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Descriptions of two new cases of Rocky Mountain spotted fever in Panama, and coincident infection with *Rickettsia rickettsii* in *Rhipicephalus sanguineus* s.l. in an urban locality of Panama City, Panama

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Abstract

The clinical and pathologic characterisation of two fatal cases of tick-borne rickettsiosis in rural (El Valle) and urban (City of Panama) Panama are described. Clinical and autopsy findings were non-specific, but the molecular analysis was used to identify *Rickettsia rickettsii* in both cases. No ticks were collected in El Valle, while in the urban case, *R. rickettsii* was detected in *Rhipicephalus sanguineus* s.l., representing the first molecular finding in this tick in Panama and Central America.

Introduction

Tick-borne rickettsioses (TBR) include nearly 20 diseases caused by different species of spotted fever group rickettsiae, placing them among the most significant zoonoses worldwide [1]. Of these, in the Americas, *Rickettsia rickettsii* causes the most severe cases of TBR and has a high mortality rate in untreated patients [1–3]. Human infections with *R. rickettsii* have been reported throughout the Americas; it is known as Rocky Mountain spotted fever (RMSF) in North America, but also has regional names like 'fiebre maculosa Brasileira' (Brazil) or 'fiebre de Tobia' (Colombia) [4]. The ecology of its transmission varies according to the region: in North America, the most common natural vectors are *Dermacentor* ticks and peri-urban *Rhipicephalus sanguineus* s.l., in Central and South America *Amblyomma* ticks, and *R. sanguineus* s.l. is potentially a vector in Costa Rica and Brazil [4–6]. In addition, *R. rickettsii* has been reported in the ticks *Haemaphysalis leporispalutris*, *Amblyomma tenellum* (previously called *Amblyomma imitator*), *Dermacentor nitens*, *Amblyomma americanum* and *Amblyomma parvum* [7].

Panamanian cases of RMSF were first reported in 1950, with two fatal cases among five patients [8, 9]. From 2004 to 2013 seven more cases were confirmed, with six of them were fatal [10–13]. In addition, more evidence of the circulation of TBR in Panama was obtained in surveillance studies [14, 15]. Except for one case from a peri-urban area in Panama City [9] and another from a heavily forested area [12], the majority of patients came from rural agricultural regions. In Panama, *R. rickettsii* was isolated from *A. mixtum* (formerly called *Amblyomma cajenenense*) [16] and *R. sanguineus* s.l. has been considered a putative vector [9]. Since these species differ in their environmental and host preferences [13], it is important to understand their ecology in Panama in order to comprehend and prevent transmission of *R. rickettsii*. This paper reports the clinical and pathological description of two new cases of *R. rickettsii* spotted fever and presents a new urban scenario for this disease in Panama.

Case 1

The first case occurred on 28 February 2017. An 8-year-old male patient from El Valle, a rural community in Coclé Province, was admitted to Hospital Aquilino Tejera (HAT) in Coclé Province; he presented with a 5-day history of intense headaches, fever, diarrhoea, vomiting and abdominal pain. On the third day after onset of fever, a generalised skin rash appeared. Laboratory tests at the time showed a platelet count of 40 000/mm^3, leucocytes at 5000/mm^3 (93% neutrophils) and haemoglobin measured at 10.3 g/dl. The initial suspicion was dengue fever. The patient was treated at a primary health centre with antipyretics and amoxicillin, and later with ampicillin, clindamycin and gentamicin at the HAT. One day after his admission to the HAT, he was transferred to the Hospital de Especialidades Pediátricas Omar Torrijos

(HEP) in Panama City in critical condition. Laboratory tests then indicated thrombocytopenia (with a platelet count of 22 000/mm^3), leucocytes at 5800/mm^3 (88% neutrophils), and low haemoglobin (9.5 g/dl) and haematocrit 27.8%. Bilateral alveolar infiltrate was observed on the radiograph. The patient's health deteriorated until he developed respiratory insufficiency and heart failure. Despite intensive treatment, he died on 2 March 2017. Molecular analyses and culture testing for dengue and other arboviruses were negative.

The autopsy revealed petechiae and generalised oedema (Fig. 1), congestion and focal haemorrhage in multiple organs associated with generalised vasculopathy of small-medium vessels with partial fibrin microthrombi formation, pericardial, pleural and peritoneal effusion, and interstitial pneumonitis with diffuse alveolar damage (Fig. 2). Additionally, the patient had foci of endo-myocarditis, acute renal tubular necrosis and chronic hepatitis.

Multiple samples of the liver were recovered during the autopsy and were used to test for TBR by PCR. DNA was extracted using the DNeasy tissue kit (Qiagen, Hilden, Germany), following the manufacturer's instructions for tissue samples. The sample was positive by PCR for *Rickettsia*-specific primers Rr190.70 and Rr190.701 [17]. PCR products were purified using ExoSap (USB), and sequenced with an automated sequencer (Applied Biosystems model ABI Prism 3130 × l Genetic, California, USA). Sequences (GenBank MF678553) had 100% homology with *R. rickettsii* (GenBank KX363464).

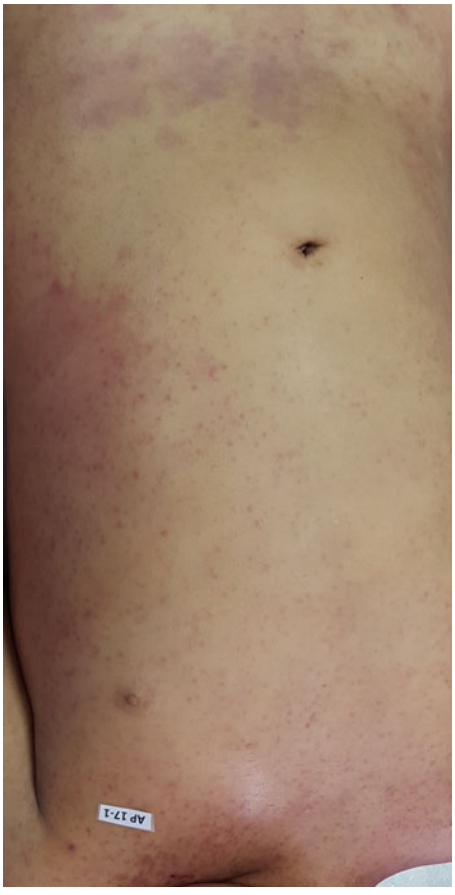

Fig. 1. Petechiae and purpuric lesions on trunk and extremities.

Relatives of the patient did not experience recent tick bites but mentioned travel to the highlands of Chiriquí Province.

The home was a concrete building surrounded by pasture. The house was visited on 4 April 2017, but no ticks were found during the visit in either the house or pasture.

Case 2

The second case occurred on 2 March 2017. A 15-year-old male patient from an urban neighbourhood of Panama City was admitted to the Complejo Hospitalario Armodio Arias Madrid, with an 8-day history of intense headaches, fever, general malaise, myalgia, fatigue, abdominal pain and 2 days of convulsions and shivers. Laboratory tests showed thrombocytopenia (low platelet count of 42 000/mm^3), normal leucocytes (6900/mm^3), with increased neutrophils (90.4%), abnormal creatinine (5.28 mg/dl), Ca^{2+} (6.9 mg/dl), inorganic phosphorus (8.0 mg/dl), Mg^{2+} (3.5 mg/dl), total serum protein (5.5 mg/gl), albumin (2.8 mg/dl), total bilirubin (3.93 mg/dl), direct bilirubin (3.3 mg/dl), lactate dehydrogenase (1077 U/l), aspartate aminotransferase (219 U/l), alanine aminotransferase (87 U/l) and a prothrombin time (14.5 s). Upon admission, serum and cerebrospinal fluid were obtained for culture, molecular and serological tests for dengue, *Leptospira* and *Rickettsia*. All tested negative. Despite intense medical efforts, the patient died on 3rd March.

In the second case, the autopsy revealed jaundice, haemorrhages in temporal muscles, brain oedema, and multiple haemorrhages in epicardium; haemorrhagic myocarditis (Fig. 3); pericardial and pleural bilateral effusions; oedema and focal haemorrhages in both lungs; ascites, hepatic lesions characterised by portal triaditis; mild endothelial proliferation and perivascular mononuclear infiltrates in the lobes and splenomegaly. Microscopic examination of haemorrhagic lesions confirmed the presence of vasculitis.

New liver samples were obtained from the autopsy and tested for hantavirus, leptospirosis and TBR (PCR). Tissues were negative for hantavirus and *Leptospira*. Using the same methods described above, the sample tested positive by PCR with *Rickettsia*-specific primers and the sequences (GenBank MF678554) showed a 100% homology with *R. rickettsii* (GenBank KX363464). One sequenced data were submitted to GenBank under the accession number MF678554.

An ecological approach was used to evaluate the house on 29 March and 4 April. This structure is a concrete building at the end

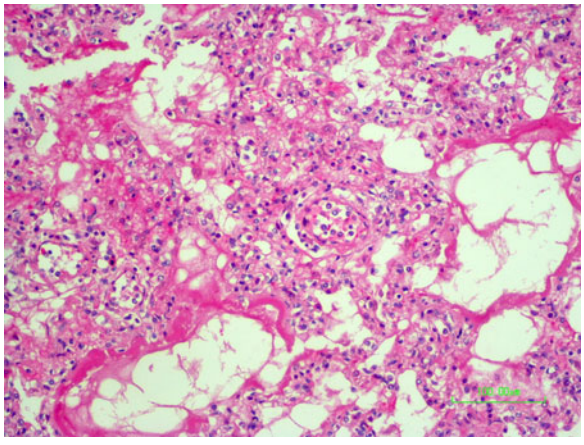

Fig. 2. Interstitial pneumonitis and diffuse alveolar damage (HE 100×).

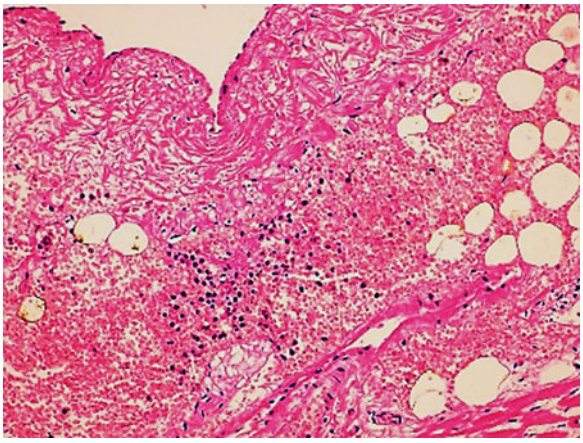

Fig. 3. Micro haemorrhagia in the myocardium (HE 10×).

of a street that borders a stream. The parents of the patient reported no travel before the onset of symptoms. Ticks were collected from internal and external walls, and from household dogs. These ticks were identified morphologically as *R. sanguineus* s.l. and 57 were analysed by PCR, using the same primers and methods described above. From these samples, DNA of *R. rickettsii* was detected in five adult ticks (8.7%). The sequenced data were submitted to GenBank under the accession numbers MF678548, MF678552. To evaluate the circulation of TBR in others in the household, blood was collected from people (4) and dogs (14) but all samples were negative by immunofluorescence assays (IFA).

Discussion

Compared with other cases of TBR, the course of disease caused by *R. rickettsii* is rapid and may be lethal if the patient does not receive appropriate antibiotic therapy early in the course of the disease. Thus, timely treatment improves the patient's chances of survival especially, in children, elderly and immunosuppressed persons [2, 18, 19]. The clinical presentations of both patients were similar to other recent cases in Panama, with sequential events being a sudden decrease in platelet counts, an increase in liver enzymes, and an abrupt deterioration of health during the fourth or fifth day after the onset of symptoms [3, 10, 11]. Oral amoxicillin, ampicillin, and clindamycin in both cases were ineffective, confirming the need to use doxycycline as early as possible [3, 12, 18, 20]. Both autopsies revealed severe damage to the organs, characterised by oedema and haemorrhage, which are common in cases of RMSF.

Two factors negatively influenced the diagnosis of these cases, which continues to be a significant challenge for public health workers. One factor is that the early clinical presentation of TBR is similar to other infectious diseases with a higher prevalence; the classic petechial rash is not always evident at initial presentation, and no epidemiological clue like ticks or tick bites was provided [10, 20]. In previous cases of RMSF in Panama and neighbouring countries, the initial clinical suspicions are mainly dengue or other arboviruses, causing tests for RMSF to be included only after these are negative. Consequently, the delay in diagnosis decreases the probability of a positive prognosis for the patient [3, 20].

Another factor is the sparse knowledge of the epidemiology of RMSF in Panama. Compared to other vector-borne diseases, there is less familiarity about the ecology and distribution of tick vectors. There is confusion about which ticks have been confirmed as vectors (e.g. *A. cajennense* complex), and other species which can be infected with *R. rickettsii*, but for which evidence is lacking for their capacity to be vectors of the disease (e.g. *H. leporispalustris* and *D. nitens*, see Miller *et al.* [21]). The epidemiology of TBR and RMSF vary considerably throughout their distribution so local experience and knowledge can be limited. These two new cases occurred in different environmental and ecological conditions.

El Valle de Antón is a rural town with conditions that support different populations of ticks, particularly *A. mixtum* and *R. sanguineus* s.l. [15, 22, 23]. El Valle and nearby regions have a recent history of circulation of TBR, based on positive serology in domestic mammals [23] and humans [15], with evidence of *R. rickettsii* infecting *A. mixtum* [13], and confirmed fatal cases of RMSF [11, 13]. In rural Panama, *A. mixtum* is the most anthropophilic species and the main vector of *R. rickettsii* [13, 16]. In fact, other species in the *A. cajennense* complex are vectors of *R. rickettsii* in rural regions of Colombia and Brazil [5, 24]. Based on these studies and considering the classification proposed by Pinter [25], this region should be classified as a transmission area for TBR, particularly RMSF.

In contrast, there are no agricultural activities or forested areas close to the home of the second victim. In Panama City, *R. sanguineus* s.l. is the most common species of tick and has been reported to use humans as hosts, although less frequently than *A. mixtum* does in rural sites [13, 15]. Panama City has a history of TBR, starting in 1952 when one case from an urban neighbourhood was linked to the presence of *R. sanguineus* s.l. In 2007, three new cases of TBR were confirmed in a rural area from this city but could not be linked to a particular species of ticks [11]. Since *R. sanguineus* s.l. is the clear vector of *R. rickettsii* in Arizona (the USA) and Mexico [19, 26] and is implicated as a potential vector in urban cases in Costa Rica [20], our findings of *R. rickettsii* infecting *R. sanguineus* s.l. inside the patient's house may be consistent with its role as a vector. The vectorial competence of this species in Panama needs further study given the complexity of the taxonomy of this tick.

Differences in the ecology of the two species of ticks should be considered when establishing control protocols. *Amblyomma mixtum* is an exophilic species that inhabits paddocks, or stables and horses appear to be its main host, but it can also be found feeding on 20 other species of vertebrates, including humans [7, 13]. In contrast, *R. sanguineus* s.l. is endophilic and can persist inside houses and on external walls; it maintains a strong parasitic relation to its normal dog host, but can easily adapt to a change of host should canines not be available [13, 19].

Although mosquito-borne diseases such as dengue have a higher prevalence than TBR in both urban and rural Panama, the wide distribution of *A. mixtum* and *R. sanguineus* s.l. in Panama needs to be considered with respect to non-specific fevers like TBR. A prompt clinical response to antibiotics such as doxycycline may both benefit the patient and confirm suspicions of a TBR infection even without laboratory confirmation [2, 18]. Further research will be needed to evaluate the ecology related to both cases, especially in the urban case given the high prevalence of dogs and *R. sanguineus* s. l. in many cities in Panama.

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Author's contribution. SEBC, AM, BM and YZ, conceived the study. AM and SEBC participated in the data collection, analysis and interpretation of data, and drafted the manuscript. GM, MA, JVP and JAS participated in the medical characterisations. GM and JVP participated in the description and analysis of the autopsies and made the pictures presents in the figures. AM, BM, CG, JM-M, YZ and SEBC participated in the conception of the molecular analysis. JBVP, LD and SEBC participated in the field works. All authors participated in the write of the text.

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