Ticks and Tick-borne Diseases 7 (2016) 653–656

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short communication

Molecular diagnosis of *Rickettsia* infection in patients from Tunisia

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ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form 5 February 2016
Accepted 6 February 2016
Available online 8 February 2016

Keywords:
Rickettsia
Skin biopsy
Swab
Cerebrospinal fluid RLB
qPCR

ABSTRACT

Diagnosis of rickettsioses had largely benefited from the development of molecular techniques. Unfortunately, in Tunisia, despite the large number of rickettsial cases registered every year, the *Rickettsia* species remain unidentified. In this study, we aimed to detect the *Rickettsia* species in clinical samples using molecular tests. A study was established to analyze skin biopsies, cutaneous swabs, and cerebrospinal fluid samples taken from clinically suspected patients to have rickettsial infection. Two molecular techniques were used to detect *Rickettsia* DNA: quantitative real time PCR (qPCR) and reverse line blot test (RLB). An analysis of the RLB hybridization assay results revealed the presence of *Rickettsia* DNA in skin biopsies (40.6%) and swabs (46.7%). *Rickettsia conorii* was the most prevalent identified species among tested samples. Other species of interest include *Rickettsia typhi* and *Rickettsia massiliae*. Using qPCR positivity rates in skin biopsies was 63.7% against 80% in swabs. *R. conorii* was the most frequently detected species, followed by *R. typhi*. The agreement between the two techniques was 68.6% (kappa = 0.33). Molecular tests, especially using specific probes qPCR, allow for a rapid, better and confident diagnosis in clinical practice. They improve the survey of Mediterranean spotted fever which is considered to be the most important rickettsial infection in humans in Tunisia.

1. Introduction

Rickettsioses are emerging infectious diseases caused by *Rickettsia* and transmitted by the bites of arthropods (ticks and fleas in particular). Although classified as neglected diseases, they continue to cause severe illnesses and death in adults and children worldwide (Parola et al., 2013). A delayed diagnosis can lead to serious complications – acute renal failure, meningoencephalitis, gastrointestinal bleeding, and multiple organ failure – and death (Boillat et al., 2008; Charra et al., 2005; Jensenius et al., 2004). An early, rapid diagnosis is the greatest clinical challenge for a successful treatment. A serology test is retrospective and infections are underestimated because control serums are rarely taken. Real-time quantitative PCR (qPCR) and reverse line blot (RLB) targeting a specific gene are currently widespread techniques with good sensitivity and specificity for detecting rickettsiosis (Jado et al., 2006; Renvoisé et al., 2012), but, few studies have compared them. We conducted a multicenter study using these two molecular techniques to identify rickettsial species in human samples and compared their diagnostic accuracy to develop a strategy to more rapidly and precisely identifying the responsible species.

2. Materials and methods

2.1. Human sampling

A study was conducted in 2012–2014 from June to October, when higher exposure to Ticks and/or fleas produces the greatest number of rickettsiosis cases. A total of 121 samples from 101 symptomatic adult patients (excluding children) with acute fever and cutaneous rashes and/or eschars in 5 hospitals (Fig. 1) was collected. The samples included 69 skin biopsies (from 35 men and

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http://dx.doi.org/10.1016/j.ttbdis.2016.02.010
1877-959X/

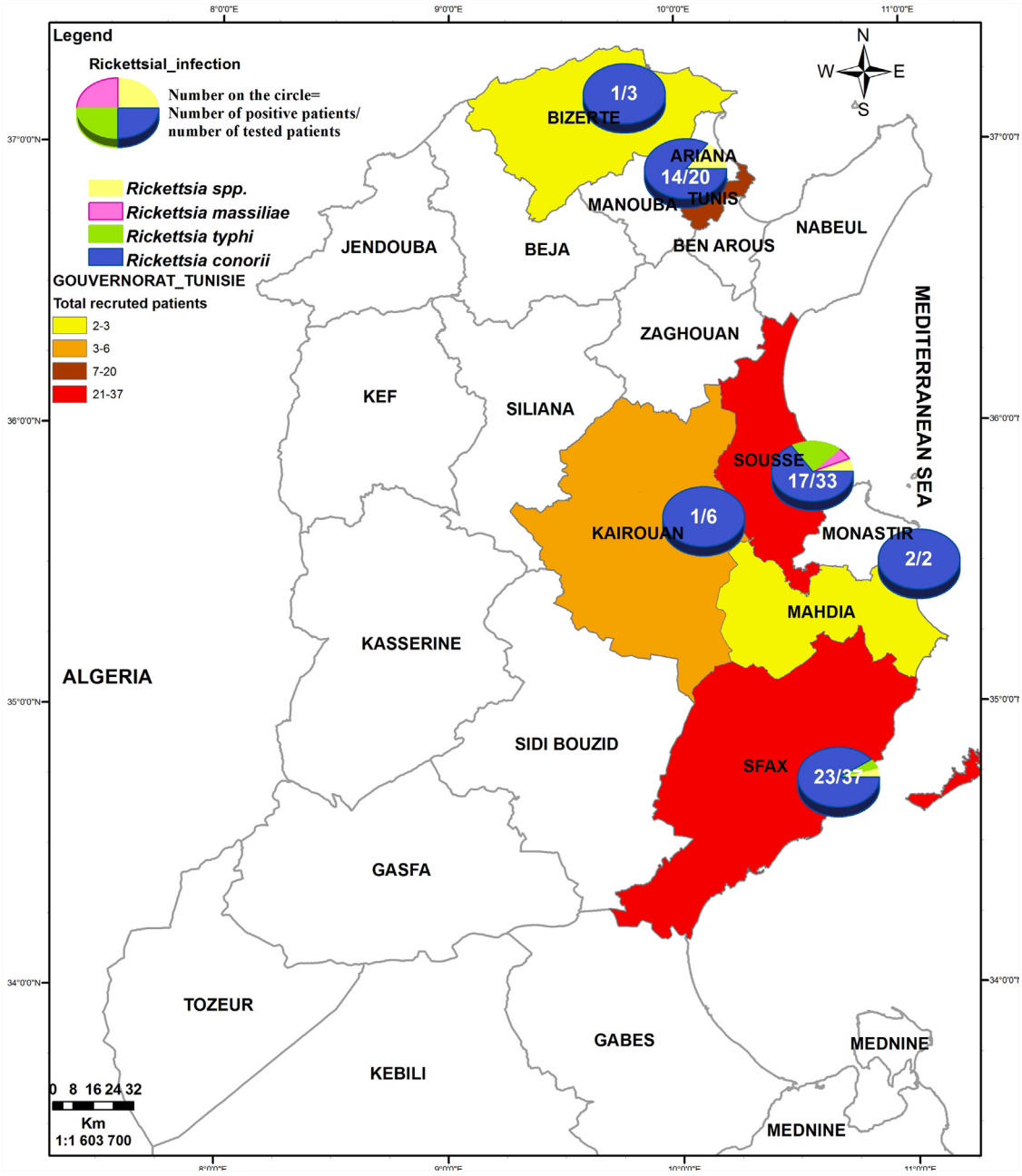

Fig. 1. Distribution of included patients and species detection in confirmed infected patients by qPCR and/or RLB.

34 women), 15 cutaneous swabs (from the eschar site (9 men and 6 women)) and 37 cerebrospinal fluid (CSF) samples (23 men and 14 women). Skin biopsies were taken using a punch (3.5 mm of diameters) from eschar, when they existed, or from the rash. The distribution of patients was mapped with ArcGIS 9 (version 9.3.1) software.

2.2. DNA extraction and QPCR amplification

DNA was extracted from all samples using the QIAamp DNA tissue extraction kit (Qiagen, Hilden, Germany) as per instructions. Biopsied tissues weighed between 18 and 20 mg. DNA was eluted in100 μl and extracts stored at −20 °C.

All samples were screened for *Rickettsia* spp. DNA using qPCR amplification (*Rickettsia* spp.-qPCR) (Renvoisé et al., 2012). The positive screening was followed by runs of qPCRs with specific probes for *Rickettsia conorii*, *Rickettsia typhi*, *Rickettsia felis* and *Rickettsia africae*, respectively (Giulieri et al., 2012; Renvoisé et al., 2012). In ambiguous cases, the *Rickettsia* infection was checked using primers and probes for the *Rickettsia* Spotted Fever Group (SFG) (RC00338) and for the Typhus group, *Rickettsia* (RP278) described by Renvoisé et al. (2012). DNA positive controls and amplification programs were used as described (Znazen et al., 2015). QPCR amplifications and product detections were carried out in the CFX96 Touch Real-time PCR Detection System (Biorad, US). To determine the quantity of DNA in each sample, five standards (10^4, 10^3, 10^2, 10, 1 copies/μl) were tested per run and the quantity subsequently measured using Bio-Rad CFX Manager software. A sample was considered positive when the Ct (cycle number at threshold of log-based fluorescence) was below 35 (≈18–35 copies of *Rickettsia* gene/PCR reaction).