

SEROEPIDEMIOLOGIC STUDY ON TICK-BORNE ENCEPHALITIS AMONG FORESTRY WORKERS AND FARMERS FROM THE LUBLIN REGION (EASTERN POLAND)

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Abstract: The paper presents the results of seroepidemiologic studies concerning tick-borne encephalitis (TBE) in 1,583 persons (1,261 forestry workers and 233 farmers) from the Lublin region (eastern Poland) occupationally exposed to ticks and in 130 healthy blood donors (a control group). The mean percentage of seropositive reactions in forestry workers amounted to 19.8% and in farmers 32.0%. Based on 5-year research (1994-1998) conducted in 5 districts of the Lublin region, the existence of endemic foci of TBE was detected in the district of Biała Podlaska, on the areas of Radzyń Podlaski and Parczew, where the percentage of seropositive reactions in forestry workers exceeded 50%. Statistical analysis showed that the frequency of seropositive reactions in forestry workers and farmers was significantly greater compared to control group ($p < 0.001$ and $p < 0.05$, respectively). It indicates that these groups are occupationally exposed to TBE virus. In the years 1994-98, a total of nine clinical cases of TBE (acute neuroinfection) in forestry workers and fourteen clinical cases in farmers were confirmed serologically. The effectiveness of specific immunization against TBE was proved on the basis of 100% seroconversion in 56 earlier seronegative forestry workers. The obtained results proved that forestry workers and farmers in Poland are under increased risk of infection with TBE virus.

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INTRODUCTION

The tick-transmitted zoonose - tick-borne encephalitis (TBE) is a viral disease of the central nervous system which may be transmitted to humans by tick bites or by ingestion of raw milk and milk products (cheese) from infected cows or goats. The TBE infection together with Lyme disease (borreliosis) are the most frequent tick-borne infections in Europe. Clinical cases of TBE in Europe are confined to special endemic regions in: Southern Germany, Slovak and Czech Republics, Croatia, Slovenia, Sweden, Switzerland, Poland, and some areas of former Soviet Union [3, 7, 10, 14, 17].

The tick-borne encephalitis virus (TBEV) persists in natural foci, where it circulates among vertebrate hosts (mainly rodents) and the arthropod hosts (ticks) [6, 14, 16, 17]. All ticks (*Ixodida*) are obligate, external temporary parasites of man and vertebrate animals. *Ixodes ricinus* and *Dermacentor reticulatus* are the most common species among Polish ticks and have the biggest epidemiologic importance [16]. Apart from temperature, tick activity is dependent on soil humidity and relative humidity. The highest tick activity in Poland is observed from spring until autumn, with the activity peaks at the end of spring (last decade of April and May) and in the end of summer (September). The example is the rhythm



Figure 1. Map of Poland, showing area of the study: five districts in the Lublin region.

of activity of *I. ricinus* and *D. reticulatus*. Ticks are frequently found on forests fringes with adjacent grasslands, glades, riverside, meadows, marshlands, forest plantations or in brushwood and shrubbery of deciduous and mixed forests [14, 16, 17].

According to the recent official data, about 250–300 clinical cases of TBE are registered in Poland annually. Most of them are noted in the north-eastern part of Poland (Białystok region) which is known as an endemic area in Poland [14, 17].

Tick-borne encephalitis occurs more frequently in people of risk groups such as: persons working in forestry and agriculture, hunters, hikers, rambblers, people engaged in outdoor sport, collectors of mushrooms and berries [10, 11, 12, 14, 17]. According to Prokopowicz, the prevalence of specific anti-TBE virus antibodies in forestry workers in Poland is estimated as 1.7–27% [14].

The aim of this research was the evaluation of the seroepidemiologic situation for TBE in the Lublin region on the basis of serologic examination and epidemiologic anamneses in people occupationally exposed to tick bites (forestry workers and farmers). A secondary goal was checking the effectiveness of immunization in an exposed group after two doses of vaccine. The preliminary results of this study have been published elsewhere [1].

MATERIALS AND METHODS

The study was conducted in the years 1994–1998 on a group of 1,261 forestry workers and 322 farmers from five districts of the Lublin region (Fig. 1) and in 130 blood donors from the city of Lublin. The forestry workers and 80 blood donors (as the first control group) were examined in the years 1994–1996, whereas the farmers and 50 blood donors (as the second control group) were examined in the years 1997–1998. In forestry

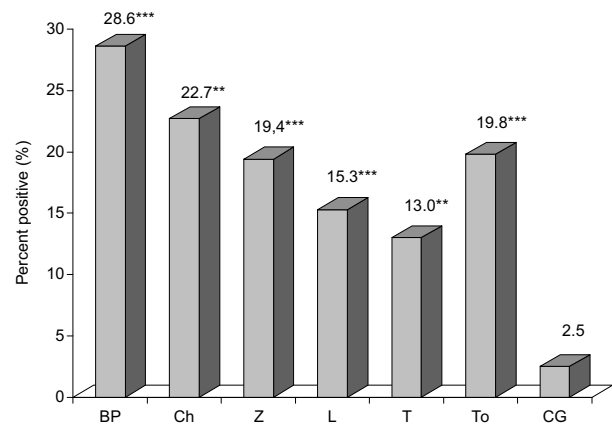


Figure 2. Frequency of positive ELISA reactions with TBE-virus antigen in forestry workers from the Lublin region and the control group. BP: Biała Podlaska district (n=300); Ch: Chełm district (n=180); Z: Zamość district (n=211); L: Lublin district (n=300); T: Tarnobrzeg district (n=270); To: total (n=1281); CG: control group (n=80).

_* Percent of positive reactions significantly higher compared to the control group: **p<0.01; ***p<0.001.

workers and farmers, epidemiologic anamneses were carried out which comprised age, period of employment (in the case of forestry workers), frequency of tick bites, contact with animals, ingestion of raw milk, history of disorders of the central nervous system and vaccination against TBE.

Mean age of forestry workers comprising 1,176 males and 85 females was 37 years, mean period of employment - 15 years. Mean age of farmers comprising 124 males and 198 females was 41 years. Mean age of blood donors comprising 119 males and 11 females was 31 years.

All the people were examined for the presence of specific IgG anti-tick-borne encephalitis virus (TBEV) antibodies by the application of an immunoenzymatic ELISA test (FSME IMMUNOZYM IgG), manufactured by Immuno AG (Vienna, Austria), in which the specific anti-TBEV antibodies from patient's serum are bound to the solid phase coated with inactivated TBEV (as an antigen). The anti-human immunoglobulins labelled with peroxidase (the conjugate) are connected to the antigen-antibody complex. Peroxidase releases oxygen from H₂O₂ which oxidises the chromogen in the substrate to a blue colour; the reaction is then halted with sulphuric acid. The colour intensity in the last stage of the immunoenzymatic reaction is directly proportional to the concentration of specific antibodies in human serum. The optical density was measured at a wave length of 450 nm in an ELISA reader. Using the reference curve, the level of anti-TBEV IgG antibodies was quantitatively evaluated in Vienna units per ml (V.u./ml). Titres of 60 V.u./ml and above were considered as positive according to the manufacturer's instruction.

Additionally, in people suspected of acute stage of TBE, the level of specific IgM antibodies was determined by the test FSME IMMUNOZYM IgM (Immuno AG, Vienna, Austria) [4, 5, 17].

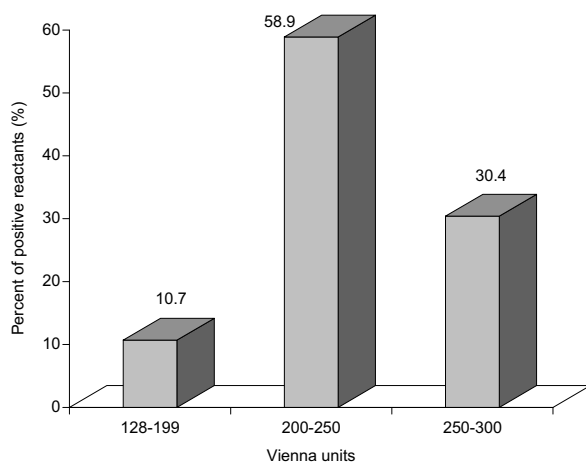


Figure 3. Seroconversion as a result of vaccination in 56 forestry workers.

The effectiveness of immunization against TBE was proved on the base of serologic results in 56 forestry workers from the Chełm district after two doses of vaccine FSME IMMUN (Immuno AG, Vienna, Austria) [17].

Statistical analysis was performed using Student's t-test.

RESULTS AND DISCUSSION

Forestry workers. Nearly all the examined forestry workers (96.0%, 1,210 persons) confirmed in epidemiologic interviews frequent tick bites and 95% of them (1,197 persons) had contact with domestic and wild animals. The mean percentage of seropositive results in 1,261 forestry workers from five districts of the Lublin region amounted to 19.8% (250 persons). People from the control group reacted positively in 2.5% (2 persons out of 80, Fig. 2). Statistical analysis has shown significant differences between forestry workers from each of the five districts (especially from Zamość and Biała Podlaska) and the control group. The highest percentage of positive results with TBEV antigen was noted in people from the Biała Podlaska district - 28.6% (Fig. 2). In some areas of the Biała Podlaska district (Radzyń Podlaski and Parczew forest inspectorates) the percentage of specific anti-TBEV antibodies was above 50%.

In one forestry worker from that region who had suffered from acute neuroinfection due to TBE-virus 20 years ago, the high positive serologic result (over 300 V.u./ml in IgG class) was still observed, but immunoglobulins of IgM class were not detectable. The very strong seropositive reactions (over 300 V.u./ml) were also observed in two persons from the Biała Podlaska district who (according to anamneses) have neither developed TBE virus infection nor been vaccinated against TBE in the past. Such a result may indicate a high stage of natural immunity in these people caused by TBEV transmission due to frequent tick bites.

The high percentage of seropositive reactions in some areas of the Biała Podlaska district may suggest an

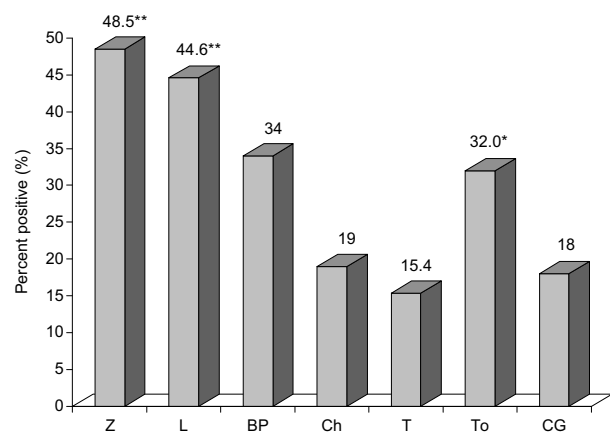


Figure 4. Frequency of positive ELISA reactions with TBE-virus antigen in farmers from the Lublin region and the control group. Z: Zamość district (n=33); L: Lublin district (n=103); BP: Biała Podlaska district (n=47); Ch: Chełm district (n=100); T: Tarnobrzeg district (n=39); To: total (n=322); CG: control group (n=50). *** Percent of positive reactions significantly higher compared to the control group (*p<0.05; **p<0.01).

existence of endemic foci of TBE on these areas which are situated in the neighbourhood of Białystok region - an endemic focus of TBE.

The percentage of seropositive results in forestry workers from the remaining four districts of the Lublin region ranged from 13.0% in the Tarnobrzeg district to 22.7% in the Chełm district. In 1995, four clinical cases of TBE as an occupational disease in forestry workers from the Tarnobrzeg district were diagnosed, however, the percentage of seropositive reactions among foresters from that area was relatively not so high (19.5%).

The mean percentage of seropositive results in forestry workers from the whole Lublin region in this study (19.8%) was about twice as high than that reported by Wróblewska-Mularczykowa *et al.* who found 8.6% of positive results in the area of the Lublin region during research conducted in 1971-1973 [18]. This fact may suggest an increasing number of ticks infected with TBE-virus in the territory of the Lublin region. Nevertheless, it must also be considered that in this study the immunoenzymatic test ELISA was applied which is more sensitive than the inhibition of hemagglutination test used by cited authors.

The percentage of seropositive reactions in foresters found in this study was much higher compared to results obtained by Moll van Charante *et al.* [11] in 312 outdoor Dutch forestry workers (1%). Cristofoloni *et al.* [2] showed only 1.07% of specific anti-TBE virus antibodies in foresters from Northern Italy. The specific anti-TBE virus antibodies were not found by Müller *et al.* [13] in 408 persons working in forestry in the Saarland (Germany). According to Marval *et al.* [8] the prevalence of seropositive results in 1,131 foresters from several cantons in Switzerland was extremely low (0.6%). Matile *et al.* [9] examined 211 forestry workers from an endemic

area in Switzerland by the inhibition of hemagglutination test and obtained 4.7% seropositive reactions.

The study by Prokopowicz *et al.* [15] concerning forestry workers from Białystok area, which is known as an endemic territory of TBE in Poland, showed 13.7% of seropositive reactions. Comparing the results of the present study and the study by Prokopowicz *et al.* to the results obtained by other European authors it may be presumed that forestry workers from some regions in Poland are more exposed to tick-borne encephalitis virus than foresters from the Netherlands, Italy, some areas of Germany and Switzerland.

In the present study, altogether in 1994-1998 nine clinical cases of TBE (acute neuroinfection) in foresters from the Lublin region were diagnosed and confirmed serologically.

Prophylactic vaccination was recommended to all seronegative forestry workers.

The study of the effectiveness of vaccination against TBE-virus infection has shown that in 56 seronegative forestry workers the positive seroconversion appeared after two doses of vaccine. In the vast majority of them (89.3%, 50 persons) a high level of specific anti-TBEV antibodies (200-300 V.u./ml) was noted (Fig. 3).

Farmers. Eighty seven percent of examined farmers (280 persons) were frequently bitten by ticks, 100% confirmed consumption of raw cow's milk and contact with domestic animals. The mean percentage of seropositive ELISA reactions to TBE virus antigen in 322 farmers from five districts of the Lublin region amounted to 32.0% (103 persons). The prevalence of specific anti-TBEV antibodies in particular districts is shown in Fig. 4. The percentage of specific anti-TBEV virus antibodies ranged from 15.4% in the Tarnobrzeg district to 48.5% in the Zamość district. People from the control group showed seropositive reactions in 18.0% (nine persons out of 50, Fig. 4). This may suggest an increase in the number of ticks infected with TBE virus in comparison with the studies from 1994, when the control group reacted positively only in 2.5%. Data from Figure 4 shows the that significant difference between farmers and the control group occurred only in relation to farmers from the Zamość and Lublin districts and to total group of farmers. All seronegative farmers were recommended to prophylactic vaccination.

It should be stressed that in 60 farmers from one village nearby Lublin the percentage of seropositive results was very high (66.0%). None of the seropositives had suffered earlier from a medically confirmed TBEV infection, or had been vaccinated against TBEV. Five of the seropositive farmers showed a very high value of specific anti-TBEV antibodies (200-300 V.u./ml). All five farmers claimed persistent headaches, two - pain in the nape of neck, one - frequent faintness, and one - some difficulties in walking. In two farmers manifesting characteristics for the first stage of borreliosis, *erythema migrans*, positive reactions with *Borrelia burgdorferi* antigen were noted.

Table 1. TBE - chronic infection in a farmer from the Lublin region.

Initials	Age	Serologic examination (ELISA test)		Data based on epidemiologic interview and clinical symptoms	
		Date	Results		
			IgG*		IgM
J.W.	56	03.08.96	270	(-)	Bitten by ticks 15 years ago. In 1994 "episode" of encephalitis, features of CNS lesions, demyelination in CNS (MRI), acroparalysis
		21.01.97	>300	(-)	
		09.05.97	>300	(-)	
		06.10.97	>300	(-)	
		20.10.97	>300**	(-)	

* - Vienna units, ** cerebrospinal fluid

The combined results of serologic tests, clinical symptoms and data from epidemiologic interview may suggest the possibility of the existence of chronic infection with tick-borne encephalitis virus and the concomitant infection with TBE and *Borrelia burgdorferi* in these farmers.

The chronic infection with TBE-virus was also confirmed in a 56-year old farmer from the Lublin district suffering from acroparalysis, and hospitalized in the Clinic of Occupational Diseases at the Institute of Agricultural Medicine in Lublin. In this patient, who had been bitten by ticks 15 years earlier, some brain changes (white matter lesions) were diagnosed with magnetic resonance investigation (MRI). The farmer reacted high seropositively with the antigen of TBEV (Tab. 1).

The acute infection with tick-borne encephalitis virus was confirmed serologically in a couple of farmers from the Lublin district ingesting raw milk from the cow being frequently attacked by ticks.

In the present study, during 1994-98, a total of 14 clinical cases of TBE (acute neuroinfection) in farmers from the Lublin region were diagnosed and confirmed serologically.

Only one report concerning the prevalence of specific anti-TBEV antibodies in farmers was found in the literature. Prokopowicz *et al.* [15] examined 40 people of the rural population from Białystok (Poland) and found a low percentage of positive reactions (2.5%). The much higher frequency of positive reactions found among farmers in the present study, performed three years later, could be explained by the increasing exposure to the TBEV-infected ticks. This hypothesis may also be applied to explain of the differences between the frequency of positive results between forestry workers and farmers, and between control groups examined in 1994 and 1997. Nevertheless, this study suggests that farmers from some areas of the Lublin region are more exposed to tick-borne encephalitis virus than forestry workers in the same region. This study will be consequently continued in the future, especially in the aspect of occupational disease in farmers.

CONCLUSIONS

1. Forestry workers and farmers from the Lublin region reacted in ELISA with tick-borne encephalitis virus antigen significantly higher compared to the control group of healthy urban dwellers. This suggests the occupational character of tick-borne encephalitis among forestry workers and farmers.

2. Data suggests the existence of an endemic area of tick-borne encephalitis in the Biała Podlaska district.

3. In the territory under study, clinical cases of TBE were found among forestry workers and farmers. The clinical picture suggests the possibility of the existence of chronic infection with TBE-virus and concomitant infection with TBEV and *Borrelia burgdorferi* in some farmers from the Lublin district.

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