TICK-BORNE ENCEPHALITIS VIRUS PREVALENCE IN EUROPEAN TICK POPULATIONS: A GEOGRAPHIC PERSPECTIVE

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Abstract

Objectives. Tick-borne encephalitis virus (TBEV) poses a significant public health threat in Europe. This systematic review investigates TBEV prevalence and expansion across 26 countries to evaluate shifting transmission risks and the emergence of new microfoci.
Material and Methods. Following PRISMA 2020 guidelines, PubMed and Scopus were searched for studies published between 2004 and 2024. To address methodological heterogeneity, data were categorized by individual prevalence, minimum infection rate (MIR), and estimated pooled prevalence (EPP).
Results. From 86 manuscripts (selected from 354 records), data synthesis revealed significant northward (65.1°N in Norway) and vertical expansion into Alpine regions (1,109 m a.s.l.). While *Ixodes ricinus* remains the primary vector, *Ixodes persulcatus* in the Baltic zone exhibited substantially higher infection rates (MIR 3.0–4.6%). In emerging zones (the UK, Belgium, Romania), tick-derived MIR was frequently <1%, whereas animal seroprevalence (2.61–15.02%) proved a more sensitive indicator of viral circulation. Genomic outliers, such as the Far-Eastern subtype in Moldova, suggest non-linear dispersal mechanisms.
Conclusion. TBEV distribution is highly dynamic, driven by climate-mediated range shifts and the silent establishment of new microfoci. Traditional tick screening often underestimates risk in emerging areas, necessitating a transition to integrated One Health surveillance. Combining entomological monitoring with veterinary seroprevalence is critical for accurate risk assessment and the implementation of targeted vaccination campaigns in the evolving European landscape.

Keywords: tick-borne encephalitis virus, TBEV, prevalence, ticks, Europe

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Introduction

Tick-borne encephalitis virus (TBEV) is a significant public health concern in Europe and Asia, causing neurological symptoms in humans (Valarcher et al., 2015). The virus is primarily transmitted by *Ixodes* ticks and maintained in small mammals, particularly rodents (Walter et al., 2020a). TBEV comprises three main subtypes: European (Eu), Siberian (Sib), and Far-Eastern (FE) (Bakhvalova et al., 2016), alongside the Baikalian subtype, a genetic bridge between Siberian and Far-Eastern strains near Lake Baikal, and the Himalayan subtype, which expands the virus's known range into high-altitude regions such as the Qinghai-Tibet Plateau (Dai et al., 2018; Sukhorukov et al., 2023). In Europe, the highest probabilities of TBEV presence are in Central Europe, southern Nordic countries, and the Baltic states (Walter et al., 2020a). The virus has been expanding to new areas, as evidenced by its recent detection in Moscow and the UK (Holding et al., 2020; Makenov et al., 2019). Infection rates vary among tick species and small mammal hosts, with mixed subtype infections more common in ticks than mammals (Bakhvalova et al., 2016). Specifically, reported infection rates for the *Ixodes ricinus* tick, which is prevalent in Central Europe, range from 0.1% to 5%. In contrast, in endemic areas of Siberia, up to 40% of *Ixodes persulcatus* ticks carry the virus. Monitoring tick populations for TBEV is essential to evaluate the risk of human transmission, and understanding the interactions between TBEV, vectors, and hosts is vital for effective control measures (Pustijanac et al., 2023; Valarcher et al., 2015).

Studies suggest that TBEV may be more widespread than previously thought, with prevalence varying by tick species, life stage, and geographical location. However, the relatively low prevalence in most areas may be influenced by sampling methods and laboratory techniques (Pettersson et al., 2014).

Despite the dynamic nature of TBEV distribution, a comprehensive systematic review quantifying tick-borne prevalence across the entire European landscape is currently lacking. This study addresses this gap by synthesizing data on TBEV infection rates in ticks across diverse European regions to better evaluate the risk of human transmission.

Material and methods

This systematic review was conducted in accordance with the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Page et al., 2021). The primary search strategy utilized the PubMed and Scopus databases, covering a twenty-year period from January 1, 2004, to December 31, 2024. A comprehensive Boolean search string was developed to ensure that the geographic scope matched the diversity of the countries analyzed: TITLE-ABS-KEY("tick-borne encephalitis virus" OR TBEV OR "encephalitis virus, tick-borne") AND TITLE-ABS-KEY(prevalence OR percentage OR rate OR incidence OR proportion OR "infection rate" OR "MIR") AND TITLE-ABS-KEY(ticks OR Ixodes OR Dermacentor OR Haemaphysalis) AND TITLE-ABS-KEY(Europe OR Germany OR France OR Sweden OR Norway OR Denmark OR "United Kingdom" OR UK OR Czechia OR Slovakia OR Slovenia OR Austria OR Switzerland OR Poland OR Estonia OR Latvia OR Lithuania OR Finland OR Romania OR Moldova OR Italy OR Bulgaria OR Serbia OR Hungary OR Croatia OR Ukraine).

The primary search was further complemented by manual reference tracking, where the bibliographies of identified primary studies and reviews were screened to capture additional relevant data. The selection process and duplicate removal were managed using the Rayyan and Elicit platforms. Eligibility was restricted to original research reporting TBEV prevalence or minimum infection rates (MIR) in field-collected or host-attached ticks within Europe. Notably, no minimum threshold was imposed for the number of ticks tested, an approach intended to maintain sensitivity for emerging or low-endemicity zones where sample sizes are frequently limited. Regarding the exclusion of vaccination-related literature, a specific distinction was applied: studies focused strictly on clinical trials or vaccine immunological efficacy were excluded, while epidemiological papers providing primary tick prevalence data-often used as context for monitoring vaccination programs-were retained to ensure a comprehensive dataset.

To address the methodological heterogeneity across the gathered literature, a clear distinction was maintained between individual prevalence, MIR, and estimated pooled prevalence (EPP) during data extraction. Individual prevalence was defined and calculated for studies where ticks were tested individually, expressed as the percentage of positive ticks relative to the total number of individuals tested. For studies employing pooled samples, two distinct metrics were utilized based on the original authors' reporting: minimum infection rate, calculated by dividing the number of positive pools by the total number of ticks tested and estimated pooled prevalence. EPP was extracted from studies that utilized maximum likelihood estimation (MLE) or other probabilistic models to provide a more precise prevalence estimate within pooled samples, particularly when pool sizes varied or infection rates were higher.

Because the reviewed studies used different methods - such as variations in tick pooling, sampling designs, and laboratory tests - the metrics reported in this review (individual prevalence, MIR, EPP, and seroprevalence) are used for descriptive purposes only. These values highlight local or regional trends and are not meant for direct statistical comparisons between different studies.

Results

The systematic search across PubMed and Scopus databases, supplemented by manual reference tracking, initially yielded 354 records. After a rigorous screening process based on the inclusion and exclusion criteria detailed in the methodology, a total of 86 manuscripts were selected for final analysis (Figure 1). These studies provided comprehensive data on TBEV prevalence and minimum infection rates across 26 European countries, covering the period from 2004 to 2024.

To account for the ecological and climatic diversity influencing tick-borne pathogens, the findings were categorized into four primary epidemiological zones: the Northern Front, the Baltic mixing zone, the Central endemic Massif, and the Southern and Balkan Foci, which were defined based on geographical distribution patterns, vector ecology, and climatic gradients. To ensure comparability with existing literature, each zone is additionally referenced using standard biogeographical descriptors (Nordic, Baltic, Central European, and Southern/Balkan regions). A comprehensive summary of these findings, including tick species, developmental stages, and viral subtypes, is synthesized in Table 1. This structured approach allows for a clearer distinction between stable endemic regions and emerging hotspots, while maintaining methodological consistency across diverse reporting formats. Specifically, the data accounts for individual prevalence, minimum infection rate, and estimated pooled prevalence, ensuring a robust comparison between studies with different sampling and statistical methodologies.

Figure 1

PRISMA 2020 Flow diagram of the study selection process

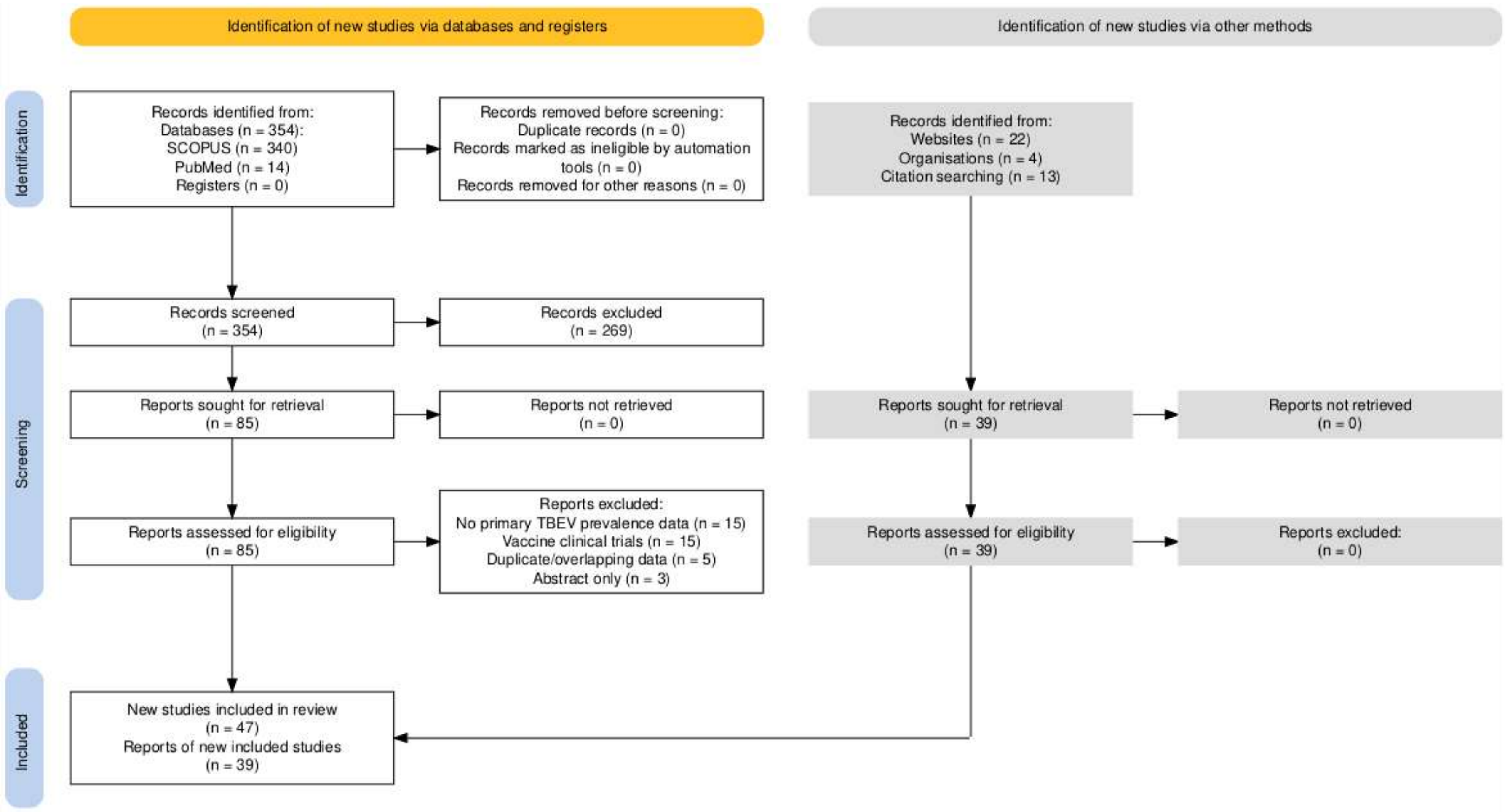

Table 1

Epidemiological metrics of Tick-Borne Encephalitis Virus in Europe (2004-2024): prevalence, minimum infection rate, and estimated pooled prevalence.

Epidemiological zone	Country	Study year	Tick species	Number of ticks	Prevalence/MIR/EPP	TBEV subtype	Citation
Northern Front (Nordic / subarctic expansion zone)	Norway	2014	*I. ricinus*	4,509	3.08% (A); 0.41% (N) (EPP)	European	Paulsen et al., 2015
	Sweden	2013	*I. ricinus*	1,500	0.3% (EPP)	European	Gondard et al., 2018
	Denmark	2019	*I. ricinus*	54	8.0% (N) (EPP)	European	Agergaard et al., 2019
	United Kingdom	2018-2019	*I. ricinus*	2,041	0.05% (MIR), 1 positive pool	European	Holding et al., 2020
Baltic mixing zone (Baltic sympatric vector zone)	Latvia	2017-2019	*I. ricinus* *I. persulcatus* *D. reticulatus*	3,840 158 595	0.65% (MIR) 0.63% (MIR) 0.67% (MIR)	European, Siberian, Far-Eastern	Capligina et al., 2020
	Estonia	2006-2009	*I. persulcatus* *I. ricinus*	3,287 2,341	4.6% (A) (MIR) 0.46% (A) (MIR)	European, Siberian	Katargina et al., 2013
	Lithuania	2017-2019	*I. ricinus* *D. reticulatus*	7,170 1,676	0.4% (MIR) 0.4% (MIR)	European	Sidorenko et al., 2021
	Finland	2015	*I. ricinus* *I. persulcatus*	878 1,326	0.2% MIR) 3.0% (MIR)	Siberian, European	Laaksonen et al., 2017
Central endemic Massif (Central European endemic region)	Germany	2020-2021	*I. ricinus, D. reticulatus*	4,230	0.58% (MIR)	European	Król et al., 2024
	Czech Republic	2011-2013	*I. ricinus*	18,721	0.096% (N); 0.477% (A) (MIR)	European	Daniel et al., 2016
	Poland	2006-2009	*I. ricinus*	2,075	0.96% (MIR)	NS	Biernat et al., 2014
	Slovakia	2016-2017	*I. ricinus*	2,308	0.56-0.6% (EPP)	European	Zubriková et al., 2024
	Switzerland	2014	*I. ricinus*	3,425	0.35% (MIR)	European	Casati Pagani et al., 2019
	France	2014	*I. ricinus*	7,488	0.24% (N) (MIR)	European	Bournez et al., 2020
	Hungary	2010-2011	*I. ricinus*	2731	0.08/0.78	European	Pintér et al., 2013
	Belgium	2011-2023	*I. ricinus*	20,000+	0% (MIR)		Adjadj et al., 2022
	Netherlands	2018-2020	*I. ricinus*	46,000+	0.02% (MIR)	European	Esser et al., 2022
Southern & Balkan foci (Southern Europe / Balkan region)	Italy	2018	*I. ricinus*	2,410	0.17% (MIR)	European	Alfano et al., 2020
	Romania	2006-2015	*I. ricinus*	NS	<1% (MIR)	European	Vladimirescu et al., 2016
	Serbia	2015	*I. ricinus*	500	0.6% (MIR)	European	Potkonjak et al., 2017
	Moldova	2010-2011	*I. ricinus* *D. reticulatus* *H. punctata*	78 77 34	3.8% (MIR) 3.9% (MIR) 8.8% (MIR)	Far-Eastern (outlier)	Ponomareva et al., 2015
	Ukraine (West)	2015-2017	*I. ricinus* *D. reticulatus*	190 124	6.3% (Individual) 14.5% (Individual)	European	Ben & Lozynskyi, 2019

Note. NS = Not specified in the source material; (A) = adults; (N) = nymphs; MIR = Minimum Infection Rate, EPP = Estimated Pooled Prevalence.

The Northern Front (Nordic / subarctic expansion zone)

The Northern Front represents the expanding latitudinal frontier of TBEV in Europe, characterized by the virus's encroachment into sub-arctic and island ecosystems previously considered at low risk or entirely non-endemic. This zone encompasses Norway, Sweden, Denmark, and the United Kingdom, where ecological shifts associated with climate change and rising mean temperatures–have facilitated the northward migration of *Ixodes ricinus* vectors (Dagostin et al., 2023).

Southern Scandinavia (southeastern Norway, southern Sweden, Denmark)

A study investigating the estimated pooled prevalence of TBEV in questing *Ixodes ricinus* nymphs across southern Scandinavia reported an overall EPP of 0.1%. Denmark recorded an EPP of 0.1%, with viral presence confirmed in 12 out of 30 sites. In southern Sweden, a slightly higher EPP of 0.2% was observed, with positive pools identified primarily in northern Skåne County. Southeastern Norway also reported an EPP of 0.1% based on eight positive results from 660 tested pools, although localized EPP values reached 0.5% and 0.7% in specific sites within the Oslofjord area. (Lamsal et al., 2023).

Norway

Norwegian studies revealed TBEV prevalence (MIR) of 3.08% in adult ticks and 0.41% in nymphs from northwestern islands (Paulsen et al., 2015). In northern Norway, TBEV was detected for the first time at latitudes up to 65.1°N, approaching the level of the Arctic Circle, demonstrating a significant northward expansion of the virus's known range (Soleng et al., 2018).

Another study investigates the distribution and prevalence of ticks, specifically *Ixodes ricinus*, along the Norwegian coastline, from Østfold County to Nordland County. The estimated prevalence of TBEV was found to be 0.3% in nymphs and 4.3% in adults, with the highest prevalence in adults detected in Rogaland and Vestfold County, and in nymphs in Vestfold, Vest-Agder, and Rogaland. This research represents one of the largest studies on TBEV distribution in Scandinavian ticks, indicating a broader distribution of the virus in Norway than previously recognized (Vikse et al., 2020).

A Paulsen study investigated the prevalence of tick-borne encephalitis virus in *Ixodes ricinus* ticks in northwestern Norway, collecting 4509 ticks in May and June 2014. Real-time PCR analysis showed a TBEV prevalence (EPP) of 3.08% in adult ticks from Sekken and 0.41% in nymphs from Hitra and Frøya, indicating that TBEV may be more widespread than currently reflected by reported human cases (Paulsen et al., 2015).

Sweden

For instance, in some regions of Sweden, the individual prevalence of TBEV in *Ixodes ricinus* ticks has been documented to be around 1-5%, but it can be higher in specific hotspots (Pettersson et al., 2014).

The exact prevalence can fluctuate due to factors such as tick population dynamics, the presence of reservoir hosts, and environmental conditions that affect tick survival and activity (Kjær et al., 2023). A large-scale survey using high-throughput microfluidic PCR across 13 European sites detected TBEV at only one Swedish location, with a prevalence of 0.3% (EPP) in nymphs (Gondard et al., 2018).

In Sweden and the Åland Islands, only the European subtype of TBEV is present, transmitted by *Ixodes ricinus* ticks. In contrast, Estonia, Latvia, and Lithuania have the Far-Eastern and Siberian subtypes, spread by *Ixodes persulcatus* ticks (Lindblom et al., 2014).

Denmark

In Denmark, TBEV has been endemic on Bornholm Island for over 60 years, with a recent study detecting the virus in ticks with an estimated prevalence of 0.6% (Andersen et al., 2019). A new tick-borne encephalitis virus hotspot was identified in Northern Zealand, Denmark in 2019, with three patients hospitalized after tick bites in Tisvilde Hegn forest. This marks the second time TBEV has been found outside Bornholm Island in Denmark. The estimated TBEV prevalence in tick nymphs was 8%, higher than in other endemic areas in Europe (Agergaard et al., 2019).

The United Kingdom

Tick-borne encephalitis virus has recently been detected in the United Kingdom, challenging previous assumptions about its absence in the region (Holding et al., 2020).

The first two human cases of TBEV acquired within the UK were confirmed in 2022, prompting enhanced surveillance (Mahase, 2023). A second probable human case of UK-acquired TBE has been reported, further confirming the virus's presence in England (Mansbridge et al., 2022).

In the United Kingdom, large-scale surveys conducted between 2018 and 2019 identified a 4% TBEV seroprevalence in deer populations across England and Scotland, with a localized focus in the Thetford Forest area. While initial entomological surveillance yielded a very low MIR of 0.05%, based on a single positive pool from 2,041 ticks, the significantly higher seroprevalence in deer provided early evidence of the virus's establishment before human cases were reported. This discrepancy highlights the superior sensitivity of animal sentinels compared to tick screening for detecting viral circulation in emerging zones, where infection rates frequently fall below the threshold of entomological detection (Holding et al., 2020).

The Baltic mixing zone (Baltic sympatric vector zone)

The Baltic mixing zone serves as a critical biological crossroads and a unique convergence zone for TBEV subtypes. Unlike most of Europe, where the European subtype predominates, this region - comprising Estonia, Latvia, Lithuania, and Finland, is characterized by the co-circulation of all three main subtypes: European, Siberian, and Far-Eastern. This zone is of paramount epidemiological importance as it marks the westernmost limit of the Siberian subtype's distribution, traditionally vectored by *Ixodes persulcatus*. The interaction between expanding *I. ricinus* populations and established *I. persulcatus* communities creates a complex risk profile, where viral prevalence is often significantly higher in the latter species, necessitating highly specific molecular surveillance to distinguish between circulating lineages.

Estonia

Estonia exhibits a high degree of subtype diversity, with both European and Siberian subtypes documented across its territory. Research has highlighted a significant disparity in viral carriage between vector species: among adult ticks, the TBEV minimum infection rate was found to be nearly ten times higher in *I. persulcatus* (4.6%) compared to *I. ricinus* (0.46%) (Katargina et al., 2013). Urban surveillance in Tallinn further confirms the virus's establishment in recreational areas, with 0.5% of ticks testing positive in locations such as the Pirita river valley and Männiku forest (Vikentjeva et al., 2024).

Latvia

Latvia represents a key transition point in the virus's geographical range, where all three main subtypes co-circulate (Lindquist, 2014).

A large-scale study in Latvia (2017-2019) identified three key tick-borne pathogens in field-collected ticks, with an overall pathogen carriage rate of 33.42%. Regarding TBEV, the virus was detected with consistent MIR across three species: *Ixodes ricinus* (0.65%), *Ixodes persulcatus* (0.63%), and *Dermacentor reticulatus* (0.67%). Geolocation analysis indicated that TBEV-positive ticks were primarily clustered in the western Kurzeme region, with significantly lower detection rates in northern and eastern areas (Capligina et al., 2020).

Lithuania

Tick-borne encephalitis virus is prevalent in Lithuania, with studies reporting infection rates in ticks ranging from 0.1% to 1.7% (Han et al., 2005; Katargina et al., 2013; Sidorenko et al., 2021).

A study conducted between 2017 and 2019 investigated the prevalence of tick-borne encephalitis virus in two tick species: *Ixodes ricinus* and *Dermacentor reticulatus*. Researchers collected a total of 7,170 *I. ricinus* and 1,676 *D. reticulatus* ticks from 81 locations. TBEV was detected in different developmental stages of *I. ricinus* and in adult *D. reticulatus*, with a minimum infection rate of 0.4% for both species. Infected ticks were identified in 16 locations across seven counties, with MIR values ranging from 0.1% to 1.0%. Notably, this research is the first to document TBEV prevalence in unfed *D. reticulatus* and *I. ricinus* larvae in Lithuania (Sidorenko et al., 2021).

Genetic characterization reveals that while TBEV strains in Lithuania are predominantly of the European subtype, the broader Baltic region serves as a crucial zone of convergence where all three main subtypes: European, Siberian, and Far-Eastern circulate. Notably, this region marks the westernmost limit of the Siberian subtype's distribution, a key transition point in the virus's geographical range (Alfano et al., 2020; Katargina et al., 2013).

Finland

In Finland, TBEV prevalence in ticks has been historically reported as relatively low, ranging from 0.23 to 1% (Jääskeläinen et al., 2010; Lindblom et al., 2014). However, a 2015 nationwide citizen-science campaign provided a more nuanced perspective on viral distribution. While over 20,000 ticks were collected for mapping, a pathogen-screening subset of 2,204 individuals revealed a significant disparity in viral carriage between species: MIR was notably higher in *I. persulcatus* (3.0%) compared to *I. ricinus* (0.2%). This study established that the Siberian subtype is establishing itself in new ecological niches across the Finnish mainland, moving beyond the previously known hotspot in the Kokkola Archipelago (Jääskeläinen et al., 2006; Laaksonen et al., 2017).

Studies have suggested an increase in tick abundance and geographical distribution in recent years, potentially impacting TBEV prevalence. Multiple TBEV subtypes and *Borrelia* species have been identified in Finnish ticks (Jääskeläinen et al., 2010; Sormunen et al., 2016). While the risk of TBEV infection after a tick bite remains low, factors such as viral load and feeding time may influence transmission (Lindblom et al., 2014).

Complementary data from the Aland Islands (AxBioTick study) showed a 0% individual tick prevalence among 425 specimens collected from volunteers, despite a high human seroprevalence of 52%, a discrepancy likely influenced by local vaccination programs and the focal nature of TBEV circulation (Carlströmer Berthén et al., 2023).

The Central endemic Massif (Central European endemic region)

The Central endemic Massif constitutes the traditional heartland of TBEV in Europe, characterized by long-standing endemicity and the overwhelming predominance of the *Ixodes ricinus* vector and the European subtype. This zone - comprising Germany, Poland, the Czech Republic, Slovakia, Austria, Hungary, Switzerland, Slovenia, France, Belgium, and the Netherlands, exhibits a highly heterogeneous and patchy distribution of viral foci. While the virus is firmly established here, recent decades (2004-2024) have seen a shift in risk profiles, marked by the identification of new microfoci in historically low-risk areas and a significant vertical expansion into higher altitudes of the Alps and Central Highlands.

Furthermore, this region faces unique public health challenges, such as high rates of alimentary transmission in certain sectors and the need for a One Health approach where tick prevalence remains low despite significant animal seroprevalence (Lang et al., 2024).

Germany

Tick-borne encephalitis virus prevalence in *Ixodes ricinus* ticks varies across different regions of Germany. Recent research has identified several new microfoci of Tick-Borne Encephalitis Virus in areas of Eastern Germany that were previously considered nonendemic. Specifically, fourteen potential TBEV microfoci were found in Germany. This indicates that TBEV is now present in regions where it was not previously recognized as endemic. The overall minimum infection rate for TBEV-positive sites in Germany was found to be 0.58% (95% Confidence Interval: 0.31–0.78) (Król et al., 2024).

From 2016 to 2018, a study conducted in Schiltach, Germany, collected a total of 4,064 *Ixodes* ticks from 12 different sites. The study found that the infection rate of these ticks in the area was 0.17% (Bestehorn et al., 2018).

In Lower Bavaria and Upper Palatinate, the overall TBEV prevalence was 0.26% (EPP), with detection at seven out of 16 high-risk sites (Zubriková et al., 2020). In contrast, a large-scale study in southwestern Germany's Odenwald Hill region reported a minimum infection rate ranging from 0 to 0.12% (mean 0.04%). Phylogenetic analyses revealed that TBEV isolates from the Odenwald Hill region belonged to the European subtype, showing a discontinuous segregation pattern with two putative lineages (Ott et al., 2020).

In western Germany, a pilot study (2011–2012) across Saarland and Rhineland-Palatinate reported a MIR of 0.1% for TBEV (4 positive results out of 4,014 ticks), marking the first regional detection of the virus and identifying *Dermacentor reticulatus* as a potential emerging vector (Mehlhorn et al., 2016). Similarly, in Lower Saxony, surveillance of areas with reported human cases involved the testing of 730 pools containing 4,242 ticks. While the pool positivity rate was 0.68%, the calculated MIR for this region was 0.12% (Boelke et al., 2019).

Tick-borne encephalitis virus has been reemerging in northeastern Germany after years of inactivity. In Mecklenburg-West Pomerania, TBEV was undetectable in ticks from 1992 to 2004, but following three autochthonous human cases post-2004, researchers confirmed its presence in ticks for the first time in 15 years (Frimmel et al., 2014). This suggests that natural TBEV foci may persist at low levels or reemerge through migrating birds. In Lower Saxony, a northward expansion of TBEV risk areas has been observed, with 18 positive tick pools found at five locations, including one previously unknown transmission focus (Topp et al., 2022).

Poland

Tick-borne encephalitis virus prevalence in ticks varies across different regions of Poland. In a study conducted in Poland from 2006 to 2009, researchers collected questing *I. ricinus* ticks (including adults, nymphs, and larvae) from 55 locations, totaling 2 075 ticks. The ticks were examined for the presence of TBEV RNA using nested RT-PCR, revealing a minimum infection rate of 0.96%. TBEV RNA was found in all developmental stages of the ticks. The prevalence of viral infection in these ticks serves as an important indicator of TBE virus circulation in the area and can aid in assessing the risk of TBE exposure in various natural habitats (Biernat et al., 2014).

In eastern Poland, *Ixodes ricinus* ticks showed a minimum infection rate of 1.6%, while *Dermacentor reticulatus* ticks had a higher rate of 10.8% (Wójcik-Fatla et al., 2011). Southern Poland exhibited lower MIR with 0.11% in nymphs (*I. ricinus*) from the Silesian Province (Cuber et al., 2015) and 0.31% in adult ticks from the highly urbanized Silesian agglomeration area (Drelich et al., 2014).

Czech Republic

Recent studies in the Czech Republic have revealed significant prevalence of tick-borne encephalitis virus and *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks. A study examined the infection rate of TBEV in *Ixodes ricinus* ticks across several regions of the Czech Republic, specifically Ústí nad Labem, Olomouc, South Bohemian, and Highlands. Researchers conducted tick flagging in optimal habitats, collecting a total of 18,721 ticks, which included 1,448 females, 1,425 males, and 15,848 nymphs. The results revealed that TBEV infection rates differed by region, influenced by local tick population density and environmental conditions. The overall prevalence (MIR) of TBEV was found to be 0.096% in nymphs and 0.477% in adult ticks at altitudes below 600 meters (Daniel et al., 2016).

Another study investigated the spatial distribution of host-seeking activity of *Ixodes ricinus* ticks and the prevalence of *Borrelia burgdorferi* sensu lato and TBEV in South Bohemia, Czech Republic, an area endemic for TBE. Researchers analyzed 30 study sites and found significant variability in tick abundance, primarily linked to vegetation cover. Out of 11,182 tick samples tested, 12% were positive for *Borrelia* DNA, with *B. afzelii* and *B. garinii* being the most common species, and the presence of *B. spielmanii* was also confirmed. For TBEV, the total prevalence in pooled samples was 0.32% (MIR), with at least one positive tick found at 21 of the 30 sites sampled (Hönig et al., 2015). The discrepancy between the high tick abundance and low viral infection rates reinforces the patchy distribution model of TBEV foci within Central Europe.

Slovakia

In Slovakia, TBEV prevalence within natural foci is highly heterogeneous, typically reaching up to 5%, though significantly higher rates have been documented in specific microfoci

(Cagnacci et al., 2012). Since the 1950s, viral isolates have been consistently recovered from both nymphs and adult *I. ricinus* ticks (Bušová et al., 2018; Cagnacci et al., 2012).

Recent investigations in the Pol'ana and Smrekovica mountains found that altitude does not significantly limit TBEV occurrence, with positive ticks identified at elevations up to 990 m a.s.l. despite low overall pooled prevalences (EPP 0.56%-0.6%) (Zubriková et al., 2024).

Human seroprevalence is estimated between 1.2% and 1.6%, with risk factors primarily linked to outdoor activities and the consumption of raw milk (Bušová et al., 2018).

Notably, Slovakia reports the highest incidence of alimentary TBE infections in Europe, a fact that underscores the unique public health challenge posed by traditional dietary habits and highlights the need for targeted preventive measures (Stanko et al., 2022).

Switzerland

In southern Switzerland, TBEV was recently identified in the Canton of Ticino, an area previously considered non-endemic. The virus was detected in *Ixodes ricinus* ticks with a MIR of 0.35%, while concurrent seroprevalence studies showed that 14% of goats tested positive for TBEV antibodies, confirming active viral circulation (Casati Pagani et al., 2019). Goats have been shown to be effective sentinel animals for TBEV detection, with studies in Valais Canton revealing new risk areas through goat serology followed by tick testing (Rieille et al., 2017).

Between 2010 and 2013, a longitudinal study of 19,331 ticks from 45 sites along the Rhône River revealed TBEV persistence at several locations, with MIR values ranging from 0.16% to a remarkable 11.11% at high-activity foci. Phylogenetic analysis revealed that all isolates belonged to the European subtype and indicated two distinct lineages, suggesting two independent colonization events in the region (Rieille et al., 2014).

Slovenia

Slovenia reports among the highest human TBE incidence rates globally, with national annual rates reaching 26.7 per 100,000 and regional spikes as high as 76.9 per 100,000 (Grgič-Vitek & Klavs, 2011). Entomological surveillance reflects this high risk, with studies in Ixodes ricinus ticks reporting a minimum infection rate of 0.47% (Durmiši et al., 2011).

This circulation is further evidenced by a 5.9% antibody seroprevalence in small mammal hosts, particularly bank voles, which serve as critical reservoirs in the region (Knap et al., 2012). These high transmission levels are consistent with findings in neighboring North-Eastern Italy, where a border-region MIR of 0.21% has been documented (D'Agaro et al., 2009).

France, Austria, and Hungary

TBEV has been present in France since at least 1968, with initial cases reported in Alsace (Hansmann et al., 2024). Studies have shown low but persistent infection rate of TBEV in ticks - 0,11% (1/944) for Foret de la Robertsau. TBEV strains from the Upper Rhine Valley exhibited genetic relationships with eastern European strains (Bestehorn et al., 2018).

A study in Alsace tested 7,488 questing *Ixodes ricinus* ticks for TBEV and found an overall minimum infection rate (MIR) of 0.11% in nymphs. The MIR was significantly lower in 2013 (0.03%) compared to 2012 (0.17%) and 2014 (0.24%), with no significant difference between 2012 and 2014. In both 2012 and 2014, nymphs had a lower MIR in the first two seasons (0.13%) than in the third season (0.92%). No adult ticks (418 tested) were found to be infected, and the minimum detectable prevalence in adults was less than 0.7% (Bournez et al., 2020). Findings suggest that TBEV is present but not widely distributed in France, with localized endemic foci primarily in eastern regions.

Historically in Austria high activity is noted in Styria (MIR 4.4/1,000), and recent data shows a high infection rate of 26.1% in horses (Rushton et al., 2013).

In Hungary, TBEV circulation has been documented even in historically low-risk areas. A study near Dévaványa (southeastern Hungary) analyzed 2,731 ticks, identifying TBEV exclusively in *Ixodes ricinus*. The reported MIR were 0.08% for questing nymphs and 0.78% for larvae collected from rodents, underscoring the critical role of small mammals in local viral maintenance. Molecular characterization confirmed that the detected strains belonged to the European subtype, sharing high homology with central European reference isolates (Pintér et al., 2013). Long-term monitoring of a specific microfocus yielded four TBEV isolates over six years, which phylogenetic analysis surprisingly linked to Finnish strains, suggesting potential trans-European dispersal (Egyed et al., 2018). Despite these findings, broader nationwide surveys suggest that TBEV prevalence in Hungarian ticks remains lower than in neighboring endemic countries, a disparity potentially influenced by specific regional climatic conditions (Egyed et al., 2012).

Belgium and Netherlands

In Belgium, TBEV detection in questing ticks remained elusive for over a decade. Large-scale screenings conducted between 2011 and 2023, involving a cumulative total of over 20,000 specimens (primarily *Ixodes ricinus*), consistently failed to detect the virus, resulting in a 0% MIR. However, local viral circulation is evidenced by animal seroprevalence in cattle, with rates ranging between 2.61% and 4.29% (Adjadj et al., 2022; Roelandt et al., 2014)

In the Netherlands, the virus was first identified in ticks and humans in 2015-2016. Subsequent surveillance found TBEV-RNA in 0.9% of rodents and identified a tick MIR of 0.02%, suggesting multiple independent introductions of the virus. Phylogenetic analysis revealed the presence of three different variants of the TBEV-Eu subtype, indicating multiple independent introductions of the virus. These findings, along with recent human cases outside known hotspots, suggest that TBEV is more widely distributed in the Netherlands than previously recognized (Esser et al., 2022).

The Southern and Balkan foci (Southern Europe / Balkan region)

The Southern and Balkan foci encompass a diverse geographical range, including the Alpine regions of Italy and the varying landscapes of Southeast Europe, from the Carpathian Mountains to the Black Sea coast. This zone is characterized by two distinct epidemiological trends: a significant vertical expansion of viral circulation into higher altitudes and a reliance on animal seroprevalence data to identify emerging risks. In the Alpine and Balkan regions, rising temperatures have facilitated the establishment of TBEV in high-altitude habitats previously considered safe. Furthermore, this zone represents a complex intersection of viral subtypes, where the European subtype predominates, but sporadic detections of the Far-Eastern subtype - likely linked to migratory bird routes - challenge established distribution models (Walter et al., 2020b).

Italy

In the Alpine region of northeastern Italy, TBEV is firmly established, with a documented MIR of 0.21% based on more than 2,300 tick samples (D'Agaro et al., 2009). Subsequent longitudinal surveillance in the Province of Trento reported a slightly lower MIR of 0.17%, while highlighting a significant emergence of new active foci and a rise in human incidence since 2012. Notably, the virus exhibits a marked vertical expansion, reaching altitudes of 1,109 m a.s.l., likely driven by climate-mediated shifts in vector distribution (Alfano et al., 2020). Both studies confirmed the circulation of the European TBEV subtype. Genetic analysis showed high variability among TBEV strains in the Trento province, suggesting multiple introductions from neighboring countries (Alfano et al., 2020; Carpi et al., 2009). The virus has also been detected in other parts of northern Italy, particularly in Turin Province, where seroprevalence study revealed antibodies in high-risk groups like hunters and wild boar breeders (Pugliese et al., 2007).

Ixodes ticks carried by migratory birds in Italy were found to harbor various tick-borne zoonotic pathogens. However, TBEV was not detected in these ticks (Grassi et al., 2023).

This evidence underscores the necessity of integrated surveillance - combining human cases, tick screening, and sentinel host monitoring - and highlights the importance of sustained vaccination and public health education in this endemic region.

Romania

Recent surveillance in Romania reveals a complex epidemiological landscape. A seroprevalence study in northwestern Romania identified low TBEV antibody rates (0.08%) in human blood donors (Coroian et al., 2022), contrasted by a significantly higher seroprevalence of 15.02% in sheep from the same region, indicating viral circulation in 80% of tested localities (Salat et al., 2017). While entomological investigations of *I. ricinus* ticks in Cluj county reported a 21.07% overall infection rate for various pathogens (including *Borrelia* spp. and *Babesia* spp.), TBEV was not detected in this specific human-attached tick population (Kalmár et al., 2020). Investigations of *I. ricinus* ticks revealed diverse pathogens, including *Borrelia* species, *Anaplasma phagocytophilum*, and *Rickettsia* spp., with co-infections common (Raileanu et al., 2017)

Nationwide screening between 2006 and 2015 confirmed *I. ricinus* as the primary vector, with TBEV minimum infection rates remaining consistently below 1% in Sibiu, Tulcea, and Giurgiu counties (Vladimirescu et al., 2016).

Historically, the virus's presence is well-documented, with nine TBEV isolates belonging to the European subtype characterized from ticks collected across five counties (Hunedoara, Tulcea, Mures, Alba, Caras-Severin) between 1985 and 1993 (Panciu et al., 2009).

Moldova

In Moldova, TBEV surveillance has yielded highly atypical results, most notably the detection of the Far-Eastern subtype RNA in ticks collected from domestic animals and agricultural landscapes. While the reported infection rates appear significant - 3.8% in *I. ricinus* (3/78), 3.9% in *D. reticulatus* (3/77), and 8.8% in *H. punctata* (3/34) (Ponomareva et al., 2015). While these figures indicate active TBEV circulation in the area, the relatively small sample sizes result in wide confidence intervals (95% CI 3.05-23.0% for the prevalence in *H. punctata* 8.8%), suggesting that these prevalence rates should be interpreted as localized snapshots of viral presence rather than stable regional indicators.

From an epidemiological perspective, these findings are considered a significant outlier. The detection of the FE subtype in Eastern Europe, particularly in vectors like *I. ricinus* and *H. punctata* rather than its traditional vector *I. persulcatus*, is highly unexpected. This occurrence may be attributed to rare independent introductions via migratory birds from distant eastern regions or accidental transport of infected ticks, rather than signifying that Moldova is an established endemic zone for the FE subtype. This perspective is consistent with earlier research; for instance, a study of archived *Ixodes ricinus* ticks from 1960 found other tick-borne pathogens but did not report TBEV (Movila et al., 2013).

While the risk of TBEV infection in Moldova is fundamentally established by the presence of competent ixodid vectors and historical human cases, the sporadic detection of the Far Eastern subtype, highlights the complex and evolving nature of the virus's circulation. This underscores the critical need to enhance surveillance efforts and public awareness to better characterize the true epidemiological status of TBE within the country (Sofronie et al., 2024).

Croatia and Serbia

In Serbia, TBEV circulation has been confirmed in *Ixodes ricinus* ticks across specific microfoci. A study examining 500 ticks (processed in 50 pools) identified three positive samples, resulting in an overall minimum infection rate of 0.6%. Notably, site-specific pool positivity rates reached up to 6.6%, particularly in areas such as the Fruška Gora Mountain, which has been identified as a high-priority zone for active surveillance. Serological evidence further supports viral endemicity, with antibodies detected in humans and various domestic and wild animals, including dogs, horses, and wild boars (Banović et al., 2023; Potkonjak et al., 2017).

Tick-borne encephalitis is a significant public health concern in Croatia, with the Central European subtype of TBEV being prevalent (Erber & Vuković Janković, 2019). Studies in Croatia have identified TBEV in ticks removed from red foxes and in spleen samples of red deer, with a prevalence of 1.6% in ticks and 1.1% in deer (Jemeršić et al., 2014). Antibodies for TBEV were found in 12.2% of horses and 9.7% of sheep (Vilibic-Cavlek et al., 2024).

Bulgaria

Tick-borne encephalitis in Bulgaria has been historically underreported, with only a few cases detected over the past 60 years (Mohareb et al., 2013). However, recent studies suggest that TBE is more widespread than previously thought. A nationwide seroprevalence study found an overall TBE virus seroprevalence of 0.6% in the Bulgarian population, with circulation detected in six districts (Christova, 2018). The identification of five confirmed TBE cases between 2009 and 2012 marked a critical turning point, highlighting that the perceived low risk was likely due to limited clinical awareness rather than viral absence (Mohareb et al., 2013). Historically, TBEV strains were isolated from *Haemaphysalis punctata* and *Dermacentor marginatus* ticks in the 1960s, with antigenic properties identical to the European subtype of TBEV (Christova, 2023). Despite the limited reported cases, the presence of TBEV in ticks and human seroprevalence indicate a potential for TBE occurrence in Bulgaria (Christova, 2018).

Ukraine

In Southern Ukraine, a retrospective molecular analysis of 15,243 ticks collected between 1988 and 1990 across nine species provided critical data on long-term viral persistence. The study identified eight TBEV strains from six sites, with site-specific MIR ranging from 0.11% to 0.81%. Phylogenetic analysis confirmed that these isolates belong to the European subtype, exhibiting up to 97.17% genetic similarity to the 'Pan' reference strain. Notably, TBEV was detected on Byriuchyj Island (Kherson Oblast) for two consecutive years, indicating stable temporal persistence

(Yurchenko et al., 2017).

In contrast, recent surveillance in Western Ukraine (Lviv region) reported significantly higher infection rates, determined through individual tick testing. The individual prevalence of TBEV-RNA was found to be 6.3% in *Ixodes ricinus* and 14.5% in *Dermacentor reticulatus* ticks. These elevated values are often associated with complex pathogen communities, as frequent co-infections with *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum* have been documented in these populations. Such data suggest intense ecological pressure and the presence of potent natural foci in the Carpathian foothills, where the use of individual prevalence provides a more precise reflection of the high local infection risk compared to large-scale pooled metrics (Ben & Lozynskyi, 2019).

Discussions

Evidence from the last two decades (2004-2024) highlights a significant dual expansion of TBEV circulation across Europe: a latitudinal northward shift and an altitudinal vertical expansion. In the Italian Alpine region of Trento, active TBE foci have been documented at elevations up to 1,109 m a.s.l. (Alfano et al., 2020), while in Slovakia's Pol'ana and Smrekovica mountains, TBEV-positive ticks reached 990 m a.s.l. (Zubriková et al., 2024). These vertical shifts, coupled with the northward movement of *Ixodes ricinus* to 65.1°N in Norway and Finland, suggest that traditional climatic barriers are increasingly permeable, likely driven by rising mean temperatures that favor vector survival and host-seeking activity at previously hostile elevations and latitudes.

The findings underscore a high degree of geographical variability and focality in TBEV prevalence. The identification of new microfoci in historically low-risk areas, such as Eastern Germany (MIR 0.58%) and the Netherlands (MIR 0.02%), indicates that the virus is silently establishing itself in new ecological niches (Król et al., 2024; Esser et al., 2022). This expansion is further complicated by the shifting ranges of primary vectors. In the Baltic mixing zone, the establishment of *I. persulcatus* has facilitated the co-circulation of European, Siberian, and Far-Eastern subtypes. The epidemiological impact is profound, as *I. persulcatus* typically exhibits significantly higher viral carriage (e.g., 3.0% MIR in Finland) compared to *I. ricinus* (0.2% MIR), drastically altering the regional risk profile (Laaksonen et al., 2017).

A critical challenge identified in this review is the methodological discrepancy between tick screening and animal seroprevalence. In emerging or low-endemicity zones like the UK, Belgium, and Romania, tick-derived MIR often remains below 1% or even at 0%, failing to reflect the true environmental pressure. In contrast, sentinel animal monitoring has proven to provide additional sensitivity. For instance, the 15.02% seroprevalence in sheep in Romania (Salat et al., 2017) and the 2.61-4.29% seroprevalence in Belgian cattle (Adjadj et al., 2022) confirm established viral circulation in areas where ticks consistently test negative. Similarly, goat serosurveys (14%) were instrumental in identifying the first TBEV foci in southern Switzerland (Casati Pagani et al., 2019). These findings highlight that a low or zero MIR in ticks does not equate to a lack of human risk, but rather reflects the virus's patchy distribution and the limitations of random tick flagging.

Furthermore, recent genomic data challenge the traditional subtype-belt model. The detection of the Far-Eastern subtype in Moldova (Ponomareva et al., 2015) and the discovery of Hungarian strains closely related to Finnish isolates (Egyed et al., 2018) suggest that long-distance dispersal, potentially via migratory birds, plays a significant role in the emergence of atypical foci. Such outliers emphasize the necessity for integrated, One Health surveillance that combines entomological data with wildlife, livestock, and human serology. Moving forward, public health strategies should consider vaccination not only in known endemic massifs but also in emerging microfoci where animal sentinels signal a rising, yet silent, TBEV presence.

Limitations and future perspectives

This systematic review acknowledges several limitations that reflect the inherent complexity of TBEV ecology and surveillance. Primarily, the significant methodological heterogeneity across the 26 countries, ranging from diverse tick collection techniques (questing vs. host-attached) to varying laboratory protocols, complicates direct comparisons of viral prevalence. The coexistence of different statistical metrics, such as minimum infection rate, individual prevalence, and estimated pooled prevalence, necessitates caution when synthesizing data into a unified European risk map. Furthermore, the limited sample sizes in several emerging or low-endemicity zones (e.g., n<100 in specific Moldovan and Southern Polish studies) result in wide confidence intervals, meaning these data should be viewed as localized snapshots rather than definitive regional benchmarks. While animal seroprevalence serves as a robust proxy for viral presence in areas where tick screening fails, it lacks the spatio-temporal precision required to identify the exact moment or micro-location of viral introduction.

Lastly, significant surveillance gaps persist in Eastern and Southern Europe, where TBEV may remain historically underreported due to low clinical awareness and limited diagnostic infrastructure. Future surveillance strategies must move beyond traditional borders by adopting a comprehensive One Health framework. This approach should integrate human clinical data with large-scale veterinary serosurveys and standardized entomological monitoring. A key perspective for future research is the standardization of molecular protocols, favoring high-sensitivity techniques and genomic sequencing to track the cross-border movement of viral subtypes, such as the atypical detection of the Far-Eastern subtype in Eastern Europe. Collaborative efforts among public health officials, ecologists, and veterinarians are essential to develop dynamic risk models that account for climate change and host migration patterns. Ultimately, these integrated insights will be vital for designing effective preventive measures, including targeted vaccination campaigns and enhanced public health education in newly identified hotspots (Gonzalez et al., 2022).

Conclusion

The systematic analysis of tick-borne encephalitis virus prevalence in Europe between 2004 and 2024 indicates a marked shift in the geographical and ecological footprint of this pathogen. Data suggest a dual expansion characterized by the emergence of new transmission foci at high latitudes, reaching the Arctic Circle in Norway, and at altitudes exceeding 1,100 meters in the Alpine and Carpathian ranges. This dynamic is further evidenced by the recent establishment of the virus in the United Kingdom, the identification of microfoci in Lower Saxony and Eastern Germany, and the detection of the pathogen in previously non-endemic regions such as the Canton of Ticino in Switzerland and Northern Zealand in Denmark.

The Baltic region remains a pivotal convergence zone where Siberian and Far-Eastern subtypes co-circulate with the European lineage, exhibiting generally higher reported infection rates in *Ixodes persulcatus* compared to *Ixodes ricinus*. In emerging areas where tick prevalence is often low or fragmented, animal seroprevalence in species such as deer, sheep, or goats appears to provide a far more sensitive indicator of viral circulation than traditional entomological screening. This evolution underscores the limitations of monitoring based solely on reported human cases and highlights the necessity of adopting a One Health framework. Integrated surveillance that combines human clinical data with veterinary serosurveys and environmental factor modeling is critical for anticipating risks in new ecological niches. Adapting public health strategies through the implementation of zonal monitoring and targeted vaccination programs holds potential to mitigate the impact of this virus in a rapidly changing European landscape.

Declaration on the use of Artificial Intelligence

During the preparation of this manuscript, the authors utilized AI-assisted technologies (specifically Gemini) solely to improve the linguistic clarity, grammatical accuracy, and structural organization of the text.

Ethical Approval

Ethical approval was not required for this study because it is based exclusively on previously published literature.

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