

Neglected vector-borne bacterial diseases and arboviruses in the Mediterranean area

R. N. Charrel¹, J.-M. Berenger², M. Laroche², N. Ayhan¹, I. Bitam², P. Delaunay^{3,4} and P. Parola²

1) Unite des Virus Emergents, IRD 190, INSERM 1207, IHU Méditerranée Infection, Aix-Marseille Université, Marseille, France, 2) IRD, AP-HM, SSA, VITROME, IHU Méditerranée Infection, Aix-Marseille Université, Marseille, France, 3) Laboratory of Parasitology and Mycology, Centre Hospitalier Universitaire de Nice, Hôpital de l'Archet, Nice, France and 4) MIVEGEC, UMR IRD224-CNRS5290, Université de Montpellier, Montpellier, France

Abstract

Arthropod vectors can transmit pathogenic microorganisms from one vertebrate to another during their blood meal. Although some vector-borne diseases have been eradicated in the Mediterranean area, such as malaria and dengue, recent endemic microorganisms (Toscana virus, *Rickettsia* spp.) remain neglected even though they cause many more cases. New diagnostic tools and innovative tools for the identification and characterization of vector species and microorganisms have been developed at IHU Méditerranée Infection, either internally or through collaborative and integrated projects. We have detected *Rickettsia slovacica* as a human pathogen and have described the disease; we have shown that *Rickettsia felis* can be transmitted by *Anopheles* mosquitoes; we have emphasized the increasing importance of bedbug (*Cimex lectularius*) as a potential vector of *Bartonella quintana*; and we have described the Toscana virus, a major agent of meningitis and meningoencephalitis which was disseminated in North Africa and Central and Eastern Europe, where it frequently cocirculates with a large number of newly described phleboviruses transmitted by sand flies.

© 2018 The Author(s). Published by Elsevier Ltd.

Keywords: Arbovirus, arthropods, fleas, lice, phlebovirus, sand fly, ticks, Toscana virus, virus

Original Submission: 20 March 2018; **Revised Submission:** 19 August 2018; **Accepted:** 21 August 2018

Article published online: 30 August 2018

Corresponding author: R. Charrel, Unite des Virus Emergents, IRD 190, INSERM 1207, IHU Méditerranée Infection, 19-21 blvd Jean Moulin, 13005 Marseille, France

E-mail: remi.charrel@univ-amu.fr

Introduction

Some arthropods can actively transmit pathogenic microorganisms (virus, bacteria, parasites) from one vertebrate to another during their blood meal; such arthropods are known as vectors. Mosquitoes and ticks are the main vectors of human infectious diseases. In the Mediterranean area, major mosquito-borne diseases such as malaria and dengue have been considered to be eradicated for decades. However, Lyme disease, the well-known human tick-borne disease, has not been described in the Mediterranean area because the major vector,

Ixodes ricinus, is not prevalent in places with a Mediterranean climate, even if the exact limits of the geographic distribution of the tick vector and the disease are not precisely known [1]. Among vector-borne viral diseases, the scientific and medical literature emphasize epidemic diseases. Despite this, these diseases usually have a lesser long-term medical impact on exposed populations than endemic diseases. As a consequence, endemic diseases are commonly neglected compared to the epidemic ones, which benefit from public interest and media attention. Newspaper focus has been recently directed towards chikungunya virus, Zika virus and dengue virus, even in geographic areas where these viruses have played a negligible role in public health in Europe [2,3]. In contrast, Crimean-Congo hemorrhagic fever, Rift Valley fever and Toscana virus (TOSV) infections have not been popularized. For instance, TOSV, which is the most prevalent arthropod-borne virus in Europe and in the Mediterranean area, is totally unknown—not only by the public but also by physicians [4].

West Nile virus (WNV) holds a peculiar situation. Undoubtedly, WNV is an emerging virus in the New World, which it stunningly entered in the fall of 1999 before spreading over North America [5]. In the Old World, it is endemic in Africa. The first noticeable outbreak in Europe was described in 1996 in Romania. Since then, WNV circulation has clearly increased in Europe over the last two decades with, special emphasis on Italy and Greece; however, other western European countries, such as Portugal, Spain and France, have not reported the same trend [6].

At the University Hospital Institute Méditerranée Infection in Marseille, France, vector-borne diseases represent a major axis of research and surveillance [7]. We have been involved in the surveillance of imported cases of major tropical vector-borne diseases (e.g. malaria) and arboviral infections (e.g. dengue, chikungunya and Zika), and more specifically in the surveillance of autochthonous transmission and even local outbreaks after importation [8]. This has been illustrated by several sentinel reports of autochthonous malaria cases and a local chikungunya outbreak [9,10]. However, our main contribution in the field of vector-borne diseases in the Mediterranean area concerns neglected vector-borne bacterial diseases and phleboviruses.

As a reference centre for rickettsiology, we have been involved in major contributions to the knowledge on rickettsioses in the Mediterranean area through clinical, microbiologic and entomologic studies [11]. Indeed, although tick-borne rickettsioses, caused by obligate intracellular bacteria belonging to the spotted fever group (SFG) of the genus *Rickettsia*, are among the oldest known vector-borne diseases, the scope and importance of the recognized tick-associated rickettsial pathogens have dramatically increased, making this complex of diseases a paradigm of emerging diseases in the Mediterranean area. Until 1990, only Mediterranean spotted fever, caused by *Rickettsia conorii conorii*, had been described in this area. In the past decades, we added to the knowledge of clinical, epidemiologic and entomologic aspects of Mediterranean spotted fever in France and Algeria, including atypical and serious life-threatening presentations or the explanation of the peak of Mediterranean spotted fever during warmer months related to a warming-mediated increase in the aggressiveness of *Rhipicephalus sanguineus* ticks to bite humans [12–14].

With the use of arthropods as specimen to study the repertoire of tick-associated rickettsia and the development and expansion of molecular tools such as PCR and sequencing on arthropods, we identified several new pathogens in ticks collected from North Africa, and we therefore complemented the knowledge already collected on arthropod-borne pathogens circulating in the Mediterranean area, particularly in France, Italy and North Africa. We detected *Rickettsia slovaca*, the causative agent of tick-borne lymphadenopathy, also known as SENLAT syndrome (scalp eschar and neck lymphadenopathy

after a tick bite), and contributed to the description of the clinical presentation of the disease. We also detected in ticks and/or patients several other emerging agents of SFG rickettsioses, including *R. aeschlimanni*, *R. massiliae*, *R. monacensis*, *R. helvetica*, *R. sibirica mongolotimonae*, *R. conorii israelensis*, *R. conorii caspia*, *R. conorii indica* and many other SFG rickettsia of unknown pathogenicity [15]. New approaches in the diagnosis have also been reported, such as swabbing of eschars to obtain material to be tested by PCR [11,16] or the use of molecular tools and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as a new tool to identify the tick that had bitten the patient as well as potential pathogens in ticks [17,18]. In addition to tick-borne rickettsia and rickettsioses studies, we detected one of the causative agents of Lyme disease, *Borrelia garinii*, in *Ixodes ricinus* ticks collected in Algeria, where the epidemiology of the disease is not known [19].

When studying fleas, we expanded the knowledge of *R. felis*, an emerging agent of rickettsioses, and also detected *Rickettsia typhi*, the agent of murine typhus in several areas. Several cases of these infections were diagnosed [16,20]. Experimental models highlighted the role of fleas in the transmission of *Bartonella* spp., and interestingly we have also shown that *R. felis* might also be transmitted by mosquitoes [21]. Finally, we have long been involved in surveying and managing infections in the homeless population, particularly louse-borne diseases such as trench fever. We have continued to study other louse-associated bacteria [22]. We have investigated strategies to eradicate lice in the homeless through study of the resistance of lice to pyrethroids and clinical trials on infested clothes [23]. More recently, and in the context of increasingly recognized infestations of human houses by bedbugs (*Cimex lectularius*), we have begun to study the potential vectorial capacity of these arthropods. We have shown their potential to transmit *Bartonella quintana*, the agent of trench fever, which is known to be transmitted by body lice [24]. A relationship has been established between three research structures of southeastern France: IRD-Montpellier, IHU-Marseille and CHU-Nice. It allowed us to evaluate the presence of this insect at the national level [25], to evaluate its genetics [26] and to look for the presence of *Wolbachia* bacteria in its gut [27].

Toscana virus

Discovered in 1971 in sand flies (*Phlebotomus perniciosus*, *Phlebotomus perfiliewi*) collected in the Tuscany region of Central Italy, TOSV was an orphan virus for 14 years before it was first recognized as the causative agent of neuroinvasive infections in southwestern Europe [28,29]. Soon after the first case report—based evidence that TOSV was causing meningitis and encephalitis, several large studies were conducted in central

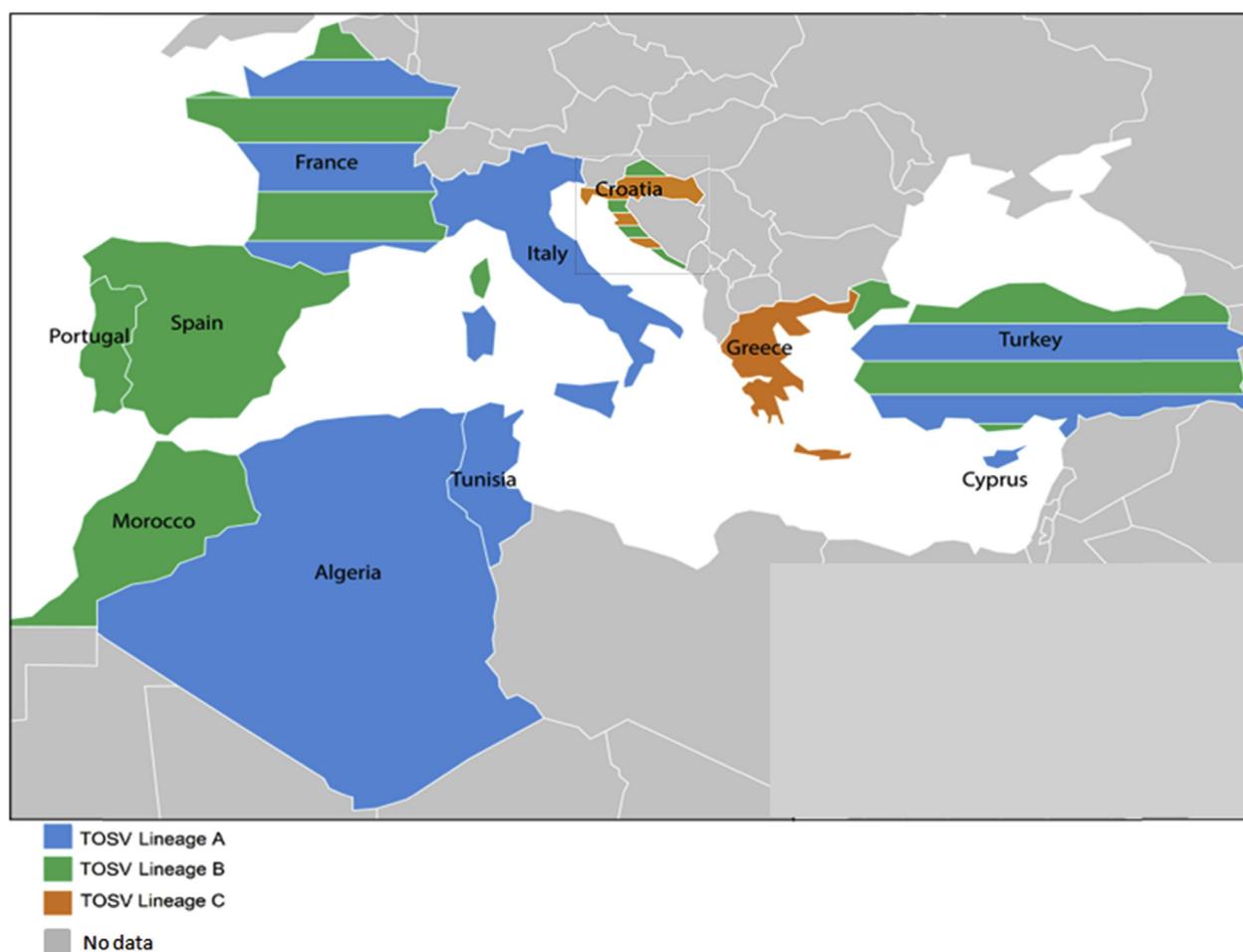


FIG. 1. Countries where Toscana virus is present.

Italy on adults and children with central nervous system infection [30–35].

Researchers and physicians in Marseille became interested in this virus in 2003, when two patients without a relevant travel history were diagnosed with TOSV infection. One patient presented with aseptic meningitis, the other with febrile illness only [36]. Investigations conducted in the Marseille region and in coastal southeastern France indicated that (1) TOSV strains could be detected and isolated in sand flies present in the region [37], (b) autochthonous human TOSV cases may be caused by strains belonging to two different lineages (A and B), demonstrating that both types of strains cocirculate in southeastern France [37] (Fig. 1) and (c) other sand fly-borne phleboviruses genetically and antigenically related to but clearly distinct from TOSV are transmitted by sand flies belonging to the same species, such as Massilia virus [38].

Studies conducted by our research group outside of France in collaboration with local researchers have demonstrated that TOSV is present in North Africa with undisputable evidence in

Algeria [39], Tunisia [40] and Morocco [41]; and that TOSV can be transmitted by sand flies belonging to species distinct from *P. perniciosus* and *P. perfiliewi*, such as *P. neglectus* in the Balkans [42], *P. sergenti* in Morocco [41] and *Sergentomyia minuta* in France [43] (Fig. 2).

We demonstrated that seroprevalence studies must be based on techniques with high specificity to provide undisputable rates of TOSV antibodies and to avoid overestimation due to techniques prone to cross-reactions, such as ELISA or immunofluorescence. From these studies, it is clear that exposure of human populations to TOSV is much higher in northern Africa (Algeria 30%, northern Tunisia 42%) than in Southern Europe, where rates are consistently lower, at 10% to 15% in high exposure areas, and commonly <5%. [39,40].

We also discovered several new viruses belonging to the *Phlebovirus* genus and transmitted by phlebotomines, such as Punique virus and Medjerda Valley virus in Tunisia [44,45], Massilia virus in France [38], Adana, Toros and Zerdali viruses in Turkey [46,47] and Dashli virus in Iran [48].

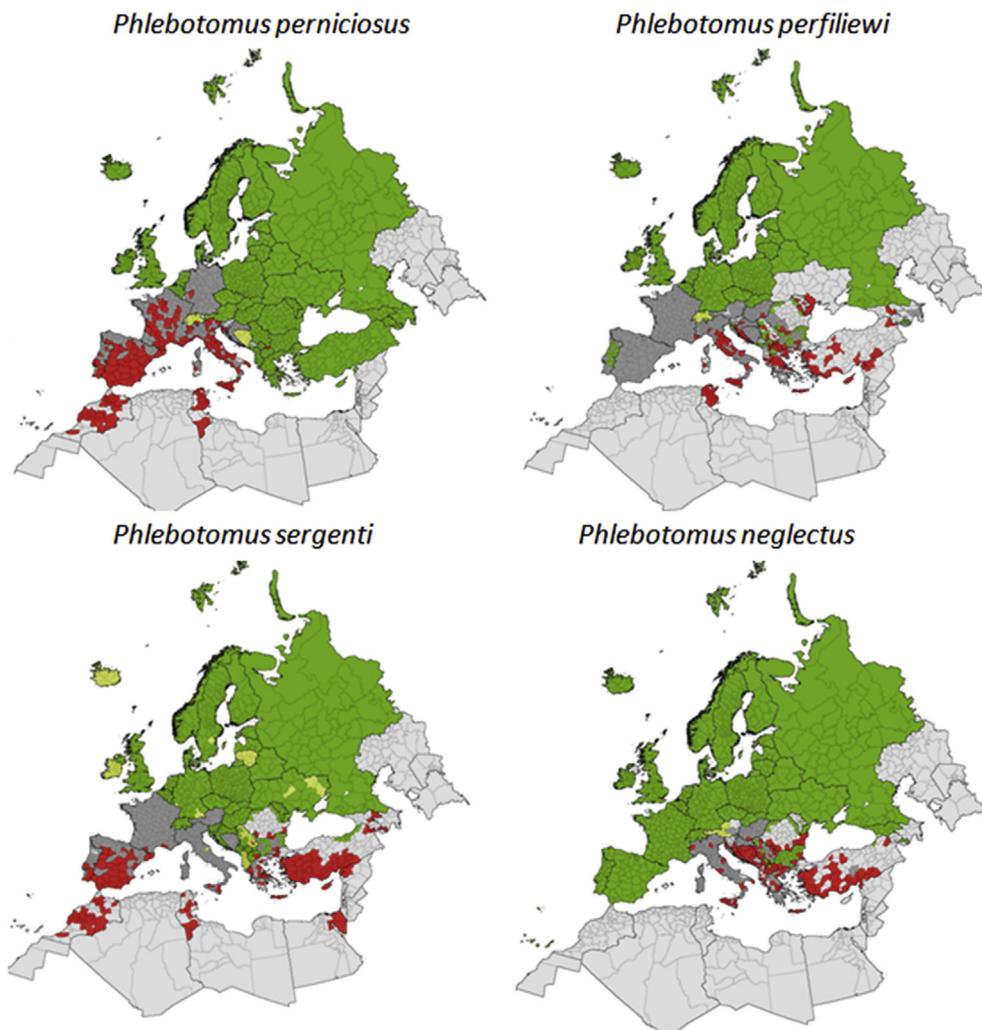


FIG. 2. Current known distribution of sand fly species transmitting Toscana virus.

Together with veterinarians, we have demonstrated that the sand fly fever Sicilian virus circulates at a high rate in various regions of the Mediterranean Basin, such as Greece and Cyprus [49] and Portugal [50].

Molecular diagnostic tools for TOSV

Because one of the major options to improve the awareness about TOSV is to disseminate molecular diagnosis to clinical microbiology laboratories, we developed a real-time reverse transcriptase PCR assay [51]. At the beginning of the 21st century, TOSV was the most prominent cause of aseptic meningitis during summertime in central Italy—far ahead of enteroviruses and other causes such as WNV, herpes simplex viruses 1/2, varicella zoster virus and lymphocytic choriomeningitis virus. At this time there were no data to assess the

medical impact of TOSV in other areas such as central south Europe (Croatia, Greece), Eastern Europe (Turkey) or North Africa (Morocco, Algeria, Tunisia). Evidence that TOSV is also circulating in those countries at rates that are at least equivalent (although frequently much higher) were provided after 2006 [39,41,42,52–57] (Fig. 1). Clinical data indicate that TOSV can cause meningitis, meningoencephalitis or encephalitis. Clinically, there is no marker to distinguish TOSV from other causes. Laboratory-based diagnosis using direct or indirect methods are therefore the only possibility to identify TOSV. Interestingly, encephalitis is frequently seen in central nervous system infections caused by TOSV, although the outcome is favourable [58–61]. Detection of TOSV RNA using real-time molecular techniques is now the reference standard.

In the Mediterranean area, an estimated 250 million people are exposed to TOSV, with seroprevalence rates higher than 40% in certain regions (Fig. 1). In France, >8 million people are at

risk of TOSV infection. TOSV is the most prevalent arthropod-borne virus in Europe. However, there are only 295 references using the keyword 'Toscana virus' in the National Center for Biotechnology Information PubMed database for a virus that was discovered almost 50 years ago. In comparison, for the same period of time (1971–2018), West Nile, Zika and chikungunya viruses have about 6500, 3360 and 3000 references, respectively. This is undisputable evidence that TOSV remains a neglected pathogen. Researchers in Marseille, working in research groups that have joined the IHU Méditerranée Infection, have produced 66 articles in the field; they now hold the leading position according to the Web of Science for studies related to viruses transmitted by sand flies in the Mediterranean Basin.

Conflict of interest

None declared.

References

- [1] Chomel B. Lyme disease. *Rev Sci Tech* 2015;34:569–76.
- [2] Paixão ES, Teixeira MG, Rodrigues LC. Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. *BMJ Glob Health* 2018;3(Suppl. 1), e000530.
- [3] Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. *N Engl J Med* 2015;372:1231–9.
- [4] Charrel RN, Gallian P, Navarro-Mari JM, Nicoletti L, Papa A, Sánchez-Seco MP, et al. Emergence of Toscana virus in Europe. *Emerg Infect Dis* 2005;11:1657–63.
- [5] David S, Abraham AM. Epidemiological and clinical aspects on West Nile virus, a globally emerging pathogen. *Infect Dis (Lond)* 2016;48:571–86.
- [6] Rizzoli A, Jimenez-Clavero MA, Barzon L, Cordioli P, Figuerola J, Koraka P, et al. The challenge of West Nile virus in Europe: knowledge gaps and research priorities. *Euro Surveill* 2015;20:21135.
- [7] Laroche M, Bérenger JM, Delaunay P, Charrel R, Pradines B, Berger F, et al. Medical entomology: a reemerging field of research to better understand vector-borne infectious diseases. *Clin Infect Dis* 2017;65(Suppl. 1):S30–8.
- [8] Vega-Rúa A, Lourenço-de-Oliveira R, Mousson L, Vazeille M, Fuchs S, Yébakima A, et al. Chikungunya virus transmission potential by local *Aedes* mosquitoes in the Americas and Europe. *PLoS Negl Trop Dis* 2015;9, e0003780.
- [9] Calba C, Guerbois-Galla M, Franke F, Jeannin C, Auzet-Caillaud M, Grand G, et al. Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017. *Euro Surveill* 2017;22(39).
- [10] Florescu SA, Popescu CP, Calistru P, Ceausu E, Nica M, Toderan A, et al. *Plasmodium vivax* malaria in a Romanian traveller returning from Greece, August 2011. *Euro Surveill* 2011;16:19954.
- [11] Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev* 2013;26:657–702.
- [12] Parola P, Socolovschi C, Jeanjean L, Bitam I, Fournier PE, Sotto A, et al. Warmer weather linked to tick attack and emergence of severe rickettsioses. *PLoS Negl Trop Dis* 2008;2:e338.
- [13] Mouffok N, Parola P, Lepidi H, Raoult D. Mediterranean spotted fever in Algeria—new trends. *Int J Infect Dis* 2009;13:227–35.
- [14] Socolovschi C, Gaudart J, Bitam I, Huynh TP, Raoult D, Parola P. Why are there so few *Rickettsia conorii conorii*-infected *Rhipicephalus sanguineus* ticks in the wild? *PLoS Negl Trop Dis* 2012;6:e1697.
- [15] Kernif T, Socolovschi C, Bitam I, Raoult D, Parola P. Vector-borne rickettsioses in North Africa. *Infect Dis Clin North Am* 2012;26:455–78.
- [16] Delord M, Socolovschi C, Parola P. Rickettsioses and Q fever in travelers (2004–2013). *Travel Med Infect Dis* 2014;12:443–58.
- [17] Yssouf A, Almeras L, Raoult D, Parola P. Emerging tools for identification of arthropod vectors. *Future Microbiol* 2016;11:549–66.
- [18] Aubry C, Socolovschi C, Raoult D, Parola P. Bacterial agents in 248 ticks removed from people from 2002 to 2013. *Ticks Tick Borne Dis* 2016;7:475–81.
- [19] Benredjem W, Leulmi H, Bitam I, Raoult D, Parola P. *Borrelia garinii* and *Rickettsia monacensis* in *Ixodes ricinus* ticks, Algeria. *Emerg Infect Dis* 2014;20:1776–7.
- [20] Angelakis E, Mediannikov O, Parola P, Raoult D. *Rickettsia felis*: the complex journey of an emergent human pathogen. *Trends Parasitol* 2016;32:554–64.
- [21] Dieme C, Bechah Y, Socolovschi C, Audoly G, Berenger JM, Faye O, et al. Transmission potential of *Rickettsia felis* infection by *Anopheles gambiae* mosquitoes. *Proc Natl Acad Sci USA* 2015;112:8088–93.
- [22] Mana N, Louni M, Parola P, Bitam I. Human head lice and pubic lice reveal the presence of several *Acinetobacter* species in Algiers, Algeria. *Comp Immunol Microbiol Infect Dis* 2017;53:33–9.
- [23] Benkouiten S, Drali R, Badiaga S, Veracx A, Giorgi R, Raoult D, et al. Effect of permethrin-impregnated underwear on body lice in sheltered homeless persons: a randomized controlled trial. *JAMA Dermatol* 2014;150:273–9.
- [24] Leulmi H, Bitam I, Berenger JM, Lepidi H, Rolain JM, Almeras L, et al. Competence of *Cimex lectularius* bed bugs for the transmission of *Bartonella quintana*, the agent of trench fever. *PLoS Negl Trop Dis* 2015;9, e0003789.
- [25] Jourdain F, Delaunay P, Bérenger JM, Perrin Y, Robert V. The common bed bug (*Cimex lectularius*) in metropolitan France. Survey on the attitudes and practices of private- and public-sector professionals. *Parasite* 2016;23:38.
- [26] Akhoundi M, Kengne P, Cannet A, Brengues C, Berenger JM, Izri A, et al. Spatial genetic structure and restricted gene flow in bed bugs (*Cimex lectularius*) populations in France. *Infect Genet Evol* 2015;34:236–43.
- [27] Akhoundi M, Cannet A, Loubatier C, Berenger JM, Izri A, Marty P, et al. Molecular characterization of *Wolbachia* infection in bed bugs (*Cimex lectularius*) collected from several localities in France. *Parasite* 2016;23:31.
- [28] Ehrnst A, Peters CJ, Niklasson B, Svedmyr A, Holmgren B. Neurovirulent Toscana virus (a sandfly fever virus) in Swedish man after visit to Portugal. *Lancet* 1985;1(8439):1212–3.
- [29] Calisher CH, Weinberg AN, Muth DJ, Lazuick JS. Toscana virus infection in United States citizen returning from Italy. *Lancet* 1987;1(8525):165–6.
- [30] Braito A, Ciufolini MG, Pippi L, Corbisiero R, Fiorentini C, Gistri A, et al. Phlebotomus-transmitted Toscana virus infections of the central nervous system: a seven-year experience in Tuscany. *Scand J Infect Dis* 1998;30:505–8.
- [31] Braito A, Corbisiero R, Corradini S, Fiorentini C, Ciufolini MG. Toscana virus infections of the central nervous system in children: a report of 14 cases. *J Pediatr* 1998;132:144–8.
- [32] Braito A, Corbisiero R, Corradini S, Marchi B, Sancasciani N, Fiorentini C, et al. Evidence of Toscana virus infections without central nervous system involvement: a serological study. *Eur J Epidemiol* 1997;13:761–4.

- [33] Nicoletti L, Verani P, Caciolli S, Ciufolini MG, Renzi A, Bartolozzi D, et al. Central nervous system involvement during infection by phlebovirus Toscana of residents in natural foci in central Italy (1977–1988). *Am J Trop Med Hyg* 1991;45:429–34.
- [34] Valassina M, Meacci F, Valensin PE, Cusi MG. Detection of neurotropic viruses circulating in Tuscany: the incisive role of Toscana virus. *J Med Virol* 2000;60:86–90.
- [35] Valassina M, Cuppone AM, Bianchi S, Santini L, Cusi MG. Evidence of Toscana virus variants circulating in Tuscany, Italy, during the summers of 1995 to 1997. *J Clin Microbiol* 1998;36:2103–4.
- [36] Hemmersbach-Miller M, Parola P, Charrel RN, Paul Durand J, Brouqui P. Sandfly fever due to Toscana virus: an emerging infection in southern France. *Eur J Intern Med* 2004;15:316–7.
- [37] Charrel RN, Izri A, Temmam S, Delaunay P, Toga I, Dumon H, et al. Cocirculation of 2 genotypes of Toscana virus, southeastern France. *Emerg Infect Dis* 2007;13:465–8.
- [38] Charrel RN, Moureau G, Temmam S, Izri A, Marty P, Parola P, et al. Massilia virus, a novel *Phlebovirus* (*Bunyaviridae*) isolated from sandflies in the Mediterranean. *Vector Borne Zoonotic Dis* 2009;9:519–30.
- [39] Alkan C, Allal-Ikhlef AB, Alwassouf S, Baklouti A, Piorkowski G, de Lamballerie X, et al. Virus isolation, genetic characterization and seroprevalence of Toscana virus in Algeria. *Clin Microbiol Infect* 2015;21:1040. e1–9.
- [40] Sakhria S, Bichaud L, Mensi M, Salez N, Dachraoui K, Thirion L, et al. Co-circulation of Toscana virus and Punique virus in northern Tunisia: a microneutralisation-based seroprevalence study. *PLoS Negl Trop Dis* 2013;7:e2429.
- [41] Es-Sette N, Ajaoud M, Bichaud L, Hamdi S, Mellouki F, Charrel RN, et al. *Phlebotomus sergenti*, a common vector of *Leishmania tropica* and Toscana virus in Morocco. *J Vector Borne Dis* 2014;51:86–90.
- [42] Ayhan N, Alten B, Ivovic V, Martinkovic F, Kasap OE, Ozbel Y, et al. Cocirculation of two lineages of Toscana virus in Croatia. *Front Public Health* 2017;5:336.
- [43] Charrel RN, Izri A, Temmam S, de Lamballerie X, Parola P. Toscana virus RNA in *Sergentomyia minuta* flies. *Emerg Infect Dis* 2006;12:1299–300.
- [44] Zhioua E, Moureau G, Chelbi I, Ninove L, Bichaud L, Derbali M, et al. Punique virus, a novel phlebovirus, related to sandfly fever Naples virus, isolated from sandflies collected in Tunisia. *J Gen Virol* 2010;91(Pt 5):1275–83.
- [45] Bichaud L, Dachraoui K, Alwassouf S, Alkan C, Mensi M, Piorkowski G, et al. Isolation, full genomic characterization and neutralization-based human seroprevalence of Medjerda Valley virus, a novel sandfly-borne phlebovirus belonging to the Salehabad virus complex in northern Tunisia. *J Gen Virol* 2016;97:602–10.
- [46] Alkan C, Erisoz Kasap O, Alten B, de Lamballerie X, Charrel RN. Sandfly-borne phlebovirus isolations from Turkey: new insight into the sandfly fever Sicilian and sandfly fever Naples species. *PLoS Negl Trop Dis* 2016;10, e0004519.
- [47] Alkan C, Alwassouf S, Piorkowski G, Bichaud L, Tezcan S, Dincer E, et al. Isolation, genetic characterization, and seroprevalence of Adana virus, a novel phlebovirus belonging to the Salehabad virus complex, in Turkey. *J Virol* 2015;89:4080–91.
- [48] Alkan C, Moin Vaziri V, Ayhan N, Badakhshan M, Bichaud L, Rahbarian N, et al. Isolation and sequencing of Dashli virus, a novel Sicilian-like virus in sandflies from Iran; genetic and phylogenetic evidence for the creation of one novel species within the *Phlebovirus* genus in the *Phenuiviridae* family. *PLoS Negl Trop Dis* 2017;11, e0005978.
- [49] Alwassouf S, Christodoulou V, Bichaud L, Ntais P, Mazeris A, Antoniou M, et al. Seroprevalence of sandfly-borne phleboviruses belonging to three serocomplexes (sandfly fever Naples, sandfly fever Sicilian and Salehabad) in dogs from Greece and Cyprus using neutralization test. *PLoS Negl Trop Dis* 2016;10, e0005063.
- [50] Alwassouf S, Maia C, Ayhan N, Coimbra M, Cristovao JM, Richet H, et al. Neutralization-based seroprevalence of Toscana virus and sandfly fever Sicilian virus in dogs and cats from Portugal. *J Gen Virol* 2016;97:2816–23.
- [51] Brisbarre N, Plumet S, Cotteaux-Lautard C, Emonet SF, Pages F, Leparç-Goffart I. A rapid and specific real time RT-PCR assay for diagnosis of Toscana virus infection. *J Clin Virol* 2015;66:107–11.
- [52] Ergünay K, Saygan MB, Aydoğan S, Lo MM, Weidmann M, Dilcher M, et al. Sandfly fever virus activity in central/northern Anatolia, Turkey: first report of Toscana virus infections. *Clin Microbiol Infect* 2011;17:575–81.
- [53] Bahri O, Fazaa O, Ben Alaya-Bouafif N, Bouloy M, Triki H, Bouattour A. Role of Toscana virus in meningo-encephalitis in Tunisia. *Pathol Biol (Paris)* 2011;59:e125–7.
- [54] Santos L, Simões J, Costa R, Martins S, Lecour H. Toscana virus meningitis in Portugal, 2002–2005. *Euro Surveill* 2007;12:E3–4.
- [55] Punda-Polić V, Mohar B, Duh D, Bradarić N, Korva M, Fajs L, et al. Evidence of an autochthonous Toscana virus strain in Croatia. *J Clin Virol* 2012;55:4–7.
- [56] Es-Sette N, Nourilil J, Hamdi S, Mellouki F, Lemrani M. First detection of Toscana virus RNA from sand flies in the genus *Phlebotomus* (*Diptera: Phlebotomidae*) naturally infected in Morocco. *J Med Entomol* 2012;49:1507–9.
- [57] Papa A, Paraforou T, Papakonstantinou I, Pagdatoglou K, Kontana A, Koukoubani T. Severe encephalitis caused by Toscana virus, Greece. *Emerg Infect Dis* 2014;20:1417–9.
- [58] Dupouey J, Bichaud L, Ninove L, Zandotti C, Thirion-Perrier L, de Lamballerie X, et al. Toscana virus infections: a case series from France. *J Infect* 2014;68:290–5.
- [59] Jaijakul S, Arias CA, Hossain M, Arduino RC, Wootton SH, Hasbun R. Toscana meningoencephalitis: a comparison to other viral central nervous system infections. *J Clin Virol* 2012;55:204–8.
- [60] Pierro A, Ficarelli S, Ayhan N, Morini S, Raumer L, Bartoletti M, et al. Characterization of antibody response in neuroinvasive infection caused by Toscana virus. *Clin Microbiol Infect* 2017;23:868–73.
- [61] Vocale C, Bartoletti M, Rossini G, Macini P, Pascucci MG, Mori F, Tampieri A, et al. Toscana virus infections in northern Italy: laboratory and clinical evaluation. *Vector Borne Zoonotic Dis* 2012;12:526–9.