Epidemiologie 4-011 22.12.2011 14:18 Str. 135
proLékaře.cz | 9.11.2026

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Sixty Years of Research of Tick-borne Encephalitis – a Basis of the Current Knowledge of the Epidemiological Situation in Central Europe

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SUMMARY

Tick-borne encephalitis (TBE) virus was isolated for the first time in Central Europe in 1948 from both a patient and *Ixodes ricinus* ticks collected in the area where the patient had been tick bitten (the Beroun area – Central Bohemia) and concomitantly from a TBE patient in Moravia (the Vyškov area). Another priority discovery was alimentary transmission of TBE virus via the milk from tick infected grazing goats that was made during a TBE outbreak in Rožňava (SE Slovakia). This outbreak of 660 cases has been the largest of its kind. Both of these discoveries were a challenge to multidisciplinary research into the natural focality of TBE. The results obtained were published by Czech and Slovak authors in the first European TBE monograph (1954) and were the stimulus for further research in this area. From the epidemiological point of view, among others, the impact of meteorological factors (on TBE incidence associated with *I. ricinus* host-seeking activity) and recreational nature of TBE were clearly defined then. At the same time, TBE became a notifiable disease (since 1971 laboratory confirmed TBE cases only). In the following decades, the phenomenon of natural focality of TBE (including anthropic impacts) was extensively studied and the determinants of high-risk areas in the field were analyzed. The results were used in the creation of *I. ricinus* and TBE risk prediction maps for the Czech Republic generated for the first time in Europe using LANDSAT 5 satellite data and GIS technology (1990). In the early 1990s (in particular since 1993), similarly to other countries, the Czech Republic reported a sharp rise in TBE cases that continues, with some fluctuations, until now. The cooperation with climatologists in the analysis of historical data, current epidemiological observations, and study of *I. ricinus* in the field have shown a decisive impact of the ongoing climate change. The analysis of the socio-economic conditions in high-risk areas for TBE has not revealed any impact of these conditions on TBE morbidity. The recreational factor that is influenced by the weather changes has a considerable impact.

The seasonal trend of TBE cases shows large fluctuations as were seen in 2006, 2009, and 2010, also as a result of weather changes with seasons. This clearly implies the need for using long time series of data, covering at least a decade, to be able to draw general conclusions as is the case in the present study (2001–2010). The data broken down by Administrative Region display substantial interregional differences. Of 14 Administrative Regions of the Czech Republic, three exhibits a linear trend in TBE incidence, with a minimum deviation from the baseline, four Regions show different downward linear trends, but seven Regions display different upward trends. The upward trend is most obvious in the Highlands (Vysočina) Region where it is associated with the prevalent orographic conditions and increase in the incidence of *I. ricinus* ticks at higher altitudes.

The knowledge of the area where the patient was tick bitten that is entered in the Epidat database as the „probable area of TBE infection acquisition" is helpful in identifying high-risk areas for TBE. By matching the area of TBE acquisition with that of the patient's domicile we revealed that TBE patients had to travel to areas other than their area of domicile to acquire TBE and thus also significance of the areas of TBE acquisition at the country level. The population of the Prague Region (NUTS3 CZ010) can be used as an illustrative example, with 37.7% of TBE cases only reported to be acquired in the Prague Region while 33.4% of TBE cases were associated with travel to the Central Bohemian Region and 13.9% of TBE cases were imported from the South Bohemian Region (the rates of TBE cases imported from other Regions were less than 5%). And conversely, the residents of the South Bohemian Region (CZ031), with the highest number of TBE cases ever in the Czech Republic, acquired TBE in the region of domicile at a rate of 99.5 %. These rates are clearly associated with the recreational potential of various Regions. The probable area of TBE acquisition is identified by cadastral community. In the light of the natural focality of TBE, the analysis of the local environmental factors involved in the circulation of TBE virus in the wild environment is required to determine the high-risk areas and local risk level. Although outbreaks of TBE cases in humans are indicative of TBE natural foci, more data is needed to delineate such areas. And similarly, the absence of TBE cases in humans over a period of time may not be indicative of a no risk area.

Keywords: **tick-borne encephalitis – epidemiology – distribution – natural focality**

Epidemiologie 4-011 22.12.2011 14:18 Str. 136
proLékaře.cz | 9.11.2026

SOUHRN

Daniel M., Beneš Č., Danielová V., Kříž B.: Šedesát let výzkumu klíšťové encefalitidy – cesty k analýze současné epidemiologické situace ve střední Evropě

Virus klíšťové encefalitidy (KE) byl ve střední Evropě izolován poprvé v roce 1948 na území České republiky z pacienta i z klíšťat *Ixodes ricinus* sebraných v místech, kde jimi byl pacient napaden (Berounsko – střední Čechy) a současně nezávisle také z pacienta na Moravě (Vyškovsko). Dalším prioritním objevem byl alimentární přenos viru KE mlékem pasených koz napadených infikovanými klíšťaty, učiněný při epidemii KE ve městě Rožňava (JV Slovensko). Tato epidemie (660 nemocných) je dosud největší svého druhu. Oba objevy podnítily rozsáhlý výzkum, který soustředil odborníky všech oborů nutných pro komplexní analýzu přírodní ohniskovosti KE. Výsledky pětileté práce byly publikovány českými a slovenskými autory v první evropské monografii KE (1954), a vytvořily tak základ znalostí pro další výzkum v tomto prostoru. Z hlediska epidemiologie, byl mimo jiné již tehdy jasně definován vliv meteorologických faktorů (ovlivňujících incidenci KE prostřednictvím aktivity *I. ricinus*) a rekreační charakter této nákazy. Současně se KE stala povinně hlášenou nákazou (od roku 1971 pouze laboratorně potvrzené případy). V dalších desetiletích byl intenzivně studován fenomén přírodní ohniskovosti KE (včetně působení antropických vlivů) a byly analyzovány faktory vhodné pro vymezení rizikových oblastí v terénu. Výsledky byly využity při tvorbě predikčních map zvýšeného rizika výskytu *I. ricinus* a onemocnění virem KE v ČR realizované poprvé v Evropě na základě družicových dat LANDSAT 5 a s využitím technologií GIS. Začátkem 90. let minulého století (zejména od roku 1993) došlo v ČR (shodně s dalšími zeměmi rozšíření KE) k prudkému vzestupu incidence KE, jejíž vysoká úroveň s určitými výkyvy se udržuje dodnes. Spolupráce s klimatology při analýzách historických dat, aktuálních epidemiologických pozorováních i při výzkumu *I. ricinus* v terénu, ukázala rozhodující vliv probíhajících modifikací klimatu. Rozbor socio-ekonomických podmínek v rizikových oblastech neprokázal vliv na nemocnost KE. Výrazný je vliv rekreačního faktoru, který je ovlivňován průběhem meteorologických změn.

Sezonnost incidence KE podléhá velkým výkyvům (demonstrováno na příkladu 2006, 2009 a 2010) rovněž pod vlivem povětrnostních podmínek jednotlivých sezon. Z toho plyne nutný požadavek používat pro závěry obecného rázu dostatečně dlouhé časové řady vstupních dat, minimálně desetiletého období, jak tomu je v předložené práci (2001–2010). Data prezentovaná podle krajů dokládají podstatné regionální rozdíly. Ze 14 krajů (na něž je ČR administrativně rozčleněna) mají 3 regiony lineární trend incidence KE s minimální odchylkou od výchozích hodnot. Čtyři kraje vykazují v různé míře sestupný lineární trend, avšak 7 regionů demonstruje v různé míře vzestupný trend. Nejzřetelnější je tato situace v kraji Vysočina, kde souvisí s orografickými podmínkami převažujícími v této oblasti a vzestupem výskytu *I. ricinus* ve vyšších polohách.

Místo, kde byl pacient napaden *I. ricinus*, registrované v databázi Epi-Dat jako „místo pravděpodobné nákazy" pomáhá při stanovení rizikových oblastí. Porovnání s místem bydliště pacienta naznačuje pohyb obyvatelstva do těchto oblastí, a tak i jejich význam v celostátním měřítku. Nejzřetelnější je to u obyvatel regionu Praha (NUTS3 CZ010), kde v rámci území vlastního regionu bylo infikováno jen 37,7 % pacientů, zatímco při návštěvě Středočeského kraje 33,4 % a Jihočeského kraje 13,9 % (v dalších krajích jen jednotlivá procenta nebo jejich zlomky). Na rozdíl od toho rezidenti Jihočeského kraje (CZ031, který je na prvním místě v početnosti případů KE v ČR) byli infikováni v 99,5 % na vlastním teritoriu. Je tu zřejmá souvislost s rekreačním potenciálem jednotlivých krajů. Místo pravděpodobné nákazy je registrováno s určením přesnosti katastru obce. Přesnost této informace se ztrácí podle typu použitého kartogramu a základní plošné jednotce, k níž je incidence vztažena.

Vzhledem k přírodní ohniskovosti KE je ke stanovení rizikových oblastí a úrovně místního rizika nutná analýza lokálních environmentálních faktorů umožňujících koloběh viru KE ve volné přírodě. Výskyt lidských případů existenci přírodního ohniska KE indikuje, ale sám o sobě není dostačující. Stejně tak delší období bez výskytu lidských případů nemusí vymezovat bezrizikové území.

Klíčová slova: **klíšťová encefalitida – epidemiologie – rozšíření – přírodní ohnisko**

Introduction

The objective of this study is to provide to the present generation of vector-borne disease (VBD) researchers an insight into the circumstances of the discovery and evolution of the knowledge of one of the most serious tick-borne infections in Central Europe since the very moment of the identification of the causative agent in the late 1940s to the present. This discovery not only elucidated the etiology of tick-borne encephalitis but also opened the era of a better understanding of a wide range of other human infections that originate in the animal kingdom and spread in natural ecosystems independently of humans [1].

The characteristics of TBE and of *I. ricinus* as its key vector and their relationships are summarized in a publication [2].

The TBE virus was first isolated in Central Europe by Czech specialists in 1948 [3, 4] from both a patient and *I. ricinus* ticks. The fact that the causative agent could be identified as well as the vector, area and route of infection was a result of a team effort of a clinician, an

Epidemiologie 4-011 22.12.2011 14:18 Str. 137
proLékaře.cz | 9.11.2026

epidemiologist, a medical entomologist and a virologist, from the hospital in Beroun and the National Institute of Public Health in Prague. In the same year, and completely independently, the TBE virus was isolated in Moravia (the Vyškov area) from patients with a history of a tick bite [5, 6, 7] and then from ticks as well [8]. An unexpected outcome was due to a systematic comprehensive approach (with veterinary surgeons and zoologists joining the team) to the analysis of an outbreak of meningoencephalitis in Rožňava in SE Slovakia in 1951. It was demonstrated that the causative agent was the TBE virus that caused alimentary infection in persons who had consumed raw milk [9]. In a dairy plant with an inoperative pasteurization system, the cow's milk was added with the goat's milk from animals that had been bitten by infected ticks while grazing and then the virus was excreted into the milk. The discovery of the alimentary transmission of the TBE virus, previously unknown in arbovirus infections, was a result of the knowledge and systematic application of the theory of natural focality to the practice.

Both the priority discoveries, shortly apart from each other, had a great repercussion and drew a great interest of the leading Czech and Slovak specialists as documented in the first monograph entitled "Czechoslovak encephalitis" that appeared as early as in 1954 [1]. It was called so because of the then known geographic distribution of TBE prior to the detection of TBE cases in other central European countries. The attention focused by the Czech and Slovak authors on the research of TBE is documented by an overview of 998 articles from 1949 to 1985 [10].

However, even before 1948, unclear summer cases of viral meningoencephalitis, attributable in part to the TBE virus, were reported e.g. by J. Pelnář 1922,1928, Z. Servít 1940, K. Henner and H. Šikl 1942 or J. Šňupárek 1947 [10].

Although a proportion of these papers were local case reports and observations, a substantial part of them presented relevant data on TBE in Central Europe and provided an overview of methodical guidelines, mainly for the field work. Great attention should also be paid to laboratory experiments that ruled out other groups of biting and blood sucking arthropods as possible vectors of TBE virus. Slonim and Kramář focused on the mosquito species found in the then known natural foci of TBE [11, 12]. Smetana conducted experiments with fleas [13].

These activities clearly influenced the research in other European countries. An important contribution in this regard were international conferences and symposiums on TBE organized in former Czechoslovakia since the 1950s. A highlight among these events was the Symposium on theoretical aspects of natural focality [14] held in Prague in 1965 with the participation of the leading advocates of this approach from both the Eastern and Western spheres of influence in the bipolar world of the Cold War of that time. Moreover, in the 1950s and 1960s, the research into natural focality of TBE was initiated and started in South East European countries (Yugoslavia, Bulgaria, and Albania) through the cooperation with Czech and Slovak researchers directly in the field [15, 16, 17]. At the international level, sharing the working methods with the university researchers (Universitäts Nervenklinik) in Köln am Rhein (Cologne) was an important step towards the isolation of the TBE virus from *Ixodes ricinus* ticks in the Federal Republic of Germany of that time [18, 19, 20, 21].

At that time, the main TBE research centres in the Czech Republic (CZ) were the Institute of Parasitology of the Czechoslovak Academy of Sciences, National Institute of Public Health, and Institute of Virology of the Czechoslovak Academy of Sciences, joined in their activities by the Institute for Research on Vertebrates of the Czechoslovak Academy of Sciences, Botanical Institute of the Czechoslovak Academy of Sciences, and National Museum in Prague (Department of Zoology).

In CZ, selected infectious diseases are notifiable by law. The first fully operational notification system of nine selected infectious diseases was laid down by Edict No. 20 604 of the Ministry of Interior of the Czech Kingdom within the Austro-Hungarian Monarchy in 1888. Over the following decades, the range of notifiable infectious diseases was progressively extended. Since 1954, any infectious disease is notifiable. The initial notification system using printed forms to be sent to the authorities was updated in 1982, i.e. converted to the Communicable Disease Information System (CDIS) that enabled basic incidence analysis. Since 1993, the Epi-Dat programme has been put in place, derived from the Epi-Info software of the World Health Organization and Centres for Diseases Prevention and Control (CDC) in Atlanta. Other connected programmes were designed by the Epi-Dat SW commission of the Public Health Services and National Institute of Public Health established in 1996.

In the following years, characterized by a downward trend in TBE cases, attention was paid to the existence of natural foci of TBE under modified environmental conditions (in urban and

Epidemiologie 4-011 22.12.2011 14:18 Str. 138
proLékaře.cz | 9.11.2026

industrial areas) and in various recreation areas as well as to the vertical spread and possible penetration to other areas [22, 23, 24, 25, 26, 27]. In the early 1990s, a pioneering approach was put in place as a result of long-term cooperation with the Laboratory for Remote Sensing of the Czech Technical University in Prague and later also with the Chair of Geoinformatics and Cartography, Faculty of Science, Charles University in Prague, making it possible to use LANDSAT 5 satellite data for the identification of high-risk areas for the occurrence of *I. ricinus* ticks as the vector for TBE in the Czech Republic. The first results were published in 1990 as the first study of this kind in Europe [28, 29, 30]. Within the project of the World Health Organization and European Commission „Climate change and adaptation strategies for human health in Europe“ (WHO/EC cCASHh), an atlas of high-risk areas for TBE infection ranked by risk level in the Czech Republic was created based on satellite data in 2002 in cooperation with the National Institute of Public Health in Prague (electronic and print versions 1 : 200 000) [31]. The participation of the National Institute of Public Health in this project in 2000 resulted in a very intensive cooperation with the Czech Hydrometeorological Institute in Prague that was very helpful in an in-depth analysis of the interrelationships between the climate change and both historical and recent TBE trends. Another result of the cooperation is the TICKPRO software that, based on the routine weather forecast, predicts the host-seeking activity level of the *I. ricinus* population [32]. This forecast has been accessible to the public for four years at the web sites of the Czech Hydrometeorological Institute and National Institute of Public Health in Prague.

In the early 1990's, non-viremic transmission of TBE virus between co-feeding *I. ricinus* ticks was proved in collaboration with the Institute of Virology NERC (Oxford) using ticks and TBE virus strain from Central Bohemia [33, 34].

The research conducted on a long-term basis made it possible for the Czech researchers to react properly to the sharp rise in TBE cases in the early 1990's not only in the Czech Republic but also across the European range of the TBE virus [35, 36]. Therefore, it was surprising when S. Randolph and D. J. Rogers [37] with nearly zero experience in the field of TBE assumed the rise in TBE cases in the Czech Republic to result from political changes brought about by the collapse of communism alongside with the poverty of the Czech population. Without the knowledge of the situation (and any tangible data), they said the following: *"This was a time of great political changes in Eastern Europe. The collapse of communism resulted in de-collectivization of agriculture, with active governmental encouragement of individuals to keep flocks of sheep and goats, often grazed on roadside verges harbouring ticks, and to use their milk products. Clusters of TBE cases have been recorded in the Czech and Slovak Republic within families or villages well known for their cheese making (M. Daniel and M. Labuda, personal communication). At the same time, increased poverty arising from the collapse of centralized welfare has forced many poor people to supplement their diet with fruits gathered from tick-infested forests."*

Moreover, that piece of absurdity was presented as a "personal communication" one of the authors of the present paper; however, without his knowledge or authorization.

Unfortunately, our attempt to issue a disclaimer was not successful. To disprove the above mentioned hypothesis, a socio-economic study of TBE patients was conducted [38] and later, an analysis of alimentary infections was performed [39]. The detailed criticism is presented in one of our papers [40]. Nevertheless, S. E. Randolph continued repeating her "political" remarks, and moreover, she added, as a supportive argument, a quote from F. Engels, the pioneer of the 19th century Marxist theory [41].

"According to Engels, the threat posed by infectious disease in the industrial slums stimulated the rise of communism in the 19th century (cited in /1/). In a curious twist of history, it appears that the fall of communist rule at the end of the 20th century may have stimulated a dramatic rise in infectious disease in parts of Central and Eastern Europe".

From the references presented, without further comments, the reader can draw his/her own conclusions about the quality of the opinions expressed

Material and methods

TBE laboratory diagnosis

1. Detection of IgM antibodies in the serum or cerebrospinal fluid (CSF) by enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence assay (IIFA).
2. Detection of seroconversion or significant increase in IgG or total antibodies by ELISA, IIFA or complement fixation reaction (CFR).
3. The laboratory is properly equipped to perform the following analyses: neutralisation test (NT), polymerase chain reaction (PCR), and electron microscopy (EM).

In patients recently vaccinated against TBE, yellow fever, Japanese encephalitis and in recent travellers to endemic areas of the respective causative viruses and of Dengue fever

Epidemiologie 4-011 22.12.2011 14:18 Str. 139
proLékaře.cz | 9.11.2026

virus (DENV) and West Nile virus (WNV), the results need to be confirmed by virus neutralization test (VNT).

Since 1971, only clinically manifest, laboratory confirmed TBE cases have been notifiable, i.e. inflammation of the meninges (meningitis), inflammation of the brain parenchyma (meningoencephalitis or meningoencephalomyelitis) and the most serious bulbocervical form (affecting the cervical spinal cord and medulla oblongata). TBE surveillance was laid down by Regulation No. 275/2010, Annex No. 28.

The data are reported by the diagnosing physicians to the respective public health authorities after obtaining positive findings from biological specimens. Epidemiologists of these institutions add specific data on the circumstances of infection transmission, in particular on the area of tick bite, which should be as accurate as possible.

The data obtained are monitored and analysed by the regional public health authorities.

An upward linear trend in TBE cases is seen countrywide in 1991–2010.

Data on all cases of infection are monitored and analyzed on an ongoing basis by the regional public health agencies and on a national level, at the National Reference Centre for Epidemiological Data Analysis, Department of Biostatistics and Informatics, National Institute of Public Health and at the Infectious Diseases Epidemiology Department, Centre for Epidemiology and Microbiology, National Institute of Public Health.

The epidemiological data are presented on a weekly basis – internal service data, on a monthly basis to the general public at http://www.szu.cz/publikace/data/infekce-v-cr, and in the Bulletin of the Centre for Epidemiology and Microbiology (National Institute of Public Health).

Results

Notification of laboratory confirmed TBE cases only was implemented by the Regulation of the Ministry of Health in the former Czechoslovakia in 1971. In 1971–1990, TBE cases in CZ showed a downward trend with year-to-year fluctuations ranging from 1.4/100 000 and 5.8/100 000 population (Fig. 1).

The epidemiological situation of TBE changed in the early 1990s and TBE incidence has shown an upward trend. The sharp rise in the first half of the 1990s peaking in 1995 was followed by a four-year drop in TBE cases and another peak in the year 2000. The highest number of TBE cases was reported in 2006: 1048 TBE cases (10.0/100 000) (Fig. 2). The data of 2011 are predictive of a high TBE incidence in this year again.

In 2006, the incidence of TBE was the highest, with a usual seasonal increase observed in the spring months peaking in calendar week 29 (July week 3), followed by a steep drop in calendar week 32 and another peak in calendar week 36 (September decade 1), even by nine cases higher than that of calendar week 29. In 2009, TBE incidence was characterized by an unusually rapid increase in the spring months, peaking in calendar weeks 28 and 30, followed by a drop, with only a hint of a second autumn wave. The winter months of 2010 were characterized by cold weather with unusual long lasting snow cover in most of the country persisting up to the end of March 2010 or even longer in some areas and followed by a period of below average spring temperatures. This had a negative impact on different stages of the life cycle of ticks and thus the first summer peak was unusually low and the main peak in TBE

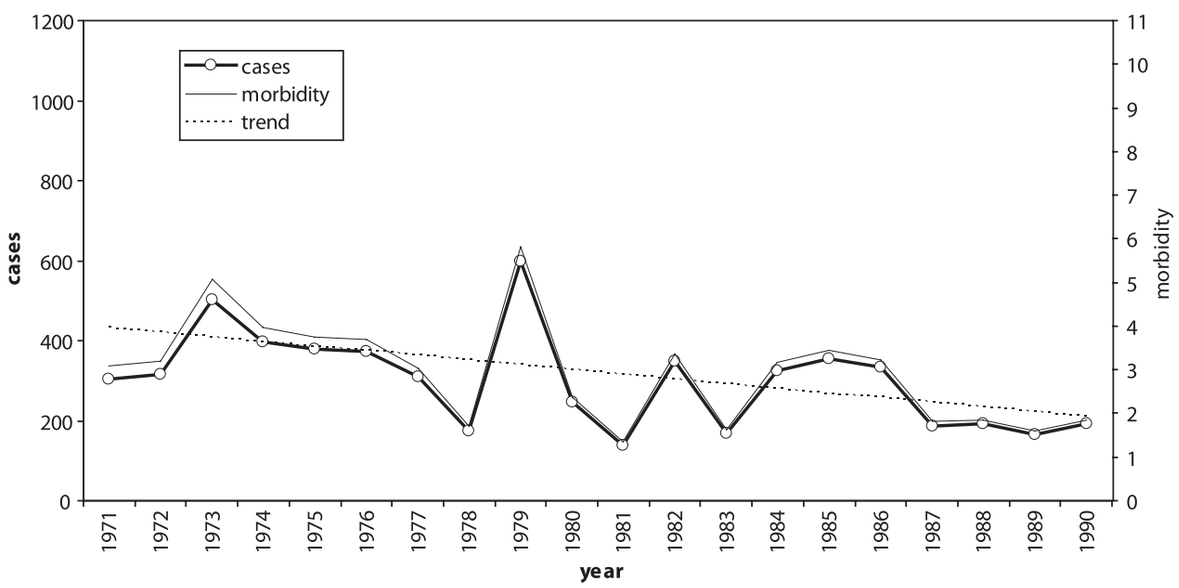


Fig. 1. Tick-borne encephalitis, Czech Republic 1971–1990, cases and morbidity per 100 000 population

Graf 1. Klíšťová encefa1itida, Česká republika, 1971–1990, počet případů a nemocnost na 100.000 obyvatel

Epidemiologie 4-011 22.12.2011 14:18 Str. 140
proLékaře.cz | 9.11.2026

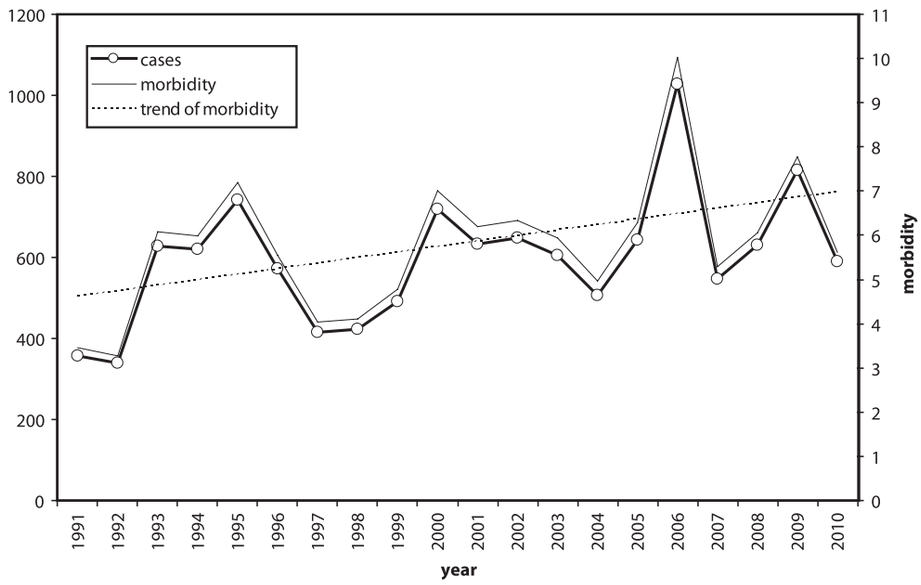


Fig. 2. Tick-borne encephalitis, Czech Republic 1991–2010, cases and morbidity per 100 000 population

Graf 2. Klíšťová encefalitida, Česká republika, 1991–2010, počet případů a nemocnost na 100.000 obyvatel

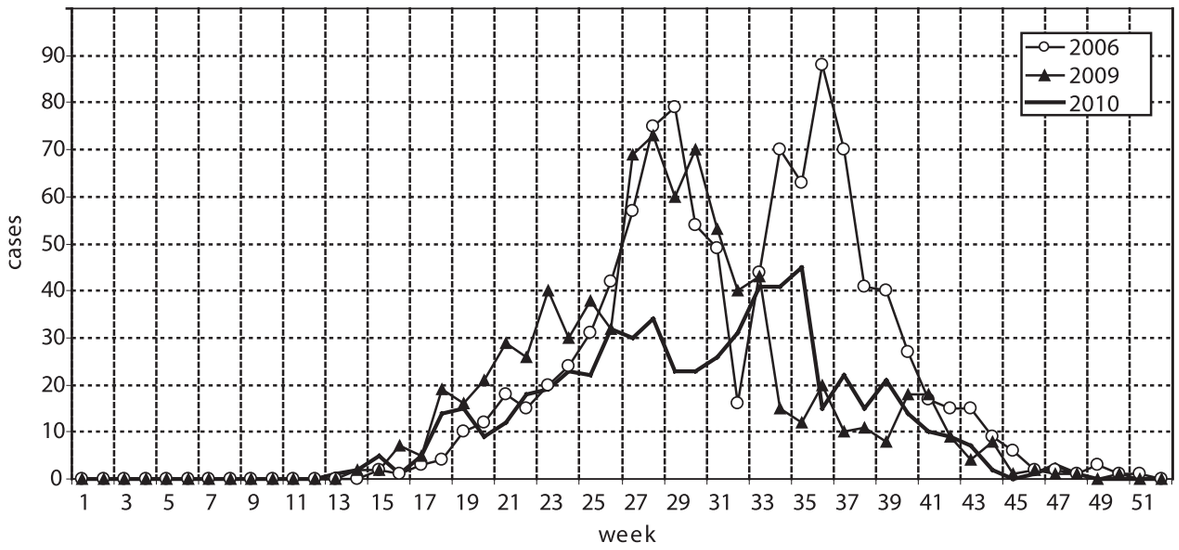


Fig. 3. Tick-borne encephalitis, Czech Republic, 2006, 2009 and 2010, number of cases by weeks of onset of the disease

Graf 3. Klíšťová encefalitida, Česká republika, roky 2006, 2009 a 2010, počet případů podle týdne onemocnění

cases was only observed in calendar week 35 (September week 1), followed by a steep drop (Fig.3).

On the other hand, climatic conditions of the winter and spring months in 2011 were favourable to the development of ticks which manifested by their high activity and a sharp rise in TBE cases reported since the end of May, the number of which was about twice as high in comparison with 2010. By the end of July 2011, as many as 296 TBE cases were notified in comparison with 152 TBE cases in the same period of 2010. The tick bite risk predicted by the TICKPRO national forecast system, designed by the Czech Hydrometeorological Institute and National Institute of Public Health, reached the highest level since May week 2.

More TBE cases have long been reported in men than in women (1.5 : 1). It is noteworthy that this ratio varies significantly across age groups. In 2010, there were only small differences between males and females in the incidence of TBE cases in the age groups 5–9, 20–24, and 25–29. The TBE incidence rates were more than

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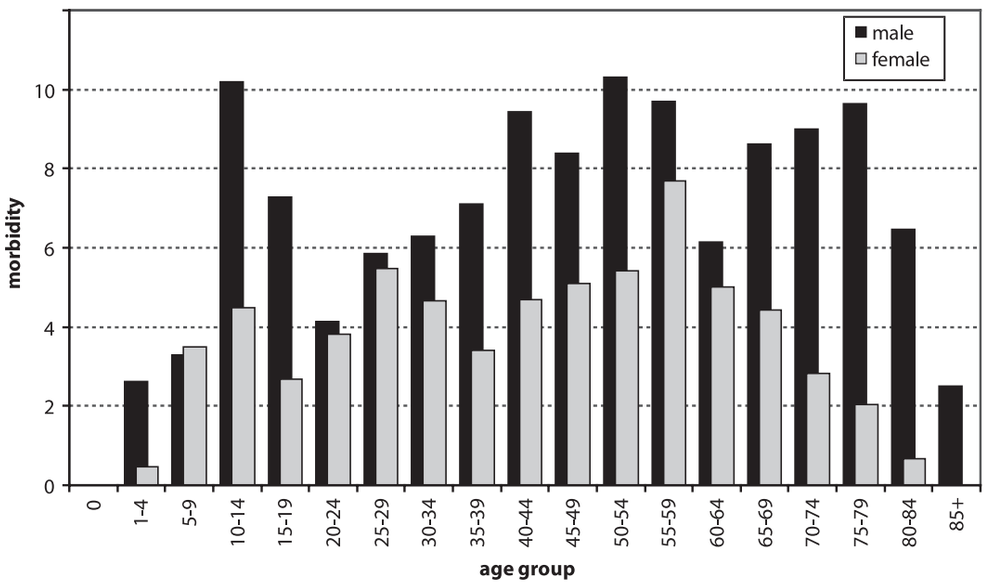


Fig. 4. Tick-borne encephalitis, Czech Republic, year 2010 morbidity per 100 000 population of males and females

Graf 4. Klíšťová encefalitida, Česká republika, rok 2010, nemocnost na 100.000 obyvatel, muži-ženy

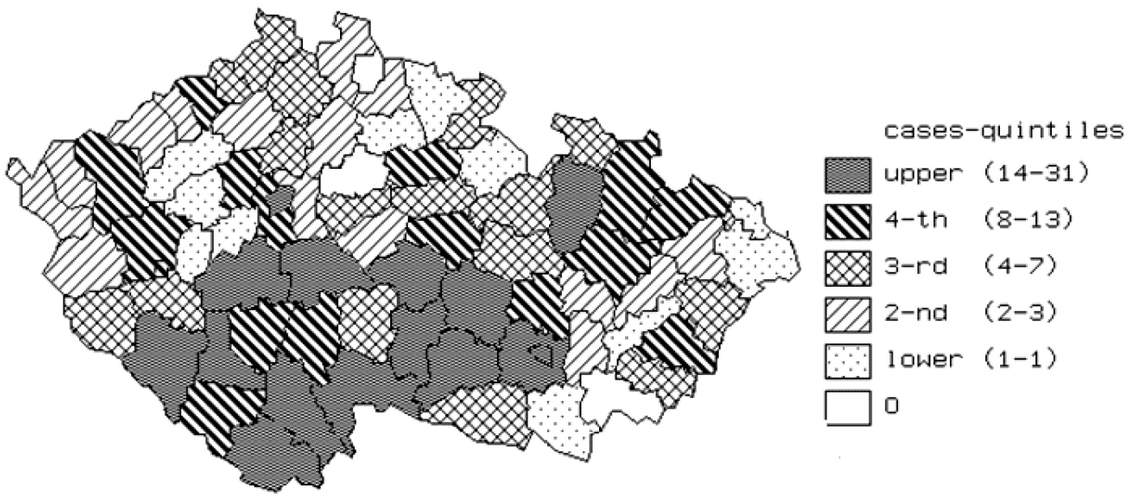


Fig. 5. Tick-borne encephalitis, Czech Republic, 2010, by district of infection

Graf 5. Klíšťová encefalitida, Česká republika, 2010, případy podle okresu infekce

twice as high in males as in females of the age groups 10–14 years (10.2/100,000) and 15–19 years (7.3/100,000). The former rate was the second highest age-specific TBE rate ever, which is alarming (Fig.4).

The distribution of TBE cases in 2010 by tick-bite district is represented in Figure 5. The highest numbers of TBE cases were acquired in districts of the South Bohemian, Highlands and Plzeň Regions. No TBE case was acquired in the districts Nymburk, Jablonec nad Nisou and Hodonín.

The average annual TBE incidence rates by age in 1991–2000 and 2001–2010 are shown in Figure 6. The age-specific TBE incidence rate in the first decade 1991–2000 reached 5.6/100,000 in 10–14-year olds and had a stable trend until the age group 60–64 years, ranging from 5.5/100,000 to 6.3/100,000. It was progressively decreasing in the older age groups to reach 0.7/100,000 in the 85+ year-olds. Similar trends, with increase in children and adolescents, followed by plateau and minimal

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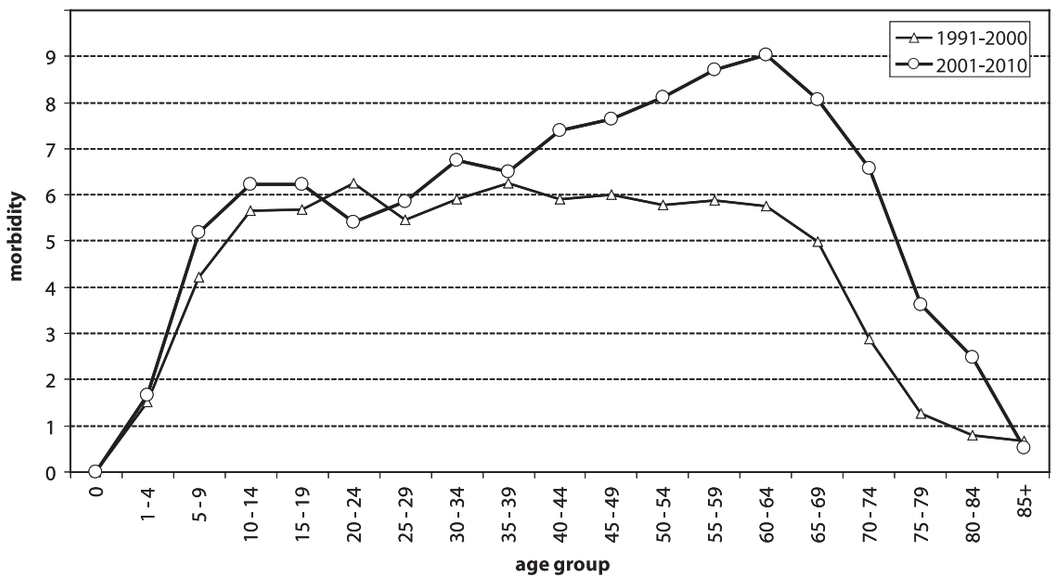


Fig. 6. Tick-borne encephalitis, Czech Republic, 1991–2000 and 2001–2010, average morbidity per 100 000 population

Graf 6. Klíšťová encefalitida, Česká republika, 1991–2000 a 2001–2010, průměrná roční nemocnost na 100.000 obyvatel

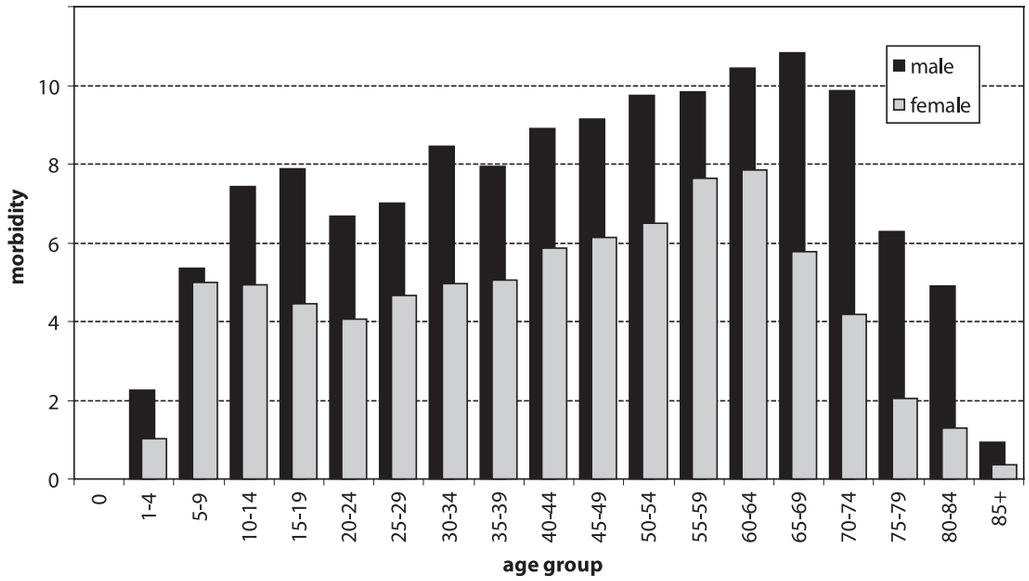


Fig. 7. Tick-borne encephalitis, Czech Republic, 2001–2010, average yearly morbidity per 100 000, males-females

Graf 7. Klíšťová encefalitida, Česká republika, 2001–2010, průměrná roční nemocnost na 100.000 obyvatel, muži-ženy

variations until the age of about 60 years and then by a drop, were observed in the previous decades as well.

In the second decade, 2001–2010, the average TBE rates in the age groups 10–14 years and 15–19 years reached 6.2/100,000. The TBE incidence rates showed a slow upward trend starting from the age group 20–25 years (5.4/100,000) to peak in the 60–64-year-olds. The peak was followed by a declining trend in the older age groups up to the age of 85+ (0.5/100,000) – see Fig. 6. The marked change in the trend in this decade was mainly due to high incidence of TBE cases in the South Bohemian Region with the highest average overall TBE rate of 23.3/100,000.

The average annual TBE incidence rates in males and females in 2001–2010 are shown in Figure 7. In males, the peak rate of 7.9/100,000 was recorded in 15–19-year-olds. It was increasing starting from the age of 20–24 years (6.7/100,000) to peak in the 65–69-year-olds with

Epidemiologie 4-011 22.12.2011 14:18 Str. 143
proLékaře.cz | 9.11.2026

Table 1. Tick-borne encephalitis by Regions, Czech Republic, 2001–2010

Tabulka 1. Klíšťová encefalitida podle kraje, Česká republika 2001–2010, počet případů a nemocnost na 100 000 obyvatel

		Cases 2001-2010 by place of infection			Average morbidity per 100000 population and year		
Region	**NUTS3**	**inside of the region**	**outside of the region**	**total**	**inside of the region**	**outside of the region**	**total**
Hl.m.Praha	CZ010	247	414	661	2.1	3.5	5.6
Středočeský	CZ020	685	50	735	5.9	0.4	6.3
Jihočeský	CZ031	1456	7	1463	23.2	0.1	23.3
Plzeňský	CZ032	601	26	627	10.9	0.5	11.4
Karlovarský	CZ041	79	5	84	2.6	0.2	2.8
Ústecký	CZ042	404	47	451	4.9	0.6	5.5
Liberecký	CZ051	128	19	147	3.0	0.4	3.4
Královéhradecký	CZ052	108	12	120	2.0	0.2	2.2
Pardubický	CZ053	252	23	275	5.0	0.5	5.4
Vysočina	CZ063	596	7	603	11.7	0.1	11.8
Jihomoravský	CZ064	484	82	566	4.3	0.7	5.0
Olomoucký	CZ071	316	16	332	4.9	0.3	5.2
Zlínský	CZ072	148	11	159	2.5	0.2	2.7
Moravskoslezský	CZ080	391	33	424	3.1	0.3	3.4
Total	CZ0	5895	752	6647	5.8	0.7	6.5

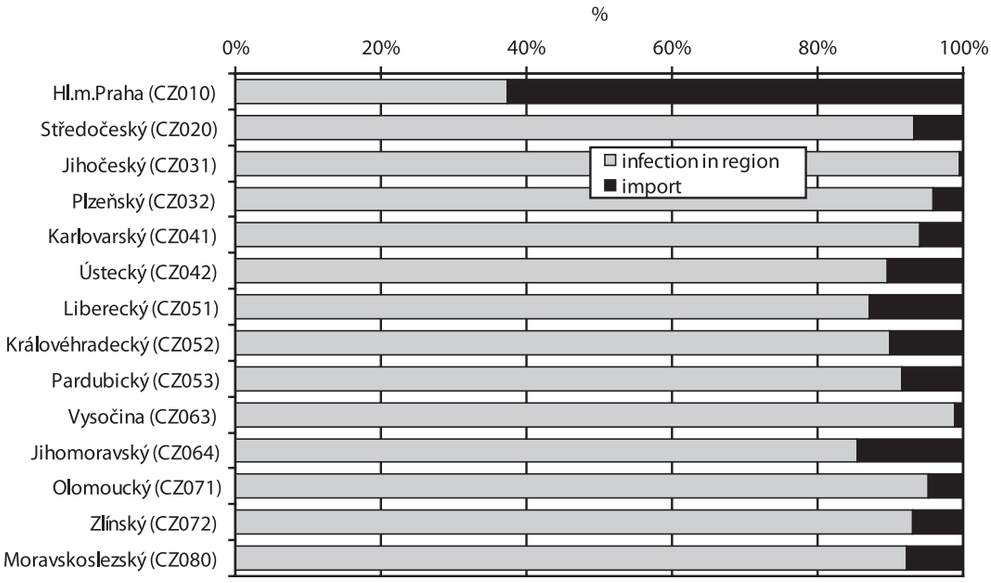


Fig. 8. Tick-borne encephalitis, Czech Republic, 2001–2010, occurence in percentage of cases by Region of domicile and Region where infection was acquired

Graf 8. Klíšťová encefalitida, Česká republika, 2001–2010, procentuální výskyt případů onemocnění podle kraje bydliště a podle infekce v kraji a mimo kraj

10.8/100,000 and to decline in the older age groups. Still in the age group 85+, the rate was 0.9/100,000.

In females, the average annual TBE incidence rate was the highest in the 5–9-year-olds (5.0/100,000), then showed a slowly declining trend until the age group 20–24 years. An upward trend was observed between this age group and that of 60–64 years (7.9/100,000) and was followed by a drop in older age groups. Among the population over 65 years of age, the more or less stable male to female ratio changed, with TBE incidence being three times as low in females as in males.

The geographic distribution of the average annual TBE incidence rates by region in

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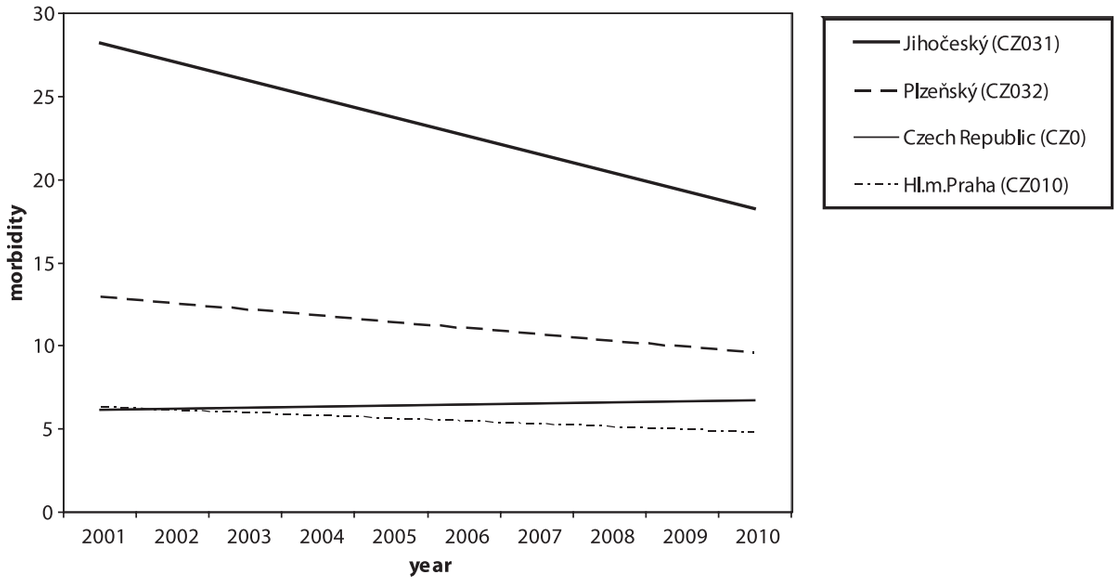


Fig. 9. Tick-borne encephalitis, Czech Republic, 2001–2010, morbidity per 100 000 population, a linear trend by region of domicile of cases (Regions Praha, Jihočeský, Plzeňský)

Graf 9. Klíšťová encefalitida, Česká republika, 2001–2010, průměrná roční nemocnost, lineární trend podle místa bydliště v krajích – vzestupný trend

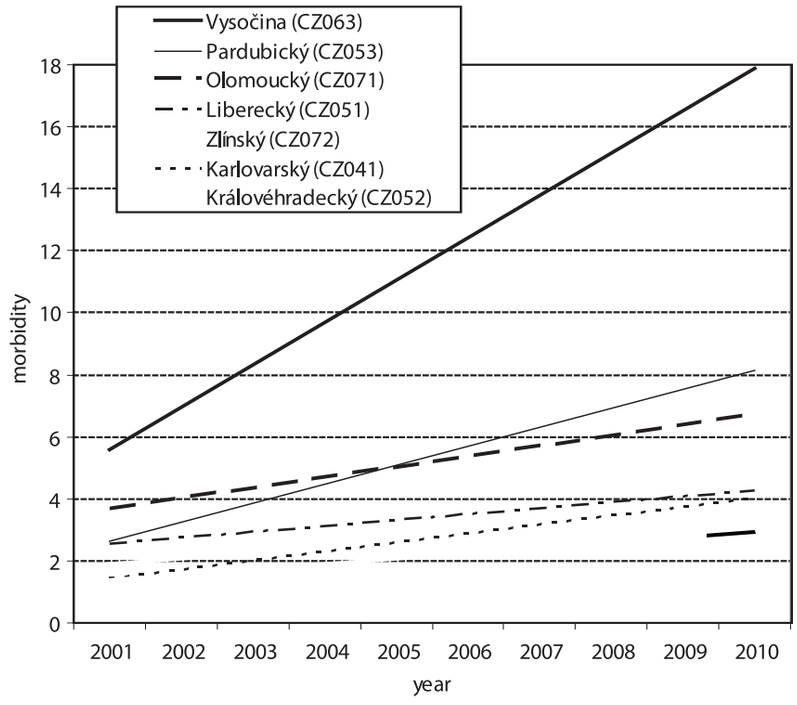


Fig. 10. Tick-borne encephalitis, Czech Republic 2001–2010 average yearly morbidity, linear trends by region of domicile of patients upward trends

Graf 10. Klíšťová encefalitida, Česká republika, 2001–2010, průměrná roční nemocnost, lineární trend podle místa bydliště v krajích – vzestupný trend

2001–2010 is presented in Table 1. The highest TBE incidence rates were recorded in the following Regions: South Bohemian – 23.3/100,000, Highlands – 11.8/100,000, Plzeň – 11.4/100,000 and Central Bohemian – 6.3/100,000, while the lowest TBE incidence rates were reported in the Regions Hradec Králové – 2.2/100,000, Zlín – 2.7/100,000, and Karlovy Vary – 2.8/100,000. The average annual TBE incidence rate in CZ in 2001–2010 was 6.5/100,000. In this table, TBE

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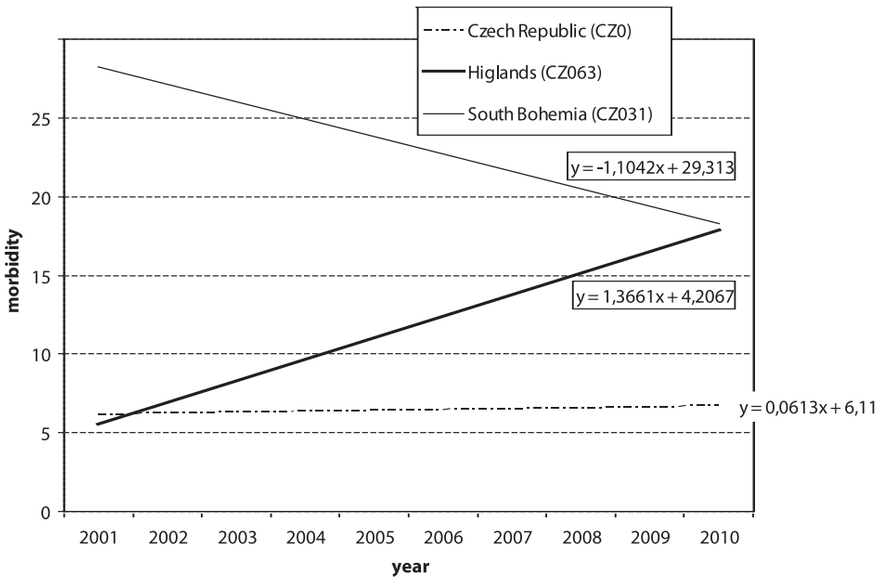


Fig. 11. Tick-borne encephalitis, Czech Republic, 2001–2010, morbidity per 100 000 population a linear trends by region of domicile of cases, Regions Jihočeský and Vysočina

Graf 11. Klíšťová encefalitida, Česká republika, 2001–2010, lineární trendy nemocnosti na 100.000 obyvatel v Jihočeském kraji a Kraji Vysočina (podle kraje bydliště nemocného)

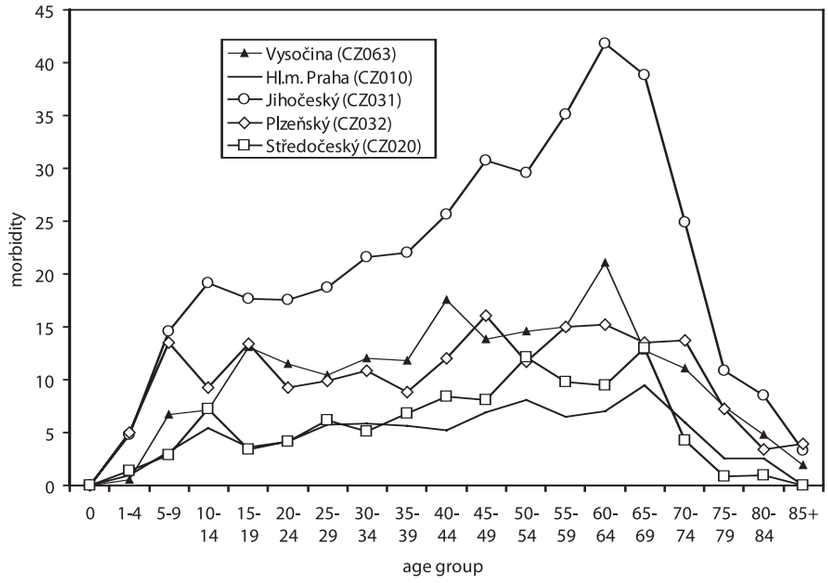


Fig. 12. Tick-borne encephalitis, Czech Republic, 2001–2010, morbidity per 100 000 population, selected Regions, morbidity per 100 000 populations by age groups

Graf 12. Klíšťová encefalitida, Česká republika, 2001–2010, vybrané kraje, nemocnost na 100.000 obyvatel podle věkových skupin

cases are divided into those acquired in the Region of domicile and those imported from other Regions (see Table 1).

The distribution of TBE cases by Region of domicile and Region of TBE acquisition are shown in Figure 8. The greatest proportion of lack of match between these two Regions was found for the population of Prague (CZ010) where 37.7% of TBE cases only were acquired in the Region of domicile while 33.4 % of TBE cases were imported from the Central Bohemian Region (CZ020), 13.9% from the South Bohemian Region (CZ031), and 3.4 % from the Plzeň Region (CZ032). TBE cases acquired in other administrative Regions accounted for 0–2% of the total reported. In contrast, the proportion of matches between the Region of domicile and the Region of TBE acquisition was the highest in the South Bohemian Region (CZ031) where 99.6% of

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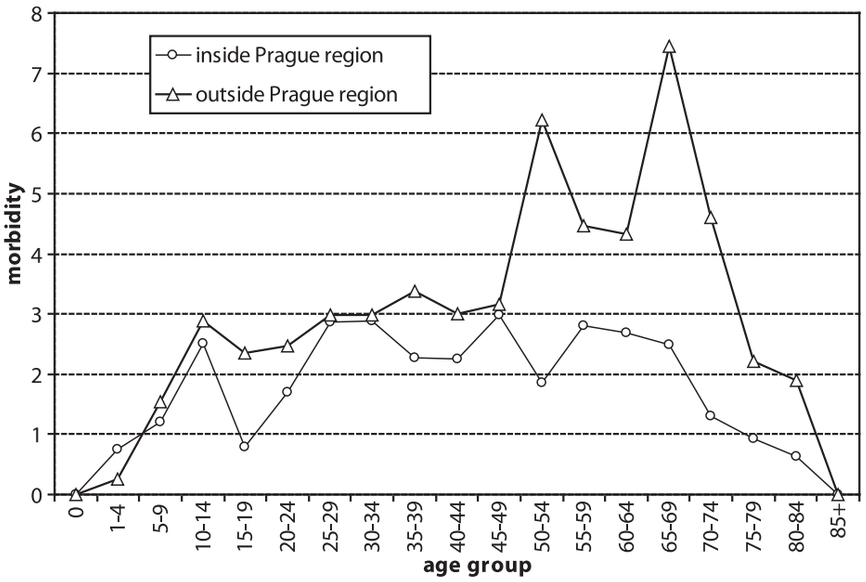


Fig. 13. Tick-borne encephalitis in Prague (CZ010), 2001–2010, average morbidity per 100 000 population age and place of infection in Prague region and outside Prague Region

Graf 13. Klíšťová encefalitida u obyvatel h1. m. Prahy (CZ010), 2001–2010, nemocnost na 100.000 obyvatel a rok, podle věku a místa infekce

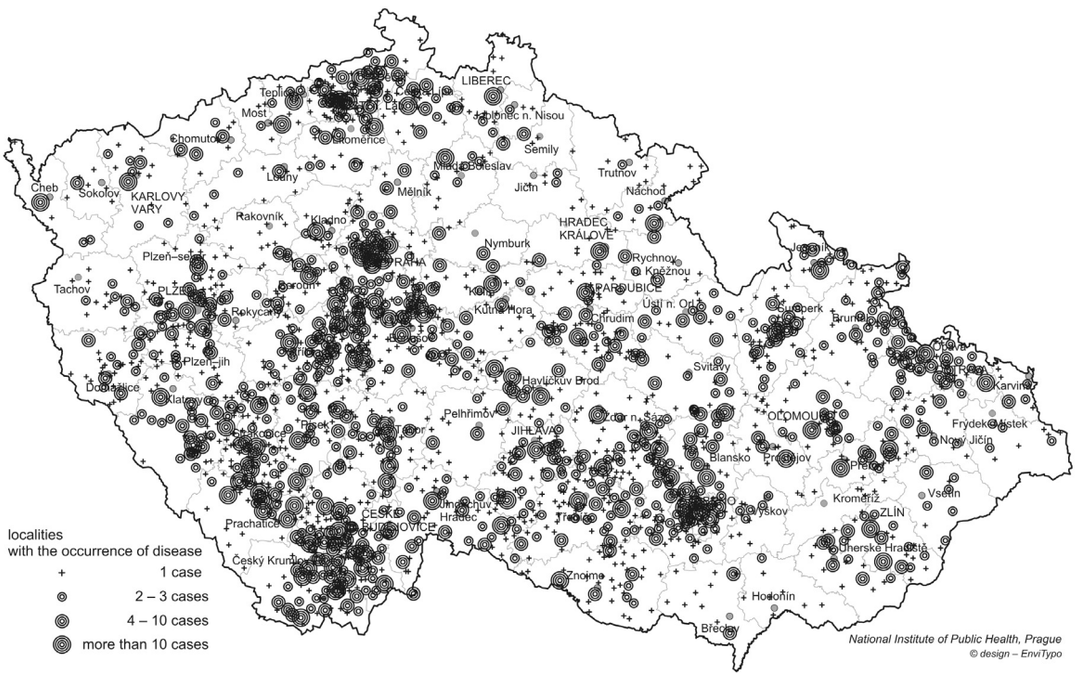


Fig. 14. Tick-borne encephalitis, Czech Republic, 2001–2010 by areas of infection

Graf 14. Klíšťová encefalitida, Česká republika, 2001–2010 případy podle místa infekce

TBE cases had been acquired in the Region of domicile. In the remaining administrative Regions, the proportion of TBE cases acquired in the Region of domicile ranged between 85.5% in the South Moravian Region (CZ064) and 98.8% in the Highlands Region (CZ063)

The diagrammatic representation of these trends in TBE incidence by Region of domicile in 2001–2010 is shown in the following figures. A linear trend with a minimum deviation from the baseline TBE incidence rate was observed in the following administrative Regions: Ústí nad

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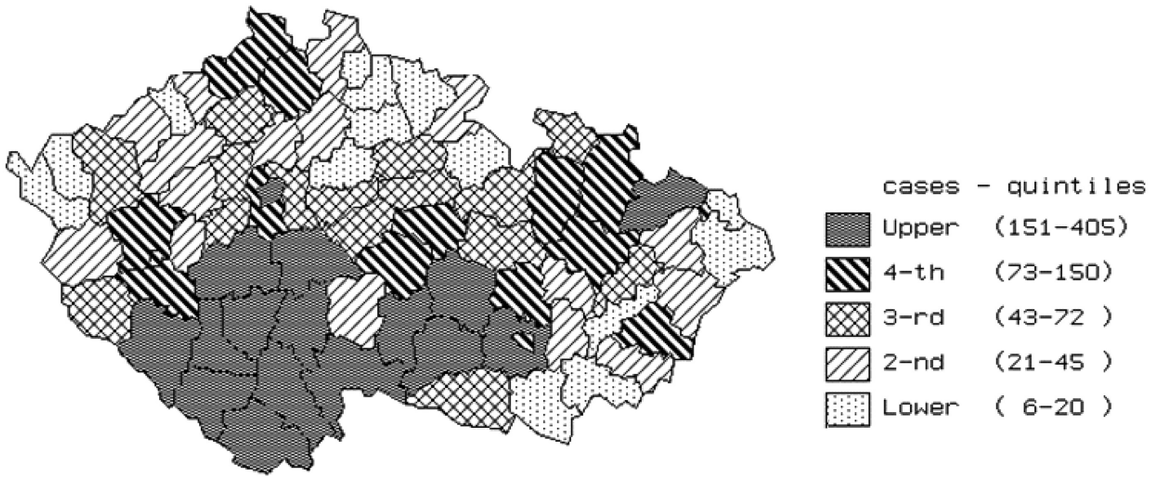


Fig. 15. Tick-borne encephalitis, Czech Republic, 2001–2010 by district of infection

Graf 15. Klíšťová encefalitida, Česká republika, 2001–2010, případy podle okresu infekce

Labem, Central Bohemian and Moravian-Silesian (not shown). A declining trend in TBE incidence was seen in the following administrative Regions: South Bohemian, Plzeň, South Moravian, and Prague. A declining trend was typically found in the Regions with a baseline higher rate of TBE cases where much more attention is traditionally paid to TBE surveillance and greater awareness of TBE vaccination benefits is achieved (Fig. 9).

An upward trend in TBE cases was observed in the following administrative Regions: Highlands, Pardubice, Karlovy Vary, Hradec Králové, Liberec, Zlín, and Olomouc (Fig. 10). The comparison of the administrative Regions with an extreme drop or rise in TBE cases with the average TBE incidence rate in CZ is shown in Figure 11.

The average age-specific TBE incidence rates in the selected administrative Regions most affected by TBE in 2001–2010 expressed by age-specific trends vary. While in the Plzeň Region, the curve is flat with relatively small fluctuations and little variation in the highest rates between the younger and older age groups, the trends in the age-specific TBE incidence rates in the remaining Regions show hints, to different extents, of upward trend in TBE incidence, with the greatest (more than double) difference between the peaks of the younger and older age groups and a sharp upward trend in the South Bohemian Region (Fig. 12).

The trend in TBE cases acquired in the Prague Region differs substantially from those in TBE cases acquired in other regions. The TBE incidence curves for children of different age groups up to 10–14 years who acquired TBE in the Prague Region and elsewhere are nearly identical. Nevertheless, the TBE incidence rates in the age groups 15–19 and 20–24 are substantially lower in the patients who acquired TBE in the Prague Region. The reason is most probably differences in the leisure time activities, as in Prague, these age groups widely prefer indoor entertainment activities to outdoor activities. The TBE incidence rates in patients of the age group 25–34 who acquired TBE infection in Prague and elsewhere are very close. The TBE incidence rates in the following two age groups are somewhat lower in Prague. Nevertheless, the TBE incidence rates in the age groups 50+ differ significantly ($chi^2 = 8.1$, $p < 0.0044$) between patients who acquired TBE in the Prague Region and elsewhere. While the TBE incidence in patients who acquired TBE in the Prague Region shows a declining trend in the age group 55+, the TBE incidence rates in patients aged over 50 years who acquired TBE in the Regions other than Prague are substantially higher until the age of 84 years, peaking in the age groups 50–54 and 65–69, with the TBE incidence rates being three times as high as those in patients who acquired TBE in the Prague Region (Fig. 13).

An essential part of the epidemiological surveillance is the geographical location of the area where infection was acquired. It is doubly true of the infections with natural focality. The more accurately a natural focus where the infection was acquired is defined, the higher public awareness of the high-risk areas can be achieved. Ideal tools are the satellite navigation, accurate geographical location, number of cases and other relevant data. The infected patients can be residents of the given area as well as

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Fig. 16. Tick-borne encephalitis, Czech Republic, average 2001–2010 morbidity per 100 000 population by Region of domicile

Graf 16. Klíšťová encefalitida, Česká republika, 2001–2010, podle kraje bydliště, průměrná nemocnost na 100.000 obyvatel a rok

travellers to it. The incidence rates reported from districts or larger areas (Regions) only refer to the cases in individuals residing in these administrative units.

The geographical representation of the summary TBE incidence rates in CZ in the last decade is shown in three figures 14, 15, 16. Different modes of diagrammatic representation were intentionally chosen to compare their illustrative potentials. It is evident that the best illustrative is defining the area where TBE was acquired as accurately as possible (Fig. 14).

The distribution of TBE cases by district can also be considered, to a certain extent, as an important quantitative indicator for TBE risk (Fig. 15), although in some instances, TBE cases accumulate in a relatively small area close to the district boundary (cf. Figures 14 and 15). The distribution of TBE cases by administrative Region (NUTS 3) is not adequately informative of TBE risk from the point of view of natural focality since an administrative Region may include areas where the risk level vary widely from high to low or even negative (Fig. 16).

The NUTS2 distribution of TBE cases by area larger than a Region where two to three administrative Regions are put together to form an entity is even less informative than the distribution of TBE cases by administrative Region (NUTS 3).

TBE causes three deaths every year on average. In 1997-2010, 40 TBE cases were fatal. The highest numbers of deaths caused by TBE were reported in the 50+ year-olds. However, TBE caused death even in a 15-year-old patient (2004). The TBE case fatality rates were over 1% in the age group 60+, 3% in the 75-79-year-olds, and nearly 5% at the age of 80+.

Discussion

Tick-borne encephalitis (TBE) is a zoonotic infection in wild vertebrates caused by the TBE virus transmitted from animal to animal, independently of humans, by ticks in general and in Europe, by the *Ixodes ricinus* species in particular. TBE is a typical infection with the phenomenon of natural focality. Humans acquire infection when moving to certain areas where ticks occur and when accidentally are attacked by infected vectors. From the point of view of human medicine, it is the staring point of TBE (that may be asymptomatic, have various clinical symptoms or even a fatal outcome); nevertheless, from the point of view of the circulation of the TBE virus in nature, it is a blind alley. The natural focus of TBE continues to exist (as well as the risk in the respective area) at the same level. TBE differs principally from other arthropod-borne infections where the zoonotic circulation of the pathogen (e.g. malaria) is absent and the interconnection of the infection with the human environment is different in nature. This difference is also crucial when selecting approaches to epidemiological analyses and infectious risk assessment.

Epidemiologie 4-011 22.12.2011 14:18 Str. 149
proLékaře.cz | 9.11.2026

An important link of the processes taking place in a natural focus of TBE is the *I. ricinus* tick. This three-host species, with each developmental stage requiring a blood meal on a different host from a different ecological group including pet animals, involves in the circulation of the TBE virus a wide range of hosts which is also enabled by the transstadial and transovarial transmission of the TBE virus during the life cycle of the tick. Like this, the circulation of the TBE virus is involved in the processes taking place in the entire ecosystem, an integral part of which is the TBE virus. The life cycle of the *I. ricinus* tick is long and thus the tick is not only a vector but also a reservoir of the TBE virus in nature. A substantial part of its life cycle takes place outside any host in the wild where it is fully influenced by changes in the environment in general, and by the microclimatic conditions in particular. On the one hand, the impact of these factors is long-term (climate changes), and on the other hand, short-term (day-to-day weather changes). These factors also influence the levels of the transstadial and transovarial transmission. They also underlie the interannual variation in the *I. ricinus* population density and TBE virus infection rate and thus also the TBE risk level in the respective natural focus.

The *I. ricinus* tick is the only link between the zoonotic circulation of the TBE virus and TBE cases in humans, even the alternative alimentary infection is a result of a tick bite in the lactating grazing animal. The tick bite in humans is closely associated with the host-seeking activity of ticks, governed by weather changes. Another factor is the human behaviour which enables the direct contact with the tick. Due to their limited mobility, ticks only come into contact with humans when entering their environment. In the Czech Republic (CZ), the main reason for such a contact to occur are outdoor leisure and recreation activities that are also strongly influenced by weather conditions in different years. The recreational nature of TBE was recognized early in the research on TBE in CZ [1] and has been confirmed by the following analyses.

The numbers of TBE cases reported in CZ since 1971 (since the notification of the laboratory confirmed TBE cases only) varied at irregular intervals with a general declining trend until the early 1990s. After four years of sharp rise in TBE cases (1993–1995), a drop was observed (1996–1999), but not below the level of the early 1990s, followed by another less important drop in 2003–2005. However, the overall upward trend in TBE cases persisted. This phenomenon was influenced by weather conditions in individual years [42]. It is alarming that despite the awareness raising campaigns on TBE vaccination organized in CZ particularly in the last decade, the upward trend in the TBE incidence continues, although varying with Region. In 2006, the highest number of TBE cases were reported [43]. It turned out to be a result of exceptionally modified, interconnected activities of ticks and humans (in particular during the mushroom season), moreover influenced by the weather conditions.

Sharp changes in the TBE incidence rates in 2006–2010 are associated with substantial modifications in the seasonal trend in the TBE cases. Three different seasonal trends in TBE cases plotted against the calendar weeks of the onset of symptoms are shown for 2006, 2007, and 2010. In 2006, the curve had two peaks, but unlike the two-peak curve observed previously (with the main peak in late spring/early summer) it displayed the main peak in early autumn, much higher than the spring/summer one, with the two peaks being separated by a summer drop in TBE cases. The year 2009 was characterized by a single-peak curve with a pronounced spring peak and only a slight hint of an autumn peak. In 2010, the TBE incidence curve followed the pattern of that of 2006 (with the late summer peak being more pronounced than the spring one), but the reported numbers of TBE cases were generally considerably lower in comparison with two previous seasons. It can be explained by the impact of unusual and sharp changes in weather conditions in the years compared (unpublished data) influencing, in turn, *I. ricinus* activity and human behaviours.

Unlike most recreational activities (camping, living in secondary residences in the countryside, hiking, hunting, etc.) that show low interannual variation, mushrooming, a favourite leisure time activity with a long tradition in CZ, varies widely from year to year depending on the harvest of mushrooms. While mushrooming, people enter high risk areas for TBE with the abundance of infected ticks (border areas of forest habitats called ecotones). Moreover, the mass media reports on wild mushroom pickers' records raise the interest in mushrooming and thus also the numbers of visitors to high-risk areas, since mushrooming is a national tradition and sport in CZ, something like a matter of prestige.

The comparison of these three principally different TBE seasons observed over a five-year period clearly shows that to be able to draw general conclusions concerning a given area, time series of input data as long as possible are needed to be evaluated. Therefore, in the present study, the focus is on the decade that can be considered

Epidemiologie 4-011 22.12.2011 14:18 Str. 150
proLékaře.cz | 9.11.2026

as the shortest acceptable time interval. When considering the reasons underlying these substantial year to year differences in 2006–2010, we can exclude demographic and socio-economic factors that did not undergo such dramatic changes that would match the fluctuations in the TBE incidence in CZ over the period studied. From the perspective of human behaviours, only outdoor recreation activities influenced by weather conditions in each individual season are to be considered.

When analyzing factors possibly involved in the variability of TBE incidence, attention also should be paid to the zoonotic circulation of the TBE virus and changes in the *I. ricinus* population density and host-seeking activity. Over the period discussed, no substantial changes occurred in the landscape ecology: neither land cover nor land use changed to cause them. Of the whole complex of environmental conditions, only weather conditions showed dramatic fluctuations across the seasons. Weather conditions had both year-to-year and day-to-day impact on the tick populations and thus modified the seasonal trends in the TBE incidence.

The study of the comprehensive phenomenon obviously needs a multidisciplinary team approach of epidemiologists, meteorologists, parasitologists specializing in the ecology of ticks, and possibly also of medical zoologists focusing on the ecology of wild animal hosts of ticks and TBE virus. It is crucial to draw the attention of the clinicians to the need for complete and reliable patient history including the data on the suspected area and date of TBE acquisition (tick bite). The data on the onset of TBE symptoms and their nature are also relevant, as in most cases, the date of admission to hospital corresponds to the onset of meningoencephalitis symptoms.

Unusual fluctuations in the TBE incidence during the season can be predicted 3–4 weeks ahead. Our experience gained with the TICKPRO software (that has been used for four years in 2011) that predicts the level of seasonal host-seeking activity of *I. ricinus* based on the routine weather forecast provides evidence of it [32].

Detailed knowledge of the area where TBE infection was acquired and its relationship to the area of the patient's domicile are relevant to the identification of high-risk areas and high-risk human behaviours for acquiring TBE in various regions of CZ. Baze for further considerations is matching the area of TBE acquisition to the area of the patient's domicile. Unlike the other administrative regions of CZ, the Prague Region shows the lowest rate of matches of TBE acquisition area and that of the patient's domicile. The vast majority of TBE patients domiciled in Prague imported TBE infection from other regions as a result of outdoor recreation activities. The well-known TBE natural foci are located in the Central Bohemian Region within commuter reach of Prague. The TBE virus was isolated from *I. ricinus* ticks and TBE patients for the first time in Central Europe in the Beroun area, i.e. in one of the Central Bohemian TBE foci, in 1948 [3]. In addition to the environmental conditions suitable for the emergence of the natural foci of TBE, another important contributor to the contact with infected ticks is recreation in secondary residences [44]. In CZ, the Central Bohemian Region has the highest density of secondary residences designed specifically for seasonal, recreation or occasional use. The trend started in the 1930s to peak in the 1970s and 1980s [44]. It is important to note that most recreation activities take place during the period of peak host-seeking activity of ticks. Apart from the secondary residences intended for recreational purposes, another contributor to the contact with infected ticks is the emergence of new green residential areas on the outskirts of Prague.

Another source of TBE infection for the Prague population is the South-Bohemian Region which is also linked to the recreational potential of this region, with some differences arising from the distance from the capital. A specific feature of this region is the landscape type, mainly the countryside landscape, 70% classified as "the countryside landscape with the potential for tourism" [44] while the urbanized area only accounts for 4%. The South Bohemian countryside landscape, characterized by a distinctive mosaic of small islands of forest, often in close contact with human dwellings, provides favourable conditions for the existence of stable *I. ricinus* tick populations and thus also for the existence of TBE natural foci [45]. The phenomenon of recreation in secondary residences is also obviously present in the South Bohemian Region. The secondary residences in South Bohemia differ in nature from those in Central Bohemia [44]. Starting from the early 1950, in small remote villages and settlements hard to reach from the main roads, many traditional dwellings were converted to recreation residences. In addition to recreation activities that are commonplace across CZ, water sports such as riverside canoeing, kayaking and rafting with camping and diverse use of the recreational potential of the Lipno Dam area have become very popular in the South Bohemian Region. Again, it is to be underlined that these activities take place mainly during the period of peak host-seeking activity of ticks.

Residents of the South Bohemian Region where

Epidemiologie 4-011 22.12.2011 14:18 Str. 151
proLékaře.cz | 9.11.2026

the highest number of TBE cases are reported at the national level acquire TBE infection primarily in their region of domicile. This suggests that their outdoor recreation (and possibly other) activities take place primarily in their Region of domicile in the period of host-seeking activity of ticks (except travel to TBE-free regions in other countries).

The South Bohemian Region should be considered as another, although more distant recreation area for a large part of the Prague population where many of them acquired TBE. In this connection it is to be noted that the South Bohemian Region - still with the highest TBE incidence rate – is the only administrative Region that shows an obvious declining trend in the TBE incidence among the local population that might be misinterpreted as a result of the reduction of TBE risk in this Region that may imply decrease in TBE cases in travelers to this region. Such a conclusion would be erroneous. The two phenomena are independent of each other. The observed declining trend in TBE cases in South Bohemian residents results from the higher vaccination rate in the local population, i.e. from an effective prevention in individuals that has no impact on the zoonotic circulation of the TBE virus in the nature. The TBE risk in natural foci remains unchanged. Recreational travel and outdoor activities of the Prague residents are directed to other Regions as well, mainly to the North-East part of the country with popular recreation areas, i.e. the Jizera Mountains and the Giant Mountains and their foothills [46].

When considering the areas of TBE acquisition for the Prague patients, it is important to add comments on 38% (247) TBE cases of "local origin". It is to be admitted that this number may also include TBE patients who were unaware of the tick bite and therefore were unable to identify where TBE infection had been acquired. Nevertheless, there are areas with stable tick populations and confirmed zoonotic circulation of the TBE virus in the capital. No general research has been conducted to date and only isolated case reports have been available [47, 48]. Attention was focused on this issue in 1977–1980 [49] when 12,657 *I. ricinus* ticks (11,007 nymphs, 803 females and 847 males) were collected in larger parks located in Prague and Prague outskirts and analysed in isolation tests. Even though this research was conducted in the period of an overall drop in the TBE incidence in Central Europe, one strain of TBE virus was isolated [25] and the prerequisites for tick infestation of the urban greenery were defined in detail [22, 50].

In the light of the issue of the occurrence of *I. ricinus* ticks in the Prague greenery, it is necessary to draw attention to the current trend in creating new park areas, that in the outskirts of Prague often extend heathland areas, as well as to their organized recreational use. From these areas *I. ricinus* is accidentally imported to gardens of the adjacent residential districts.

In 1991–2010, a rise in TBE cases was reported countrywide. In the last decade the linear trend in TBE cases is flat and no peak is seen. Nevertheless, this general conclusion is not applicable to any single Region. While three administrative Regions (Ústí nad Labem, Central Bohemian and Moravian-Silesian) show linear trends with minimal fluctuations from the baseline, four administrative Regions (South Bohemian, Plzeň, South Moravian, and Prague) show various declining trends. The steepest drop in TBE incidence was recorded in the South Bohemian Region where the highest number of TBE cases in CR had been reported previously. In the light of the fact that these Regions displayed the highest rates of TBE cases in the past, the declining trends can be explained by the increased population awareness of the disease as a result of awareness-raising and vaccination promotion campaigns and by the higher vaccination coverage achieved. Nevertheless, it is alarming that an inverse, i.e. upward, trend was observed in the other administrative Regions, with the sharpest rise always reported by the Highlands Region (CZ063) that used to rank among the least affected by TBE.

When analysing the situation in more depth, it turns out that starting from 1997, TBE cases are on rise in the Highlands Region (23.9 per 100,000), with the rate being twice as high as the national average (10.0 per 100,000) while in 1971–1996 the rate of TBE cases in the Highlands Region was below the national average [51]. The rise in TBE cases still continues, with small fluctuations, and is observed in any district of this region. It is important to note that in this region, no marked changes occurred in landscape use or socio-economic conditions that might increase the high-risk group of the population. Factors implicated in the local rise in TBE cases need to be sought in other environmental conditions. As orographically most of the area of the Highlands Region is part of the Bohemian-Moravian Highlands system, it can be assumed that the reason for the rise observed in the cadastral areas at altitudes above 500 m is increasing density of *I. ricinus* tick populations and thus also increasing circulation of the TBE virus in the nature associated with the temperature increase recorded. A long-term field experiment in an area of the Giant Mountains [52, 53] clearly showed the impact of climate

Epidemiologie 4-011 22.12.2011 14:18 Str. 152
proLékaře.cz | 9.11.2026

warming (particularly in the months of peak host-seeking activity of ticks) on the life cycle of this vector. *I. ricinus* ticks not only move to higher altitudes above the limit of their previously known vertical range but also the density of tick populations close to the limit altitude increases. *I. ricinus* becomes more abundant in vast areas at higher altitudes.

The age distribution of TBE patients can be a base for considerations on the impact of vaccination on TBE incidence in different age groups and on their behaviours resulting in contact with ticks. The distribution of TBE cases by age in the South Bohemian Region is different in comparison with other administrative Regions. It shows a marked increase in TBE cases in the 60–64-year-olds, even twice as high as in the under 39-year-olds. This phenomenon is influenced by two factors. One is the vaccination coverage in the youngest population as well as in the productive age groups. The elderly still tend to underestimate the risk and refuse to get vaccinated, as they believe to have become progressively immunized against TBE virus as a result of their long-term presence in the high-risk area. A part of this population may have acquired TBE infection without developing clinical symptoms and may have produced antibodies, but only low levels of antibodies, that do not protect against future infection. As the South Bohemian Region was among the most affected in CZ during the entire TBE monitoring period, from the age distribution of TBE cases it can be concluded that the TBE virus infection is not able to induce lifelong herd immunity. The other factor is contact with the nature associated with a high TBE risk in the South Bohemian Region. Given the high recreational potential of this Region, most TBE cases were acquired in the Region of domicile. The age groups 55–69 years in particular (accounting for the peak TBE incidence) include retirees in good health living in secondary residences from the early spring throughout the period of host-seeking activity of ticks, no matter how modified it may be in various seasons. This conspicuous peak recorded in the South Bohemian Region is also reflected in the countrywide curve of age specific TBE incidence. The productive age groups (and parents with children) time their holidays with the school summer holidays, with the host-seeking activity of ticks being substantially reduced in August. Moreover, these summer holiday stays in secondary residences are much shorter in comparison with retirees. The ever upward trend in seaside holidays in other countries taken primarily by the productive age population should also be taken into account. The age distribution of TBE cases in Prague is analyzed in detail. The comparison of TBE cases acquired in the Prague Region and outside of it shows clear differences in the outdoor activities between age groups. The curve representing the TBE cases acquired within the Prague Region follows the pattern of the countrywide TBE incidence in the decade 1991–2000, with a sharp rise in the 10–14-year-olds, followed by a plateau until the age 60–64 years. The Prague curve shows two distinct drops. The one in the age group 15–19 years, continuing in part also in the 20–24-year olds, can be explained by the lifestyle of the adolescents and young adults who tend to spend their leisure time indoors, thus avoiding contact with high-risk areas infested by *I. ricinus*. Nevertheless, given that a similar (although relatively smaller) drop is seen in this age group in TBE cases acquired outside the Prague Region, it is evident that another factor is the TBE vaccination coverage in the young age groups. Nevertheless, a question arises as to why more TBE cases are reported in the age group 10–14 years. The explanation may be the participation of these teenagers in leisure time outdoor activities during the summer holidays organized in recreation areas of the Prague Region. It would be desirable that organizers of such activities should be aware of TBE risk and proceed accordingly when preparing and running such events. In this context, the public health authorities authorizing and monitoring such events should pay great attention to the TBE risk. The other drop seen in the age group 50–54 years for TBE cases acquired in the Prague Region is mirrored by TBE cases acquired outside the Prague Region and they are likely to influence each other. The two curves diverge diametrically at the age of 50+. It means that the recreational behaviour of the younger and middle generations follows the pattern observed in the South Bohemian population. The trend toward seaside holiday stays in other countries seems to be more pronounced at the expense of longer summer stays in CZ. In contrast, the population at the retirement age (50+) clearly tend to live in secondary residences particularly in the Central Bohemian and South Bohemian Regions. And again, the tendency toward underestimating TBE risk and the resulting low vaccination coverage in senior age groups need to be taken into consideration.

A primary aim of epidemiological analyses of TBE incidence is to identify, as accurately as possible, the high risk areas and high risk population groups. In general, there are two approaches to this issue. The starting point for both of them is the fact that the infectious process

Epidemiologie 4-011 22.12.2011 14:18 Str. 153
proLékaře.cz | 9.11.2026

in humans starts with a tick bite that takes place in the very environment of the tick (suitable vegetation formation) while accidentally entered by humans. Without this contact, no infection develops. When identified, a TBE natural focus is informative of the existing TBE risk but not of the TBE incidence in humans that is impossible to determine without the knowledge of the frequency with which human visitors enter the defined area during the host-seeking activity period of ticks.

The first approach is based on direct detection of the landscape elements which, in accordance with the results of long-term field studies, are indicators of the presence of suitable ecological conditions for the *I. ricinus* tick and its animal hosts and, at the same time, for the circulation of TBE virus among the vector and its hosts. Such indicators are particularly plant communities as previously reported in both the Czech and international literature (for more details see [28, 29, 30]). Remote sensing with the potential for large-scale use of satellite data and geographic information system (GIS) technologies applied to the epidemiology are useful tools in determining the TBE risk level in a defined part of the landscape analyzed [29]. The atlas of TBE in CZ was generated this way as the first TBE atlas in Europe and was published within the cCASHh project [31]. On the one hand, it contains maps of TBE cases in humans in 1971–2000 by tick bite areas (with an accuracy of cadastral community) and on the other hand, maps of high-risk areas for TBE due to the occurrence of *I. ricinus* ticks. This approach is comprehensive and multidisciplinary and the outcomes are presented as theme maps derived from larger-scale geographical maps.

The other approach applied in the present study is based on detailed analysis of patient medical history (PMH) data and the outcomes are presented as cartograms derived from administrative areas that are the subject of study. This diagrammatic representation at a medium or smaller scale does not provide topographical and physico-geographical details of the landscape but does provide relevant information on high-risk population groups for TBE and high-risk human behaviour along with other demographic data such as age and sex. High quality PMH data is crucial for obtaining relevant results. In the Czech reporting system EPIDAT, the data is entered on probable area of TBE acquisition which is indicative of the patient's travel activities and behaviour and of other details related to TBE acquisition.

Three cartograms are included in this study to illustrate three modes of the presentation of the same data. The figure where the cadastral communities of TBE acquisition are indicated shows a mosaic pattern of the reported TBE cases that is consistent with the distribution of local environmental conditions. Noteworthily, when the distribution of TBE cases is shown by administrative region, the information on the actual high-risk areas for TBE is lost. The reports of TBE cases by administrative Region cannot be considered as a basis for a detailed epidemiological analysis and identification of high-risk areas for TBE. To obtain more reliable results in this regard, it would be desirable to group together the districts characterized by closely similar TBE incidence rates on a long-term basis (and not based on their administrative location) to form greater entities with distinct environmental conditions (including land cover and land use), and the recreational potential – which are important factors in identifying and planning preventive measures.

The length of the time series of TBE cases reported is also highly relevant to getting a reliable idea on the geographical spread of these cases. This issue is to be considered in the geographical areas with low TBE incidence rates. For instance the cartogram summarizing the areas of TBE acquisition in 2010 displays three districts with zero TBE incidence; however, based on summary data for 2001-2010, these districts are part of the areas with TBE incidence, although in the lowest quintile. In our opinion, a decade as used in the present study is the shortest still reliable period that can be used for this purpose.

Conclusion

The complex nature of the phenomenon of natural focality of TBE was a challenge for an actual multidisciplinary team cooperation of clinicians, epidemiologists, virologists, parasitologists, zoologists, veterinary experts, botanists, landscape ecologists, climatologists and other specialists in the past. Accurate diagnosis including the collection of reliable patient history data plays a crucial role. Starting point for the adoption of effective preventive measures is the analysis of relevant data on local populations of ticks, their conditions to thrive and TBE virus prevalence in ticks. The collection of the input data in the field cannot be replaced by questionnaire surveys and their formal statistical analysis. The TBE risk assessment should be based on the analysis of sufficiently long time periods, as the activity of natural foci of TBE is

Epidemiologie 4-011 22.12.2011 14:18 Str. 154
proLékaře.cz | 9.11.2026

subject to interannual variation depending on the long (even several-year) life cycle of ticks which is considerably affected by changing climatic conditions. Moreover, it is to be underlined that, both from the factual and ethical perspectives, respect needs to be shown to the classical literature, even if it dates back to the era before electronic databases, as it provides an invaluable basis for the current knowledge.

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Epidemiologie 4-011 22.12.2011 14:18 Str. 155
proLékaře.cz | 9.11.2026

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