Current Comments

Rickettsial diseases in Brazil and Portugal: occurrence, distribution and diagnosis

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Abstract
The present study is an update review on the occurrence and diagnosis of rickettsial diseases in Brazil and Portugal, aiming at promoting their epidemiological surveillance in both countries. A literature review was carried out and unpublished data of laboratories and surveillance systems were presented. The results described the occurrence of rickettsial diseases and infections in Brazil and Portugal, including other new and still poorly understood rickettsial infections. Current diagnostic methods were discussed. As in many other countries, rickettsial diseases and infections seem to be an emerging public health problem. Treated as a minor problem for many decades, the interest in these infections has increased in both countries but further studies are needed to establish their role as a public health problem.

INTRODUCTION

The initiative for an update emerged from a collaboration between institutions working on rickettsial disease research in Brazil and Portugal. The main purpose of this study was to further knowledge on the main rickettsial diseases found in both countries, aiming mostly at developing new diagnostic approaches and control strategies. Therefore, it is sought to promote increased knowledge exchange between both countries in this field.

There is a growing concern in public health about rickettsial diseases as they are transmitted by arthropod vectors (ticks, fleas, lice and mites) and exist worldwide in endemic foci.[6]

They are caused by bacteria of the family *Rickettsiaceae*, comprising the genera *Rickettsia, Orientia, Coxiella, Bartonella (*formerly *Rochalimaea*), *Ehrlichia*, and *Anaplasma.*[6] The present review focused on the genus *Rickettsia* only.

There is an increasing worldwide concern with human infections caused by the genus *Rickettsia*. In recent years new species have been identified in several countries, mostly because of improved detection power of molecular biology techniques. Rickettsial infections have very similar and overlapping clinical manifestations, making it difficult their etiological diagnosis. Until recently no laboratory resources were available to accurately differentiate rickettsial species. Species differentiation is not even possible using *in vitro* culture, which is not available in most laboratories, as different rickettsia species have nearly identical morphological and biochemical characteristics.[6,17,40]

Public health experts have showed increasing interest not only in identifying several new species and describing their particular clinical manifestations but

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also because they have acknowledged rickettsial infections have a higher occurrence and distribution than previously thought.[6,17,40]

ETIOLOGY

Rickettsiae are Gram-negative obligate intracellular microorganisms. Only few years ago, they were considered to be "large viruses" as these bacteria survive only inside cells, cannot be isolated in artificial media and have incomplete enzyme systems. Humans are accidental hosts and they become the final link in these bacteria life cycle, except in cases of exanthematous typhus when humans act as their reservoir.[6,17,40]

The genus *Rickettsia* is divided into two groups. The first one, the typhus group, comprises three species: *R. prowazekii*, the pathogen of epidemic exanthematous typhus; *R. typhi*, the pathogen of murine typhus; and *R. canadensis*, which has been isolated from ticks but not known to cause human infection.[5,6]

The second one, the spotted fever group, currently comprises many serotypes. New rickettsiae have been isolated in arthropods worldwide as well as in humans (*R. africae* and *R. japonica)*. The species often categorized in this group are: *R. rickettsii*, the pathogen of the Rocky Mountain spotted fever and the Brazilian spotted fever; and *R. conorii*, which causes the Mediterranean spotted fever, also known as boutonneuse fever. The species *R. sibirica*, *R. australis* and *R. slovaca* are among those not yet associated to any particular clinical manifestations.[4,6,19,26,32]

It has been proposed to move the species *R. canadensis* from the exanthematous typhus group to create a new ancestral group, which would also include *R. bellii.*[34]

OCCURRENCE AND DISTRIBUTION OF RICKETTSIAL DISEASES IN BRAZIL AND PORTUGAL

Human rickettsial diseases that have been described in Brazil and Portugal can be didactically classified into three groups: classical, atypical, and new or recently described.

Patients with classical rickettsial diseases present with high fever of sudden onset and mostly exanthema. Some of them are considered cosmopolite diseases, such as epidemic exanthematous typhus and endemic or murine typhus being borne by vectors such as body lice and flea, respectively. The epidemic exanthematous typhus occurs in high altitudes of Latin America (from Mexico to South America) and Africa. Outbreaks of this disease form are easily identified and the literature has also described isolated cases in travelers of endemic areas, which are detected only when causing severe manifestations. Murine typhus is an endemic form found in many islands and port areas worldwide. In South America, it is associated with the existence of rats as reservoirs and humans are sporadically infected after being bitten by the rat flea *Xenopsylla cheopis.*[5,6]

Epidemic exanthematous typhus, one of the first rickettsial diseases described in humans, has never been reported in Brazil. Brill-Zinsser disease, a recurrent form of epidemic exanthematous typhus, was identified in Eastern European war refugees in the early 1950's. Reports from the late 19th century and early 20th century suggest their clinical manifestations to be consistent with recurrent typhus, but no laboratory confirmation is available.[11,24]

Only murine typhus of the exanthematous typhus group has been described in Brazil in the states of Minas Gerais, São Paulo and Rio de Janeiro.[2,15,25,30,36,38,39]

Among rickettsial diseases of the spotted fever group, the Brazilian spotted fever is the most prevalent and most lethal form. Their etiologic agents are spotted fever group rickettsiae, and they are borne by the anthropophilic tick *Amblyomma cajennense*. This disease was first reported in 1929 in the state of São Paulo,[31] and cases have also been reported in Minas Gerais, Rio de Janeiro, Espírito Santo, and Bahia.[11,13-15,20-22,35,38,*,**] Only the states of Minas Gerais and São Paulo have implemented a disease surveillance system.

Minas Gerais had an incidence rate of 0.35 cases per 100,000 population between 1990 and 1994. From 1981 to 1994, the disease occurred mostly among males aged five to 14 in the month of October and had 10% fatality. Between 1995 and 2003, 106 cases were confirmed with a fatality rate of 18%.[13,14,*]

First described in the 1920's in São Paulo, the first disease focus was identified in an urban expansion area, where today lie Sumaré and Perdizes neighborhoods. Later, foci were reported in the suburban area of the Greater São Paulo area, such as in Mogi das

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Cruzes and Santo Amaro, but these foci disappeared or became inactive as urban expansion progressed. Today, in the state of São Paulo, there is only one well-known focus in the area of Campinas (municipalities of Campinas, Pedreira, Jaguariúna and Santo Antonio de Posse), in the basin of Atibaia and Jaguari rivers, which has been expanding into the basin of Piracicaba river.[31,38,*]

Until recently, only two cases had been described outside the Campinas focus: one in Botucatu and the other in Mogi das Cruzes. In the last years, however, cases have been reported in the southern region of Greater São Paulo, in the same areas where there were reported cases in the 1950's.[11,13-15,20-22,35,38,**.***]

Mandatory disease reporting was established for the Campinas area in São Paulo in 1996 and nationwide in 2002. From 1985 to 2002, 76 cases were confirmed, 36 deaths reported with 47.6% fatality.*

In Portugal, among classic rickettsial diseases, endemic or murine typhus[3,****] and, in the spotted fever group, Mediterranean spotted fever –also known as escharo-nodular fever of Ricardo Jorge[10,****] – has been described. Murine typhus is endemic in the Madeira archipelago, island of Porto Santo, and is rarely diagnosed in the European continent.[1,3,5,8-10,12,37]

The only rickettsial disease of public health concern in Portugal, Mediterranean spotted fever reporting has been mandatory since 1950. It is known to be caused by *R. conorii*, which has been isolated, and the disease is borne by the dog tick *Rhipicephalus sanguineus*.[1,3,5,8-10,12,37]

From 1989 to 2000, the incidence rate of the Mediterranean spotted fever in Portugal was 9.8 cases per 100,000 population, which is one of the highest rates in the Mediterranean basin. It has been evenly distributed among men and women and the highest rates have been reported among those aged one to four, i.e., 60 cases per 100,000 population. While most cases are benign, severe cases and deaths have increased in recent years in some districts. In 1999, the disease fatality rate was 2.8%.[7,10]

The malign form of the Mediterranean spotted fever has increased in Portugal in the last three years and has been identified in some hospital services. Several bacteria have been isolated and it was possible to identify a strain of the *R. conorii* complex, previously found only in Israel. The Mediterranean spotted fever usually has a gradual onset as a flu-like illness, no eschar is seen at the inoculation site and skin exanthema is not typical as that found in *R. conorii* infection. Multiorgan failure is a common finding.[1,7,37]

The Center for Vector and Infectious Diseases Studies of Dr. Ricardo Jorge National Health Institute is the national reference rickettsial laboratory in Portugal. Over the last eight years (1995–2002) around 4,868 tests for *R. conorii* were performed using indirect immunofluorescence, of which 10% were positive. During the same time, 453 whole blood samples were tested and 31 strains of *R. Conorii* complex[7,9,10] were isolated.

Atypical rickettsial diseases have ill-defined clinical manifestations. The so-called apathogenic rickettsiae would likely produce atypical manifestations and would go unnoticed not being either clinically or laboratorially diagnosed. Most spotted fever group rickettsiae are considered apathogenic as they have not already been isolated in humans.[4,6]

Of these, *R. bellii* and *R. amblyommii* have been detected in Brazil through molecular biology techniques and tick isolation. Other molecular studies in ticks and in human biological specimens from endemic areas have allowed detecting and describing spotted fever group rickettsiae.[14,18,27,*****]

The third category comprises new rickettsial diseases, namely: vesicular rickettsial infection (rickettsialpox), DEBONEL/TIBOLA (*Dermacentor*-borne necrosis erythema and lymphadenopathy/tick-borne lymphadenopathy), perimyocarditis and *Rickettsia felis* rickettsiosis. Rickettsialpox has not yet been described in either Brazil or Portugal. It is a benign illness developing about a week after the host is bitten by a parasite mite of *Mus musculus* mouse. A non-tender erythematous papule develops at the bite site, later forming a vesicular lesion. Local lymphadenopathy and sudden fever with chills may be associated with vesicular exanthema, similar to the rash of varicella.

Rickettsia felis rickettsiosis has recently been described in humans in the state of Minas Gerais. The cases have been confirmed using serology and molecular biology and the pathogen has been detected in the vector

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***Nascimento EMM. [Isolation and molecular detection of spotted fever group rickettsia from *Amblyomma cajennense* (Fabricius, 1787) and human biological samples, from endemic areas of the state of São Paulo] (Master's dissertation). São Paulo: Instituto de Ciências Biomédicas da Universidade de São Paulo; 2003.
****Bacellar F. [Rickettsias isolated in Portugal. Contribution to strain identification and classification] (doctorate thesis). Évora: Universidade de Évora; 1996.
*****Labruna MB, Bouyer DH, McBride JW, Luis Marcelo A, Camargo EP, Walker DH. Rickettsia species infecting *Amblyomma* ticks in Rondonia, Western Amazon, Brazil. In: 18th Meeting American Society for Rickettsiology; 2003; Cumberland, MD. Maryland; 2003.

through molecular biology. The etiologic agent is *R. felis*, which is borne by a flea of the genus *Ctenocephalides*. This rickettsial disease is included in the spotted fever group due its phylogenetic characteristics, but it has not yet been isolated in humans.[4,9,14,19,27]

DEBONEL/TIBOLA or tick-borne lymphadenopathy is caused by *Dermacentor* tick bite and has been recently described by Raoult et al[32] in France, Lakos[19] in Hungary and Oteo et al[28] in Spain. Its pathogen, *R. slovaca*, is borne by the *Dermacentor marginatus* vector. In Portugal, this tick is often parasited by rickettsia and frequently found in cows and wild boars. Clinical manifestations in humans are characterized by local rash at the bite site, commonly in the scalp, associated with cervical and submandibular lymphadenopathy. The rash starts as a papule or vesicle and develops into an exudative necrotic lesion later progressing into a crust and alopecia. Low fever can persist for months and alopecia can persist for years.

Perimyocarditis is caused by *Rickettsia* spp. and, in Sweden, it has been described as causing death in young adults.[26] The etiologic agent seems to be *R. helvetica* and its vector *Ixodes ricinus*, both found in Portugal.*

LABORATORIAL DIAGNOSIS

Since Weil & Felix breakthrough in 1921, the laboratorial diagnosis of rickettsial diseases relies on relatively unspecific serology testing. Diagnosis is made through an agglutination reaction with sera of epidemic exanthematous typhus patients and strains of *Proteus* sp. and, following the 1987 World Health Organization (WHO) recommendation, indirect immunofluorescence with specific antigens.[40,**]

Direct methods can be used for detecting rickettsiae in tissue biopsies or organ autopsies by isolating them from cell cultures, histochemistry, and genotype identification using molecular biology.

SEROLOGY DIAGNOSIS

The Weil-Felix reaction is a low-cost and easy-to-perform method. It detects agglutinating antibodies in patients sera, which react with different *Proteus* strains or species. Each *Proteus* species has antigen epitopes similar to membrane lipopolysaccharides of different group rickettsiae. The agglutinins, which are detectable from five to ten days after symptom onset, are IgM immunoglobulins. Exanthematous typhus group rickettsiae preferentially react with *Proteus vulgaris* OX19, while most spotted fever group rickettsiae, except for *R. akari*, preferentially react with *Proteus* OX2. This diagnostic method cannot be used in Brill-Zinsser patients – recrudescent epidemic typhus – as they do not produce IgM. In addition, as antigens are unspecific, cross-reaction have been described, mostly in sera of patients who have already been infected by both *Proteus* and other α-proteobacteria with similar antigen epitopes, such as *Legionella* spp. and *Brucella* spp.[40]

Recommended by the WHO as the gold standard for rickettsial disease diagnosis, indirect immunofluorescence (IIF) use species-specific antigens of *Rickettsia*. IgM detection strongly evidences an active rickettsial infection but its diagnosis can be masked by the prozone phenomenon, and affected by rheumatoid factor and autoantibodies. Besides, there are also cross-reactions between exanthematous typhus and spotted fever group rickettsiae. IgG antibodies can be detected about a week after disease onset, they are specific in their biogroup and can persist up to four years.[40]

The use of antibiotics, whether specific or not, to treat rickettsial diseases can also interfere with the results, though to a lesser extent than in the agglutination reaction. Low or even undetectable antibody titers in dilutions used as a positive cut-off can produce false negative results. However, IIF can cross-react with species of the same group, making it difficult to accurately identify species either of exanthematous typhus or spotted fever group. In this case, one has to rely on clinical and epidemiological evidences for diagnostic confirmation.[40]

Other serology testing has been used for diagnosing rickettsial diseases, but they are hardly reproducible, e.g., ELISA and Western blot. These techniques require purified antigens, which are not available in many public health laboratories. Western blot test is considered more sensitive than IIF providing early IgM detection. Presumably, it is a more specific method, recommended for epidemiological studies investigating actual infection prevalence. However, the amount and purity of antigen required hinder its use as a routine method.**

DIRECT METHODS

After the etiologic agent is isolated, it can be identified through several methods. This can be performed by laboratories using viral isolation from *in vitro* cell cultures. The safety level required for rickettsiae are similar to that for viruses (Biohazard-II). As few

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rickettsiae may remain viable when isolated, samples should be collected before starting with antibiotic treatment and be kept refrigerated and tested as soon as possible. Samples include blood with anticlotting agents, plasma, and biopsy and autopsy material. The most common method used is called "shell vial assay", adapted from the rickettsia study by the Unité des Rickettsies de Marseille group. The inconvenience for the patient is the time elapsed between sample collection and diagnosis. Definitive diagnosis takes about 15 days because this is a lengthy procedure.[23,*]

Rickettsia detection and identification using molecular biology require properly collected samples. This technique can also be used for identifying isolated strains and consists of the amplification of a genoma segment shared by all rickettsiae codifying the 17-kDa antigen gene, and/or the citrate synthase enzyme (*gtl*A) gene, which condenses acetyl-CoA with oxaloacetate to form citrate in the citric acid cycle. Gene sequences codifying the membrane surface proteins *Omp* found in the spotted fever group rickettsiae, such as rOmp A and rOmp B, can also be used. After amplification, restriction enzymes break down gene segments and species-specific fragment maps are produced.[23] Technology advancements have virtually made all these procedures automated and allowed for direct sequencing of nucleotide bases of each segment and quickly and accurately identifying every rickettsia species. Such identification is possible through comparison of genomic database, such as *GeneBank*.[33,*] There are, however, many deleterious factors associated with sample collection and preservation, e.g., action of DNAses, RNAses, inhibition by Fe^{+2} or heparin, which yield false negative results.

Molecular biology techniques will be likely be the first choice in the near future when equipment and reagents should be more affordable, and sample collection and preservation conditions would be properly optimized.

Immunohistochemistry can be experimentally used in several reference laboratories. But, if needed, any clinical pathology laboratory can implement it. Histological sections of skin or other tissues are subjected to the action of anti-rickettsiae (monoclonal or polyclonal) antibodies produced by laboratory animals. Antibody binding to antigen can be seen by peroxidase staining or fluorescence.[16,29,*]

Final considerations

As a conclusion, many techniques can, and should be, used in the laboratorial diagnosis of rickettsial diseases as well as effective epidemiological surveillance of cases. In some geographical areas, where the occurrence of certain rickettsiae is still unknown, this approach presumably requires searching for these bacteria in arthropod vectors. In cases of atypical rickettsial diseases with strong epidemiological suggestion of infection, all known diagnostic methods should be applied, even in the face of no clinical evidence. Using antigens from strains locally isolated increase serology specificity. In countries such as Brazil and Portugal, where suspected clinical cases of rickettsiae are reported every year, rapid and effective diagnosis is key and crucial to avoid treatment delays. It is emphasized, however, that clinical evaluation is still the fastest and the most valuable diagnostic tool available.

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