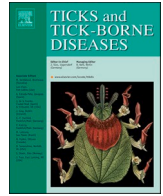





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Short communication

Novel variants of tick-borne encephalitis virus from patient and tick samples in Norway

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ABSTRACT

The annual number of tick-borne encephalitis (TBE) cases in Norway has increased dramatically from 1 case in 1998 to 113 in 2023. Characterization of TBE virus (TBEV) genomes from both clinical samples and tick vectors is necessary to understand disease severity and transmission dynamics. However, clinical samples with intact virus are rare because TBE is usually diagnosed by serology in the post-viremic phase, when the viral load is low and undetectable by molecular methods such as polymerase chain reaction (PCR). To date, Mandal-2009 is the only TBEV sequence from Norway with complete virus genome, sequenced directly from the tick vector. We used a combined approach with newly designed overlapping primer pairs and nanopore sequencing together with Sanger sequencing to obtain nearly complete TBEV genomes from both patient and tick samples from Norway. The patient had severe TBE complicated with hemophagocytic lymphohistiocytosis (HLH). The patient and tick samples were collected 16 km apart, from Telemark and Vestfold Counties, respectively. Pairwise genomic comparison showed 99.7 % identity, and phylogenetic analysis revealed that these sequences were closely related to the TBEV strain from Kumlinge in Åland, Finland, rather than to the previously published Norwegian variant Mandal-2009. These findings confirm the existence of novel TBEV variants in the endemic areas of Telemark and Vestfold Counties of Norway. Our findings highlight the need for continuous monitoring and characterization of novel TBEV genomes in Norway and Europe.

1. Introduction

Tick-borne encephalitis virus (TBEV) is the causative agent of tick-

borne encephalitis (TBE), an infectious disease of the central nervous system (Taba et al., 2017). TBEV is mainly transmitted to human and animals through *Ixodes* spp. tick bites (Licková et al., 2021; Wondim

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et al., 2022). The main reservoir and vector of the TBEV European subtype (TBEV-Eu) to humans is the castor bean tick, *Ixodes ricinus*. Ticks can be transported across long distances and extensive geographical barriers, e.g. by migratory passerine birds and larger mammals like cervids, leading to the introduction of different TBEV genetic variants to new geographical locations (Hasle et al., 2009; Mehl, 1983; Paulsen et al., 2020).

TBEV belongs to the genus *Orthoflavivirus*, and its genome consists of approximately 11 kb of positive-sense single-stranded genomic RNA. The virus genome contains a single open reading frame (ORF) flanked by 5' and 3' non-coding regions (NCRs). The ORF encodes a single polyprotein of about 3,400 amino acids, comprising three structural proteins: Capsid (C), precursor Membrane (prM) and Envelope (E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Paulsen et al., 2021; Ruzek et al., 2019). Differences have been observed in the 3' NCR sequences of TBEV strains isolated from ticks and patient samples. These changes might have occurred during adaptation of the virus from the tick vector to the mammalian host (Asghar et al., 2014). Most of the available TBEV-Eu sequences are derived from ticks, and very few studies have sequenced complete TBEV genomes from clinical samples (Haglund et al., 2003; Saksida et al., 2005; Smura et al., 2019; Zakotnik et al., 2022). TBEV sequences from human clinical samples are rare because the infection is usually diagnosed during the post-viremic phase and the viral loads are often low (Zakotnik et al., 2022).

The aim of this study was to sequence and phylogenetically characterize novel TBEV genetic variants circulating in Norway. We used the published protocols (Paulsen et al., 2021; Quick et al., 2017), to amplify 2,000 bp long overlapping amplicons before sequencing them by the Oxford nanopore technology (ONT). We sequenced and characterized nearly complete TBEV genomes from a patient from Telemark County and tick samples from Vestfold County of Norway and identified the presence of different TBEV variants.

2. Materials and methods

2.1. Ethical approval

The study was approved by the South-Eastern Regional Ethical Committee (Study no 96,505) and the Data Protection Office at all the participating centers. Written informed consent was obtained from the patient. Additional ethical approval and consent for publication is not applicable based on the rules and regulation from the Norwegian ministry of Health and Care Services for Reference functions of infectious agents.

2.2. Case report of patient

In late May 2022, a man in his 60s was hospitalized presenting with fever, dysarthria, tremor, dysphasia, diarrhea, mental slowness, and ataxia. He had a history of spontaneous venous thrombosis in 2007 and was not on any medication as well as unvaccinated against TBEV.

He reported a tick bite in Sandøya, Telemark County, eight days before hospitalization, followed by fever and muscle pain three days later (day 1), which worsened until admission. Following suspicion of having TBE, a lumbar puncture was performed, revealing normal number of white cells, protein, and normal cerebrospinal fluid (CSF) to serum ratio of glucose and albumin in CSF. TBE specific antibodies were negative.

Blood tests showed thrombocytopenia, leukopenia, elevated d-dimer, and elevated liver transaminases. He was treated with antibiotics according to the guidelines for central nervous infections of unknown origin. Microbiological tests for infection in feces were negative. Additionally, the bacterial cultures on CSF, blood, urine were also negative.

Magnetic resonance imaging of the brain was unremarkable, including angiography which ruled out sinus venous thrombosis.

Electroencephalography indicated diffuse cerebral dysfunction. Computer tomography scans of the thorax, abdomen, and pelvis were conducted, revealing enlarged abdominal lymph nodes. Microscopy of bone marrow aspirate revealed lymphoid infiltration and suspected phagocytosis. Flow cytometry of the bone marrow showed findings indicating B-cell lymphoma, which later was confirmed in lymph node biopsy.

Five days after admission, he developed a swollen epiglottis leading to airway obstruction, requiring intubation. PCR of nasopharyngeal aspirate was negative for airborne viruses and bacteria. Blood tests showed markedly raised levels of serum ferritin, interleukin 2 receptor, and triglycerides. Due to his deteriorating condition, suspected hemophagocytic lymphohistiocytosis (HLH), and an unclear diagnosis, he was referred to the tertiary hospital.

After 10 days of symptom debut TBEV was detected through PCR in CSF and serum. He met criteria for HLH, receiving corticosteroids and intravenous immunoglobulin. He was further diagnosed with hospital-acquired pneumonia, and microbiological findings in bronchoalveolar lavage fluid included growth of *Aspergillus fumigatus*, positive Galactomannan test and positive *Pneumocystis jirovecii* PCR. He responded well to targeted antibiotic treatment and required mechanical ventilation for 9 days.

TBEV IgM turned positive on day 29, and he was transferred to rehabilitation department on day 77 of symptom debut. Both IgM and IgG were positive by day 87 and he was discharged home after 113 days. One year later, neurological examination was normal except for nystagmus, though he reported some memory issues. The lymphoma did not require any treatment during hospitalization and is currently under observation. The clinical case timeline of the patient is summarized in Fig. 1, and detailed case report with additional laboratory test results is addressed in Supplement S1.

In this study, we analyzed and sequenced the patient's serum and CSF samples from three different days after symptom debut (Table 1, Fig. 1, Supplement S1).

2.3. Ticks

In September 2021, 74 pools of 10 nymphs each were collected near Farris Lake, Larvik, Vestfold County (SL4 location in Fig. 2), which is located 16 km from Sandøya where the patient got tick bite, as part of a separate tick-survey project. The ticks were collected by dragging a flannel cloth, sorted, pooled, and stored at -80°C , all collected ticks were morphologically identified as *Ixodes ricinus* (Filippova, 1977, 2017; Kjær et al., 2019; Røed et al., 2016; Vikse et al., 2020).

2.4. RNA extraction and cDNA synthesis

A pool of ten nymphs was homogenized in 500 μL Minimum Essential Medium Eagle using MP FastPrep-24 bead beating grinder and lysis system (MP biomedical, Santa Ana, California, USA) and total RNA were extracted by RNeasy Mini kit on QiaCube extractor (QIAGEN GmbH, Hilden, Germany). Viral RNA was extracted manually from patient serum and CSF samples by QIAamp Viral RNA mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The viral RNA was immediately reversely transcribed to cDNA by Superscript IV reverse transcription (RT) kit (ThermoFisher Scientific, Waltham, Massachusetts, USA) using random primers and RNase inhibitor (Applied Biosystems, Foster City, California, USA) according to the manufacturer's protocol. The presence of TBEV in patient and tick samples was analysed by In-house real-time RT-PCR (Andreassen et al., 2012). The PCR-positive samples were confirmed by sequence analysis (SQA) pyrosequencing on a PyroMark Q48 system (Qiagen, Hilden, Germany) according to the manufacturers protocol optimized for the Norwegian strain of TBEV-Eu (Andreassen et al., 2012).

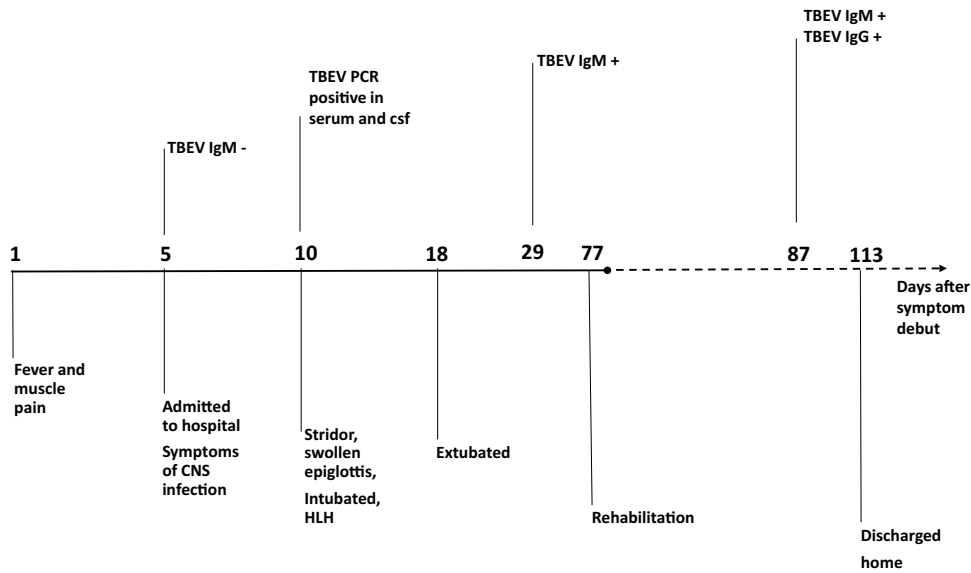


Fig. 1. Timeline of TBE clinical case in a man in 60s in Norway, 2022. The timeline summarizes events from the day of development of symptoms (day 1) to discharge of patient from hospital (day 113).

Table 1
Patient samples collected on different days after symptom debut.

Patient Sample ID	Sample Type	Days after symptom debut
A1	Serum	10
A2	Serum	11
A3	CSF	11
A4	Serum	39

2.5. Whole genome sequencing

For the overlapping amplification of TBEV genome, two primer sets were used: JK (Paulsen et al. 2021) and KUPA (this study) (Supplement

Table S2). To sequence the ORF, PCR was performed with the Q5 high fidelity polymerase enzyme as described in Quick et al. (2017). Library preparation and amplification were performed using Rapid PCR Bar-coding Kit 24 V14 (SQK-RPB114.24; version 14). Sequencing was performed using R10.4.1 flow cell (FLO-MIN114) with the GridION platform (Oxford Nanopore Technology, Oxford, UK), following manufacturer’s recommendations.

Nested PCR was performed to amplify and sequence the ORF termini and NCRs of TBEV genome using KOD Hot Start Master Mix (Sigma-Aldrich) and 0.3 μM of each set of primers as previously described (Asghar et al., 2014). Nested PCR comprised an initial amplification using a first pair of outer primers, followed by a second amplification of a second pair of inner primers. PCR products were confirmed by gel



Fig. 2. Map of Norway indicating sites where the patient got infected (Sandøya, Porsgrunn), and the collection site of ticks (SL4 near Farris Lake, Larvik) in Telemark County and Vestfold County, respectively. The distance between Sandøya and location SL4 is approximately 16 km. Map credit: Kartverket (Norwegian Mapping Authority).

electrophoresis and purified by QIAquick gel extraction kit (Qiagen). Purified amplicons were sent for Sanger sequencing with appropriate primers (Eurofins Genomics, Ebersberg, Germany). The amplified PCR fragments of the 3' NCR were cloned into pcDNA3.1/V5-His-TOPO (Invitrogen) (Asghar et al., 2014) and fifteen individual clones were sequenced from each sample to examine fragment diversity. The experimental workflow performed for genome sequencing is detailed in Supplement Figure S3.

2.6. Sequence data analysis

The sequencing reads from the overlapping amplification of all 3 samples were mapped against all sixty genomes representing the European TBEV genetic diversity were retrieved from NCBI GenBank (criteria: complete CDS and whole genome TBEV-Eu sequences) (Supplement Table S4) by minimap2 version 2.24, with settings "data type: Oxford Nanopore (more sensitive)" in Geneious Prime version 2022.2.2 (Geneious, Auckland, New Zealand). The reference sequence of the Kumlinge A52 strain (GenBank accession no: GU183380) showed the most number of mapped reads and were consequently used for the construction of consensus sequences of the samples. The consensus sequences were generated from the patient and tick samples with settings "Threshold: 25 % Majority, Assign Quality: Total (Call "-" if coverage < 20)". Bases matching at least 25 % of the reads were considered and the entire region had >1000 coverage. When performed BLAST search on the consensus sequences the best match was Kumlinge A52. The consensus sequences obtained from ONT, and Sanger sequencing were merged to reconstruct the whole genome of TBEV.

2.7. Phylogenetic analysis

Multiple sequence alignment was performed by Clustal Omega on TBEV consensus sequences from the patient and tick samples, and genomes listed in Supplement Table S4. Identity matrix summarizing the percentage of identity between the samples and their closest relative was calculated from this sequence alignment. Nucleotide model selection was performed in ModelFinder integrated in IQ-TREE 1.6.12 (Nguyen et al., 2015), followed by maximum-likelihood phylogenetic tree reconstruction with the GTR+*F* + *I* + G4 evolutionary model as best describing the process of evolution. The resultant phylogenetic tree was drawn and annotated in FigTree version 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). The Louping ill virus (GenBank accession number: NC_001809.1, strain 369/T2), a closely related flavivirus, was used as an outgroup.

3. Results

3.1. TBEV detection by PCR

Among the four patient samples, only three tested positive for TBEV by In-house real-time RT-PCR, namely A1-serum (Ct = 28.2), A2-Serum (Ct = 22.3) and A3-CSF (Ct = 27.7), which indicates that these samples were collected during the viremic phase. One of 74 pools was confirmed as TBEV PCR-positive, with a higher viral load (Ct = 16) compared to the patient samples.

3.2. TBEV sequencing

Two sets of primers were used (JK and KUPA) to obtain nearly complete TBEV genome from the patient (A2-Serum and A3-CSF) and tick samples using the overlapping amplicon method. Due to limitation in the overlapping amplicon method obtaining whole genome sequence, we included nested PCR and Sanger sequencing to complete the 5' and 3' NCRs.

The sequences from the overlapping amplicon method, and the nested PCR with Sanger sequencing were assembled to construct the

nearly complete genome of TBEV. The length of TBEV ORF sequences from both patient and tick samples was 10,245 nt. The TBEV sequences from A3-CSF and tick samples (final length 11,057 nt) were submitted to GenBank under accession number PQ614182 and PQ614181, the primer sequences used in the nested PCR and Sanger sequencing (Fwd1_primer and Rev1_primer) were not included in these sequences. The consensus TBEV sequence from A2-Serum was identical to A3-CSF, but due to incomplete sequencing we were unable to retrieve the complete sequence of 5' NCR and 3' NCR and not submitted to GenBank.

When comparing the nearly complete TBEV sequences from A3-CSF and tick samples, we found 99.7 % pairwise identity (Table 2) and identified a total of 30 mutations. Among these, 27 SNPs were in the ORF and 3 in the 3' NCR. The analysis with deduced amino acid sequences of A3-CSF and tick samples revealed 6 amino acid substitutions in the complete ORF (Supplement Table S6). Our TBEV genome sequences were also compared with the reference sequence of Kumlinge A52 strain (GenBank accession number: GU183380) used for assembly to check for identity and genetic mutations. Both A3-CSF and tick TBEV sequences had 99.6 % pairwise identity with Kumlinge A52. The comparison revealed a difference between the A3-CSF and Kumlinge A52 sequence of 42 mutations: 39 in ORF and 3 in 3NCR, while the tick samples and Kumlinge A52 sequence differed with 46 mutations: 44 in ORF, 2 in 3NCR.

3.3. Variation in polyA tract of CSF sample

Sequencing the 3' NCR of TBEV genome from the tick sample showed a short 6A (AAAAAA) internal polyA tract. Whereas in case of patient A3-CSF sample, Sanger sequencing failed at internal polyA tract indicating presence of more than one variant in the patient sample. We cloned the 3' NCR from A3-CSF and tick samples into a cloning vector. Sanger sequencing of 15 individual clones from the patient sample (A3-CSF) showed a variable number of A's in the repeat tract between 6A to 9A (AAAAAAAA) long, whereas all the 15 clones from the tick sample contained only 6A tract. Since the A3-CSF sample showed variation within polyA tract, we also sequenced the 3' NCR by ONT that confirmed the presence of both 6A (70 %) and 8A (AAAAAAAA) (30 %). ONT could not confirm the 9A tract.

3.4. Phylogenetic analysis

In phylogenetic analysis, the genomic sequences from the patient and tick samples clustered with three TBEV isolates from *I. ricinus* in Kumlinge, Finland (Fig. 3). The Norwegian TBEV sequence Mandal-2009 clustered with the four TBEV isolates from *I. ricinus* in United Kingdom and Denmark.

4. Discussion

4.1. Clinical characterization

A patient with severe disease and a monophasic TBE course was enrolled in May 2022 at Telemark Hospital Trust, Norway. The monophasic disease course has recently been described in nearly half of the TBE patients in a Norwegian cohort (Skudal et al., 2024). This is consistent with previous findings in Åland (Wahlberg et al., 2006). In contrast, earlier studies from Europe describe biphasic course in around

Table 2

Identity matrix summarizing the percentage of identity between Kumlinge A52 strain, and TBEV genomic sequences from tick and patient samples.

	A3-CSF	Kumlinge A52	Ticks sample
A3-CSF		99.6 %	99.7 %
Ticks sample	99.7 %	99.6 %	
Kumlinge A52	99.6 %		99.6 %

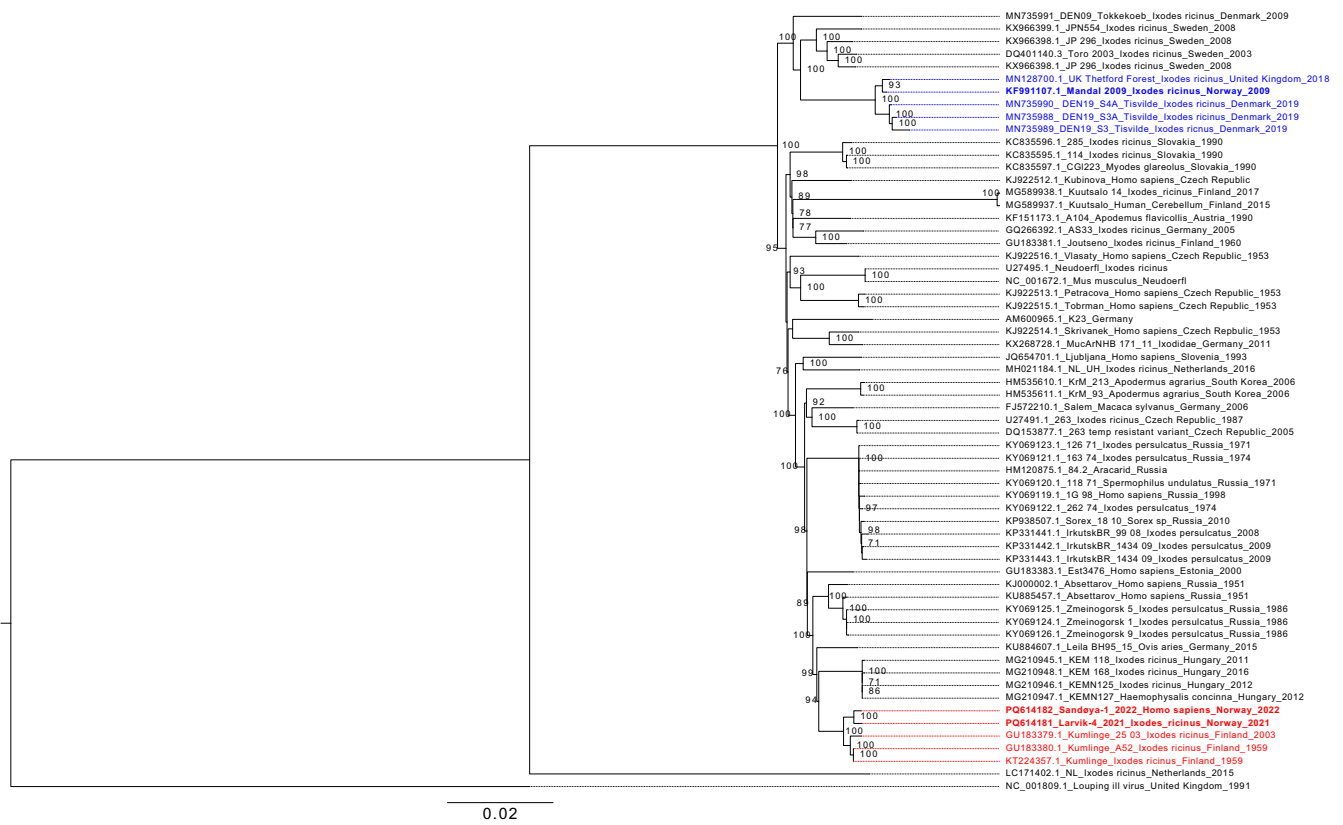


Fig. 3. Maximum Likelihood tree showing the TBEV-Eu diversity of 60 strains. The evolutionary history was tested with ML method based on GTR+*F* + *I* + *G4* as the best-fit substitution model. The phylogenetic tree was drawn and annotated in Fig Tree version 1.4.4. Node numbers represent bootstrap values (SH-aLRT support (%), only bootstrap values ≥ 70 are shown. Scale bars show the number of nucleotide changes. Genomic sequences (GenBank accession number: PQ614182 and PQ614181) from this study (bold, red) and closest cluster shown in red whereas the cluster with Norwegian TBEV sequence Mandal-2009 (bold, blue) is shown in blue.

70 % of patients (Barp et al., 2020; Bogovič et al., 2021; Dobler and Stoma, 2023; Griška et al., 2024). Interestingly, monophasic course has been associated with more severe disease in a previous study (Bogovič et al., 2021).

In this case, the encephalitis was complicated by HLH, a hyper-inflammatory syndrome with subsequent immunodeficiency that can be caused by infections, malignancy and autoimmune disease. HLH has previously been reported in only one case of TBE (Chmelík et al., 2016). Bicytopenia (leukopenia, thrombocytopenia) is a frequent finding in TBE during the first disease phase (Barp et al., 2020), but in this case, the condition worsened dramatically with the development of HLH. Bicytopenia is one of the criteria for HLH diagnosis. The patient in our study was also infected with *A. fumigatus* and *P. jirovecii*, which are per se opportunistic infections and define an immunocompromised status. These infections in the patient are most likely due to treatment of HLH with steroids, but also the dysregulation of the immune system in HLH could contribute to immunodeficiency. The etiology of the patient's epiglottitis remains unknown, but the presentation under ongoing treatment with cefotaxime and ampicillin (according to Norwegian guidelines for CNS-infection of unknown origin) and negative PCR results for airborne microbes, suggest most likely an abnormal immunoreaction. The patient had subclinical lymphoma at presentation, and this could contribute to a weakened and/or dysregulated immune response leading to both a more severe course of TBEV and a delayed antibody response. The corticosteroids given for his HLH could further delay the antibody response as has been shown in reported in other immunocompromised patients with TBE (Czarnowska et al., 2024).

4.2. Sequencing and characterization of TBEV

Four samples; A1-Serum, A2-Serum, A3-CSF, and A4-Serum were collected from the patient at different times points during the disease course (Table 1). The first three samples were positive for TBEV PCR whereas no TBEV was detected in A4-serum. The lack of TBEV in A4-Serum could be due to elimination of the virus from the bloodstream during the late, post-viremic phase of TBE. This is in accordance with a study by Saksida et al. (2005) who reported PCR positive samples from TBE patients with fever, after a recent tick bite, when they are in the early phase of the disease.

In this study, we sequenced the nearly complete TBEV genome from a TBE patient and tick samples by ONT and Sanger sequencing. We were successful to sequence the nearly complete TBEV genome from the tick samples and A3-CSF. The sequences from the A1-Serum and A2-Serum were incomplete, this could be due to a comparatively low viral load or quality. Despite higher Ct value, whole genome of TBEV was retrieved from CSF sample while only 93.6 % of genome was obtained from lower Ct value serum sample. This is the first TBEV genomic sequence reported from a human sample in Norway. We would like to highlight that the TBEV genome of A3-CSF was 99.7 % identical to the TBEV genome from the tick samples. The ticks were collected in 2021 from Larvik whereas the patient was infected in Sandøya, Porsgrunn in 2022 and the two sampling sites are 16 km apart. The differences in geographical location, sampling time and host species of these TBEV strains indicate the potential endemic presence of this TBEV strain in nearby regions within southern Norway, which requires further surveillance.

The phylogenetic analysis revealed that these TBEV isolates originated from same node, and they showed >99 % identity with the TBEV Kumlinge A52 strain from a small island municipality in Åland, Finland.

Kumlinge A52 strain was originally from *I. ricinus*, the TBEV RNA was extracted from virus cultivated in suckling NMRI mice and sequenced (Uzcategui et al., 2012). The novel variant differ from the previously characterized Norwegian sequence Mandal-2009 (Fig. 3) by having a longer 3NCR and some nucleotide differences. Mandal-2009 clustered with the four *I. ricinus* TBEV isolates from United Kingdom and Denmark. We emphasize that the Mandal-2009 and the present genomic sequences are different variants of TBEV though Mandal in Agder County is geographically close (distance of approximately 173 km) to Vestfold and Telemark Counties. The distance between Kumlinge, and Vestfold and Telemark counties is >750 km across the Bothnian bay and the Swedish mainland. Thus, it is likely that migratory birds have transported the ticks from Kumlinge to Norway and established the new TBEV foci in Vestfold and Telemark Counties. The migrating birds increase the possibility of geographic spreading of ticks and TBEV (Waldenström et al., 2007). There is a possibility that birds might import TBEV-infected tick larvae which molt to nymphs and spread the virus to local ticks (Hasle, 2013). A study by Waldenström et al. (2007) found 3.4 % of 13,260 migrating birds infested with ticks in Sweden. Most of those ticks were identified as *I. ricinus*. Four birds, each of a different species, carried TBEV-infected ticks (Waldenström et al., 2007). Our findings highlight the need for investigating new TBEV foci and novel genetic variants, along with surveillance of migrating birds to gain insights of ticks and TBEV transmission in Norway.

TBEV is known to exist as a pool of quasispecies in both tick and mammals and switching between different hosts lead to rearrangements in the quasispecies pool for selection of the best-fit variants (Asghar et al., 2014; Romanova et al., 2007; Růžek et al., 2008). Several TBEV-Eu strains contain variable length of the internal polyA tract within the 3NCR. The Neudoerfl strain has the longest known TBEV genome with a 30–250 nt long polyA tract (Asghar et al., 2017; Wallner et al., 1995). Earlier studies have shown that the polyA tract plays an important role in the replication, diversity of the quasispecies pool, and pathogenicity of TBEV (Asghar et al., 2016; Sakai et al., 2015). In this study, we identified TBEV variants with different lengths of the polyA tract, in the patient sample (A3-CSF) whereas TBEV from the tick sample showed a stable 6A long polyA tract. The diversity of TBEV heterogeneity of A3-CSF might be the reason behind the severe clinical symptoms observed in the current patient compared to the less severe TBE cases in Norway. The presence of TBEV variants with longer polyA tract in the patient sample in comparison to the tick sample could be due to the switching from invertebrate host with lower body temperature to vertebrate hosts with 37 °C body temperature. This phenomenon has also been proposed for TBEV strain Saringe 2009 (GenBank accession number: KF991106), sequenced from a tick after >60 h of blood-feeding on a 72-year-old male in Sweden (Asghar et al., 2014). This heterogeneity may affect the replication of the virus and pathogenesis which might explain the severe clinical symptoms observed.

5. Conclusion

This study presents, for the first time, a Norwegian patient derived TBEV sequence and a highly identical TBEV sequence from a tick collected in a neighboring county. The genomic ORF was sequenced by large overlapping amplicons with ONT, and non-coding regions were sequenced by Sanger sequencing. The patient had severe and monophasic TBE complicated with HLH and lymphoma. Notably, he did not seroconvert until 12 weeks (IgG+) after the onset of symptoms. This delayed seroconversion may have been crucial for the prolonged presence of the virus and the subsequent detection of the whole genome. His disease course highlights the potential severity of TBE, especially in patients with immunodeficiency. During sequencing the CSF sample showed a diversity of heterogeneity with varying polyA tails. The observations of new phylogenetic clusters of Norwegian TBEV variants emphasizes the importance of further surveillance of ticks and patients.

CRediT authorship contribution statement

Urusha Maharjan: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Hilde Kristin Skudal:** Visualization, Writing – original draft, Writing – review & editing. **Naveed Asghar:** Writing – review & editing, Writing – original draft, Formal analysis. **Arnulf Soleng:** Writing – review & editing, Visualization, Resources. **Magnus Johansson:** Writing – review & editing, Formal analysis. **Heidi Elisabeth Heggen Lindstedt:** Writing – review & editing, Visualization, Resources. **Anita Koskela von Sydow:** Writing – review & editing, Formal analysis. **John H.-O. Pettersson:** Writing – review & editing, Formal analysis. **Wenche Johansen:** Writing – review & editing, Supervision. **Børre Fevang:** Writing – review & editing, Formal analysis. **Randi Bjerkreim:** Writing – review & editing. **Suyog Basnet:** Writing – review & editing, Visualization. **Rose Vikse:** Writing – review & editing, Conceptualization. **Åshild K. Andreassen:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition. **Kristian Alfsnes:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tbd.2025.102501.

Data availability

The two complete TBEV genomes originating from a patient and a tick sample are available at NCBI GenBank with accession numbers: PQ614182 and PQ614181.

References

- Andreassen, A., Jore, S., Cuber, P., Dudman, S., Tengs, T., Isaksen, K., Hygen, H.O., Viljugrein, H., Ånestad, G., Ottesen, P., 2012. Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. *Parasit. Vectors*. 5, 1–12. <https://doi.org/10.1186/1756-3305-5-177>.
- Asghar, N., Lee, Y.-P., Nilsson, E., Lindqvist, R., Melik, W., Kröger, A., Överby, A.K., Johansson, M., 2016. The role of the poly (A) tract in the replication and virulence of tick-borne encephalitis virus. *Sci. Rep.* 6, 1–13. <https://doi.org/10.1038/srep39265>.
- Asghar, N., Lindblom, P., Melik, W., Lindqvist, R., Haglund, M., Forsberg, P., Överby, A. K., Andreassen, Å., Lindgren, P.-E., Johansson, M., 2014. Tick-borne encephalitis virus sequenced directly from questing and blood-feeding ticks reveals quasispecies variance. *PLoS One* 9, e103264. <https://doi.org/10.1371/journal.pone.0103264>.

- Asgar, N., Pettersson, J.H.-O., Dinnetz, P., Andreassen, Å., Johansson, M., 2017. Deep sequencing analysis of tick-borne encephalitis virus from questing ticks at natural foci reveals similarities between quasispies pools of the virus. *J. Gen. Virol.* 98, 413. <https://doi.org/10.1099/jgv.0.000704>.
- Barp, N., Trentini, A., Di Nuzzo, M., Mondardini, V., Francavilla, E., Contini, C., 2020. Clinical and laboratory findings in tick-borne encephalitis virus infection. *Parasite Epidemiol. Control* (10), e00160. <https://doi.org/10.1016/j.parepi.2020.e00160>.
- Bogovič, P., Lotrič-Furlan, S., Avšič-Županc, T., Korva, M., Kastrin, A., Lusa, L., Strle, K., Strle, F., 2021. Comparison of clinical, laboratory and immune characteristics of the monophasic and biphasic course of tick-borne encephalitis. *Microorganisms* 9, 796. <https://doi.org/10.3390/microorganisms9040796>.
- Chmelfík, V., Chrdele, A., Růžek, D., 2016. Fatal tick-borne encephalitis in an immunosuppressed 12-year-old patient. *J. Clin. Virol.* 74, 73–74. <https://doi.org/10.1016/j.jcv.2015.11.029>.
- Czarnowska, A., Groth, M., Okrzeja, J., Garkowski, A., Kristoferitsch, W., Kulakowska, A., Zajkowska, J., 2024. A fatal case of tick-borne encephalitis in an immunocompromised patient: case report from Northeastern Poland and review of literature. *Ticks Tick Borne Dis* 15 (1), 102273. <https://doi.org/10.1016/j.ttbdis.2023.102273>. Epub 2023 Nov 18. PMID: 37984275.
- Dobler, G., Stoma, I., 2023. TBE in special situations. In: Dobler, G., Erber, W., Broker, M., Schmitt, H.J. (Eds.), *The TBE Book*. Global Health Press, Singapore, p. 83.
- Filippova, N., 1977. Arachnida class: ixodid ticks of the subfamily Ixodidae.
- Filippova, N., 2017. History of the species range of ixodid ticks, vectors of pathogens with natural nidality (Acari, Ixodidae), as a prerequisite of their intraspecific biodiversity. *Entomological Review* 97, 255–275.
- Griška, V., Pranckevičienė, A., Pakalnienė, J., Gabrijolavičiūtė, D., Veje, M., Studahl, M., Ahlberg, J., Schwieler, L., Lindquist, L., Mickienė, A., 2024. Long-term neurological and neurocognitive impairments after tick-borne encephalitis in Lithuania—a prospective study. *Infect. Dis.* 56, 732–742. <https://doi.org/10.1080/23744235.2024.2346793>.
- Haglund, M., Vene, S., Forsgren, M., Günther, G., Johansson, B., Niedrig, M., Plyusnin, A., Lindquist, L., Lundkvist, Å., 2003. Characterisation of human tick-borne encephalitis virus from Sweden. *J. Med. Virol.* 71, 610–621. <https://doi.org/10.1002/jmv.10497>.
- Hasle, G., 2013. Transport of ixodid ticks and tick-borne pathogens by migratory birds. *Front. Cell. Infect. Microbiol.* 3, 48. <https://doi.org/10.3389/fcimb.2013.00048>.
- Hasle, G., Bjuene, G., Edvardsen, E., Jakobsen, C., Linnehol, B., Røer, J.E., Mehl, R., Røed, K.H., Pedersen, J., Leinaas, H.P., 2009. Transport of ticks by migratory passerine birds to Norway. *J. Parasitol.* 95, 1342–1351. <https://doi.org/10.1645/GE-2146.1>.
- Kjær, L.J., Soleng, A., Edgar, K.S., Lindstedt, H.E.H., Paulsen, K.M., Andreassen, Å.K., Korslund, L., Kjelland, V., Slettan, A., Stuen, S., 2019. Predicting and mapping human risk of exposure to *Ixodes ricinus* nymphs using climatic and environmental data, Denmark, Norway and Sweden, 2016 Euro Surveill 24, 1800101. <https://doi.org/10.2807/1560-7917.ES.2019.24.9.1800101>.
- Ličková, M., Fumačová Havlíková, S., Sláviková, M., Klempa, B., 2021. Alimentary infections by tick-borne encephalitis virus. *Viruses* 14, 56. <https://doi.org/10.3390/v14010056>.
- Mehl, R., 1983. Distribution and host relations of Norwegian ticks (Acari, Ixodidae). *Fauna Norv. Ser. B* 30, 46–51.
- Nguyen, L.-T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32, 268–274. <https://doi.org/10.1093/molbev/msu300>.
- Paulsen, K.M., Das Neves, C.G., Granquist, E.G., Madslie, K., Stuen, S., Pedersen, B.N., Vikse, R., Rocchi, M., Laming, E., Stiasny, K., 2020. Cervids as sentinel-species for tick-borne encephalitis virus in Norway—A serological study. *Zoonoses Public Health* 67, 342–351. <https://doi.org/10.1111/zph.12675>.
- Paulsen, K.M., Lamsal, A., Bastakoti, S., Pettersson, J.H.O., Pedersen, B.N., Stiasny, K., Haglund, M., Smura, T., Vapalahti, O., Vikse, R., Alfsnes, K., Andreassen, Å.K., 2021. High-throughput sequencing of two European strains of tick-borne encephalitis virus (TBEV). *Hochosterwitz and 1993/783. Ticks Tick Borne Dis.* 12, 101557. <https://doi.org/10.1016/j.ttbdis.2020.101557>.
- Quick, J., Grubaugh, N.D., Pullan, S.T., Claro, I.M., Smith, A.D., Gangavarapu, K., Oliveira, G., Robles-Sikisaka, R., Rogers, T.F., Beutler, N.A., 2017. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nat. Protoc.* 12, 1261–1276. <https://doi.org/10.1038/nprot.2017.066>.
- Romanova, L.I., Gmyl, A.P., Dzhibanian, T.I., Bakhtmutov, D.V., Lukashev, A.N., Gmyl, L.V., Rummyantsev, A.A., Burenkova, L.A., Lashkevich, V.A., Karganova, G.G., 2007. Microevolution of tick-borne encephalitis virus in course of host alternation. *Virology* 362, 75–84. <https://doi.org/10.1016/j.virol.2006.12.013>.
- Růžek, D., Gritsun, T.S., Forrester, N.L., Gould, E.A., Kopecký, J., Golovchenko, M., Rudenko, N., Grubhoffer, L., 2008. Mutations in the NS2B and NS3 genes affect mouse neuroinvasiveness of a Western European field strain of tick-borne encephalitis virus. *Virology* 374, 249–255. <https://doi.org/10.1016/j.virol.2008.01.010>.
- Ruzek, D., Županc, T.A., Borde, J., Chrdele, A., Eyer, L., Karganova, G., Kholodilov, I., Knap, N., Kozlovskaya, L., Matveev, A., 2019. Tick-borne encephalitis in Europe and Russia: review of pathogenesis, clinical features, therapy, and vaccines. *Antiviral Res.* 164, 23–51. <https://doi.org/10.1016/j.antiviral.2019.01.014>.
- Røed, K.H., Kvie, K.S., Hasle, G., Gilbert, L., Leinaas, H.P., 2016. Phylogenetic lineages and postglacial dispersal dynamics characterize the genetic structure of the tick, *Ixodes ricinus*. *Plos one* 11 (12), e0167450.
- Sakai, M., Muto, M., Hirano, M., Kariwa, H., Yoshii, K., 2015. Virulence of tick-borne encephalitis virus is associated with intact conformational viral RNA structures in the variable region of the 3'-UTR. *Virus Res.* 203, 36–40. <https://doi.org/10.1016/j.virusres.2015.03.006>.
- Saksida, A., Duh, D., Lotrič-Furlan, S., Strle, F., Petrovec, M., Avšič-Županc, T., 2005. The importance of tick-borne encephalitis virus RNA detection for early differential diagnosis of tick-borne encephalitis. *J. Clin. Virol.* 33, 331–335. <https://doi.org/10.1016/j.jcv.2004.07.014>.
- Skudal, H., Lorentzen Å, R., Stenstad, T., Quist-Paulsen, E., Egeland, J., Fevang, B., Jaioun, K., Hansen, B., Solheim, A.M., Tveten, Y., Veje, M., Eikeland, R., Kersten, H., 2024. Clinical characteristics and factors affecting disease severity in hospitalized tick-borne encephalitis patients in Norway from 2018 to 2022. *Eur. J. Clin. Microbiol. Infect. Dis.* 43, 1355–1366. <https://doi.org/10.1007/s10096-024-04855-2>.
- Smura, T., Tonteri, E., Jääskeläinen, A., von Troil, G., Kuivaniemi, S., Huitu, O., Kareinen, L., Uusitalo, J., Uusitalo, R., Hannila-Handelberg, T., Voutilainen, L., Nikkari, S., Sironen, T., Sane, J., Castrén, J., Vapalahti, O., 2019. Recent establishment of tick-borne encephalitis foci with distinct viral lineages in the Helsinki area. *Finland. Emerg. Microbes. Infect.* 8, 675–683. <https://doi.org/10.1080/22221751.2019.1612279>.
- Taba, P., Schmutzhard, E., Forsberg, P., Lutsar, I., Ljøstad, U., Mygland, Å., Levchenko, I., Strle, F., Steiner, I., 2017. EAN consensus review on prevention, diagnosis and management of tick-borne encephalitis. *Eur. J. Neurol.* 24, 1214–1261. <https://doi.org/10.1111/ene.13356>.
- Uzcategui, N.Y., Sironen, T., Golovljova, I., Jääskeläinen, A.E., Välimaa, H., Lundkvist, Å., Plyusnin, A., Vaheri, A., Vapalahti, O., 2012. Rate of evolution and molecular epidemiology of tick-borne encephalitis virus in Europe, including two isolations from the same focus 44 years apart. *J. Gen. Virol.* 93, 786–796. <https://doi.org/10.1099/vir.0.035766-0>.
- Vikse, R., Paulsen, K.M., Edgar, K.S., H.-O. Pettersson, J., Ottesen, P.S., Okbaldet, Y.B., Kiran, N., Lamsal, A., Lindstedt, H.E.H., Pedersen, B.N., 2020. Geographical distribution and prevalence of tick-borne encephalitis virus in questing *Ixodes ricinus* ticks and phylogeographic structure of the *Ixodes ricinus* vector in Norway. *Zoonoses Public Health* 67 (4), 370–381.
- Wahlberg, P., Carlsson, S.-A., Granlund, H., Jansson, C., Lindén, M., Nyberg, C., Nyman, D., 2006. TBE in Åland Islands 1959–2005: kumlinge disease. *Scand. J. Infect. Dis.* 38, 1057–1062. <https://doi.org/10.1080/00365540600868297>.
- Waldenström, J., Lundkvist, A., Falk, K.I., Garpmo, U., Bergström, S., Lindgren, G., Sjöstedt, A., Mejlom, H., Fransson, T., Haemig, P.D., Olsen, B., 2007. Migrating birds and tickborne encephalitis virus. *Emerg. Infect. Dis.* 13, 1215–1218. <https://doi.org/10.3201/eid1308.061416>.
- Wallner, G., Mandl, C.W., Kunz, C., Heinz, F.X., 1995. The flavivirus 3'-noncoding region: extensive size heterogeneity independent of evolutionary relationships among strains of tick-borne encephalitis virus. *Virology* 213, 169–178. <https://doi.org/10.1006/viro.1995.1557>.
- Wondim, M.A., Czupryna, P., Pancewicz, S., Kruszczyńska, E., Groth, M., Moniuszko-Malinowska, A., 2022. Epidemiological trends of trans-boundary tick-borne encephalitis in Europe, 2000–2019. *Pathogens* 11, 704. <https://doi.org/10.3390/pathogens11060704>.
- Zakotnik, S., Knap, N., Bogovič, P., Zorec, T.M., Poljak, M., Strle, F., Avšič-Županc, T., Korva, M., 2022. Complete genome sequencing of tick-borne encephalitis virus directly from clinical samples: comparison of shotgun metagenomic and targeted amplicon-based sequencing. *Viruses* 14, 1267. <https://doi.org/10.3390/v14061267>.