste*, las cuales ante la falta de hospedadores naturales primarios, se han adaptado exitosamente al perro. Las cantidades de perros se han incrementado al aumentar los asentamientos, actuando como hospedador primario alternativo para la garrapata. Estos factores han propiciado que las poblaciones de garrapatas se multipliquen y que los pobladores locales se quejen continuamente de las picaduras sufridas, aumentando notablemente los diagnósticos de casos de rickettsiosis.

La otra rickettsiosis por Fiebre manchada cuya re-emergencia posee una claro patrón antropogénico es la Fiebre Manchada de las Montañas Rocosas también conocida como Fiebre Maculosa Brasilera (FMB) o Fiebre de Tobia, causada por *R. rickettsii* siendo la rickettsiosis más letal del mundo, presentándose en varios países de Latinoamérica.[3]

Es una enfermedad que está bajo control epidemiológico en Brasil, y en este país la misma también se ha visto favorecida por cambios producidos por el hombre, especialmente en el estado de São Paulo, aunque la situación puede darse en otros estados de la misma región como Minas Gerais y Rio de Janeiro, en el sureste del país. En Brasil, la mortalidad puede alcanzar entre el 30 al 40%.[28] En esta región el principal vector de *R. rickettsii* es *Amblyomma cajennense*, una garrapata extremadamente agresiva y que parasita frecuentemente al humano, y aunque *R. sanguineus* y *A. aureolatum* también han sido involucradas en la transmisión de la enfermedad, lo son en mucho menor grado.[3,29] Si bien *A. cajennense* puede parasitar una amplia variedad de hospedadores, los equinos y carpinchos (*H. hydrochaeris*) favorecen a mantener las poblaciones de esta garrapata.[29]

Uno de los problemas se debe a que en Brasil, las poblaciones del carpincho se han visto favorecidas por varios factores, los cuales han influido directamente en el aumento de casos de rickettsiosis por *R. rickettsii* en ciertas regiones. Gracias a la legislación ambiental brasilera, el carpincho se encuentra protegido por lo que su caza es prohibida y también existen planes de conservación de montes o bosques asociados a cursos de agua, lo cual favorece su protección. Además, en las proximidades de su hábitat se han desarrollado cultivos como el de la caña de azúcar que le sirven como alimento. Todos estos factores han llevado a que la población de carpinchos aumentara en forma importante.[29]

Por lo que el carpincho que además de actuar como hospedador para *A. cajennense* también se comporta como amplificador del patógeno. Esto determina que en las zonas donde este desequilibrio ecológico ocurre, las posibilidades de contraer rickettsiosis aumentan, por lo que la enfermedad puede ser tratada como re-emergente, por causas claramente antrópicas.

Agradecimientos y colaboradores: Este trabajo forma parte de la Red Iberoamericana de Investigación y Estudio de las Enfermedades Rickettsiales (RIICER), financiada por el programa Iberoamericano de Ciencia y Tecnología para el desarrollo (CYTED).

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ISSN 0001-6012/2013/55/3/48-59
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Rickettsiosis: pathogenesis, inmunidad y desarrollo de vacunas

(Rickettsioses: pathogenesis, immunity, and vaccine development)

Gustavo Valbuena

Resumen

Varias especies dentro del género *Rickettsia* son altamente patogénicas; por ejemplo *R. rickettsii* (el agente de la fiebre manchada de las Montañas Rocosas) y *R. prowazekii* (el agente del tifus epidemémico). Muchas de las rickettsiosis son prevalentes a lo largo de América Latina; sin embargo, estas enfermedades son desatendidas porque rara vez son consideradas en el diagnóstico diferencial de enfermedades febriles en los trópicos. Esto se explica parcialmente por el hecho de que todas las infecciones causadas por *Rickettsia* son difíciles de diagnosticar, debido a la presentación clínica no-específica inicial, sospecha clínica ausente, y la falta de pruebas diagnósticas sensibles y específicas que se pueden utilizar durante la presentación aguda. Además, la confusión diagnóstica con infecciones virales es la regla, y esto es un problema crítico ya que estas infecciones pueden tratarse con antibióticos apropiados. Con esta revisión, esperamos contribuir al conocimiento y la conciencia de estas importantes enfermedades dentro de los profesionales científicos y de salud en América Latina.

Descriptores: *Rickettsia*, patogénesis, inmunidad, vacuna

Abstract

Several species within the genus *Rickettsia* are highly pathogenic; for example, *R. rickettsii* (the agent of Rocky Mountain spotted fever) and *R. prowazekii* (the agent of epidemic typhus). Many of the rickettsioses are prevalent throughout Latin America; however these diseases are neglected because they are seldom considered in the differential diagnosis of febrile diseases in the tropics. This is partly explained by the fact that all infections caused by *Rickettsia* are difficult to diagnose due to the initial non- specific clinical presentation, absent clinical suspicion, and the lack of sensitive and specific diagnostic tests that can be deployed during the acute presentation. Furthermore, diagnostic confusion with viral infections is the rule, and this is a critical problem because these infections can be treated with appropriate antibiotics. With this review, we expect to contribute to increase knowledge and awareness of these important diseases among scientists and health care professionals in Latin America.

Keywords: *Rickettsia*, pathogenesis, immunity, vaccine

Rickettsia are the etiologic agents of two of the most lethal infections known to man, Rocky Mountain spotted fever (*Rickettsia rickettsii*) and epidemic typhus (*R. prowazekii*). Moreover, epidemic typhus has shaped History due to the massive epidemics that it produced during times of war until World War I.[1] Both agents are select agents because of their potential use as bioweapons.[2] On the other hand, several new pathogenic *Rickettsia* have been discovered in the last few decades; new rickettsioses are certainly emerging and old rickettsioses are re-emerging.[3]

Members of the genus *Rickettsia* (family *Rickettsiaceae*, order *Rickettsiales*) are α-proteobacteria that share the following general characteristics: 1) they have closely related A/T-

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rich small genomes, a consequence of evolutionary loss of genes encoding proteins that participate in various biosynthetic pathways;[4-6] 2) they can only survive in the cytoplasm of eukaryotic cells where they obtain needed metabolic substrates that they cannot synthetize themselves (they are strict obligate intracellular parasites); 3) most of the well-known rickettsiae reside within arthropods. Indeed, hematophagous insects and ticks transmit rickettsiae that are pathogenic to humans and other vertebrates (they are zoonoses); 4) in humans, rickettsiae preferentially target endothelial cells, the cells that line vascular and lymphatic vessels (except for *Rickettsia akari*, the agent of rickettsialpox, which specially targets monocytes and macrophages).[7]

The transmission of rickettsia by hematophagous arthropod vectors was established early in the 20th century. In 1906 WW King[8] and HT Ricketts[9] described their experiments with guinea pigs in which they demonstrated that ticks transmit Rocky Mountain spotted fever (RMSF). At the time, Ricketts and others recognized that the clinical presentation of Rocky Mountain spotted fever closely resembled that of epidemic typhus; however, it was not yet known that closely related organisms caused the two diseases. What was clear then was that the human body louse was the vector of typhus.[10] Charles Nicolle received the 1928 Nobel Prize for this discovery.

In 1914, H. Plotz reported the identification of a gram-positive bacillus in the blood of patients with typhus as well as their lice.[11] H. da Rocha-Lima confirmed these findings in 1916;[12] he named the organism *Rickettsia prowazekii* in honor of Ricketts and Stanislaus von Prowazek, both of whom died of typhus acquired in the course of their investigations. In 1916, SB Wolbach studied samples from guinea pigs with Rocky Mountain spotted fever and identified very small gram-negative organisms in vascular vessels.[13,14] Subsequently, in 1917, he confirmed this finding as well as the vascular nature of the infection in autopsies of human patients with Rocky Mountain spotted fever.[15] The integration into a single genus, *Rickettsia*, would not be proposed until 1943.[16] By the late 1960s and early 1970s a more modern conception began to be synthetized.[17,18]

At the present moment, there are 22 entries for *Rickettsia* genomes in the database of NCBI. They are *R. rickettsii*, *R. prowazekii,*[19] *R. conorii,*[20] *R. typhi*, *R. massiliae*, *R. canadensis*, *R. slovaca*, *R. bellii*, *R. africae*, *R. sibirica*, *R. peacockii*, *R. akari*, *R. felis*, *R. montanensis*, *R. rhipicephali*, *R. australis*, *R. parkeri*, *R. philipii*, *R. japonica*, *R. heilongjiangensis*, *Candidatus Rickettsia amblyommii,* and *Rickettsia endosymbiont of Ixodes scapularis.* Based on the analysis of a subset of these data,[21,22] new phylogenetic relationships were proposed. Accordingly, there are four groups: 1) the non-pathogenic ancestral group (*R. bellii* and *R. canadensis*), which diverged earlier; 2) typhus group (*R. typhi* and *R. prowazekii*); 3) spotted fever group (*R. rickettsii*, *R. parkeri, R. conorii*, and several others); and 4) transitional group (*R. akari*, *R. australis,* and *R. felis*). A more recent analysis proposes to split the ancestral group in two with one *Rickettsia* in each group (i.e., *R. bellii* and *R. canadensis*) and to include the transitional group within the spotted fever group (SFG).[23] According to this new scheme, the SFG group is divided in four subgropus: 1) the *R. rickettsii* subgroup (*R. rickettsii*, *R. conorii*, *R. africae*, *R. parkeri*, *R. sibirica*, *R. slovaca, R. honei, R. japonica, R. heilongjiangensis*, and a few others); 2) *R. massiliae* subgroup (*R. massiliae* , *R. montanensis*, *R. aeschlimannii* and *R. rhipicephali*, *R. raoultii* and others); 3) *R. helvetica* subgroup (*R. helvetica, R. asiatica, R. tamurae, R. monacensis*); 4) *R. akari* subgroup (*R. akari, R. australis, and R. felis*). A phenotypic characteristic of the *R. rickettsii* subgroup is its susceptibility to rifampin, while the *R. massiliae* subgroup is resistant to this antibiotic.[24] For a long time, the serological response was the main criterion used to classify rickettsiae in only two groups,[25,26] spotted fever and typhus; using those criteria, *R. canadensis* was included in the typhus group at that time. Also, until 1995,[27] *Orientia tsutsugamushi*, the etiologic agent of scrub typhus, was included in the genus *Rickettsia* (i.e., *Rickettsia tsutsugamushi*) and considered a third group.

In temperate regions of the globe, the seasonality of SFG rickettsioses is explained by the activity of the tick vectors, particularly the adults, which are more active during the spring and early summer. There is also a periodicity in a timeframe of decades that has not been appropriately explained yet. It is possible that climate change may affect the behavior of tick vectors.[28] One of the recent peaks of reporting of Rocky Mountain spotted fever (RMSF) occurred during the early 2000s.[29] This may be related to increased disease activity but also to renewed interest not only in the United States but also throughout the Americas (RMSF occurs only in the Americas). The disease has now been documented in almost all countries of Latin America.[30-40] Even more importantly, new SFG rickettsioses have been discovered. For instance, *R. parkerii*, which was considered a non-pathogenic *Rickettsia* for a very long time, was recently shown to produce a mild spotted fever with an eschar and local lymphadenopathy.[41-44] Other recently described *Rickettsia* associated with eschars and relatively mild disease include *Rickettsia* 364D[45] and *R. massiliae.*[46,47]

One of the consequences of the non-specific initial febrile syndrome and the lack of commercially available diagnostic methods that are sensitive and specific during the acute presentation of the rickettsioses is that the disease is frequently underreported and diagnosed as a viral illness.[48] In Latin America, the umbrella diagnosis of dengue is frequently applied to cases of rickettsiosis.[49]

Pathogenesis

Rickettsioses are systemic febrile diseases that affect individuals of any age independently of their immune status.[48,50-53] Although the pathogenetic mechanisms are shared, not all rickettsioses are equally severe, which is explained by differences in virulence of the individual species and vector-related factors.

The entry of *Rickettsia* into host cells is an active process that requires energy from both the host and the rickettsiae.[54] There is evidence that rickettsiae use surface cell antigen 0 (sca0 or rOmpA)[55] and sca 1[56] to attach to target cells (these and the other rickettsial sca proteins are autotransporters). Subsequent to attachment, which is mostly a passive process, endocytosis of rickettsia is actively triggered when the rickettsial outer

membrane protein B (rOmpB or sca5) binds to the host cell membrane form of Ku70.[57] Since blocking of this interaction only inhibits about 50% of rickettsial entry, other ligands and receptors must be present; sca2[58] and adr2[59] appear to be some of those bacterial ligands.

The necessary cytoskeletal rearrangements that produce the zipper-like entry mechanism of *Rickettsia* spp. involve multiple host pathways that activate the Arp2/3 complex[60] with the participation of Cdc42, cofilin, c-Cbl, clathrin, and caveolin 2.[61] *Rickettsia* may also enter phagocytic cells such as monocytes and macrophages (which are a secondary target of most *Rickettsia*) by antibody-mediated opsonization.[62] Within a short period of time after endocytosis, rickettsia escapes into the cytosol. The rickettsial genes *pld*, which encodes an enzyme with phospholipase D activity,[63] and *tlyc*, which encodes a hemolysin[64] are believed to be effectors of this function. This conclusion is based on the ability of the normally vacuolar *Salmonella enterica* to escape into the cytosol when it expresses rickettsial *tlyc* or *pld*.[65] In addition, rickettsial proteins with phospholipase A activity were confirmed[66, 67] but only in the typhus group *Rickettsia*. That activity underlies the phenomenon of hemolysis produced by these rickettsiae in vitro.[68,69]

Once *Rickettsia* escapes the phagocytic vacuole, it acquires multiple metabolic substrates from the host cytoplasm. The availability of those substrates allowed genome reduction through loss of many genes including, among many others, those for nucleotide synthesis and enzymes for sugar metabolism.[70] Multiple transporters of substrates from the host cytoplasm, including ATP,[71] compensated for these gene losses.[72] The mechanisms of transport are active and include the use of the transmembrane electrical potential.[73]

Typhus group *Rickettsia* grow until they burst the host cell[74] while spotted fever group *Rickettsia* rapidly spread from cell to cell[75] due to their actin propulsion. Of course, host cells are damaged in the process;[76] the mechanisms may involve the production of free radicals[77,78] and phospholipase activity.[79] On the other hand, there is experimental evidence that rickettsiae can maintain their cellular niche through inhibition of apoptosis,[80] and that pathogenic *Rickettsia* can inhibit autophagy.[81]

The main target cells of most *Rickettsia*, with the exception of *R. akari* are endothelial cells, the cells that line all vascular vessels in the body. These cells have important regulatory functions in angiogenesis, hemostasis, permeability and solute exchange, vascular tone, and inflammation.[82-84] Thus, their targeting by rickettsiae explains many of the clinical features of the diseases including systemic involvement and leakage of intravascular fluid. Rickettsial infection of endothelial cells induces cellular damage leading to detachment. Those infected endothelial cells circulate in the blood[85,86] and are likely to be the source of new foci of infection once they lodge in distal capillaries.

Several mechanisms are likely to contribute to the increased vascular permeability observed in clinical cases. They include production of vasoactive prostaglandins as a consequence of increased expression of COX-2,[87] endothelial production of nitric oxide,[88] effects of inflammatory cells and their mediators,[89] and endothelial detachment and denudation of vessels. Such damage may be caused by phospholipase activity,[79] mechanical damage to the membrane caused by exiting rickettsiae under actin propulsion,[90] or lipid peroxidation of the cell membrane.[76,77,91,92] The most severe clinical presentations are a consequence of endothelial damage in the lungs and brain and include noncardiogenic pulmonary edema, interstitial pneumonia, adult respiratory distress syndrome, meningoencephalitis, seizures, and coma;[93-97] involvement of these organs explains the majority of the mortality, which is observed particularly with Rocky Mountain spotted fever and epidemic typhus (the reported mortality without antibiotics ranges from 10 to 60%). However, it should be emphasized that reliance on serological methods for diagnostic confirmation may lead to underestimation the actual case-fatality rate. This was well illustrated in a recent report of nine fatal cases with negative serological results that were confirmed by immunohistochemical demonstration of the antigen in tissues.[51] At the other end of the clinical spectrum are several rickettsioses; murine typhus, with a mortality of less than 2%, is the most important of them because of its global distribution.[98]

Although multiple coagulation abnormalities have been described during the course of clinical and experimental rickettsiosis,[99] disseminated intravascular coagulation occurs only rarely in lethal cases and is not a common feature of rickettsiosis.[100]

The cells that are infected immediately after inoculation have not been identified. Many of the rickettsiae that result in less severe disease also produce an eschar (area of necrosis with a rich inflammatory infiltrate and local rickettsial proliferation) at the bite site.[101] When an eschar is present, another frequent clinical finding is local lymphadenitis, suggesting initial spread through lymphatics. Rocky Mountain spotted fever, the most severe of the spotted fever rickettsioses, does not manifest with an eschar or local lymphadenitis. This could be due to a more rapid hematogenous dissemination.

The recommended antibiotic treatment for all rickettsioses is doxycycline.[102] This antibiotic has the advantage of covering other tick-borne bacterial infections. Rickettsiae are resistant to many antibiotics.[103] Other antibiotics, including chloramphenicol and fluoroquinolones may be effective, although there is evidence that they may have deleterious effects.[104,105] The antibiotic resistance of *Rickettsia* combined with the non-specific initial clinical presentation and lack of commercially available laboratory tests for confirmatory diagnosis during the acute presentation, lead to delayed diagnosis and inappropriate treatment; the consequence is excessive mortality.[104]

Rickettsial virulence

Many rickettsial genes have been predicted to participate in virulence based on bioinformatics analyzes;[72] several toxin-antitoxin systems are examples. One of them, encoded by the *vapB/C* genes was shown to be functional; *E. coli* transformed with rickettsial *vapC* significantly decrease their growth, while

VapB formed a complex with VapC to inhibit its RNase activity.[106] More importantly, microinjection of VapC to mammalian cells induced apoptotic death.

A large number of intracellular bacteria use type IV secretion systems to inject proteins into the host in order to produce a favorable niche. Interestingly, genomic analysis showed that multiple genes with the potential to encode a reduced type IV secretion system are conserved in *Rickettsia*.[107] Whether the system is actually functional or not remains to be tested.

The phospholipase D encoded by the gene *pld*, a likely mediator of phagosomal escape, is a virulence factor as suggested by the milder disease produced in guinea pigs infected with *R. prowazekii* with a mutated *pld*.[108] This study used homologous recombination for targeted knockout of a rickettsial gene. Previous studies using the difficult techniques of genetic manipulation of *Rickettsia*, including transposon-mediated mutagenesis, indicated that mutation of the open reading frames (ORFs) 243, 294, and 689 of *R. prowazekii* do not produce an observable phenotypic difference.[109] Thus, these genes may be non-essential genes (at least for growth in mouse cell line *in vitro*). Also, *R. rickettsii* mutants lacking expression of sca2, which participates in actin polymerization, do not cause apparent illness in guinea pigs.[110]

Loss of regulation due to genome decay has also been proposed as a mechanism of increased virulence;[111] however, this argument does not explain why *R. rickettsii* and *R. prowazekii* are almost equally pathogenic and the radical difference in virulence between the two typhus group rickettsiae, *R typhi* and *R. prowazekii*.

In the absence of genetic approaches that work well and consistently for *Rickettsia*, other methods have been introduced to identify virulence factors. One example is the comparison of the genomes of closely related *Rickettsia* with different pathogenicity. The *Dermacentor andersoni* endosymbiont *R. peacockii* was compared to virulent *R. rickettsii*; it was found that it had a plasmid, multiple transposons with intact transposase sequences, and many deletions, nonsense mutations, and split genes.[112] The authors proposed that some of the absent or mutated genes in *R. peacockii* might explain the lack of pathogenicity. Those genes include *DsbA* (a catalyzer of disulfide bond formation), *RickA*, *Sca0, Sca1*, a gene encoding Protease II, and a gene encoding a putative phosphoethanolamine transferase that could play a role in the formation of the prominent slime layer found in the pathogenic spotted fever-group rickettsiae. Interestingly, the hypothetical protein A1G_05165 of a virulent strain of *R. rickettsii* (strain Sheila Smith) is deleted in *R. peacockii* and it is also not present in other non-pathogenic rickettsiae. This hypothetical protein has ankyrin repeats; similar proteins in other members of this order (i.e., *Anaplasma*) appear to play a role in virulence through binding of host DNA and altered host gene regulation. A1G_05165 is also mutated in a non-pathogenic strain of *R. rickettsii* (strain Iowa). In addition, the genomic study that compared the pathogenic strains R and Sheila Smith with strain Iowa also found 23 deletions within predicted ORFs of *R. rickettsii* Sheila Smith and 24 deletions within predicted ORFs of *R. rickettsii* Iowa.[113] One of the genes deleted in *R. rickettsii* Iowa is the adhesin rOmpA (sca0). Also, *rompB* has four single nucleotide polymorphisms (SNPs) that may explain the defective processing of this important membrane protein in strain Iowa.[114] Finally, it should be emphasized that there is a good opportunity to understand virulence by comparing the genomes, transcriptomes, and proteomes of the two typhus group *Rickettsia* since they have very closely related genomes but very different virulence in humans, with *R. prowazekii* producing a much more severe infection (epidemic typhus) than *R. typhi* (murine or endemic typhus).

Another system to study the physiology of *Rickettsia* in the absence of more efficient genetic systems is the use of *E. coli*-based assays. For example, to identify proteins transported out of the rickettsial cytoplasm, bioinformatic tools were used to uncover predicted secreted proteins (based on the presence of N-terminal signal peptides). The signal peptides of those proteins from *R. typhi* were then fused to the *E. coli* alkaline phosphatase *phoA* gene (lacking an intrinsic signal peptide sequence) to test if those signal peptides provided information to translocate PhoA into the periplasm of *E. coli*.[115] Eighty-four functional signal peptides were identified suggesting that those rickettsial proteins might be secreted using the rickettsial Sec system. Those proteins include sca1-3, sca5, Pld, and proteins that are believed to be part of a type IV secretion system.

Immunity and vaccines

An often overlooked but critical factor in the pathogenesis of rickettsial diseases is the transmission by arthropod vectors because their saliva is not a passive vehicle for transmission.[116-118] In fact, the tick saliva modifies the host environment in order to successfully complete the blood feeding, which occurs during extended periods (several days for nymph and adult ticks). Proteins in the tick saliva modulate host hemostasis, innate and adaptive immuntiy, complement activation,[119] angiogenesis, and extracellular matrix regulation.[120,121] Evidently, all of those factors could determine the final outcome of the infection. Furthermore, tick saliva can modulate the physiology of endothelial cells, the main target cells of *Rickettsia*. For example, salivary gland extracts from *D. andersoni* reduce the upregulation of ICAM-1 induced by TNF-α on a mouse endothelial cell line.[122] This change could contribute to reduce the migration of leukocytes into tick bite sites.

Endothelial cells are not passive actors in the anti-rickettsial immune response. Upon rickettsial infection, the transcription factor NFκB (a critical stimulating factor of the immune system) becomes activated in endothelial cells.[123-125]Other critical signaling mediators become activated as well. They include STAT1, STAT3,[126] and p38 MAPK.[127-129] As a consequence of the activation of these various signaling systems, endothelial cells respond by expressing a variety of chemokines,[130,131] cytokines such as IL-1 α, and IL-6,[132,133] adhesion molecules such as E-selectin, VCAM-1, ICAM-1,[134-136] and αVβ3 integrin,[137] and secretion of prostanoids.[87,138]

NK cells are early producers of IFN-γ after infection with *Rickettsia*.[139,140] This cytokine is important because, together with TNF-α, it activates the bactericidal functions of the

endothelium.[141,142] Those functions are performed in part through expression of indoleamine-2,3-dioxygenase (IDO), which leads to tryptophan starvation.[143] Animal studies have demonstrated the importance of a T helper 1 (Th1) response in effective immunity against rickettsiae[144] with a particularly important role for CD8+ T cells.[145,146] In fact, T cells are sufficient to mediate protection against a lethal rickettsial challenge, even in the context of a heterologous challenge where anti-typhus group T cells protect against a lethal challenge with SFG *Rickettsia* and vice versa.[147]

Despite the fact that rickettsiae are intracellular parasites and that cellular adaptive immunity is critical during a primary infection, there is clear evidence that the humoral immune response is very important in preventing the development of disease during secondary infections or after a lethal challenge following passive serum transfer. In fact, it was Ricketts himself who demonstrated this fact.[148] The anti-rickettsial humoral immune response is cross-reactive within rickettsiae of the same group but not across groups (e.g., between typhus and SFG groups).[149,150] The most abundant surface protein of *Rickettsia* is rOmpB (Sca5), which is an autotransporter. It is an immunodominant protein and antibodies against it are protective.[151]

Inactivated vaccines for *R. rickettsii* and *R. prowazekii* were produced early from a variety of sources including their vectors but they were very reactogenic and protection was incomplete. Later on, inactivated vaccines were produced from *Rickettsia* cultivated in eggs but antigenicity was variable and protection was poor.[152-155] In the 1950s a very effective vaccine for epidemic typhus was produced. It was an attenuated strain denominated Madrid E;[156] however, spontaneous reversion to a virulent phenotype precluded further development and testing.[157,158]. We now know that the attenuation is explained, at least in part, by a point mutation in the gene encoding a S-adenosulmethionine-dependent methyltransferase.[159] Given the nature of the mutation, it is not surprising that reversion was not an uncommon occurrence. Deletion of the entire gene would permit the production of a safer vaccine. Alternatively, strains with multiple genetic differences could prove to be safe vaccines. In this regard, it is interesting to note that the strain Iowa of *R. rickettsii*, which is attenuated and has multiple genetic differences when compared with virulent strains, can protect guinea pigs against a challenge with virulent *R. rickettsii*.[113]

Other recent efforts have focused on the production of a subunit vaccine. Fragments of rickettsial proteins that may trigger protective immunity were tested. They included rOmpA[160,161] and rOmpB[162-164] and results were encouraging; however, these approaches are limited and biased because of their focus on proteins that elicit a strong humoral response. A major effort for identification of immunogenic antigens is clearly needed, and the antigen discovery effort will need new tools to identify relevant conserved antigens recognized by T cells.

It will be possible to produce vaccines that cover more than one species of *Rickettsia* given the evidence of cross-protective immunity within the typhus or spotted fever groups[165-171] or even across groups.[147] The production of an effective anti-*Rickettsia* vaccine is a public health priority for several reasons. Firstly, some rickettsioses are highly lethal not only to humans but also to companion animals (i.e., dogs). Secondly, clinical diagnosis of rickettsioses is very difficult due to the non-specific initial clinical presentation. Thirdly, there are no commercially available diagnostic tests that can be used during the acute stage when antibiotic intervention is helpful.

The contemporary development of a vaccine has two initial essential aspects, namely identification of the relevant antigens and definition of immunological correlates of protection to guide the selection of vehicles, vectors, schedules, and adjuvants. In the case of infections caused by *Rickettsia*, due to the availability of excellent murine models, relevant correlates of protective immunity can be derived from the characterization of experimental infections because animals (as well as humans) that survive the infection become solidly immune to reinfection.

In regard to immunological correlates of protection, the magnitude of a response assessed by a single parameter (e.g., IFN-γ for intracellular pathogens, as frequently reported), is not enough. Now we know that there is functional heterogeneity of the T cell effector responses (including cytokine secretion, cytolytic activity, and development of various memory phenotypes) and that there are particular subsets of T cells, which express unique combinations of effector functions, that are more protective.[172-174] We probably should approach the definition of correlates of protective immunity in a way that parallels the complexity of physiological immunity, which is a multifaceted and integrated response that includes many different cells, receptors, ligands, and signaling modules that function in a combinatorial mode. For infections in which cellular immunity plays a predominant role, there is evidence from experimental models that multifunctional T cells are the best correlate of protection described thus far. More importantly, this has been demonstrated in humans as well.[175-177]

The technologies for understanding the integrated functioning of the immune system are now available and accessible. Those are the tools of Systems Biology, the "omics" methods and bioinformatics tools that permit the analysis of complex interactions in biological systems through the investigation of massively parallel data acquired from each experimental condition.[178] The application of Systems Biology to vaccinology is already identifying transcriptional signatures of protective immune responses that include sub-signatures of appropriate innate and adaptive responses.[179] Moreover, early predictive signatures of appropriate adaptive immune responses immediately after vaccination have been defined and verified using the Yellow fever (17D) vaccine as a model.[180] It is expected that such knowledge will provide paradigms for the development of novel vaccines for which limited data from humans is currently available. That is certainly the case for infections caused by *Rickettsia* because it is unlikely that we will be able to collect sufficient human samples from clinical cases with diverse outcomes in order to define broad signatures of

protective immunity. A promising solution to this problem is to use our current understanding of well-known effective immune responses as guiding principles. The study of the response to two of the most successful human vaccines in history, the yellow fever vaccine[177,181] and the smallpox vaccine,[176] is likely to yield relevant paradigms that we could use as guiding posts in rickettsiology.

From the perspective of antigen identification for vaccine development, until recently it was almost exclusively biased towards the humoral immune response. This bias was partly due to the effectiveness of antibodies in protection against almost all of the currently approved vaccines for human use, the relative technical simplicity of working with serum and antibodies, and the methodical challenges of working with T-cells. Presently, the barriers to identify potent vaccine antigens recognized by T-cells need to be addressed because most of the vaccines that remain to be produced require a strong T-cell component to afford significant protection. In particular, there is an urgent need to develop appropriate techniques to identify antigens recognized by T-lymphocytes because antigen discovery is the most important aspect of any vaccine development project; without appropriate antigens, a vaccine is unlikely to succeed.

Given the evidence that $CD4^{+}$T cells and $CD8^{+}$T cells target different antigens,[182] it is clear that antibody-based screening methods are not suitable to identify antigens recognized by $CD4^{+}$T cells or, particularly, $CD8^{+}$T cells. Several approaches to more directly identify antigens recognized by T-cells have been used; many of them rely on Reverse Vaccinology, a branch of Systems Biology that analyses entire microbial genomes to predict immunogenic proteins based on predefined rules derived from the analysis of large empirical datasets.[183] On the other hand, the predicting power of those immunoinformatic strategies has not been thoroughly tested by direct experimentation. Moreover, at least for bacterial proteins, known protective antigens actually have less predicted epitopes than randomly selected bacterial protein sets used as a control.[184]

Empirical methods for identification of antigens recognized by T-lymphocytes rely on T-cells from animals or individuals that are immune to the pathogen. Those memory T-cells had been selected during the physiological immune response to persist and recognize a limited number of antigens (i.e., immunodominant antigens). Thus, methods that use memory T-cells for antigen identification are more likely to miss potentially protective subdominant antigens. One strategy for T-cell antigen identification that is not biased towards immunodominant antigens is genomic immunization or Expression Library Immunization (ELI).[185] In this technique, pools of eukaryotic expression vectors with cloned pathogen genes are used to directly immunize animals. The animals are then challenged with lethal doses of the microbial pathogen. The gene pools that trigger protection are subsequently deconvoluted by testing each component of the pool one at a time. This method allows the priming of naïve T-cells by the expressed cloned microbial genes regardless of whether they are subdominant or dominant during a natural infection as long as the appropriate T-cell receptors are present. Although ELI has been successfully used,[186] it has its own problems as it relies on a DNA immunization strategy; thus, antigen expression is not guaranteed in all cases. Accordingly, it is not possible to know which pathogen genes were not screened validly; a negative response can be due to lack of an immunological response or to failed expression of the microbial gene.

As an alternative, we produced a new *in vivo* screening platform; the idea is to easily produce antigen presenting cells (APCs) expressing individual open reading frames (ORFs) from any sequenced *Rickettsia* and use them for immunization of naive mice. Immunization with pooled APCs containing 4 to 5 rickettsial ORFs is followed by challenge with live virulent pathogen and measurement of an indicator of protection such as decreased bacterial load. Once protective pools are identified, each member of the pool is tested individually to identify ORF(s) responsible for a protective immune response. With this platform, one can easily test for cross-protective responses by immunizing with the ORFs of one species of *Rickettsia* and challenging with another. Importantly, the proposed methodology is not biased by immunodominance because T cells from immune animals are not used to select antigens. This aspect is potentially important for vaccine development because subdominant or cryptic antigens have been shown to elicit protective immune responses in other systems.[187-189] The ability of our platform to discover relevant antigens for vaccine development independently of their ranking in the natural hierarchy of immunodominance dramatically expands the universe of possible antigens; thus, this platform offers a possible solution to the identification of protective antigens that are conserved among different strains of a microbe or even different species within a genus.

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Resúmenes de conferencias magistrales

Avances en el conocimiento de los principales vectores de *Rickettsia* en Latinoamérica

(Advances in the knowledge of the principal vectors of *Rickettsia* in Latin America)

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Las garrapatas son ectoparásitos hematófagos causantes de perjuicios por el parasitismo *per se* o por la transmisión de agentes patógenos que pueden provocar enfermedades en animales y humanos. Dentro de este grupo de parásitos, tres de las especies con mayor importancia sanitaria son *Rhipicephalus sanguineus* sensu lato, *Amblyomma cajennense* y *Amblyomma triste*. Estudios recientes han demostrado que los taxones *R. sanguineus* s.s y *A. cajennense* en realidad constituyen complejos de especies. Particularmente en Sudamérica, existen evidencias que indican que el complejo *R. sanguineus* está formado por al menos dos especies, mientras que *A. cajennense* es un complejo de la menos seis especies. *Amblyomma cajennense* ha sido incriminada como una de las especies con mayor relevancia sanitaria no solo por el efecto deletéreo causado por el parasitismo *per se*, sino también por su capacidad para transmitir agentes patógenos a los humanos. Una de las enfermedades en la que las garrapatas del complejo *A. cajenense* se constituyen en el principal vector es la fiebre manchada por *Rickettsia rickettsii*. Esta es la rickettsiosis humana transmitida por garrapatas más importante de Latinoamérica. Ha sido reportada en México, Panamá, Costa Rica, Colombia, Brasil y Argentina, con casos fatales en la mayoría de estos países. Hasta el momento, todos los casos fatales por rickettsiosis en humanos reportados en Latinoamérica fueron provocados por *R. rickettsii*. Las garrapatas del complejo *R. sanguineus* pueden actuar como vectores y reservorios de *Ehrlichia canis*, el agente causal de la erlichiosis canina, y son también vectores potenciales de otros agentes rickettsiales como *R. rickettsii*, *R. massiliae* y *Anaplasma platys*. Este nuevo escenario taxonómico referido a *A. cajennense* s.s y *R. sanguineus* s.s conlleva implicancias ecológicas y epidemiológicas relacionadas a diferencias en la dinámica estacional, distribución y capacidad vectorial entre las especies que forman los dos complejos. *Amblyomma triste* es una garrapata filogenéticamente también presenta una amplia distribución, desde el sur de Estados Unidos al sur de Uruguay y centro de Argentina. Sin embargo, las poblaciones de *A. triste* involucradas en la transmisión de *Rickettsia parkeri* a humanos están restringidas a localidades de la Cuenca del Plata, en Argentina, Brasil y Uruguay. Estudios publicados recientemente y en curso han mostrado que *A. triste* presenta una distribución más amplia que la usualmente reconocida. Asimismo, se han establecido diferencias morfológicas asociadas a poblaciones con distinto origen geográfico. En esta presentación se detallan y discuten los avances mencionados sobre sistemática y ecología de estas tres garrapatas con importancia sanitaria en la región Neotropical.

Vigilancia de enfermedades rickettsiales en poblaciones animales

(Surveillance of rickettsial diseases in animal populations)

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The Brazilian Spotted Fever (BSF) is the most severe tick-borne-disease in Brazil. It is caused by infection with the bacterial organism *Rickettsia rickettsii*. In the last 10 years, approximately 80 cases of BSF have been reported annually and over 50% lethality. In the last ten years, several other *Rickettsia* species have been detected infecting ticks in South America, for most of them the potential threat to human being is yet to be confirmed. The disease epidemiology is strongly associated with the life cycle and ecology of the tick vectors, and therefore with the behavior of their hosts.

Detecting the presence of rickettsial agents in a tick population is a very important step to evaluate the risk that the human population is exposed in a determined local and an important surveillance tool to categorize areas in regard to the risk of disease transmission. This ought to be used by local public health services in order to focus efforts only upon the areas which are infested by infected ticks. Besides it can be used as a screening approach to areas where no information is available.

In order to categorize a specific area it is necessary to determine whether rickettsial agents are circulating among the ticks and whether it is pathogenic to humans or not. A direct survey for rickettsia on the tick population through techniques such as PCR or cell culture isolation is very assertive but is limited by a very high financial cost, thus it cannot be used in surveillance protocols for large areas, although it must be encouraged in research projects for rickettsial agents characterization.

Indirect protocols are a better choice for surveillance, specifically the serosurvey on sentinel vertebrate hosts. Since ticks are strict haematophagous parasites, the vertebrate hosts living in a specific area are highly exposed to that tick population and consequently to the rickettsial agents.

The elected vertebrate host species to take part of the survey must be primary hosts for that specific tick species found in this area. Part of the host population must be accessed in order to have a sample of blood individually collected, labeled and stocked until the moment of processing. The number of animals that must be tested depends on the size of the population and can be calculated by the simple random sample for prevalence determination, expect prevalence must be set up to 50%, significance to 95% and acceptable error to 10%.

Sera collected from sampled animals must be tested for presence of anti-*Rickettsia* antibodies through Indirect Immunofluorescence Assay (IFA), this technique depends on glass slides prepared with cell cultured *Rickettsia*. IFA test shows a cross reaction among all *Rickettsia* species from the spotted fever group. It is possible to determine the likely homologous reaction if each individual serum is tested against different *Rickettsia* species, if the title obtained against one species is four times higher than the other tested species, it is highly probable that it is an homologous reaction, whereas if just one or few species of *Rickettsia* is tested the positive outcome may only be informative to Spotted Fever Group *Rickettsia*.

The serosurvey outcome must be used to classify a restricted area in regards to the threat to the human population. Suggestions on area classification to vector-borne-diseases have been proposed for several authors, but most of those are intended to insect vectors and may not be suitable to tick vectors.

An example of this difference in terminology is the areas where the vector is well established but laboratory tests yielded negative for presence of pathogens. In case of insect-borne-diseases this area is classified as "predisposed" because insect vector complete several generation per year and can disperse very easily; therefore, populations can be overtaken or mixed with other migrant populations. On the other hand, most ticks complete one or two generations per year and ecology of the hosts and environment play a very important role to regulate the presence or absence of a pathogen, which makes tick populations more stable. Thus, the absence of a pathogen in a tick population may show that the ecological conditions that this population is exposed to might create a refractory profile to infection. In fact, when a tick population is found free of pathogenic rickettsial agents the question should not be "When will a rickettsial agent be introduced?" but rather, "What prevents this population from being infected for so long?". An exception may be the tick species *Rhipicephalus sanguineus*, because this species is extremely anthropophilic and population of this tick can rise very fast and be suitable to have rickettsial agent introduced in by hosts that are also fed on by native tick species, such as hunting dogs that go into forests and dwell nearby or in human houses, which may cause an epizootic profile and eventually human infection, but this situation is not natural and especially different from the expected behavior of New World endemic tick species.

As a result, the terminology "predisposed areas" might not be used, but "alert areas" is suggested instead.

The full classification for areas created upon serosurvey results in regards to tick-borne-rickettsial agents is suggested below:

-"Silence areas", when no information about presence or absence of tick species is known.

-"Non-infested areas", when repeated searches for ticks yielded negative.

-"Alert areas", presence of competent vector tick species but absence of a pathogenic rickettsial agent.

-"Risk areas", presence of competent vector tick species and presence of a pathogenic rickettsial agent, but no human cases of disease have been reported.

-"Transmission areas", presence of competent vector tick species, presence of a pathogenic rickettsial agent, and human cases of disease have been reported within the last five years.

To confirm a specific area as a "Risk Area", the seropositive prevalence threshold value for a sampled group of animal depends of some variables such as the average age and animal species, and the higher the average title obtained by the IFA the higher the chance of a recent rickettsial epizootic event. Different studies on dogs and horses in South-eastern Brazil have shown a seroprevalence higher than 40% for endemic areas and smaller than 5% for non-endemic areas.

The parameters of a surveillance program must be determined specifically for each Country and ought to be an important research focus for the forthcoming years.

Ecología de las rickettsiosis en America Latina

(Ecology of spotted fever rickettsioses in Latin America)

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Spotted fever rickettsioses are caused by bacteria of the genus *Rickettsia,* which are primarily transmitted to humans through the bite of infected ticks. These bacteria belong to the so called spotted fever group (SFG), currently composed by over 20 valid species distributed through the world. While some SFG species are agents of human illness (SFG rickettsioses), others have been described only from ticks, and

are considered non-pathogenic or of unknown pathogenicity. In Latin America, at least four tick-borne SFG rickettsiae have been reported to cause illness in humans: *Rickettsia rickettsii, Rickettsia parkeri, Rickettsia massiliae,* and strain Atlantic rainforest (a *R. parkeri*-like agent). *Rickettsia rickettsii* is the agent of Rocky Mountain spotted fever, a severe, acute disease caused by the bacterium *Rickettsia rickettsii,* which has been reported in Canada, the United States, Mexico, Costa Rica, Panama, Colombia, Argentina, and Brazil. In this later country, the disease was named as Brazilian spotted fever (BSF). Current fatality rates of BSF are between 30-40% in Brazil. In the state of São Paulo, southeastern Brazil, *R. rickettsii* is transmitted mainly by *Amblyomma cajennense* in the country side, and by *Amblyomma aureolatum* in the Metropolitan area. For both ticks under natural conditions, *R. rickettsii*-infection rates are usually very low, below 1%. Interestingly, laboratory studies have shown that while *A. aureolatum* ticks are highly susceptible to *R. rickettsii* (usually 100% of the ticks become infected after feeding on rickettsemic guinea pigs), *A. cajennense* are partially refractory (only ≈20% of the ticks become infected after feeding on rickettsemic guinea pigs). In addition, transovarial transmission of *R. rickettsii* is highly efficient in *A. aureolatum,* and very low in *A. cajennense.* Thus, populations of *A. cajennense* might not be capable to sustain *R. rickettsii* infection though successive generations, unless new cohorts of infected ticks are frequently created through the feeding on rickettsemic amplifier hosts, such as capybaras (*Hydrochoerus hydrochoeris*), a common host of *A. cajennense* in BSF-endemic areas in the country side of the state of São Paulo. In fact, experimental studies have shown that capybaras are competent amplifier hosts of *R. rickettsii* for *A. cajennense* ticks. Despite of the high susceptibility of *A. aureolatum* to *R. rickettsii* infection, this tick also might not be able to sustain rickettsial infection for a long term due to the deleterious effect that *R. rickettsii* elicits to engorged females. In this case, the participation of amplifier hosts (yet to be determined) might also be needed for maintenance of infection amongst *A. aureolatum* populations in the metropolitan area of São Paulo, although in a lesser extent. Anyhow, BSF has occurred with similar incidences, always low, in both the country side and the metropolitan areas. In this case, low number of cases in the metropolitan area might occur because the highly competent vector (*A. aureolatum*) only rarely bites human; on the other side, even that *A. cajennense* frequently bites humans, the low incidence of the disease in the country side might be a result of the low vector competence of this tick, since it is partially refractory to *R. rickettsii* infection. Finally, a recent study showed that *R. rickettsii* infection among *A. aureolatum* populations in the São Paulo metropolitan area was significantly associated with degraded Atlantic forest fragments, especially in the southern part of the metropolitan area. The agent *Rickettsia parkeri* has been reported infecting ticks in the United States, Peru, Bolivia, Brazil, Argentina, and Uruguay. In South America, human cases of *R. parkeri*-caused rickettsioses have been reported only in Uruguay and Argentina, where the agent is transmitted by *Amblyomma triste* ticks. In the Atlantic coast of Brazil, *Rickettsia* sp. strain Atlantic rainforest (SARF) causes a disease very similar to *R. parkeri.* In fact, this strain is genetically very similar to *R. parkeri,* raising the possibility that both agents could be strains of a single species. SARF is primarily transmitted by the tick *Amblyomma ovale,* although *A. aureolatum* might also be important in some areas where both tick species coexist. While both *R. parkeri* and SARF are usually found infecting around 10% of the ticks in nature, nothing is known about transovarial and transstadial transmission of these agents in ticks, and neither if there is any amplifier vertebrate host for the bacteria in nature. Finally, recent studies have reported the presence of *R. massiliae* infecting *Rhipicephalus sanguineus* ticks in Argentina, from where at least one case of *R. massiliae*-caused rickettsioses was reported. Since *R. massiliae* is considered a pathogenic species in Europe, where it is transmitted by ticks of the *R. sanguineus* group, it is possible that the presence of this agent in the south cone of South America is much broader than currently recognized.

Resúmenes de ponencias

Ponencias en Modalidad Oral

1. Caracterización molecular de agentes rickettsiales y pulgas de perros y gatos en Colombia

(Molecular characterization of rickettsial agents and fleas from Colombian dogs and cats)

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Background: Serological evidence obtained for Colombians from Caldas has recently suggested that both *Rickettsia typhi* and *R. felis* infections may be arising from contact with fleas which are common on both dogs and cats in this area. We describe here the molecular characterization of rickettsial agents found in fleas, collected during a survey of clinical and epidemiological aspects of flea infestations of 140 dogs and 30 cats at Caldas Veterinary Hospital. We also performed molecular identification and characterization of the fleas.

Methods: A *glt*A duplex TaqMan was used to test DNAs from 1318 individual fleas from 14 cats and 73 dogs for the presence of *R. felis* and *R. typhi* DNAs. At least one *R. felis*-infected flea per animal was genetically typed with both plasmid and chromosomal markers. Six genes (ITS1, ITS2, 18S rRNA, 28S rRNA, COII, and EF1a were sequenced from selected fleas.

Results: *R. felis* DNA was detected in 1 of 17 *Pulex irritans* and in 45.7% of *Ctenocephalides felis* from cats (n=186) and 27.6% of *C. felis* from dogs (n=1125) while no *R. typhi* DNA was detected. No fleas from 3 cats and 17 dogs were infected. The quantity of *R. felis* DNA per infected flea was rather similar and did not depend on their animal host or vary appreciably with the percentage of infected fleas on either animal. Most *R. felis* infections were of URRWXCal2 *glt*A genotype (3 fleas had a new 1 nucleotide variant) but only 2 of the 63 *R. felis*-infected fleas tested were RF2125 type. All five less frequent variants of *R. felis* were in fleas from different dogs. Sequencing of the flea DNAs confirmed the morphological identity of the fleas but detected little genetic heterogeneity. No epidemiological or clinical factors were useful in predicting which animals would have infected fleas.

Conclusions*:* *Rickettsia felis* but not *R. typhi* was commonly present in cat fleas obtained from dogs and cats from Caldas; the high rate of flea infection and high infection level per flea found with *R. felis* may pose a significant risk of infection for their owners. Human exposure to *R. typhi* may be due to its presence in other species of fleas in Colombia.

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2. Serie de casos de pacientes con fiebre manchada de las Montañas Rocosas por *Rickettsia rickettsii* en Jujuy, Argentina

(Case series of patients with Rocky Mountain spotted fever by *Rickettsia rickettsii* in Jujuy, Argentina)

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Dos enfermedades asociadas a picaduras de garrapatas en nuestra provincia fueron reportadas por primera vez en Argentina; Primero la Fiebre Maculosa por *Rickettsia rickettsii* en 1999 y recientemente la parálisis por picadura de garrapatas "tick paralysis" en 2012. Posteriormente, en la región pampeana de nuestro país se reporta *Rickettsia parkeri.* Sin embargo, desde 1919 hasta 1946 existen datos sobre la presencia de enfermedades rickettsiales en Argentina. En nuestra provincia existen 4 focos de *R. rickettsii* confirmados. Jujuy se encuentra al norte de Argentina en una región subtropical, limitando con Bolivia y Chile.

Casos clínicos: Se describen 8 casos clínicos causados por *R. rickettsii.* Se describen y comparan sus signos clínicos y análisis de laboratorio. El nivel sospecha del equipo de salud de la provincia era casi inexistente y la mortalidad alta del 62.5%, como suele ocurrir al reportar los primeros casos. Todos fueron del sexo masculino, predominaron adolescentes y niños, solo un adulto. Compromiso del SNC presente en todos los casos; depresión del sensorio y clínica de encefalitis fueron los más frecuentes. Transaminitis y trastornos de coagulación fueron también frecuentes. La especie de garrapata prevalente en muestreos fue *Amblyomma cajennense***,** vector ya demostrado para FMMR en nuestra provincia en 2008.

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3. Detección de anticuerpos IgG contra *Rickettsia* spp. en pacientes con síndrome febril inespecífico en el Uraba Antioqueño, Colombia

(Detection of IgG antibodies against *Rickettsia spp* for patients with nonspecific febrile syndrome in the Urabá region of Antioquia, Colombia)

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Justificación: la presentación de síndromes febriles agudos es común en el Urabá Antioqueño, donde usualmente se diagnostican enfermedades como malaria, dengue y leptospirosis, las cuales presentan manifestaciones clínicas similares. La rickettsiosis hace parte de los diagnósticos diferenciales para este síndrome y poco se tiene en cuenta, aunque haya evidencia de circulación del agente y se hayan presentado dos brotes (2006 y 2008) en esta región. Considerando lo anterior, es importante identificar la frecuencia de infecciones por *Rickettsia* spp en los casos de síndrome febril y realizar un acercamiento al entorno epidemiológico de la enfermedad.

Métodos: Se tomaron sueros en fase aguda y convaleciente de 200 pacientes febriles negativos para malaria durante los años 2007 y 2008, procedentes tanto de área rural como urbana de los municipios de Necoclí, Turbo y Apartadó. Se realizaron pruebas serológicas para Rickettsiosis (IFI IgG), Dengue (ELISA IgM) y Leptospirosis (IFI IgM e IgG). Se evaluaron signos clínicos, variables sociodemográficas, medioambientales y espaciales para explorar posible asociación con estas enfermedades mediante una prueba estadística de regresión logística.

Resultados: Se encontró una frecuencia de Rickettsiosis del 3.0% (6/200), cuatro casos para el municipio de Turbo y un caso para Apartadó y Necoclí, respectivamente. A pesar del bajo número de casos, se evidenció un 20.1% (46/200) de reactividad serológica para *Rickettsia spp* sin cambio de título de anticuerpos, que se distribuyó homogéneamente entre los tres municipios. Para dengue y leptospirosis se encontraron frecuencias del 37,3% y 14,1% respectivamente. Se presentaron 12 casos de co-infección entre *Leptospira* spp y dengue, dos casos entre *Rickettsia* spp y dengue, además de un individuo con co-infección entre los tres agentes.

Conclusión: Se reafirma la importancia de *Rickettsia* spp, el virus del dengue y *Leptospira* spp como agentes causantes del síndrome febril agudo en Urabá. Debido al bajo número de diagnósticos definitivos por serología (con cambio cuádruple en el título de anticuerpos) que se obtuvo para *Rickettsia* spp, no fue posible encontrar asociaciones con las variables seleccionadas. Sin embargo, llama la atención el alto número de sueros que presentaban reactividad serológica para IgG (sin cambio de titulo), lo cual sugiere una exposición continua a este agente, y podría indicar que en los municipios de Apartado, Turbo y Necoclí, la rickettsiosis puede presentarse en forma endémica y debe ser tenida

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4. Rickettsiosis del grupo de las fiebres manchadas en pacientes con síndrome febril agudo que consultaron al Hospital Salazar de Villeta, Colombia

(Rickettsiosis of spotted fever group in patients with acute febrile syndrome who visited the Salazar Hospital of Villeta, Colombia)

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Justificación: En Colombia, el municipio de Villeta ha sido clasificado como una zona endémica para rickettsiosis del grupo de las fiebres manchadas (GFM) dados los casos de mortalidad por *Rickettsia rickettsii* en 1935, en el periodo 2003-2004 y los altos porcentajes de seropositividad para el GFM tanto en humanos como en animales domésticos evidenciados en estudios posteriores. A pesar de esto, después del año 2004 no se han vuelto a reportar casos probables o confirmados de rickettsiosis en pacientes que asisten al Hospital Salazar de Villeta, siendo la entidad hospitalaria de referencia para el propio municipio y municipios aledaños. Es así como el objetivo de este estudio fue determinar el número de casos probables de infección por rickettsias del GFM y otros diagnósticos diferenciales en pacientes con síndrome febril agudo que consultaron al Hospital Salazar en un periodo de tiempo determinado.

Métodos: Entre el mes de noviembre de 2011 y diciembre de 2012 se recolectaron muestras de suero pareadas (diferencia mayor a 15 días pero menor a 2 meses entre la muestra de fase aguda y la de fase convaleciente) en pacientes con síndrome febril agudo (diagnóstico presuntivo de infección por Virus Dengue) que consultaron al Hospital Salazar de Villeta. Mediante Inmunofluoerescencia indirecta (IFI) se determinaron anticuerpos de tipo IgG contra *Rickettsia rickettsii* y *R. amblyommii* en las muestras pareadas, teniendo en cuenta como positivo una dilución ≥ 1:64 y definición de caso probable con seroconversión ≥ a 4 títulos o 2 veces la dilución. A su vez, a estos mismos pacientes se les realizó IgM para Virus Dengue y Leptospira en la muestra de fase aguda.

Resultados: Un total de 293 pacientes con síndrome febril agudo consultaron al Hospital Salazar durante el periodo referenciado. En 95 pacientes (32%) se logró obtener muestras de suero pareadas. De estos, 14 pacientes (14,7%) fueron casos probables de rickettsiosis del GFM (8 *R. rickettsii* y 6 *R. amblyommii*). Por su parte, 38 pacientes (40%) presentaron IgM positiva para Virus Dengue y 12 pacientes (12,6%) IgM positiva para Leptospira. Cabe destacar que de los 95 pacientes, 62 (65%) presentaron títulos de anticuerpos ≥ 64 en por lo menos una de las muestras de suero, para por lo menos una especie de Rickettsia del GFM, sin presentar seroconversión diagnóstica.

Conclusión: Las rickettsiosis del GFM hacen parte de la etiología del síndrome febril agudo en pacientes que consultaron al Hospital Salazar de Villeta en el periodo referenciado.

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5. Rickettsiosis en la República Argentina

(Rickettsiosis, Argentina)

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Justificación: Están establecidos dos complejos patogénicos: provincias del noroeste (NOA), Salta y Jujuy, involucra a *Rickettsia rickettsii* y *Amblyomma cajennense*; Delta Paranaense, Buenos Aires y Entre Ríos, asociado con *R parkeri* y *Amblyomma triste*. Casos en otras regiones, zonas aledañas a la Bahía de Samborombon (BS) (centro y costa de Buenos Aires), Córdoba y Chaco, plantean una extensión mayor. Objetivo: describir características epidemiológicas y clínicas de rickettsiosis en Argentina.

Métodos: Análisis retrospectivo de pacientes asistidos en Zoonosis, Hospital Muñiz de Buenos Aires con rickettsiosis. La sospecha se basó en clínica compatible y antecedentes de riesgo epidemiológico. Se confirmó mediante métodos moleculares (PCR y secuenciación) en biopsia de piel o seroconversión con microinmunofluorescencia.

Resultados: Se diagnosticaron 15 pacientes. Adquisición: 7 Delta, 4 BS, 3 Córdoba, 1 Chaco. Edad promedio 50 años (38-76) Sexo masculino 10/14. Todos menos uno presentaron escara de inoculación. 8/14 en región craneana. Frecuencia de síntomas: fiebre y exantema 14/14 (vesicular 10, maculopapular 15, purpúrico 2), cefalea y mialgias fueron frecuentes. Todos menos uno presentaron la tríada fiebre, exantema y escara. Ningún paciente tuvo compromiso sistémico. El paciente chaqueño presentó exantema petequial, múltiples mordeduras de garrapata, sin escara. Diagnóstico: 4 por secuenciación de fragmento de *R parkeri* en biopsia (BS, Córdoba y Delta), 11 conversión serológica.

Conclusión: La enfermedad tuvo presentación homogénea, con exantema febril de evolución benigna. La detección de *R parkeri* en BS y Córdoba representa la primera identificación. En BS se identificó *R parkeri* en ejemplares de *A triste* (datos no publicados), por lo cual podría describirse como una extensión del área endémica delta, compartiendo vector y características fitogeográficas. Diferente acontece en Córdoba, donde la fitogegrafía no representa un ecosistema propicio para *A triste*, involucrando probablemente a otra garrapata. El paciente de Chaco con diferencias clínicas y epidemiológicas, representaría una extensión del complejo NOA. Es probable que el inicio precoz del tratamiento haya evitado una evolución grave.

La rickettsiosis por *R parkeri* presenta una amplia distribución en la región litoral y central de Argentina, abarcando al menos tres provincias. Restan estudios para identificar vectores involucrados en la región central. Es probable que el área NOA se extienda a más provincias de las descriptas. Es importante insistir en la relevancia de extremar las medidas para arribar al diagnóstico etiológico en los pacientes con cuadros clínicos compatibles, así como realizar estudios en vectores que ayuden al conocimiento de la ecología de la enfermedad en Argentina.

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6. *Rickettsia* spp. del grupo fiebres manchadas asociadas con perros (*Canis lupus familiaris*) de sitios urbanos con casos de fiebre manchada de las Montañas Rocosas en San José, Costa Rica

(Spotted fever group *Rickettsia* spp. associated with dogs (*Canis lupus familiaris*) from urban sites with reports of human Rocky Mountain spotted fever in San Jose, Costa Rica)

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Background: Rickettsiae are obligate intracellular bacteria, many of which cause zoonotic diseases worldwide. *Rickettsia rickettsii* is responsible for Rocky Mountain spotted fever (RMSF) in humans, and disease has also been reported in dogs. In the past 5 years, 3 cases of RMSF have been diagnosed in San Jose, Costa Rica. No animals or tick species were associated with these cases, which poses new challenges regarding the local epidemiology of RMSF. The aim of this study was to analyze the role of dogs and their ectoparasites in transmission cycles of rickettsiae in areas associated with human cases of RMSF from San José.

Methods: For each RMSF human case, dogs were identified within a radius of approximately 100 m from specific sites where transmission may have occurred. Blood samples were drawn and ectoparasites were collected from dogs. Presence of IgG antibodies to SFG *Rickettsia* was evaluated by immunofluorescence using *R. rickettsii*, *Rickettsia amblyommii*, and *Rickettsia felis* antigen. Samples with titers ≥ 1:32 were considered positive, and an end titer was determined by two-fold serial dilutions. Seroreactive samples were further analyzed using antigen of *Rickettsia bellii, Rickettsia rhipicephali*, and *Rickettsia parkeri*. Ectoparasites were pooled and analyzed by a PCR targeting the citrate synthase gene (*gltA*) of *Rickettsia* spp.

Results: Antibodies to SFG *Rickettsia* were present in dogs from all sites. A total 21.4% (36/168) of dogs were positive, but seroreactivity varied from 6.5% (4/62) to 45.8% (11/24) between the 5 sites sampled. Antibodies to *R. rickettsii* and *R. amblyommii* were detected more frequently and with higher titers. Seroreactivity to *R. felis*, *R. rhipicephali*, and *R. parkeri* was also detected, although with much lower end titles than for *R. amblyommii* and *R. rickettsii* in the same sample. End titers varied from 1:32 to 1:2048. *Ctenocephalides felis* and

Rhipicephalus sanguineus were the most common ectoparasites collected. Sequencing identified DNA of *Rickettsia felis* in 23.1% of fleas (18/78).

Conclusions: These results demonstrate the occurrence of SFG rickettsia infection in dogs from sites in San Jose associated with human cases. Considering that *R. sanguineus* and *C. felis* are common and that they are capable of transmitting *R. rickettsii* and *R. felis* to humans, the possible role of dogs and their ectoparasites in the maintenance of these pathogenic rickettsiae in urban environments requires further investigation. Moreover, we propose dogs as urban sentinels to study the epidemiology of *Rickettsia rickettsii* in humans and animals of urban areas.

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7. Enfermedades transmitidas por garrapatas en EEUU: incremento en incidencia e identificación/control de nuevo foco de FMMR

(Tick-borne diseases in the USA: Increasing incidence and identification/ control of a novel focus of RMSF)

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Background: Evaluate incidence of rickettsial tick-borne diseases in the USA and identification of a focus of disease in the USA involving the brown dog tick and canids.

Methods: The CDC surveillance data includes 3 tick-borne rickettsial diseases listed as nationally notifiable: spotted fever group (SFG) rickettsiae, human anaplasmosis, and human ehrlichiosis. A focus of Rocky Mountain spotted fever (RMSF) was identified, characterized, and a pilot prevention project initiated.

Results: Cases of SFG rickettsiae, ehrlichiosis and anaplasmosis increased dramatically from 1130 cases in 2001 to 6,498 cases in 2011. In particular, SFG rickettsial infections increased from <1,000/year prior to 2002 to 2,802 and 3825 cases in 2011 and 2012, respectively. Additional changes in SFG rickettsial disease reports include the identification of novel human-disease causing agents (*R. parkeri*, 364D), a decrease in the number of confirmed cases, and the identification of an epidemic of RMSF in the southwest USA. While *Dermacentor variabilis* and *D. andersoni* tick species have generally been implicated as the vectors of *R. rickettsii* in the USA, *Rhipicephalus sanguineus* (brown dog) ticks were responsible for transmission in the southwest USA. In southwestern communities affected by RMSF, the incidence rate for 2010-2011 was 130 cases per 100,000 persons (national rate <1 case per 100,000). Likewise, the RMSF case-fatality rate was 7%, compared to <1% for the rest of the USA. Brown dog ticks are present throughout the Americas and have been implicated as the vector during several recent RMSF outbreaks in Mexico. In 2012 the CDC collaborated with local public health authorities in the southwest USA to perform a pilot RMSF prevention project on a single community of approximately 600 homes. This project involved dog control, acaracide application on dogs and in the environment , and public education, and resulted in a significant reduction in the tick population with <1% of dogs in the pilot community harboring ticks compared to >60% of dogs in neighboring non-treated communities.

Conclusion: Cases have increased significantly for spotted fever group rickettsiae, human anaplasmosis, and human ehrlichiosis within the past decade. The agents of these diseases are transmitted by different tick species, and often in different regions of the USA, suggesting a general increase in tick populations and a corresponding increase in human risk. The brown dog tick and canids were implicated in an outbreak of RMSF and likely play a prominent role in disease transmission throughout the Americas.

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8. *Rickettsia* spp. en *Amblyomma ovale* de Colombia

(*Rickettsia* spp. in *Amblyomma ovale* from Colombia)

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Justificación: La fiebre de Tobia (causada por *Rickettsia rickettsii*), reportada por Patiño y cols. en 1937, fue el primer brote de enfermedad rickettsial en Colombia, el cual fue seguido por un largo silencio epidemiológico hasta el año 2003 cuando Hidalgo y cols. reportaron algunos casos aislados de rickettsiosis. Posteriormente, se presentaron dos nuevos brotes en los años 2006 y 2008 en los municipios de Necoclí y Turbo, respectivamente, ubicados en la zona de Urabá, noroeste de Colombia en los cuales se confirmaron 28 casos, 9 de ellos fatales. El objetivo de este trabajo fue buscar especies de *Rickettsia* en garrapatas de animales domésticos y silvestres en los dos municipios donde se reportaron estos últimos brotes ocasionados por *Rickettsia* spp.

Métodos: Se recolectaron garrapatas de diferentes especies de animales domésticos y silvestres de los municipios de Necoclí y Turbo durante los años 2010 y 2011. Las garrapatas fueron transportadas al laboratorio Centauro de la Universidad de Antioquia en el mínimo tiempo para garantizar viabilidad. A todas las muestras se les realizó extracción de ADN, PCR con los genes *gltA, ompB* y *ompA*, y posterior secuenciación para la búsqueda de bacterias del género *Rickettsia* y análisis

filogenéticos de los productos obtenidos utilizando el programa MEGA 5.1.

Resultados: Se recolectaron 60 garrapatas adultas de la especie *Amblyomma ovale*, de las cuales 37 (61,6%) llegaron vivas al laboratorio y fueron congeladas, 23 (38,4%) llegaron muertas y se almacenaron en isopropanol. Todas las garrapatas adultas fueron recolectadas de perros. Se recolectaron 20 ninfas también *A. ovale*, 16 de ellas en roedores silvestres, 3 en marsupiales y una en un canino. Diez (27%) de las garrapatas vivas y dos (9%) de las muertas fueron positivas por PCR para el gen *gltA*. Los análisis filogenéticos de dicho gen mostraros que circulan dos especies de *Rickettsia* en *A. ovale,* una de ellas relacionada con *Rickettsia bellii* y otra con secuencia idéntica a *Rickettsia* sp Atlantic rainforest, especie patógena reportada en Brasil en la misma especie de garrapatas.

Conclusión: Este es el primer reporte de circulación de *Rickettsia* spp. en *A. ovale* en Colombia y el segundo reporte de una *Rickettsia* potencialmente patógena del grupo de las fiebres manchadas en este país.

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9. Identificación molecular de *Ehrlichia chaffeensis* en perros y animales silvestres de Costa Rica

(Molecular identification of *Ehrlichia chaffeensis* in dogs and wildlife animals of Costa Rica)

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Justificación: *Ehrlichia chaffeensis* es el agente etiológico de la ehrlichiosis monocítica humana (CME), asimismo es causante de enfermedad en el perro. En el ciclo de trasmisión se involucra como principal agente reservorio el venado cola blanca, *Odocoileus virginianus.* Entre otros reservorios implicados se citan el coyote, el zorro, el lobo, el mapache y la cabra. El vector principal de transmisión es la garrapata *Amblyoma americanum.* En Costa Rica se ha demostrado la presencia de *Ehrlichia canis* en perros por medio de frotis sanguíneo, reacción en cadena de la polimerasa (PCR) y serología. La imposibilidad de diferenciar por microscopía la *E.canis* de la *E.chaffeensis* y ante el hallazgo de mórulas en frotis sanguíneos de perros sintomáticos, pero negativos por PCR a *E.canis*, condujo a sospechar de la presencia de *E.chaffensis* en estos animales. El objetivo del presente estudio fue identificar *E.chaffensis* en perros y en reservorios naturales como el venado cola blanca (*Odocoileus virginianus),* perezosos *(Bradypus variegatus* y *Choloepus hoffmanni),* mapaches *(Procyon lotor),* por medio de PCR anidado.

Métodos: El estudio se realizó en 39 perros de diferentes razas, sexo y procedencia, con síntomas clínicos de erhlichiosis, presencia de mórulas y alteraciones hematológicas pero negativos a *E.canis* por PCR. Los perros eran pacientes del Hospital de Especies Menores y Silvestres (HEMS) de la Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica. Se muestrearon también 35 animales silvestres procedentes de diferentes zonas geográficas de Costa Rica, (28 venados, 4 mapache y 3 perezosos). La identidad de ADN amplificado para *E. chaffeensis* se confirmó por secuenciación y alineamiento múltiple de una región parcial del gen 16S ARN ribosomal.

Resultados: Se demostró la presencia de *E.chaffeensis* en 23 (59%) perros, 13 (46%) venados, 3 (100%) perezosos y 3 (75%) mapaches. Los productos amplificados fueron similares a cepas de *E.chaffeensis* reportadas en el GenBank (AF416764.1 y EU181140.1).

Conclusiones: Los hallazgos del presente estudio evidencian por primera vez la presencia de *E.chaffeensis* en perros, venados cola blanca, perezosos y mapaches de Costa Rica. El carácter zoonótico de la rickettsia y el estrecho contacto del humano con las mascotas, hace imperativo reforzar las medidas profilácticas y sanitarias en el país, entre ellas el control de ectoparásitos. El diagnóstico de ehrlichiosis canina debe realizarse por medio de PCR simultáneamente para *E.canis* y *E.chaffeensis* para lograr así la debida identificación del agente y evitar falsos negativos de infección. La presencia en animales silvestres de *E.chaffeensis* subraya la necesidad de controlar la fuga de reservorios silvestres a áreas urbanas.

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10. Detección molecular de *Ehrlichia chafeensis* en una población humana de Costa Rica

(Molecular detection of *Ehrlichia chaffeensis* in a human population from Costa Rica)

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Justificación: La ehrlichiosis en Costa Rica se informó por primera vez en 1995, y desde entonces, una amplia distribución de la de infección por *E. canis* en perros ha sido reportada en base a los resultados hematológicos y serológicos. Recientemente, la detección molecular de *E. canis* ha sido implementada exitosamente en Costa Rica. En humanos, hay pocos reportes de ehrlichiosis, basados únicamente en el diagnóstico hematológico. En vista que las herramientas diagnósticas para ehrlichiosis humana son limitadas y con baja sensibilidad, se hace necesario optimizar una técnica molecular sensible y relativamente rápida para el estudio de casos sospechosos.

Métodos: En este trabajo se estudió una población humana con antecedentes clínicos y epidemiológicos sugestivos de contacto con *Ehrlichia*, y se recolectaron 20 muestras de sangre total

para su análisis por medio de PCR anidado. 17 pacientes (85 %) fueron previamente diagnosticados como positivos mediante citología hematológica.

Resultados: Un total de 3 muestras (15 %) resultaron positivas para el producto esperado de amplificación, y la secuenciación posterior confirmó la homología con el segmento de ARNr 16S de *Ehrlichia chafeensis*.

Conclusión: La detección molecular demostró ser altamente específica para la confirmación de casos sospechosos en humanos, al contrario del diagnóstico citohematológico. La optimización de esta técnica para uso diagnóstico en humanos, en conjunto con los datos clínicos y epidemiológicos, provee una herramienta útil y confiable al servicio de la salud pública en Costa Rica.

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11. Seroprevalencia de *Ehrlichia canis* y *Anaplasma phagocytophilum* en perros que visitan parques recreativos de Costa Rica – estudios preliminares

(Seroprevalence of *Ehrlichia canis* and *Anaplasma phagocytophilum* of dogs that visit recreational areas in Costa Rica – preliminary results)

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Justificación: En estudios anteriores se ha logrado determinar la presencia de *Ehrlichia canis* y *Anaplasma phagocytophilum* en perros y garrapatas de Costa Rica, sin embargo se desconoce la seroprevalencia de estos agentes en perros que visitan parques recreativos. La importancia de determinar la prevalencia en perros sanos radica, en que estos animales pueden ser utilizados como animales centinelas, para estimar el riesgo de infección que tiene la población humana. El objetivo del presente trabajo fue establecer la seroprevalencia de *E. canis* y *A. phagocytophilum* que visitan parques recreativos de Costa Rica y, adicionalmente, comparar dos técnicas de diagnóstico serológico para *E. canis*.

Métodos: Durante el 2011 y 2012 se visitaron 15 parques recreativos (La Sabana, Desamparados, La Paz, Barrio México Aserrí, Monte de la Cruz, Agricultor, Ciudad Colón, Instituto Tecnológico, Vargas-Asís Esna, Guápiles, La Fortuna, Quebrada Ganado y Cañas) en las siete provincias y se recolectaron 429 sueros de perros (31 animales en promedio por parque, mínimo 16 y máximo 62). Se han analizado 290 sueros mediante Inmunocromatografía de Membrana (IM) para la detección de anticuerpos contra *E. canis* (Speed Ehrli, Virbac®), y mediante Inmunofluorescencia Indirecta (IFI) para la detección de anticuerpos contra *E. canis* y *A. phagocytophilum* (*E. canis* and *A. phagocytophilum* MIF Canine IgG Antibody Kit, Fuller Laboratories®).

Resultados: De 290 muestras analizadas, 87 (30,0%) resultaron positivas para *E. canis* con IM; 41(14,1%) muestras tuvieron resultado indeterminado. En contraste, IFI detectó 70 (24,1%) muestras positivas para *E. canis* (punto de corte 1:80); 12 (4,1%) tuvieron resultado indeterminado. Se encontraron anticuerpos contra *A. phagocytophilum* en 11 (3,8%) muestras, mientras que en 8 (2,7%) no se determinó resultado utilizando IFI. La IM e IFI para *E. canis* tuvieron concordancia en el resultado de 241 sueros (83,1%), determinándose, para la IFI, una sensibilidad de 70,9%, especificidad de 96,1%, VPP de 91,0%, VPN de 85,6% (IM estándar de oro). De las muestras seropositivas, 20 (16 seropositivos a *E. canis*, 3 seropositivos a *E. canis* y *A. phagocytophilum* y 1 seropositivo a *A. phagocytophilum*) mostraron alteraciones en los valores del hemograma (hematocrito, hemoglobina y conteo plaquetario menores al valor de referencia y linfocitos mayores al valor de referencia).

Conclusión: Los resultados de este estudio preliminar muestran una alta y baja seroprevalencia para *E. canis* y *A. phagocytophilum*, respectivamente, en caninos que visitan parques recreativos de Costa Rica.

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12. Determinación de *Ehrlichia canis* y *Anaplasma platys* en perros de Costa Rica

(Determination of *Ehrlichia canis* and *Anaplasma platys* in dogs from Costa Rica)

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Justificación: La ehrlichiosis canina (EC) y la anaplasmosis son enfermedades provocadas por las bacterias intracelulares *Ehrlichia canis* y *Anaplasma platys*, respectivamente; y transmitidas principalmente por la garrapata *Rhipicephalus sanguineus*. La EC se ha reportado con alta prevalencia a nivel mundial. Al contrario, la anaplasmosis solo se ha determinado en algunos países del continente americano. Ambas infecciones comprometen la salud de los animales, y aumenta si existe coinfección con los dos patógenos. Este trabajo busca determinar la presencia de *E. canis* y *A. platys* en perros de diferentes zonas de Costa Rica.

Métodos: Se efectuó un muestreo no probabilístico a conveniencia de 146 perros de las zonas de San Ramón, Punta Morales, Talamanca y Liberia, en Costa Rica. Se obtuvo muestras de sangre en EDTA de cada animal y se extrajo el ADN con un kit comercial. El diagnóstico molecular se realizó mediante la técnica de la reacción en cadena de la polimerasa

(PCR) convencional que amplifica un fragmento de 345 bp del gen *16S* de *Ehrlichia* spp. y *Anaplasma* spp. Las muestras fueron reevaluadas por medio de un PCR tiempo real acoplado con análisis de desnaturalización de los productos (“*melt analysis*”), que detecta un fragmento de 150 bp del gen *16S* de *E. canis*.

Resultados: Se obtuvo un 34,2% (50/146) de perros positivos por *Ehrlichia canis* y 9,6% (14/146) por *Anaplasma platys,* mediante el uso de ambas técnicas. Sorpresivamente, se encontró que los primeros empleados en el PCR convencional, amplificaron también *Wolbachia* sp. presente en las muestras. El PCR tiempo real detectó más muestras positivas por *E. canis* que el PCR convencional. Además, con el empleo de ambos ensayos se pudo demostrar la co-infección con *E. canis* y *A. platys* en 2,7% (4/146) de las muestras analizadas. Por último, Liberia y Punta Morales mostraron la mayor presencia de *E. canis* (58% ambos), en comparación con San Ramón (17%; $p<0.001$) y Talamanca (4,7%; $p<0.001$).

Conclusión: Este trabajo demuestra una alta presencia de *E. canis* en perros de Costa Rica. Adicionalmente, representa el primer reporte de coinfección con *A. platys* y *E. canis* en perros de este país. Por ende, es importante aumentar la vigilancia de enfermedades transmitidas por vectores, para así reducir el impacto producido por estos y otros patógenos.

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13. Anaplasma sp. y *Ehrlichia* sp. en garrapatas de bovinos, equinos y caninos del área tropical de Córdoba, Colombia

(Anaplasma sp. and *Ehrlichia* sp. in ticks collected from cattle, horses and dogs in a tropical area of Cordoba, Colombia)

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Justificación: En Colombia, no se han comfirmado casos de anaplasmosis ni de ehrlichiosis en humanos. Sin embargo, estudios serológicos demuestran la presencia de anticuerpos en personas del Caribe colombiano (Córdoba). La búsqueda de estos agentes en garrapatas permitirá determinar las especies y la posible asociación con casos humanos en el área. Se pretende detectar por PCR la presencia de *Ehrlichia* sp. y *Anaplasma* sp. en garrapatas de caninos, equinos y bovinos de 7 municipios del departamento de Córdoba.

Métodos: Estudio descriptivo-prospectivo de corte transversal, 2011-2012, realizado en los meses de verano y lluvias. Se capturaron garrapatas de la familia *Ixodidae* de bovinos, equinos y caninos en siete municipios de Córdoba (Montería, Planeta rica, Los Córdoba, Ciénaga de oro, Sahagún, Carrizal y Pelayito). Para la detección molecular de *Anaplasma* sp. se amplificó un fragmento del gen 16s ARNr, con los iniciadores GE2: F (5-GTTAGTGGGAGACGGGTGAGT-3) y HE3: R (5-TATAGGTACCGTCATTATCTTCCCTAT-3). Que amplifican un fragmento de 360pb. Para *Ehrlichia* sp. se amplificó un fragmento del gen *dsb* con los iniciadores; Dsb 330 (5-GATGATGTCTGAAGATATGAAACAAAT-3)F y Dsb 728 (5-CTGCTCGTCTATTTTACTTCTTAA AGT-3) R. que amplifican un fragmento de 409pb. Los productos fueron secuenciados y analizados (BLAST) para determinar la identidad con otras especies.

Resultados: Se recolectaron 1.105 garrapatas de 226 bovinos, 87 caninos y 19 equinos de los 7 municipios, los ectoparásitos se agruparon en 332 grupos. Las especies fueron clasificadas como: *Rhipicephalus microplus* 679 (61.5%), *Rhipicephalus sanguineus* 353 (32%) y *Dermacentor nitens* 73 (6,6%). De los 332 grupos analizados por PCR, once (3.3%), resultaron positivos para *Ehrlichia* sp. tres grupos de garrapatas eran de la especie *Rh. sanguineus,* de caninos y 8 grupos pertenecían a *Rh. microplus* de bovinos. Para *Anaplasma* sp. 8 grupos (2.4%) resultaron positivos, 7 grupos de la especie *Rh. microplus* de bovinos y 1 de la especie *D. nitens* de equinos. Los análisis preliminares de las secuencias muestran que para *Ehrlichia* las secuencias tienen una identidad entre el 99 - 100% con las especies *Ehrlichia ewingii*, *E. chaffensis* y *E. canis*. Para *Anaplasma*, siete secuencias tienen una identidad entre el 99 – 100% con *A. marginale*. La muestra restante tiene un porcentaje de identidad del 99% con *A. phagocytophilum*.

Conclusión: Este es el primer estudio que demuestra en el Caribe colombiano la presencia de *Ehrlichia* y *Anaplasma* en garrapatas. Los resultados demuestran que existe un riesgo potencial de transmisión a los humanos y que es necesario establecer una vigilancia epidemiológica de la infección por estos agentes.

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14. Distribución geográfica de *Amblyomma cajennense* y *Amblyomma ovale* en Colombia basada en modelos de nicho ecológico

(Geographic distribution of *Amblyomma cajennense* and *Amblyomma ovale* in Colombia based on ecological niche modeling)

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Justificación: El grupo de las fiebres manchadas del genero *Rickettsia* spp. reviste particular importancia por contener especies patógenas (por ejemplo *Rickettsia rickettsii)* y por estar asociado a vectores como las garrapatas de la familia *Ixodidae*. La distribución geográfica de estas garrapatas está determinada en parte por elementos bióticos como la vegetación y abióticos como temperatura y humedad relativa, entre otros. Entender las condiciones que determinan la distribución permite identificar

áreas potenciales de riesgo para la transmisión de *Rickettsia* spp. El objetivo de este estudio es predecir áreas potenciales de distribución geográfica de las garrapatas *A. cajennense* y *A. ovale* en Colombia.

Métodos: Se generaron modelos de distribución potencial basada en modelado de nicho ecológico, utilizando Sistemas de Información Geográfico y el software Maxent 3.3.3k, tomando 70 puntos de presencia georreferenciados, reportados en la literatura y colectados en campo por el grupo de investigación. Se utilizaron 19 variables ambientales de la base de datos WorldClim y 4 variables topográficas. Los modelos se validaron mediante la curva ROC y el área bajo la curva (AUC).

Resultados: Para *A. cajennense* el modelo predice distribución en el departamento de Nariño y en zonas de los valles interandinos, especialmente del rio Magdalena y en la zona noroeste del país. El modelo para *A. ovale* detecta zonas de alta probabilidad de presencia en los valles interandinos, zona noroeste y región Caribe. Las variables que más aportaron a los modelos son temperatura, precipitación y elevación. Se observa en ambos modelos, baja probabilidad de presencia en las regiones de los Llanos Orientales y Amazonia.

Conclusión: los modelos obtenidos muestran que las condiciones ambientales de los valles interandinos y la región noroeste de Colombia son favorables para la distribución de *A. cajennense* y *A. ovale,* y pueden ser consideradas, potenciales áreas de riesgo para la transmisión de *Rickettsia* spp. La ausencia de datos en la zona oriental y sur del país puede explicar la baja probabilidad de presencia obtenida en los modelos.

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15. Rickettsia y garrapatas en roedores pequeños de un parque urbano, Uberlândia, Minas Gerais, Brasil

(Rickettsia and ticks in small rodents from an urban park, Uberlândia, Minas Gerais, Brazil)

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Background: Large rodents like capybaras are already known to be amplifying hosts for *Rickettsia* spp. However, small rodents are important hosts of the immature stages of ticks, especially the genera *Ixodes* and *Amblyomma* and its role in the epidemiology of rickettsioses is still poorly known. Serology of rodents may indicate *Rickettsia* circulation in a region. The Parque do Sabiá is an important area for sports and environmental preservation, with a history of numerous tick bites in humans. Thus, a survey was conducted with serum from small rodents to detect *Rickettsia* circulation in Parque do Sabiá, Uberlândia city, Minas Gerais state.

Methods: Small mammals were captured between March 2011 and December 2012, every two months, with Sherman and Tomahawk cages spread on trails within the forest. The rodents were anesthetized by intramuscular injection of Ketamine-Xylazine combination, identified to species according to field guides, examined for the presence of ticks, submitted to blood collection by caudal vein puncture, banded, and released at the capture site. The blood was centrifuged to obtain serum, labeled and stored at -20 ° C until the realization of the immunofluorescence assay (IFA) for *R. rickettsii*, *R. parkeri*, *R. amblyommii*, *R. rhipicephali* and *R. bellii*. The slides containing the specific antigens of *Rickettsia* spp. were provided by FMVZ/USP, São Paulo. Titres >= 64 were considered positive. Positive sera were titrated to the endpoint titres by dilution in 2-fold increments.

Results: There were fifty-nine capture events of four rodent species: forty nine *Oecomys bicolor* (Thomas 1906), six *Rhipidomys macrurus* (Tschudi 1844), three *Rattus norvegicus* (Berkenhout, 1769) and one *Oligoryzomys* sp. (Bangs 1900). Eleven animals were infected with ticks (18,6%). Nine *O. bicolor* hosts six nynphs of *Ixodes loricatus*, nine larvae of *Amblyomma* sp. and six larvae of *Ixodes* sp. Four larvae of *Amblyomma* sp. were found in two *R. macrurus*.

The serological survey was realized in forty-eight rodents. Seven rodents displayed anti-*Rickettsia* antibodies (11,8%). *R. norvegicus* had the higher endpoint titres (1:4096 to *R. parkerii*, 1:2048 to *R. rickettsii*, *R. amblyommii*, *R. rhipicephali* and *R. bellii*). Four *O. bicolor* reacted to *R. rhipicephali* (1:128), *R. amblyommii* and *R. bellii* (1:64).

Conclusion: Results suggest *Rickettsia* circulation in the Parque do Sabiá. The participation of small rodents as reservoirs or amplifying hosts in nature has not been established and therefore they are important targets for further investigation.

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16. Caninos silvestres (*Pseudalopex* sp.) como posibles hospederos de *Rickettsia andeanae* en Chile

(Wild canids (*Pseudalopex* sp.) as possible hosts of *Rickettsia andeanae* in Chile)

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Justificación: *Rickettsia andeanae* ha sido identificada en garrapatas en algunos países de América y recientemente en varias regiones de Chile. La ecología, hospederos y ciclo natural de este nuevo agente no están aún descritos.

Objetivos: Determinar presencia de *Rickettsia andeanae* en garrapatas de perros y zorros y de anticuerpos séricos anti-*Rickettsia* spp. en estos animales, para evaluar si los caninos silvestres pudieran ser hospederos de este agente.

Métodos: Estudio transversal en la Región de Coquimbo, norte de Chile. Se efectuó muestreo domiciliario de perros (uno por vivienda) en la ciudad de Coquimbo y localidades rurales cercanas, en primavera-verano 2011-2012, y de zorros en áreas rurales, capturados mediante cepos acolchados, sedados para la toma de muestras y posteriormente liberados. Se recolectaron garrapatas si estaban presentes y una muestra de sangre de cada animal. Todos los sitios de muestreo fueron georeferenciados. Se realizó análisis taxonómico de las garrapatas y amplificación y secuenciación de los genes gltA y ompA en ellas. Considerando la reactividad cruzada entre las especies, se determinaron anticuerpos séricos contra *Rickettsia* utilizando antígenos de *R. amblyommi* y *R. parkeri,* mediante un test de inmunofluorescencia *in house*.

Resultados: se examinaron 226 perros y 25 zorros: 20 zorros chilla (*Pseudalopex griseus*) y 5 zorros culpeo (*Pseudalopex culpaeus*). En la ciudad, 53% (60/114) de los perros tenían garrapatas, todas correspondían a *Rhipicephalus sanguineus.* En localidades rurales, 62% (69/112) de los perros tenían garrapatas: *Rhipicephalus sanguineus* (84%) y *Amblyomma tigrinum* (26%). 92% (23/25) de los zorros tenían garrapatas, todas ellas *Amblyomma tigrinum.* Se identificó *Rickettsia andeanae* en *Amblyomma tigrinum* de 8/18 perros rurales (44%) y de 20/23 zorros (87%). Siete de los 8 perros con garrapatas con *Rickettsia andeanae* vivían en el área donde se muestrearon los zorros. No se encontró *Rickettsia andeanae* en *Rhipicephalus sanguineus*. La seroprevalencia en perros urbanos, rurales y zorros anti-*Rickettsia parkeri* fue de 23%, 58% y 72%, respectivamente y anti-*Rickettsia amblyommi*, de 22%, 29% y 84%.

Conclusión: La exclusividad de la presencia de *Amblyomma tigrinum* y de *Rickettsia andeanae* en zonas rurales, la coincidencia geográfica de perros y zorros infestados con estos agentes y la gradiente creciente del parasitismo por *Amblyomma tigrinum*, de la infección de esta garrapata por *Rickettsia andeanae* y de los anticuerpos anti-*Rickettsia* spp. en perros urbanos, rurales y zorros, indican al zorro como un posible hospedero de este agente rickettsial y sugieren una direccionalidad de la infección desde zorros a perros de zonas rurales.

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17. Infecciónes por rickettsia en garrapatas de aves silvestres de Paraguay

(Rickettsial infections in ticks collected from wild birds in Paraguay)

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Background: There is no evidence on the presence of *Rickettsia* in Paraguay, although in neighbor countries Brazil, Argentina and Bolivia, the presence of pathogenic *Rickettsia* for humans has been confirmed. Our objective was to conduct a study to identify *Rickettsia* spp. in ticks collected on wild birds in Paraguay.

Methods: Wild birds were caught in San Rafael, Agripino Enciso, and Tres Gigantes in Paraguay during summer 2012 and subjected to examination for the presence of ticks. All collected ticks were identified and individually tested for the presence of *Rickettsia* by PCR using primers targeting a fragment of the *gltA* and a fragment the *ompA* genes.

Results: Ticks collected on birds were identified as *Amblyomma calcaratum* (two larvae, 20 nymphs), *Amblyomma longirostre* (17 larvae, three nymphs), *Amblyomma parvum* (seven nymphs), *Amblyomma aureolatum* (one nymph), *Amblyomma ovale* (one nymph), *Amblyomma tigrinum* (one larva), and *Amblyomma* spp. (four larvae). Two (12%) out of 17 *A. longirostre* larvae were found infected with "*Candidatus* Rickettsia amblyommii" and two (33%) out of six *A. parvum* nymphs were infected with "*Candidatus* Rickettsia andeanae".

Conclusion: We present here the first report of rickettsial infections among the Paraguayan tick fauna.

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18. Infección experimental de caballos con *Rickettsia rickettsii* y evaluación de la transmisión a garrapatas *Amblyomma cajennense*: resultados preliminares

(Experimental infection of horses with Rickettsia rickettsii and evaluation of transmission to Amblyomma cajennense ticks - preliminary results)

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Background: In Brazil, one of the vectors for the bacterium *Rickettsia rickettsii* is the tick *Amblyomma cajennense*. Horses are one of the preferred hosts for the tick, but there is no information about the role of these animals as amplifier host for the agent. The study aimed to evaluate: possible clinical signs in horses experimentally infected with *R. rickettsii;* the occurrence and duration of rickettsemia; the anti-*R. rickettsii* antibody curve; and the occurrence of transmission of the bacterium from horses to *A. cajennense* ticks.

Methods: among three serologically negative horses for *Rickettsia* spp, two were infested with *R. rickettsii*-infected *A. cajennense* adult ticks, and the third was inoculated intraperitoneally with a homogenate of guinea pig organs

infected with *R. rickettsii*. All horses were infested with larvae, nymphs and adults of uninfected *A. cajennense*. The three horses were monitored for clinical signs and collection of engorged ticks for 30 days post-infection. Within this period, blood samples were taken every 2 days for hemogram, real-time PCR of whole blood for the detection of *Rickettsia* spp, biological test by inoculating whole blood in guinea pigs, and indirect fluorescent antibody test (IFAT) for the detection of anti-*R. rickettsii* antibodies. Additionally, serum biochemistry was performed every 6 days. After 30 days, only the IFAT was performed for 180 days post-infection. Ticks recovered from horses, following molting or oviposition, were fed on rabbits and were subjected to real-time PCR for detection of *Rickettsia* spp.

Results: no horse showed clinical, hematologic or serum biochemistry abnormalities. All blood samples were negative for *Rickettsia* spp in real-time PCR. No guinea pigs inoculated with the horse blood showed clinical signs and all of them remained negative by IFAT at 21 days post inoculation. Horses developed anti-*R. rickettsii* IgG antibodies at 10 or 12 days post-infection, and remained positive up to 180 days, when the serological monitoring was stopped. Rabbits infested with ticks previously fed on the horses showed no clinical signs and remained seronegative to rickettsia. All ticks recovered from rabbits were negative by real-time PCR.

Conclusion: our results indicate that the infection by *R. rickettsii* does not cause illness or clinical abnormalities or detectable rickettsemia in horses, but induces seroconversion. In addition, infected horses did not serve as infection source for *A. cajennense* ticks, suggesting that horses have no role as amplifier host for *R. rickettsii* in nature.

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19. Infección de *Rickettsia parkeri* en *Amblyomma ovale* Koch, en perros de la costa oeste de Sao Paulo, Brazil

(Rickettsia parkeri infection in *Amblyomma ovale* Koch ticks from dogs, northeast coast of the Sao Paulo State, Brazil)

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Background: *Amblyomma ovale* is an important human-biting tick in Brazil and the main vector of *Rickettsia parkeri*. Adult specimens of *A. ovale* parasitize Carnivora including domestic dogs, and Rodentia appears to be the major host for immature stages. Here there is a report of partial results of a three-year rickettsial survey in coastal area located at low altitude areas among the Serra do Mar State Park, where thousands of tourists visit annually.

Methods: The study was conducted in two communities in the Ubatuba municipality, Sao Paulo State, southern Brazil during two years totalizing 13 visits (one visit in two months). Ticks were collected from domestic dogs and identified to the species. About 30% of ticks collected during each visit were individually tested for the presence of *Rickettsia* by PCR using primers targeting a fragment of the *gltA* and a fragment the *ompA* genes. The *gltA* and *ompA-PCR* amplicons of the expected size were submitted to direct DNA sequencing. The BLAST program was used to compare appropriate similarities of the rickettsial partial sequences generated in the current study. In total, 38 canine blood samples were collected during the study. All dog sera were tested by the immunofluorescence assay (IFA) using Vero cells infected with *Rickettsia parkeri* as crude antigen.

Results: A total of 1074 adult *A. ovale* (520 males, 554 females) were collected. The mean prevalence was 56,4% and mean intensity 5,4 tick/dog. Of 211 ticks only 29 (13.8%) tested were found infected with *Rickettsia parkeri*. However, the prevalence varied form 5.8% in January 2013 to 32.2% in March 2011. According to RIFI results, the total of 24/38 (63.2%) of the dogs was exposed to SFG rickettsiae.

Conclusion: This is the first long-time study of the *A. ovale* seasonality, we showed that adult ticks can be found on dog throughout the entire year, therefore a threaten for human beings regardless the season.

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20. Detección de anticuerpos contra *Rickettsia rickettsii* en algunas especies de animales y garrapatas seleccionados en el Valle del Río Piracicaba, Sao Paulo, Brasil

(Detection of *Rickettsia rickettsii* antibodies in some species of animals and survey of ticks on the fauna of Piracicaba River Basin, State of Sao Paulo, Brazil)

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Background: Tick-borne diseases are a public health problem of global importance. Some wild animals play a role as *Rickettsia rickettsii* amplifiers, contributing to the maintenance and dissemination of this agent in nature. These animals in the phase of rickettsemia infect ticks that feed on them. The objective of this study was to iinvestigate the prevalence of antibodies against *Rickettisia rickettsii* and study the fauna of ticks in the Piracicaba River Basin, State of Sao Paulo, Brazil.

Method: 218 capybaras (*Hydrochaeris hydrochaeris)*, 109 opossums (44 *Aurita didelphis* and 65 *Didelphis albiventris*), and 178 small rodents were captured in the riparian forest and peri domiciles of regions that are part of the Piracicaba river basin, state of São Paulo. The captured animals were

sedated and a blood sample was collected. After centrifugation and obtaining serum, they were processed by the indirect immunofluorescence (RIFI) technique against *R. rickettsii* antigen. Those with titers ≥1/64 were considered reactive sera. Ticks collected were identified using a taxonomic key.

Results: The seroprevalence found for *R. rickettsii* was 32% in capybaras, 37% in opossums and 7.7% in small rodents. Were collected 7514 ticks, 87.7% of these in capybaras, 12,3% in opossums, and none in small rodents. The most prevalent vectors were *Amblyomma cajennense* with 1640 (24.9%), *Amblyomma dubitatum,* with 2095 (27.9%), larvae of *Amblyomma* sp. with 1350 (18%), nymphs of *Amblyomma sp.* with 2415 (32.1%), and *Ixodes loricatus* with 13 (0.2%) specimens. The predominant species of ticks collected coincide with those already described in the literature, as well as a higher prevalence of immature forms of *Amblyomma* sp. parasitizing small animals.

Conclusion: With this data it is possible to conclude that the small rodents studied in this region are not important as amplifying hosts of Rickettsiae. Capybaras and opossums participate in the epidemiological chain of Brazilian spotted fever as potential amplifying hosts of *R. rickettsii* in nature, leading to an increase in the number of infected ticks, thus making transmission to humans possible.

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21. Un estudio de ectoparásitos en animales peridomésticos guatemaltecos sobre agentes rickettsiales

(A survey of ectoparasites from Guatemalan peridomestic animals for rickettsial agents)

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Background: An outbreak of febrile rickettsial illness occurred in 2007 in Moyuta, Guatemala, a farming community near the border with El Salvador. Ectoparasites were collected from horses, cattle, dogs and cats from peridomestic sites located in South-East to South-Central Guatemala in November 2008 to determine the prevalence of rickettsial agents in these hosts.

Methods: Ticks, fleas and lice were identified morphologically. A representative sample of ticks was analyzed to confirm their identity by sequencing part of their 12S rDNA. DNAs were extracted and the ticks and fleas were assayed with an EvaGreen *omp*A assay or *glt*A PCR assay, respectively, to detect spotted fever group rickettsiae. Larger amplicons of *omp*A or the *glt*A products were sequenced to identify the species of *Rickettsia* that had been detected.

Results: Three genotypes of *Dermacentor* ticks were found in abundance (138) in the ears of 8 of 11 horses from 6 locations. The bodies of four horses from two locations had smaller numbers of *Amblyomma cajennense* (22) and single *Rhipicephalus (Boophilus) microplus* was found on two horses. 483 *R. microplus* were collected from 15 cows in 5 locations. Two cats from two sites both only had cat fleas, while 45 of 47 dogs from 12 sites had lice (5), fleas (37) and/or ticks (29). Three different *Amblyomma* species were found on dogs (4 ticks on 3 dogs) but *Rhipicephalus sanguineus* was far more prevalent (172). *Boophilus* and *Amblyomma* were not restricted to specific hosts, unlike *Dermacentor* (horse ears) and *R. sanguineus* (dogs). *Rickettsia amblyommii* was detected in *Amblyomma auricularium*. However, the prevalence of *Rickettsia* was surprisingly low in this sample of ticks. About 10% of the fleas (222) had *R. felis* and 2 of 23 that were sequenced were RF2125 genotype which had not been previously found in Guatemala.

Conclusions*:* Although *Rickettsia felis* was detected frequently in fleas from both dogs and cats, its distribution was highly sporadic and its prevalence did not correlate to the degree of infestation with any of the ectoparasites found on dogs. While rickettsial agents are certainly present in Guatemala as found here, their seasonal prevalence, host associations, and the extent of risk which they pose for human health will require more extensive investigations.

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22. Evaluación del potencial patogénico de *Rickettsia amblyommii* en cobayos (*Cavia porcellus*) e inmunidad protectora contra *Rickettsia rickettsii*

(Evaluation of the pathogenic potential of *Rickettsia amblyommii* in guinea pigs (*Cavia porcellus*) and protective immunity against *Rickettsia rickettsii*)

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Justificación: *Rickettsia amblyommii* pertenece al grupo de las fiebres manchadas (GFM), que incluye patógenos de humanos y animales transmitidos por artrópodos. Aunque en un único estudio en modelo animal se estableció que *R. amblyommii* probablemente no es patógena, existe evidencia serológica de posible infección y enfermedad leve en humanos. Además, experimentos anteriores han demostrado inmunidad protectora contra *R. rickettsii* en cobayos inoculados previamente con rickettsias del GFM pero no específicamente con *R. amblyommii.* El objetivo de este estudio fue evaluar el potencial patogénico de *R. amblyommii* en cobayos y determinar su capacidad inmuno-protectora ante una infección subsecuente con *R. rickettsii.*

Métodos: Se inocularon 6 cobayos con *R. amblyommii* vía intraperitoneal y 2 controles con medio de cultivo. Se efectuaron necropsias por duplicado de los infectados al día 2 y 4, y de infectados y controles al día 13. Se realizó seguimiento de temperatura, peso y se tomaron muestras de sangre a los días 0, 1, 2, 3, 4, 7, 9, 11 y 13. La sangre y los tejidos se procesaron por PCR para detectar el gen *gltA* y se determinó el título de IgG anti-*R. amblyommii* por inmunofluorescencia indirecta. Para evaluar inmunidad protectora, se inocularon otros 5 cobayos con *R. amblyommii*; 4 semanas después se inoculó una cepa patógena de *R. rickettsii* en este grupo de 5 inoculados (GI) y en otros 3 no inoculados previamente como control positivo (GC). A todos los animales se les evaluó el título de IgG anti-*Rickettsia* y los signos clínicos.

Resultados: Se evidenciaron títulos de 1/512 anti-*R. amblyommii* al día 13 post-inóculo. *Rickettsia amblyommii* se detectó por PCR en testículos al día 2. Algunos cobayos desarrollaron orquitis, sin otros signos de enfermedad. En el ensayo de inmunidad protectora, se obtuvieron títulos finales IgG anti-*Rickettsia* menores en cobayos GI que en GC luego de infección con *R. rickettsii*. *Rickettsia rickettsii* se detectó mediante PCR sólo en testículos del GC. Cobayos GI solamente presentaron fiebre transitoria, mientras cobayos GC exhibieron signos de enfermedad severa y murieron dos.

Conclusión: Se evidenció infección, desarrollo de anticuerpos y patología leve en cobayos ante una infección experimental con *R. amblyommii* de Costa Rica. Aunque son necesarios más estudios, se confirma su potencial patogénico y no se debe descartar aún como agente causante de enfermedad. Además, por su capacidad de generar inmunidad protectora, *R. amblyommii* podría influir en la epidemiología y severidad de infecciones por *R. rickettsii* en zonas donde ambas coexistan.

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Ponencias en Modalidad de Cartel. Sesión A

A-1. *Rickettsia felis* en ectoparásitos de las zarigüeyas (*Didelphis aurita*) en Brasil

(*Rickettsia felis* in ectoparasites from opossums (*Didelphis aurita*) in Brazil)

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Background: Rickettsial diseases are common in Brazil involving some species of Rickettsia as the agent of these diseases, being transmitted by transovarian transmission in the tick host and transient horizontal transmission in mammalian hosts. The purpose of this study was to identify *Rickettsia* spp. in ectoparasites of opossums from Santa Cruz do Escalvado municipality, Minas Gerais State, Brazil. This city is considered an old focus for Brazilian spotted fever and was chosen for this study in order to investigate an area of low endemicity for Rickettsiae.

Methods: The capture of opossums was performed during 2005-2007, with quarterly frequency. The opossums were collected in nearby dwellings, including garages, sheds, stockpiles of food, plantations of corn, bamboo thickets, waste deposits and near the homes (100 traps/collection). Ectoparasites were collected from opossums and screened using molecular techniques (PCR) to detect the presence of *Rickettsia* spp. PCR was performed using primers CS-62 and CS-462 to amplify a fragment of the gene encoding a protein citrate synthase (*glt*A), specific for genus *Rickettsia*. PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide, and examined under UV light. PCR products of positive samples were sequenced directly by using a dideoxynucletotide cycle sequencing method with an automated sequencer (ABI PRISM 310; Perkin-Elmer). Sequences obtained in the present study were compared with the corresponding sequences deposited in GenBank by using the BLAST program.

Results: 278 samples of ectoparasites were collected from the 38 opossums of the specie *Didelphis aurita*. Among the ectoparasites 31 were ticks (27 *Amblyomma* sp. and 4 *Riphicephalus sanguineus*), 225 fleas (195 *Ctenocephalides canis*, 25 *Ctenocephalides felis* and 5 *Xenopsylla cheopis*) and 22 mites (*Rhapslopsyllus* sp.). The DNA of *Rickettsia* spp. was detected in fleas of five animals (13,2%). Using the BLASTn application against the biological sequence database GenBank, it was found that all sequences showed homology or identity of 100% with *Rickettsia felis* already deposited by other authors.

Conclusion: These results suggest that the presence of this agent in opossums in the study area may represent a potential threat to humans, and the public health impact of these findings should be further investigated.

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A-2. Diferencias en el crecimiento de las cepas RF2125 y URRWXCal2 de *Rickettsia felis* en dos líneas celulares

(Differential growth of *Rickettsia felis* strains RF2125 and URRWX Cal2 in two cell lines)

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Justificación: En las últimas décadas, bacterias emergentes del género *Rickettsia* han cobrado importancia dentro de las zoonosis causadas por bacterias intracelulares obligadas. *Rickettsia*

felis es considerado uno de estos patógenos emergentes en Latinoamérica, pues ha sido implicado en casos de enfermedad en humanos. En Costa Rica, recientemente se han desarrollado estudios en los cuales ha sido posible aislar dos cepas diferentes de *Rickettsia felis* en cultivo celular. El objetivo de este estudio fue determinar las características de crecimiento de las cepas RF2125 y URRWXCal2 en líneas celulares Vero y C6/36.

Métodos: Dos aislamientos de *Rickettsia felis* cepa RF2125 y uno de la cepa URRWXCal2 provenientes de *Ctenocephalides felis* de Costa Rica fueron inoculados a una concentración de $1.4x10^6$ bacterias/ml en botellas con monocapas confluentes de células C6/36 en RPMI al 2.5% de suero fetal bovino y células Vero en MEM al 4% de suero de bovino recién nacido. Se evaluó el crecimiento en ambas líneas celulares con o sin suplemento al 2% de triptosa fosfato a 28 °C y 32 °C. Se evaluó crecimiento bacteriano semanalmente y por un periodo de un mes, utilizando una escala semicuantititativa de 1 a 4 cruces (+ a ++++) según la cantidad de bacterias por célula observadas con tinción de Giménez.

Resultados:

Ambos aislamiento de *R. felis* RF2125 crecieron bien (+++ a ++++) en células Vero con y sin triptosa a ambas temperaturas, aunque el crecimiento fue más lento en uno de ellos en medio sin triptosa a 32 °C. El crecimiento de los aislamientos RF2125 en C6/36 a las 4 semanas fue mínimo (+) en medios con y sin triptosa a ambas temperaturas. El aislamiento de URRWXCal2 creció moderadamente (+++) en células C6/36 a 28 °C con y sin triptosa, así como a 32 °C con triptosa. Su crecimiento fue menor (++) a 32 °C sin triptosa y en células Vero sólo hubo crecimiento leve (+) a 28 °C con triptosa.

Conclusión: Ambos aislamientos de *R. felis* RF2125 mostraron características similares de crecimiento, el cual fue mejor en células Vero. El aislamiento de la cepa *R. felis* URRWXCal2 fue completamente diferente y mostró un mejor crecimiento en células C6/36 que en Vero. El suplemento de triptosa en el medio favoreció levemente el crecimiento de ambas cepas en cultivo celular. Estos resultados demuestran que existen diferencias metabólicas o a nivel de receptores entre ambas cepas que deben ser evaluadas con más detalle.

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A-3. Caso clínico de fiebre manchada de las Montañas Rocosas con p24 Ag -HIV y HBs Ag-HVB falsos positivos durante la fase aguda

(Clinical case of Rocky Mountain spotted fever with p24 Ag -HIV and HBs Ag-HVB false positives during the acute phase)

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Caso clínico: Paciente varón de 18 años de edad, heterosexual, procedente de zona rural, El Palmar, Dpto. San Pedro, de la Provincia de Jujuy, Argentina, expuesto a picaduras de garrapatas, sin antecedente de consumo de drogas ilegales ni etilismo. Previamente sano, inicia cuadro 5 días antes de su internacion, con fiebre, astenia, mialgias, rash. Al día posterior a su ingreso, refiere cefalea presenta hipotensión y exantema macular morbiliforme que luego se hace petequial, no presenta adenomegalias si hepato-eslenomegalia, examen cardiopulmonar normal. Evoluciona con depresión del SNC, dolor abdominal y disnea, se le realizo Punción Lumbar y pasa a UTI, se inicia Doxiciclina y luego se cambia Cloranfenicol EV y se inicia tratamiento antiviral para infección aguda por HIV. El laboratorio muestra, ligera hemoconcentración, plaquetopenia, leucopenia. Muestra prolongación de Tiempo de Quick y KPTT, transaminitis leve. El LCR: 6 células con hiperproteinorraquia y cultivos negativos. EAB y O2 dentro de valores normales. Hemocultivos para bacterias negativos. Serologia: Ag P24 +, Hbs Ag HVB + (Relación de Positividad: 250). HBc Ig M, Ig G, HBe Ag y Anti HBe Ag negativos para HVB: HVC negativo. La inmunoflorescencia indirecta para *Rickettsia rickettsii* RRI-IgG dio positivo, con título de 256, realizado en CDC-USA. La Rx. de tórax normal. ECO: muestra hepato-esplenomegalia. ECG y valoración cardiaca normal. El paciente tuvo buena respuesta clínica y recuperación con el Cloranfenicol. Los diagnósticos planteados fueron: meningoccocemia, rickettsiosis, sepsis bacteriana, infección aguda por HIV, ehrlichiosis, dengue, leptospirosis.

Comentarios: El motivo de presentar este caso clínico, es por su cuadro clínico también compatible con otras patologias endémicas y con la infección aguda por HIV y con presencia de Ag. P-24 positivo. Luego al ser negativa la detección de RNA para HIV por PCR se suspende el HAART. Por técnica de ELISA de detección de anticuerpos para HIV y técnica de confirmación de HIV por WB fueron negativos en su seguimiento, al igual que el Hbs Ag +. Se interpretó que tanto la detección de Hbs Ag + y del Ag P24 fueron falsos positivos durante la etapa aguda y su convalecencia inmediata de la infección por *R. rickettsii.* La literatura menciona otras enfermedades infecciosas como causas de Ag. P 24 y de HBs Ag falsos positivos, pero no parece haber datos publicados que también la infección aguda por *R. rickettsii* sea una causa más. El paciente actualmente se encuentra en buen estado de salud y no es portador de HVB ni HIV.

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A-4. Factores de riesgo asociados a la transmisión de fiebre maculosa en el Valle del Rio Piracicaba, São Paulo, Brasil

(Risk factors associated with Brazilian spotted fever transmission in Piracicaba river basin, State of São Paulo, Brazil)

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Background: In the Americas, spotted fever disease has been reported in Canada, U.S.A., Mexico, Costa Rica, Panama, Colombia, Brazil and Argentina. It started to be informed more frequently in the state of São Paulo in 1980, with high rates of lethality, and 428 cases were registered in the State from 1998 to 2010, 80% occurred in 31 municipalities belonging to region of Campinas. This region of the hydrographic basin of Piracicaba river is the area with greater notification of cases, located northeast of State of São Paulo with an approximate extension of 370 km. The objective of this study was to analyze risk factors associated to the confirmed cases of Brazilian spotted fever that occurred from 2003 to 2011 in the Piracicaba river basin, state of São Paulo.

Methods: The totality of the cases in the area of study (n = 478) were notified and laboratory confirmed by Epidemiological Surveillance System. During an epidemiological investigation at the probable site of infection, demographic and environmental variables related to vector species and its main hosts were collected. Temporal distribution (onset of symptoms) and characteristics of the cases were described, and a logistic regression model was adjusted using laboratory confirmed cases of Brazilian spotted fever as the dependent variable, and cases disposed as controls. Estimates were considered significantly associated with p values ≤ 10.

Results: There was a seasonal pattern of disease, with higher occurrence between July and November. The age group of 20 to 59 years was the most affected (60% of cases). Variables associated with spotted fever in the multiple regression model were: age (OR = 1,020, CI 90%: 1,01-1,024), proximity to the riparian (OR= 1,403, CI 90%: 1,101-1,97), number of ticks on horses (OR = 1,16, CI 90%: 1,07-1,26), amount of larvae collected at the suspected infection site (OR = 0,997, CI 90%: 0,995-0,999).

Conclusion: The proximity to water courses with presence of riparian as a predictor of disease reinforced the importance of this information. Horses may play a role as sentinels of infection risk. The predominance of larvae of *Amblyomma* as a "protective" factor for the occurrence of cases may indicate that the period of greatest abundance of these immature forms is unfavorable for transmission, probably due to biological characteristics and displacement of the vector in larval form and the reduced chance of being infected by *Rickettsia* at this stage of development.

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A-5. Rickettsiosis por *Rickettsia parkeri* en un viajero que regresa de Uruguay: Un caso confirmado por PCR

(*Rickettsia parkeri* rickettsiosis in a traveller returning from Uruguay: A PCR-confirmed case)

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Background: The first case of human rickettsiosis caused by *Rickettsia parkeri*, a spotted fever group *Rickettsia*, was reported in The United States in 2004. At the same time, *R. parkeri* was first detected in *Amblyomma triste* ticks from Uruguay by our team, suggesting its pathogenic role as the etiological agent of rickettsioses in that country. To our knowledge, only two human cases of infection caused by this *Rickettsia* species and confirmed by polymerase chain reaction (PCR) have been published in South America (Argentina). Herein, we report a case of spotted fever rickettsiosis caused by *R. parkeri* confirmed by molecular methods in a traveller returning from Uruguay.

Clinical case: A 54-year-old man returned to Spain on December 16, 2012 following a 7-day trip to Uruguay (Colonia Suiza). He did not notice any arthropod-bites. Two days after arrival in Spain, he noticed two crusted lesions on the inner side of the left ankle. The following day he presented fever, chills and an erythema surrounding both lesions. He was treated with amoxicillin-clavulanic acid and mupirocin cream for four days by a primary care physician, but his symptoms persisted. On December 25, he was admitted to the Hospital San Pedro in La Rioja (Spain) with the presumptive diagnosis of cellulitis with petechial rash after probable arthropod-bite. Examination showed fever (39ºC) and two eschars surrounded by an indurated, erythematous halo on the inner side of the left ankle. EDTA-treated blood and cutaneous swab specimens were collected to investigate the presence of *Rickettsia* spp. using PCR assays for *gltA* and *ompA* rickettsial genes. In addition, acute and convalescent sera specimens (collected two weeks after the onset of the illness) were tested by immunofluorescence assay (IFA) using *Rickettsia conorii* as antigen. Fragments of *gltA* and *ompA* rickettsial genes were amplified from the swab sample. Partial *gltA* (285/285 bp) and *ompA* (535/536 bp) sequences showed 100 and 99.8% identity to the corresponding sequences of *R. parkeri*. Diagnostic antibodies against spotted fever group rickettsiae were not detected in the acute serum specimen but the convalescent one was positive for IgG at a titer of 4096. Doxycycline (100mg/12h) was administered for 7 days and the patient fully recovered.

Conclusion: This finding confirms that *R. parkeri* is an etiological agent of human cases of spotted fever in Uruguay.

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A-6. Aislamiento de *R. typhi* a partir de sangre de un paciente en fase aguda en Mérida Yucatán México

(*Rickettsia typhi* isolation from blood obtained of an acute febrile patient in Mérida, Yucatán, México)

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Justificación: *R. typhi* es una bacteria intracelular causante del tifo murino, su distribución es universal siendo endémico en extensas áreas geográficas de los cinco continentes donde se ha considerado una enfermedad re-emergente. En Yucatán, *R. typhi* se ha encontrado en diversos hospederos. En el Laboratorio de Enfermedades Emergentes y RE-emergentes del Centro de Investigaciones Regionales Dr. Hideyo Noguchi, acude un paciente de 23 años de edad con sintomatología sugestiva a enfermedad Rickettsial con presencia de erupción exantemática de forma petequial en extremidades superiores, extremidades inferiores y torso, respetando cabeza y rostro. El paciente no refiere fiebre, malestar general o alguna otra sintomatología. Se le realizan biometría hemática, química sanguínea arrojando resultados dentro de los parámetros normales y serología de dengue negativo. Se realiza pruebas de reacciones febriles resultando positivo a proteus OX19 1:164, como dato adicional refiere convivencia con animales domésticos gatos y perros. Como objetivo general fue determinar la presencia del agente Rickettsial, caracterización de la especie y su aislamiento a partir de sangre periférica.

Métodos: El diagnóstico del paciente fue realizado por PCR de un fragmento del gen de 17 KDa, citrato sintasa (*gltA*) y *ompB* y su caracterización se realizó por medio de secuenciación de los mismos genes empleando un secuenciador ABIPRISM 310 (APPLIED BIOSYSTEM), posteriormente se realizó el aislamiento de *R. typhi* empleando células VERO (ATCC) crecidas en placas de 24 pozos y mantenidas a 33 °C en 5% de CO_2, la identificación del aislado se realizó empleando la tinción de Giménez y por PCR para la amplificación de 17 kDa, gen de citrato sintasa (*gtlA*) y *ompB.*

Resultados: Se logró el aislado de la especie Rickettisial de la sangre periférica del paciente, la tinción de Giménez permitió la visualización del aislado en el cultivo. La secuencia de los fragmentos de 17kDa y de *gltA* se analizaron en el BLAST dando como resultado un 100% de identidad con *R. typhi* disponible en el GenBank.

Conclusión: En nuestra entidad la presencia de diversas especies de rickesttias se han identificado en sangre de pacientes. La importancia de *R. typhi* en nuestra entidad y la gravedad que genera al ser confundida con otras enfermedades con sintomatología similar podrían sugerir su subdiagnóstico. El aislamiento de esta especie favorecerá la generación de nuevos estudios encaminados a determinar estudios de patogenicidad.

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A-7. Evaluación de la PCR en tiempo real para el diagnóstico de la Fiebre Manchada Brasileña

(Evaluation of real-time PCR for the diagnosis of Brazilian Spotted Fever)

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Background: In Brazil, since May 2011 real-time PCR has been successfully employed in the routine diagnosis of fatal cases of Brazilian Spotted Fever (BSF) in the state of São Paulo, where the disease is usually severe; fatality rate in 2012 was 54.4. In the state of Santa Catarina, where there is high incidence of BSF, the disease course is milder, with no fatality recorded so far. In this study real-time PCR was used as diagnostic tools to detect fatal cases, and non-fatal cases from São Paulo and Santa Catarina States.

Methods: Between 2009 and 2012, 36 cases of fatal BSF, from São Paulo (confirmed by immunohistochemistry or bacterial isolation in cell culture), and 46 nonfatal cases from São Paulo and 38 from Santa Catarina (confirmed by seroconversion by indirect immunofluorescence in paired samples) were selected for this study. Patient acute phase serum was used for nucleic acid extraction and 3 real-time PCR protocols were applied, one targeting the genus *Rickettsia*, one the spotted fever group rickettsiae, and one the RnaseP as endogenous internal control.

Results: All fatal cases were confirmed by real-time PCR with CT average of 31.26 ± 3.17; among nonfatal cases of São Paulo 15 (32.61%) cases were detected by real-time PCR with CT average of 35.1 ± 2,78; no case from Santa Catarina was detected by FMPCR.

Conclusion: These data show that real-time PCR in serum have high predictive value only in severe cases of BSF, who develop acute vasculitis and possibly release a larger amount rickettsiae in circulating blood. That would increase the sensitivity of real-time PCR on blood or serum samples. In State of Santa Catarina real-time PCR performance could be improved by using biopsy samples of eschar or exanthema lesions.

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A-8. Evidencia serológica de exposición a rickettsias del grupo de las fiebres manchadas en bovinos del municipio de Villeta, Colombia

(Serological evidence of exposure to rickettsiae of spotted fever group in cattle of Villeta, Colombia)

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Justificación: En Colombia, el municipio de Villeta ha sido clasificado como una zona endémica para rickettsiosis del grupo de las fiebres manchadas (GFM) dados los casos de mortalidad por *Rickettsia rickettsii* en 1935, en el periodo 2003-2004 y

los altos porcentajes de seropositividad para el GFM tanto en humanos como en animales domésticos (caninos y equinos) evidenciados en estudios posteriores. A pesar de la detección de especies de rickettsias del GFM en garrapatas como *Amblyomma cajennense* y *Rhipicephalus* (*Boophilus*) *microplus*, los cuales son ectoparásitos de bovinos en Suramérica, no se conoce evidencia serológica por parte de estos rumiantes, ni su posible papel en la epidemiología de las rickettsiosis del GFM en esta región de las Américas. Es así como el objetivo de este estudio fue determinar la seropositividad contra rickettsias del GFM en bovinos del municipio de Villeta, Colombia.

Métodos: Se realizó un muestreo por conveniencia durante los meses de noviembre y diciembre de 2011 en 22 veredas y la cabecera municipal del municipio de Villeta, con el fin de obtener muestras de suero de bovinos. En la muestra de suero de cada individuo se determinó por medio de inmunofluorescencia indirecta (IFI), anticuerpos de tipo IgG contra *R. rickettsii* y *R. amblyommii*, considerando como positivo una dilución ≥ 1:64. Como control positivo se utilizó un suero de un bovino infectado con *R. africae* (cortesía Dr. Patrick Kelly, Ross University School of Veterinary Medicine, Basseterre, San Cristóbal, Islas del Caribe).

Resultados: En total se obtuvieron muestras de suero de 62 bovinos pertenecientes a 14 veredas de las 22 del municipio. Veintinueve bovinos (46,7%) presentaron anticuerpos de 1:64 contra *R. rickettsii*, dos bovinos (3,2%) de 1:128 contra *R. rickettsii* y un bovino de 1:64 (1,6%) contra *R. amblyommii*; esta última muestra no presentó reacción cruzada con *R. rickettsii.*

Conclusión: La seropositividad total para rickettsias del GFM en bovinos del municipio de Villeta fue de 51,6%. Dados estos resultados se hace evidente la exposición a especies de rickettsias del GFM por parte de los bovinos evaluados y plantea nuevas hipótesis acerca del posible papel de estos animales en la epidemiologia de las rickettsiosis del GFM en el municipio de Villeta, Colombia.

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A-9. *Rickettsia* spp. del grupo fiebres manchadas asociadas con perros (*Canis lupus familiaris*) en refugios del Área Metropolitana, Costa Rica

(Spotted fever group *Rickettsia* spp. associated with dogs (*Canis lupus familiaris*) from shelters in the Metropolitan Area of Costa Rica)

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Justificación: Las rickettsiosis son zoonosis causadas por bacterias intracelulares obligadas, que se clasifican dentro del género *Rickettsia*. En Costa Rica, recientemente se han reportado casos humanos de fiebre manchada de las Montañas Rocosas en la Gran Área Metropolitana. Los perros pueden ser hospederos para especies de *Rickettsia*, representando un riesgo zoonótico por el estrecho contacto que ellos y sus ectoparásitos mantienen con humanos. El objetivo de esta investigación fue determinar la presencia de anticuerpos IgG contra *Rickettsia* spp. del grupo de fiebres manchadas en perros de refugios del Área Metropolitana en Costa Rica y detectar la presencia de *Rickettsia* spp. en los ectoparásitos de esos perros.

Métodos: Se tomaron muestras de sangre y se recolectaron ectoparásitos de perros provenientes de 3 refugios del Área Metropolitana. Anticuerpos tipo IgG contra *R. rickettsii*, *R. amblyommii* y *R. felis* se detectaron mediante inmunofluorescencia indirecta y títulos ≥1/32 se consideraron positivos. Todos los sueros positivos fueron llevados a título final empleando diluciones seriadas. Los ectoparásitos de cada perro fueron procesados en "pooles" para detección del gen *gltA* de *Rickettsia* spp. por PCR.

Resultados: De un total de 139 perros, se obtuvo 27% de seropositividad para una o más de las especies evaluadas. En general los perros presentaron títulos finales mayores para *R. amblyommii* y/o *R. rickettsii*, sin que se pudiera diferenciar entre ellas. Para *R. amblyommii* y *R. rickettsii*, el 16% mostró un título final de 1/32 y el 5,8% un título final entre 1/64 y 1/512. Para *R. felis*, un 7,9% del total presentó título final de 1/32 y sólo 2 perros (1,4%) presentaron títulos finales mayores (1/64 y 1/128). Los ectoparásitos más frecuentes fueron *Ctenocephalides felis* y *Rhipicephalus sanguineus,* presentes en el 18% y 15% de los perros, respectivamente, pero también se colectaron *Ixodes boliviensis* y *Pulex simulans*. Se detectó *Rickettsia* spp. en el 47% de los "pooles" de ectoparásitos, específicamente en el 50% de "pooles" de pulgas y el 75% de garrapatas.

Conclusión: Este estudio demuestra una alta frecuencia de anticuerpos en los perros y de la bacteria en los ectoparásitos. El hallazgo a nivel epidemiológico es importante para Costa Rica, ya que la seroprevalencia en el grupo evaluado indica alto riesgo de transmisión entre perros y posiblemente hacia humanos. Por lo tanto, las autoridades de salud humana y animal en el Área Metropolitana deben considerar las rickettsiosis en la vigilancia y el diagnóstico diferencial de la enfermedad febril.

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A-10. *Rickettsia* spp. del grupo fiebres manchadas asociadas con perros (*Canis lupus familiaris*) de parques metropolitanos de Costa Rica.

(Spotted fever group *Rickettsia* spp. associated with dogs (*Canis lupus familiaris*) of recreational areas from Costa Rica)

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Justificación: En Costa Rica, actualmente la situación epidemiológica sobre la prevalencia de *Rickettsia* spp. se encuentra en investigación. En el ambiente urbano, esta zoonosis está asociada a perros, los cuales por su relación estrecha con los humanos podrían ser una fuente importante de infección. En este estudio se pretende, mediante la detección de la seroprevalencia en perros de parques metropolitanos y la presencia de la bacteria en ectoparásitos vectores encontrados en los mismos, estimar la exposición del ser humano a este agente.

Métodos: Se realizó un muestreo de sueros caninos, así como una colecta de ectoparásitos en diversos parques públicos del Valle Central (La Sabana, Desamparados, La Paz, Barrio México Aserrí, Monte de la Cruz, Agricultor, Ciudad Colón y TEC) y parques de otros poblados del país (Vargas, Asís Esna, Guápiles, La Fortuna, Quebrada Ganado y Cañas). Se examinaron los animales y se recolectaron un total de 429 sueros, 173 pooles de pulga (*Ctenocephalides felis, Pulex simulans y Echidnophaga gallinacea*) y 129 pooles de garrapatas (*Rhipicephalus sanguineus, Amblyomma cajennense, Amblyomma ovale e Ixodes boliviensis*). Los sueros fueron analizados por inmunofluorescencia indirecta para la detección de anticuerpos contra las especies *Rickettsia rickettsii*, *R. amblyommii* y *R. felis*, así como su respectivo título. Los pooles de ectoparásitos, se analizaron por técnicas moleculares, para la detección del gen *gltA* de *Rickettsia* sp.

Resultados: De los 429 sueros tamizados, se obtuvo un 14.5% de positividad frente a alguna de las rickettsias, o reacción simultanea contra *R. felis* (5 muestras). En 21 sueros la reacción fue mayor contra *R. rickettsii*, y en 27 hacia *R. amblyommii.* Los títulos variaron entre 1:16 a 1:256.

En los ectoparásitos, se detectó ADN de *Rickettsia* spp. en el 32.0% de los pooles de pulgas y en el 30.8% de los pooles de garrapatas. Siete de los perros con serología positiva presentaron alteraciones en el hemograma aparentando proceso infeccioso, mientras que sólo uno de ellos presentó síntomas claros de enfermedad (debilidad, astenia, petequias, artralgia, fiebre) con un título significativo hacia *R. rickettsii.*

Conclusión: Con el presente estudio se demuestra la circulación de *Rickettsia* en los ectoparásitos vectores encontrados en los perros de entornos urbanos que a su vez muestran respuesta inmunológica posterior a una infección. Esto sugiere una probabilidad de exposición y transmisión hacia el ser humano**,** generando una alerta epidemiológica a las autoridades de salud humana y animal.

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A-11. Análisis serológico de caninos domésticos residentes en el área endémica de rickettsiosis humana en Uruguay: resultados preliminares

(Serological analysis of domestic dogs living in an endemic area of human rickettsiosis in Uruguay: preliminary results)

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Justificación: En Uruguay hasta el momento se han reportado dos especies del género *Rickettsia* de importancia sanitaria: *Rickettsia felis,* en las pulgas *Ctenocephalides canis* y *Ctenocephalides felis;* y *Rickettsia parkeri,* en adultos de *Amblyomma triste,* especie de garrapata que más frecuentemente parasita a humanos en el territorio uruguayo. *Rickettsia parkeri* es la responsable de provocar los casos de rickettsiosis humana, siendo el sur del país considerado como área endémica de la enfermedad, presentándose casos clínicos todos los años. La presencia de *R. parkeri* ha sido detectada en *A. triste* de diferentes localidades incluidas dentro del área endémica mediante la utilización de técnicas moleculares, lo que permitió determinar el vector para la rickettsiosis humana en el Uruguay. El estadio adulto de esta garrapata parasita principalmente a caninos domésticos, los que conviven estrechamente con el hombre. El objetivo de este trabajo fue aportar información sobre la epidemiología de la rickettsiosis en Uruguay a través de un análisis serológico de caninos domésticos residentes en el área endémica de la enfermedad.

Métodos: Se empleó la técnica de Inmunofluorescencia Indirecta con antígenos correspondientes a tres especies de *Rickettsia* spp.: *R. rhipicephali, R. felis,* y *R. parkeri.* Para la determinación de la especie implicada, se consideró aquella que presentó un título al menos cuatro veces mayor al de la especie siguiente.

Resultados: Se analizaron hasta el momento 672 muestras de suero canino, correspondiendo a cuatro Departamentos del área considerada endémica en el territorio uruguayo: Canelones, Maldonado, Montevideo y Rocha. La prevalencia de *Rickettsia* spp. en dichas muestras fue de 18.76 % (126/672). A través de la titulación de los sueros positivos para *Rickettsia,* fue posible la adjudicación de la especie involucrada en 85 casos, correspondiendo en todos ellos a *R. parkeri.* Los caninos serológicamente positivos correspondieron a caninos residentes en los cuatro Departamentos muestreados en este trabajo, y a su vez, pudo confirmarse por los valores de titulaciones de anticuerpos, a *R. parkeri,* como el patógeno responsable de la infección en todos los Departamentos muestreados.

Conclusión: Este trabajo ha permitido determinar un valor aproximado de prevalencia de *Rickettsia* spp. en caninos domésticos por primera vez en Uruguay y adjudicar como especie involucrada a *R. parkeri,* aportando información sobre la epidemiología de la enfermedad en el área considerada endémica en Uruguay.

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A-12. Infección rickettsial en caninos domésticos y garrapatas de áreas urbana y rural del estado de Maranhao, Noreste de Brasil

(Rickettsial infection in dogs and ticks of urban and rural areas of the State of Maranhao, Northeastern Brazil)

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Background: Domestic dogs are often exposed to different tick species, what makes these animals good sentinels for rickettsial diseases that affect humans. The state of Maranhão is located in the northeastern region of Brazil, in a transition area from Amazon to Cerrado biomes. In this context, the present study aimed to evaluate rickettsial infection in dogs and ticks from this state.

Methods: During 2011 to 2012, blood serum samples were randomly collected from 1080 domestic dogs from urban and rural areas of six municipalities of Maranhão: Balsas, Grajaú, Barreirinhas, São Bento, Cururupu, and Caxias. Samples were tested by indirect imunofluorescence assay against 5 *Rickettsia* species: *R. rickettsii*, *R. parkeri*, *R. amblyommii*, *R. rhipicephali* and *R. bellii.* Serum showing to a *Rickettsia* species titer at least 4-fold higher than those observed for the other *Rickettsia* species was considered homologous to the first *Rickettsia* species. When present, ticks were collected from dogs and individually tested by PCR targeting the rickettsial genes *gltA* and *ompA*.

Results: Overall, 17.7% (191/1080) of the dogs were seroreactive to *Rickettsia* spp. One hundred twenty-five (125/760) sera showed titers to *R. amblyommii*, *R. rhipicephali* and *R. bellii* at least 4-fold higher than those observed to the other rickettsial antigens. In this way, we considered that these dogs were naturally infected by *R. amblyommii* (30 sera), *R. rhipicephali* (4 sera) and *R. bellii* (3 sera) with titers ranging from 128 to 16.384. The following tick species were collected from dogs: *Rhipicephalus sanguineus, Amblyomma cajennense, A. ovale*, *A. oblongoguttatum*, *A. parvum*, and *Haemaphysalis juxtakochi.* 240 ticks were processed by PCR plus DNA sequencing, which detected "*Candidatus* Rickettsia andeane" in 9 *A. parvum,* and *R. bellii* in 3 *A. ovale* specimens.

Conclusion: These results suggest that *R. amblyommii*, *R. rhipicephali*, *R. bellii* and/or close-related strains such as "*Candidatus* Rickettsia andeane" are infecting dogs in Maranhão state.

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A-13. *Rickettsia* spp. en ectoparásitos de perros de una comunidad rural de Yucatán, México

(*Rickettsia* sp. in dog ectoparasites from a rural community of Yucatan, Mexico)

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Justificación: En Yucatán se ha documentado la circulación de bacterias del género *Rickettsia*, mas no se conoce la gama de hospederos y vectores involucrados en la transmisión ni las especies que pudieran estar presentes. Este estudio tuvo como objetivo identificar la diversidad de ectoparásitos de perros de una localidad rural y determinar la frecuencia de ectoparásitos infectados con *Rickettsia.*

Métodos: El estudio se realizó en Dzidzilché, Yucatán, México (21°08'N, 89°41'W). Se realizó un censo de perros en la localidad y con la autorización de los propietarios se revisó a cada animal para colectar los ectoparásitos visibles. Se identificó taxonómicamente cada uno de los ejemplares por morfología. Se hicieron "pools" de ectoparásitos por perro y por especie para el diagnóstico molecular por PCR. Se usaron los cebadores Fw 17KDa1 y Rv 17KDa2 y luego un procedimiento de PCR anidado con el producto del PCR de 17 KDa y los cebadores Fw $17KDaN_1$ y Rv $17KDaN_2$. Los 10 grupos de muestras que dieron positivos se enviaron a secuenciar empleando un secuenciador ABIPRISM 310 (APPLIED BIOSYSTEM) en el Instituto de Síntesis y Secuenciación de ADN, Instituto de biotecnología de la Universidad Nacional Autónoma de México.

Resultados: Se revisaron 64 perros de los cuales 62 tuvieron ectoparásitos. Se recolectaron 417 ectoparásitos (337 pulgas y 80 garrapatas). Se identificaron tres especies de garrapatas y dos de pulgas: *Rhipicephalus sanguineus* (70/80), *Amblyomma parvum* (8/80), *A. cajennense* (2/80), *Ctenocephalides felis* (332/337) y *Pulex porcinus* (5/337). Se analizaron 103 pools de ectoparásitos (34 de *R. sanguineus*, 8 de *A. parvum*, 2 de *A. cajennense,* 5 de *P. porcinus* y 54 de *C. felis*), de los cuales resultaron 10 pools positivos (9.7%). La frecuencia de infección en los ectoparásitos fue de 87.5% para *A. parvum*, 50% para *A. cajennense*, 20% para *P. porcinus* y 1.7% para *C. felis.* El análisis de los productos secuenciados resultó en la identificación de tres especies: *Rickettsia rickettsii* en *A. cajennense*, *R. felis* en *C. felis* y *R. typhi* en *P. porcinus* y *A. parvum.*

Conclusión: Los resultados muestran la circulación de diferentes especies de *Rickettsia* en los ectoparásitos de una misma población de perros. Resalta la necesidad de realizar estudios epidemiológicos formales en la región. Es notoria la ausencia de *Rickettsia* en *R. sanguineus.* Se reporta por primera

vez para la península de Yucatán a *P. porcinus* y *A. parvum* infestando perros domiciliados e infectados con *Rickettsia.*

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A-14. Dos agentes rickettsiales identificados en ectoparásitos de perros de cuatro ecoregiones de Chile

(Two rickettsial agents in canine ectoparasites from four ecoregions in Chile)

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Justificación: Las rickettsiosis constituyen infecciones emergentes, de las cuales existe poca información en Chile. En países sin identificación de casos en humanos, los estudios iniciales deben enfocarse a la búsqueda de los agentes en vectores. Las mascotas pueden ser afectadas y además ser fuente de estas infecciones para humanos. **Objetivos:** Determinar presencia de agentes rickettsiales en ectoparásitos recolectados de perros de diferentes ecoregiones de Chile.

Métodos: estudio transversal descriptivo realizado en cuatro ciudades de Chile, de norte a sur: Arica, Coquimbo, Santiago (Región Metropolitana, RM) y Angol. Se efectuó muestreo domiciliario aleatorizado en zonas urbanas y por conveniencia en localidades rurales cercanas a cada ciudad. Tamaño muestral calculado: 97 perros en cada localidad. En viviendas con mascotas caninas y cuyos dueños accedieron a participar, un veterinario examinó a un perro y recolectó ectoparásitos si estaban presentes. Luego de su identificación taxonómica los ectoparásitos fueron sometidos a amplificación y secuenciación de los genes gltA y ompA.

Resultados: se examinaron 921 perros en las 8 localidades estudiadas. Un 53% de los perros tenían garrapatas (rango 31 a 76%), identificándose 3 especies: *Amblyomma triste* en localidades rurales de Arica, *Amblyomma tigrinum* en áreas rurales de Coquimbo y Angol y *Riphicephalus sanguineus* en todas las localidades, constituyendo la principal garrapata del país. Un 39% de los perros tenía pulgas (rango 7 a 83%), las especies identificadas fueron *Ctenocephalides canis, Ctenocephalides felis, Pulex irritans* y *Echidnophaga gallinacea,* en proporciones variables. Se identificaron dos especies de rickettsias en los ectoparásitos: *Rickettsia felis* y *Rickettsia andeanae. Rickettsia felis* fue identificada en pulgas de seis de las ocho localidades estudiadas, destacando un elevado grado de positividad en pulgas de Arica y RM urbana (43% de las pulgas). Además se identificó *Rickettsia felis* en garrapatas *Rhipicephalus sanguineus* provenientes de Arica urbano y RM rural. *Rickettsia andeanae* fue encontrada en *Amblyomma triste* de uno de 3 de perros de Arica rural y en *Amblyomma tigrinum* de 8/16 perros de Coquimbo rural y de 3/8 perros de Angol rural.

Conclusión: Se identifican dos especies de *Rickettsia* en ectoparásitos de perros en Chile, con diferente localización geográfica: *Rickettsia felis* predominantemente en pulgas pero también en *Rhipicephalus sanguineus,* de perros de viviendas urbanas y rurales; y *Rickettsia andeanae*, exclusivamente en garrapatas del género *Amblyomma* de perros de zonas rurales. Los resultados pueden ayudar a sospechar casos de rickettsisosis en mascotas y personas, así como a programar medidas de control de estos vectores.

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A-15. Evidencia serológica de infección por rickettsias del grupo de la fiebre manchada y *Rickettsia bellii* de pequeños mamíferos en zona periurbana de Uberlândia, Minas Gerais

(Serological evidence for Spotted-Fever group *Rickettsia* and *Rickettsia bellii* infection of small mammals from the peri-urban region of Uberlândia, Minas Gerais, Brazil)

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Background and aim: Epidemiology of rickettsial diseases is increasingly unrevealed but it is still poorly understood worldwide. Many *Rickettsia* species are associated to distinct ecological background linked to vectors and its hosts. Ticks are important *Rickettsia* vectors and small mammals are important hosts for immature stages. The aim of this study was to evaluate seropositivity of small mammals against five species of *Rickettsia* in periurban region of Uberlândia, MG, Brazil.

Methods: Small mammals were captured from July 2011 to August 2012 with Sherman traps at cages in seven locations around Uberlândia, Minas Gerais, Brazil. Animals were identified to species according to field guides, examined for the ticks, and blood collected. Seroreactivity of each animal was tested by Immunofluorescence assay (IFA) for *R. rickettsii*, *R. parkeri*, *R. amblyommii*, *R. rhipicephali* and *R. bellii.*

Results: Overall 416 animals representing 13 species of wild rodents and marsupials were captured. Of these, 48 (11.5%) were infested with ticks of the genus *Ixodes* and *Amblyomma*, and 70 (16.8%) of the animals were seropositive for *Rickettsia*

spp.. Higher titers against four *Rickettsia* species were found (*R. rickettsii*, *R. parkeri*, *R. rhipicephali* and *R. bellii*).

Conclusions: Small mammals in the peri-urban area of Uberlândia, Minas Gerais are exposed to *Rickettsia* and further investigation shall determine Rickettsia species as well as assess human infection risk.

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A-16. Ocurrencia de anticuerpos de *Rickettsia* spp. en roedores silvestres del Parque Nacional Grande Sertão Veredas, Minas Gerais, Brasil

(Antibodies occurrence of *Rickettsia* spp. in wild rodents from Grande Sertão Veredas National Park, Minas Gerais, Brazil)

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Background: The Brazilian savannah is the second largest biome in South America and is considered as one of the "hotspots" of global biodiversity, presenting extreme abundance of endemic species but never before explored regarding of ticks and their associated pathogens. The Grande Sertão Veredas National Park (PNGSV) is one of the last natural reserves of Brazilian savannah, located in the northwest of Minas Gerais State, Brazil. The research that is being conducted in this area intends to bring a major contribution to the natural history of ticks and rickettsia associated with ticks. The preliminary results are presented herein for rickettsial infection among wild rodents evaluated by serological technique.

Methods: Wild rodents were captured between May 2012 and February 2013 using live traps, anesthetized and then blood samples were collected through intracardiac puncture, being further processed and stored at -20 °C until be tested by immunofluorescence assay (IFA ≥ 64) against six rickettsial antigens: *R. rickettsii*, *R. parkeri*, *R. amblyommii*, *R. rhipicephali*, *R. bellii* and *R. felis*.

Results: Samples of 31 wild rodent specimens were obtained: *Oxymycterus delator* (13), *Thrichomys apereoides* (11), *Calomys tener* (3), *Cerradomys* sp (1), *Dasyprocta* sp (1), *Galea spixii* (1) and *Olygorizomys* sp (1). Of the 31 wild rodent sera analyzed 14 (45.2%) were IFA positive with titer varying from 64 to ≥ 1024. Positive samples were found for all six rickettsias tested: 10 to *R. parkeri* (6 *O. delator*; 2 *C. tener*; 1 *Dasyprocta* sp; 1 *T. apereoides*), 6 to *R. amblyommii* (5 *O. delator*; 1 *Dasyprocta* sp), 5 to *R. rickettsii* (4 *O. delator*; 1 *Dasyprocta* sp), 4 to *R. rhipicephali* (3 *O. delator*; 1 *Dasyprocta* sp), 8 to *R. bellii* (3 *T. apereoides*; 2 *O. delator*; 1 *C. tener*; 1 *Dasyprocta* sp; 1 *Galea spixii*) and 1 to *R. felis* (1 *O. delator*). The highest titers were found for *R. parkeri* (128 to ≥ 1024) and *R. rickettsii* (256 to ≥ 1024) in *O. delator*, suggesting that the homologous antigen is likely to be *R. parkeri* by the difference found in titration.

Conclusion: The results indicate that the PNGSV wild rodents are exposed to ticks infected by rickettsia from Spotted fever group.

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A-17. Detección de *Rickettsia* spp. en garrapatas (Ixodidae) retiradas de tapires de montaña, vacas y vegetación en un área protegida de Ecuador

(Detection of *Rickettsia* spp. in ticks (Ixodidae) removed from mountain tapirs, cattle and vegetation in a protected area from Ecuador)

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Background: The genus *Rickettsia* is represented by Gram-negative intracellular bacteria transmitted by arthropods. Among them, the spotted fever group (SFG) *Rickettsia* is mostly associated with ticks and may cause human diseases. Surveillance researches for *Rickettsia* spp. in ticks are required to understand which species are present in a region and to establish the potential risk for human health. Up to our knowledge, there are no data concerning the presence of SFG *Rickettsia* in ticks from Ecuador. Our study aimed to investigate rickettsial infection in ticks removed from wild and domestic animals (mountain tapirs and cattle) and vegetation using molecular biology techniques in a protected area from Ecuador.

Methods: A total of 151 ticks from mountain tapirs (n=74), cattle (n=61) and vegetation (n=16) collected in Antisana Ecological Reserve and Cayambe-Coca National Park (Ecuador) were studied at the Center of Rickettsioses and Arthropod-Borne Diseases (Spain). PCR assays targeting fragments of the rickettsial genes *gltA* (primer pair: CS-78/CS-323) and *ompA* (Rr190.70p/Rr190.701n for the first round, and Rr190.70p/Rr190.602n for the second round) were used as screening tests. Positive samples were subsequently tested for portions of *ompB* (primers rompB-OF/rompB-OR) and *sca4* (primers D1f/D928r) rickettsial genes. Obtained amplicons were compared with those available in GenBank using BLAST analysis.

Results: From a total of 151 ticks, 3 *Rhipicephalus microplus* specimens collected from two cows at different livestock yielded

positive results for *gltA* fragment gene. Two of these samples were also positive for *ompA*. Nucleotide sequences obtained for each fragment gene were identical each other. The *gltA* partial sequence was closest (99% identity) to the corresponding sequences of *Rickettsia monacensis, Rickettsia tamurae* and *Rickettsia asiatica*. The *ompA* partial sequence showed highest identity (96%) with the corresponding sequences of *R. monacensis* and *R. tamurae*. A sequence was obtained for the *ompB* gene, which showed to be 99 and 96.9% identical to corresponding sequences of *R. monacensis* and *R. tamurae*, respectively. Trials to amplify *sca4* fragment gene were unsuccessful for these 3 samples. Since our rickettsial isolate seems to be genetically distinct from other validly published *Rickettsia* species, a potential new *Candidatus* status is suggested. One *R. microplus* specimen was co-infected with *Rickettsia* spp. and *Anaplasma* spp. (data are shown in other abstract).

Conclusion: This is the first molecular evidence of the presence of *Rickettsia* spp. in ticks from Ecuador. A potential new *Candidatus* Rickettsia sp. seems to be circulating in this country.

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A-18.Infección por *Rickettsia bellii* y *Rickettsia rhipicephali* en las garrapatas de la reserva biológica Serra do Japi, São Paulo, Brasil

(*Rickettsia bellii* and *Rickettsia rhipicephali* infection in ticks from Serra do Japi Biological Reserve, São Paulo, Brazil)

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Background: Recently many rickettsiae-infected ticks have been identified and various Brazilian Spotted Fever cases have been reported in areas of the Atlantic Forest. This is the first survey to *Rickettsia* in ticks from this remaining Atlantic Forest fragment.

Methods: The Serra do Japi Biological Reserve is a 36 ha fragment of Atlantic forest located in Jundiaí municipality, 60 km away from the city of São Paulo and is under intense anthropogenic pressure. During 5 days in December 2010, questing ticks were captured through drag flannel, visual search on vegetation and human/animal hosts. Ticks were identified morphologically to the genus level following Barros-Battesti et al. (2006), whereas *Amblyomma* nymphs were identified based on the study by Martins et al. (2010). Ticks were tested individually for the presence of *Rickettsia* by polymerase chain reaction (PCR) using primers CS-78 (5`-GCA AGT ATC GGT GAG GAT GTA AT-3`) and CS-323 (5`-GCT TCC TTA AAA TTC AAT AAA TCA GGA T -3) targeting a 401-bp fragment of the rickettsial gene gltA that occurs in all *Rickettsia* species (Labruna et al., 2004). PCR products were DNA sequenced and analyzed in BLAST to determine similarities to other *Rickettsia* species (Altschul et al., 1990).

Results: Eighty ticks were collected: fifteen nymphs of *Amblyomma cajennense*, eleven nymphs and 6 adults of *Amblyomma brasiliense*, forty-four nymphs and 2 adults of *Haemaphysalis juxtakoch*, 1 nymph and 1 adult of *Amblyomma aureolatum* (fixed on domestic dog). Nineteen nymphs and three adults were fixed in humans or removed from the clothes. The gltA PCR amplicons from twenty positive ticks (1 *A. aureolatum*, 1 *A. cajennense*, eighteen *H. juxtakochi*) were sequenced and found to be identical to one another. These sequences were identical (100%, 350/350 bp) to the corresponding sequence of *Rickettsia bellii* (CP000087). Moreover, the gltA PCR amplicons from 3 other *H. juxtakochi* were sequenced and found to be identical to one another. These sequences were identical (100%, 350/350 bp) to the corresponding sequence of *Rickettsia rhipicephali* strain HJ5 (DQ865206).

Conclusion: *R. bellii* is probably the most frequent *Rickettsia* species infecting ticks in Brazil. *R. rhipicephali* was isolated from *H. juxtakochi* ticks in another Altantic Forest Reserve from the state of São Paulo (Labruna et al., 2007) and it is likely that this is a frequent interaction. Currently, the pathogenicity of *R. rhipicephali* and *R. bellii* to humans is still unknown.

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A-19. Detección Molecular de *Rickettsia* spp. (Da Rocha-Lima, 1916) en Garrapatas Recolectadas en Tres Regiones de Colombia

(Molecular Detection of *Rickettsia* spp. (Da Rocha-Lima, 1916) in Ticks Collected in Three Regions from Colombia)

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Justificación: Las bacterias del género *Rickettsia* son microorganismos patógenos transmitidos a humanos y animales principalmente por garrapatas de la familia Ixodidae. Durante los años 2005 a 2008, cuando se presentaron brotes confirmados de Rickettsiosis, en los departamentos de Córdoba, Antioquia y Cundinamarca (Colombia), fueron recolectadas garrapatas de animales y casas para el respectivo análisis epidemiológico. Hasta la fecha, no hay reporte de las especies de *Rickettsia* spp. que pudieron circular en estos artrópodos. El objetivo del presente estudio fue la identificación, por métodos moleculares, de las especies de *Rickettsia* que circularon en garrapatas colectadas durante

los brotes asociados a Rickettsiosis en los departamentos mencionados.

Métodos: Un total de 791 garrapatas fueron colectadas en los Municipios de Los Córdobas (Córdoba); Necoclí y Turbo (Antioquia) y Villeta y La Peña (Cundinamarca). Se procesaron en pools de la misma especie para la extracción de ADN con un estuche comercial y solución de lisis comercial. Cada pool fue sometido a una amplificación del gen *16S rRNA* mitocondrial de garrapata para evaluar una posible inhibición de la reacción de amplificación. Las muestras positivas a este gen, fueron posteriormente analizadas para la detección de material genético de *Rickettsia* a través de la amplificación de los genes *gltA*, *ompA* y *17kD*. Las muestras positivas fueron enviadas a secuenciación para posterior análisis de homología a través de BLAST e identificación de la especie.

Resultados: Se analizaron 240 pools de garrapatas de las especies *Amblyomma cajennense*, *Dermacentor nitens*, *Rhipicephalus sanguineus*, *R.* (*Boophilus*) *microplus* y *Amblyomma* sp. La mayoría de especímenes correspondieron a la especie *R. sanguineus* (35,8%). Un total de 15 muestras fueron positivas al gen *gltA* (6,3%) en su mayoría provenientes de los municipios de Villeta (47%) y La Peña (47%). Doce muestras fueron incluidas en la secuenciación. Once tuvieron identidad (>98%) con *R. prowazekii* y una con *R. felis* (99%) para el gen *gltA*; y una de las muestras del municipio de Villeta presentó identidad del 100% para *R. rickettsii* con el gen *17kD*.

Conclusión: Los resultados evidencian la presencia de bacterias del género *Rickettsia* y de la especie *R. rickettsii* en garrapatas asociadas a los brotes de rickettsiosis presentados entre los años 2005 y 2008 en tres regiones de Colombia.

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A-20. Identificación molecular de *Rickettsia* en *Haemaphysalis yuxtakochi y Amblyomma tapirellum* (Ixodida: Ixodidae) de fases no parasíticas en Panamá

(Molecular identification of Rickettsia on non-parasitic *Haemaphysalis juxtakochi* and *Amblyomma tapirellum* (Ixodida: Ixodidae) from Panama)

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Justificación: Las rickettsiosis son las zoonosis transmitidas por garrapatas más importantes en Panamá, las cuales son conocidas a partir de brotes en zonas rurales. Similar a otros países, en Panamá la diversidad de garrapatas está mejor representada en bosques; sin embargo, pocos estudios se han realizado en ambientes naturales. Por lo tanto, en este trabajo se presentan nuevos datos sobre la presencia de *Rickettsia* en fases de vida libre de garrapatas Ixodidae, en dos áreas boscosas cercanas a la Ciudad de Panamá.

Métodos: Durante enero-diciembre 2009, se recolectaron garrapatas en fases de vida libre en el Parque Municipal Summit y en áreas boscosas de Gamboa. Se identificaron las siguientes especies: *Amblyomma cajennense* s.l. (3), *Amblyomma naponense* (18), *Amblyomma pecarium* (2), *Amblyomma tapirellum* (12), *Haemaphysalis juxtakochi* (23) e inmaduros de *Amblyomma* (22).

Las garrapatas fueron analizadas individualmente en agrupaciones de (2-10 individuos) mediante Reacción en Cadena de la Polimerasa (PCR) utilizando cebadores que amplifican genes gltA, ompA y 17KDa.

Resultados: Se obtuvo secuencias parciales para el gen gltA en *Haemaphysalis juxtakochi* que mostró un 99.9% de identidad con Candidatus "*Rickettsia amblyommii".* Además las secuencias de ADN de los productos de PCR de *A. tapirellum* fueron 100% idénticos a *Rickettsia akarii.*

Conclusión: Estos resultados ofrecen nueva información sobre *Rickettsia* en Panamá, permitiendo conocer algunas especies de garrapatas que pudieran actuar como vectores y reservorios de agentes rickettsiales en estas áreas del país.

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A-21. Detección de una *Rickettsia* cercana a *Rickettsia monacensis* en *Ixodes boliviensis* de Costa Rica

(Detection of a *Rickettsia* closely related to *R. monacensis* in *Ixodes boliviensis* from Costa Rica)

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Background: *Rickettsia monacensis* was officially described in 2002 from *Ixodes ricinus* ticks in Germany. It has now been detected in most areas of Europe and has been associated with Mediterranean spotted fever-like human rickettsiosis in Spain and Italy. *Ixodes pacificus* and *Ixodes scapularis* from North America also contain similar *Rickettsia* sp., which have not been fully described. We report the presence of a *Rickettsia* sp. in *Ixodes boliviensis* from Costa Rica that is very closely related to *R. monacensis.*

Methods: In February 2012, ticks were collected from domestic dogs in the province of Heredia, Costa Rica. Specimens of the same species and from the same dog were pooled, and DNA was extracted. Pools were analyzed by *Rickettsia* spp. specific PCRs that detect fragments of the *gltA*, *htrA, ompA*, and *ompB* genes. Amplicons from positive pools were sequenced, and BLAST searches and phylogenetic analyses were performed.

Results: *Ixodes boliviensis* were collected from 5 of 9 dogs evaluated. DNA of *Rickettsia* spp. was detected in all 5 pools of *Ixodes boliviensis*. At least 2 of the tick pools contained *gltA* fragments that were 100% homologous with each other, and BLAST analyses determined 99.7% (365/366) homology to *R. monacensis* IrR/Munich. BLAST and phylogenetic analyses of *gltA*, *ompA, ompB*, and *htrA* amplicons confirmed that the *Rickettsia* sp. IbR/CRC present in *Ixodes boliviensis* ticks from Costa Rica groups closely with *R. monacensis* and *Rickettsia* sp. from *I. pacificus* and *I. scapularis*.

Conclusion: This is the first detection of a *Rickettsia* in *Ixodes boliviensis* ticks of Central America. Further analyses are required to determine if *Rickettsia* sp. IbR/CRC is a genotype of *R. monacensis*, a genotype of the *Rickettsia* sp. in *Ixodes* from North America, or a different species yet to be described. Considering that *I. boliviensis* can bite humans and that *R. monacensis* and other closely related species have been associated with human disease, it is important to characterize and determine the pathogenic potential of this *Rickettsia*.

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A-22. Un nuevo agente semejante a *R. parkeri* infectando la garrapata *Amblyomma parvitarsum* en Argentina y Chile

(A novel *R. parkeri*-like agent infecting the tick *Amblyomma parvitarsum* in Argentina and Chile)

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Background : The tick species *Amblyomma parvitarsum* typically inhabit the highland areas (altiplano) of Argentina, Chile, Bolivia and Peru. The present study reports for the first time the presence of a *Rickettsia* strain infecting *A. parvitarsum* ticks.

Methods: Adults of *Amblyomma parvitarsum* were collected 45 km west from San Antonio de los Cobres, Salta (4,500 m), Argentina; and from Arica and Parinacota region (4,069 m), Chile. Adult ticks were tested for rickettsial infection by PCR targeting the rickettsial genes *gltA, ompA* and *ompB.* In addition, ticks were also processed by the shell vial technique for isolation of rickettsiae in Vero cells.

Results: A total of 45 ticks from Argentina were individually tested by PCR, which yielded rickettsial DNA in 29 (64.4%) specimens. PCR products were sequenced from these ticks, which generated sequences closest to a *Rickettsia parkeri*-like strain from Brazil (strain Atlantic Forest), a novel pathogenic rickettsial strain shown to cause spotted fever illness in Brazil; the shared genetic similarities were 99.8%, 99.6%, and 99.1% identities with the partial sequences of the *gltA, ompA* and *ompB* genes, respectively. Phylogenetic analyses inferred from the three rickettsial genes showed that the *A. parvitarsum* rickettsiae is a new member of a group composed by a number of strains of *R. parkeri, Rickettsia sibirica,* and *Rickettsia africae*. Isolates of this novel *A. parvitarsum* rickettsia were established in Vero cells that had been inoculated with two ticks from Argentina, and with two ticks from Chile. The remaining Chilean ticks are still being tested by PCR in order to calculate their infection rate.

Conclusion: A novel *R. parkeri*-like agent is reported infecting *A. parvitarsum* ticks from Argentina and Chile, expanding our knowledge on tick-borne rickettsia in South America. The pathogenicity of this novel agent for humans is unknown; however, it should be regarded as potential pathogenic because of the pathogenic role of their sister genotypes, such as the pathogens *R. parkeri, R. sibirica,* and *R. africae*.

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Ponencias en Modalidad de Cartel. Sesión B

B-1. Presencia de *Ehrlichia canis* en donadores de Bancos de Sangre de Costa Rica

(Presence of *Ehrlichia canis* in donors from Blood Banks from Costa Rica)

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Justificación: Las ehrlichiosis humanas son enfermedades infecciosas emergentes, no contagiosas, zoonóticas, cuya transmisión está relacionada con picaduras de garrapatas de distintas especies. El estudio de las ehrlichiosis humanas en Costa Rica es importante, ya que su sintomatología puede ser tan simple como la de un resfriado común, o muy compleja y semejante al dengue hemorrágico, patología que se presenta con mucha regularidad en este país. El objetivo del presente trabajo es estimar la seropositividad contra *E. canis* y determinar la presencia de agentes del género *Ehrlichia* en donadores de Bancos de Sangre de Costa Rica.

Métodos: En el presente trabajo se utilizó la inmunofluorescencia indirecta (IFA) para detectar anticuerpos contra *Ehrlichia canis* en 100 sueros de donadores de Bancos de Sangre; además, se analizó la sangre mediante la técnica de Reacción en Cadena de la Polimerasa (PCR) del gen *dsb,* así como la secuenciación para determinar la presencia de ADN de *E. canis*, *E. chaffeensis* o *E. ewingii* en sangre de humanos de Costa Rica.

Resultados: La IFA detectó anticuerpos en 35 de los sueros analizados; de ellos, 30 (85,7%) evidenciaron títulos relativamente bajos (1:64 a 1:256), mientras que en 5 (14,3%) se

determinaron títulos altos (1:1024 a 1:8192). El 68,6% (24/35) de las muestras positivas correspondió al sexo femenino. En ambos sexos las edades coincidieron entre los 18 y los 35 años. Mediante PCR se detectaron 15 muestras positivas (5,3%) de un total de 280 muestras analizadas. De éstas se secuenciaron y analizaron 10 muestras por medio de BLASTn, confirmándose en ellas *E. canis*. Las muestras presentaron un porcentaje de identidad nucleotídica de 94 a 99% con respecto a otras cepas de *E. canis* depositadas en GenBank. El análisis estadístico mostró que, en forma global, no hay asociación entre el sexo de los donantes y la positividad del PCR, aunque si lo hubo en el grupo de donantes masculinos mayores de 30 años.

Conclusión: Estos resultados representan el primer reporte de la presencia de anticuerpos contra *E. canis* y la presencia de ADN de *E. canis* en humanos de Costa Rica y Centroamérica.

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B-2. Caracterización molecular de especies de *Ehrlichia* en perros, Distrito Central, Honduras.

(Molecular Characterization of *Ehrlichia* species in dogs, Central District, Honduras)

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Justificación: En Honduras el diagnóstico tradicional de la erhlichiosis canina se basa en la detección de mórulas intracitoplasmáticas en las células sanguíneas visualizadas en el frotis de sangre periférica; sin embargo su sensibilidad y especificidad es muy baja. El propósito de estudio fue caracterizar por biología molecular las especies del género *Ehrlichia* circulantes en el municipio del Distrito Central a partir de muestras de sangre total y de garrapatas extraídas en perros con sospecha clínica de ehrlichiosis

Métodos: Se colectaron 304 muestras de sangre total de igual número de caninos, estas fueron analizadas para detectar la presencia de distintas especies de *Ehrlichia* por medio de PCR-Nested. Se colectaron 781 garrapatas de 35 perros con sintomatología clínica, las que se caracterizaron mediante claves taxonómicas y en las cuales se investigó infecciones naturales con especies del género *Ehrlichia*.

Resultados: De las 304 muestras, 77 resultaron positivas a la presencia de mórulas intracitroplasmaticas y 72 muestras amplificaron para ADN de *Ehrlichia canis*, dando una prevalencia del 23.7%. No se logró detectar ADN de *Ehrlichia ewingii y Ehrlichia chaffensis*. La fuerza de concordancia entre el frotis de sangre y el PCR fue débil (Índice kappa= 0.2784; IC 95%= 0.1482 -0.4086). Con respecto a la ficha clínica los síntomas más frecuentes entre los perros que resultaron positivos a *E. canis* fueron anorexia (27.8%), debilidad (27.8%), fiebre (25.0%), mucosas pálidas (22.2%) y depresión (18.1%). Se recolectaron un total de 781 individuos (*Rhipicephalus sanguineus* 98.46% y *Amblyomma cajennense* 1.53%). Se demostró que *Rhipicephalus sanguineus* fue la especie de garrapata que más predominó y en la cual se logró demostrar la infección natural con *Ehrlichia canis* con una tasa de infección del 8%.

Conclusión: Este estudio representa el primer diagnóstico molecular de *E. canis* en sangre de perros e incrimina a *Rhipicephalus sanguineus* como el principal vector de la ehrlichiosis monocitica canina en el Municipio del Distrito Central, Honduras.

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B-3. Infección causada por Rickettsiae y Ehrlichiae en Perros en Región Semiárida del Noreste de Brasil

(Rickettsiae and Ehrlichiae Infection in Dogs in Semiarid Region of Northeast of Brazil)

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Background: *Rickettsia* and *Ehrlichia* species cause important tick-borne diseases that infect dogs worldwide, and were described in several Latin America countries, including Brazil. This study evaluated the infection by *Rickettsia* and *Ehrlichia* among dogs of Northeast of Brazil.

Methods: Dogs were sampled in urban and rural areas of Juazeiro, Bahia state, and Petrolina, Pernambuco state, within the semiarid region of Brazil. From June 2009 to April 2011, blood was collected from 504 dogs. Sera were tested by indirect immunofluorescence assay (IFA) for *Rickettsia rickettsii, R. parkeri, R. rhipicephali, R. amblyommii, R. bellii* and *Ehrlichia canis* antigens; whole blood was tested by PCR targeting fragments of the genes *gltA* for *Rickettsia*, and *dsb* for *Ehrlichia*. PCR products were DNA-sequenced. During blood collection, dogs were examined for the presence of ticks, fleas and lice. Risk factors for these tick-borne agents were also evaluated using logistic regression models.

Results: Overall, 12.1% (61/504) and 23.0 (116/504) of the dogs were seroreactive by IFA to at least one *Rickettsia* species, and to *E. canis*, respectively. Some canine sera showed titers to *R. amblyommii* (3 dogs), *R. rhipicephali* (1), *R. bellii* (11), and *R. rickettsii* (2) at least 4-fold higher than other rickettsial antigens. Molecular results showed *E. canis* infection in 8.3% (42/504) dogs, while no *Rickettsia* DNA was detected. Infestation by ticks (*Rhipicephalus sanguineus*), fleas (*Ctenocephalides felis felis*), and lice (*Hetedoroxus spiniger*)

were observed in 55.2% (278/504), 17.3% (87/504) and 2.0% (10/504) dogs, respectively. *Rickettsia* infection rates in ticks, fleas and lice were 1.1% (3/285), 40.7% (74/182), and 0 (0/18), respectively. Tick infection rate by *Ehrlichia* was 4.9% (14/285). DNA sequencing indicated infection by *Rickettsia felis* in fleas and ticks; and *E. canis* in ticks. Risk factors for presence of *Rickettsia* spp. antibodies were (i) live at Petrolina municipality; and (ii) presence of ectoparasite.

Conclusion: Considering the low prevalence of rickettsial infection by IFA; the presence of the lower pathogenic *Rickettsia* species as probable antigen; and the absence of human cases; we concluded that the study area may be classified as non-endemic for spotted fever rickettsiosis. However, canine monocytic ehlrichiosis (caused by *E. canis*) was more frequent in the study area, associated with the high prevalence of canine infestation by *R. sanguineus* ticks.

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B-4. La diversidad genética de *Ehrlichia canis* en Brasil

(Genetic diversity of *Ehrlichia canis* in Brazil)

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Background: Canine monocytic ehrlichiosis (CME) is a potentially fatal tick-borne rickettsial disease of dogs caused by *Ehrlichia canis*. CME is endemic in Brazil, highly prevalent among dogs throughout the country. Despite the high prevalence, little is known regarding the molecular characteristics of Brazilian *E. canis*. The present study investigated the genetic diversity of *E. canis* in Brazil based on amino acid sequences of TRP36 protein of new Brazilian *E. canis* isolates.

Methods: Blood samples were collected from 126 dogs assisted at Veterinary Hospitals and Zoonosis Control Centers from various regions in Brazil from January 2007 to February 2012 and were tested by PCR protocol to amplify *dsb* ehrlichial gene. *E. canis* PCR positive samples were inoculated into DH82 cells for isolation. Genomic DNA of isolates was purified and amplified by PCR with universal primers TRP36-F2 (5'-TTTAAAACAAAATTAACACACTA-3') and TRP36-R1 (5'-AAGATTAACTTAATACTCAATATTACT-3') in order to obtain full *TRP36* gene sequences. The amplicons of *TRP36* gene was sequenced and the BLAST program was used for the comparison with different *E. canis* isolates previously deposited in GenBank database. The phylogenetic relationships were determined with the MEGA Beta program.

Results: DNA of the *Ehrlichia dsb* gene was amplified from 82 (65%) dogs and *E. canis* was isolated from 13 dogs within 30 days of inoculation into cell culture. Partial sequences of the *dsb* gene amplified from the isolates had 100% identity to *E. canis dsb* sequences in GenBank. Isolates obtained were named according the city of origin; northern region (Belem, Monte Negro#15; Monte Negro#24), midwest region (Cuiaba#1; Cuiaba#16), northeast region (Petrolina), southeast region (Presidente Prudente; Atibaia#7; Atibaia#14; Nova VenéciaNv#1), and southern region (Londrina). The amplified TRP36 gene was sequenced from eight Brazilian isolates and two major genogroups were identified based on tandem repeat sequence. Isolates with TR amino acid sequence (TEDSVSAPA) identical to the previously reported TRP36 sequence were found in the midwest (Cuiabá#16), northeast and southeast regions of Brazil, and classified into the US genogroup. A novel Brazilian genotype with a different tandem repeat sequence (ASVVPEAE) was also identified in Midwest (Cuiabá#1), northern and southern regions. Other subtypes within the Brazilian genogroup were also identified using C-terminal amino acid divergence. Similarity in the N-terminal sequence of Cuiabá#16 US genogroup member with the Brazilian genogroup suggested that genomic recombination between the two genogroups may have occurred.

Conclusion: We identified two distinct major Brazilian genogroups demonstrating substantial genetic diversity within Brazilian *E. canis* strains.

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B-5. Detección molecular de *Rickettsia amblyommii* y *Ehrlichia canis* en garrapatas de vegetación y garrapatas sobre animales domésticos en Costa Rica

(Molecular detection of *Rickettsia amblyommii* and *Ehrlichia canis* in ticks from vegetation and domestic animals in Costa Rica)

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Justificación: Especies de los géneros *Rickettsia, Ehrlichia* y *Anaplasma* son agentes infecciosos transmitidos por garrapatas, que pueden ocasionar enfermedad en humanos y animales,

considerándose en la actualidad como enfermedades emergentes. Las principales especies de garrapatas que transmiten estos patógenos pertenecen a los géneros *Amblyomma, Rhipicephalus, Ixodes, Dermacentor* y *Haemaphysalis*, las cuales están presentes en Costa Rica, país que al ser tropical presenta condiciones ideales que favorecen la reproducción de las garrapatas. El objetivo del estudio fue detectar mediante técnicas moleculares la presencia de *E. canis*, *E. chaffeensis*, *E. ewingii, A. phagocytophilum* y *Rickettsia* spp. en garrapatas recolectadas en la de vegetación y sobre animales domésticos en Costa Rica.

Métodos: Se analizaron garrapatas capturadas en bosque primario, bosque secundario y potrero, y garrapatas sobre animales domésticos de 13 localidades (Hojancha, Cañas, Sarapiquí, Talamanca, Turrialba, Guápiles, Ciudad Colón, La Unión, Poás, Goicoechea, Osa, La Palma y Barbacoas) mediante la técnica de Reacción en Cadena de la Polimerasa (PCR) para determinar la presencia de *Rickettsia* spp., *Ehrlichia* spp. y *Anaplasma phagocytophilum.* Las muestras que resultaron positivas en PCR fueron sometidas a secuenciación para identificar la especie y confirmar los resultados.

Resultados: El análisis mediante PCR y secuenciación determinó que larvas y ninfas de *Amblyomma* spp. encontradas en potreros de Ciudad Colón y Hojancha, respectivamente; garrapatas *Amblyomma coelebs* en bosque primario de Sarapiquí y garrapatas *Amblyomma cajennense* en bosque secundario de Cañas y La Palma, fueron positivas a *Rickettsia amblyommii.* Las restantes garrapatas positivas a *R. amblyommii* provenían de equinos de Sarapiquí, Talamanca, Ciudad Colón, Osa y La Palma, y fueron identificadas como *A. cajennense* y *A. nitens.* Además caninos de Hojancha, Ciudad Colón y Barbacoas presentaron garrapatas *A. cajennense* y *R. sanguineus* positivas a *R. amblyommii.* Garrapatas *A. cajennense* y *R. sanguineus* recolectadas de caballo y perro de Ciudad Colón, respectivamente, resultaron positivas a *E. canis*. La garrapata *A. cajennense* proveniente del caballo de Ciudad Colón presentó infección mixta (*R. amblyommii* y *E. canis*). No se encontró *A. phagocytophilum* en las garrapatas analizadas.

Conclusión: Estos hallazgos muestran una amplia distribución de *R. amblyommii* en el país y reportan por primera vez la detección de *R. amblyommii* en *A. nitens* y *A. coelebs* recolectadas de equinos y vegetación, respectivamente; además de la presencia de *E. canis* en una garrapata recogida de un equino.

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B-6. Detección de agentes zoonóticos (*Ehrlichia* spp., *Rickettsia* spp., *Anaplasma platys,* y *Borrelia burgdorferii* s.l.) en garrapatas recolectadas de perros en Costa Rica

(Detection of zoonotic agents (*Ehrlichia* spp., *Rickettsia* spp., *Anaplasma platys,* and *Borrelia burgdorferii* s.l.) in ticks collected from dogs from Costa Rica)

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Justificación: Las condiciones propias de un país tropical como Costa Rica representan grandes ventajas para la distribución y actividad de las garrapatas. Por otro lado, la biodiversidad propia del país, facilita que existan una serie de hospedadores para este tipo de ectoparásitos. Ambas condiciones inciden en la presentación y prevalencia de enfermedades vectoriales zoonóticas. El objetivo del presente trabajo fue detectar mediante técnicas moleculares la presencia de diferentes especies de *Ehrlichia*, *Anaplasma, Rickettsia y Borrelia burgdorferi* s.l. en garrapatas de perros.

Métodos: De octubre 2006 a julio del 2007 se recolectaron 165 garrapatas de perros atendidos en clínicas veterinarias ubicadas en el Valle Central (157) y algunas residencias (8) en cinco provincias de Costa Rica. En el caso de garrapatas adultas se analizó una garrapata por perro, mientras que en el caso de ninfas se analizaron grupos de cinco. El ADN extraído de las garrapatas se sometió a diferentes técnicas de PCR para determinar la presencia de especies de *Ehrlichia, Rickettsia* y *Anaplasma platys.* En Alemania se analizaron las muestras de garrapatas con un PCR en Tiempo Real para determinar la presencia de *B. burgdorferii* s.l. Finalmente las muestras positivas fueron secuenciadas y comparadas con secuencias del GenBank mediante un Blast.

Resultados: De las garrapatas recolectadas se identificaron 160 como *Rhipicephalus sanguineus*, 4 como *Amblyomma ovale* y 1 como *Ixodes boliviensis. E. canis* y *A. platys* fueron detectados por PCR anidado en 43 (26.06%) y 5 (3.03%) garrapatas *R. sanguineus,* respectivamente, siendo éste el primer reporte de la presencia de estos agentes en garrapatas de Costa Rica. Dos garrapatas presentaron infecciones mixtas con *E. canis* y *A. platys.* Las secuencias para cada uno de estos agentes, mostraron una similitud de 99% para *E. canis* y de 98% para *A. platys. Rickettsia* spp. fue detectado mediante PCR (gltA y ompA) en 3 garrapatas *R. sanguineus* y en 1 garrapata *I. boliviensis*, la secuenciación determinó a estas como *Rickettsia amblyommii.* No se detectó la presencia de *E. chaffeensis* y *E. ewingii* y *B. burgdorferii* s.l. en ninguna de las garrapatas analizadas.

Conclusión: Se detectó por primera vez la presencia de *E. canis* y *A. platys* en garrapatas *R. sanguineus* y la presencia de *R. amblyommii* en una garrapata *I. boliviensis* de perros en Costa Rica.

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B-7. Patógenos transmitidos por garrapatas en sangre y garrapatas de venados cola blanca de Costa Rica

(Tick borne pathogens in blood and ticks of white-tailed deer from Costa Rica)

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Justificación: Un reservorio animal se define como un huésped, que aloja un patógeno, al cual le proporciona condiciones ideales para su multiplicación y el cual es capaz de transmitir ese agente mediante vías de eliminación naturales o vectores a un huésped susceptible, sin presentar signos clínicos. Diferentes estudios experimentales realizados con venados cola blanca (*Odocoileus virginianus*) indican que estos actúan como reservorios para *Ehrlichia chaffeensis*, *E. ewingii* y *Anaplasma phagocytophilum*. El objetivo del presente estudio consistió en detectar la presencia de *Ehrlichia* spp., *A. phagocytophilum y Rickettsia* spp. en sangre y garrapatas de venados cola blanca de Costa Rica.

Métodos: Se recolectaron muestras de sangre de 44 venados (Alajuela 17, Limón 8, Puntarenas 5, Guanacaste 5, Heredia 4, Cartago 3, y San José 2) y 59 garrapatas *Rhipicephalus microplus* encontradas sobre siete venados de Alajuela. Todas las muestras de sangre se analizaron en forma individual, mientras que garrapatas hembras adultas en grupos de 2-3 individuos, garrapatas adultas machos en grupos de 5 y ninfas en grupos de 5 individuos. Se utilizó la técnica de Reacción en Cadena de la Polimerasa (PCR) para determinar la presencia de *Ehrlichia* spp., *Anaplasma phagocytophilum* y *Rickettsia* spp. Las muestras positivas mediante PCR se secuenciaron para identificar la especie.

Resultados: Solamente una muestra de sangre de Alajuela resultó positiva para *A. phagocytophilum*, mientras que en cinco muestras de sangre de Alajuela (3), Heredia (1) y Puntarenas (1) se detectó *Rickettsia amblyommii*. Además 2 garrapatas de 2 venados fueron positivas a *R. amblyommii*. No se encontró *E. chaffeensis* y *E. ewingii* en las muestras de sangre y garrapatas.

Conclusiones: Se reporta el primer diagnóstico molecular de *A. phagocytophilum* y *R. amblyommii* en muestras de sangre de venados en Costa Rica. En garrapatas *R. microplus* recolectadas de venados se determinó la presencia de *R. amblyommii.* No se determinó la presencia de *E. chaffeensis* y *E. ewingii* en garrapatas y sangre de venados de Costa Rica.

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B-8. Detección molecular de *Anaplasma platys* en perros de Costa Rica

(Molecular detection of *Anaplasma platys* in dogs from Costa Rica)

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Justificación: *Anaplasma platys* es el agente causal de la trombocitopenia cíclica infecciosa canina, una enfermedad transmitida por la garrapata *Ripicephalus sanguineus.* La enfermedad ha sido reportada, hasta la fecha, solamente afectando a caninos. La infección aguda se caracteriza por parasitemia en las plaquetas, seguida por episodios de trombocitopenia; en algunos casos, se han reportado signos clínicos de mayor severidad, similares a aquellos asociados a infecciones con *E. canis.* El objetivo del presente trabajo fue determinar, mediante técnicas moleculares, la presencia de *A. platys* en perros de Costa Rica.

Métodos: Se analizaron 300 muestras sanguíneas de perros con sintomatología sospechosa de erliquiosis o trombocitopenia cíclica infecciosa canina. Todos los animales habían sido atendidos en diferentes clínicas veterinarias. Se utilizó un PCR anidado para amplificar específicamente *A. platys*.

Resultados: Un total de 19 (6,33%) muestras resultaron positivas, demostrando la presencia del agente en perros de las provincias de Alajuela (5), San José (5), Heredia (4), Guanacaste (2) y Cartago (1). Una cepa de *A. platys* fue secuenciada y mostró un 100% de similitud con una secuencia parcial del gen 16S ARNr de *A. platys* (AF156784) publicado en GenBank. Este hallazgo representa el primer reporte de la detección de *A. platys* en caninos de Costa Rica.

Conclusión: El agente infeccioso *A. platys* está presente en perros de Costa Rica y, por lo tanto, debe ser tomado en cuenta, por los médicos veterinarios practicantes en pequeñas especies, en el diagnóstico diferencial de enfermedades que afectan el sistema hematopoyético. Se recomienda realizar el diagnóstico molecular de *A. platys* mediante el PCR anidado.

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B-9. Seroprevalencia de *Dirofilaria immitis*, *Ehrlichia canis*, *Anaplasma* spp. y Borreliosis de Lyme en perros de Costa Rica

(Seroprevalence of *Dirofilaria immitis*, *Ehrlichia canis*, *Anaplasma* spp. y Borreliosis de Lyme in dogs from Costa Rica)

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Justificación: Costa Rica reviste factores particulares respecto a la epidemiología de enfermedades vectoriales. Ser un país tropical y no contar con cuatro estaciones permite que, todo el año, se mantengan poblaciones de artrópodos vectores de agentes infecciosos que afectan cánidos. Actualmente, es común la presencia de mascotas en la mayoría de los hogares

del país, principalmente perros. Es normal que estas mascotas reciban atención veterinaria, con especial interés por controlar ectoparásitos. Recientemente, por múltiples factores, sociales, económicos y culturales, se concentró una alta población canina en el Valle Central, y una población menor en costas y regiones fronterizas. El objetivo del estudio fue determinar la prevalencia de *Dirofilaria immitis*, *Ehrlichia canis*, *Anaplasma* spp. y Borreliosis de Lyme mediante una prueba serológica comercial (SNAP 4X®).

Métodos: Se muestrearon perros de clínicas veterinarias de centros urbanos de alta población humana de las siete provincias, así como dos puntos de atención veterinaria que son parte de proyectos de extensión de la EMV de la UNA. Criterios de inclusión: 1) anuencia del propietario, previo consentimiento informado, a muestrear sus mascotas, 2) mayores de un año, 3) residencia en Costa Rica durante el último año, 4) sin tratamiento con ivermectina en los últimos seis meses, 5) sin tratamiento con doxiciclina. No se excluyeron animales por presencia o ausencia de signos de enfermedad. Se muestrearon 314 perros (157 de cada sexo) de 18 comunidades. El diagnóstico se realizó mediante la prueba comercial SNAP 4X®, siguiendo las especificaciones del fabricante.

Resultados: De los perros muestreados, asistieron a control el 88%, a consulta curativa 10,5% y 1,2% por ectoparásitos. El promedio de edad fue 4,7 años (DE 1,3). Presentaron algún síntoma de enfermedad el 52,1%. El agente más detectado fue *E. canis* (37,0%), presentando perros positivos en todas las localidades. Proceder de la provincia de Puntarenas se asoció con la presencia de antígeno contra *D. immitis,* asimismo, proceder de zonas costeras presenta asociación con serología positiva a *Anaplasma* spp.

Conclusión: Se demuestra la presencia de anticuerpos contra *E. canis* en perros procedentes de las siete provincias y se detectan anticuerpos contra *Anaplasma* spp. Se evidencia *D. immitis,* principalmente en Puntarenas. Es importante informar que médico veterinario conozca sobre la distribución y características de estos agentes. Se recomienda mantener la vigilancia de enfermedades transmitidas por vectores y considerar su distribución y prevalencia para prevenir, controlar y tratar.

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B-10. Aspectos sobre el control de ectoparásitos y diagnóstico de enfermedades vectoriales a nivel de clínicas veterinarias en Costa Rica. Encuesta

(Aspects about ectoparasites control and diagnostic of vector borne diseases in veterinary clinics in Costa Rica. Survey)

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Justificación: Costa Rica reviste factores particulares respecto a la incidencia de ectoparásitos en animales de compañía. Ser un país tropical y no tener cuatro estaciones permite que, durante todo el año, se mantengan poblaciones constantes de artrópodos como pulgas y garrapatas, reconocidos vectores de agentes infecciosos que pueden afectar a cánidos. Actualmente, es común la presencia de mascotas en la mayoría de los hogares del país; principalmente perros. La atención veterinaria para estas mascotas es constante y el control de los ectoparásitos es importante para el propietario. El objetivo fue determinar mediante una encuesta telefónica, con qué frecuencia los médicos veterinarios reciben consultas por problemas con ectoparásitos, cuales son estos ectoparásitos, como se aborda el control de éstos y cuáles de las enfermedades vectoriales, se consideran en el diagnóstico y cómo se confirman.

Métodos: Gracias a una lista brindada por el Colegio de Médicos Veterinarios, donde se incluyen datos de todos los establecimientos que brindan servicios médicos veterinarios en el país, se procedió a realizar una encuesta telefónica, que debía ser respondida únicamente por el médico veterinario responsable del establecimiento.

Resultados: Se logró contactar al total de los establecimientos presentes en la lista oficial brindada, de éstos, 82 médicos veterinarios contestaron la encuesta, obteniendo respuestas de las siete provincias, mayormente de San José (35,4%); la mayoría indicaron dedicarse principalmente a la práctica de especies menores (76,8%). Al preguntarse cómo consideraban la frecuencia de consultas por problemas con ectoparásitos un 81,7% contestó "alta"; a la pregunta sobre cuál ectoparásito es por el que más frecuentemente acuden sus clientes, un 46,3% indicó garrapatas en primer lugar y 41,5% pulgas. Los tratamientos más recomendados incluyen productos "pour on" y collares. Acerca de cuál enfermedad vectorial considera principalmente en su diagnóstico un 78% respondió Ehrlichiosis y un 47,6% indicó que lo confirma mediante una prueba serológica comercial, mientras que un 30,5% lo hace mediante hemograma.

Conclusión: Se logró acceder a un grupo de médicos veterinarios practicantes, logrando de primera mano información sobre el abordaje y el control de los principales ectoparásitos que afectan a cánidos en el país. Se logró demostrar que existe una gran variedad de protocolos control y qué por lo general algunas enfermedades vectoriales no son consideradas por el médico veterinario como posible diagnóstico. Se debe valorar la posibilidad de organizar charlas o conferencias sobre el diagnóstico de enfermedades vectoriales y el control de ectoparásitos en animales de compañía.

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B-11. Rol de la *Rhipicephalus sanguineus* en la transmisión de *Anaplasma platys*

(Role of *Rhipicephalus sanguineus* in the transmission of *Anaplasma platys)*

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Justificación: *Anaplasma platys* es el agente etiológico de la trombocitopenia cíclica infecciosa y se caracteriza por parasitar las plaquetas de caninos, produciendo fiebre, decaimiento, pérdida de peso, petequias en las mucosas, lesiones hemorrágicas cutáneas y hemorragias fatales en casos graves. En la actualidad no se conoce con certeza cuál es el vector de *Anaplasma platys*. Investigadores plantean que la *Rhipicephalus sanguineus* es la principal sospechosa de la transmisión de esta enfermedad. Algunos autores lograron amplificar ADN de *Anaplasma platys* en el intestino de la *Rhipicephalus sanguineus,* sin embargo otros autores no lo han logrado. *Rhipicephalus sanguineus* puede no ser un eficiente transmisor, otra especie de garrapata puede estar involucrada. Este trabajo tiene como objeto determinar la participación de la garrapata *Rhipicephalus sanguineus* en la transmisión de *Anaplasma platys* en caninos

Métodos: La metodología empleada fue la siguiente: se inoculo un canino con una cepa de *Anaplasma platys* y una vez que desarrollo parasitemia confirmada por frotis y por PCR, se infestó con ninfas y adultos de *Rhipicephalus sanguineus*, se disecó el intestino, la glándula salival de las garrapatas alimentadas en el canino inoculado y se realizó PCR.

Resultados: No se logró amplificar ADN de *Anaplasma platys* ni en el macerado de ninfas, ni en adultos post muda sin alimentar, ni en adultos alimentados del canino inoculado experimentalmente

Conclusión: *Rhipicephalus sanguineus* no participa en la transmisión de *Anaplasma platys.*

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B-12. Detección molecular del agente zoonótico *Anaplasma phagocytophilum* en muestras de sangre de caballos, sangre y garrapatas de perros de Costa Rica

(Molecular detection of the zoonotic agent *Anaplasma phagocytophilum* in blood samples from horses, dogs and dog ticks in Costa Rica)

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Justificación: La ehrlichiosis y la anaplasmosis se consideran problemas emergentes y su importancia ha ido aumentando, sugiriendo que este agente podría estar afectando la salud humana y animal. Estudios previos realizados en nuestro país lograron detectar la presencia de *Ehrlichia canis* y *Anaplasma platys* en sangre de perros y garrapatas de perros; sin embargo sugirieron la presencia de otra especie de la misma familia (Anaplasmataceae) en perros y garrapatas de Costa Rica; posiblemente *A. phagocytophilum*. El objetivo del presente estudio fue determinar la presencia de *A. phagocytophilum* en sangre de caballos, así como en garrapatas y sangre de perros de Costa Rica.

Métodos: Mediante PCR anidado se analizó la presencia de una secuencia del gen ARN 16S de *A. phagocytophilum* en 300 muestras de sangre de caballos y un banco de ADN compuesto por 342 muestras de sangre de perros y 160 muestras de garrapatas de perros de diferentes regiones de Costa Rica.

Resultados: Ninguna de las muestras de caballo resultó positiva para el agente; tres (0.9%) muestras de sangre de perro y dos (1.25%) muestras de garrapatas *Rhipicephalus sanguineus* resultaron positivas en PCR. Una de las muestras de sangre de perro positiva al agente provenía de Jacó, Puntarenas; de las otras dos muestras no se pudo obtener su procedencia. Las muestras de garrapatas positivas provenían de perros que habían sido presentados en clínicas veterinarias de San José y Heredia. La confirmación de los resultados mediante secuenciación está en proceso.

Conclusiones: Se reporta la presencia de *A. phagocytophilum* en garrapatas *Rhipicephalus sanguineus* y sangre de perros de Costa Rica.

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B-13. Detección de *Anaplasma* spp. en garrapatas duras (Ixodidae) retiradas de tapires de montaña, vacas y vegetación en un área protegida de Ecuador

Detection of *Anaplasma* spp. in ticks (Ixodidae) removed from mountain tapirs, cattle and vegetation in a protected area from Ecuador

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Background: Tick-borne diseases (TBD) affecting wildlife are frequently unknown and can negatively influence in the survival of critically endangered animals. In addition, these animals can be reservoirs and source of infection for humans and other animals. Thus, TBD may be a threat to mountain tapirs (*Tapirus pinchaque*) in Ecuador, where these populations are in close contact with cattle. For these reasons, and due to the lack of information about the presence of Anaplasmataceae in ticks removed from animals or vegetation in Ecuador, our aim was to investigate the presence of *Anaplasma / Ehrlichia* spp.

in ticks removed from mountain tapirs, cattle and vegetation collected in a protected area from Ecuador.

Methods: A total of 151 ticks from mountain tapirs (n=74), cattle (n=61) and vegetation (n=16) collected in Antisana Ecological Reserve and Cayambe-Coca National Park (Ecuador) were analyzed using molecular biology tools at the Center of Rickettsioses and Arthropod-Borne Diseases (Spain). PCR assays and sequencing of *msp2* from *Anaplasma phagocytophilum* (primer pair: MSP2-3F/MSP2-3R) and 16S rRNA fragment genes from Anaplasmataceae (primer pairs: ge3a/ge10r; ge2/ge9f; EHR16SD and EHR16SR; GEP-s and GEP-as) were performed.

Results: Using *msp2* PCRs, *A. phagocytophilum* was found in 10 out of 151 ticks: 2 *Amblyomma multipunctum* (removed from a mountain tapir and vegetation, respectively) and 8 *Rhipicephalus microplus* (all removed from the same cow). Only 2 of these *R. microplus* specimens were also positive for nested PCR assays of 16S rRNA gene (primers ge3a/ge10r and ge2/ge9f). The 16S rRNA amplicons showed highest identity with *A. phagocytophilum.* In addition, when primers EHR16SD/EHR16SR and GEP-s/GEP-as were used in single PCR assays, 5 *R. microplus* (removed from 3 cows) evidenced the presence of *Anaplasma* spp. Nucleotide sequences obtained were identical each other and showed 100% identity with more than one *Anaplasma* species (*A. marginale, A. ovis, A. centrale* or *A. phagocytophilum*). Lastly, one *R. microplus* tick was also co-infected with *Rickettsia* sp. (these data are shown in other abstract).

Conclusion: 1.- *A. phagocytophilum* has been found in *A. multipunctum* ticks from mountain tapir and vegetation. 2.- *Anaplasma* spp. has been detected in *R. microplus* from cattle. 3.- The presence of *A. phagocytophilum* has been highlighted for the first time in Ecuador and in ticks removed from mountain tapirs.

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B-14. Infestación natural por *Ixodes* sp. (Acari: Ixodidae) en animales domésticos y en un humano en Venezuela

(Natural infestation by Ixodes sp. (Acari: Ixodidae) in domestic animal and in a human in Venezuela)

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Justificación: Las garrapatas del genero *Ixodes* comúnmente parasitan reptiles, aves y mamíferos y algunas de ellas pueden ser eficientes transmisoras de patógenos a animales domésticos, silvestres e incluso al hombre. Este género incluye aproximadamente 240 especies en el mundo, de las cuales 46 aparecen registradas en la región Neotropical. No obstante este alto número de especies en la región, la información sobre distribución y hospederos para Venezuela es relativamente escasa. Las garrapatas adultas prefieren alimentarse sobre grandes mamíferos como: perros, ganado, caballos, venados, borregos, cerdos y el hombre. Las larvas y las ninfas se alimentan principalmente de aves y pequeños mamíferos. Estas especies han sido encontradas en Europa, África y América, mientras que en Venezuela hasta la fecha se han identificado sólo en animales silvestres. *Ixodes* sp. está involucrada en la transmisión de una gran variedad de agentes infecciosos al hombre y animales tales como *Borrelia burgdorferi, Babesia divergens*, *Babesia bovis*, *Anaplasma marginale*, así como parálisis recurrente en humanos por sus toxinas. El objetivo de este estudio fue la identificación taxonómica de especies de garrapatas encontrada infestando naturalmente perros, gatos, humanos y venado.

Metodología: Las garrapatas fueron obtenidas de varias especies que permanecían en área domiciliar y peri-domiciliar. Se realizó la extracción manual de los ixodidos con la ayuda de una pinza, con un leve movimiento para su extracción completa, se colocaron en envases plásticos *ad hoc* para su traslado al Laboratorio de Parasitología del DCV-UCLA y su posterior identificación morfológica utilizando claves taxonómicas.

Resultados: Se colectaron en total 46 ejemplares de los cuales 9 fueron en perros domésticos y 1 en una persona en el estado Lara. Así como dos en gato doméstico, dos en un venado y 32 ejemplares en perros domésticos en estado Mérida. Estas fueron identificadas taxonómicamente como del género *Ixodes* basándose en sus características morfológicas más resaltantes; a saber: presencia de un surco anal anterior al ano (postriata), escudo no ornamentado, ausencia de ojos y de festones, entre otras.

Conclusión: Este estudio reporta por vez primera en Venezuela la presencia del género *Ixodes* infestando estos hospedadores, en dos regiones con características ecológicas similares de clima templado de montaña, cuyas temperaturas oscilan entre los 17-22°C y una altura alrededor de los 1500 msnm. La identificación de la especie esta siendo realizada con técnicas moleculares, porque taxonómicamente hay características que sugieren que en este estudio hay más de una especie de *Ixodes.*

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B-15. Distribución geográfica de garrapatas duras (Parasitiformes: Ixodidae) en ambiente y animales domésticos de diferentes ecotopos en Costa Rica

(Geographic distribution of hard ticks (Parasitiformes: Ixodidae) in the environment and on domestic animals from different ecotopes from Costa Rica)

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Justificación: Los estudios de garrapatas sobre animales domésticos de América Central son escasos. En Costa Rica se cuenta con un catálogo de garrapatas suaves y duras; y los estudios existentes se han enfocado en registros de especies, carga parasitaria, distribución y taxonomía de garrapatas en animales domésticos, así como de resistencia a los acaricidas y control alternativo. También se reportan estudios de garrapatas sobre perros de ambientes domiciliares, bovinos y equinos de áreas ganaderas. Sin embargo, no existe información sobre estudios de garrapatas en vegetación y de animales domésticos en ecotopos con diferencias altitudinales, por lo que se realizó el presente trabajo.

Métodos: Durante el 2009 se recolectaron e identificaron garrapatas de ambiente (bosque primario [BP], bosque secundario [BS] y potrero [P]), y garrapatas sobre animales domésticos (bovinos, equinos y caninos), procedentes de trece localidades y seis regiones de Costa Rica (Pacífico Norte, Zona Norte, Zona Atlántica, Meseta Central, Pacífico Sur y Pacífico Central). Se utilizó el método de trampeo con CO_2 y bandereo para las garrapatas de vegetación y el método de extracción manual, para las garrapatas sobre animales domésticos.

Resultados: Se recolectaron un total de 3,314 garrapatas (larvas, ninfas y adultas), 851 de vegetación (819 larvas, 16 ninfas y 16 adultas) y 2,463 de animales domésticos (8 larvas, 341 ninfas y 2114 adultas). Las garrapatas adultas recolectadas de vegetación fueron *Amblyomma cajennense* (11 BP, 4 BS y 9 P), *Amblyomma coelebs* (7 BP) y *Amblyomma dissimile* (1 BS), mientras que las larvas fueron de los géneros *Rhipicephalus* spp. (780 P) y *Amblyomma* spp. (39 P). Las garrapatas adultas recolectadas de animales domésticos pertenecieron a las especies *Anocentor nitens* (1,360), *Rhipicephalus microplus* (978), *Rhipicephalus sanguineus* (75), *A. cajennense* (30), *Amblyomma ovale* (10) y *Amblyomma imitator* (10). La especie de garrapata más frecuente en caballos fue *Anocentor nitens*, en bovinos *R. microplus* y en caninos *R. sanguineus.* Las larvas de los géneros *Amblyomma* (BP y BS) y *Riphicephalus* (P) fueron los estadíos que se recolectaron en mayor proporción en las localidades analizadas.

Conclusión: En el presente estudio se reporta por primera vez la presencia de *A. cajennense* y *A. coelebs* en bosque primario de la provincia de Heredia.

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B-16. Estudio de la competencia vectorial de *Ehrlichia canis* por cuatro poblaciones de *Rhipicephalus sanguineus*

(Study of vector competence of *Ehrlichia canis* by four populations of *Rhipicephalus sanguineus*)

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Justificación: Estudios sobre ehrlichiosis canina en América Latina indican que *E. canis* es altamente prevalente en países de América Latina tropical, siendo rara en América Latina templada (cono Sur). El objetivo del presente trabajo fue evaluar la capacidad de cuatro poblaciones de *Rhipicephalus sanguineus* Neotropicales, de transmitir la bacteria durante la alimentación de sangre de caninos saludables.

Métodos: Larvas y ninfas de garrapatas derivadas de cuatro poblaciones de *R. sanguineus* provenientes de Argentina, Uruguay, Estado de Rio Grande do Sul (América Latina templada), y de la ciudad de San Pablo (América Latina tropical) fueron expuestos a *E. canis,* alimentando se sobre caninos experimentalmente infectados, en la fase aguda de la enfermedad. Paralelamente, larvas y ninfas no infectadas de cada una de las cuatro poblaciones fueron usadas para infestar caninos sanos (grupo control). Las larvas y ninfas ingurgitadas recuperadas fueron dejadas en estufa para que mudaran a ninfas y adultos respectivamente, para su posterior uso para infestar caninos no infectados. Ejemplares de estas fases de las cuatro poblaciones fueron procesados por PCR en tiempo real para investigar la presencia de ADN de *E. canis.* Muestras de sangre de los caninos infestados se colectaron semanalmente durante dos meses, y fueron procesadas inmediatamente para hemograma, serología (técnica de inmunofluorescencia indirecta para anticuerpos anti-*E. canis*) y PCR en tiempo real.

Resultados: Solamente los caninos infestados con adultos de *R. sanguineus* de San Pablo expuesto a *E. canis* en la fase de ninfa, presentaron alteraciones en el número de eritrocitos, volumen globular, hemoglobina y plaquetas por debajo del valor mínimo de referencia para caninos sanos, títulos de anticuerpos anti-*E. canis* a partir del día 14 post-infestación, variando entre 1280 y 327680, y positividad entre 19 y 48 días para PCR en tiempo real. Ningún canino presentó fiebre. En relación a las garrapatas analizadas, solo las provenientes de San Pablo fueron positivas, siendo 1% (1 muestra positiva /100 garrapatas analizadas) de las ninfas y 28% (80/285) de los adultos.

Conclusión: Los resultados obtenidos permiten una mejor comprensión de la ausencia de casos de infección canina por *E. canis* en el cono Sur y refuerzan la hipótesis de que en estas áreas tal hecho se debe a la baja competencia vectorial de las garrapatas de la especie *R. sanguineus* presentes en esa región, al contrario de América tropical, donde las garrapatas presentes de la especie *R. sanguineus* poseen alta competencia vectorial.

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B-17. Desarrollo de un modelo de transmisión vectorial en ehrlichiosis monocitotrópica

(Development of a Vector Transmission Model for Monocytotropic Ehrlichiosis)

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Background: Ehrlichial diseases affect a broad range of mammalian hosts including humans. *Ehrlichia chaffeensis* and *E. ewingii* are important human pathogens. A new *Ehrlichia* species closely related to *E. muris* infects humans in Wisconsin and Minnesota where it is found in *Ixodes scapularis* ticks. We have developed a tick transmission model with this new species.

Methods: Acquisition of ehrlichiae was accomplished using *Ixodes scapularis* larvae feeding on mice experimentally infected with the *Ehrlichia muris*-like agent (EMLA), during the peak of the bacteremia, with 90 % efficiency. Naïve mice were infested with these nymphs for evaluation of transmission. Samples were obtained at day 9 and 45 post infection (p.i.), based on the needle-transmitted mouse model of EMLA. Tissues were collected to determine the pathology and organ distribution of the ehrlichiae. We also determined the antibody titer by ELISA using EMLA antigen. Tick transmission was compared to mice inoculated intradermally (ID) with high dose of EMLA, or with low dose intravenously (IV).

Results: Spleen, liver, lung, lymph node, kidney and brain were demonstrated to contain EMLA at 9 d.p.i. The lung was the predominant infected organ, including in the late stage of infection, suggesting that tick-transmitted EMLA induces disease persistence. Mice infected by tick transmission produced IgM during the early stage of infection. Low levels of IgG were present on day 9 p.i. with a significant increase at day 45 p.i. We have not identified severe pathological changes in infected tissues. The results obtained by tick transmission were similar to those of the ID and IV EMLA-inoculated mice. The bacterial levels in the organs at day 9 p.i. were higher than in ID inoculated animals, but lower than the IV infected mice. However, more variation was observed at day 45 p.i. with greater ehrlichial loads in different organs depending on the route of infection. The organ distribution of tick-transmitted EMLA was similar to IV inoculation during the acute phase of infection. We also demonstrated the presence of the ehrlichiae in the transmission site by IHC, suggesting high levels of ehrlichiae in the tick salivary gland.

Conclusion: With the transmission model we will be able to determine the host-vector-pathogen interactions, which have not been studied in ehrlichial diseases. Our future studies will characterize the initial target cells, local changes, and immune responses at the site of tick feeding and the characteristics of ehrlichial dissemination.

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B-18. Adquisición y transmisión de *Rickettsia prowazekii* por *Amblyomma imitator* en un Modelo Experimental con Cobayos.

(Acquisition and Transmission of *Rickettsia prowazekii* by *Amblyomma imitator* in an Experimental Guinea Pig Model)

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Background: *Rickettsia prowazekii*, the agent that causes epidemic typhus, is transmitted by *Pediculus humanus corporis* and by the ectoparasites of the flying squirrel, *Glaucomys volans*. In addition to these well-defined cycles for disease transmission, isolation of *R. prowazekii* from ticks has also been reported. In 2001 25.5% of sera collected from febrile suspected dengue patients in Nuevo Leon, Mexico demonstrated typhus group antibodies. *Rickettsia prowazekii* was isolated from *Amblyomma imitator* ticks collected in this region. We therefore hypothesize that *A. imitator* is a host and vector for *R. prowazekii*. The aim of this study is to experimentally infect *A. imitator* to study the maintenance and transmission of *R. prowazekii* by *A. imitator*.

Methods: *Amblyomma imitator* ticks were collected from the Laguna Atascosa wildlife refuge in South Texas. Ticks were fed on guinea pigs, collected after engorgement, and females were allowed to lay eggs. Portions of egg masses and post gravid females were tested by real time PCR to confirm the absence of rickettsial DNA. *Rickettsia prowazekii*-infected guinea pigs and an uninfected guinea pig were then used to feed hatched larvae. Engorged larvae and those that molted to the nymphal stage were tested by real time PCR to determine rickettsial acquisition. Infected and uninfected control nymphs were then fed on naïve guinea pigs. Engorged nymphs were collected and allowed to molt. Infected and control adult ticks were then fed on naïve guinea pigs. Guinea pig sera obtained prior to infestations and 4 weeks after infestations were tested by immunofluorescence assay to detect typhus group antibodies.

Results: DNA from portions of egg masses and post gravid females tested negative for *Rickettsia* by PCR, establishing an uninfected tick colony. Engorged larvae tested by PCR prior to molting demonstrated the presence of rickettsiae in 12% of ticks fed on infected guinea pigs versus none from the uninfected guinea pig. After molting, 4% of nymphs contained *R. prowazekii* detected by PCR. At day 28 after the collection of engorged infected nymphs and adults, guinea pigs seroconverted with at least a four-fold rise in titer. Seroconversion did not occur in guinea pigs infested with uninfected nymphal and adult controls.

Conclusions: *A. imitator* is capable of acquiring *R. prowazekii* from a rickettsemic host and maintains the infection transstadially. The feeding of infected ticks on guinea pigs results in seroconversion. The evidence suggests that *A. imitator* can serve as a reservoir and vector for *R. prowazekii*.

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B-19. Caracterización funcional de genes de *Amblyomma cajennense* modulados por una infección experimental con *Rickettsia rickettsii*

(Functional characterization of *Amblyomma cajennense* genes modulated by an experimental infection with *Rickettsia rickettsia*)

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Backgroun: The etiologic agent of Rocky Mountain Spotted Fever (RMSF) is the tick-borne obligate intracellular bacterium *Rickettsia rickettsii*. In Brazil, *R. rickettsii* is transmitted to humans by *Amblyomma cajennense* and *A. aureolatum*. Interestingly, experimental infections of ticks from laboratory colonies have shown that the prevalence rates of *R. rickettsii* in *A. aureolatum* are higher than in *A. cajennense*. These data indicate that the responses of these two species to infection are different. Therefore, the aims of the present study were: (i) to compare the transcriptomes of *A. cajennese* and *A. aureolatum* experimentally infected with *R. rickettsii*; and (ii) to evaluate the effects of the knock-down of certain up-regulated genes on the acquisitions and transmission of this bacterium by *A. cajennense*.

Methods: Total RNA of the salivary glands of infected or uninfected adult females was used to generate specific cDNA libraries by suppression subtractive hybridization (SSH). Randomly picked clones were sequenced and submitted to bioinformatics processing. Certain up-regulated genes were selected to be functionally characterized by interference RNA (RNAi) in *A. cajennense*. To that end, double strand RNA (dsRNA) for either selected genes or the membrane protein 1 of *Plasmodium falciparum* (MSP1; control) was administrated to female adults. After injection, ticks were fed on infected rabbits. The presence of *R. rickettsii* in salivary glands was evaluated by TaqMan qPCR.

Results: *R. rickettsii* infection up-regulated genes encoding mitochondrial enzymes and proteins involved in tick immune responses [for instance, Kunitz-type inhibitors and antimicrobial peptide (AMP; hebraein)] in both *Amblyomma* species. Administration of dsRNA for hebraein resulted in a silencing rate of 92% in the salivary glands of *A. cajennense*. In spite of the high silencing rate, hebraein knock-down had no effect on *R. rickettsii* acquisition.

Conclusion: According to SSH data, *A. cajennense* and *A. aureolatum* exhibit a similar transcriptional profile upon infection with *R. rickettsii*. Hebraein was selected for functional characterization by RNAi. Although a high silencing rate was obtained, hebraein knock-down had no effect on acquisition of *R. rickettsii* by *A. cajennense*. This result suggests that this AMP is not important for invasion of the salivary glands of this tick by *R. rickettsii*. We are currently investigating the effects of hebraein knock-down on transmission of the bacterium. In addition, the effects of the knock-down of one Kuntiz-type inhibitor on both acquisition and transmission of *R. rickettsii* is underway.

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B-20. Análisis comparativo de expresión génica en huevos de la garrapata *Amblyomma aureolatum* infectados y no infectados con *Rickettsia rickettsii*

(Comparative gene expression analysis in *Rickettsia rickettsii* infected and non-infected eggs of the tick *Amblyomma aureolatum*)

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Background: The Brazilian Spotted Fever is an infectious disease caused by the bacterium *Rickettsia rickettsii*, transmitted by ticks of the genus *Amblyomma*. The tick species *Amblyomma aureolatum* is one of the main vectors to human being. Recent studies showed that *R. rickettsii* is competently transovarially transmitted by *A. aureolatum*. The present study aims to understand whether *R. rickettsii* infected eggs show any diferent gene expression compared to non-infect eggs

Methods: A laboratory colony originated from field collected *A. aureolatum* ticks was used in the study. *Rickettsia*-free larvae were separated into two groups: the infected group started with ticks fed on *R. rickettsii*-infected guinea pigs; the control group started with ticks fed on uninfected guinea-pigs. Subsequent nymphs were tested to confirm infection and thereafter raised to adults. Adults from the two groups were fed on dogs, and females that naturally detached were left to lay eggs at 25°C and > 90% RH. At 25th day after the beginning of oviposition, eggs from two females from each group were collected and submitted to total RNA extraction and afterward grouped in two samples, 2.6 µg (Control-group) and 4.5µg (Infected-group) of total RNA were purified to obtain mRNA in order to product cDNA that were sequenced by Illumina platform single-end technology, yielding over 10^6 reading with 100-bp each. The reads assembled 87,000 unigenes, in major search for function, Blastx cuttoff was 10^{-5} against an ACARI database but all sequences were run against NR, SWISS-PRO, VERTEBRADA and RICKETTSIA databases. Bioinformatics were used to quantify the reading and a comparative subtractive library was created.

Results: In the infected group, no downregulated genes were detected; on the other hand a list of upregulated genes was yielded. The expression of Cythocrome P-450 was 4,000 times more abundant in the infected group, and so were a Serine-Protease Inhibitor, an Aspartyl Protease, and a Fatty Acyl-CoA elongase, all matches from *Ixodes scapularis* database. Also the *Rick*A gene from *Rickettsia* was largely

expressed in the infected group with no traces in the control group.

Conclusion: This study is the first to evaluate the interference of *R. rickettsii* in transcription modulation of *Amblyomma* tick eggs. Noteworth a cell respiratory enzyme was upregulated, what may be related to higher metabolic activity, and so was a Serine-protease that may be related to invertebrate immune response against *Rickettsia.* A quantitative Real-Time PCR in order to validate the data must still be conducted.

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B-21. Diseño de candidatos a vacunas de DNA de *R. Rickettsii* a partir de secuencias con péptidos reconocidos por los alelos de HLA I por análisis *in sílico.*

(Design of DNA vaccine candidates for *R. Rickettsii* from sequences with peptides recognized by HLA I alleles using *in silico* analysis)

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Justificación: FMMR causada por *R. Rickettsii* se presenta en Centroamérica América del Norte y del Sur. Hasta el momento no se cuenta con una vacuna efectiva y estrategias basadas en la vacunología reversa son prometedoras. El objetivo fue desarrollar candidatos a vacunas DNA a partir de secuencias de *R. Rickettsii* con epítopes reconocidos por alelos de HLA I por análisis *in sílico.*

Métodos. Se analizaron 1343 secuencias de *R. rickettsii* (NC_009882). Se seleccionaron secuencias con péptidos reconocidos por el HLA I con programas ProPred 1, RANKPEP, HLA Binding, y Epitope prediction para alelos: A0201, A24,B3501 y B3901. Se excluyeron aquellas con identidad ≥80% a secuencias humanas. Selección en base a función y expresión en sistemas procariotas. Los cebadores se diseñaron en software pDraw32. El plásmido pVAX1 fue digerido con XbaI y XhoI. Se transformaron cepas *E. coli* DH5α y se crecieron en placas con LB-Kanamicina (50μg/ml). La verificación de orientación se realizó por secuenciación.

Resultados. 317 secuencias del genoma de *R. rickettsii* analizadas presentaron al menos 1 péptido compartido por más de un alelo, 20 presentaron valores de afinidad al HLA I superior al punto de corte en cada programa. Se seleccionaron 5 con valores del 80% de afinidad: 3 proteínas hipotéticas y 2 de membrana: OmpA y OmpB. 4) Se amplificaron y clonaron 3 fragmentos (OmpB24, OmpB15 y OmpA49).

Conclusión. La bioinformática permite una aproximación teórica de secuencias antigénicas para el desarrollo de vacunas. Esta nueva estrategia de selección de secuencias permitió el diseño de 3 plasmidos vacunales que seran evaluados como futuros candidatos a vacuna necesarias en zonas vulnerables donde *R. rickettsii* es endémica.

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