Prevalence and Genotyping of Tick-Borne Encephalitis Virus in Questing *Ixodes ricinus* Ticks in a New Endemic Area in Western Switzerland

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ABSTRACT Tick-borne encephalitis virus (TBEV) is the causative agent of tick-borne encephalitis (TBE) and causes neurological disease in humans in Eurasia. TBEV is transmitted by ticks of the genus *Ixodes.* Currently 10,000–12,000 clinical cases are reported annually in ≈30 TBE endemic countries. Since 1990 the epidemiology of TBE is characterized by a global increase of clinical cases and an expansion of risk areas. Similar trends are also observed in Switzerland but few studies confirmed the emergence of new TBE foci by detecting viral RNA in field-collected ticks. In this study, free-living *Ixodes ricinus* (L.) ticks from one nonendemic and three new TBE endemic regions located in the Western part of Switzerland were screened during four consecutive years (2007–2010) for the presence of TBEV. A total of 9,868 *I. ricinus* ticks (6,665 nymphs and 3,203 adults) were examined in pools for TBEV by real-time reverse transcription polymerase chain reaction. Our results confirmed the presence of viral RNA in 0.1% (6/6120) of questing ticks collected in one new endemic region. Among TBE endemic sites, the minimal infection rate per 100 ticks tested ranged from 0.21 (1/477) to 0.95 (1/105). Four positive samples were sequenced and phylogenetic analysis of the NS5 gene showed that all TBEV nucleotide sequences belonged to the European subtype and were split into two distinct lineages originating probably independently from two distinct foci located North–East and East of the study region.

KEY WORDS tick-borne encephalitis virus, *Ixodes ricinus*, Switzerland, phylogeny

Tick-borne encephalitis (TBE) is a viral zoonosis that can affect humans after the bite of infected *Ixodes ricinus* ticks in Western Europe and *Ixodes persulcatus* in Eastern Europe and Asia. The causal agent, the tick-borne encephalitis virus (TBEV) is an arbovirus, member of the Flaviviridae family and of the *Flavivirus* genus (Mandl et al. 1997). Three TBEV subtypes are distinguished: the European, the Far Eastern and the Siberian subtypes (Ecker et al. 1999).

TBE is endemic in many Eurasian countries and has a wide geographic occurrence extending from Eastern France to Japan (Hayasaka et al. 1999). Since 1990 the number of reported cases has increased in many European countries where TBEV is present (Süss 2008, Randolph et al. 2008). For example, between 1976 and 1989, an average of 2,755 TBE cases was documented annually in Europe and Russia while between 1990 and 2007 the average was 8,755 annual cases (Süss 2008).

In Switzerland, such an increase in the number of cases was also observed with a peak of 245 cases reported in 2006 while there was a mean of 92 annual cases between 1995 and 2004 (Randolph et al. 2008). However, since 2006, the annual incidence is again around 100 cases (Swiss Federal Office of Public Health [SFOPH] 2010). Krech et al. (1969) and Spiess et al. (1969) reported the first human cases of TBE in the North–East of Switzerland in 1969. Since then and until 1999 all TBE cases were limited to the known distribution perimeter of TBE endemic areas reported by Wyler and Matile in 1984. This perimeter included the North and Eastern parts of the country with the East of the lake of Neuchâtel as western limit. However, between 2000 and 2006, TBE cases were registered West and South of the Lake of Neuchâtel, outside this perimeter (de Vallière et al. 2006, Schlaefli et al. 2007) and in the Jura Mountains (SFOPH 2007). Thereby, three new TBE endemic regions were recognized by the SFOPH in the Western part of the country (SFOPH 2007). In Switzerland, an area is considered as TBE endemic by the SFOPH when three clinical cases have been reported in this area or when TBEV has been detected in its vector *I. ricinus.* Nevertheless, very few studies have been conducted in this country on the prevalence of TBEV in *I. ricinus* ticks. The most recent studies reported TBEV in ticks from TBE endemic sites with a prevalence of the virus ranging from 0.04% to 14.3% (Wicki et al. 2000, Casati

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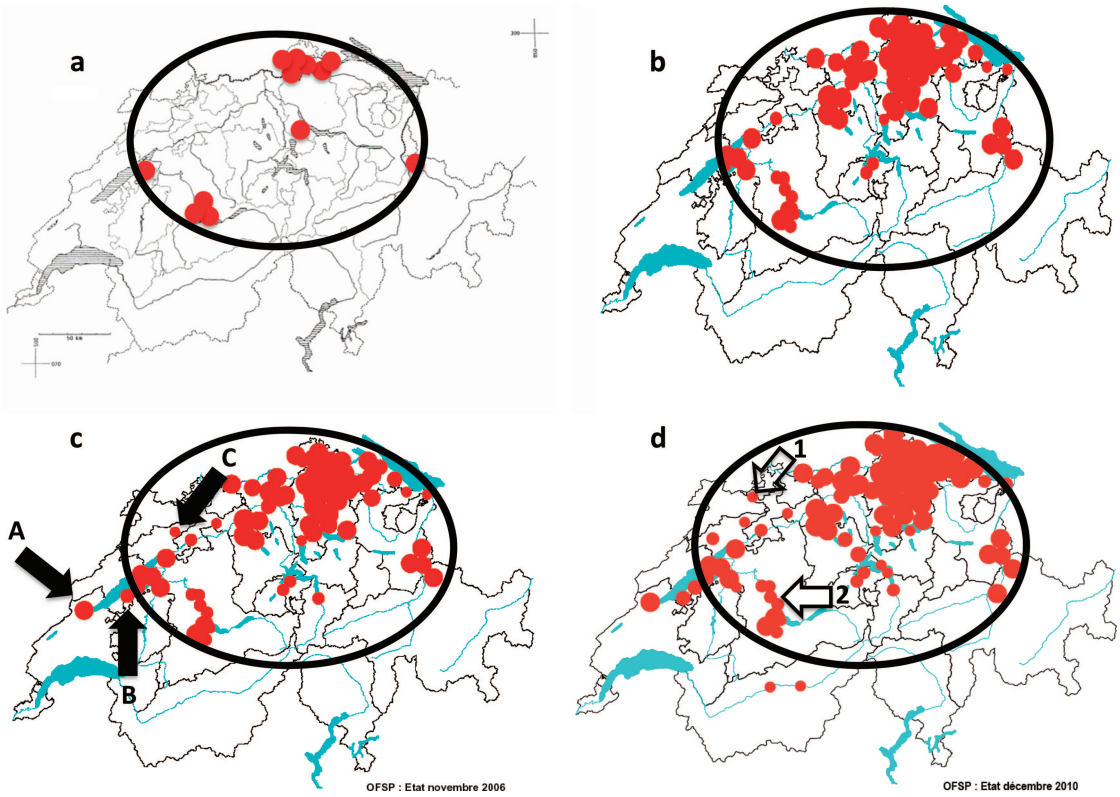

Fig. 1. Temporal evolution of the geographic distribution of TBE endemic areas in Switzerland. (a) Situation in 1984 according to Wyler and Matile (1984), (b) Situation in March 2006 (modified from SFOPH 2006), (c) Situation in November 2006 (modified from SFOPH 2007), (d) Situation in November 2010 (modified from SFOPH 2010). A circle represents the perimeter of the geographic distribution of TBE endemic areas (dots) between 1984 until March 2006. Black arrow A: Region A, black arrow B: Region B, black arrow C: Region C, empty arrow 1: Liestal (BL), empty arrow 2: Thun/Belp (BE). (Online figure in color.)

et al. 2006, Burri et al. 2011). Finally a study based on a national screening to assess prevalence of TBEV in *I. ricinus* ticks from 165 collection sites reported a mean TBEV prevalence of 0.46% (Gäumann et al. 2010).

Here, we screened for TBEV questing *I. ricinus* ticks collected at 49 sites located in the three new TBE endemic regions (SFOPH 2007) and in a neighboring nonendemic region (North of the Lake of Neuchâtel) to determine the geographic distribution of TBEV infected ticks. We also analyzed the genetic diversity of TBEV strains and their phylogeny to evaluate whether TBEV might have been imported in these new endemic regions from other parts of Switzerland.

Materials and Methods

Study Area and Tick Sampling. This study was carried out in the Western part of Switzerland in three new TBE endemic regions located outside a perimeter of distribution of TBE endemic sites delimited since 1984 by Wyler and Matile (1984) (Fig. 1). The studied regions were the following: region A (Plaine de l'Orbe), region B (South of the Lake of Neuchâtel), and region C (Jura Mountains, Moutier) (Fig. 1c, Fig. 2). In addition, investigation of TBEV in ticks was also conducted in the periurban forest of Neuchâtel, region D (North of the Lake of Neuchâtel) (Fig. 2).

Ticks were collected at 26 sites in endemic region A, 14 sites in region B and at two sites in region C (Table 1). Most of these sites were selected according to TBE cases reported by de Vallière et al. (2006), Schlaefli et al. (2007), and SFOPH (2007). In region D, seven sites were selected (Table 1). All sites were localized in mixed deciduous forests.

Questing *I. ricinus* ticks were collected with a 1-m^2 white flag in spring and summer of 2007, 2008, 2009, and 2010. Each site was prospected for TBEV between one and four times during the study period. All collected ticks were washed in ethanol 70%, dried, pooled from 1 to 25 nymphs and from 1 to 12 males or females and stored at −20°C until processed for TBEV detection.

TBEV RNA Isolation and Amplification. Ticks were crushed in pools in 300 μl of TRIzol (Invitrogen, Basel, Switzerland) containing a 3-mm ball with a mixer mill MM 300 (Retsch, Arlesheim, Switzerland) during 3 min for nymphs and 5 min for adults. RNA was extracted according to Invitrogen protocol (Chomczynski and Sacchi 1987). Incubation times were 10 min for

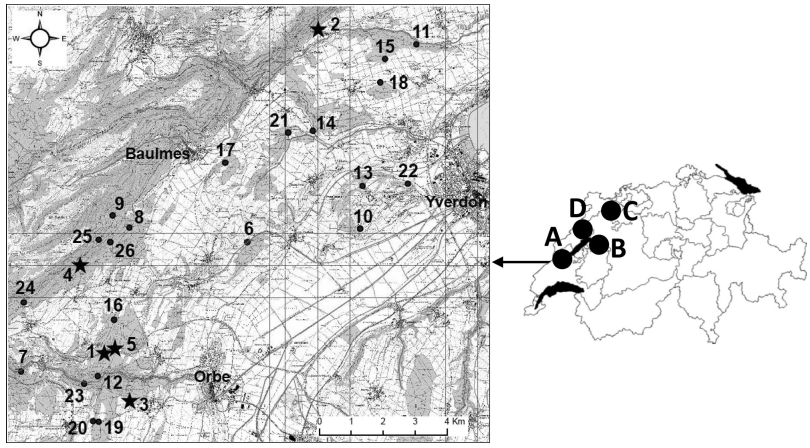

Fig. 2. Geographic location of TBE endemic regions A (West of the lake of Neuchâtel), B (South of the lake of Neuchâtel), C (Jura Mountains, Moutier), and TBE nonendemic region D (North of the Lake of Neuchâtel) where questing ticks were collected. For TBEV endemic region A, tick sampling sites are shown with black dots and stars indicate that TBE infected ticks were sampled at these sites. Sites 1: Montcherand 2; 2: Vugelles, 3: Agiez; 4: Abergement; 5: Montcherand 1; 6: Mathod; 7: Les Clées 1; 8: Le Suchet 1; 9: Le Suchet 2; 10: Suscevaz; 11: Fiez; 12: Montcherand 3; 13: Chamblon; 14: Mornens; 15: Giez; 16: La Russille; 17: Baulmes; 18: Orges; 19: Bofflens 1; 20: Bofflens 2; 21: Champvent; 22: Les Uttins; 23: Les Clées 2; 24: Lignerolle; 25: Le Gothard; 26: Conrad Bourgeois.

chloroform and 10 min for isopropanol. Centrifugation time after adjunction of isopropanol was 15 min and 6 min after adjunction of 75% ethanol. Samples were then dried for 30–40 min, eluted in 30 μl of RNase-free water (Qiagen, Hombrechtikon, Switzerland) and stored at −20°C until analysis by real-time reverse transcription (RT)-polymerase chain reaction (PCR). A negative control was included during each extraction procedure consisting of reagents without ticks.

TBEV RNA reverse transcription and amplification were performed in an iCycler (Biorad, Reinach, Basel Land, Switzerland) as described in Burri et al. (2011) and modified from Schwaiger and Cassinotti (2003). Reaction volume (25 μl) consisted in 12.5 μl of reaction mix (containing dNTPs, 0.04 mM each), primers and probes: 3 μM of F-TBE1 (5′GGG CGG TTC TTG TTC TCC 3′), 0.6 μM of R-TBE1 (5′ACA CAT CAC CTC CTT GTC AGA CT 3′), and 0.8 μM of probe TBE-WT (5′FAM-TGA GCC ACC ATC ACC CAG ACA CA-TAMRA 3′), 0.5 μl Superscript III Platinum *Taq* (Invitrogen, Basel, Switzerland, Superscript III Platinum One-step quantitative system) and 5 μl of template RNA. The TBEV RNA extract was first reverse transcribed into complementary DNA (cDNA) at 42°C for 30 min and then incubated for 10 min at 95°C. Directly after reverse transcription the noncoding region localized in 3′ (NCR3′) was amplified at 95°C for 15 s, 60°C for 1 min during 45 cycles according to Schwaiger and Cassinotti (2003). To monitor the real-time RT-PCR, a human TBEV isolate was used as positive control (provided by P. de Mendonça, Ludwig-Maximillians-Universität, München, Germany). Negative controls (5 μl RNase-free water; Qiagen) were included during the real-time RT-PCR amplification steps to exclude false-positive results.

Confirmation of Positive Real-Time RT-PCR Results and Sequencing of NS5 Gene. Amplicons that were positive by real-time RT-PCR were confirmed by amplifying the nonstructural protein NS5 using primers described in Puchhammer-Stöckl et al. (1995). Reaction mix for amplification was modified after Saksida et al. (2005). Before amplification 10 μl of RNA was transcribed into cDNA according to Invitrogen protocol. The first amplification was performed in a reaction volume of 50 μl containing 5 μl of Qiagen Buffer 10×, 200 μM of each dNTP, 0.2 μM each of primers FSM-1 and FSM-2 (Puchhammer-Stöckl et al. 1995), 1.5 U per test of DNA *Taq* polymerase (Qiagen) and 10 μl of cDNA. Amplification was performed in 40 cycles (94°C, 30 s; 40°C, 30 s; 72°C, 30 s) followed by a 5 min elongation step (72°C). For the nested PCR, a 50 μl reaction mixture consisting in 5 μl of Qiagen Buffer 10×, 200 μM of each dNTP, 0.2 μM each of inner primers FSM-1i and FSM-2i (Puchhammer-Stöckl et al. 1995), 1.5 U per test of DNA *Taq* polymerase and 2 μl of amplified DNA was used. The nested PCR program used the following conditions: 94°C, 2 min for an initial denaturation step followed by 40 cycles of amplification (94°C, 30 s; 53°C, 30 s; 72°C, 30 s) and 72°C, 5 min for the final elongation. Obtained amplified products (252 nucleotides) were visualized using an agarose gel 2% stained with red gel (Brunschwig, Basel, Switzerland) and visualized under UV light. All amplified products detected positive for TBEV were purified with a kit (Promega, Madison, WI) and sent for sequencing to Microsynth (Balgach, Switzerland).

Estimation of Infection Prevalence. TBEV infection prevalence in ticks was expressed as the minimum infection rate (MIR) per 100 tested ticks based on the

Table 1. Details on collection sites of questing ticks and TBEV infection in ticks

Sampling sites		GPS coordinates		No tested ticks	No positive pools
		Latitude	Longitude		
Region A	Les Clées 1	46°43′42″ N	6°27′15″ E	74	0
	Les Clées 2	46°43′31″ N	6°28′47″ E	96	0
	Le Suchet 1	46°46′8″ N	6°29′51″ E	139	0
	Le Suchet 2	46°46′20″ N	6°29′26″ E	96	0
	Suscevaz	46°46′10″ N	6°35′31″ E	172	0
	Fiez	46°49′16″ N	6°36′51″ E	95	0
	Montcherand 1	46°44′7″ N	6°29′31″ E	675	1
	Montcherand 2	46°44′2″ N	6°29′16″ E	901	1
	Montcherand 3	46°43′38″ N	6°29′7″ E	109	0
	Vugelles	46°49′30″ N	6°34′26″ E	459	1
	Chamblon	46°46′52″ N	6°35′34″ E	186	0
	Mornens	46°47′48″ N	6°34′20″ E	215	0
	Giez	46°49′1″ N	6°36′5″ E	156	0
	La Russille	46°44′35″ N	6°29′30″ E	283	0
	Baulmes	46°47′15″ N	6°32′11″ E	192	0
	Orges	46°48′37″ N	6°35′59″ E	97	0
	Bofflens 1	46°42′52″ N	6°29′9″ E	130	0
	Bofflens 2	46°42′53″ N	6°29′1″ E	97	0
	Champvent	46°47′46″ N	6°33′44″ E	153	0
	Agiez	46°43′14″ N	6°29′54″ E	735	2
	Les Uttins	46°46′56″ N	6°36′41″ E	125	0
	Mathod	46°45′55″ N	6°32′45″ E	137	0
	Lignerolle	46°44′51″ N	6°27′18″ E	21	0
	Le Gothard	46°45′55″ N	6°29′6″ E	169	0
	Conrad Bourgeois	46°45′53″ N	6°29′23″ E	71	0
	L'Abergement	46°45′30″ N	6°28′39″ E	537	1
Total				6,120	6
Region B	Portalban	46°55′8″ N	6°58′10″ E	179	0
	Chabrey 1	46°55′45″ N	6°59′45″ E	105	0
	Chabrey 2	46°55′43″ N	6°59′44″ E	32	0
	Chabrey 3	46°55′30″ N	6°59′57″ E	114	0
	Chabrey 4	46°55′25″ N	6°59′35″ E	70	0
	Chabrey 5	46°55′59″ N	7°0′21″ E	10	0
	Salavaux 1	46°54′7″ N	7°2′58″ E	18	0
	Salavaux 2	46°54′21″ N	7°2′35″ E	505	0
	Salavaux 3	46°54′55″ N	7°1′60″ E	7	0
	Salavaux 4	46°54′16″ N	7°2′40″ E	98	0
	Cudrefin 1	46°56′13″ N	6°59′8″ E	81	0
	Cudrefin 2	46°58′13″ N	7°2′41″ E	433	0
	Cudrefin 3	46°58′16″ N	7°2′35″ E	17	0
	Cudrefin refuge	46°56′7″ N	7°0′14″ E	12	0
Total				1,681	0
Region C	Moutier 1	47°16′57″ N	7°23′25″ E	43	0
	Moutier 2	47°17′17″ N	7°22′15″ E	156	0
Total				199	0
Region D	Bevaix	46°55′11″ N	6°47′2″ E	226	0
	Hauterive 1	47°0′60″ N	6°58′18″ E	61	0
	Hauterive 2	47°1′11″ N	6°58′30″ E	448	0
	Cadolles	47°0′6″ N	6°55′26″ E	484	0
	Bois de l'Hôpital	47°0′19″ N	6°56′54″ E	283	0
	La Coudre	47°0′46″ N	6°57′37″ E	332	0
	Colombier	46°58′28″ N	6°51′16″ E	34	0
Total				1,868	0
Total				9,868	6

assumption that at least one tick was positive within a positive pool.

Phylogenetic Analyses. NS5 sequences of different TBEV strains and a closely related flavivirus chosen from the NCBI GenBank database were used for genetic comparison: Omsk hemorrhagic fever (GenBank accession number AY323489); Siberian strain Vaslichenko (AF069066); Russian strains Zausaev (AF527415), 886–84 (EF469662), 178–79 (EF469661), and Glubinnoe 2004 (DQ862460); Finnish strains Kokkola-8 (DQ451298), Kokkola-84 (DQ451305), Kokkola-86 (DQ451307), Kokkola-4 (DQ451297), Kokkola-39 (DQ451302), and Kumlinge (AJ298321); Sofjin-HO strain (AB062064); MDJ-01 (AY217093); Senzhang (AY182009); Turkish strain (DQ235151); Greek strain (DQ235153); Slovenian strains Stefanja Gora (EU057639), Sodraica (EU057638), and Kamnik EU057641; Czech Republic strain (DQ153877); Austrian strains Hypr (U39292), Neudoerfl (U27495), and U27491; Italian strains FVG Pt12 (FJ159007), FVG BM Forni di Sotto (FJ159002), and FVG ML Raccolana (FJ159003); Lithuanian strains Lith413 (DQ112086), Lith414 (DQ112087), and Lith418 (DQ112088); German strain K23 (AM600965); and Swiss strains NETBE1 (HM450136), NETBE2 (HM450137),

Table 2. Details on TBEV detected in ticks collected at five sites located in region A

Sites[a]	Locality	Sampling year	No infected/ analyzed ticks	MIR[b]	95% CI	NS5 sequence name	Accession no
1	Montcherand 2	2007	1/170	0.59	0.02–3.23	—	—
2	Vugelles	2007	1/339	0.29	0.01–1.63	NETBE8	HQ883373
3	Agiez	2008	1/105	0.95	0.02–5.19	NETBE9	HQ883374
3	Agiez	2009	1/477	0.21	0.01–1.16	NETBE10	HQ883375
4	Abergement	2009	1/281	0.36	0.01–1.97	—	—
5	Montcherand 1	2010	1/249	0.40	0.01–2.22	NETBE11	HQ883376

[a] Site numbers refer to site numbers shown on Fig. 2.
[b] Prevalence rate is expressed as the minimal infection rate (MIR) per 100 ticks tested.

NETBE3 (HM450138), NETBE4 (HM450139), NETBE5 (HM450140), NETBE6 (HM450141), NETBE7 (HQ883372).

Phylogenetic analyses were performed using Bioedit (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and PHYLIP 3.69 (http://evolution.gs.washington.edu/phylip/getme.html) (Felsenstein 1993). First 1000 bootstrap replicates of the sequence data (SEQBOOT) were executed. Then, distance matrices were calculated by using Kimura's two-parameter model (DNADIST) and analyzed by the neighbor-joining algorithm (NEIGHBOR). Alternatively, the DNAPARS program was used to find the trees with maximum parsimony. The bootstrap support percentages of particular branching points were calculated from these trees (CONSENSE). The resulting phylogenetic tree was presented using the program TreeView 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) (Page 1996). Alignment of the sequences was performed using ClustalW2.0.12 (Thompson et al. 1994).

Results

Between 2007 and 2010, a total of 9,868 *I. ricinus* ticks (6,665 nymphs, 1,699 males, and 1,504 females) collected at 49 sites (Table 1) in four different regions (A, B, C, and D) located in the Western part of Switzerland (Fig. 2) were tested for the presence of TBEV by pools.

In region A, a total of 6,120 ticks (3,965 nymphs and 2,155 adults) collected at 26 sites were tested for TBEV in 602 pools (265 pools of 1–25 nymphs and 337 pools of 1–12 adults) (Table 1). TBEV-specific RNA was detected in 6/602 pools of ticks collected at 5/26 sites (Table 2; Fig. 2). Two pools of eight and 20 nymphs were infected in 2007, one pool of 10 females in 2008, two pools of 10 and 20 nymphs in 2009 and one pool of 11 nymphs in 2010. Overall, the MIR per 100 tested nymphs for TBEV was 0.13 (5/3965 [95% CI: 0.04–013]) and 0.1 for females (1/1023 [95% CI: 0–0.54]) corresponding to a global MIR of 0.1 (6/6120 [95% CI: 0.04–0.21]). No male was found infected with TBEV. Among the five sites where TBEV-specific RNA was detected in ticks, the MIR per 100 tested ticks ranged from 0.21 (1/477 [95% CI: 0.01–1.16]) (site 3, in 2009) to 0.95 (1/105 [95% CI: 0.02–5.19]) (site 3, in 2008) (Table 2) and the mean of MIR was 0.37 (6/1621 [95% CI: 0.14–0.8]) per 100 tested ticks. At one site (site 3), we could detect TBEV in ticks over two consecutive years (2008 and 2009) whereas in all other sites the virus was detected only sporadically in ticks over the study period although at two sites (sites 1 and 5) ticks were collected during four consecutive years (Fig. 2; Table 2). No TBEV could be detected in ticks collected in TBE endemic regions B (n = 1,681) and C (n = 199) nor in the nonendemic region D (n = 1,868).

Sequencing of the TBEV NS5 gene was possible for 4/6 positive samples and resulted in 209–211 nt amplicons (corresponding to the 252 nt bands without primers sequences). The NS5 gene sequences were submitted to the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/PubMed/) under the following accession numbers: HQ883373 (NETBE8), HQ883374 (NETBE9), HQ883375 (NETBE10), and HQ883376 (NETBE11) (Table 2). All TBEV sequences obtained in this study belonged to the European subtype relating them closely to Neudoerfl strain (U27495) but none of them were 100% identical to Neudoerfl strain. To relate the obtained TBEV nucleotide sequences with other TBEV sequences available in GenBank, a phylogenetic tree was constructed on the basis of the NS5 gene (Fig. 3). Two different lineages of TBEV sequences were clearly distinguishable in this region. First, a group of three genetically identical sequences (from sites 3 and 5, NETBE9, NETBE10, and NETBE11) showed 100% homology with a previously identified sequence (strain NETBE7, HQ883372) (Fig. 4) obtained from ticks collected at Liesberg, Canton Basel Land (BL), a new endemic region situated inside the perimeter of TBEV distribution known since 1984, North East of region A (Fig. 1d) (SFOPH 2010). Second, one single sequence (from site 2, NETBE8) segregated in a group of strains (HM450136, HM450137, HM450138, HM450140, HM450141; Burri et al. 2011) identified in ticks collected at Thun/Belp, Canton Bern, an endemic region located East of region A (Fig. 1d) and also included in the perimeter of TBEV distribution known since 1984, although this strain showed a divergence of 2.4% with the other sequences (Figs. 3 and 4).

Discussion

TBE is an increasing health problem in Eurasian countries with thousands of cases reported annually (Süss 2003). The distribution maps are generally elaborated with registered autochthonous cases of the

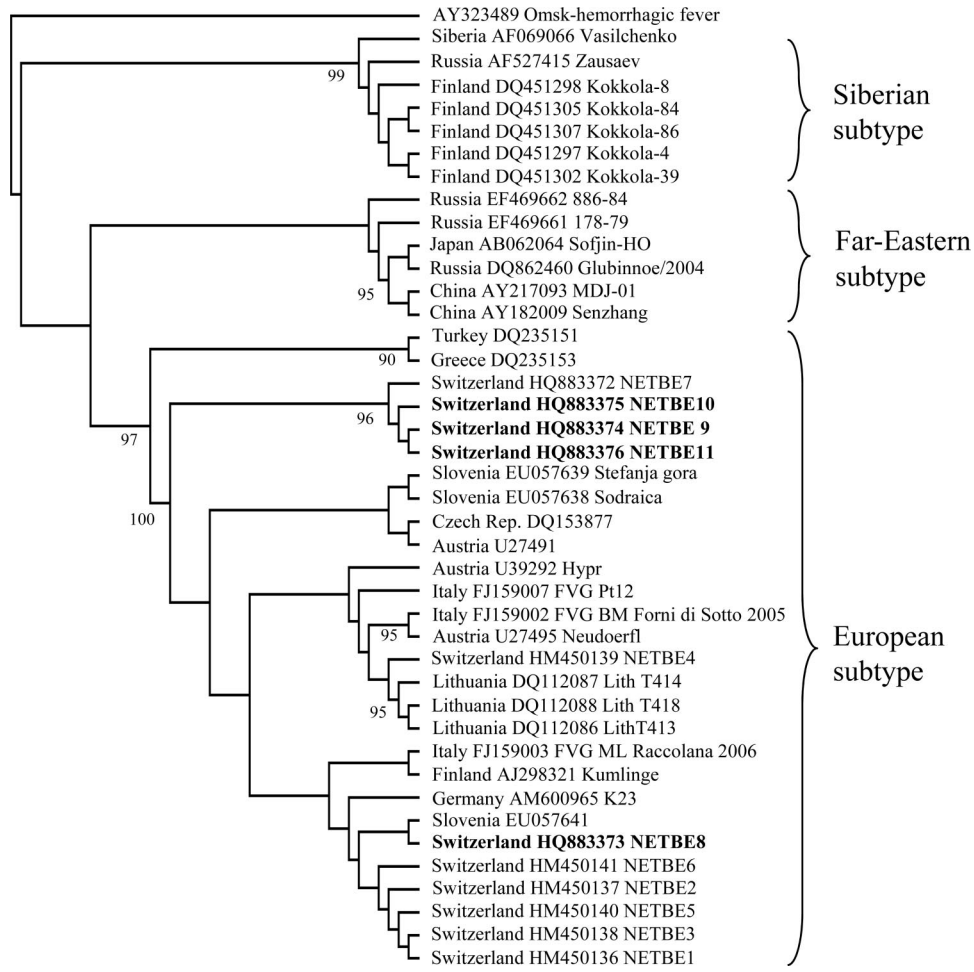

Fig. 3. Phylogenetic tree based on the NS5 gene. Only bootstrap values ≥90% are shown. The tree is rooted by the Omsk-hemorragic fever virus. The TBEV sequences obtained in this study are shown in bold.

disease and few studies investigated the prevalence of TBEV in questing ticks. In Switzerland recent studies estimated the TBEV prevalence in free-living ticks (Wicki et al. 2000, Casati et al. 2006, Gäumann et al. 2010, Burri et al. 2011). During the last decade new TBE endemic areas emerged in the Western part of the country following the recording of clinical cases (SFOPH 2007). Therefore, we focused our attention on these new TBE endemic regions (regions A, B, and C) as well as on a neighboring region (region D). We investigated questing *I. ricinus* ticks for TBEV during four consecutive years to evaluate TBE risks. In this study, TBEV could not be detected in ticks collected in two out of three endemic regions (B and C) nor in the nonendemic region D. In region B, clinical cases were recently reported by de Vallière et al. (2006) and in 2010, Gäumann et al. detected TBEV in one pool of ticks with a local prevalence of 0.2% (1/500, Cudrefin). An explanation for this discrepancy could be that our sampling site did not exactly overlap the TBE site investigated by Gäumann et al. (2010). In fact, it has been reported that within a focus TBEV infected ticks are not uniformly distributed (Blaskovic and Nosek 1972). Clinical cases were reported in region C (Jura Mountains, Moutier) (SFOPH 2006) but we were not able to confirm the presence of TBEV in ticks. Similarly, Gäumann et al. (2010) were not able to detect TBEV in ticks collected in this region. Part of explanation can be that the sampling sizes in both studies were too small ($n = 199$ in this study and $n = 392$ in Gäumann et al. 2010) to detect TBEV in ticks all the more so because the prevalence of TBEV in ticks is low. Nonendemic region D, located North of the Lake of Neuchâtel, was considered in this study because it is adjacent to known TBE endemic areas (SFOPH, http://www.bag.admin.ch/themen/medizin/00682/00684/01069/index.html?lang = fr). As no clinical TBE cases have been reported in this region and no TBEV has been detected in ticks ($n = 1868$) we conclude that currently the risk of TBEV infection is either very low or even absent in this region.

The presence of TBEV in questing ticks could be confirmed in one endemic region (region A, Plaine de l'Orbe), situated West of the Lake of Neuchâtel, with

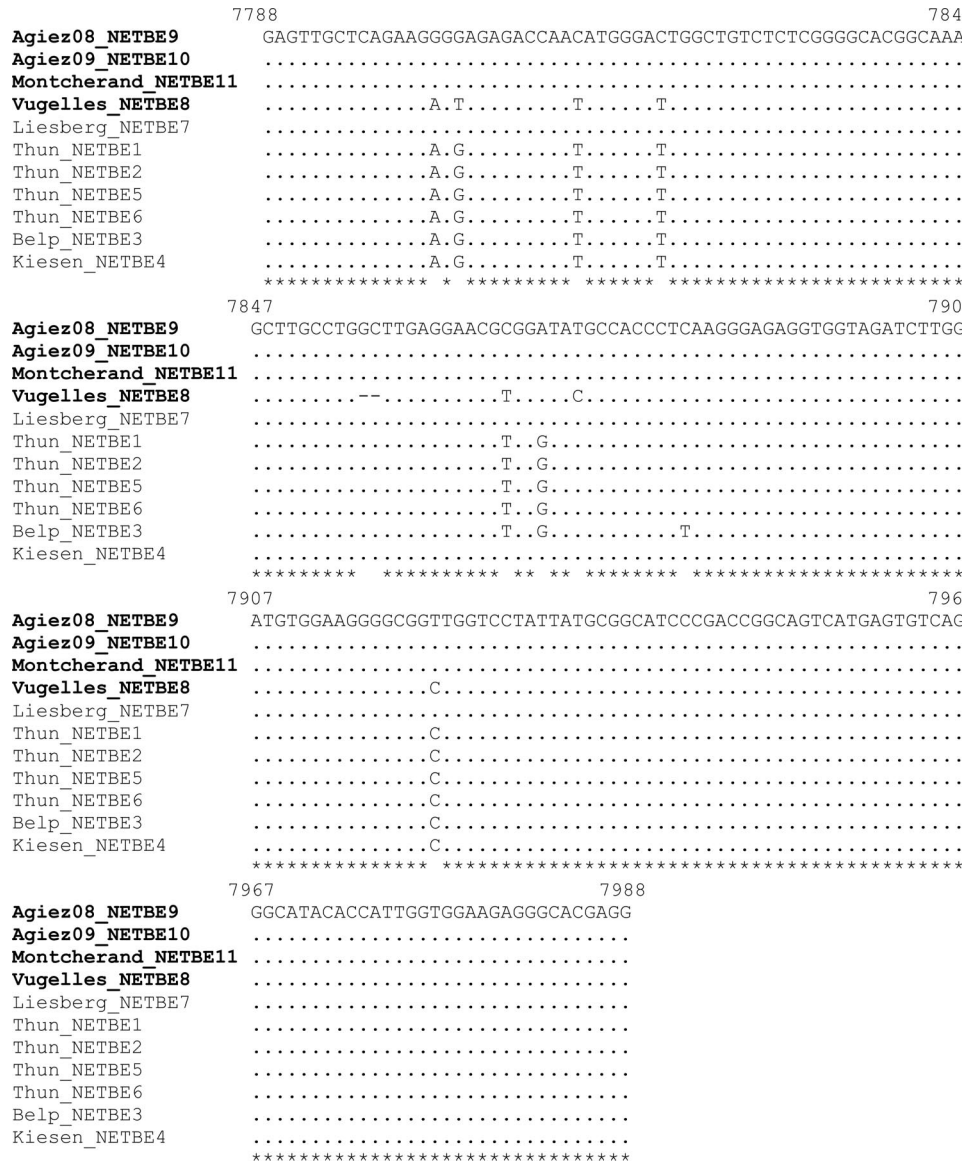

Fig. 4. Comparison of NS5 gene encoding sequences of TBEV strains obtained in this study (in bold) with previously identified Swiss strains (nt 7,788–7,988).

a global MIR of 0.1 per 100 tested ticks (6/6120). This prevalence is in accordance with the study of Burri et al. (2011) carried out in a known endemic region located near Bern (Switzerland) and is also in line with TBEV prevalence recorded in Europe (Durmisi et al. 2011, D'Agaro et al. 2009, Gäumann et al. 2010, Han et al. 2001).

In region A, TBEV infected ticks were detected at 5/26 sites. We could not detect viral RNA in ticks collected at the other investigated sites located in region A, even if they had been reported as endemic by de Vallière et al. (2006), Schlaefli et al. (2007) and SFOPH (2007). That could be explained by the low number of analyzed ticks at each of these sites (<300 ticks) in relation to the low mean TBEV MIR in natural foci (0.37 per 100 tested ticks). This explanation seems to be consistent because at each site where TBEV was detected in ticks at least 450 ticks were screened (Table 1). This is exemplified by results from Gäumann et al. (2010) who reported 2/555 TBEV infected ticks at site six (Fig. 2) whereas we could not detect the virus in the 137 ticks we analyzed.

Phylogenetic analysis provided evidence that TBEV strains circulating in endemic region A belonged to the European subtype and that among them two different clusters were distinguishable. Each of these clusters included strains originating from two geographical distant areas (Liestal, BL and Thun/Belp, BE) in Switzerland located in the perimeter of TBE distribution known since 1984 (Wyler and Matile

1984) (Fig. 1d). The presence of two different lineages in region A, located outside the perimeter, suggests that TBE foci (at site 2, and at sites 3 and 5) emerged independently of each other after the introduction of TBEV infected ticks probably infesting migrating birds coming from the North-East, for example. This is supported by studies reporting that birds may carry TBEV infected ticks (Ernek et al. 1968, Waldenström et al. 2007).

Our results confirm the presence of TBEV in ticks collected in a new TBE endemic region in the Western part of Switzerland and underline risk of exposure to TBEV infected ticks in this region. Phylogenetic analysis showed that TBEV strains obtained from this region belonged to the European subtype. They were closely related to Swiss strains previously identified in the North and East of the country that could be explained by the introduction of TBEV infected ticks via migrating birds into regions where biotic and abiotic conditions were favorable for the maintenance of TBEV in ticks and vertebrate hosts.

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