RMMG
Revista Médica de Minas Gerais

REVIEW ARTICLE

Spotted fever: early diagnosis and its relevance

Febre maculosa: diagnóstico precoce e sua relevância

Adelina Machado de Carvalho Nogueira[1], Cristielen Andrade Dantas Colen[1], Paola Valeska da Silva Cupertino[1], Josiane da Silva Quetz[1]

ABSTRACT

Introduction: Brazilian spotted fever (BSF) is a neglected zoonotic disease, with compulsory notification, high mortality rates, since adequate diagnosis and treatment usually begin at a late stage. The main etiological agent of BSF is *Rickettsia rickettsii*, one of the most lethal when compared to others of its kind. The vectors of this organism are the different species of ticks, which contributes to a wide transmission area. **Objective:** This work aims to analyze the challenges of clinical and laboratorial diagnosis of BSF, addressing epidemiological aspects and describing the signs and symptoms presented by patients affected by the disease in clinical cases described in the scientific literature, with the aim of highlighting the importance of an early clinical diagnosis, as well as exploring alternatives for laboratorial diagnosis that could help reduce the time for correct identification of this zoonosis. **Methods:** A narrative review was carried out on BSF and its clinical and epidemiological aspects, as well as its laboratory diagnosis methods, consulting the databases of the Scientific Electronic Library Online (SCIELO), Latin American and Caribbean Literature on Health Sciences (LILACS), Virtual Health Library (BVS) and PubMed. The articles were selected through the delimitation of keywords by the Descriptors in Health Sciences (DeCS-BIREME) described in the keywords. Relevant articles were selected, published between 2011 and 2022, written in Spanish, English or Portuguese. **Results and Discussion:** The laboratory diagnosis of BSF can be performed by Indirect Immunofluorescence Assay (IFA), Enzyme Immunoassay (ELISA), Immunohistochemistry (IHC), Polymerase Chain Reaction (PCR) or Culture with bacterial isolation. Complementary exams include blood count, coagulogram, evaluation of enzymes such as lactic dehydrogenase (LDH), liver transaminases (ALT and AST), creatine kinase (CK) and pancreatic enzymes. The procalcitonin test (PCT) can be useful to select suspected cases, since procalcitonin is an important laboratory marker in systemic bacterial infections and its elevation can guide the clinical diagnosis. **Conclusion:** Factors such as delay in laboratory diagnosis or inaccuracy in the clinical diagnosis of this neglected disease result in high lethality, which remains a challenge for public health institutions. The present work demonstrated that IFA is considered the gold standard test for laboratory diagnosis, but there are problems such as cross-reactions with other exanthematous diseases, which causes inaccuracy in the results. The data obtained underscore the need to implement new technologies for clinical and laboratory diagnosis in order to speed up the diagnosis and appropriate treatment.

Keywords: Brazilian spotted fever; Diagnostics; *Rickettsia rickettsii*.

[1] Newton Paiva University Center, Minas Gerais, Brazil.

Responsible Editor:
Dr. Alexandre Moura
Holy House of Mercy of Belo Horizonte
Belo Horizonte, MG, Brazil.

Corresponding Author:
Adelina Machado de Carvalho Nogueira
E-mail: adelinamcnogue@yahoo.com.br

Institution that research was developed:
Centro Universitário Newton Paiva.

Sources of support:
There were no sources of support.

Ethics Committee:
Not applicable.

Clinical Trial Registration:
Not applicable.

Conflict of Interests:
The authors declare that there is no conflict of interest.

Received on: July 23, 2022
Approved on: February 08, 2023
Publication Date: August 08, 2023.

DOI: 10.5935/2238-3182.2022e33208-en

RESUMO

Introdução: A febre maculosa (FM) é uma doença zoonótica negligenciada, de notificação compulsória, com elevadas taxas de mortalidade, visto que o diagnóstico e o tratamento adequados costumam ser tardios. O principal agente etiológico da FM é *Rickettsia rickettsii*, um dos mais letais quando comparado aos outros de seu gênero. Os vetores desse organismo são as diversas espécies de carrapatos, o que contribui com uma ampla expansão de transmissão geográfica. **Objetivo:** Este trabalho tem como objetivo analisar os desafios do diagnóstico clínico e laboratorial da FM, abordando aspectos epidemiológicos e descrevendo os sinais e sintomas apresentados por pacientes acometidos pela doença em casos clínicos descritos na literatura científica, com o intuito de salientar a importância de um diagnóstico clínico precoce, bem como explorar alternativas para o diagnóstico laboratorial que poderiam auxiliar na diminuição do prazo para identificação correta dessa zoonose. **Métodos:** Foi realizada uma revisão narrativa sobre a FM e seus aspectos clínicos e epidemiológicos, assim como seus métodos de diagnóstico laboratorial, consultando as bases de dados *Scientific Electronic Library Online* (SciELO), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS), Biblioteca Virtual em Saúde (BVS) e PubMed. Foram selecionados artigos através da delimitação das palavras-chave pelos Descritores em Ciências da Saúde (DeCS-BIREME) descritos nas palavras-chave. Foram selecionados artigos relevantes, publicados entre 2011 e 2022, escritos nos idiomas espanhol, inglês ou português. **Resultados e Discussão:** O diagnóstico laboratorial da FM pode ser realizado por Reação de Imunofluorescência Indireta (RIFI), Ensaio Imunoenzimático (ELISA), Imunohistoquímica (IHQ), Reação de Cadeia Polimerase (PCR) ou Cultura com isolamento da bactéria. Exames complementares incluem o hemograma, coagulograma, avaliação de enzimas como desidrogenase lática (LDH), transaminases hepáticas (ALT e AST), creatinoquinase (CK) e enzimas pancreáticas. O exame de procalcitonina (PCT) pode ser útil para selecionar os casos suspeitos, visto que a procalcitonina é um marcador laboratorial importante nas infecções bacterianas sistêmicas e sua elevação pode direcionar o diagnóstico clínico. **Conclusão:** Fatores como atraso no diagnóstico laboratorial ou imprecisão no diagnóstico clínico dessa doença negligenciada resultam em alta letalidade, o que continua sendo um desafio para as instituições de saúde pública. O presente trabalho demonstrou que a RIFI é o teste considerado padrão ouro para o diagnóstico laboratorial, porém ocorrem problemas como as reações cruzadas com outras doenças exantemáticas, o que causa imprecisão nos resultados. Os dados obtidos ressaltam a necessidade de implantação de novas tecnologias para diagnóstico clínico e laboratorial, com o intuito de agilizar o diagnóstico e o tratamento adequados.

Palavras-chave: Febre maculosa; Diagnóstico; *Rickettsia rickettsii*.

INTRODUCTION

Brazilian spotted fever (BSF) is a neglected zoonotic disease, with compulsory notification, high mortality rates, since adequate diagnosis and treatment are usually late[1,2]. The main etiological agent of BSF is *Rickettsia rickettsii*, one of the most lethal when compared to others of its kind. The vectors of this organism are the different species

of ticks, which contributes to a wide transmission area[2,3]. In Brazil, the main vector of *Rickettsia rickettsii* are ticks of the *Amblyomma cajennense* species, known as *carrapato-estrela* (star-tick), one of the most common and significant species when it comes to diseases that can be transmitted to humans, usually as accidental hosts. Ticks parasitize horses, rodents, capybaras, possums, rabbits, dogs and other domestic and wild animals[1]. Scarce knowledge about the disease tends to delay diagnosis, resulting in a number of deaths that can reach up to 80% of untreated cases. In Brazil, this disease tends to be more frequent in the States of Santa Catarina, São Paulo, Minas Gerais, Rio de Janeiro and Espírito Santo[1,4].

The clinical diagnosis can be masked by nonspecific symptoms similar to other exanthematous diseases. The main symptom is rash, but it is not always present in patients affected by the disease, which leads to a complexity and delay in clinical diagnosis[1-4]. Characterized as an acute febrile condition, it has nonspecific symptoms, such as intense myalgia, high fever, general malaise, nausea, vomiting, abdominal pain and diarrhea, which can cause confusion with other exanthematic diseases that have similar symptoms, such as: dengue, leptospirosis, malaria and meningoencephalitis[4,5]. In more severe cases, hemorrhagic manifestations such as cutaneous, digestive and pulmonary bleeding can be noticed[4].

The laboratory diagnosis of BSF occurs through specific exams (direct investigation of rickettsia, investigation of genetic material by molecular biology and investigation of anti-rickettsial antibodies) associated with complementary nonspecific laboratory evaluation (hematological exams and quantification of serum enzymes). Currently, the serological test of Indirect Immunofluorescence Assay (IFA) is the gold standard for diagnosing rickettsiosis. However, IgM antibodies are easily triggered in cross-reaction with other diseases and IgG antibodies are only detected from 7 to 10 days after the appearance of signs and/or symptoms, requiring the collection of a serum sample in the first days of the disease and another serum sample within a period of 14 to 21 days after the first collection[4].

This work aims to analyze the challenges of clinical and laboratorial diagnosis of BSF, addressing epidemiological aspects and describing the signs and symptoms presented by patients affected by the disease in clinical cases, with the aim of highlighting the importance of an early clinical diagnosis, as well as exploring alternatives for laboratory diagnosis, with the aim of reducing the period for correct identification of this zoonosis.

Methods

The present study is a narrative review about BSF and its clinical and epidemiological aspects, as well as its laboratory diagnosis methods. The following databases were used for consultation: Scientific Electronic Library Online (SciELO), Latin American and Caribbean Literature in Health Sciences (LILACS), *Biblioteca Virtual em Saúde* (BVS) and PubMed. Articles were selected by the delimitation of keywords by *Descritores em Ciências da Saúde* (DeCS-BIREME). Key words were: Brazilian Spotted Fever (D012373); diagnosis (D012698); *Rickettsia rickettsii* (D012284); Rickettsia infections (D012282) and Spotted Fever Group Rickettsiosis (D000073605). The articles were selected according to the year, language and relevance of the subject. Articles published between 2011 and 2022 were selected. There were few works in Portuguese, so it was decided to also include articles in English and Spanish. Epidemiological bulletins were used in order to contribute with aspects about the expansion of information about the disease. Other inclusion criteria involved research on case reports that illustrated the high lethality of BSF, compulsory notification, areas of coverage, research on the etiological agent and vector, research on clinical and laboratorial diagnosis, in addition to possible technologies for early diagnosis. Exclusion criteria were: articles published outside the American continent, duplicate articles, articles without free access and articles outside the chosen time range.

Pathophysiology of BSF

Infection occurs through the introduction of the bacterium *Rickettsia rickettsii*, under the posterior dermis, adhesion to the endothelial surface and dissemination of the infection by the endothelial cells. The bacterium is gram-negative, bacillary in shape, has an approximate size of 0.3 to 0.5µm by 0.8 to 2.0µm and is obligatorily intracellular[4,6,7]. As it is an infectious agent with tropism for endothelial cells, BSF can be considered a kind of systemic vasculitis, as it is associated with a disseminated inflammatory vascular state, triggering the phagocytic defense cells, which are directed to the focus of the infection, resulting in the activation of procoagulatory vasoactive mediators and proinflammatory cytokines that increase vascular permeability and other effects that can culminate in hemorrhage, microthrombi and, in many cases, rapid evolution to generalized edema and multiple organ failure. By infecting endothelial cells, the bacterium induces the activation of proteins of the complexes responsible for the innate immune response, which is directly linked to the process of autophagy, and, paradoxically, the beginning of this process favors the replication of the bacteria[6].

Transmission

Rickettsia rickettsii is found in the salivary glands and ovaries of BSF - transmitting arthropods. The main transmitting ticks are those of the genus *Amblyomma*, of the species *A. sculptum*, formerly known as *Amblyomma cajennense*. This tick species has a wide distribution in America. The vector has a preferred host, the horse, but in the larval and nymph stages, the parasite specificity is low, which can lead to the occurrence of other hosts/reservoirs, such as: oxen, sheep, goats, dogs, pigs, deer, capybaras, wild dogs, rabbits, agoutis, raccoons, armadillos and anteaters. The vectors are popularly known as "star-tick", "horse-tick" or "rodoleiro", which are always hematophagous

and become infected the moment they suck wild animals. They are considered reservoirs, since they can transmit the bacteria in eggs and larvae and/or transmission between vector generations, in order to continue the disease cycle. Transmission to the host occurs through the bite of the infected tick, and it is not necessary to take into account the length of time the tick remained adhered to the dermis. After the bite, the time for the installation of the bacteria in the endothelial cells can vary from six to ten hours[7-11].

Epidemiology and clinical aspects of BSF

The Brazilian Ministry of Health determined BSF would be an infectious disease of compulsory notification in 2001 and, subsequently, of immediate compulsory notification in 2014[10]. A "suspected case" of BSF is defined as any patient who presents nonspecific initial signs and symptoms, such as: acute high fever of sudden onset, headache, severe myalgia, general malaise, nausea, vomiting, appearance of maculopapular rash between the second and sixth day after the onset of infection, hemorrhagic manifestations and/or patient who has reported a tick bite or who has visited areas considered at risk in the last 15 days. These are important points to take into account during the patient's anamnesis. The summary of clinical manifestations is described in Chart 1 below[4,8-10].

SINAN - *Sistema de Informação de Agravos de Notificação* (Notifiable Diseases Information System) is the official system to report cases by the Brazilian Ministry of Health. It collects information from records of confirmed cases, clinical suspicions and notifications expressed in a number of days. For the definition of a "confirmed case", laboratory criteria are necessary, in addition to the inclusion of individuals who present the signs, symptoms, past history that fulfill the definitions of a "suspected case", in addition to confirmation of *Rickettsia* infection in one of the diagnostic tests: Immunofluorescence Assay (IFA), Immunohistochemistry (IHC), Isolation in culture of the etiologic agent and/or Molecular biology techniques (PCR). In cases where death occurred without clinical/laboratory confirmation, investigative clinical-epidemiological criteria are used where individuals are scored; who have presented signs and symptoms compatible with the disease; frequented BSF transmission areas; presented tick bites; who have reported contact with domestic and wild animals; contact with people who have had laboratorial confirmation of the infection. Considering the confirmed cases in Brazil, the predominance occurs in the South and Southeast regions[4,10-12].

Case Report

In order to discuss better the laboratorial diagnosis of BSF, a preliminary analysis of case reports was carried out in different places of the American continent, being notorious the severity and lethality of the disease, which is often confused with other conditions due to the nonspecific symptoms presented by the patients affected by BSF, which can be seen in Table 1[13-19].

In an endemic region, where individuals have tick bites, rash, fever and headaches, occurring during the expected season (dry season in Brazilian Southeast Region), the clinical diagnosis is easier. On the other hand, for the reported cases in patients who present the disease in the initial phase, without previous history of contact with ticks, not showing a rash, all these factors can cause confusion with other viral diseases. Because it is a disease that has nonspecific symptoms, it is difficult to have a definitive clinical diagnosis, and also the patient may be symptomatic with clinical manifestations compatible with other diseases, or with inconsistent characteristics and even patients who recover without treatment, with subclinical infections or even there may be asymptomatic patients. In all these examples, incorrect diagnosis may occur[1,4,7,8,11].

Chart 1. Clinical manifestations and symptoms of patients with BSF.

Manifestations	Symptoms
Hemorrhagic	Petechiae; Mucocutaneous, digestive, and pulmonary bleeding.
Gastrointestinal	Nausea; vomits; abdominal pain; diarrhea.
Renal	Oliguria; Acute kidney failure.
Pulmonary	Cough; Pulmonary edema; Pneumonia; Pleural leakage.
Others	Maculopapular rash; Lower limbs edema; Hepatosplenomegaly; Nerological manifestations; Myalgia; Headache; Arthralgia; Meningoencephalitis.

Source: Elaborated by authors.

Table 1. BSF case reports detailed, with major clinical and laboratory findings.

Literature	Studied period	Countries	Patients (n)	Symptoms	Laboratorial findings
Bradshaw et al. (2019)[13]	2007 - 2014	USA	19	Meningoencephalitis, seizures, mental confusion, decreased level of consciousness, agitation, aphasia, cranial nerve palsies, ataxia, focal weakness, increased tone, Babinski sign, ankle clonus, and extensor posturing or neuroimaging abnormality; fever, rash, headache.	Pleocytosis (CSF); high CSF protein (>50mg/dL); hypoglycorrhachia (glucose <45mg/dL). Leukocytosis: (>11,772/mm³); Thrombocytopenia (<150,000/mm³), elevated levels of aminotransferases (>40U/L).
Gil-lora et al. (2019)[14]	2013 - 2014	Colombia	149	Headache, jaundice, myalgia, nausea, vomiting, abdominal pain, petechiae.	Thrombocytopenia (approximately 80,000 platelets/mm³)
La Mora et al. (2018)[15]	2013 - 2016	Mexico	47	Petechial rash, headache, myalgia.	Leukocytosis (>10,000 WBCs/mm³) with neutrophilia; lymphopenia and important thrombocytopenia (<19,000/mm³); Prolonged Prothrombin Time and Active Partial Thromboplastin; elevation of serum creatinine and serum urea; hyperbilirubinemia (total bilirubin: 4.04mg/dL; direct bilirubin 3.10mg/dL); moderately elevated AST and ALT (around 280UI/L and 95IU/L, respectively); hypoproteinemia; hypoalbuminemia, hypoglobulinemia and hyponatremia; Increased lactic dehydrogenase (LDH) (>1,200.0U/L); Elevated serum procalcitonin: 22.98ng/ml (ref. 0-0.5ng/ml).
Armitano et al. (2019)[16]	2016	Argentina	01	Acute fever, generalized arthralgia, abdominal pain and diarrhea.	Pancytopenia: Anemia (hematocrit: 27%; hemoglobin: 8.3g/dL); Leukocytosis (>10,000/mm3); Neutrophility: 7,700/mm³; Significant thrombocytopenia: 71,000/mm³; Prolonged prothrombin time; Elevated serum creatinine: 1.31mg/dL; Serum urea: 69.0mg/dL; Hyperbilirubinemia (total bilirubin: 4.04mg/dL; direct bilirubin 3.10mg/dL); Mild elevation of liver transaminase levels (AST: 57.0U/L; ALT: 48.0U/L).

Literature	Studied period	Countries	Patients (n)	Symptoms	Laboratorial findings
Drexler et al. (2017)[17]	2017	Mexico and USA	01	Fever, chills, hypotension, hepatomegaly, splenomegaly, acute renal and hepatic failure, altered mental status, respiratory failure, dark purple rash, bilateral necrosis (hands and feet), gangrene and intense edema.	Elevated levels of creatinine, hepatic transaminases and hyperbilirubinemia, acidosis and thrombocytopenia.
	2017	Mexico and USA	01	Fever, diarrhea, nausea, vomiting, petechiae, encephalopathy, cardiomyopathy, and acute renal failure.	Disseminated intravascular coagulation with pancytopenia, prolonged clotting time and increased levels of liver transaminases.
Drexler et al. (2017)[17]	2017	Mexico and USA	01	Fever, cough, dyspnoea, diarrhea, nausea, vomiting, abdominal pain, mottled rash and pulmonary infiltrates, in addition to respiratory failure.	Leukopenia and thrombocytopenia.
	2017	Mexico and USA	01	Fever, headache, myalgia, fatigue and arthralgia abdominal pain, rash, headache and extreme fatigue, generalized petechial rash; cardiac, pulmonary and hepatic perivascular inflammation; petechiae; haemorrhage in the epicardium and pulmonary pleura.	Leukocytosis, thrombocytopenia, elevated levels of liver transaminases and pancreatic enzymes.
Sánchez et al. (2018)[18]	2018	Argentina	01	Febrile seizures, rash, progressing to multiple organ dysfunction, progressing to septic shock.	Leukocytosis: 16,400/mm^3; hematocrit: 47%; severe thrombocytopenia: 58,000/mm^3; elevation of serum creatinine: 2.11mg/dL; Hyperbilirubin (total bilirubin: 9.39mg/dL; direct bilirubin 8.63mg/dL); AST: 188.0IU/L; ALT: 198.0IU/L; Hyponatremia 127.0mEq/L; increased lactic dehydrogenase (LDH): 2,323.0U/L.
Fraga, da Silva, Soares (2021)[9]	2019	Brazil	01	Fever, myalgia, prostration and irritability, neck stiffness, diffuse maculopapular rash and petechiae, abdominal pain, hepatosplenomegaly, edema of extremities, jaundice hypotension, tachycardia, bilateral pleural effusion, sedation and onset of vasoactive amines	Pancytopenia, Anemia (hematocrit: 25%; hemoglobin: 8.6g/dL); Thrombocytopenia: (around 38,000/mm^3); Metabolic acidosis.

Literature	Studied period	Countries	Patients (n)	Symptoms	Laboratorial findings
Bolaños, Chácon (2019)[19]	2019	Costa Rica	01	General malaise, fever, headache, chills, jaundice, myalgia in lower and upper limbs and maculopapular outbreak.	Leukocytosis (>10,000/mm³), Thrombocytopenia: 66,000/mm³; Elevated serum creatinine: 1.4mg/dL; Urea: 32mg/dL; AST: 155IU/L; ALT: 175IU/L; GGT:195IU/L; Alkaline Phosphatase: 146IU/L; Procalcitonin: 15.52ng/mL

Legend: ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; GGT = Gamma glutamyl transferase; USA = United States of America. Reference Values (RV) for adults (plasma/serum levels): ALT (RV: <31U/L); Albumine (RV: 3,80-5,40g/dL); AST (RV: <32U/L); direct Bilirrubin (BD; RV: <0,25mg/dL); indirect bilirrubine (BI; RV: <0,85mg/dL); total Bilirrubine (BT; RV: <1,10mg/dL); Creatinin (<1,3mg/dL); Lactic desidrogenase (RV: 240-400U/L); Glucose in cerebrospinal fluid, CSF (>45mg/dL); Globulin (RV: <3,80g/dL); Hematocrit (RV: 35-45%); Hemoglobin (RV for men:15,0-18,0g/dL); Leucocytes (RV: 4.600-10.200/mm³); Limphocytes (RV. 1.000-4.000/mm³); Neutrofils (RV: 2.800-5.200/mm³); Sodium (RV: 136,0-146,0mEq/L); Platelets (RV: 150.000-450.000/mm3); Procalcitonin (RV: <0,50ng/mL); Proteins in CSF (RV: <50,0mg/dL); Total proteins (RV: 6,40-8,30 mg/dL); Prothrombin time (RV: 11-14s); Thromboplastin time (RV: 20-40s); Urea (RV:<48,5mg/dL)[9,13-19]. Source: Elaborated by authors.

Adding to the difficulty in clinical and laboratorial diagnosis, another aggravating factor – which can be seen on Table 2 – has to do with incorrect treatment. The delay of clinical suspicion directly impacts the laboratory diagnosis. In the case reports, serological and molecular tests, for the most part, only yielded results when the patients had already died or were in an advanced stage of the disease, which led to a delay in correct treatment. Such delay in starting appropriate antibiotic therapy is related to the difficulty/delays in concluding the results of laboratory tests, which, in most cases, can lead to death due to the delay in treatment or failure administrating doxicicline during the first days of the infection[9,14-19].

Results and Discussion

1. A disease with challenges in clinical and laboratory diagnosis

Currently, the laboratorial diagnosis of BSF is performed by Indirect Immunofluorescence Assay (IFA); also the following methods may be used: Immunoenzymatic Assay (ELISA), Immunohistochemistry (IHC), Polymerase Chain Reaction (PCR) or Culture with isolation of the bacterium. Complementary laboratory tests, other than serological, can help to define or elucidate certain hematological or biochemical parameters presented by the affected patients such as blood count, clotting tests, evaluation of enzymes such as lactate dehydrogenase (LDH), liver transaminases (AST, ALT), creatine kinase (CK), and pancreatic enzymes[20].

Diagnosis using IFA reaction has some limiting characteristics, namely: immunoglobulins M (IgM) are easily triggered in cross-reaction with other diseases, which can lead to false positive results, with low sensitivity and specificity, due to reactions with non-specific bacterial lipopolysaccharides. IgG-type antibodies are detected from the 7th day of infection onwards, requiring paired serum collections: an initial one in the first days of the disease and another sample between the 14th and 21st day after the first collection[3]. IFA can show low specificity for the detection of anti-*Rickettsia rickettsii* specific antibodies due to the sharing of similar surface proteins with other species; therefore, antibodies that react with different antigens can be detected, and, in this way, false-positive results may occur[20].

Traceability studies to detect anti-*Rickettsia rickettsii* antibodies (IgG) in confirmed cases, demonstrated that despite being seropositive by the IFA method with a title equal to or greater than 1:64, the laboratory diagnosis of BSF might represent a challenge to different population profiles. For example, confounders were found among cases notified in the period from 2010 to 2015, in blood donors in 2016, and in people showing acute cases of infection by Dengue and Chikungunya among individuals with seropositivity for anti-*Rickettsia rickettsii* antibodies, being difficult to confirm the disease by IFA[20-22] (Table 3).

Regarding the other laboratory methods for BSF's laboratory diagnosis, ELISA is also a serological method that presents similar challenges presented by the IFA assays, related to the low specificity for the detection of anti-*Rickettsia rickettsii* antibodies, in addition to the possibility of cross-reactivity with other pathogens, since ELISA detects antibodies formed after pathogen exposure[4,20-23].

IHC, in turn, search for *Rickettsia rickettsii* antigens, and because of this, presents an even more important complicating factor than the serological tests: it requires tissue samples obtained from biopsy of skin lesion, necropsy or fragments of organs and tissues. In most of the cases, the execution of an invasive procedure to obtain not completely well located tissues (that needs to be in some way compromised by the pathogen) is impracticable and even unnecessary[4,20-23].

Table 2. Antibiotic treatment for patients described in some case reports.

Main Literature	Age (years)	Gender	Treatment	Outcome
Drexler et al. (2017)[17]	22	M	Penicillin; Vancomycin e Piperacillin.	Death
	52	F	Vancomycin e Metronidazol.	
	39	M	Vancomycin; Imipenem; Azitromycin; Metronidazole; Doxycillin (7th day).	
	18	F	Palliative for fever; Cefalexin.	
Armitano et al. (2019)[16]	50	F	Ciprofloxacin; Ampicillin-sulbactam; Vancomycin; Doxycillin.	
Sánchez et al. (2018)[18]	17	M	None (death in 48h).	
Bolaños e Chácon (2019)[19]	52	M	Doxycillin.	Survival
Fraga et al. (2021)[9]	5	-	Doxycillin.	

Legend: Gender of the patients: M = Masculine; F = Feminine.

Table 3. Results of individuals with traceable antibodies (IgG) anti-*Rickettsia rickettsii*.

Studied population	IFA≥1:64*	Notable characteristics
16,807 notified cases fever involving thick-borne pathogens (2010-2015)[20]	167	From 16,807 notified cases, only 1% (167) were *Rickettsia* confirmed by molecular or serological tests.
3.004 blood donors in USA (2016)[20]	261	From 3,004 blood donors tested for the presence of anti-Rickettsia rickettsia antibodies, 8.7% (261) were positive, without confirmation of *Rickettsia* infection.
222 febrile patients testing for Dengue virus in Ecuador city (2014-2015)[22]	55	From 222 febrile patients testing for Dengue virus infection, 55 patients (25%) presented anti-*Rickettsia rickettsia* antibodies, while 17 of these patients (7.7%) presented also coinfection by Dengue virus or Chikungunya virus.
243 patients with IFA reagent in USA (2010-2015)[20]	243	From 243 positive IFA, 28% filled up to clinical criteria (n=68), 19% were asymptomatic (n=46), and 53% presented unspecific symptoms (n=129).

IFA ≥ 1:64** = Number of positive samples with IgG titers higher than 1:64.
Source: Elaborated by authors.

Molecular techniques, such as PCR, use whole blood samples as the starting material, or clots formed after centrifugation of the collected blood, or biopsy, or necropsy tissues. PCR can be very useful for the characterization of the Rickettsia groups, mainly in the context of research laboratories. However, for the routine laboratory diagnosis, these molecular techniques present important difficulties, such as the standardization of the *primers* and the establishment of adequate positive control samples[4,20-23].

Bacteriological culture of samples (clotted blood, or tissue, or organ fragments) followed by the isolation of the pathogen – a method that would presumably be considered as ideal for laboratory diagnosis – has the limiting effect of empiric treatment with antibiotics inhibiting the

bacterial growth. Besides this, in many cases, the biosafety requirements for this cultivation would be an obstacle in many Brazilian territorial regions that lack the adequate laboratory structure and the properly trained staff for interpreting the results[4,20-23].

Either way, confirmatory assays in tropical settings will probably only be available in zoonotic diseases Reference Centers. That being said, confirmatory tests, as serology or nucleic acid amplification, may take weeks to return and, in most cases, will not help to direct the acute treatment. Therefore, providers must use clinical and epidemiological clues to make presumptive diagnosis and start empiric antibiotic treatment while awaiting confirmatory test results[24].

Another aggravating factor in obtaining an accurate laboratory diagnosis is empiric treatment, which can mask the disease, reducing the production of antibodies detectable by IFA, or even preventing the appearance of skin eruptions and/or typical signs of BSF. On the other hand, empiric treatment can reduce the severity and duration of the disease, and help to save lives[19,20,24].

2. Important laboratory tests in the diagnosis of BSF

The implementation of new methods or technologies to be used concomitantly with the serological methods of diagnosing BSF may improve the speed and accuracy of the laboratory diagnosis of the disease, since it is an infectious disease of compulsory notification, with high lethality and late diagnosis. In a molecular study carried out using the direct immunofluorescence technique, with the aim of unveiling the mechanistic actions involved in a specific signalization pathway, the presence of the *Rickettsia rickettsia* were in the lysate of endothelial cells infected by it, proving that, with a specific spot for samples collection, it is possible to retrieve the pathogen antigens[6]. The direct method, nevertheless, is still restricted to molecular research, and, if transposed from bench to bed hospital, the main difficulty would be obtaining representative biological material, since the pathogen is necessarily intracellular. Precise ways to obtain the most representative area with the possible presence of the pathogen need further investigations.

Another promising laboratory test, although still not very explored, comprises the procalcitonin (PCT) test, since this marker can be useful to better discriminate suspected cases: PCT is an important laboratory marker that has been studied as a serological marker of systemic infection and sepsis. PCT is a 116 amino acid peptide that can be elevated by many orders of magnitude in systemic inflammation accompanying sepsis. In systemic bacterial infections, PCT elevation would rule out viral infections. PCT levels above 1ng/mL indicate systemic bacterial infections or sepsis[15,19,23].

Conclusion

Factors such as the delay in laboratorial diagnosis and inaccuracy in the clinical diagnosis of BSF, a neglected disease, result in high mortality, which remains a challenge for public health institutions. The present work demonstrated that IFA, even though it is the gold standard for laboratory diagnosis, is dependent on the generation of antibodies by the host, which hinders the speed of the diagnostic process, in addition to the possibility of cross-reactions with other exanthematic diseases, causing inaccuracy in the results. The clinical manifestations are nonspecific and can be easily confused with some arboviruses, making the diagnosis even more difficult. Other situations include the fact that it is an endemic disease, however, contact with the vector is not necessarily reported, which results in delayed treatment. The data obtained underscore the need for studies of new diagnosis technologies, preferably for direct detection of the pathogen, in addition to PCT evaluation, with the aim of helping in the earlier diagnosis of the disease and, therefore, in adequate treatment. Late adequate antibiotic therapy is associated with worse prognosis for patients with severe bacterial infection.

Acknowledgements

We would like to thank Otto Hanriot for his help in translating this paper.

Authors' Contribution

We describe contributions to the papers using the taxonomy (CRediT) provide above: Conceptualization, Investigation, Methodology, Visualization & Writing – review & editing: Carvalho AM; Colen CAD; Cupertino PVS. Project administration, Supervision & Writing – original draft: Quetz, JS. Validation & Software: Not applicable. Resources & Funding acquisition: There is not. Data curation & Formal Analysis: Not applicable.

References

1. Araújo RP, Navarro MBMA, Cardoso TAO. Febre maculosa no Brasil: estudo da mortalidade para a vigilância epidemiológica. Cad Saúde Colet. 2015;24:339-46.
2. Oliveira RC, Fernandes J, Gonçalves-Oliveira J, Guterres A, Lemos ERS. Out of the shadows, into the spotlight: Invisible zoonotic diseases in Brazil. Lancet Regional Health – Americas. 2022;8:100202.
3. Blanton LS. The rickettsioses: a practical update. Infect Dis Clin North Am. 2019 Mar;33(1):213-29.

4. Ministry of Health (BR). Secretaria de Vigilância em Saúde. Guia de Vigilância em Saúde. Brasília (DF): Ministry of Health; 2019.

5. Amâncio FF, Amorim VD, Chamone TL, Brito MG, Calic SB, Leite AC, et al. Epidemiological characteristics of Brazilian spotted fever in Minas Gerais State, Brazil, 2000-2008. Cad Saúde Pública. 2011;27:1969-76.

6. Sahni A, Narra HP, Sahni SK. Activation of mechanistic target of rapamycin (mTOR) in human endothelial cells infected with pathogenic spotted fever group Rickettsiae. Int J Mol Sci. 2020;21(19):71-79.

7. Oliveira HMR, Paixão LMMM, Toledo PMP. Boletim da Vigilância em Saúde - Febre Maculosa Brasileira (FMB). Belo Horizonte (MG): Secretaria Municipal de Saúde; 2019.

8. Durães LS, Bitencourth K, Ramalho FR, Nogueira MC, Nunes EC, Gazêta GS. Biodiversity of Potential Vectors of Rickettsiae and Epidemiological Mosaic of Spotted Fever in the State of Paraná, Brazil. Front Public Health. 2021;9:161.

9. Fraga KS, Silva ACMP, Sant'anna FS, Soares NK. Choque séptico por febre maculosa: relato de caso. Revista de Saúde. 2021;12(1):27-9.

10. Ministry of Health (BR). Gabinete do Ministro. Portaria n. 1.271, de 6 de junho de 2014. Define a lista nacional de notificação compulsória de doenças, agravos e eventos de saúde pública nos serviços de saúde e privados em todo território nacional, nos termos do anexo, e dá outras providências. Brasília (DF): Ministry of Health; 2014.

11. Muchon JD, Forte GV, Siqueira LD, Arruda JT, Paludo RLR. Aspectos epidemiológicos, diagnóstico e tratamento para febre maculosa brasileira. Res Soc Dev. 2021;10(16):1-16.

12. Oliveira SV, Angerami RN. Timeliness in the notification of spotted fever in Brazil: evaluating compulsory reporting strategies and digital disease detection. Int J Infect Dis. 2018;72:16-8.

13. Bradshaw MJ, Byrge KC, Ivey KS, Pruthi S, Bloch KC. Meningoencephalitis due to Spotted Fever Rickettsioses, including Rocky Mountain Spotted Fever. Clin Infect Dis. 2020;71(1):188-95.

14. Gil-Lora EJ, Patiño-Gallego JJ, Acevedo-Gutiérrez LY, Montoya-Ruiz C, Rodaz-González JD. Infección y enfermedad por Rickettsia spp. del grupo de las fiebres manchadas en pacientes febriles del Urabá antioqueño, Colombia. Latreia. 2019;32(3):167-76.

15. La Mora JDD, Licona-Enríquez JD, Leyva-Gastélum M, La Mora DDD, Rascón-Alcantar A, Álvarez-Hernández G. A fatal case series of Rocky Mountain spotted fever in Sonora, México. Biomédica. 2018;38(1):69-76.

16. Armitano RI, Guillemi E, Escalada V, Govedic F, Lopez JL, Farber M, et al. Fiebre manchada en Argentina. Descripción dos casos clínicos. Rev Argent Microbiot. 2019:51(4):339-44.

17. Drexler NA, Yaglom H, Casal M, Fierro M, Kriner P, Murphy B, et al. Fatal rocky mountain spotted fever along the United States-Mexico Border, 2013-2016. Emer Infect Dis. 2017;23(10):1621-6.

18. Sánchez AP, Verduguez MH, Asis E, Barrojo G, Ortega M, Fuente R. Fiebre manchada de evolución fatal en la provincia de Salta. Medicina (Buenos Aires). 2018;78(5):356-9.

19. Bolaños P, Chacón M. Respecto a un caso de Rickettsia rickettsii en Costa Rica. Med Leg Costa Rica. 2019;36(1):14-21.

20. Straily A, Stuck S, Singleton J, Brennan S, Marcum S, Condit M, et al. Antibody titers reactive with Rickettsia rickettsii in blood donors and implications for Surveillance of Spotted Fever Rickettsiosis in the United States. J Infect Dis. 2020;221(8):1371-8.

21. McClain MT, Sexton DJ. Surveillance for Spotted Fever Group Rickettsial Infections: Problems, Pitfalls, and Potential Solutions. J Infect Dis. 2020;221(8):1238-40.

22. Farovitch L, Sippy R, Beltrán-Ayala E, Endy TP, Stewart-Ibarra AM, Leydet BF. Detection of antibodies to Spotted Fever Group Rickettsiae and Arboviral Coinfections in Febrile Individuals in 2014-2015 in Southern Coastal Ecuador. Am J Trop Med Hyg. 2019;101(5):1087-90.

23. Assink-de Jong E, Lange DW, Oers JA, Nijsten MW, Twisk JW, Beishuizen. Stop Antibiotics on guidance of Procalcitonin Study (SAPS): a randomised prospective multicenter investigator-initiated trial to analyse whether daily measurements of procalcitonin versus a standard-of-care approach can safely shorten antibiotic duration in intensive care unit patients – calculated sample size: 1816 patients. BMC Infectious Diseases. 2013;13(178):1-7.

24. Lowery K, Rosen T. Probable African tick bite fever in the United States. Yale J Biol Med. 2020;93(1):49-54.