Infectious Medicine 2 (2023) 338–342

Contents lists available at ScienceDirect

Infectious Medicine

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

Case Report

Use of eschar swab DNA to diagnose *Rickettsia conorii* subspecies *conorii* infection in Crimea: A case report

Emine E. Alieva[a], Muniver T. Gafarova[b,*], Eugene I. Bondarenko[c], Igor A. Dovgan[a], Irina I. Osiptchuk[d], Marina E. Eremeeva[e]

[a] *Federal State Budgetary Institution 'N.I. Pirogov Saki Military Clinical Sanatorium', Ministry of Defense of Russia, Saki 296500, Russia*
[b] *S.I. Georgievsky Medical Academy (Academic Unit), V.I. Vernadsky Crimean Federal University, Simferopol 295051, Russia*
[c] *Vector-Best, Novosibirsk 630117, Russia*
[d] *Saki Regional Hospital, Saki 296500, Russia*
[e] *Jiann-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, GA 30458, USA*

ARTICLE INFO

Keywords:
Eschar
Mediterranean spotted fever
PCR
Rickettsia conorii
Swab

ABSTRACT

Mediterranean spotted fever (MSF) has been diagnosed clinically in the Crimean Peninsula since the 1930s. We describe the recent illness of an elderly patient from Crimea who had developed a classic triad of MSF symptoms consisting of fever, maculopapular rash, and eschar. Clinical diagnosis of rickettsiosis was confirmed using real-time PCR and sequencing of 4 *Rickettsia* protein genes. The strain causing clinical illness was characterized as *Rickettsia conorii* subspecies *conorii* Malish 7. This report corroborates the utility of eschar swab material as a source of DNA for PCR-based diagnostics that enables timely patient treatment and management.

1. Introduction

Mediterranean spotted fever (MSF) has been known on the Crimean Peninsula since the mid-1930s. The disease was first described in 1936 in the city of Sevastopol, and later in the coastal western and northwestern steppe regions, and in the coastal regions of the south and southeast of the peninsula [1,2]. Subsequently, the epidemiological association of the disease with tick bites and contact with dogs was shown [3], particularly the role of the brown dog tick, *Rhipicephalus sanguineus*, as a carrier and natural reservoir of the pathogen of the MSF on the peninsula [4]. It was also determined quickly that the disease is caused by *Rickettsia conorii* [1,5]. Several clinical and tick isolates were obtained over the years; however, they were only typed using classic biological procedures (cited in [5]).

A regional increase in the incidence of MSF began in 1991. It impacted the city of Sevastopol and the nearby Saki region in the west and the Kerch Peninsula in the east, where the disease episodes coincided with a growing number of stray dogs and frequent reports of *R. sanguineus* infestations [2,5,6]. The Saki district experienced its largest outbreak of MSF in 1996 consisting of 26 diagnosed cases including one fatality [5]. Nowadays, clinical cases of MSF are reported annually across the entire Crimean Peninsula; however, the diagnosis is largely based on clinical signs and symptoms and a history of tick exposure, and only rarely confirmed using the insensitive complement fixation test [7]. Clinically, most cases manifest with the spotted fever rickettsiosis classic triad of fever, eschar, and rash; 30% of patients also develop lymphadenitis. Most patients have a mild disease and fully recover following tetracycline or doxycycline treatment [5]. However, MSF can also have a more malignant course associated with multiorgan failure and death [8–10]. Access to prompt and accurate laboratory diagnosis key for optimal patient management and timely recovery.

We present here a case of MSF diagnosed in Crimea using contemporary molecular methods.

* Corresponding author.
E-mail address: muniver@mail.ru (M.T. Gafarova).

https://doi.org/10.1016/j.imj.2023.09.004
Received 16 July 2023; Received in revised form 15 September 2023; Accepted 24 September 2023

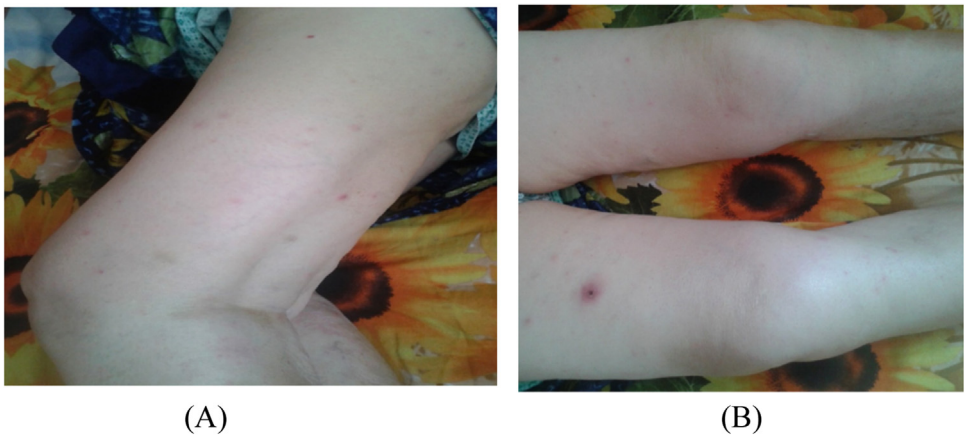

Fig. 1. Skin manifestations in a patient diagnosed with Mediterranean spotted fever in this study. (A) maculopapular rash on lower extremities; (B) eschar on the inner surface of the right thigh.

2. Case presentation

A 78-year-old woman was admitted on July 24 of 2019, to the Saki Regional Hospital with a history of 8-day illness manifesting with high fever, headache, skin rash (maculopapular rash on the skin, including the palms and soles of the feet), myalgia on the lower extremities, generalized weakness, and reduced appetite. The patient has a dog and lives in a private residence in Saki district surrounded by undeveloped land with abundant vegetation characteristic of the steppe zone. Patient complained about an ulcer-like skin lesion on the inner surface of the right thigh, the site of a tick attachment which she removed on July 14. Three days later (July 17) she developed a fever (38 °C) and self-medicated with paracetamol (acetaminophen), a skin rash appeared on day 5 of fever (July 21), and 3 days later the patient sought medical care.

The generalized maculopapular rash of the skin, including the palms and soles of the feet observed on admission to the hospital is shown in Fig. 1A, and the eschar of 6–7 mm diameter on the inner surface of the right thigh in Fig. 1B. Physical examination revealed an asthenic febrile woman in moderate to severe condition, with no meningeal or gastrointestinal symptoms. Liver and spleen were of normal size, and no lymphadenopathy was noted. Respiration rate was 20 per minute with heavy breathing, pulse of 100 per minute, and blood pressure 100/60 mmHg. EKG revealed sinus tachycardia, first-degree atrioventricular block and left ventricular hypertrophy.

Clinical blood tests determined slight leukocytosis (9.5×10^9 per L), increased level of stab neutrophils (26%, norm 1%–6%) and decreased hemoglobin level – 104 g/L (120–140 g/L), as well as increased creatinine (163 μM/L) and cholesterol (6.7 mM/L). AST, ALT and bilirubin levels were within their normal range.

Tick-borne rickettsiosis was suspected, and oral doxycycline was prescribed (100 mg twice a day for 7 days). Patient also received 2 g ceftriaxone (cephalosporin), 10 mg asparkam, 8 mg dexamethasone (cortisol) intravenously, and 3 mL diclofenac intramuscular. Patient's condition first improved after 3 days of treatment (July 26) with rash bleached significantly, although she complained about knee pain. She returned to her normal level of physical activity in another 24 hours and was discharged from the hospital the next day for ambulatory treatment and observation during her convalescence period.

3. Laboratory investigation and results

On day 2 of treatment (July 25) a scab was removed from the eschar and the underlying area was sampled using a dry sterile cotton swab by gentle rotation in the wound area and transferred into a sterile container containing 200 μL of sterile PBS and stored at −20 °C. Two separate DNA extractions were performed each using 100 μL of swab material using "Real Best Extraction 100" reagent kit according to the manufacturer's instructions ("Vector-Best", Novosibirsk), purified DNA was eluted using 100 μL of the elution buffer as described previously [11,12]. Each DNA was first tested in triplicates for *Rickettsia glt*A fragment using "Real Best DNA Rickettsia species" reagent kit ("Vector-Best") and corresponding positive and negative controls included with the kit. Amplification and sequencing larger portions of *Rickettsia glt*A, *omp*A, *omp*B and *sca*4 was done according to the previously described protocols [11,12]. Primers used are listed in Supplementary Table S1. Consensus sequences were assembled using Sequencer and analyzed using NCBI BLASTn. Four gene fragments were concatenated, and phylogenetic analysis was performed using MEGA X [13]. The nucleotide sequences of the fragments amplified from the patient sample were deposited in the NCBI database under the following accession numbers: OQ184719–OQ184722.

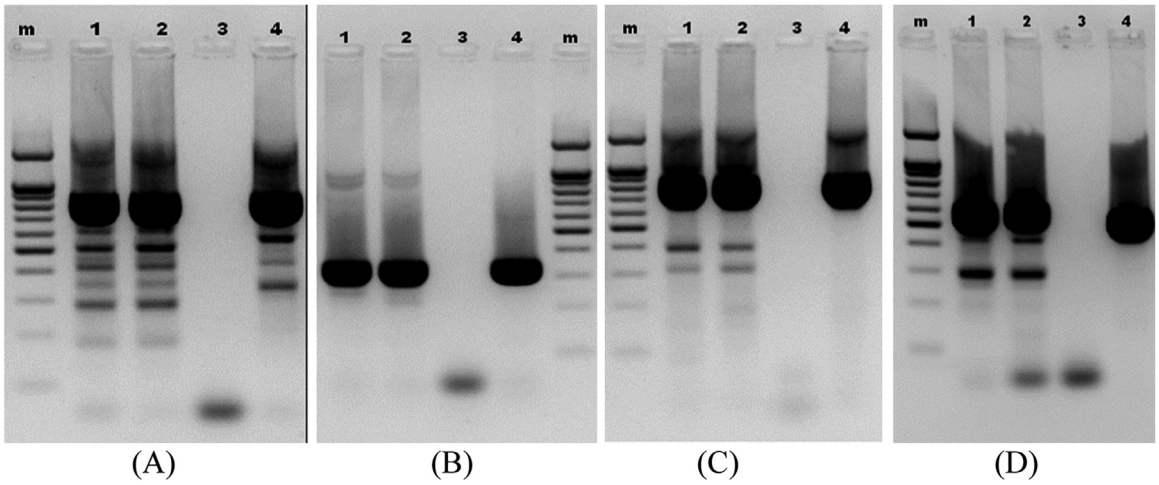

Fig. 2. Amplification of 4 *Rickettsia* gene fragments used for identification of the etiological agents of clinical case studied. (A) Amplification of a 837-bp fragment of the *glt*A using primers RS-F8 and RS-R1; (B) Amplification of a 316-bp fragment of the *omp*A using primers RSp-F14 and RSp-R2; (C) Amplification of a 813-bp fragment of the *omp*B using primers PKO-ompB-F1 and PKO-ompB-R2; (D) Amplification of a 576-bp fragment of the *sca*4 using primers R.Sca4-F3 and R.Sca4-R1. On each panel, lines 1 and 2 correspond to 2 replicate DNA extracted from the patient swab specimen, line 3 – sterile distilled water (no template control), line 4 – *R. sibirica* positive control; line m – DNA ladder.

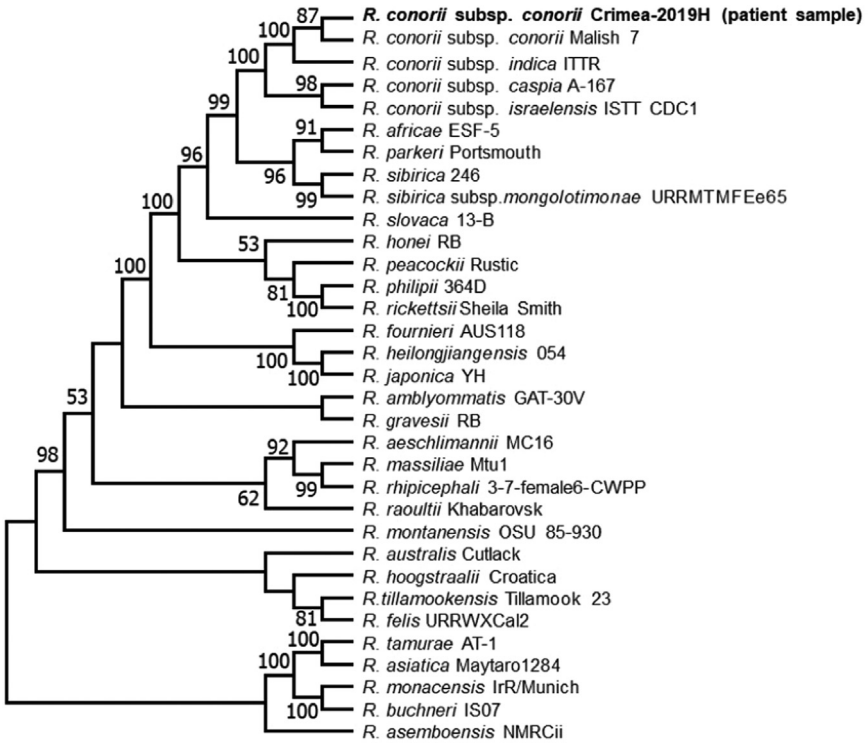

Fig. 3. Relationship of *Rickettsia* species identified in patient sample from Crimea using the Maximum Parsimony method. Analysis of *omp*A-*glt*A-*omp*B-*sca*4 concatenated fragments was conducted in MEGA X [13]. Homologous sequences of the type strains of the *Rickettsia* species with standing in nomenclature were retrieved from their whole genome sequences available in the NCBI GenBank. The most parsimonious tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. This analysis involved 33 nucleotide sequences. There were a total of 2376 positions in the final dataset.

Patient DNA tested positive by *glt*A qPCR with consistent Ct = 27 cycles. Further PCR testing for larger fragments of *Rickettsia glt*A, *omp*A, *omp*B and *sca*4 resulted in corresponding amplicons of expected size (Fig. 2). BLAST analysis of 4 sequenced gene fragments determined the highest genetic similarity to homologous gene fragments of *R. conorii* subspecies *conorii* Malish 7 including 100% similarity between their *sca*4 and *omp*A fragments, and 1 single nucleotide polymorphism (SNP) for each *glt*A and *omp*B fragments. Furthermore, nucleotide sequences of the *omp*A and *sca*4 from patient's sample had 100% sequence similarity to homologous sequences of *R. conorii* Crimea-2016-1 and *R. conorii* Crimea-2016-2 which were previously identified in *R. sanguineus* from Crimea but

had 1 SNP difference in its *glt*A sequence [11]. Phylogenetic analysis of *omp*A-*glt*A-*omp*B-*sca*4 concatenated gene fragments further confirmed identification of the strain isolated from the patient as *R. conorii* subspecies *conorii* (Fig. 3).

4. Discussion

Several tick-borne spotted fever group rickettsiae have been identified in Crimea; however, MSF is the only rickettsiosis that has been diagnosed in the peninsula [11,12,14]. Previously, we tested *R. sanguineus* collected from different parts of Crimea and identified DNA of *R. conorii* subspecies *conorii* in ticks collected in different locales including Saki district [11,12]. In this study, we molecularly confirmed a case of SFGR disease in Crimea caused by *R. conorii* subspecies *conorii* and corroborated the utility of eschar swab material as a source of DNA for PCR-based diagnostics.

The strain causing clinical illness was characterized as *R. conorii* subspecies *conorii* Malish 7-type, the largest of the 3 genotypes of *R. conorii* subspecies *conorii* identified based on multigene sequence typing with coding genes [15]. Two other types are Moroccan and M1 types; these isolates have deletions in their corresponding *omp*A sequences [15,16]. Follow-up studies will be necessary to further analyze the genetic diversity of *R. conorii* isolates circulating in the Crimean Peninsula and other countries of the Black Sea basin using multispacer typing. This approach identified 27 different genotypes among 39 geographically diverse isolates of *R. conorii* and revealed that contemporary *R. conorii* isolates from the European foci of MSF are distinct from *R. conorii* subspecies *conorii* Malish 7 from South Africa [15]. Although such in depth analysis of *R. conorii* is not necessary for clinical practice, multispacer typing may be useful for identifying the source of a strain during outbreak investigations.

Whole blood, white blood cells, plasma and serum have been evaluated in numerous studies for their suitability for molecular diagnosis of rickettsioses; however, the rickettsial DNA load in human blood depends on *Rickettsia* species and severity of the illness [17,18]. Only a limited number of rickettsia cells are present in human blood during a short period of time, and this drops significantly in response to antibiotic therapy. Furthermore, it was also shown that rickettsia quantity in patients suffering from Rocky Mountain spotted fever has a circadian rhythm and the largest numbers are present during early morning hours [19]. Therefore, a skin biopsy of eschar or skin rash is considered more informative and reliable specimens for confirmatory diagnosis of rickettsioses by PCR, culture isolation or pathological evaluation [20,21]. Unfortunately, collecting these samples requires more invasive procedures which are not as acceptable for pediatric patients or for eschars found on the face or other sensitive areas of the skin [9,22,23].

Wang et al. reported their experience with using eschar swabs for molecular diagnosis of SFG rickettsioses due to *R. australis* and *R. africae* [23]. Subsequently, Bechah et al. [22] applied the same approach to confirm acute infection by other rickettsiae causing eschars - *R. conorii, R. slovaca* and *R. sibirica mongolotimae*, and demonstrated experimentally that rickettsia DNA can be detected in swabs starting on day 3 and until day 12–20 of infection. Furthermore, eschar scab is also a good source of rickettsial DNA and can be used to confirm clinical diagnosis of SFG rickettsiosis and determine its etiology [24]. It is expected that broader availability of PCR-based diagnostics and its use in regular clinical practice will improve routine diagnosis of spotted fever group rickettsioses and permit more effective treatment of these diseases in the region.

Fundings

No special funding was available for this study.

Author contributions

E.E.A.: concept and study design, sample collection and processing, data analysis, writing draft and editing final manuscript. M.T.G.: concept and study design, sample collection and processing, data analysis, project coordination and supervision, writing draft and editing final manuscript. E.I.B.: sample collection and processing, data analysis and interpretation, editing manuscript. I.A.D.: approval of the study, editing and approval of the final manuscript. I.I.O.: approval of the study, editing and approval of the final manuscript. M.E.E.: data analysis and interpretation, writing and editing final manuscript.

Acknowledgments

None.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data available statement

Data sharing not applicable to this article as no specific datasets were generated or analyzed during the current study. Nucleotide sequences generated as a part of this study were deposited at the NCBI GenBank.

Ethics statement

None.

Informed consent

Patient provided informed voluntary consent to medical intervention and processing of personal data.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imj.2023.09.004.

References

[1] A.Y. Alymov, Marseille fever, Soviet Med. 13 (1939) 30–33 (in Russian).
[2] M.F. Pakshin, Materials on the study of Marseille fever in the city of Sevastopol for 45 years (1947-1992), In: M.F. Pakshin, A.M. Nikitin (Eds.), The collection of materials of the scientific-practical conference dedicated to the 70th anniversary of the sanitary-epidemiological service of the city of Sevastopol, December 16, 1992, Sevastopol 1992, pp. 87-92. (in Russian)
[3] E.A. Verbenets, Modern epidemiological and clinical features of Marseilles fever at the present stage, optimization of epidemiological surveillance, Master's thesis, L. V. Gromashevsky Institute of Epidemiology and Infectious Diseases, Kiev, Ukraine, 2013.
[4] K.D. Maly, I.Y. Andrukhiv, N.N. Tovpinets, et al., Marseille fever, Tauride Med. Biol. Bullet. 3-4 (2000) 240–243 (in Russian).
[5] M.T. Gafarova, Marseille Fever (Epidemiology, Clinic, Diagnostics), Tarpan, Simferopol, 2004, p. 98. (in Russian).
[6] I.V. Nebogatkin, N.N. Tovpinets, The outbreak of the bloody tick *Rhipicephalus sanguineus* (Ixodidae) on the Kerch Peninsula, Vestnik. Zool. 4 (1997) 81 (in Russian).
[7] Anonymous. On the state of sanitary and epidemiological well-being of the population in the Republic of Crimea and the federal city of Sevastopol in 2019: state report. Moscow: Federal Service for Supervision of Consumer Rights Protection and Human Welfare, 2020. Available at: http://82.rospotrebnadzor.ru/s/82/files/documents/Gosdoklad/148365.pdf. Accessed June 30, 2023. (in Russian).
[8] E. Botelho-Nevers, C. Rovery, H. Richet, et al., Analysis of risk factors for malignant Mediterranean spotted fever indicates that fluoroquinolone treatment has a deleterious effect, J. Antimicrob. Chemother. 66 (8) (2011) 1821–1830, doi:10.1093/jac/dkr218.
[9] N. Mouffok, P. Parola, H. Lepidi, et al., Mediterranean spotted fever in Algeria: new trends, Int. J. Infect. Dis. 13 (2) (2009) 227–235, doi:10.1016/j.ijid.2008.06.035.
[10] C. Rovery, P. Brouqui, D. Raoult, Questions on Mediterranean spotted fever a century after its discovery, Emerg. Infect. Dis. 14 (9) (2008) 1360–1367, doi:10.3201/eid1409.071133.
[11] E.E. Alieva, E.I. Bondarenko, K.D. Maliy, et al., The role of *Rhipicephalus sanguineus* ticks parasitizing dogs in the spread of tick-borne rickettsial pathogens in the city of Sevastopol, New Microbes New Infect. 36 (2020) 100704, doi:10.1016/j.nmni.2020.100704.
[12] M.T. Gafarova, E.I. Bondarenko, K.D. Maliy, et al., Prevalence of causative agents of transmissive tick-borned ricketsious in the Crimean Peninsula, Klin. Lab. Diagn. 67 (3) (2022) 170–176 (in Russian), doi:10.51620/0869-2084-2022-67-3-170-176.
[13] S. Kumar, G. Stecher, M. Li, et al., MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (6) (2018) 1547–1549, doi:10.1093/molbev/msy096.
[14] M.Y. Kartashov, S.N. Tikhonov, T.P. Mikryukova, et al., Genotyping of rickettsial isolates circulating in the Crimean peninsula, Mol. Genet. Microbiol. Virol. 33 (2) (2018) 110–117 (in Russian), doi:10.3103/S0891416818020088.
[15] P.E. Fournier, Y. Zhu, H. Ogata, et al., Use of highly variable intergenic spacer sequences for multispacer typing of *Rickettsia conorii* strains, J. Clin. Microbiol. 42 (12) (2004) 5757–5766, doi:10.1128/JCM.42.12.5757-5766.2004.
[16] V. Roux, P.E. Fournier, D. Raoult, Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA, J. Clin. Microbiol. 34 (9) (1996) 2058–2065, doi:10.1128/jcm.34.9.2058-2065.1996.
[17] M. Leitner, S. Yitzhaki, S. Rzotkiewicz, et al., Polymerase chain reaction-based diagnosis of Mediterranean spotted fever in serum and tissue samples, Am. J. Trop. Med. Hyg. 67 (2) (2002) 166–169, doi:10.4269/ajtmh.2002.67.166.
[18] E.M. Mendes do Nascimento, S. Colombo, T.K. Nagasse-Sugahara, et al., Evaluation of PCR-based assay in human serum samples for diagnosis of fatal cases of spotted fever group rickettsiosis, Clin. Microbiol. Infect. 15 (suppl 2) (2009) 232–234, doi:10.1111/j.1469-0691.2008.02153.x.
[19] C. Kato, I. Chung, C. Paddock, Estimation of *Rickettsia rickettsii* copy number in the blood of patients with Rocky Mountain spotted fever suggests cyclic diurnal trends in bacteraemia, Clin. Microbiol. Infect. 22 (4) (2016) 394–396, doi:10.1016/j.cmi.2015.12.019.
[20] H.M. Biggs, C.B. Behravesh, K.K. Bradley, et al., Diagnosis and management of tick-borne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis - United States, MMWR Recomm. Rep. 65 (2) (2016) 1–44, doi:10.15585/mmwr.rr6502a1.
[21] P. Brouqui, F. Bacellar, G. Baranton, et al., Guidelines for the diagnosis of tick-borne bacterial diseases in Europe, Clin. Microbiol. Infect. 10 (12) (2004) 1108–1132, doi:10.1111/j.1469-0691.2004.01019.x.
[22] Y. Bechah, C. Socolovschi, D. Raoult, Identification of rickettsial infections by using cutaneous swab specimens and PCR, Emerg. Infect. Dis. 17 (1) (2011) 83–86, doi:10.3201/eid1701.100854.
[23] J.M. Wang, B.J. Hudson, M.R. Watts, et al., Diagnosis of Queensland tick typhus and African tick bite fever by PCR of lesion swabs, Emerg. Infect. Dis. 15 (6) (2009) 963–965, doi:10.3201/eid1506.080855.
[24] S.H. Johnston, C.A. Glaser, K. Padgett, et al., *Rickettsia* spp. 364D causing a cluster of eschar-associated illness, California, Pediatr. Infect. Dis. J. 32 (9) (2013) 1036–1039, doi:10.1097/INF.0b013e318296b24b.