PAHO/WHO EXPERTS CONSULTATION ON
RICKETTSIOSIS IN THE AMERICAS

FINAL REPORT

Ouro Preto, Minas Gerais – Brazil
18-19 September 2004

Pan American Health Organization
Regional Office of the World Health Organization

DISEASES PREVENTION AND CONTROL AREA
Veterinary Public Health Unit – PAHO/WHO
Pan American Foot-and-Mouth Disease Center

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PREFACE

Zoonoses represent a serious threat to the health and welfare of the entire world's population. Several genera and species of the Rickettsiaceae family maintain zoonotic cycles in nature, which represent a great menace to public health because they are agents of malignant fever, typhus fever and scrub typhus in vast areas of the countries in the Americas.

Fortunately, these countries have professionals who made significant contributions to the knowledge of such illnesses, and who have provided most effective and efficient alternatives for monitoring, controlling and preventing them.

PAHO brought together a group of such experts to analyze the current situation and to implement mechanisms for the improvement and dissemination of knowledge and progress attained in diagnosis, surveillance, prevention and control of Rickettsiosis.

The panel produced a set of recommendations and working documents, which are presented here, to serve as an input and a stimulus for actions to protect the people who are exposed to such illnesses.

ALBINO BELOTTO
Chief of Veterinary Public Health Unit - PAHO/WHO
Pan American Health Organization
World Health Organization

PAHO/WHO EXPERTS CONSULTATION ON RICKETTSIOSIS IN THE AMERICAS

BACKGROUND

Several genera and species of the Rickettsiaceae family have zoonotic cycles in nature, representing a great threat to Public Health. Rickettsial type bacteria are most frequently transmitted by ectoparasites.

International trade, tourism, and migration are among the factors contributing to the reintroduction of infectious agents, unknown in the areas where the disease did not exist.

Microorganisms of the rickettsial type are grouped in spotted fevers, typhus and scrub typhus, being a public health problem in the various countries in the American continent.

OBJECTIVES OF THE CONSULTATION

General

Gather specialists on the theme aiming to increase awareness and generate recommendations to the countries of the Americas.

Specific

- Discuss and update technical and scientific information on Rickettsiosis.
- Review the situation of Rickettsiosis in the Americas.
- Analyze the impact of Rickettsiosis on humans and animals, and also its social and economic impact.
- Establish an epidemiological surveillance network.
- Prepare recommendations for the countries of the region.

PLACE AND AGENDA

The meeting was held in the conference hall of the Estalagem Hotel in the city of Ouro Preto, State of Minas Gerais, Brazil, on 18-9 September 2004.

AGENDA

Saturday, 18 September

08:30 - 09:00 Registration of participants
09:00 - 09:30 Inauguration
09:30 - 10:00 Theme 1: Rickettsiosis as a Public Health Problem in South America
Speaker: Dr. Márcio Antônio Moreira Galvão - Brazil
10:00 - 10:30 Theme 2: Current Situation and Trends of Rickettsiosis in the Americas
Speaker: Dr. David H. Walker – United States
10:30 - 11:00 Recess
11:00 - 11:30 Theme 3: Tick-borne Disease in Humans
Speaker: Dr. Luis Jacintho da Silva - Brazil
11:30 - 12:00 Theme 4: Rickettsiosis Research: Diagnosis and Advances
Speaker: Dr. Elba Lemos - Brazil and Dr. Donald Bouyer – United States
12:00 - 12:30 Theme 5: Prevention and Control of Rickettsiosis in Peru
Speaker: Dr. Elizabeth Irene Anaya Ramírez – Peru
12:30 - 14:00 Lunch
14:00 - 17:00 Working Groups
Group 1 (Research and Diagnosis)
Group 2 (Control and Prevention)

Sunday, 19 September

09:00 - 10:00 Presentation and discussion of activities of Group 1
10:00 - 11:00 Presentation and discussion of activities of Group 2
11:00 - 11:30 Recess
11:30 - 12:30 Conclusions and Recommendations

PARTICIPANTS

Guest specialists and Pan American Health Organization/World Health Organization (PAHO/WHO) (see Appendix 2)

DEVELOPMENT OF THE CONSULTATION

Opening and historical background

At the opening, Dr. Márcio Antonio Moreira Galvão, Head of the Dean's Office of the Federal University of Ouro Preto, on behalf of the University's Dean gave a welcome speech and presented a summary of the exchange of Rickettsiosis scientific information and research among scientists from several countries of the

Americas, under the leadership of Dr. David H. Walter, of the Center for Biodefense and Emerging Infectious Diseases of the University of Texas. He highlighted the importance of the cooperation of PAHO and noted that this was not the first meeting on the subject because in 1986 PAHO/WHO gave support to the holding of the first national meeting on the topic, since then Brazil began to deal with the problem.

The second presentation was made by Professor Dr. Luiz Jacinto da Silva, Superintendent of Endemics Control – SUCEN, Department of Health of the State of São Paulo, Brazil. Dr. Luiz Jacinto emphasized that this technical consultation represents the end of one stage and the beginning of new phase of cooperation, integration, incorporation, formation of human resources and collaboration of health ministries in dealing with these diseases in their working schedules. He recalled that in 1981, Rickettsiosis were included in the list of mandatory reporting diseases in the State of Minas Gerais, thanks to the effort made by Dr. Márcio Galvão. It took place in São Paulo in 1985. He pointed out that, after this consultation, the "XIII Veterinary Parasitology Congress and I Latin American Rickettsiosis Symposium" would start, which means a great advance. He ended his intervention by stressing his hope that at the end of the conference, recommendations for the countries and for the Pan American Health Organization would be available, to contribute to the strengthening of Rickettsiosis control in the region.

Dr. Rosely Oliveira, a representative of the Brazilian Ministry of Health, welcomed the participants, and on behalf of Dr. Jarbas Barbosa, Secretary of Epidemiological Surveillance, she congratulated and thanked the organizers for the initiative in having convened this PAHO/WHO Experts Consultation on Rickettsiosis in the Americas. She recalled that for many years in Brazil, zoonosis control was restricted to rabies. Since 2001, advances have been made in Rickettsiosis treatment and it was incorporated to the group of diseases under epidemiological surveillance. Furthermore, Dr. Oliveira said that she hopes, after this meeting, that working guidelines will start being outlined for control of the disease in the Americas.

Dr. Albino Belotto, on behalf of Dr. Mirta Roses Periago, Director of the Pan American Health Organization / World Health Organization (PAHO/WHO); and the Representative of PAHO/WHO in Brazil, thanked the Federal University of Ouro Preto for the collaboration in organizing the Consultation and the specialists for having accepted the invitation and presented the terms of reference of the work meeting. He commented that although Rickettsiosis have not had the importance of other diseases in the media, in some areas of the region they are a problem and PAHO/WHO and the countries are concerned about and involved in it. Dr. Belotto stressed the importance that this accomplishment has had so far and the importance of the multi-sector and multi-disciplinary network that is already being operated, undertaking on behalf of PAHO/WHO to support the expansion and cohesion of this network. Furthermore, he expressed that the results of the meeting and its recommendations will certainly contribute to the solution of several problems existing in rickettsiosis control, particularly in the exchange of experience and information between the countries.

Presentations on specific themes

The texts of the presentations are enclosed in Appendix 1.

RECOMMENDATIONS

The group gathered in Ouro Preto during the PAHO/WHO Experts Consultation on Rickettsiosis in the Americas recognizes the economic and social importance that these diseases have, probably constituting the most important health problem currently known, and present the following recommendations to the countries of the Americas and for the Pan American Health Organization:

Recommendations for the countries

1. Implement and improve specific rickettsiosis surveillance systems respecting local epidemiological characteristics and, accordingly, when applicable, emphasize: typhus, spotted fever and scrub typhus.

   Implementation of these systems will imply: relying on sufficient diagnosis means, including these diseases in the differential diagnosis of fever syndromes, building, strengthening and/or coordinating diagnosis and research laboratories of rickettsiosis in humans, vectors, and vertebrate reservoirs, including the capacity of producing their own inputs. This network could operate under criteria similar to those of existing networks, with collaborating and reference centers.

   Epidemiological surveillance should focus on less known rickettsiosis such as ehrlichiosis, *Rickettsia felis*, *Rickettisa parkeri* and others. This surveillance should be proactive and not only passive, as it is currently done.

2. Include the rickettsiosis problem in public health and research priority schedules.

3. Disseminate knowledge of rickettsiosis and their impact on health through universities and other health professional education centers, incorporating rickettsiosis as an item in professional education curriculum.

   This will imply the coordination of discussion and supporting forums to prepare human resources.

4. Support and encourage courses aimed at disseminating knowledge on Rickettsiosis in their different aspects.

5. Characterize transmission areas of different rickettsioses, particularly typhus, spotted fever and scrub typhus.

6. Improve natural history knowledge of these diseases in the Americas, with particular emphasis of the ecology of the vectors and the role of vertebrate reservoirs.

7. Support and encourage evaluation studies of control measures with emphasis on vectors and environmental management.

Development of vaccines for human and veterinary use is included, as well as anti-tick vaccines and epidemic typhus vaccine.

Recommendations for PAHO/WHO

1. Recommend and coordinate the implementation of a Regional Control Program, and eventually eradication, of epidemic typhus in the Americas.
2. Sensitize national authorities so that they will include the rickettsiosis problem in their public health and research priority activities.
3. Sensitize national authorities to the present epidemiological situation of zoonoses leading them to implement epidemiological surveillance programs in “silent” areas and implement vector control programs.
4. Disseminate knowledge on rickettsiosis and its impact on public health to national health services and agencies that train health professionals, leading them to incorporate rickettsiosis in professional training curriculum, which implies coordination of discussion forums and human resources training.
5. Support and encourage discussion on rickettsiosis-related issues through existing means, such as Virtual Health Libraries (VHL).
6. Promote cooperation among countries aimed at developing research capacity in different aspects of rickettsiosis, with emphasis on the development and production of appropriate diagnosis means, as an immediate measure to enable the supply of laboratory inputs for diagnosis.

APPENDIX 1

PRESENTATIONS

RICKETTSIOSIS AS A PUBLIC HEALTH PROBLEM IN SOUTH AMERICA

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INTRODUCTION

The epidemiological parameters for a disease to be considered a public health problem are magnitude, vulnerability and importance. The first one represents the incidence of the disease, the second one the capacity of the disease to be attacked by an effective control mechanism such as vaccines and the third one, the recognized importance of the disease, including the point of view of the population. In order to analyze rickettsiosis by these parameters, we share attempt first try to study these diseases in South America to understand their history and the roles they play in Public Health.

RICKETTSIOSIS IN SOUTH AMERICA

Rickettsiosis in Brazil during the 1930 – 1950s

Rickettsia rickettsii was first described in São Paulo by Piza (1) in 1929, as the agent of Brazilian Spotted Fever (BSF) transmitted by the tick *Amblyomma cajennense*. At that time, the similarity of this disease to Rocky Mountain Spotted Fever was also demonstrated. In 1939, BSF and murine typhus were described in Minas Gerais state by Dias & Martins (2) and *Rickettsia typhi* was isolated for the first time from a São Paulo human patient by Travassos (3) in 1949.

From this last date until 1980, there was an epidemiological silence with no cases of BSF described in the medical literature. Interviews of active physicians during this period revealed only rare cases of BSF during that time.

Rickettsiosis in Brazil during the 1980 – 1990s

In 1981, BSF cases were described in Rio de Janeiro State by Gonçalves (4), and Galvão (5) described the reemergence of BSF in Minas Gerais State with a fatality rate of 50% in the epidemic episode.

Outbreaks of BSF occurred again in Minas Gerais State in 1984, 1992, 1995 and 2000. Although there were several fatal cases in these outbreaks, the diagnoses were not well documented by laboratory methods. The frequent hidden mortality of BSF appears to exist because of a low proportion of autopsies and deaths (6).

From 1985 to 2002, 76 cases of BSF were confirmed in São Paulo state with a fatality rate of 47,6%, and *R. rickettsii* was isolated from a patient's skin biopsy in the rural area of São Paulo state by Melles (7) in 1992. Isolation of spotted fever group *Rickettsia* sp. in *Amblyomma cooperi* ticks collected from capybaras (*Hydrochaeris hydrochaeris*) occurred in the same state, as per Lemos et al (8) in 1996.

In 1993, a BSF focus of infection was recognized in a new endemic region in Espírito Santo State by Sexton et al (9).

Rickettsiosis in Brazil during the 2000s

Rickettsia felis human infections cases were described in Brazil for the first time by Raoult et al (10) in 2001, and *R. felis* was identified by PCR in *Ctenocephalides* sp. fleas by Oliveira & Galvão et al (11) in 2002.

In 2004, Calic & Galvão et al (12) described the first clinical human cases in Brazil suspected of being caused by the genus *Ehrlichia* sp. and Labruna et al (13, 14) described *Rickettsia bellii*, *Rickettsia parkeri* from *A. cooperi* by PCR and isolation and *Rickettsia amblyommi* from *Amblyomma longirostre* by PCR.

Rickettsiosis in Uruguay

Conti-Diaz et al (15) diagnosed by indirect immunofluorescence serology cases of a disease caused by a spotted fever group *Rickettsia* sp. in Uruguay in 1990. The suspected vector collected from pets was *Amblyomma triste*.

In 2004, Venzal et al (16) suggest that *A. triste* is a host of SFG *Rickettsia* in Uruguay and *R. parkeri* could be the causative agent of human cases of Rickettsiosis in Uruguay.

Rickettsiosis in Argentina

In 1999, spotted fever group *Rickettsia* sp. infection was diagnosed by Ripoll et al (17) in a patient from Jujuy Province, and a serosurvey in the same area and period of time detected antibodies reactive with *R. rickettsii, Ehrlichia chaffeensis* and *R. typhi*. The suspected vector was *A. cajennense* collected from horses and pets in the same area.

Rickettsiosis in the last 20 years in Peru

Epidemic louse-borne typhus has continued in Peru during the last twenty years. An epidemic typhus outbreak occurred in Cuzco in two rural communities in 1985 (18). During 1989-1999, there were substantial notifications of epidemic typhus cases distributed in the departments of Ancash, Arequipa, Cuzco, Huanuco, Piura, and Puno. Almost 70% of cases of epidemic typhus are concentrated in two provinces of Cuzco State, Quispicanchis and Paucartambo, with a combined population of 120,000 people (19). Three typhus outbreaks between May 1997 and Apri11998 were investigated in the province of Quispicanchis (20).

Other rickettsiosis were investigated in Peru, such as murine typhus described in patients from Huari province, Department of Ancash (21) and *R. felis* was identified in *Ctenocephalides canis* fleas from domestic animals and relatives of suspected murine typhus cases in Peruvian Andes domiciles (22).

Rickettsiosis in Colombia

Nothing has been known or published about Rickettsiosis in Colombia since 1937 when Luis Patiño Camargo published the report of an epidemic caused by *R. rickettsii* and known then as "Fiebre de Tobia" (23).

We have noticed that two fatal cases or Rocky Mountain Spotted Fever occurred in Colombia in 2004. A publication about these cases is being prepared.

CONCLUSIONS

In South America, Rickettsiosis occurring both in developed areas at Campinas region in São Paulo - Brazil, and in poor areas in some regions of Brazil, Peru and other countries.

The ecologic transformation and the low socioeconomic condition of the population have collaborated to maintain and reintroduce Rickettsiosis in many areas of South America.

Even with a low magnitude and vulnerability of these diseases, their importance expressed by the occurrence of outbreaks and family clusters with high case-fatality rate can define them as a public health problem.

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CURRENT SITUATION AND TRENDS OF RICKETTSIOSIS IN THE AMERICAS

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BACKGROUND

Eight human rickettsiosis and ehrlichiosis cases have been documented to occur in the Western Hemisphere (Table 1).

CURRENT SITUATION

Rocky Mountain Spotted Fever (RMSF) has been diagnosed in only eight countries ranging from Canada to Argentina. Outbreaks with extremely high mortality are sporadically recognized. The actual geographic distribution, incidence, and case fatality rates are not known owing to lack of attention and unavailability and infrequent application of effective diagnostic methods. RMSF has periodic waves of progressively increased incidence extending over decades. Another period of increasing incidence is likely to occur. The ecologic basis of increased or decreased tick transmission is unknown. Although a relatively rare infection, RMSF has a case fatality rate (23%) among untreated previously healthy persons, among the highest of any infection.

Recently *Rickettsia parkeri* has been documented to cause a human infection in the U.S. The clinical manifestations (eschars, fever, headache, myalgias/arthralgias, and lymphadenopathy) closely resemble those of African tick-bite fever and the spotted fever group (SFG) rickettsiosis of Uruguay. *Rickettsia parkeri* and *R. africae* are so closely related that they could be considered a single species. These rickettsiae have been identified in *Amblyomma triste* and *A. cooperi* ticks in Uruguay and Brazil, respectively. The complete distribution of *R. parkeri* in the Americas is not known.

Outbreaks of louse-transmitted *R. prowazekii* occur regularly in Andean Peru and historically were present in Mexico, Central America, and South America. Because typhus fever results in long-term latent non-symptomatic infection of survivors who may suffer recrudescent infection even decades later, epidemics can be ignited in populations in which typhus occurred in the past and human body lice are prevalent. The geographic distribution and incidence of latent *R. prowazekii* infection in the Americas are not known, but likely reflect some migration of latently infected persons. A recent study in Mexico suggested the continued presence of seropositive persons in a population with a low prevalence but presence of *Pediculus humanus corporis.*

Infections by tick-borne *Ehrlichia* sp. were until 1987 the sole province of veterinary medicine. Currently both *E. chaffeensis* and *E. ewingii* are known to cause human infections in the U.S. with serologically diagnosed

cases also in Mexico and Brazil. Human infection with *E. canis* has been documented in Venezuela. Usually recognized because of the infrequent presence of microcolonies of bacteria in the target monocytes or neutrophils, contemporary more sensitive diagnostic methods are polymerase chain reaction detection of ehrlichial DNA and convalescent serology. Leucopoenia, thrombocytopenia, and elevated serum hepatic transaminases are useful diagnostic clues. *Ehrlichia chaffeensis* infection (human monocytotropic ehrlichiosis) is a life threatening multisystem disease that manifests as a toxic shock-like illness, adult respiratory distress syndrome, or meningoencephalitis in the most gravely ill cases. Active prospective surveillance has demonstrated a much higher incidence (11 to 100 per 100,000 population) than passive surveillance of this difficult-to-diagnose illness has suggested.

CONCLUSION

The epidemiology of RMSF, *R. parkeri, R. felis, R. typhi,* and *R. akari* infections and louse-borne typhus are quite incompletely known in the Americas. The public health impact of these infections will not be appreciated until appropriate diagnostic methods and epidemiological surveillance are effectively employed.

Just as the human ehrlichioses were not recognized in the U.S. two decades ago, their occurrence, etiologic agents, incidence, clinical severity, and geographic distribution are unknown currently in Latin America.

PROPOSED ACTION/RECOMMENDATIONS

1. Establish effective diagnostic methods and active surveillance for SFG and typhus group rickettsiae throughout the Americas.
2. Educate physicians regarding clinical manifestations, diagnostic methods, and treatment for *Rickettsia parkeri* infections and ehrlichioses.
3. Identify populations at risk for recrudescent typhus-ignited epidemics.
4. Establish surveillance system in the at-risk populations with appropriate acute diagnostic methods.
5. Determine ecologic factors responsible for increased populations of *R. rickettsii*-infected ticks and periodic increases and decreases in incidence of RMSF.
6. Investigate *Amblyomma* ticks in all geographic regions for *R. parkeri* and *Ehrlichia* infections to determine the distribution and risk of infection.
7. Develop and administer an effective vaccine to non-immune persons in populations at risk of louse-borne typhus infection.
8. Develop a method for clearance of *R. prowazekii* from latently infected persons.
9. Basic scientists should develop effective vaccines against rickettsiosis and ehrlichiosis.
10. Scientists in the fields of rickettsiology, epidemiology, veterinary science, and acarology should elucidate the vulnerable points in the zoonotic maintenance cycles of *R. rickettsii* and *E. chaffeensis* and develop an effective, environmentally acceptable intervention to decrease the infected tick populations.

Table 1
Human rickettsiosis and ehrlichiosis documented in the Western Hemisphere.

Agent	Disease	Arthropod Host	Established Geographic Distribution	Critical Vertebrate Hosts
R. rickettsii	Rocky Mountain spotted fever, Brazilian spotted fever	*Dermacentor variabilis, D. andersoni, Amblyomma cajennense, Rhipicephalus sanguineus, Haemaphysalis leporispalustris* ticks	U.S., Mexico, Canada, Costa Rica, Panama, Colombia, Argentina, Brazil	Not known
R. akari	Rickettsialpox	*Liponyssoides sanguineus* mites	U.S.	*Mus musculus* mice
R. felis	Flea-borne spotted fever	*Ctenocephalis felis* fleas	U.S., Mexico, Brazil, Peru	Not known
R. parkeri	African tick-bite fever	*Amblyomma maculatum, A. americanum, A. triste, A. cooperi, A. variegatum* ticks	U.S., Brazil	Not known
R. prowazekii	Louse-borne typhus	*Pediculus humanus corporis* and *Neohaematopinus sciuropteri* lice, *Orchopeas howardii* fleas	U.S., Peru	Humans, *Glaucomys volans* flying squirrels
R. typhi	Murine typhus	*Xenopsylla cheopis, Ct. felis,* and other fleas and lice	U.S., probably many countries in South and Central America and Caribbean	*Rattus* sp. and Didelphis sp.
E. chaffeensis	Human monocytotropic ehrlichiosis	*A. americanum, D. variabilis, Ixodes pacificus* ticks	U.S.	White-tailed deer, canids
E. ewingii	Ehrlichiosis ewingii	*A. americanum*	U.S.	White-tailed deer, canids

TICK-BORNE DISEASES IN HUMANS

Occurrence, distribution and impact on public health, with emphasis on the State of São Paulo.

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INTRODUCTION

Recognition that ticks can transmit diseases dates from the 19th century; however, the importance attributed to these diseases, and in consequence to the tick itself, has always been very limited. It was not until the last two decades of the 20th century that the importance of ticks as a disease vector was perceived in a more realistic light. Not only was the almost cosmopolitan distribution of these diseases better evaluated but also new trick-borne diseases were described.

It cannot be denied that the description of Lyme disease, a borreliosis transmitted by ticks in the northeast of the United States, in New England, was of fundamental importance in bringing the tick and the diseases transmitted by it to the center of attention in public health and medical science.

Today, an extensive variety of diseases is known; viral, bacterial and parasitic, transmitted by ticks in different parts of the world, both in developed countries such as North America and Western Europe to developing countries such as Central Africa. Recognition of the importance of these diseases is reflected in the large number of recent articles in the public health literature on the occurrence and impact of these diseases in different parts of the world.[i]

In Brazil, the importance of human tick-borne diseases, and even the existence of some of them has yet to be adequately evaluated.

TICK-BORNE DISEASES

Ticks today make up the second group in importance of vectors of infectious diseases. There are some 800 species found in every kind of ecosystem. Ticks can infest a wide variety of species, with the exception of fish.

The importance of ticks as transmitters of disease was initially recognized in veterinary science. In 1886 Theobald Smith described what was then known as Texas Cattle Fever, known today as babesiosis. Some years later, Smith himself and Frederick Kilborne demonstrated transmission of the disease by ticks. In the beginning of the 20th century, Ricketts studies in the USA demonstrated transmission by ticks of Rocky

Mountain Spotted Fever, a rickettsial disease. Later, encephalitis transmitted by ticks, an infection by flavivirus was recognized as a public health problem from Central Europe to Siberia. In 1929, Piza and Gomes described Paulista exanthematic typhus, known today as Brazilian Spotted Fever, a rickettsial disease.

Since then, innumerable diseases have been found to be transmitted by ticks. Interest in ticks as transmitters of infectious agents has grown. Undoubtedly, the appearance of Lyme disease, a borreliosis, in the USA was a preponderant factor. Lyme disease showed that even in a highly developed region like the northeast of the USA, diseases transmitted by ticks can represent a significant public health problem. The number of infectious agents recently described as transmitted by ticks has grown significantly in recent years.

Almost all rickettsial infections of interest in human medicine are transmitted by ixodo ticks, with the exception of endemic recurring fevers, or recurring fever transmitted by other species of ticks. This disease, caused by *Borrelia duttoni*, has never been described in Brazil, but has been found practically everywhere else in the world.

Tick-borne diseases known today form an extensive list. As opposed to the older concept, these diseases are not restricted to given areas, although they are characteristically focused, but rather have been found practically everywhere where there have been studies to direct them.

Human [iii] tick-borne diseases are caused by:

Virus: tick-borne encephalitis, Congo-Crimea hemorrhagic fever, Omsk hemorrhagic fever, Colorado tick borne fever, Powassan encephalitis, Langat encephalitis, louping ill encephalitis.

Bacteria: Gram-negative bacillus - tularemia

Ehrlichias – monocytic ehrlichiosis and granulocytic ehrlichiosis

Rickettsias – spotted fevers

Borrelias – Lyme disease, recurring tick-borne fever

Protozoan: babesiosis

Ticks as vectors and reservoirs of disease

Ticks are arachnideous arthropods, ectoparasites of terrestrial vertebrates including amphibians. There are some 850 species of ticks in the world. Of these, 650 belong to the Ixodidae family and 170 to the Agarsidae family.[ii]

Ticks are more than simple vectors of diseases and act as reservoirs, transmitting the infection to their progeny by transovarial infection. They are the main vectors of animal diseases, second only to the mosquito as vectors of human diseases.

Tick-borne diseases are usually focused, since their mobility is restricted except when transported by vertebrates found in rural or forest areas, since their adaptation to urban areas is limited. On the other hand, due to their resistance to the outside environment, longevity and their capacity for transovarial infection,

continuity in transmission of diseases is indefinite, especially since control of tick populations is extremely difficult.

Diseases caused by ticks do not occur in rapid surges or epidemics, as they are only occasional ectoparasites of humans, and generally only feed on blood once in each life stage.

As opposed to winged vectors such as mosquitoes or diptera, ticks move very little and the great majority of diseases transmitted by ticks are not transmitted between humans, and the reservoir is essential to keep the natural focus active. This means that diseases transmitted by ticks, as a general rule, do not come in strong epidemics or surges, and occurrences are generally focal and sporadic.

Human diseases transmitted by ticks in Brazil

In Brazil, although the importance of ticks in veterinary medicine has received extensive attention, for several decades, their role in human public health has been neglected. Until recently the only known human disease transmitted by ticks was Brazilian Spotted Fever. Studies on the epidemiological dynamics of the disease are scarce. The classic *Geografia Médica do Brasil*, published by Lacaz, Baruzzi and Siqueira Jr., dedicates only one paragraph of four lines of its 568 pages to ticks and does not cite any reference.

Apart from Brazilian Spotted Fever, descriptions of diseases transmitted by ticks are sporadic. Babesiosis and ehrlichiosis are known mostly to veterinarians but there are few descriptions of human cases, so that descriptions of distribution and incidence of these infections, like the borreliosis, are practically unknown in Brazil.

Lyme disease, which is today the main disease transmitted by the vector in the USA, has been described in Brazil. However, its agent, *Borrelia burgdorferi,* has never been isolated, either in humans or in ticks or in reservoir animals. The evidence available for its existence is limited to clinical, serum and epidemiological data. It is possible and even probable that Lyme disease in Brazil, like that found in Europe, is caused by borrelia other than *B.burgdorferi*, as the rates of antibodies found in patients are low compared with those found in the USA. Cases of borreliosis in São Paulo, however, have been detected with increasing frequency, suggesting that the relative rarity of these cases is due more to problems with the surveillance systems, with limited of access to laboratory resources for its diagnosis.

Tick-borne viral diseases that cause encephalitis are relatively common in extensive areas of the northern hemisphere, both in America and in Europe and Asia. In Brazil, they have never been described.

BRAZILIAN SPOTTED FEVER

Like other diseases transmitted by ticks, transmission of Brazilian spotted fever is focused. Described initially in the 1920s in São Paulo, the first focus recognized was in an area of urban expansion, what today are the Sumaré and Perdizes neighborhoods. Later, foci were found in the outskirts of the greater São Paulo metropolitan region such as Mogi das Cruzes and Santo Amaro, however, with the expansion of the urban

area these foci gradually disappeared or became less active. The best-known foci in the State of São Paulo is in the region of Campinas (municipalities of Campinas, Pedreira, Jaguariúna and Santo Antonio de Posse), in the Atibaia and Jaguari river basins. There is evidence that these focuses are expanding, with cases reported in Piracicaba and Araras.

Other foci have been described in the States of Minas Gerais, Espírito Santo and Rio de Janeiro. Transmission of spotted fever seems to be more intense and extensive in Minas Gerais. There have recently been reports of cases of rickettsial diseases, most probably Brazilian Spotted Fever, in the State of Santa Catarina.

The extent of the focus in São Paulo is known from the occurrence of clinical cases, but it has never been properly defined, nor has its real importance to public health been recognized. The occurrence of human cases appears to be a purely biological accident, its infrequency not reflecting absence or lower intensity of *R. rickettsi* in nature.

Little is known about the reservoirs, the species of transmitting ticks or the real extent of the transmission area. The capybara has been identified as an important reservoir. Dogs and horses have also been identified as reservoirs or amplifying hosts. *Amblyomma cajennense* has been considered the main vector for Brazilian spotted fever ever since the first studies in the 1930s. The few studies that exist on infection by *R. rickettsi* in ticks in Brazil suggest that other ticks are vectors but *A. cajennense* is the most important in infections to humans. *A. cooperi* was recently identified as a vector in Mogi das Cruzes, in the eastern greater São Paulo metropolitan region.

Human ehrlichioses

Although infection by bacteria of the genus *Ehrlichia* sp. has long been known in veterinary medicine, the first human cases were only recognized in 1987. The species associated with diseases in humans are the Ehrlichia sp..

The genus *Ehrlichia* sp. belongs to the tribe Ehrlichieae, family Rickettsiaceae, order Rickettsiales. They are gram-negative bacteria, small and spherical (coccid), of obligatory intracellular lives, and transmitted by ticks. Ehrlichias mainly invade leucocytes, so that human illnesses caused by ehrlichieae are divided into two groups:

- Granulocytic ehrlichiosis
- Monocytic ehrlichiosis

The genus *Ehrlichia* sp. includes seven recognized species: *E. canis, E. chaffeensis, E. equi, E. phagocytophila, E. risticii, E. ewingii,* and *E. sennetsu*, and at least four others whose names are still pending.

The ehrlichias multiply inside leucocytes, forming characteristic structures in agglomerations called morulae, which are visible with optical microscopes.

The taxonomy of the ehrlichias was a matter of great controversy. In modern studies molecular biology methods have been used to classify the group. Currently, ehrlichias are classified into three “genogroups”, which include some bacteria that previously did not belong to the group.

Most human infections described are from the USA, possibly reflecting fewer limits of awareness, as it is mandatory that the illness be reported in some North American States. Human cases have been reported in Japan and in Europe.

The ehrlichioses clinical symptoms in humans are not sufficiently characteristic to make simple clinical diagnoses. Its symptoms are easily confused with other infections disease, spotted fever amongst them.

Human cases of ehrlichias infections have not yet been described in Brazil. As the clinical symptoms are not characteristic, and the infection is not a major concern either of the official health authorities or of most researchers, it is perfectly possible that these cases are simply not perceived.

TICK-BORNE VIRUS DISEASES

Virus diseases transmitted by ticks show at least three distinct kinds of clinical manifestations: encephalitis, hemorrhagic fever and dengue-like diseases, as in figure 1.

Figure 1

Main tick-borne viral diseases

DISEASE	FAMILY	GENUS	CLINICAL CHARACTERISTICS	DISTRIBUTION
Colorado tick-fever	Reoviridae	Coltivirus	Dengue-like	Mountain regions of Western USA and Canada
Louping ill	Flaviviridae	Flavivirus	Encephalitis	British Isles
Encephalitis transmitted by ticks	Flaviviridae	Flavivirus	Encephalitis	Central Europe
Russian Spring and Summer encephalitis	Flaviviridae	Flavivirus	Encephalitis	Eastern Russia
Kyasanur Forest Disease	Flaviviridae	Flavivirus	Hemorrhagic fever	India
Omsk hemorrhagic fever	Flaviviridae	Flavivirus	Hemorrhagic fever	Siberia
Powassan	Flaviviridae	Flavivirus	Encephalitis	Northeast USA and Southeast Canada
Congo-Crimea hemorrhagic fever	Bunyaviridae	Nairovirus	Hemorrhagic fever	Africa, Middle East, Balkans, Caucasus

The first group contains the encephalitis transmitted by ticks (known in the English literature as TBE, tick-borne encephalitis), a group of encephalitis found in an extensive area from the British Isles (louping ill encephalitis), through continental Europe (tick-borne Central European encephalitis), to the extreme east of Russia (Spring–Summer Russian Encephalitis). The severity of this encephalitis seems to increase from the west to the east.

Powassan encephalitis is an uncommon disease found in northwestern USA and areas adjacent to Canada. Little more than twenty cases have been described. While rare, this encephalitis is particularly serious.

The tick-borne hemorrhagic fevers have clinical characteristics similar to mosquito-borne hemorrhagic fevers or those acquired from contact with rodents or their excreta.

Congo-Crimea encephalitis was initially described in Crimea but later appeared in the Middle East, Central and Southern Africa. More interesting was a recent occupational surge reported among workers at an ostrich slaughterhouse in South Africa.

Of greater interest, perhaps is Colorado tick fever, which was described in 1850 in the mountainous western areas of the USA. It was only in the 1930 decade that this disease was separated from Rocky Mountain Spotted Fever, another tick-borne disease with similar clinical and epidemiological characteristics. The history of tick-borne diseases in Colorado illustrates how it is possible for a viral disease to be transmitted by ticks and go unnoticed for a long time.

Tick-borne virus pathogens have never been described in Brazil.

BABESIOSIS

Babesias are protozoa very similar to malaria, especially in their invasion of blood cells.

There are about 100 known species but only three have been identified as causing human diseases. It is, however, a disease of well–known veterinary interest, especially in cattle.

Described in 1891 by the Hungarian parasitologist Babes it was only in 1957 that the first human case was reported.

Human cases were described in the USA and in Europe. The species associated with human infection were: *B. microti, B. gibsoni* and *B. divergens* (*B. bovis*).

The clinical symptoms are usually discrete but can be serious in patients with no spleen. Mortality is generally 5%. Association with HIV infection is an aggravating factor.

LYME DISEASE AND OTHER BORRELIOSES

Borrelias are spirochetes, filamentary and spiral bacteria belonging to the family Treponemataceae. The genera *Treponema* sp*., Borrelia* sp*., Leptospira* sp. and *Spirillum* sp. include species that are pathogenic to humans. Borrelias are more elongated and less spiral shaped than other spirochetes. Of interest for developing vaccines is the fact that the determining genes in its external membrane are plasmids.

Human diseases caused by borrelias are: Lyme disease, recurring fever caused by lice and tick-borne recurring fever, of which the only one of interest in Brazil is Lyme disease.

Lyme disease

Lyme disease is caused by *Borrelia burgdorferi* sensu lato. *B. burgdorferi* was isolated in 1981. Sensu lato means that there are genetic variations in the species according to the region.

Eight genospecies of the genus *Borrelia* sp. were identified using molecular biology methods (DNA hybridization), of which four are causal agents for Lyme disease. *B. burgdorferi sensu strictu* is predominant in North America. Mixed infections have been described in Europe and there is coexistence of *B. burgdorferi* sensu strictu*, B. garinii*, and *B.afzelii (7).*

Varieties recently described, *B. valaisiana, B. lusitaniae,* and *B. japonica*, were only found in Europe and Japan. *B. garinii* is recognized as the ancestor of the entire group and is very probably responsible for most neurological manifestations in the cases acquired in Europe (7, 15).

A new species, *B. lonestari*, not cultivable so far, was identified in the USA, and is associated with a syndrome similar to Lyme.

Lyme disease itself has not been identified in Brazil, or even in the southern hemisphere, but undoubtedly, very similar clinical manifestations caused by other borrelias are more common than have been identified up to the present. Cases described in Brazil as Lyme disease had only clinical and serum diagnoses and are

considered Lyme-like. The occurrence of borrelia infection, however, whether in ticks or mammals must be common, it being only a matter of looking.

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ii Ticks are divided into 2 large groups: argasids and ixodes. The second have rigid exoskeletons and feed on blood slowly as opposed to argasids.

Ticks have 4 stages: larva, nymph, with six legs and with 8 legs, and adult. They are parasites of only one host at each stage.

iii The great majority of these illnesses are zoonoses, i.e. illnesses of animals that attack humans only occasionally.

RICKETTSIOSIS RESEARCH: DIAGNOSIS AND ADVANCES

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The clinical diagnosis of rickettsiosis, mainly spotted fever and typhus group rickettsiosis and scrub typhus, must rely on epidemiological suspicion and suggestive clinical manifestations such as high fever, myalgia, arthralgia, headache and a typical rash. Because these diseases can be life-threatening, it is important that physicians consider rickettsiosis in their differential diagnosis of a febrile disease in all patients with history of contact with animals and arthropods and that appropriate therapy is administrated early, in order to reduce mortality.

Accurate and efficient diagnosis of rickettsiosis is important for confirmation of the diagnosis, to differentiate rickettsiosis from other diseases such as dengue, and leptospirosis, and for the clinical management of patients with severe and atypical disease. The diagnosis is also important for surveillance support and pathogenesis studies.

Rickettsia diagnosis can be performed through serological techniques, isolation, genome and antigen detection.

Serology is currently the most widely applied test in routine diagnosis, although diagnostic titers of antibodies IgM and IgG appear only after 7 to 10 days of the disease. According to some studies, by day 7 to 10 of illness, 50-60% of cases have detectable antibodies, and by day 10 to 15, 75% of cases have detectable antibodies that do not persist with elevated titers for any longer than 3-4 months.

Although several tests, such as latex agglutination, enzyme immunoassay (EIA), Weil-Félix test, indirect hemagglutination, microagglutination, complement fixation, indirect immunoperoxidase assay, line blot, and Western immunoblotting can be used, the indirect immunofluorescence assay (IFA) has become the most widely used. In the last two decades, expensive kits have been commercialized for use in clinical laboratories. These include an ELISA, a dipstick format (a cellulose strip chemically impregnated so it is more sensitive to protein, glucose or other urine substances) and latex agglutination.

Rickettsial isolation is performed in few laboratories. Guinea pigs, rats and voles, as well as yolk sac of embryonated chicken eggs were used in the past. Nowadays, isolation of rickettsia by cell cultures such as Vero cells lines, using the centrifugation shell vial technique, represents an important contribution to rickettsia diagnosis.

Rickettsemia is short and usually observed only in the first five-seven days of the disease. Therefore, samples such as buffy coat of heparinized blood, whole blood, triturated coat, plasma, and necropsy or biopsy tissues must be taken in the first seven days of the disease.

In recent years, PCR (polymerase chain reaction) has allowed the diagnosis of acute cases of rickettsial infections as well as the characterization of new species of rickettsia in different regions of the World. Rickettsia DNA has been detected by PCR in blood, skin biopsy, necropsy tissues and arthropods, using several protocols that vary in the genomic localization of primers, and in their specificity and sensitivity.

Immunohistochemical techniques such as immunoperoxidase and alkaline phosphatase staining have been shown to be useful for rickettsia antigen detection in formalin-fixed paraffin-embedded tissue samples, although this method is not widely used for diagnosis of rickettsiosis.

ADVANCES IN THE DIAGNOSIS OF RICKETTSIOSIS IN THE AMERICAS

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ABSTRACT

Rickettsial diseases that are transmitted by ticks, fleas, and lice are rapidly achieving recognition among physicians as an important, often clinically indistinguishable, set of diagnostic and clinical management problems such as delays or misdiagnosis. The current status for diagnosis of rickettsial diseases is based upon the four “cornerstones” of isolation, immunological detection, genetic analysis and the gold standard, serology. However, there are efforts underway to develop sensitive, accurate assays for rickettsial diseases as the prototypes for what is envisioned as becoming clinically available, automated batteries of tests. These tests will yield timely, clinically useful results for the diagnosis of febrile diseases in tick- or cat flea-exposed patients.

BACKGROUND

Rocky Mountain Spotted Fever occurs throughout the United States with 600 cases reported annually, of which 5% are fatal (1). It is likely that there are three times as many unreported cases including a similar rate of hidden mortality. Murine typhus (*R. typhi*), cat-flea typhus (*R. felis*), rickettsialpox (*R. akari*), and flying squirrel-associated typhus (*R. prowazekii*) occur in the US with unknown incidence of diagnosed and misdiagnosed cases, and a surprising number of imported infections with *R. conorii* and *R. africae* occur. Clinical diagnosis is deceptively difficult with delay of missed diagnosis leading to preventable deaths (2). The only assays that are diagnostically effective during the acute stage of illness, namely immunohistochemistry of skin biopsies or immunocytochemistry of circulating infected endothelial cells for the presence of rickettsiae, are not generally available (3). The existing serologic tests are based on detection of reaction with a crude mixture of bacterial antigens such as *Proteus vulgaris* strains OX-19 and OX-2 or *R. rickettsii* by agglutination, indirect immunofluorescence (IFA), or enzyme immunoassay. Some of the assays are nonspecific: none provide reliable, sensitive, accurate, detection of specific antibodies at the time when therapeutic decisions should be made. This is a particular challenge with serological based assays in which anti-rickettsial antibodies are usually detected during convalescence. However, these assays that will be discussed in further detail have proven to be effective in the diagnosis of rickettsiosis and will continue to be relied upon until the development of more accurate and sensitive assays.

ISOLATION

Perhaps the definitive diagnosis for a rickettsial disease is isolation of the organism in culture. Rickettsial isolation is performed in few laboratories and can be used for clinical samples such as blood and tissues and in many cases, arthropods. Cumbersome historic methods such as inoculation of adult male guinea pigs, mice, or the yolk sac of embryonated chicken eggs have been supplanted by cell culture methods utilizing Vero, L-929, HEL, and MRC5 cells in antibiotic-free media to isolate rickettsiae (4, 5, 6, 7). For the isolation of rickettsiae from blood, the sample should be obtained in a sterile heparin-containing vial prior to the administration of antimicrobial agents that are active against rickettsiae (4, 5, 6). The blood can be stored temporarily at 4^{o} C but it should be processed as promptly as possible. If inoculation of cell culture or animals must be delayed for more than 24 hours, plasma, buffy coat, or whole blood should be frozen rapidly and stored at -70^{o} C or in liquid nitrogen.

If arthropods or tissues are the starting material, sterile handling of the materials is essential. Arthropods should be surface sterilized using a three step procedure of using a solution containing 1% bleach, followed by washing with 70% alcohol, followed by a thorough rinse with sterile phosphate-buffered saline of water. Clinical samples should be collected in an aseptic manner. The samples containing 0.5 ml of triturated material mixed with 0.5 ml of tissue culture medium are inoculated as promptly as possible onto 3.7 ml shell vials with 12 mm round cover slips having a confluent layer of cells and centrifuged at 700 x g for 1 hr at room temperature to enhance attachment and entry of rickettsiae into host cells (5, 6, 8). After removal of the inoculum, the shell vials are washed with PBS and incubated with minimal essential medium containing 10% fetal calf serum in an atmosphere containing 5% CO_2 at 34^{o} C. At 48 and 72 hrs, a cover slip is examined by Giemsa or Gimenez stain or by immunofluorescence with antibodies against SFG and typhus group rickettsiae. Detection of 4 or more organisms is interpreted as a positive result. In France, this method has yielded a diagnosis in 59% of samples from patients with boutonneuse fever who had neither been treated nor developed antibodies to *R. conorii* prior to collection of the sample (5). Rickettsiae were detected at 48 hours of growth in 82% of the positive samples.

The three main drawbacks to rickettsial detection by isolation are: 1) it is not the best option for rapid diagnosis; 2) rickettsiae cannot be isolated from improperly stored samples; and 3) due to the potential risk of aerosol exposure the isolation procedure should be performed within the confines of a biocontainment laboratory (BSL-3) in a laminar flow biosafety cabinet with personal protective equipment (gloves, gown and mask) worn by the clinician or researcher. Although the quantity of rickettsiae in the cell culture is relatively low, avoidance of aerosol, internal, or contact exposure should be taken as reports have indicated that less than 10 organisms can cause disease by inhalation (9, 10)

IMMUNOLOGIC DETECTION

The diagnoses of Rocky Mountain Spotted Fever, boutonneuse fever, murine typhus, louse-borne typhus, and rickettsialpox have been established by immunohistochemical detection of rickettsiae in cutaneous

biopsies of rash and eschar lesions (4, 11, 12, 13, 14, 15, 16). Direct imunofluorescence staining with a fluorescein conjugated polyclonal antiserum that is reactive with *R. rickettsii*, *R. conorii*, and *R. akari* has been applied successfully to frozen sections and formalin-fixed, paraffin-embedded sections of maculopapular rash lesions and eschars Eschar biopsies are a sensitive specimen for the diagnosis of SFG rickettsioses that manifest that lesion and should be considered for diagnostic evaluation in patients suspected to have rickettsialpox, boutonneuse fever, and African tick bite fever. The drawbacks are that there is a lack of antibody conjugate on the market and that there are small numbers of reference labs for consultation (3).

GENETIC DETECTION

PCR has been applied to the amplification of the DNA of *R. rickettsii*, *R. conorii*, *R. japonica*, *R. typhi*, *R. prowazekii*, *R. africae, R. felis*, *R. helvetica, R. slovaca,* and *O. tsutsugamushi*, usually from peripheral blood, buffy coat, or plasma, but occasionally from fresh, frozen, or paraffin-embedded tissue or arthropod vectors from patients (1, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). For all pathogenic *Rickettsia* spp., the 17 kDa lipoprotein gene is the principal target, employing the primers CATTACTTGGTTCTCAATTCGGTand GTTTTATTAGTGGTTACGTAACC, which amplify a 231 base pair DNA fragment (22). The citrate synthase, 16S rRNA, and OmpA genes have also been amplified diagnostically with the *Rickettsia* being identified through either restriction fragment length polymorphism analysis (RFLP) using *Alu* I and *Xba* I or sequencing of the PCR product (22). Although PCR is a good tool for diagnosis, problems may arise with false positives due to DNA or PCR contamination, insufficient DNA yield from sample preparations, and the presence of PCR inhibitors such as chitin or heme in the DNA preparation.

SEROLOGIC DETECTION

The gold standard of rickettsial diagnosis is the immunofluorescence assay (IFA) The IFA contains all the rickettsial heat-labile protein antigens and group-shared lipopolysaccharide antigen and, thus, provides group-reactive serology. IFA reagents are available commercially for SFG and typhus group rickettsiae from Pan Bio, Inc., Baltimore, MD; Focus Technologies, Cypress, CA; and Bio-Mérieux, France. In cases of Rocky Mountain spotted fever, the IFA detects antibodies at a diagnostic titer of ≥64, usually in the second week of illness. Effective antirickettsial treatment of RMSF must be initiated by day 5 of illness to avoid a potentially fatal outcome. Other rickettsioses prevalent in the US and Europe allow more leeway except in patients with particular risk factors for severe disease. For boutonneuse fever, a diagnostic IFA titer ≥40 occurs in 46% between days 5 and 9 of illness, in 90% between 20 and 29 days, and in 100% thereafter (28). In murine typhus, diagnostic IFA titers are present in 50% of cases by the end of the first week of illness and in nearly all cases by 15 days after onset (29). In endemic areas for particular rickettsial diseases, a higher diagnostic cut-off titer is required. For example, for the IFA diagnosis of scrub typhus in patients residing in endemic zones, an IFA titer to *O. tsutsugamushi* of ≥400 is 96% specific and 48% sensitive with sensitivity rising from

29% in the first week to 56% in the second week (27). Lowering the diagnostic cut-off titer to 100 only raises the sensitivity to 84% and reduces the specificity to 78%. These considerations are not as important when testing patients who have visited endemic regions for only a short period. The stated cutoff titer may serve as a guide, but each laboratory performing the test should establish its own cutoff titers for the patient population of the region and the microscope and reagents used and the laboratory judgment of the minimal positive signal.

Indirect immunoperoxidase assays for scrub typhus, murine typhus, boutonneuse fever, and presumably other rickettsioses yield results similar to IFA when the IgG diagnostic titer is set at 128 and that of IgM at 32 (27). Advantages include the use of a more generally available light microscope rather than an ultraviolet microscope and the production of a permanent slide result.

The assays that have been most widely used for the diagnosis of rickettsial diseases are agglutination of the OX-19 and OX-2 strains of *Proteus vulgaris* for rickettsioses and the OX-K strain of *Proteus mirabilis* for *O. tsutsugamushi* infections. These assays have been largely discredited owing to their poor sensitivity and specificity (14, 30). They should be replaced by more accurate serologic methods such as IFA. However, there are situations in developing countries where the choice is between the *Proteus* agglutination tests and none at all for the detection of important public health problems such as outbreaks of louse-borne typhus. In fact, the evidence leading to the recent recognition of some emerging infectious diseases such as Japanese spotted fever and Flinders Island spotted fever included *Proteus* agglutinating antibodies.

RECENT DEVELOPMENTS

Immunocytochemical detection of *R. conorii* in circulating endothelial cells has been accomplished by capture of the endothelial cells from blood samples using magnetic beads coated with a monoclonal antibody to a human endothelial cell surface antigen followed by immunofluorescent staining of the intracellular rickettsiae (5). Over a six-year period, this method achieved a sensitivity of 50% and a specificity of 94%. Rickettsiae were detected in 56% of untreated patients and in 29% of patients receiving anti-rickettsial treatment

Labruna and others recently developed a real-time PCR assay for the quantification of *Rickettsia* sp. species in ticks that can detect one copy of *R. rickettsii*. This assay has been shown to detect both spotted fever and typhus group rickettsiae (31). The potential commercialization of this technology will provide benefits to point of care treatment of suspected patients in the acute stages.

NEW HORIZONS

Recent advances in the field of proteomics, microarray analysis, and the information provided by the complete genome sequencing of several *Rickettsia* sp. species have provided opportunities for scientists to develop assays that are more sensitive than what is currently in use today and can be used during the early stages of infection. One such effort involves the creation of recombinant antibodies against *Rickettsia* sp.

species that can be used to “capture” any circulating organisms that may be found in a patient’s blood or serum sample. This complex could then be detected using a protein-based microarray system, which in turn could amplify the signal to a measurable level. Another protein-based assay in development is the identification of the “biosignature” of rickettsial infections. This is based on the hypothesis that specific microbial agents produce specific and individual serum analyte patterns during infection. A similar approach has been tested with cancer patients and has been found to have some success in the identification of specific types of tumors.

The last approach is the use of microarrays based upon the completed genomes of the recently sequenced *Rickettsia* sp. species to analyze gene expression patterns during the various stages of infections. This methodology will allow us to determine which proteins are expressed during the early phase of infection and to develop methods to detect them.

CONCLUSIONS

Until the newer technologies are fully developed and clinically evaluated, diagnosis of rickettsial diseases will continue to be done utilizing IFA as the gold standard. The uniform standard titers for clinical situations should be 64 (IgG) and 32 (IgM) unless regional variances are determined by the laboratory. Also, when possible, both acute and convalescent sera should be collected from the patients to look for a change in titer. Lastly, attempts should be made to isolate the rickettsial organism from the infected patient or from the arthropod vector to assist in the identification of the agent.

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PREVENTION AND CONTROL OF RICKETTSIOSIS IN PERU

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INTRODUCTION

Endemic typhus was responsible for millions of deaths in the pre-antibiotic era. Worldwide, the disease continues to affect thousands of people. According to WHO statistics, the great majority of cases occur in African countries such as Ethiopia, Burundi, Zimbabwe, Zambia, Rwanda and Nigeria. Other countries reporting epidemic typhus cases include Peru, Bolivia, and Central American countries. In Peru, it is reported that more than 50% of worldwide exanthematic typhus cases are coming reported from endemic zones in the southern and central mountains, and in the regions of Cuzco, Apurímac, Ayacucho, Puno; and Arequipa, confirmed by laboratory exams.

In Peru, diseases produced by rickettsias are commonly named typhus. From 1918 to 1934 exanthematic typhus prophylaxis consisted of isolating the sick person and eliminating lice; the employed treatment scheme was based on the use of Nicolle's anti-exantemathic serum or Neosalvarsan, which was a chemical arsenic treatment. Later pigments such as chrome mercury and rivanol were used.

Currently (1998-2004) exanthematic typhus is concentrated in Calca-Cuzco (20%). In order to obtain adequate prevention and control of exanthematic typhus it is necessary to study the diseases, associated risk factors and establish an active surveillance system.

BACKGROUND

Different zones of Cuzco, Arequipa and Puno are endemic for Exanthematic Typhus, thus constituting an important regional public health problem linked to episodes of war, overcrowding, lack of hygiene, etc., such as for example in the A Breña Campaign with Andrés Avelino Cáceres (Canta-1881).

Furthermore, the formation of Heath Brigades with "rijchary" (Puno-1930) community participation represented at their time an example of disease prevention by employing as strategy fairs and Sunday markets to promote hygiene and provide basic health care. Dr. Manuel Núñez Butrón did a similar work with indigenous brigades.

In Cuzco about 70% of the cases were concentrated in two provinces, Quispicanchis and Paucartambo, which have a total population of 120,000 inhabitants. In a collaborative study funded by the Fogarty

International Center, which involved the Peruvian National Health Institute, Cuzco Regional Health Directorate, General Epidemiology Office, and the University of Texas Medical Branch, three typhus foci occurred between May 1997 and April 1998 were studied in Quispicanchis province, the highest number of cases corresponding to Ccatcca district, followed by Calca and Chilihuani. Using active or passive surveillance systems confirmed cases of rickettsiosis were detected in 17 costal, mountain and forest cities. This surveillance is considered incomplete since that the most affected population belongs to very poor, isolated Andean communities, often without access to health services, added by the scarcity of laboratories with experience in rickettsias diagnosis.

CURRENT SITUATION

In Peru, where typhus is an endemic disease, there are focuses located in the young population. The classic history of a typhus focus in the endemic zone begins when news of a focus with several deaths, which is similar to typhus, reaches the health authority. The health Sector sends a team to investigate and control the focus. By the time the team arrives at the site focus, generally delayed by travel, distance problems, etc., new cases have occurred. Patients have been treated and homes sprayed with insecticides once or more times in a short period, without being acquainted with lice sensitivity to the insecticide. The foci cease by control measures or simply by the seasonal pattern, but there must be more cases in addition to those reported. This practice of limiting typhus control to sporadic disease focuses as they are occurring, has several disadvantages:

(a) it does not recognize all occurring typhus-related diseases,

(b) it only recognizes typhus when the population becomes seriously ill,

(c) the vector is not eradicated and resistance to insecticide may increase,

(d) no permanent or significant change has been made in the epidemiological scope and in guidelines for basic or potential typhus control.

Some of the major factors that contribute to endemicity and that interfere with optimum application of control measures include:

- Poverty, ignorance, lack of cleanliness, unhealthy dwelling with living conditions leading to infestation by chronic and endemic lice.
- Difficulty in communications, both in notifying health authorities about the disease occurrence and in the accessibility of the population to quick, repeated application of control measures.
- Limited economic resources, qualified personnel, transportation, etc.
- Little developed case reporting systems and unsuitable conditions in the region for laboratory diagnosis.
- Application of control measures based on experiences of the developed countries and wanted to be applied directly and inappropriately to certain chronic, endemic conditions in developing countries.

- All diseases transmitted by *Rickettsia* sp. are not included in the immediate notification surveillance system.

Prevention actions of humans in Peru

- Individual prophylaxis: frequent baths and clothes washing.
- Improvement of dwelling hygiene conditions.
- Avoid overcrowding and promiscuity.
- Social reality analysis and educational programs project. Establishment of multi-sector committees to fight exanthematic typhus.
- Entomological surveillance for exanthematic typhus: capture, taxonomic identification of human body lice and detection by molecular tests.
- Immediate reported by card of probable cases, according to the definition of exanthematic typhus case.
- Sample collection of probable case.
- Treatment (without laboratory results) with doxicicline or chloranphenicol.
- Apply insecticide on clothes and bed linen of sick people and their contacts, improve hygiene habits.
- Investigation of contacts by multidisciplinary teams at central, regional and local levels to take immediate action of an outbreak of exanthematic typhus focus. These multidisciplinary teams should include: physician, laboratory professional, technical personnel for insecticide handling.
- The team should determine: Affected area and population exposed to risk – Implicated risk factors.
- The survey should include geographic information of the zone.

CONCLUSIONS

The Metaxenic Program (MINSA-Peru) includes several diseases transmitted by vectors except Rickettsiosis.

The Official Technical Document issued by MINSA and INS-Peru in 2001 is only for exanthematic typhus (mandatory report disease).

The circulation of *R.typhi* (Typhus Group) and *R.felis* (Spotted Fever Group) in Peru is evident; therefore it is necessary to update the Technical Document published in 2001.

RECOMMENDATIONS

- Consider all rickettsial diseases detected in Peru within the immediate notification program.
- That rickettsial diseases reported in Peru be included in the Health Ministry's Metaxenic Program.

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