



HAL
open science

Tick-borne encephalitis virus in *Ixodes ricinus* (Acari: Ixodidae) ticks, Tunisia

Wasfi Fares, Khalil Dachraoui, Seifedine Cherni, Walid Barhoumi, Talel Ben Slimane, Hend Younsi, Elyes Zhioua

► To cite this version:

Wasfi Fares, Khalil Dachraoui, Seifedine Cherni, Walid Barhoumi, Talel Ben Slimane, et al.. Tick-borne encephalitis virus in *Ixodes ricinus* (Acari: Ixodidae) ticks, Tunisia. *Ticks and Tick-borne Diseases*, 2021, 12 (1), pp.101606. <10.1016/j.ttbdis.2020.101606>. <hal-03492959>

HAL Id: hal-03492959

<https://hal.science/hal-03492959v1>

Submitted on 21 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

1 Ticks and Tick-borne Diseases

2 Short Communication

3 Tick-borne encephalitis virus in *Ixodes ricinus* (Acari: Ixodidae) ticks, Tunisia

4

5 Wasfi Fares^a, Khalil Dachraoui^a, Seifedine Cherni^a, Walid Barhoumi^a, Talel Ben Slimane^a,

6 Hend Younsi^b, and Elyes Zhioua^{a*}

7

8

9 ^a *Vector Ecology Unit, Institut Pasteur de Tunis, Tunis, Tunisia*

10 ^b *Institut Supérieur des Sciences Biologiques Appliquées, Tunis, Tunisia*

11

12

13

14

15

16 *Corresponding author

17 Email address: elyes.zhioua@gmail.com (E. Zhioua)

18

19

20 ABSTRACT

21 Tick-borne encephalitis virus (TBEV) is the etiologic agent of tick-borne encephalitis (TBE)
22 and transmitted by *Ixodes ricinus* (Linnaeus, 1758) in Europe. The geographical distribution
23 of *I. ricinus* in the Palearctic region covers also northern Africa, including northwestern
24 Tunisia. While the eco-epidemiology of TBE in Europe is well documented, no data
25 concerning TBEV from northern Africa are available. We investigated whether TBEV is
26 circulating in Tunisia. A total of 877 adult *I. ricinus* collected from northwestern Tunisia were
27 examined in pools for the presence of TBEV by nRT-PCR. Viral RNA was detected in one
28 pool of three engorged ticks, yielding a minimum infection rate of 0.11% (1/877).
29 Phylogenetic analysis showed that Tunisian TBEV strain belongs to the European lineage.
30 We report for the first time the presence of TBEV in *I. ricinus* from northern Africa. These
31 preliminary data led us to suspect the presence of TBE in Tunisia. Therefore, more studies are
32 needed to assess the public health importance of TBEV in northern Africa.

33

34 *Key words:* Tick-borne encephalitis virus; Ticks; Tunisia

35

36 **1. Introduction**

37 Tick-borne encephalitis virus (TBEV) is the etiologic agent of tick-borne encephalitis (TBE)
38 which is the most important viral tick-borne disease in Europe (Süss, 2010). Five main
39 lineages of TBEV are recognized: European (TBEV-Eu), Siberian (TBEV-Sib), Far Eastern
40 (TBEV-FE), Baikalian (TBEV-Bkl) and Himalayan (Ecker et al., 1999; Zlobin et al., 2001;
41 Demina et al., 2012; Kovalev et al., 2017). Case-fatality rate for TBEV-Eu and TBEV Sib is
42 low (1 to 2%) while for TBEV- FE is up to 30% (Gritsun et al., 2003). While the TBEV-Eu
43 is transmitted mainly by *Ixodes ricinus* (Linnaeus, 1758) (Lommano et al., 2012), TBEV-Sib
44 and TBEV-FE are transmitted by *Ixodes persulcatus* Schulze (Kholodilov et al., 2019).

45 *Ixodes ricinus* has a very wide geographic distribution throughout Europe and in parts
46 of northwestern Africa (Gern and Humair, 2002; Piesman and Gern, 2008). In Tunisia, *I.*
47 *ricinus* is principally located in oak forests, in humid to semi-humid microclimatic zones of
48 the Kroumirie Mountains in the northwest (Zhioua et al., 1989, 1999; Younsi et al., 2005;
49 Dsouli et al., 2006). In previous studies, we reported for the first time the presence of *Borrelia*
50 *lusitaniae* (Zhioua et al., 1999) and *Borrelia garinii* (Younsi et al., 2005) in *I. ricinus*
51 collected from northwestern Tunisia. While *I. ricinus* is considered the main vector of TBEV
52 in Europe (Lommano et al., 2012; Alfano et al., 2020), this arbovirus has never been studied
53 before in northern Africa. Therefore, this study was undertaken to assess the presence of
54 TBEV in *I. ricinus* from northwestern Tunisia by molecular and phylogenetic analysis.

55 **2. Materials and Methods**

56 *2.1. Study area, tick sampling, and tick identification*

57 Ticks were collected from the oak forest of EL Jouza (36°58'N, 9°05'E), located in
58 northwestern Tunisia by flagging and from grazing cattle during the period from November
59 2015 through February, 2016, a period corresponding to the peak activity of only adult *I.*
60 *ricinus* in Tunisia (Dsouli et al., 2006). Larvae and nymphs are active on vegetation from

61 March to August (Dsouli et al., 2006). Ticks were identified to species level by using
62 available adult *I. ricinus* morphological identification keys (Perez, 2007; Estrada-Peña et al.,
63 2004). It is of major importance to point out that a new tick species *Ixodes inopinatus*
64 (Estrada-Peña et al., 2014), closely related to *I. ricinus* has been described by Estrada-Peña et
65 al. (2014). *Ixodes inopinatus* is distributed mainly in the dry areas of the Mediterranean region
66 (Estrada-Peña et al., 2014). Both species are sympatric in northwestern Tunisia (Younsi et al.,
67 2019), and in Central Europe (Chitimia-Dobler et al., 2018). Identifying adult ticks is difficult
68 as both species have intermediate morphological characters, and therefore, molecular analysis
69 of 16SrRNA sequences (Mangold et al., 1998) was used to differentiate between *I. ricinus*
70 and *I. inopinatus* (Estrada-Peña et al., 2014; Chitimia-Dobler et al., 2018; Younsi et al., 2019;
71 Hauck et al., 2019). Taking into account that both species cannot be differentiated based
72 solely on morphological characters (Younsi et al., 2019), and since no molecular analysis was
73 performed to confirm morphological identification, we are aware that many *I. inopinatus* have
74 been misclassified as *I. ricinus*. Therefore, bearing in mind the limited scope of this
75 preliminary work, we considered both species as *I. ricinus*.

76 2.2. Extraction of nucleic acid, detection and identifying TBEV infections

77 Ticks were pooled according to the collecting date with a maximum of 10 specimens per pool
78 for the non-engorged ticks and up to 5 per pool for engorged ticks, and directly stored at -
79 80°C until use. Ticks were homogenized in 500 µl of Minimum Essential Medium (MEM)
80 using a PreCellys.24-Bertin instrument. Total RNA was extracted according to the Qiagen
81 RNA Mini Kit (Qiagen, Hilden, Germany) from 140 µl of tick's supernatant. During the RNA
82 extraction, no positive control was used to preclude any risk of contamination. Instead, only
83 negative control consisting of water solution was used. The presence of TBEV RNA was
84 determined by conventional flavivirus reverse transcription heminested PCR
85 (Scaramozzino et al., 2001) using the SuperScript One-Step RT-PCR with Platinum Taq

86 (Invitrogen Life Technologies) according to the manufacturer's instructions. West Nile virus
87 pre-extracted RNA was used as positive control. PCR products were visualized in a 2%
88 TBE/agarose electrophoresis gel and sequenced in both directions using the Big Dye
89 Terminators v3.1 kit (Applied Biosystems). Resulting sequence was firstly submitted to the
90 NCBI BLASTN and BLASTX programs (www.ncbi.nlm.nih.gov/BLAST/) for identification.
91 A partial NS5 polymerase fragment of 218-nt was used for phylogenetic analysis by MEGA
92 v.5.22 (Tamura et al., 2001). Phylogenetic tree was obtained according to the maximum
93 likelihood analysis method and the tamura-3 model with general time-reversible rates among
94 sites. The tree topology was supported by 500 bootstrap replicates. Available Louping ill
95 virus (LIV) sequences were added to the TBEV collected database. The Omsk hemorrhagic
96 fever virus (OHFV) sequence was used as an out-group with TBEV sequences. Genetic
97 distances were calculated using the neighbor joining and p-distance methods.

98 **3. Results and Discussion**

99 Of a total of 882 adult ticks collected during the capture season from November 2015
100 to February 2016, 877 ticks were identified as *I. ricinus*. Only 5 ticks were identified as
101 *Hyalomma marginatum*. Of a total of 120 pools, including 92 pools from non-engorged and
102 28 pools from engorged ticks, only one pool of three engorged females *I. ricinus* was positive
103 by reverse transcription heminested PCR. Subsequently, the minimum infection rate (MIR) is
104 0.11% (1/877). The sequence of the heminested positive PCR product was identified as
105 TBEV by BLAST analysis (GenBank accession number: KY412768).

106 Phylogenetic analysis showed that five main lineages of TBEV are described (Figure
107 1). While the Baikalian and Himalayan lineages were represented by one sequence each, the
108 European, the Far Eastern and the Siberian subtypes are more represented (Figure 1), and they
109 seem to share the same properties in their genetic diversity with 2.8%, 4.4% and 3.7%,
110 respectively. The newly detected Tunisian TBEV sequence is included in the TBEV European

111 subtype, supported by a 99% bootstrap value. The European sequences within the European
112 subtype were genetically very close to each other (0–3.7% divergence) and clearly different
113 from the Tunisian TBEV sequence (6–8.3% divergence).

114 While there are substantial data of TBEV in *I. ricinus* from Europe, there are no data
115 concerning this arbovirus from northern Africa. To our knowledge, this is the first report of
116 TBEV in *I. ricinus* from northern Africa. The 0.11% MIR observed in Tunisia is in line with
117 TBEV prevalence rates in ticks reported from European countries. The overall TBEV
118 prevalence rates in unfed nymphal and adult *I. ricinus* from endemic areas in northern Italy
119 varied from 0.17% (Alfano et al., 2020) to 0.21% (D'Agaro et al., 2009). The minimal
120 infection rates of questing nymphal and adult *I. ricinus* with TBEV collected from a new
121 endemic area in western Switzerland ranged from 0.95% to 0.21% (Lommano et al., 2012).

122 It is important to note that the detection of RNA corresponding to a region of the
123 TBEV polymerase in a pool of three engorged female ticks may indicate that the virus could
124 have been present in the blood meal and not actually infecting the ticks. However, the
125 detection TBEV provide strong evidence of the circulation of the virus in this natural biotope
126 of *I. ricinus* where *B. burgdorferi* sensu lato have been detected previously (Zhioua et al.,
127 1989, 1999; Younsi et al., 2005; Dsouli et al., 2006).

128 Taking into account that Tunisia is positioned on the flight path of the Palearctic-
129 African bird migration route, and migratory birds play an important role in spreading TBEV
130 infected ticks (Waldenström et al., 2007; Lommano et al., 2014), it is not excluded that TBEV
131 might be introduced in northwestern Tunisia, a hotspot of biodiversity where oak forests offer
132 suitable habitat for *I. ricinus* and favorable conditions for the maintenance of this arbovirus in
133 ticks and vertebrate hosts. This is supported by the fact that both immature stages of *I. ricinus*
134 are active in synchrony in Tunisia (Dsouli et al., 2006), a prerequisite for efficient co-feeding

135 transmission of TBEV (Randolph et al., 1999), which is a mechanism of maintenance of the
136 virus in nature that could not be excluded (Labuda et al., 1993).

137 The Tunisian strain of TBEV belongs to the European subtype of TBEV but distant
138 phylogenetically from European strains. This divergence could be explained by the strong
139 genetic differentiation between Eurasian and North-African populations of *I. ricinus* (De
140 Meeûs et al., 2002; Noureddine et al., 2011), which in turn may modify the epidemiological
141 landscape of the pathogens vectored by this tick species in northern Africa. Further studies are
142 needed to clarify this hypothesis.

143 In North Africa, including Tunisia, the problem of arboviral infections has been
144 neglected in the past except for West Nile virus infection (Fares et al., 2016). With the recent
145 report of the first autochthonous fatal case of TBE from southern Italy (Spagnolo et al., 2018),
146 a region acting as natural bridge connecting continental Europe to northern Africa and a
147 common pathway of migratory birds infested mainly with *I. ricinus* (Falchi et al., 2012), the
148 detection of TBEV in *I. ricinus* support the suspicion of the presence of TBE in northwestern
149 Tunisia. Thus, more studies are needed to attempt isolating the virus from field-collected *I.*
150 *ricinus* and to ascertain the circulation of TBEV in animals and human populations at risk
151 through serosurveys.

152

153 **Acknowledgements**

154 The authors would like to thank Dr. Stuart Dowall and Prof. Roger Hewson (Public Health
155 England, Porton Down, UK) for their significant contribution in performing this work. This
156 work was supported by the U.S. Civilian Research Fund and Development Foundation grant
157 OISE-16-62883-1.

158

159 **Conflict of interests**

160 The authors declare that they have no competing interests

161

162 **References**

- 163 Alfano, N., Tagliapietra, V., Rosso, F., Ziegler, U., Arnoldi, D., Rizzoli, A., 2020. Tick-
164 borne encephalitis foci in northeast Italy revealed by combining virus detection in ticks,
165 serosurvey on goats and human cases. *Emerg. Microbes Infect.* 9, 474-484.
166 <https://doi.org/10.1080/22221751.2020.1730246>.
- 167 Chitimia-Dobler, L., Rieß, R., Kahl, O., Wölfel, S., Dobler, G., Nava, S., Estrada-Peña, A.,
168 2018. *Ixodes inopinatus* occurring also outside the Mediterranean region. *Ticks Tick-*
169 *borne Dis.* 9, 196-200.
- 170 D'Agaro, P., Martinelli, E., Burgnich, P., Nazzi, F., Del Fabbro, S., Iob, A., Ruscio, M.,
171 Pischutti, P., Campello, C., 2009. Prevalence of tick-borne encephalitis virus in *Ixodes*
172 *ricinus* from a novel endemic area of north eastern Italy. *J. Gen. Virol.* 81, 309-316.
- 173 De Meeûs, C., Beati, L., Delay, C., Aeschlimann, A., Renaud, F., 2002. Sex-biased dispersal
174 in the vector of Lyme disease *Ixodes ricinus*. *Evolution* 59, 1802-1807.
- 175 Demina, T. V., Dzhioev I. P., Kozlova, I. V., Verkhozina, M. M., Tkachev, S. E.,
176 Doroshchenko, E. K., Lisak, O. V., Paramonov, A. I., Zlobin, V. I., 2012. Genotypes 4
177 and 5 of the tick-borne encephalitis virus: features of the genome structure and
178 possible scenario for its formation. *Vopr. Virusol.* 57, 13-19 (in Russian).
- 179 Dsouli, N., Younsi-Kabachii, H., Postic, D., Gern, L., Bouattour, A., 2006. Reservoir role of
180 the lizard, *Psammodromus algirus*, in the transmission cycle of *Borrelia burgdorferi*
181 *sensu lato* (Spirochaetaceae) in Tunisia. *J. Med. Entomol.* 43, 737-742.
- 182 Ecker, M., Allison, S.L., Meixner, T., Heinz, F.X., 1990. Sequence analysis and genetic
183 classification of tick-borne encephalitis viruses from Europe and Asia. *J. Gen. Virol.* 80,
184 179-185.

- 185 Estrada-Peña, A., Bouattour, A., Camicas, J.L., Walker, A.R., 2004. Ticks of domestic
186 animals in the Mediterranean region, a guide to identification of species. University of
187 Zaragoza, Zaragoza, Spain.
- 188 Estrada-Peña, A., Nava, S., Petney, T., 2014. Description of all stages of *Ixodes inopinatus* n.
189 sp. (Acari: Ixodidae). Ticks Tick-borne Dis. 5, 734-743.
- 190 Falchi, A., Dantas-Torres, F., Lorusso, V., Malia, E., Lia, R.P., Otranto, D., 2012.
191 Autochthonous and migratory birds as a dispersion source for *Ixodes ricinus* in
192 Southern Italy. Exp. Appl. Acarol. 58, 167-174.
- 193 Fares, W., Dachraoui, K., Cherni, S., Bosworth, A., Barhoumi, W., Dowall, S., Chelbi, I.,
194 Derbali, M., Zoghalmi, Z., Beier, J.C., Zhioua, E., 2016. West Nile virus in Tunisia,
195 2014: First isolation from mosquitoes. Acta Trop. 159, 106-110.
- 196 Gritsun, T.S., Lashkevich, V.A., Gould, E.A., 2003. Tick-borne encephalitis. Antiviral Res.
197 57, 129-146.
- 198 Gern, L., P. F. Humair, P. F., 2002. Ecology of *Borrelia burgdorferi* sensu lato in Europe. In:
199 Gray, J., Kahl, O., Lane, R.S., Stanek, G. (Eds.), Lyme Borreliosis: Biology,
200 Epidemiology and Control. CABI International, Oxon, United Kingdom, pp. 149- 174.
- 201 Hauck, D., Springer, A., Pachnicke, S., Schunack, B., Fingerle, V., Strube, C., 2019. *Ixodes*
202 *inopinatus* in northern Germany: occurrence and potential vector role for *Borrelia* spp.,
203 *Rickettsia* spp., and *Anaplasma phagocytophilum* in comparison with *Ixodes ricinus*.
204 Parasitol. Res. 118, 3205-3216. <https://doi.org/10.1007/s00436-019-06506-4>.
- 205 Kholodilov, I., Belova, O., Burenkova, L., Korotkov, Y., Romanova, L., Morozova, L.,
206 Kudriavtsev, V., Gmyl, L., Belyaletdinova, I., Chumakov, A., Chumakova, N., Dargyn,
207 O., Galatsevich, N., Gmyl, A., Mikhailov, M., Oorzhak, N., Polienko, A., Saryglar, A.,
208 Volok, V., Yakovlev, A., Karganova, G., 2019. Ixodid ticks and tick-borne encephalitis

- 209 virus prevalence in the South Asian part of Russia (Republic of Tuva). *Ticks Tick-borne*
210 *Dis.* 10, 959-969.
- 211 Kovalev, S. Y., Mukhacheva, T. A., 2017. Reconsidering the classification of tick-borne
212 encephalitis virus within the Siberian subtype gives new insights into its evolutionary
213 history. *Infect. Genet. Evol.* 55, 159-165.
- 214 Labuda, M., Nuttall, P., Kozuch, O., Eleckova, E., Willians, T., Zuffova, E., Sabo, A., 1993.
215 Non-viraemic transmission of tick-borne encephalitis virus: a mechanism for arbovirus
216 survival in nature. *Experientia* 49, 802-805.
- 217 Lommano, E., Burri, C., Maeder, G., Guerne, M., Bastic, V., Patalas, E., Gern, L., 2012.
218 Prevalence and genotyping of tick-borne encephalitis virus in questing *Ixodes ricinus*
219 ticks in a new endemic area in Western Switzerland. *J. Med. Entomol.* 49, 156-164.
- 220 Lommano, E., Dvorák, C., Vallotton, L., Jenni, L., Gern, L., 2014. Tick-borne pathogens in
221 ticks from breeding and migratory birds in Switzerland. *Ticks Tick-borne Dis.* 5, 871-
222 882,
- 223 Mangold, A. J., Bargues, M. D., Mas-Coma, S., 1998. Mitochondrial 16S rRNA sequences
224 and phylogenetic relationships of *Rhipicephalus* and other tick genera among
225 *Metastricata* (Acari: ixodidae). *Parasitol. Res.* 84, 478-484.
- 226 Nouredine, R., Chauvin, A., Plantard, O., 2011. Lack of genetic structure among Eurasian
227 populations of the tick *Ixodes ricinus* contrasts with marked divergence from north-
228 African populations. *Inter. J. Parasitol.* 41, 183-192.
- 229 Pérez, C., 2007. *Les Tiques: Identification, Biologie, Importance Médicale et Vétérinaire.*
230 Lavoisier, Paris, France.
- 231 Piesman, J., Gern, L., 2008. Lyme Borreliosis in Europe and North America. In: Bowman, A.,
232 Nuttall, P. (Eds.), *Ticks: Biology, Disease and Control.* Cambridge University Press,
233 Cambridge, pp. 220-253.

- 234 Randolph, S. E., Miklisova, D., Lysy, J., Rogers, D.J., Labuda, L., 1999. Incidence from
235 coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne
236 encephalitis virus. *Parasitology* 118, 177-186.
- 237 Spagnolo, E.V., Mondello, C., Rocuzzo, S., Cardia, L., Raffino, C., 2018. A lethal tick-borne
238 encephalitis (TBE) due to TBE virus in Sicily (Italy): a case of IgG+/IgM- response?
239 *Clin. Ter.* 169, e145-148.
- 240 Scaramozzino, N., Crance, J.M., Jouan, A., DeBriel, D.A., Stoll, F., Garin, D., 2001.
241 Comparison of flavivirus universal primer pairs and development of a rapid, highly
242 sensitive heminested reverse transcription-PCR assay for detection of flaviviruses
243 targeted to a conserved region of the NS5 gene sequences. *J. Clin. Microbiol.* 39, 1922-
244 1927.
- 245 Süss, J., 2011. Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in
246 Europe and Asia-an overview. *Ticks Tick-borne Dis.* 2, 2-15.
- 247 Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5.
248 Molecular evolutionary genetics analysis using maximum likelihood, evolutionary
249 distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731-2739.
- 250 Waldenström, J., Lundkvist, A., Falk, K.I., Garpmo, U., Bergström, S., Lindgren, G., Sjöstedt,
251 A., Mejlom, H., Fransson, T., Haemig, P. D., Olsen, B., 2007. Migrating birds and tick-
252 borne encephalitis virus. *Emerg. Infect. Dis.* 13, 1215-1218.
- 253 Younsi, H., Sarih, M., Jouda, F., Godfroid, E., Gern, L., Bouattour, A., Baranton, G., Postic,
254 D., 2005. Characterization of *Borrelia lusitaniae* Isolates collected in Tunisia and
255 Morocco. *J. Clin. Microbiol.* 43, 1587-1593.
- 256 Younsi H., Fares, W., Cherni, S., Dachraoui, K., Barhoumi, W, Najjar, C., Zhioua, E., 2019.
257 *Ixodes inopinatus* and *Ixodes ricinus* (Acari: Ixodidae) are sympatric ticks in North
258 Africa. *J. Med. Entomol.* 57, 952-956.

- 259 Zhioua, E., Gern, L., Aeschlimann, A., 1989. Isolement d'un spirochète à partir d'*Ixodes*
260 *ricinus* de Tunisie. Bull. Soc. Franç. Parasitol. 7, 107-110.
- 261 Zhioua, E, Bouattour, A., Hu, C.M., Gharbi, M., Aeschlimann, A., Ginsberg, H.S., Gern, L.,
262 1999. Infection of *Ixodes ricinus* (Acari: Ixodidae) by *Borrelia burgdorferi* sensu lato in
263 North Africa. J. Med. Entomol. 36, 216-218.
- 264 Zlobin, V. I., Demina, T. V., Mamaev, L. V., Butina, T. V., Belikov, S. I., Gorin, O. Z.,
265 Dzhioev I, P.,Verkhozina, M. M., Kozlova, I. V., Voronko, I. V., Adel'shin, R. V.,
266 Grachev, M. A., 2001. Analysis of genetic variability of strains of tick-borne
267 encephalitis virus by primary structure of a fragment of the membrane protein E gene.
268 Vopr. Virusol. 46, 12-16 (in Russian).
- 269
- 270
- 271

272 Figure Caption

273 Figure 1. Phylogenetic analysis was performed on the partial polymerase 218-nt aligned
274 sequences of 24 LIV strains, 74 TBEV strains retrieved from the GenBank database and the
275 Tunisian TBEV sequence (KY412768, in bold) obtained during this study. The Omsk
276 hemorrhagic fever virus (OHFV) sequence was used as an out-group with TBEV sequences.
277 Phylogenetic tree was obtained according to the maximum likelihood analysis method and the
278 tamura-3 model with general time-reversible rates among sites. The tree topology was
279 supported by 500 bootstrap replicates.

280

281

282

283

284

