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## Article

# The influence of Biogeographic Diversity, Climate and Wildlife on Tick-Borne Encephalitis in Croatia

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**Abstract:** Tick-borne encephalitis (TBE) is the most significant arboviral infection in Croatia. The aim of the study was to analyse and correlate 17-year TBE seroprevalence data with winter temperature, precipitation and wildlife abundance to identify possible patterns that can be predictive of TBE incidence. TBE diagnosis was based on IgM/IgG anti-TBE antibodies and individual clinical interpretation of the results. Of the 19,094 analysed patients, 4.2% had acute TBE, significantly more often in older age ( $p < 0.001$ ) and in men ( $p < 0.001$ ). Overall seroprevalence of TBE among the tested population was 5.8%, and varied annually from 2.8% to 10.7%. The mean acute TBE incidence rate was 1.1 per 100,000 population with significant regional differences: 1.7 in the continental vs. 0.2 and 0.5 in the Mediterranean and Alpine regions, respectively. Particularly high incidence of 3.1 was recorded in northern Croatia. TBE displayed a seasonal pattern, peaking in June and July. Moderate negative correlations were observed between TBE acute cases and winter temperatures from December to February ( $r = -0.461$ ;  $p = 0.062$ ), relative rodent abundance ( $r = -0.414$ ;  $p = 0.098$ ) and yearly precipitation from one year before ( $r = -0.401$ ;  $p = 0.123$ ). Considerable efforts are needed to understand the impact on TBE incidence to improve disease prevention.

**Keywords:** tick borne encephalitis virus; Croatia; TBE incidence; TBE seroprevalence; biogeographic diversity; climate; precipitation; wildlife

## 1. Introduction

Tick-borne encephalitis (TBE), caused by the tick-borne encephalitis virus (TBEV), a neurotropic arbovirus from the genus *Flavivirus*, family *Flaviviridae*, is a potentially life-threatening central nervous system infection prevalent in eastern, central and northern Europe, including Croatia [1,2]. The European subtype virus (TBEV-Eu) is the etiological agent of TBE in central Europe, and its primary arthropod vector is the highly prevalent hard tick species *Ixodes ricinus* [3]. Small rodents, eg. mice and voles, especially the yellow-necked mouse (*Apodemus flavicollis*) and the bank vole, (*Clethrionomys glareolus*) are crucial for the maintenance of TBEV within the tick population. Several species of larger vertebrates, most notably the roe deer (*Capreolus capreolus*) are considered tick maintenance hosts, as their abundance correlates with higher tick density [4].

As with other high important zoonotic diseases, a multifaceted, integrated approach is required to formulate effective and economically viable measures against human infection and disease caused by TBEV. This is partly due to the fact that the dynamic of TBEV transmission is a result of interaction between several ecological and environmental factors: arthropod vector populations, zoonotic vertebrate hosts, geography, vegetation, climate and others [4]. Along with that, determinants of human behaviour leading to increased exposure to infected ticks are a key component of TBE epidemiology [5–8]. The epidemiology of tick-borne diseases is subject to a wide range of influencing

factors that intertwine with each other. The influence of one parameter on the change in TBE incidence is not easy to define.

In order to guide the establishment of effective TBE infection prevention programs, risk areas need to be identified, geographically defined and monitored over time, ideally through standardized continuous surveillance [9]. This is especially important for areas with a high level of biogeographic diversity which result in large differences in disease risk between close geographic areas, like in Croatia. EU-level standard case definitions and a reporting system have been established and updated, but they have yet to be fully and consistently adopted by individual member states [9]. Underreporting and underdiagnosis of TBE cases have been reported, which may negatively impact surveillance [9–13].

TBEV transmission to humans is known to occur within relatively small endemic foci, where the environmental requirements for the enzootic cycle of TBEV are met [4]. Endemic hotspots have been observed in the same locations for decades, but it has been shown that new ones appear over time in new locations, and some of the existing ones may disappear [14–16]. Climate change has been implicated as a driving factor in some of the observed shifts in the geographical distribution of tick activity, and, potentially, TBEV endemicity [17,18]. A more precise analysis of local TBEV endemicity can be established directly, through focused surveillance of TBE cases in humans and seroepidemiological studies, or through surveillance of TBEV transmission, detection of TBEV in ticks or anti-TBE antibodies in small vertebrate hosts or domestic animals [19–23]. Monitoring vector density and abundance of specific vertebrate hosts may enable the development of risk prediction models for TBE, while considering the deep complexity of all the factors influencing the TBEV enzootic cycle and the epidemiology of TBE [24–26].

The aim of the study was to present, analyse and correlate 17-year data on TBE human cases in Croatia with temperature, precipitation and wildlife abundance parameters. We sought to identify possible patterns in climate and environmental data that could be useful in predicting future TBE risk. Our hypothesis was that warmer winters enable better survival and more efficient reproduction of the tick population in the following season, and that precipitation, which likely influences both tick biology and human behaviour-potentially leading to greater exposure to vectors-may be linked to the incidence of TBE.

## 2. Materials and Methods

### 2.1. Data Collection

#### 2.1.1. TBE Cases

This retrospective study included data of patients diagnosed for TBE from 2002 to 2018 at the University Hospital for Infectious Diseases Zagreb, Croatia, a leading national medical center for infectious diseases. The diagnosis of TBE was defined according to IgM and IgG anti-TBE antibodies in blood followed by individual clinical and patient history data interpretation. Commercial IgM and IgG anti-TBE enzyme-linked immunosorbent assays (ELISA, FSME/TBE IgG/IgM ELISA, Virotech Diagnostics, Germany) were used according to the manufacturer's instructions. Acute TBE diagnosis was confirmed by specific IgM in the acute serum and/or a significant increase in the IgG titre in follow-up serum samples. Positive IgG anti-TBE antibody titres less than four times cut-off value were considered as past infection or post-vaccination. Defined individual TBE acute cases were used for the analysis. Patient age, sex, sampling date and sample collection site, specified to the level of county were included in the dataset.

#### 2.1.2. Rodent and Common Game Abundance

Game data was obtained from a publicly available hunting record e-database of the Ministry of Agriculture [27]. Total numbers of shootings per year for roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), wild boar (*Sus scrofa*) and common pheasant (*Phasianus colchicus*) were used for the analysis. Relative abundance of rodents was calculated as number of trapped animals per number of

trap nights. Rodents were trapped in lowland and highland forests as part of routine rodent monitoring using snap traps during which guidelines by Gannon et al. were followed [28].

### 2.1.3. Winter Temperatures and Precipitation

The temperature data for the City of Zagreb representing the continental regions with the largest number of reported acute TBE were analysed for period 2002-2018. The following temperature parameters were included: average and minimum temperature in January, average temperature of three winter months (December, January, February), and minimum annual temperatures. Monthly precipitation data (rainfall in mm) from 12 different meteorological stations scattered through continental Croatia was obtained. All weather data was acquired from the Croatian Meteorological and Hydrological Service (DHMZ).

### 2.2. Statistical Methodology and Analysis

Acute TBE case age groups were defined as follows: 0-4, 5-14, 15-24, 25-44, 45-64 and 65 years and older, and sexes were defined as male and female. Total population numbers for all six age groups and both sexes were obtained. Pearson's Chi-squared test for independence was used to determine whether the distribution of acute TBE cases was significantly different across the age groups and between sexes.

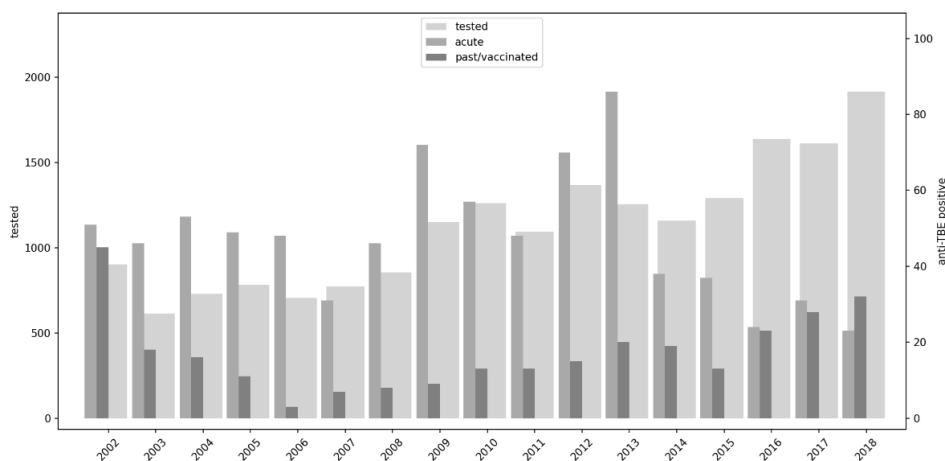
One-way ANOVA was conducted to assess the differences in the mean number of acute TBE cases across all 12 months for the period 2002-2018. A post-hoc analysis using the Least Significant Difference (LSD) test was performed to identify specific months that differed significantly in their mean TBE case counts.

TBE incidence rates per 100,000 population were calculated for each year from 2002 to 2018. for both Croatia as a whole and for each individual county. A map of Croatia (Figure 2) was generated showing biogeographical regions: continental, mediterranean and alpine region with ArcGIS® 9.3. Incidences of TBE in individual counties are shown by the diameter of the circle approximated to the seat of each county.

The correlation between acute TBE cases and population numbers of rodents and common game species, the winter temperature parameters, annual and mean monthly precipitation was tested with the Pearson's correlation coefficient. Demographic data required for the calculations was obtained from Croatian Bureau of Statistics [29,30]. All statistical analysis in this paper was done in Statistica Version 14.0.0.15 TIBCO Software Inc.

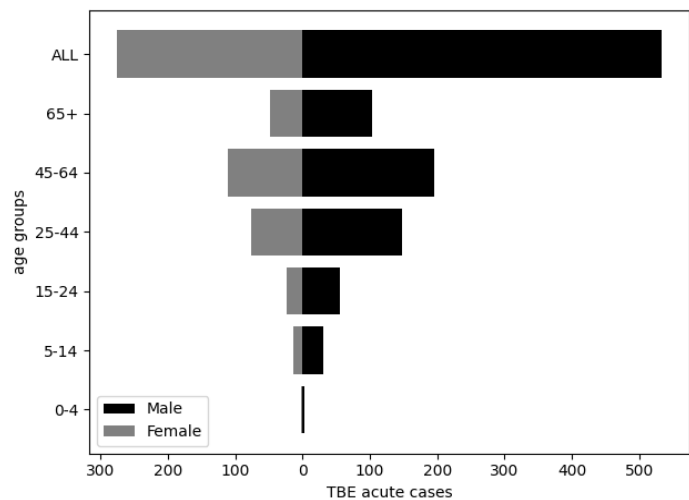
## 3. Results

A total of 19,094 patients in 17-year period from 2002 to 2018 were tested for IgM and IgG anti-TBE antibodies. Anti-TBE IgM and/or IgG antibodies were positive in 1103 (5.8 %) subjects. Annual seroprevalence among the tested population ranged from 2.8 % to 10.7 %. 810 patients had acute TBE, which accounted for 4.2% of tested and 73.4 % of all positive patients. Residual anti-TBE IgG antibodies were found in 293 (1.5 %) subjects. The mean number of acute TBE cases per year was 47.7 (SD  $\pm$  17.00). The annual number of acute TBE cases varied from 23 to 86 (range 63) (Figure 1).



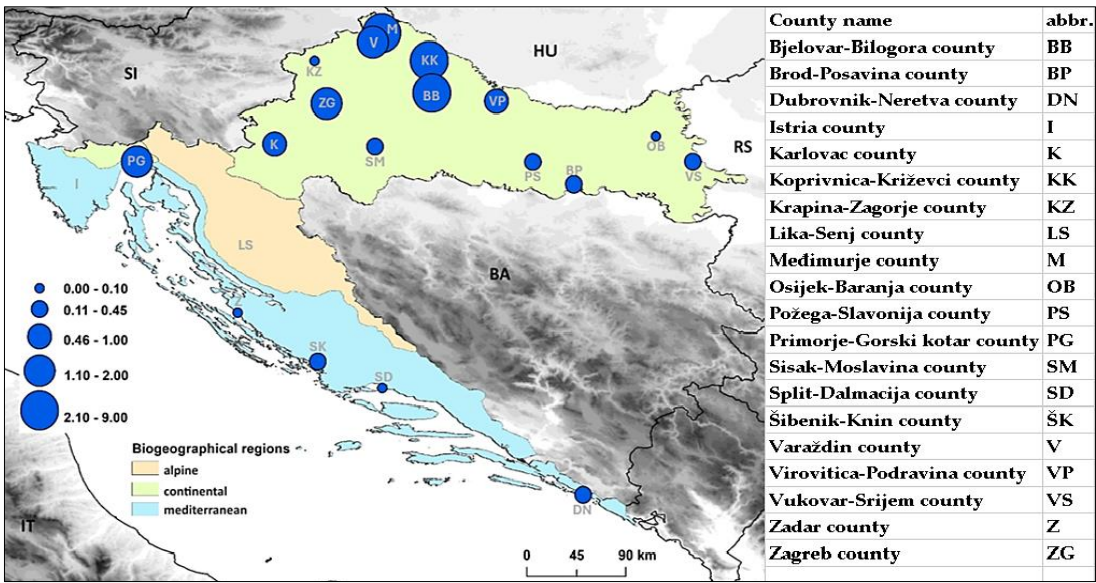
**Figure 1.** TBE cases from 2002-2018 in Croatia: total tested persons (Y-axis left), acute TBE and residual anti-TBE IgG antibodies (Y-axis right).

The number of acute TBE was statistically different between age groups ( $\chi^2 = 78.471$ ,  $p < 0.001$ ) and genders ( $\chi^2 = 64.479$ ,  $p < 0.001$ ). The highest proportion of acute cases was detected in the older age groups, especially in the age group 45-64 (37.8%). Male to female ratio of acute TBE cases was 1.9/1. Men accounted for 65.9 % of acute cases (Figure 2).

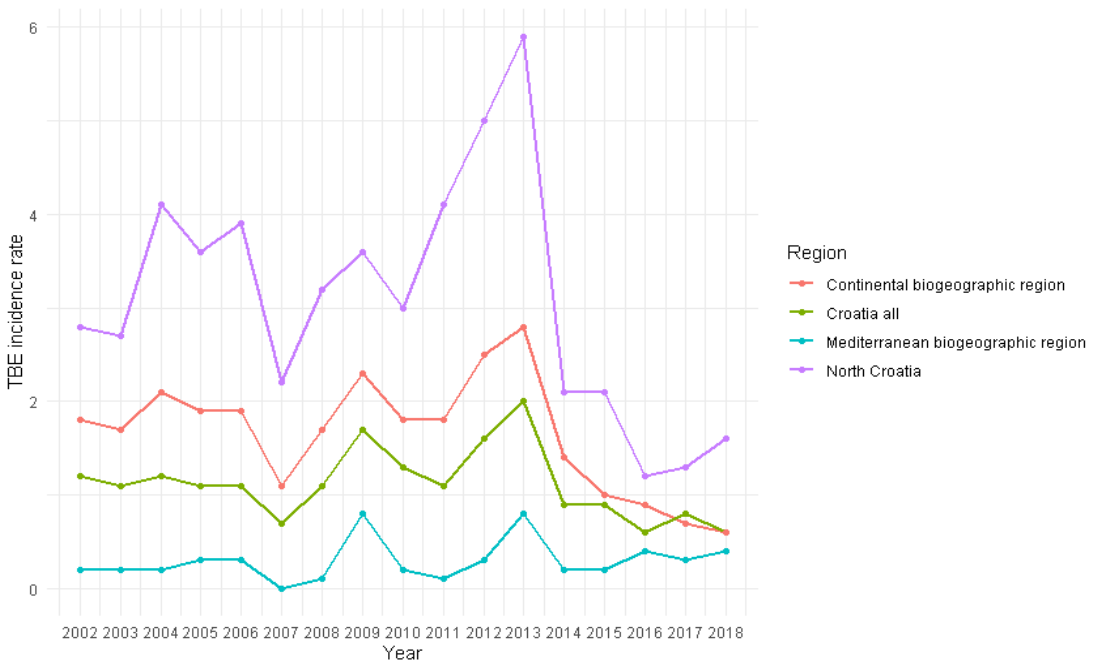


**Figure 2.** Age-sex pyramid chart for acute TBE cases, 2002-2018.

The overall incidence of TBE for the 2002-2018 timeframe was 1.12/100.000 population (SE 0.10). The highest incidence was recorded within the continental biogeographical region (1.65/100.000 population, SE 0.15) (Figure 3, green areas). Of the 810 acute TBE, 787 (97.16%) cases were in the continental biogeographical region, particularly in the North Croatia region (consisting of Zagreb, Koprivnica-Križevci, Varaždin, Međimurje and Krapina-Zagorje counties; 3.09/100,000 population, SE = 0.31). (Figures 3 and 4)

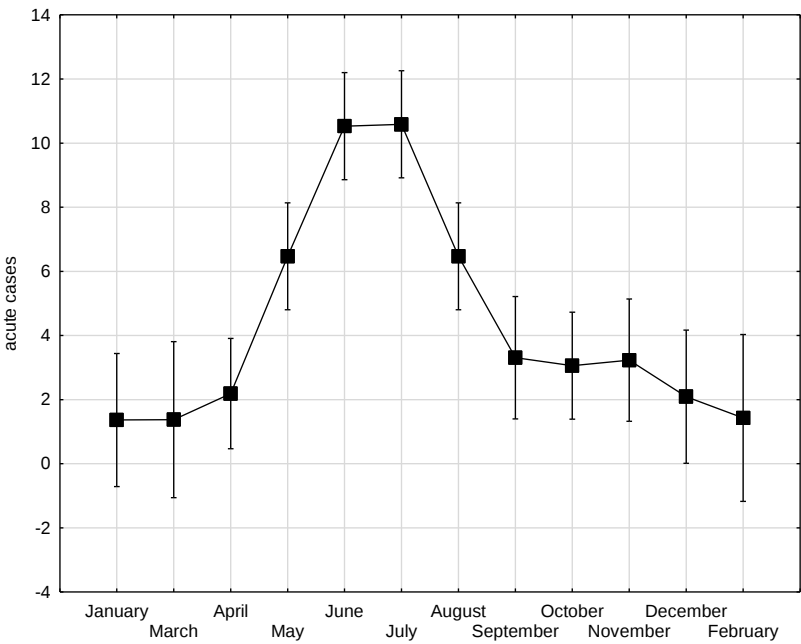


**Figure 3.** Biogeographical regions in Croatia with confirmed acute TBE cases per 100 000 population for each county for the 2002–2018 timeframe. Each circle and county abbreviation are placed approximately to the position of county seats.



**Figure 4.** Acute TBE incidence rates per 100,000 population for each year, 2002-2018. .

Typical seasonality with highest numbers of acute TBE cases from May to August was recorded. The ANOVA analysis of the variance of mean monthly TBE case numbers from 2002 to 2018 indicates a statistically significant difference in the means across the months ( $F(11, 152) = 13.674, p < 0.001$ ). Post-hoc analysis reveals notable peaks in mean monthly TBE cases in July ( $10.59 \pm SE 0.84$ ) and June ( $10.53 \pm SE 0.84$ ). Conversely, the lowest number of acute TBE cases was observed during the winter months of December, January, and February (Figure 5).



**Figure 5.** Distribution of acute TBE by month from 2002-2018. (Vertical bars denote 0.95 CI).

No significant correlation was found between all four temperature parameters and the number of acute TBE both in Zagreb and throughout Croatia. Similar results were found when temperatures from year back were correlated with acute TBE cases (Table 1). However, a moderate negative correlation was observed between winter temperatures and acute cases of TBE for the whole of Croatia (December, January and February) ( $r=-0.461$ ;  $p=0.062$ ). Moderate positive correlation was found for minimum January temperatures from one year back and acute TBE cases in Zagreb ( $r=0.430$ ;  $p=0.085$ ).

Correlation results of yearly game abundance for each of four game species (roe deer, red deer, wild boar and common pheasant) and acute TBE cases showed no significance (Table 2). There was a moderate negative correlation between relative rodent abundance and TBE cases ( $r=-0.414$ ;  $p=0.098$ ).

**Table 1.** Correlation between acute TBE cases and average (mean) and minimum temperatures in winter seasons 2002-2018 for Zagreb county and whole Croatia.

| Period<br>Temperature                                             | JANUARY<br>mean |          | DECEMBER-FEBRUARY<br>mean |          | JANUARY<br>minimum |          | YEARLY<br>minimum |          |
|-------------------------------------------------------------------|-----------------|----------|---------------------------|----------|--------------------|----------|-------------------|----------|
|                                                                   | r*              | p- value | r                         | p- value | r                  | p- value | r                 | p- value |
| ZAGREB COUNTY acute TBE cases                                     | -0.251          | 0.330    | -0.340                    | 0.181    | -0.048             | 0.852    | -0.173            | 0.506    |
| CROATIA acute TBE cases                                           | -0.303          | 0.236    | -0.461                    | 0.062    | -0.122             | 0.640    | -0.170            | 0.512    |
| ZAGREB COUNTY acute TBE and one year back temperature correlation | 0.207           | 0.425    | 0.023                     | 0.928    | 0.430              | 0.085    | -0.013            | 0.958    |
| CROATIA acute TBE and one year back temperature correlation       | 0.169           | 0.515    | -0.132                    | 0.613    | 0.364              | 0.151    | -0.061            | 0.814    |

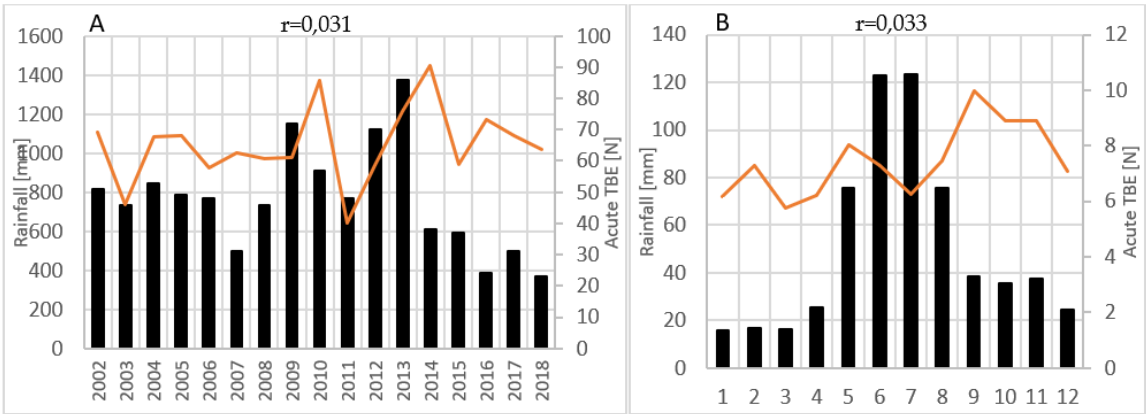
\* Pearson's correlation coefficient.

**Table 2.** Correlation of annual acute TBE cases rodents and most common game species population numbers for 2002-2018. .

| Game species                                       | Rodents |          | Roe deer |          | Red deer |          | Wild boar |          | Common pheasant |          |
|----------------------------------------------------|---------|----------|----------|----------|----------|----------|-----------|----------|-----------------|----------|
|                                                    | r*      | p- value | r        | p- value | r        | p- value | r         | p- value | r               | p- value |
| Acute TBE                                          | -0.414  | 0.098    | -0.208   | 0.422    | -0.334   | 0.189    | -0.248    | 0.337    | -0.354          | 0.163    |
| Acute TBE<br>and one year back data<br>correlation | -0.206  | 0.443    | -0.206   | 0.443    | -0.186   | 0.488    | -0.280    | 0.293    | -0.251          | 0.347    |

\* Pearson’s correlation coefficient.

For the entire period from 2002-2018, total annual precipitation did not show any correlation with acute TBE cases ( $r=0.031$ ;  $p=0.905$ ) (Figure 6 A). There was a moderate negative correlation found between annual precipitation from one year before and incidence of acute TBE ( $r=-0.401$ ;  $p=0.123$ ). There was no significant correlation found between monthly precipitation and monthly acute TBE cases ( $r=0.033$ ;  $p=0.637$ ) for the entire period from 2002-2018. (Figure 6B). Same result was obtained for correlation of monthly acute TBE cases and rainfall from previous month ( $r=-0.025$ ;  $p=0.726$ ) (Figure 6B).



**Figure 6.** (A) Annual precipitation and number of TBE cases. (B) Mean monthly precipitation and number of acute TBE cases. (Pearson’s correlation coefficient ( $r$ ). Orange line = rainfall / precipitation; black bars= acute TBE).

**4. Discussion**

Despite its small area, Croatia is extremely biogeographically diverse, which is reflected in the heterogeneous endemicity of TBE. The largest known TBE hotspots are located between the Sava and Drava river and in Gorski Kotar (Primorje-Gorski Kotar county) [31,32]. Our data is consistent with previous analyses with an incidence of 1.64 per 100,000 population in the period from 2002-2018 for the continental part of Croatia. Most notably, within the continental biogeographic region, the highest number of patients was recorded in the counties of north Croatia: Koprivnica-Križevci, Bjelovar-Bilogora and Međimurje (8.2, 4.1 and 4.0 respectively per 100,000 inhabitants). These areas represent a south-eastern extension of the Slovenian and Austrian TBE endemic areas and thus rival the incidences of known Central European risk areas and TBE distribution, with the southernmost border approximately corresponding to the 45th parallel [31,32]. The overall incidence of TBE in Croatia in the period from 2002-2018 was 1.12 per 100,000 population. In comparison with the EU/EEA data (reporting at the EU/EEA level started in 2012), the incidence in Croatia according to our recalculated data for the period 2012-2018 was 1.05 per 100,000 population and is slightly higher than the average

EU/EEA TBE incidence (0.73 per 100,000 population), but it is significantly lower than that in neighbouring Slovenia (6.73 per 100,000 population) [33].

TBE was first reported in Croatia in 1953 and has been a notifiable disease since 1987 [32,34]. TBE reporting is mandatory, ECDC case definitions are used, and it is usually tasked to the physician treating the patient [35]. There is heterogeneity in the surveillance and reporting systems between EU countries, which somewhat hinders the inter-comparability of national TBE data [9,36]. TBE is assumed to be an underreported disease and the true burden of TBE in EU underestimated [9]. This study is based on the results of diagnosis and treatment from a tertiary medical center for infectious diseases where the largest number of patients with central nervous system infections, including TBE, are diagnosed and treated. In seventeen years of follow-up, acute TBE was diagnosed in 810 patients, while only 466 of them were officially reported, which further confirms the problem of insufficient reporting. All presented results were interpreted individually for each subject in accordance with appropriate epidemiological and clinical data to confirm acute TBE diagnosis.

The TBE distribution over age groups was similar to the EU/EEA-wide data reported by ECDC and was the highest in the 45-64 age group (37.78% and 37.98%, respectively). A slightly higher proportion of acute TBE cases was reported in the 15-24 age group compared to ECDC data (9.9% vs. 7.9%), as well as a higher male to female ratio (1.9/1 vs. 1.5/1, respectively) [36]. This could indicate a more pronounced difference in the infection risk between genders in Croatia, possibly due to occupational exposure.

The incidence of TBE has a regular seasonal pattern that was confirmed in our analysis. Most cases were detected in the period from April to September, with the most prominent peaks in June and July. The annual TBE upsurge throughout the observed period was statistically significant for the months of May, June, July and August. A small TBE increase was observed during autumn (October, November), creating a discrete bimodal pattern. In the EU/EEA-wide analysis, TBE cases usually peaked in July and August, and a bimodal pattern also occurs [36,37]. Tick activity is typically highest in late spring, and sometimes can have a bimodal dynamic, with a minor second peak in autumn [38,39]. This smaller autumn increase could be due to an increase in precipitation which provides the moisture to support tick host seeking behaviour [7]. During the warmer months, human activity also increases and leads to an increased risk of tick bites further fuelling the annual increase in TBE incidence [8]. The characteristic biphasic clinical course of TBE should be considered: after the tick bite and incubation phase (median of 8 days), a non-specific prodromal phase occurs, lasting approximately 5 days, which, after a variable asymptomatic period (1-21 days), is followed by the second disease stage with CNS involvement, which is when diagnosis is usually made [3]. The increase in TBE cases is accompanied by a seasonal increase in tick activity, but with a lag of 2-4 weeks, approximately accounting for the time between infection and diagnosis [3].

The effect of climate change on the distribution of ixodid tick activity and the extent of transmission of TBEV and other vector-borne agents has attracted much attention during recent decades. A warmer climate potentially means an earlier onset and longer tick activity, and milder winters allow propagation of tick populations into the next season [40]. The progressive increase in temperatures has resulted in the expansion of tick habitats into areas previously deemed tick-free, eg. higher altitudes in mountainous regions and cold areas of northern Europe. In a 1981-1983 field study in the Czech Republic, *Ixodes ricinus* populations were stable up to 700 m above sea level. In a later study from 2002, the tick activity up to 1100 m was confirmed. In Sweden, a trend of tick habitat expansion towards the north was observed [17-19]. Milder winters, which are expected to occur more often due to climate warming allow tick to be maintained for a longer part of the year, which can lead to an increase in their numbers throughout the year [41,42]. Analysing data over a 38-year period, a Swedish study showed a correlation between a combination of temperature variables, including two consecutive milder winter seasons, and an increased incidence of TBE [42]. Although we found no statistically significant correlation between the winter temperatures and TBE incidence, a weak positive correlation with the January minimum temperature from the year before was noted. A milder winter increases the chance of survival of cold-sensitive hibernating larvae and their melting into nymphs during the warmer season in the same year. Those nymphs can then hibernate during

the coming winter and become active the following spring [42]. The winter of 2006/2007 was one of the warmest ever recorded in Zagreb, followed by another warm winter, which preceded the increase in TBE incidence in 2009.

Observing the temporal dynamics of TBE incidence during the analysed period, two peaks were recorded: in 2009 and 2013, and a decline in incidence in the period 2015-2018 [35]. Neighbouring Slovenia also reported increased incidence in 2009 and 2013 [43]. A large increase in the number of cases in Slovenia and several other European countries was observed in 2006, but was less pronounced or absent in other countries, including Croatia [7,43,44]. Randolph et al found that the weather induced changes in tick abundance alone did not account for the increase in TBE cases in 2006. The variance in incidence in 2006 between countries despite similar weather patterns was probably mostly due to factors associated with human behaviour like outdoor recreation and activities like mushroom picking and harvest of wild berries [44]. Daniel et al concluded that in the Czech Republic, the peak of TBE incidence in 2006 was partly related to an unusually rainy August, which stimulated tick host-seeking activity in late summer/early autumn [7].

Precipitation is possibly related to the occurrence of TBE in humans, as it affects the biology of components of the TBEV enzootic cycle as well as human behaviour that leads to greater exposure to ticks [44,45]. Several authors have presumed the influence of rainfall on TBE incidence and have included precipitation data into prediction models [7,44–46]. Danielova et al. found that during the TBE season, a period of increased rainfall is often followed by a period of increased TBE incidence with a latency corresponding to the incubation period of TBE. It is argued whether the influence of increased relative humidity on the activity of Ixodid ticks combined with increased human activity after the end of the rainy season can result in a short-term increase in TBE cases [45]. Haemig et al found a correlation between TBE incidence and precipitation in December of the previous year, but not in other months. A tentative theory for this correlation could be the protective effect of snowfall on the survival of hibernating ticks [46]. The protective effect of snow cover on the winter survival of ticks has been previously hypothesized, but the data is limited [47]. If we expand on this, in Croatia, the winter of 2012/2013, marked by heavy snowfall, was followed by an exceptionally warm period in late April and early May, which coincided with the highest recorded annual incidence of TBE in this study. Our data did not prove a relationship between rainfall and TBE incidence, but this does not mean that it does not exist and should not be further studied.

A network of ecological relationships is fundamental for effective local TBEV transmission, and it involves many animal species. Large vertebrate hosts, especially roe deer, are a major source of blood meal for ticks and have a positive effect on tick numbers, but being dead-end hosts, they are not directly involved in TBEV transmission. In extremely large numbers, deer may divert ticks away from TBE transmission capable hosts such as mice and voles, potentially reducing TBEV burden rather than increasing it [48–50]. We found a weak negative correlation between the rodent abundance and TBE incidence that could possibly be explained by a dilution effect, especially in environments with a lot of biodiversity and abundance of rodent species that are less competent TBEV reservoirs, as observed in Lyme borreliosis [51]. The slight negative correlation we found between forest rodents and TBE acute cases in the same year should be subjected to a more detailed analysis because the number of forest rodents declines in years after the population outbreaks associated with acorn and beech mast, and therefore the forest rodent monitoring could be a potential tool for predicting possible TBE outbreaks [52,53].

We are aware of the limitations of this study and that the analysed period of seventeen years is still not enough to draw major conclusions, but we believe that it contributes to the improvement of knowledge about TBE, especially since there is a lack of similar research in Croatia. The great issues in case reporting in individual counties are short-term migrations: a person diagnosed with TBE at one site could have been infected within a different geographical region. Patients from counties neighbouring a larger medical centre, eg Zagreb, often gravitate toward that centre and register there, which results in an overflow of the case numbers. Despite this, the regional distribution remains. It is known that the complex multifactorial interaction between the tick and the reservoir, with the influence of various environmental factors, makes the assessment of the possible effect of a single

variable on the incidence of TBE difficult. Although we used temperature data from a single location (Zagreb), we believe that due its central position it is representative for of the continental region and sufficient for a preliminary analysis. Further research should certainly include more detailed local data analysis. In Croatia there are well-known endemic areas between the Sava and Drava rivers and in the Gorski Kotar northeast of the Istrian peninsula-areas which differ in terms of fauna. Therefore, the role of the number of animals which we analysed for the entire country, even though most hunting grounds were related to continental areas with the highest number of TBE, would be worth investigating in narrower local areas.

## 5. Conclusions

The geographical distribution of TBE in Croatia is highly variable within the continental region itself, and especially in relation to the mediterranean and alpine biogeographic areas, so the generalized data in the ECDC reports may deviate significantly from reality for certain regions. Regional data should be as accurate as possible because they have an impact on public health and national and international efforts to develop strategies to assess, manage and predict the TBE risk at the local level based on incidence reports. The analysis of a 17-year period indicates the possible influence of climate and environmental factors on the occurrence of TBE. In order to enable modelling and support a robust One Health approach, continuous long-term monitoring of epidemiological, environmental and meteorological data is needed to define local high-risk areas in which to target possible preventive measures.

**Author Contributions:** Conceptualization, O.Đ.R., J.B. and L.B.; methodology, O.Đ.R., L.B. and J.B.; formal analysis, L.B. and J.B.; investigation, N.C.B., O.Đ.R., L.B., M.V., K.T. and J.M.; writing—original draft preparation, L.B. and J.B.; writing—review and editing, O.Đ.R., L.B., J.B., and J.M.; supervision O.Đ.R.; funding acquisition, O.Đ.R. and J.M. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Patient consent was waived due to the retrospective nature of the study.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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