

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. cajennense* 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 América 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 through 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.