



Opinion

Broader Geographical Distribution of Toscana Virus in the Mediterranean Region Suggests the Existence of Larger Varieties of Sand Fly Vectors

Nazli Ayhan ^{1,2,*}, Jorian Prudhomme ^{3,*}, Lison Laroche ³, Anne-Laure Bañuls ^{3,†} and Remi N. Charrel ^{1,‡}

¹ Unité des Virus Emergents (Aix-Marseille Univ–IRD 190–Inserm 1207–IHU Méditerranée Infection), 13005 Marseille, France; remi.charrel@univ-amu.fr

² Unité de Virologie EA7310 Bioscope, Université de Corse Pasquale Paoli (UCPP), 20250 Corte, France

³ UMR MIVEGEC (IRD—CNRS—Université de Montpellier), 911 avenue Agropolis, F34394 Montpellier, France; lisonlaroché@hotmail.fr (L.L.); anne-laure.banuls@ird.fr (A.-L.B.)

* Correspondence: nazliayhann@gmail.com (N.A.); jorian.prudhomme@hotmail.fr (J.P.); Tel.: +33-782-202794 (N.A.); +33-621-504351 (J.P.)

† These authors contributed equally to the work.

‡ These authors contributed equally to the work.

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Abstract: Toscana virus (TOSV) is endemic in the Mediterranean basin, where it is transmitted by sand flies. TOSV can infect humans and cause febrile illness as well as neuroinvasive infections affecting the central and peripheral nervous systems. Although TOSV is a significant human pathogen, it remains neglected and there are consequently many gaps of knowledge. Recent seroepidemiology studies and case reports showed that TOSV's geographic distribution is much wider than was assumed a decade ago. The apparent extension of the TOSV circulation area raises the question of the sandfly species that are able to transmit the virus in natural conditions. *Phlebotomus (Ph.) perniciosus* and *Ph. perfiliewi* were historically identified as competent species. Recent results suggest that other species of sand flies could be competent for TOSV maintenance and transmission. Here we organize current knowledge in entomology, epidemiology, and virology supporting the possible existence of additional phlebotomine species such as *Ph. longicuspis*, *Ph. sergenti*, *Ph. tobbi*, *Ph. neglectus*, and *Sergentomyia minuta* in TOSV maintenance. We also highlight some of the knowledge gaps to be addressed in future studies.

Keywords: Toscana virus; sand fly; *Phlebotomus*; *Sergentomyia*; Mediterranean area; *Phenuiviridae*; Bunyavirales; Sandfly fever Naples phlebovirus; arbovirus; arthropod-borne; sandfly; phlebotomine

1. Introduction

The genus *Phlebovirus* includes 58 viruses classified into a ten-species complex: Bujaru, Candiru, Chilibre, Frijoles, Punta Toro, Rift Valley fever, Salehabad, Sandfly fever Naples, Severe fever with thrombocytopenia syndrome, and Uukuniemi phleboviruses. The Sandfly fever Naples phlebovirus species comprises thirteen viruses, including Toscana virus (TOSV) [1]. TOSV has a tropism for central and peripheral nervous systems and is responsible for meningitis and encephalitis in the Mediterranean region [2].

Phleboviruses are transmitted to humans by the bite of an infected female sand fly during the blood meal. There is still little information on the development cycle of phleboviruses in the vector [3].

During the last decade, the known geographical area where TOSV circulates has increased considerably. With recent entomological studies, seroepidemiological studies, and case reports, the known distribution of TOSV is now extended in Central and Eastern Europe, North Africa, and Turkey.

We suggest that the existence of a larger diversity of sand fly vectors may explain the TOSV circulation in different geographical regions, which is supported by the recent identification of the virus in phlebotomine species that were not previously considered as TOSV vectors.

Here we present a comprehensive analysis of recent data suggesting that additional species of sand flies are involved in natural cycles to explain the recently revealed broader geographical distribution of TOSV in the Mediterranean region.

2. Toscana Virus

2.1. Overview on Toscana Virus

TOSV is an enveloped, tri-segmented RNA arbovirus which belongs to the genus *Phlebovirus*, the family *Phenuiviridae*, and the order *Bunyavirales* [4]. At the family level, genomes are comprised of three unique molecules of negative or ambisense single-stranded RNA, designated L (large), M (medium), and S (small) to a total of 11 to 19 kb. Within each genus, viruses share similar segment and structural protein sizes, as well as characteristic terminal sequences at the 3' and 5' ends of each of the three segments. TOSV distribution and abundance are highly dependent on its arthropod vector. Human cases are observed during the warm season, with a peak during the hottest months in relation with sand fly activity. This virus shows a peculiar neurotropism and causes central nervous system (CNS) diseases in infected individuals [2,5]. Like other arboviruses, most TOSV infections in humans are asymptomatic [6]. So far, 864 symptomatic human cases (834 autochthonous and 30 imported) have been reported in residents of different Mediterranean countries and in tourists and travelers returning from endemic regions [7–10].

Genetic studies have identified the existence of three lineages of TOSV (A, B, and C) [11] (Figure 1). To date, there is no difference in virulence or clinical manifestations depending on the genetic lineage. At least two lineages co-circulate in France [12], Turkey [13], and Croatia [14]. Further studies are required in order to have more information about the genotype distribution and their association with vector distribution. The known geographical distribution of TOSV in the Mediterranean basin raises the question of whether the demonstrated vectors, *Phlebotomus (Ph.) perniciosus* and *Ph. perfiliewi*, can explain this distribution or whether other species could be implicated.

The knowledge of the vectors and geographical distribution of TOSV is increasing in parallel with the number of studies. The current TOSV distribution includes South European countries (Italy, Spain, France, and Portugal) [16–19], East Mediterranean countries (Turkey and Cyprus) [13,20], Balkan countries (Greece, Croatia, Bosnia and Herzegovina, Kosovo, and Bulgaria) [21], and North African countries (Tunisia, Morocco, and Algeria) [22]. The geographical distribution of TOSV in the Mediterranean basin raises the following question: Can the known vectors, *Ph. perniciosus* and *Ph. perfiliewi*, explain this distribution, or are other species implicated?

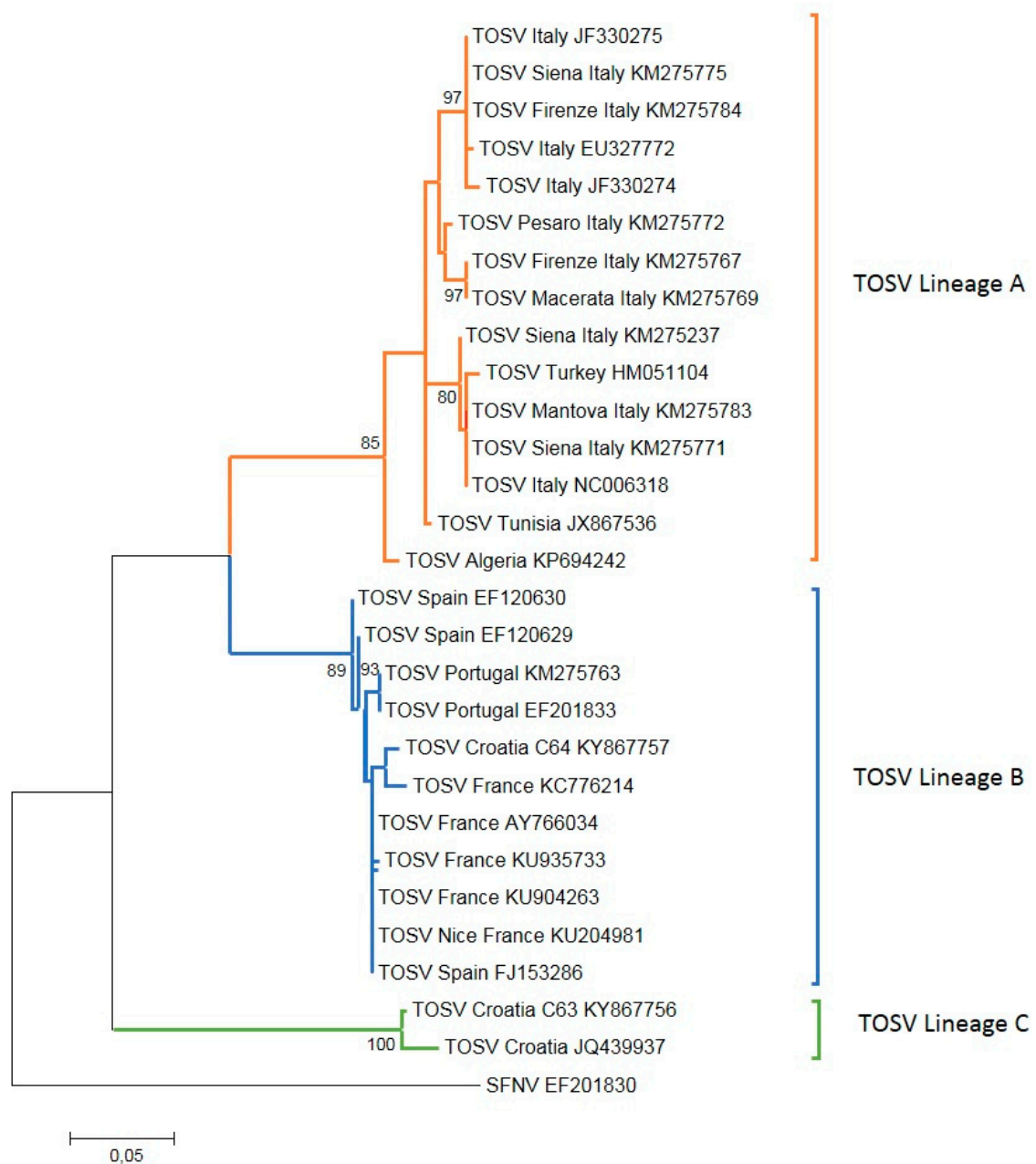


Figure 1. Phylogenetic analysis of Toscana virus (TOSV) based on the nucleocapsid protein gene. Maximum likelihood analysis at nucleotide level was performed using the MEGA 6.06 software program [15]. Bootstrap values are indicated and correspond to 1000 pseudoreplications.

2.2. Epidemiology of Toscana Virus

Seroprevalence studies demonstrate that human populations are exposed to TOSV in Italy, Spain, France, Portugal, Turkey, Malta, Cyprus, Greece, Croatia, Bosnia and Herzegovina, Kosovo, Bulgaria, Tunisia, and Algeria [7,22–36]. Together, the results of serological surveillance studies and case reports showed that the TOSV geographic distribution is much wider than believed a decade ago; in particular, the presence of TOSV has been recently documented in North Africa and the Balkan countries [31,32,34–36] (Figure 2).

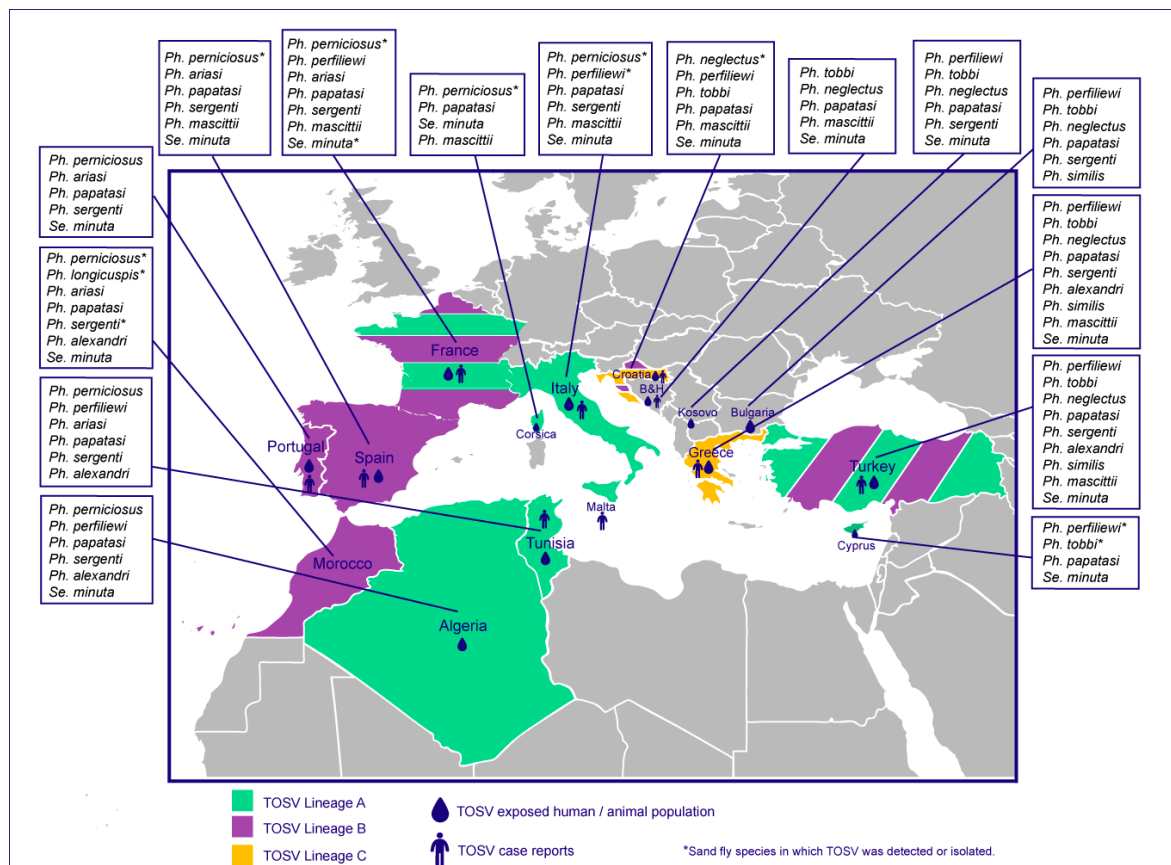


Figure 2. Geographical distribution of Toscana virus (TOSV) and reported distribution of predominant sand fly species in the countries where TOSV is present.

High rates of TOSV-neutralizing antibodies (17.2–59.4%) have been identified in the healthy population of Tunisia, with geographical variation [35,36]. Similar results were obtained in Algeria with a prevalence up to 50% in Draa El Mizan [22]. Although the studies are geographically limited within Tunisia and Algeria, the high rates of neutralizing antibodies emphasize the massive circulation of TOSV in these countries (Figure 2).

In Kosovo, 5.5% of blood donors possess IgG against TOSV [32]. Higher rates were reported in healthy residents of Croatia, with the highest values in coastal areas and Croatian islands [31]. Another study identified the presence of TOSV antibodies in patients from Bosnia and Herzegovina [33]. Recently, anti-TOSV IgGs were found in 24.4% of healthy residents of Bulgaria [34] (Figure 2).

2.3. Pathways of TOSV Transmission and Maintenance

Transovarial and venereal pathways of TOSV transmission have been demonstrated experimentally with *Ph. perniciosus* colonies [37–39]. These experimental results were complemented by the observation in nature of equal rates of TOSV infection in male and female sand flies, suggesting the existence of transovarial (vertical) and/or venereal (horizontal) transmission during mating [40–43]. Additionally, the survival of TOSV in overwintering *Ph. perniciosus* larvae underlined the capability of TOSV for maintenance during diapause [37]. Co-feeding transmission of TOSV during sugar meal was also suggested experimentally in males and females since it was demonstrated in *Ph. perniciosus* with Massilia virus, a relative of TOSV also transmitted by *Ph. perniciosus* in nature [44]. Future studies need to be conducted with TOSV and other species belonging to the *Phlebotomus* genus in order to confirm that viral transmission is possible with other species.

3. Sand Flies

3.1. Overview on Sand Flies

Sand flies are tiny blood-feeding (hematophagous) insects belonging to the order Diptera, family Psychodidae, and subfamily Phlebotominae. Approximately 900 sand fly species are described, of which 70 have been identified as potential vectors of *Leishmania* [45] and a few species were associated with *Phlebovirus* and other viruses [46]. Each species can be identified by the characteristic shape of their cibarium, pharynx, and reproductive organs [47].

Both genders feed on honeydew, plant sap, and aphid secretions [48], but only females take a blood meal, necessary for egg maturation. Sand flies present a crepuscular and nocturnal activity [49]. They are very abundant in warm regions (e.g., Mediterranean basin, Asia, Africa, South America), but their range is very wide (between 50° N and 40° S). They occur on all continents but have not been reported in New Zealand or the Pacific Islands. Their altitudinal distribution ranges from sea level to 3500 m above sea level (Afghanistan: *Ph. rupester*) [50–52]. Modeling studies suggest an expansion of the range of different *Phlebotomus* species in Europe due to the influence of environmental and climate change (*Ph. ariasi*, *Ph. mascittii*, *Ph. perniciosus*, *Ph. neglectus*, and *Ph. perfiliewi*) allowing the colonization of new habitats [53,54].

However, this insect remains relatively unknown from a biological and ecological point of view (unknown breeding sites, poorly known food preferences, etc.).

3.2. Sandflies as TOSV Vectors

Sandfly activity is seasonal in the Mediterranean countries. They are present from May to October due to low temperatures in winter. The average annual temperatures and the latitude influence the emergence periods and the seasonal dynamics of these insects (with a peak in July and August) [49]. Their activity and abundance are dependent on environmental and climatic conditions and may vary depending on the species and the location of capture.

The first isolation of TOSV was obtained from *Ph. perniciosus* in central Italy, in 1971 [55]. Subsequently, other strains were isolated from *Ph. perfiliewi* [56]. The isolation or detection of TOSV in sand flies started to be reported not only in Italy but also in other Mediterranean countries. *Ph. perniciosus* was identified as vector species of TOSV in Southern France [12], the South-West region of Madrid, Spain [57], and Morocco [58]. Additionally to *Ph. perniciosus*, *Ph. longicuspis* and *Ph. sergenti* are identified as potential vectors of TOSV in Morocco [59]. Another study reported TOSV presence in *Se. minuta* in France which feeds preferentially on cold-blooded vertebrates [60]. More recently, TOSV was detected from two pools of *Ph. neglectus* in Croatia [14]. In Cyprus, TOSV was detected in one pool of *Ph. perfiliewi* and in two pools of *Ph. tobbi* [61].

In some other studies, the infected vectors were identified at the genus level only, thus preventing implication of a given species. This was the case in Spain (where almost 70% of captured insects were *Ph. perniciosus*) [62], Corsica (where *Ph. perniciosus* and *Se. minuta* were largely predominant in equal proportions) [63], Algeria (where six species were morphologically identified: *Ph. perfiliewi* (51.4%), *Ph. perniciosus* (36.7%), *Ph. longicuspis* (2.6%), *Ph. papatasi* (6.5%), *Ph. sergenti* (0.5%), and *Se. minuta* (2.3%)) [22], and in Tunisia (where *Ph. perniciosus* was the most abundant species (71.74%)) [64] (Figure 2).

To date, only TOSV lineage A has been recorded in *Ph. perfiliewi* [56]. However, at this stage of knowledge, the correlation of TOSV genetic lineage and phlebotomine species is poorly supported (Figure 2). *Ph. perniciosus* can transmit TOSV strains belonging to lineage A and lineage B [12,55,57,58]. Lineage A was also detected once in *Se. minuta* [60]. In addition to *Ph. perniciosus*, *Ph. longicuspis*, *Ph. sergenti*, and *Ph. neglectus* are possible vectors of TOSV strains belonging to lineage B [11,59]. So far, TOSV strains belonging to lineage C have only been recorded in Croatia and Greece, where they are assumed to be transmitted by *Ph. neglectus* [14] (Figure 2).

In regions where known vector species (e.g., *Ph. perniciosus* and *Ph. perfiliewi*) are in the minority or even totally absent (e.g., Bosnia and Herzegovina), seroprevalence rates observed in humans and animals suggest that alternative species could be competent (e.g., *Ph. neglectus* in Bosnia and Herzegovina). It is therefore important to study the specificities for each country and species in order to better understand the current expansion of TOSV together with its demonstrated and putative vectors.

Climate change affects the geographical distribution of many sand fly species [65,66]. As a consequence, the geographical distribution of sand flies has expanded towards Northern Europe during the last decade. For instance, populations of *Ph. perniciosus* are now present in new regions in Germany [54,67]. Consequently, TOSV could also spread to these newly colonized regions where competent species are present [68].

Despite the lack of knowledge for many species of sand flies, those belonging to the genus *Phlebotomus* have benefited from recent efforts from the European Center for Disease Prevention and Control (ECDC) in terms of species inventory and mapping. However, these efforts should be pursued and completed by abundance studies [69]. Regarding *Sergentomyia* species, very little is known, nevertheless these species deserve more attention due to their possible association with TOSV transmission [60] and their putative role in *Leishmania* transmission [70–73].

4. Vertebrates as Possible Reservoir or Amplification Host

The conditions required for a given species to be an efficient reservoir or amplifying host are (i) to be able to generate high and/or sustained viremia for transmission to the competent sand fly species and (ii) to present a geographical distribution equal to, or larger than, that of the disease.

In humans the short duration of viremia together with the absence of persistent infection preclude any role in TOSV maintenance in nature. TOSV was isolated once from the brain of one wild caught bat (*Pipistrellus kuhlii*) in Italy where TOSV-infected *Ph. perniciosus* and *Ph. perfiliewi* were present [56]. This is the first and the only record of TOSV identification from bats.

Many seroprevalence studies testing vertebrate sera have described the presence of variable rates of antibodies directed against TOSV. These studies showed that TOSV circulates in the following countries: Greece [74], Spain [75], Portugal [76,77], France (Corsica island) [78], Tunisia [79], Algeria [80], and Kosovo [81], and can infect dogs, cats, cattle, and sheep. One study reported the presence of TOSV RNA detection in dogs in Portugal [76] and another study detected TOSV RNA in goat serum in southern Spain [75]. These results are very likely to be anecdotic. So far, there is no evidence for a vertebrate species acting as reservoir or amplifying host in the natural cycle of TOSV. Considering that dogs are a reservoir for sand-fly-borne *Leishmania infantum*, they are good candidates as natural reservoir hosts for TOSV. The fact that neutralizing antibodies against TOSV have been repeatedly described in dogs (4.3–8.4% in studies conducted in France, Portugal, Tunisia, Algeria, Greece, and Cyprus) is likely to reflect exposure rather than being indicative of a more peculiar role in the natural cycle [74,76–80]. However, two studies conducted in dogs from Mediterranean Anatolia (Turkey) reported the presence of TOSV RNA in the blood of approximately 3% and 10% of tested dogs, respectively, of which some were co-infected with *Leishmania* parasites [82,83]. Co-infection could contribute to this apparent active replication of TOSV. More recently, TOSV was detected in the brain and kidney from a greater flamingo (*Phoenicopterus roseus*), a great white pelican (*Pelecanus onocrotalus*), and a black stork (*Ciconia nigra*) in Turkey [84]. Nevertheless, the role of these animals in the life cycle of TOSV remains unknown. Currently, no data support the hypothesis that humans and/or any other vertebrates are the reservoirs of sand-fly-borne phleboviruses, due to the short duration of the viremia and the lack of persistent infection. The consideration of the vector as the reservoir of phleboviruses is currently under debate [60].

5. Conclusions

Here we highlight that there is very limited information on the biology and epidemiology of TOSV, its reservoirs, and its vectors. As there is neither a vaccine nor a specific treatment, the control of TOSV infections can only be achieved through sand fly control measures (indoor and outdoor residual spraying, attractive toxic sugar baits, etc.) or personal protection against bites (skin repellents, impregnated bed nets, etc.). In order to be able to target regions where these measures could be promoted, it is necessary to know the geographical distribution of populations at risk through seroepidemiological studies and the surveillance of neuro-invasive TOSV infection cases. The recent demonstration that TOSV circulates in a much broader area than suspected a decade ago has raised questions about the possibility that additional phlebotomine species (at least *Ph. longicuspis*, *Ph. sergenti*, *Ph. tobbi*, *Ph. neglectus*, and *Sergentomyia minuta*) may be involved in the TOSV natural cycle. Whether these species or others could be competent for the transmission of TOSV merit further study. Accordingly, it is necessary to set up specific studies to address this question (i) in the natural environment and (ii) under experimental conditions using sand fly colonies in insectarium.

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References

1. Maes, P.; Alkhovsky, S.V.; Bao, Y.; Beer, M.; Birkhead, M.; Briese, T.; Buchmeier, M.J.; Calisher, C.H.; Charrel, R.N.; Choi, I.R.; et al. Taxonomy of the family *Arenaviridae* and the order *Bunyavirales*: Update 2018. *Arch. Virol.* **2018**, *163*, 2295–2310. [[CrossRef](#)]
2. Charrel, R.; Bichaud, L.; De Lamballerie, X. Emergence of Toscana virus in the mediterranean area. *World J. Virol.* **2012**, *1*, 135. [[CrossRef](#)] [[PubMed](#)]
3. Tesh, R.B.; Modi, G.B. Studies on the biology of phleboviruses in sand flies (Diptera: Psychodidae). I. Experimental infection of the vector. *Am. J. Trop. Med. Hyg.* **1984**, *33*, 1007–1016. [[CrossRef](#)] [[PubMed](#)]
4. King, A.M.Q.; Lefkowitz, E.J.; Mushegian, A.R.; Adams, M.J.; Dutilh, B.E.; Gorbalenya, A.E.; Harrach, B.; Harrison, R.L.; Junglen, S.; Knowles, N.J.; et al. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2018). *Arch. Virol.* **2018**, *163*, 2601–2631. [[CrossRef](#)] [[PubMed](#)]
5. Charrel, R.N.; Gallian, P.; Navarro-Mari, J.M.; Nicoletti, L.; Papa, A.; Sanchez-Seco, M.P.; Tenorio, A.; de Lamballerie, X. Emergence of Toscana virus in Europe. *Emerg. Infect. Dis.* **2005**, *11*, 1657–1663. [[CrossRef](#)] [[PubMed](#)]
6. Braitto, A.; Corbisiero, R.; Corradini, S.; Marchi, B.; Sancasciani, N.; Fiorentini, C.; Ciufolini, M.G. Evidence of Toscana virus infections without central nervous system involvement: A serological study. *Eur. J. Epidemiol.* **1997**, *13*, 761–764. [[CrossRef](#)] [[PubMed](#)]
7. Schultze, D.; Korte, W.; Rafeiner, P.; Niedrig, M. First report of sandfly fever virus infection imported from Malta into Switzerland, October 2011. *Euro. Surveill.* **2012**, *17*. [[CrossRef](#)]
8. Jaijakul, S.; Arias, C.A.; Hossain, M.; Arduino, R.C.; Wootton, S.H.; Hasbun, R. Toscana meningoencephalitis: A comparison to other viral central nervous system infections. *J. Clin. Virol.* **2012**, *55*, 204–208. [[CrossRef](#)] [[PubMed](#)]
9. Nougairède, A.; Bichaud, L.; Thiberville, S.D.; Ninove, L.; Zandotti, C.; de Lamballerie, X.; Brouqui, P.; Charrel, R.N. Isolation of Toscana virus from the cerebrospinal fluid of a man with meningitis in Marseille, France, 2010. *Vector Borne Zoonotic Dis.* **2013**, *13*, 685–688. [[CrossRef](#)]

10. Karunaratne, K.; Davies, N. Toscana virus meningitis following a holiday in Elba, Italy. *Br. J. Hosp. Med.* **2018**, *79*, 292. [[CrossRef](#)]
11. Echevarria, J.M.; de Ory, F.; Guisasola, M.E.; Sanchez-Seco, M.P.; Tenorio, A.; Lozano, A.; Cordoba, J.; Gobernado, M. Acute meningitis due to Toscana virus infection among patients from both the Spanish Mediterranean region and the region of Madrid. *J. Clin. Virol.* **2003**, *26*, 79–84. [[CrossRef](#)]
12. Eitrem, R.; Vene, S.; Niklasson, B. Incidence of sand fly fever among Swedish United Nations soldiers on Cyprus during 1985. *Am. J. Trop. Med. Hyg.* **1990**, *43*, 207–211. [[CrossRef](#)] [[PubMed](#)]
13. Hemmersbach-Miller, M.; Parola, P.; Charrel, R.N.; Paul Durand, J.; Brouqui, P. Sandfly fever due to Toscana virus: An emerging infection in southern France. *Eur. J. Intern. Med.* **2004**, *15*, 316–317. [[CrossRef](#)] [[PubMed](#)]
14. Ayhan, N.; Charrel, R.N. Emergent Sand Fly–Borne Phleboviruses in the Balkan Region. *Emerg. Infect. Dis.* **2018**, *24*, 2324–2330. [[CrossRef](#)]
15. Alkan, C.; Allal-Ikhlef, A.B.; Alwassouf, S.; Baklouti, A.; Piorkowski, G.; de Lamballerie, X.; Izri, A.; Charrel, R.N. Virus isolation, genetic characterization and seroprevalence of Toscana virus in Algeria. *Clin. Microbiol. Infect.* **2015**, *21*, 1040. [[CrossRef](#)]
16. Ayhan, N.; Charrel, R.N. Of phlebotomines (sandflies) and viruses: A comprehensive perspective on a complex situation. *Curr. Opin. Insect Sci.* **2017**, *22*, 117–124. [[CrossRef](#)]
17. Charrel, R.N.; Izri, A.; Temmam, S.; Delaunay, P.; Toga, I.; Dumon, H.; Marty, P.; de Lamballerie, X.; Parola, P. Cocirculation of 2 genotypes of Toscana virus, southeastern France. *Emerg. Infect. Dis.* **2007**, *13*, 465–468. [[CrossRef](#)] [[PubMed](#)]
18. Ergunay, K.; Ayhan, N.; Charrel, R.N. Novel and emergent sandfly-borne phleboviruses in Asia Minor: A systematic review. *Rev. Med. Virol.* **2017**, *27*. [[CrossRef](#)]
19. Ayhan, N.; Alten, B.; Iovic, V.; Martinkovic, F.; Kasap, O.E.; Ozbek, Y.; de Lamballerie, X.; Charrel, R.N. Cocirculation of Two Lineages of Toscana Virus in Croatia. *Front. Public Health* **2017**, *5*, 336. [[CrossRef](#)]
20. Tamura, K.; Stecher, G.; Peterson, D.; Filipinski, A.; Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [[CrossRef](#)]
21. Peyrefitte, C.N.; Devetakov, I.; Pastorino, B.; Villeneuve, L.; Bessaud, M.; Stolidi, P.; Depaquit, J.; Segura, L.; Gravier, P.; Tock, F.; et al. Toscana virus and acute meningitis, France. *Emerg. Infect. Dis.* **2005**, *11*, 778–780. [[CrossRef](#)] [[PubMed](#)]
22. Mendoza-Montero, J.; Gamez-Rueda, M.I.; Navarro-Mari, J.M.; de la Rosa-Fraile, M.; Oyonarte-Gomez, S. Infections due to sandfly fever virus serotype Toscana in Spain. *Clin. Infect. Dis.* **1998**, *27*, 434–436. [[CrossRef](#)] [[PubMed](#)]
23. Anagnostou, V.; Papa, A. Seroprevalence of Toscana virus among residents of Aegean Sea islands, Greece. *Travel Med. Infect. Dis.* **2013**, *11*, 98–102. [[CrossRef](#)] [[PubMed](#)]
24. De Lamballerie, X.; Tolou, H.; Durand, J.P.; Charrel, R.N. Prevalence of Toscana virus antibodies in volunteer blood donors and patients with central nervous system infections in southeastern France. *Vector Borne Zoonotic Dis.* **2007**, *7*, 275–277. [[CrossRef](#)] [[PubMed](#)]
25. Eitrem, R.; Stylianou, M.; Niklasson, B. High prevalence rates of antibody to three sandfly fever viruses (Sicilian, Naples, and Toscana) among Cypriots. *Epidemiol. Infect.* **1991**, *107*, 685–691. [[CrossRef](#)]
26. Brisbarre, N.; Attoui, H.; Gallian, P.; Di Bonito, P.; Giorgi, C.; Cantaloube, J.F.; Biagini, P.; Touinssi, M.; Jordier, F.; de Micco, P. Seroprevalence of Toscana virus in blood donors, France, 2007. *Emerg. Infect. Dis.* **2011**, *17*, 941–943. [[CrossRef](#)]
27. Ergunay, K.; Aydogan, S.; Ilhami Ozcebe, O.; Cilek, E.E.; Hacıoglu, S.; Karakaya, J.; Ozkul, A.; Us, D. Toscana virus (TOSV) exposure is confirmed in blood donors from Central, North and South/Southeast Anatolia, Turkey. *Zoonoses Public Health* **2012**, *59*, 148–154. [[CrossRef](#)]
28. Papa, A.; Andriotis, V.; Tzilianos, M. Prevalence of Toscana virus antibodies in residents of two Ionian islands, Greece. *Travel Med. Infect. Dis.* **2010**, *8*, 302–304. [[CrossRef](#)]
29. Santos, L.; Cardoso, M.J.; Marinho, A.S.; Guimaraes, T.; Sarmiento, A. [Seroprevalence survey of Toscana virus infection in Oporto region]. *Acta Med. Port.* **2011**, *24*, 479–482.
30. Terrosi, C.; Olivieri, R.; Bianco, C.; Cellesi, C.; Cusi, M.G. Age-dependent seroprevalence of Toscana virus in central Italy and correlation with the clinical profile. *Clin. Vaccine Immunol.* **2009**, *16*, 1251–1252. [[CrossRef](#)]
31. Punda-Polic, V.; Jeroncic, A.; Mohar, B.; Sisko Kraljevic, K. Prevalence of Toscana virus antibodies in residents of Croatia. *Clin. Microbiol. Infect.* **2012**, *18*, E200–E203. [[CrossRef](#)] [[PubMed](#)]

32. Venturi, G.; Marchi, A.; Fiorentini, C.; Ramadani, N.; Quaglio, G.; Kalaveshi, A.; Bertinato, L.; Putoto, G.; Benedetti, E.; Rezza, G.; et al. Prevalence of antibodies to phleboviruses and flaviviruses in Peja, Kosovo. *Clin. Microbiol. Infect.* **2011**, *17*, 1180–1182. [[CrossRef](#)]
33. Hukic, M.; Salimovic-Besic, I. Sandfly—Pappataci fever in Bosnia and Herzegovina: The new-old disease. *Bosn. J. Basic Med. Sci.* **2009**, *9*, 39–43. [[CrossRef](#)] [[PubMed](#)]
34. Christova, I.; Panayotova, E.; Trifonova, I.; Taseva, E.; Gladnishka, T.; Ivanova, V. Serologic evidence of widespread Toscana virus infection in Bulgaria. *J. Infect. Public Health* **2019**. [[CrossRef](#)] [[PubMed](#)]
35. Fezaa, O.; Bahri, O.; Alaya Bouafif, N.B.; Triki, H.; Bouattour, A. Seroprevalence of Toscana virus infection in Tunisia. *Int. J. Infect. Dis.* **2013**, *17*, e1172–e1175. [[CrossRef](#)] [[PubMed](#)]
36. Sakhria, S.; Bichaud, L.; Mensi, M.; Salez, N.; Dachraoui, K.; Thirion, L.; Cherni, S.; Chelbi, I.; De Lamballerie, X.; Zhioua, E.; 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. [[CrossRef](#)] [[PubMed](#)]
37. Tesh, R.B.; Lubroth, J.; Guzman, H. Simulation of arbovirus overwintering: Survival of Toscana virus (Bunyaviridae: *Phlebovirus*) in its natural sand fly vector *Phlebotomus perniciosus*. *Am. J. Trop. Med. Hyg.* **1992**, *47*, 574–581. [[CrossRef](#)]
38. Maroli, M.; Ciufolini, M.G.; Verani, P. Vertical transmission of Toscana virus in the sandfly, *Phlebotomus perniciosus*, via the second gonotrophic cycle. *Med. Vet. Entomol.* **1993**, *7*, 283–286. [[CrossRef](#)]
39. Tesh, R.B.; Modi, G.B. Maintenance of Toscana virus in *Phlebotomus perniciosus* by vertical transmission. *Am. J. Trop. Med. Hyg.* **1987**, *36*, 189–193. [[CrossRef](#)]
40. Peyrefitte, C.N.; Grandadam, M.; Bessaud, M.; Andry, P.-E.; Fouque, F.; Caro, V.; Diancourt, L.; Schuffenecker, I.; Pagès, F.; Tolou, H.; et al. Diversity of *Phlebotomus perniciosus* in Provence, Southeastern France: Detection of Two Putative New Phlebovirus Sequences. *Vector Borne Zoonotic Dis.* **2013**, *13*, 630–636. [[CrossRef](#)]
41. Zhioua, E.; Moureau, G.; Chelbi, I.; Ninove, L.; Bichaud, L.; Derbali, M.; Champs, M.; Cherni, S.; Salez, N.; Cook, S.; et al. Punique virus, a novel *phlebovirus*, related to sandfly fever Naples virus, isolated from sandflies collected in Tunisia. *J. Gen. Virol.* **2010**, *91*, 1275–1283. [[CrossRef](#)] [[PubMed](#)]
42. Alkan, C.; Alwassouf, S.; Piorkowski, G.; Bichaud, L.; Tezcan, S.; Dincer, E.; Ergunay, K.; Ozbel, Y.; Alten, B.; de Lamballerie, X.; 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*, 4080–4091. [[CrossRef](#)] [[PubMed](#)]
43. Remoli, M.E.; Fortuna, C.; Marchi, A.; Bucci, P.; Argentini, C.; Bongiorno, G.; Maroli, M.; Gradoni, L.; Gramiccia, M.; Ciufolini, M.G. Viral isolates of a novel putative phlebovirus in the Marche Region of Italy. *Am. J. Trop. Med. Hyg.* **2014**, *90*, 760–763. [[CrossRef](#)] [[PubMed](#)]
44. Jancarova, M.; Bichaud, L.; Hlavacova, J.; Priet, S.; Ayhan, N.; Spitzova, T.; Volf, P.; Charrel, R.N. Experimental infection of sand flies by Massilia virus and viral transmission by co-feeding on sugar meal. *Viruses* **2019**, *11*, 332. [[CrossRef](#)] [[PubMed](#)]
45. Ready, P. Biology of Phlebotomine Sand Flies as Vectors of Disease Agents. *Annu. Rev. Entomol.* **2013**, *58*, 227–250. [[CrossRef](#)]
46. Rueda, L.M.; Pecor, J.E.; Wolkoff, M.; Pecor, D.; Benyamin, S.; Bousses, P.; Debboun, M. New records, distribution, and updated checklists of old world Phlebotomine sand flies, with emphasis on Africa, southwest Asia, and central Asia. *US Army Med. Dep. J.* **2017**, *1–17*, 6–85.
47. Abonnenc, E. *Les Phlébotomes de la Région Éthiopienne (Diptera, Psychodidae)*; Service Central de Documentation de l'ORSTOM: Bondy, France, 1972; pp. 1–290.
48. Muller, G.; Schlein, Y. Nectar and honeydew feeding of *Phlebotomus papatasi* in a focus of *Leishmania major* in Neot Hakikar oasis. *J. Vector Ecol.* **2004**, *29*, 154–158.
49. Alten, B.; Maia, C.; Afonso, O.; Campino, L.; Jiménez, M.; González, E.; Molina, R.; Bañuls, A.L.; Prudhomme, J.; Vergnes, B.; et al. Seasonal dynamics of phlebotomine sand fly species proven vectors of Mediterranean leishmaniasis caused by *Leishmania infantum*. *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004458. [[CrossRef](#)]
50. Killick-Kendrick, R. The biology and control of phlebotomine sand flies. *Clin. Dermatol.* **1999**, *17*, 279–289. [[CrossRef](#)]
51. Lane, R. Sandflies (Phlebotominae). In *Medical Insects and Arachnids*; Springer: Dordrecht, Germany, 1993; pp. 78–119.

52. Alten, B.; Ozbel, Y.; Ergunay, K.; Kasap, O.; Cull, B.; Antoniou, M.; Velo, E.; Prudhomme, J.; Molina, R.; Bañuls, A.; et al. Sampling strategies for phlebotomine sand flies (Diptera: Psychodidae) in Europe. *Bull. Entomol. Res.* **2015**, *105*, 664–678. [CrossRef]
53. Fischer, D.; Moeller, P.; Thomas, S.; Naucke, T.; Beierkuhnlein, C. Combining Climatic Projections and Dispersal Ability: A Method for Estimating the Responses of Sandfly Vector Species to Climate Change. *PLoS Negl. Trop. Dis.* **2011**, *5*, e1407. [CrossRef] [PubMed]
54. Naucke, T.; Menn, B.; Massberg, D.; Lorentz, S. Sandflies and leishmaniasis in Germany. *Parasitol. Res.* **2008**, *103*, 65–68. [CrossRef]
55. Verani, P.; Lopes, M.; Nicoletti, L.; Balducci, M. Studies on *Phlebotomus*-transmitted viruses in Italy. I. Isolation and characterization of a sandfly fever Naples-like virus. *Arboviruses Mediterr. Ctries. Stuttg. Gustav Fischer Verl.* **1980**, Suppl. 9, 195–201.
56. Verani, P.; Ciufolini, M.G.; Caciolli, S.; Renzi, A.; Nicoletti, L.; Sabatinelli, G.; Bartolozzi, D.; Volpi, G.; Amaducci, L.; Coluzzi, M.; et al. Ecology of viruses isolated from sand flies in Italy and characterized of a new Phlebovirus (Arabia virus). *Am. J. Trop. Med. Hyg.* **1988**, *38*, 433–439. [CrossRef] [PubMed]
57. Remoli, M.E.; Jimenez, M.; Fortuna, C.; Benedetti, E.; Marchi, A.; Genovese, D.; Gramiccia, M.; Molina, R.; Ciufolini, M.G. Phleboviruses detection in *Phlebotomus perniciosus* from a human leishmaniasis focus in South-West Madrid region, Spain. *Parasit. Vectors* **2016**, *9*, 205. [CrossRef] [PubMed]
58. Es-Sette, N.; Nourlil, 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*, 1507–1509. [CrossRef]
59. Es-Sette, N.; Ajaoud, M.; Anga, L.; Mellouki, F.; Lemrani, M. Toscana virus isolated from sandflies, Morocco. *Parasit. Vectors* **2015**, *8*, 205–208. [CrossRef]
60. Charrel, R.N.; Izri, A.; Temmam, S.; de Lamballerie, X.; Parola, P. Toscana virus RNA in *Sergentomyia minuta* files. *Emerg. Infect. Dis.* **2006**, *12*, 1299–1300. [CrossRef]
61. Ergunay, K.; Kasap, O.E.; Orsten, S.; Oter, K.; Gunay, F.; Yoldar, A.Z.; Dincer, E.; Alten, B.; Ozkul, A. *Phlebovirus* and *Leishmania* detection in sandflies from eastern Thrace and Northern Cyprus. *Parasit. Vectors* **2014**, *7*, 575. [CrossRef]
62. Sanbonmatsu-Gamez, S.; Perez-Ruiz, M.; Collao, X.; Sanchez-Seco, M.P.; Morillas-Marquez, F.; de la Rosa-Fraile, M.; Navarro-Mari, J.M.; Tenorio, A. Toscana virus in Spain. *Emerg. Infect. Dis.* **2005**, *11*, 1701–1707. [CrossRef]
63. Bichaud, L.; Izri, A.; Lamballerie, X.; Moureau, G.; Charrel, R. First detection of Toscana virus in Corsica, France. *Clin. Microbiol. Infect.* **2014**, *20*, O101–O104. [CrossRef] [PubMed]
64. Bichaud, L.; Dachraoui, K.; Piorkowski, G.; Chelbi, I.; Moureau, G.; Cherni, S.; De Lamballerie, X.; Sakhria, S.; Charrel, R.N.; Zhioua, E. Toscana virus isolated from sandflies, Tunisia. *Emerg. Infect. Dis.* **2013**, *19*, 322–324. [CrossRef] [PubMed]
65. Chalghaf, B.; Chemkhi, J.; Mayala, B.; Harrabi, M.; Benie, G.B.; Michael, E.; Ben Salah, A. Ecological niche modeling predicting the potential distribution of *Leishmania* vectors in the Mediterranean basin: Impact of climate change. *Parasit. Vectors* **2018**, *11*, 461–470. [CrossRef] [PubMed]
66. Koch, L.K.; Kochmann, J.; Klimpel, S.; Cunze, S. Modeling the climatic suitability of leishmaniasis vector species in Europe. *Sci. Rep.* **2017**, *7*, 13325. [CrossRef] [PubMed]
67. Mencke, N. The importance of canine leishmaniosis in non-endemic areas, with special emphasis on the situation in Germany. *Berl. Munch. Tierarztl. Wochenschr.* **2011**, *124*, 434–442.
68. Depaquit, J.; Grandadam, M.; Fouque, F.; Andry, P.-E.; Peyrefitte, C. Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: A review. *Euro. Surveill.* **2010**, *15*, 19507.
69. European Centre for Disease Prevention and Control. Phlebotomine Sand Flies Maps. Available online: <https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/phlebotomine-maps> (accessed on 1 October 2019).
70. Senghor, M.W.; Niang, A.A.; Depaquit, J.; Ferte, H.; Faye, M.N.; Elguero, E.; Gaye, O.; Alten, B.; Perktas, U.; Cassan, C.; et al. Transmission of *Leishmania infantum* in the Canine Leishmaniasis Focus of Mont-Rolland, Senegal: Ecological, Parasitological and Molecular Evidence for a Possible Role of *Sergentomyia* Sand Flies. *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004940. [CrossRef]

71. Jaouadi, K.; Ghawar, W.; Salem, S.; Gharbi, M.; Bettaieb, J.; Yazidi, R.; Harrabi, M.; Hamarsheh, O.; Ben Salah, A. First report of naturally infected *Sergentomyia minuta* with *Leishmania major* in Tunisia. *Parasit. Vectors* **2015**, *8*, 649. [[CrossRef](#)]
72. Maia, C.; Depaquit, J. Can *Sergentomyia* (Diptera, Psychodidae) play a role in the transmission of mammal-infecting *Leishmania*? *Parasite* **2016**, *23*, 55. [[CrossRef](#)]
73. Bravo-Barriga, D.; Parreira, R.; Maia, C.; Afonso, M.O.; Blanco-Ciudad, J.; Serrano, F.J.; Perez-Martin, J.E.; Gomez-Gordo, L.; Campino, L.; Reina, D.; et al. Detection of *Leishmania* DNA and blood meal sources in phlebotomine sand flies (Diptera: Psychodidae) in western of Spain: Update on distribution and risk factors associated. *Acta Trop.* **2016**, *164*, 414–424. [[CrossRef](#)]
74. Alwassouf, S.; Christodoulou, V.; Bichaud, L.; Ntais, P.; Mazeris, A.; Antoniou, M.; Charrel, R.N. 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. [[CrossRef](#)] [[PubMed](#)]
75. Navarro-Mari, J.M.; Palop-Borras, B.; Perez-Ruiz, M.; Sanbonmatsu-Gamez, S. Serosurvey study of Toscana virus in domestic animals, Granada, Spain. *Vector Borne Zoonotic Dis.* **2011**, *11*, 583–587. [[CrossRef](#)] [[PubMed](#)]
76. Alwassouf, S.; Maia, C.; Ayhan, N.; Coimbra, M.; Cristovao, J.M.; Richet, H.; Bichaud, L.; Campino, L.; Charrel, R.N. Neutralization-based seroprevalence of Toscana virus and sandfly fever Sicilian virus in dogs and cats from Portugal. *J. Gen. Virol.* **2016**, *97*, 2816–2823. [[CrossRef](#)] [[PubMed](#)]
77. Maia, C.; Alwassouf, S.; Cristovao, J.M.; Ayhan, N.; Pereira, A.; Charrel, R.N.; Campino, L. Serological association between *Leishmania infantum* and sand fly fever Sicilian (but not Toscana) virus in sheltered dogs from southern Portugal. *Parasit. Vectors* **2017**, *10*, 92. [[CrossRef](#)] [[PubMed](#)]
78. Dahmani, M.; Alwassouf, S.; Grech-Angelini, S.; Marie, J.L.; Davoust, B.; Charrel, R.N. Seroprevalence of Toscana virus in dogs from Corsica, France. *Parasit. Vectors* **2016**, *9*, 381. [[CrossRef](#)]
79. Sakhria, S.; Alwassouf, S.; Fares, W.; Bichaud, L.; Dachraoui, K.; Alkan, C.; Zoghalmi, Z.; De Lamballerie, X.; Zhioua, E.; Charrel, R.N. Presence of sandfly-borne phleboviruses of two antigenic complexes (*Sandfly fever Naples virus* and *Sandfly fever Sicilian virus*) in two different bio-geographical regions of Tunisia demonstrated by microneutralisation-based seroprevalence study in dogs. *Parasit. Vectors* **2014**, *7*, 476.
80. Tahir, D.; Alwassouf, S.; Loudahi, A.; Davoust, B.; Charrel, R.N. Seroprevalence of Toscana virus in dogs from Kabylia (Algeria). *Clin. Microbiol. Infect.* **2016**, *22*, e16–e17. [[CrossRef](#)]
81. Ayhan, N.; Sherifi, K.; Taraku, A.; Berxholi, K.; Charrel, R.N. High Rates of Neutralizing Antibodies to Toscana and Sandfly Fever Sicilian Viruses in Livestock, Kosovo. *Emerg. Infect. Dis.* **2017**, *23*, 989–992. [[CrossRef](#)]
82. Dincer, E.; Karapinar, Z.; Oktem, M.; Ozbaba, M.; Ozkul, A.; Ergunay, K. Canine Infections and Partial S Segment Sequence Analysis of Toscana Virus in Turkey. *Vector Borne Zoonotic Dis.* **2016**, *16*, 611–618. [[CrossRef](#)]
83. Dincer, E.; Gargari, S.; Ozkul, A.; Ergunay, K. Potential animal reservoirs of Toscana virus and coinfections with *Leishmania infantum* in Turkey. *Am. J. Trop. Med. Hyg.* **2015**, *92*, 690–697. [[CrossRef](#)]
84. Hacıoglu, S.; Dincer, E.; Isler, C.T.; Karapinar, Z.; Ataseven, V.S.; Ozkul, A.; Ergunay, K. A Snapshot Avian Surveillance Reveals West Nile Virus and Evidence of Wild Birds Participating in Toscana Virus Circulation. *Vector Borne Zoonotic Dis.* **2017**, *17*, 698–708. [[CrossRef](#)] [[PubMed](#)]

