Acta Tropica 178 (2018) 40–45

Contents lists available at ScienceDirect

Acta Tropica

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

Potential exposure of humans to *Rickettsia felis* in Greece

Dimosthenis Chochlakis[a,b], Antonios Germanakis[c], Georgios Chaliotis[d], Stefania Kapetanaki[e], Loukia Kalogeraki[e], Elliona Gkika[a], Nikolaos Partalis[f], Georgia Polymili[f], Yannis Tselentis[a], Anna Psaroulaki[a,b,*]

[a] Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, School of Medicine, University of Crete, Greece
[b] Regional Laboratory of Public Health of Crete, School of Medicine, University of Crete, Greece
[c] Department of Internal Medicine, General Hospital of Agios Nikolaos, Crete, Greece
[d] Department of Internal Medicine, General Hospital of Chalkida, Evoia, Greece
[e] B' Department of Internal Medicine, General Hospital of Chania, Crete, Greece
[f] A' Department of Internal Medicine, General Hospital of Chania, Crete, Greece

ARTICLE INFO

Keywords:
Rickettsia felis
Ctenocephalides felis
IFA

ABSTRACT

Rickettsia felis is a flea-transmitted pathogen however, in Greece, much work has been done on another flea-borne pathogen, *R. typhi*; human cases have been described and high-risk areas have been characterized. Nevertheless, little is known about human infections caused by *R. felis* in the country since human cases are not routinely tested for antibodies against this pathogen.

During the past seven years, we have set up a protocol at the National Reference Centre in order to improve the testing of tick-borne diseases in Greece.

Based on this protocol, *R. conorii*, *R. typhi R. slovaca*, *R. felis*, and *R. mongolotimonae* have been added into the routine analysis; during these last years, eight (8) cases of potential exposure to *R. felis* were identified by serology. On an environmental investigation carried out at the residences of the patients, the pathogen was detected in *C. felis* only.

The demonstration of *R. felis* potential presence highlights the need for better testing and surveillance of the pathogen.

1. Introduction

Rickettsia felis was first described in 1990 (Adams et al., 1990) and was originally named ELB. It was first classified as a member of typhus group (TG) for over 10 years until it was considered as a newly described species of the spotted fever group rickettsiae (SFGR) (Bouyer et al., 2001). Nevertheless, novel molecular tools have suggested that additional groups exist within the genus *Rickettsia*, including a sister clade of the SFG now known as the transitional group (TRG), at which *R. felis* has now been classified (Gillespie et al., 2007). The first human case was described in 1994 in the United States (Schriefer et al., 1994), while the first autochthonous human case in Europe was reported in 2002 (Richter et al., 2002). Cultivation of this rickettsial pathogen is very difficult since it requires low temperature and that is why isolation of *R. felis* has been achieved only once (La Scola et al., 2002). Clinical manifestations of human *R. felis* infection mostly include fever, fatigue, headache, maculopapular rash, and eschar (Brouqui et al., 2007). The main vectors of *the pathogen* (as with *R. typhi*) are fleas; in fact, only *Ctenocephalides felis has been classified as a proven vector of the pathogen* because it is able to transmit the pathogen transovarially (Azad et al., 1992). The world-wide distribution of *R. felis* is probably due to the co-migration of humans and domestic animals harbouring this flea species. However, recently there have been reports revealing the possible role of other flea species such as *C. canis*, *Anomiopsyllus nudata*, *Archaeopsylla erinacei*, *Ctenophthalmus* sp, and *Xenopsylla cheopis, tick species such as Haemaphysalis flava*, *Rhipicephalus sanguineus* sensu lato, and *Ixodes ovatus*, or even mites, as potential vectors of *R. felis* (Perez-Osorio et al., 2008). These findings may reflect the role of a vertebrate host, for example of a dog, in a possible horizontal transmission from flea to tick (*R. sanguineus s.l.)* (Oliveira et al., 2008).

Apart from its worldwide distribution, *R. felis* human cases have been described in France (Raoult et al., 2001; Renvoise et al., 2009), Germany (Richter et al., 2002), Spain (Oteo et al., 2006) and Sweden (Lindblom et al., 2010; Nilsson et al., 2014). So far in Greece there had

* Corresponding author at: Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine. School of Medicine, University of Crete, Heraklion – Crete, 71100, Greece.
E-mail address: annapsa@med.uoc.gr (A. Psaroulaki).

http://dx.doi.org/10.1016/j.actatropica.2017.10.020
Received 5 May 2017; Received in revised form 23 October 2017; Accepted 23 October 2017
Available online 24 October 2017
0001-706X/

been no descriptions of *R. felis* infections, although there are regions that are endemic for *R. typhi*, partly because antibodies against the pathogen were not tested at a routine basis leaving this type of human infection both underestimated and poorly characterized.

At least two regions, Evoia (central Greece) and Chania (Crete, Southern Greece), have been characterized as endemic to *R. typhi*. As regards Evoia, studies that were carried out in the past have demonstrated the presence of the flea-borne pathogen in rats (Chaniotis et al., 1994; Tselentis et al., 1996). In a surveillance study (Chaliotis et al., 2012), 174 patients were evaluated with suspicion of murine typhus (MT), of which 90 (51.7%) were confirmed as *R. typhi* infections. As regards Chania, ~10 humans of MT infection are described annually.

Until 2010, only *R. typhi* and *R. conorii* were routinely tested in human samples arriving at the National Reference Centre under the suspicion of rickettsial infection. However, the identification of more than these two rickettsia species in ticks, urged towards the addition of *R. slovaca*, *R. felis* and *R. mongolotimonae* into the routine analysis.

The results of this protocol, corresponding to the possible exposure of humans to *R. felis*, are described herein.

2. Materials and methods

2.1. Human sampling

Samples are generally being sent from all Hospitals of the Greek territory but mostly from Hospitals of Crete. In some cases, particularly when suspicion for rickettsial disease was put at the early stage of the disease and certainly before antibiotic administration, serum samples were accompanied by whole blood samples as well. In exceptional cases, eschar is, also, included into the samples for testing. Samples are transported at 4 °C and always following centrifugation. The minimum symptoms required are fever, headache, rash and absence of other findings that could be attributed to some other infection (eg community acquired pneumonia, urinary tract infection or gastrointestinal infection). For each patient who turns out positive, demographic and epidemiologic data are collected such as age, gender, job, residence, tick- or flea-bite, contact with animals and travel abroad two weeks before the onset of symptoms. Data corresponding to clinical signs and symptoms, complications during hospitalization, laboratory results, time until fever convalescence and therapy, are, also, recorded.

Serum and whole blood samples (together with eschars, where possible) were received from each patient, the 1st on admission and the 2nd three weeks later. Sera were initially tested by immunofluorescence assay (IFA) against *R. conorii* and *R. typhi*. As from 2010, immunoglobulin (Ig)G and IgM antibody titers are being determined for the presence of antibodies against *Rickettsia* spp using a slide that can test against *R. conorii, R. mongolotimonae, R. slovaca, R. felis, R. massiliae and R. typhi* as individual antigens (Fuller laboratories, California, USA), by an immunofluorescence antibody assay (IFA) that is based on the targeting of the rLPS. Sera were tested at titers beginning from 25 and from 60 against immunoglobulins (Ig)G and M antibodies respectively. Single sera at IgG titers of ≥960 or IgM titers of ≥200 were considered as positive. Sera where seroconversion [4-fold difference; increase in case of IgG titers and increase or decrease (depending on time elapsed from the time of infection) in case of IgM antibodies] between acute and convalescent phase was recorded, were, also, considered as positive. The cut-offs were selected following a survey on SFGR on blood donors in Crete (unpublished data). In that survey, a total of 20.3% of the sera tested were positive for IgG at titers ≥120, while 12.9% of the sera were tested positive at titers ≥100 for IgM antibodies. Based on these results, a relative exposure to *Rickettsia* antigens was noted, thereafter the decision was made to use the above described cut-off points in order to avoid false-positive results. Sera from patients already described as positive for *R. conorii*, *R. typhi* and *R. mongolotimonae* were used as positive controls. The positive controls are kept at the library of the laboratory as part of its duties as a reference center. The negative controls used were those provided by the manufacturer of the diagnostic kit.

Negative sera that corresponded to patients with high suspicion of rickettsial infection were further tested for the presence of IgG and IgM antibodies against *Rickettsia* spp using a slide that could separately test against *R. aeschlimanii, R. siberica mongolotimonae, R. slovaca, R. felis,* and *R. massiliae* as individual antigens (Fuller laboratories, California, USA), by an IFA method that is based on the targeting of a spotted fever group-specific lipopolysaccharide (rLPS) extracted for each of the above mentioned pathogens.

DNA was extracted from whole blood samples and eschars using the PureLink Genomic DNA kit (Invitrogen) according to the manufacturer's recommendations and was stored at −20 °C until further analysis. The molecular analysis was carried out by amplification of the *gltA* gene either by PCR (up until 2010) (Roux et al., 1997) or by Real-time PCR (from 2010 onwards) (Stenos et al., 2005). *Rickettsia conorii* kept in culture in Vero cells at the laboratory, was used as positive control.

All samples were tested at the Regional Laboratory of Public health of Crete (branch of the Hellenic CDC) situated at the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, University of Crete.

2.2. Environmental investigation

An epidemiological survey was carried out at the patients' environment; rats were trapped using bags while fleas were collected either from trapped rats or by use of large bags placed at areas where cats were present; in the continuity rats were anaesthetized with the use of ether in order to draw blood samples and where then set free, while fleas were placed in separate 1.5 ml eppendorf tubes in 70% ethanol and stored at −80 °C until further testing.

Sera and whole blood samples were collected from animals of veterinary importance (sheep, goats) and from dogs. Ticks and fleas parasitizing the above mentioned animals were, also, removed and treated as above.

Before further testing, the collected fleas and ticks were identified using morphologic criteria used in past studies of the laboratory (Ioannou et al., 2011; Tsatsaris et al., 2016). In the continuity, each flea or tick was immersed in a solution of 70% ethanol/0.2% iodine for five minutes, washed three times (five minutes per wash) in sterile distilled water, and crushed individually in sterile eppendorf (Hamburg, Germany) tubes using the tip of a sterile pipette.

2.3. PCR-sequencing analysis

DNA was extracted by using the QIAamp Tissue Kit (Qiagen, Hilden, Germany) in case of fleas, ticks or eschars and the QIAamp DNA mini kit (Qiagen, Hilden, Germany) in the case of blood samples, according to the manufacturer's instructions.

During the routine procedure, all samples arriving at the laboratory are initially screened by Real-time PCR targeting the *gltA* gene of Rickettsia species (Stenos et al., 2005). Positive samples are further amplified by PCR targeting the outer membrane protein (*ompB*) (Roux and Raoult, 2000) and citrate synthase (*gltA*) (Roux et al., 1997) genes. In the current case, after purification of the obtained PCR amplicons (QIAquick Spin PCR purification kit; Qiagen), sequencing analysis (sequencer CEQ 8000; Beckman Coulter, Athens, Greece) was performed and DNA sequences obtained were compared for similarity with sequences in GenBank (http://www.ncbi.nlm.nih.gov/BLAST). Chromas version 1.49 (Technelysium Pty. Ltd., Holland Park, Queensland, Australia) and ClustalW37 software was used for processing identified sequences.