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Identification of new endemic tick-borne encephalitis foci in Poland – a pilot seroprevalence study in selected regions

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Abstract

In Poland, large-scale serologic surveys carried out in 1965–1972 revealed regions of Poland with particularly high prevalences of antibodies against tick-borne encephalitis virus (TBEV). The information provided by the routine surveillance of communicable diseases during 1970–2005 indicated, however, that the geographic distribution of the disease is limited to a few eastern and south-western regions of Poland (defined as endemic for the purpose of this study). In the present serologic survey, 1498 human serum samples collected in 1996–2005 were randomly selected from a serum bank, and 358 goat serum samples were collected from milk-producing farms in selected areas of Poland 2002–2006. Thirty-nine human samples were positive for anti-TBEV antibodies, with an overall seroprevalence of 2.6%. Seroprevalence in endemic provinces ranged from 0.8% to 4.3%, and seroprevalence in non-endemic provinces ranged from 1.9% to 4.3%. In endemic, compared to the non-endemic provinces, the highest seroprevalence was found in the age group >60 years (7% vs. 1%) and in inhabitants of villages (3.9% vs. 1.8%). In non-endemic, compared to endemic provinces, the highest seroprevalence was detected in the age groups 30–39 years (5% vs. 2%) and 40–49 (4% vs. 0%), and in inhabitants of large towns inhabited by >100,000 people (4.1% vs. 2.5%). Out of 358 goat samples, 17 (4.7%) were positive for anti-TBEV antibodies. Seroprevalence in goats reached 14/151 (9.3%) in endemic, and 3/207 (1.4%) in non-endemic provinces. The present study indicates the possible existence of endemic foci in north-western provinces of Poland, in which barely any cases were reported during 1970–2005. The socio-demographic profile of seropositive subjects in non-endemic regions suggests that they might have been exposed to TBEV during travels to known endemic regions. This would mean, however, that they were missed by the surveillance system. A thorough review of diagnostic protocols in non-endemic regions and work towards an improvement of the TBE surveillance sensitivity is necessary.

Keywords: Tick-borne encephalitis; Seroprevalence; Poland

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Introduction

Central European tick-borne encephalitis (TBE) is a viral disease of the central nervous system, which persists endemically in several Central-European countries (Dumpis et al., 1999). Although the main route of transmission of tick-borne encephalitis virus (TBEV) is through tick bites, TBE outbreaks were linked to the consumption of raw milk (Matuszczyk et al., 1997; Kerbo et al., 2005). TBE is a mandatory reportable disease in Poland since 1970. The administrative division of Poland and the geographic distribution of registered TBE incidence per 100,000 inhabitants in 1994–2005 are presented in Fig. 1. The routine surveillance data indicate clustering of TBE incidence in two north-eastern voivodships, Podlaskie and Warminsko-mazurskie, and only sporadic case reports from isolated districts in other voivodships (Fig. 1b). Before the introduction of the mandatory surveillance, large-scale serologic surveys were performed in 1965–1972 among more than 20,000 foresters and 17,000 other subjects representing the general population (Wroblewska et al., 1968, 1973). Antibodies against TBEV detected using the hemagglutination inhibition test were found in 0.5–6.5% of the population and in 7.0–27.0% of the foresters in different regions. Regions with a particularly high prevalence of antibodies against TBEV have been identified, including areas in the northern and eastern parts of Poland (Fig. 2). Further evidence on the presence of active TBE foci was provided by virological studies of ticks in selected regions and regional seroprevalence studies (Prokopowicz et al., 1995; Cisak et al., 1998, 2002). In these studies, evidence on the presence of active foci in the Pomorskie, Podlaskie, and Lubelskie voivodships was strengthened. In general, the extent of the disease incidence as notified within the surveillance system is not compatible with the seroprevalence distribution established in field studies.

The aim of the present study was to perform a serologic survey among humans assessing the prevalence of antibodies against TBEV in selected regions of Poland classified as endemic and non-endemic. Additional objective evidence of the endemic status of selected regions was obtained from a parallel serologic survey among goats.

Materials and methods

The present seroprevalence study among humans was based on serum samples ($n = 1498$) selected from a serum bank collected by the Dept. of Virology, National Institute of Public Health, in 1996–2005. The samples were obtained from subjects referred to local health departments for testing against different pathogens not related to neuroinfections, including HBV, food-borne pathogens, etc. A stratified sample without replacement, weighted on age group, gender, and geographic location was selected from sera collected in 3 'endemic' voivodships and 4 'non-endemic' voivodships (encompassing a total of 148 administrative districts). Endemic status was assigned to regions from which at least 1 autochthonous case of TBE was reported in each 3-year period during 1995–2005. The 7 provinces were selected based on the availability of samples appropriately referenced to administrative districts. Testing was performed using

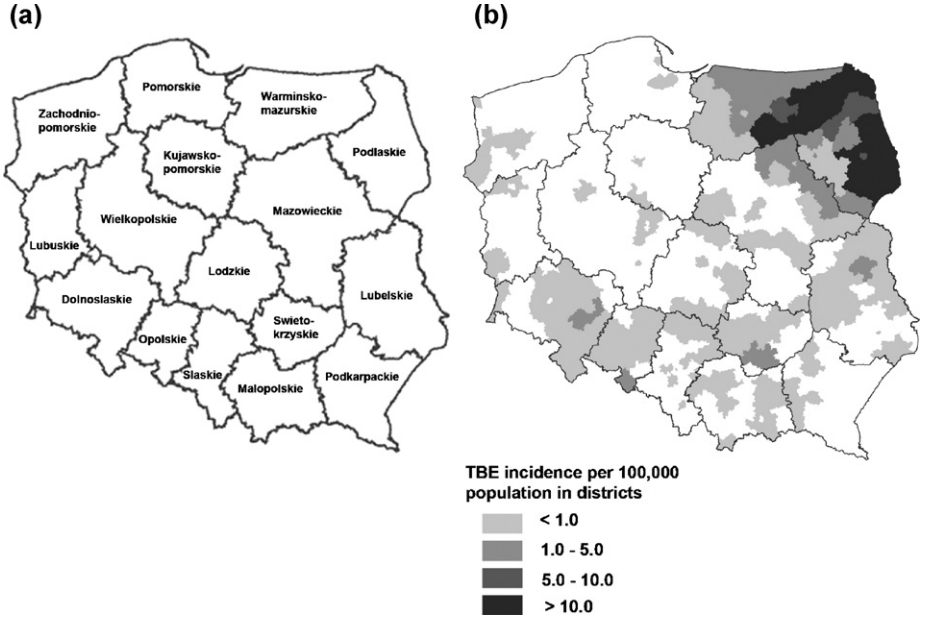

Fig. 1. Division of Poland into administrative units (a), and mean number of reported tick-borne encephalitis cases per 100,000 population in districts in 1994–2005 (b).

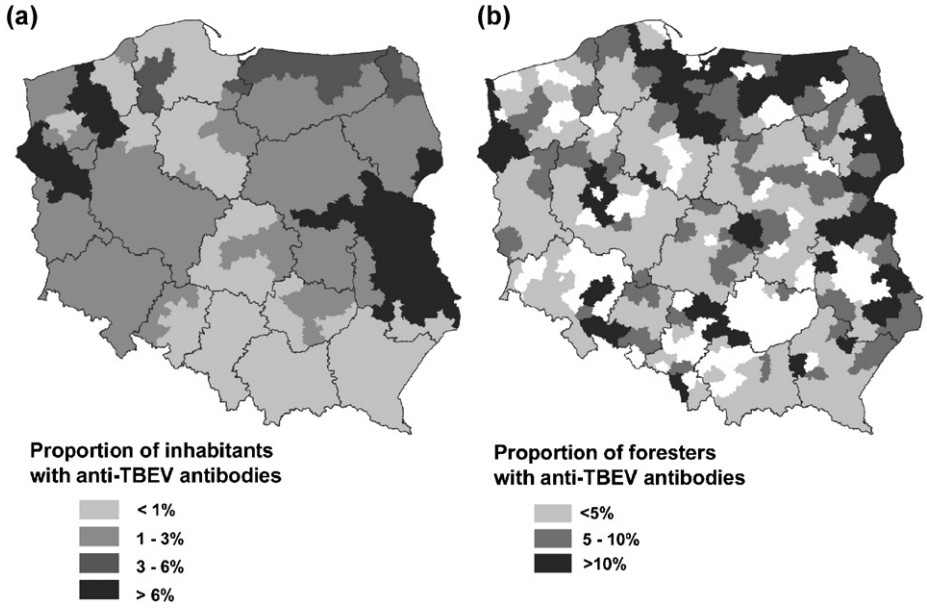

Fig. 2. Prevalence of antibodies against tick-borne encephalitis virus in Poland; in the general human population in 1968 (a), among foresters in 1971 (b).

the FSME ELISA IgG test (Genzyme Diagnostics Virotech, Germany). Positive human sera were confirmed by indirect immunofluorescence (IIF) and hemagglutination inhibition (HI) tests, performed in an independent laboratory in Hungary. Because the sensitivity of both tests differs from the ELISA test, a positive result of either IIF or HI was taken as a confirmation of an ELISA-positive result. Association of seropositive results with age, gender, residence type, and geographic location was assessed. To facilitate efficient presentation of the obtained results, districts included in the study were merged in groups of 7–10, and the local anti-TBEV antibody prevalence was calculated as number of positive results in a particular area divided by the number of tested samples. This approach was chosen due to often small number of samples tested in particular districts.

Also the seroprevalence survey among goats was based on serum samples ($n = 358$), selected from a serum bank collected from milk-producing goat farms by the Warsaw University of Life Sciences in 2002–2006. Only goats aged $\geqslant 1$ years, which had been freely feeding on pastures, were selected for the study. Testing of material from goats was performed using the FSME ELISA IgG test (Genzyme Diagnostics Virotech), modified for competition blocking test (blocking reactivity of human serum by goat antibodies), validated for this purpose. The results of the validation procedure will be published elsewhere.

The authors decided not to exclude ambiguous results, which were ELISA-positive but negative in both HI and IIF tests. This decision was based on a relatively high repeatability of ELISA confirmation (92%) in an independent laboratory. Possible reasons for the lack of confirmation could be a low level of IgG antibodies or the presence of antibodies against another flaviviruses.

Results

Out of 1498 human samples tested, 39 were positive for anti-TBEV antibodies, giving an overall seroprevalence of 2.6%. Out of 38 re-tested samples, 35 were confirmed with TBEV-specific tests. However, only 7 out of the 10 re-tested sera from Podlaskie voivodship, 3/10 from Kujawsko-Pomorskie, 1/5 from Pomorskie, and merely 4 out of the 14 tested sera from the remaining voivodships (Lubelskie, Zachodniopomorskie, Wielkopolskie, Dolnośląskie) indicated earlier infection based on IIF and HI results. All the remaining ELISA-positive sera with low or medium antibody titre could result from earlier immunisation (against TBEV or another flavivirus) or more distant flavivirus infection. Seroprevalence in endemic voivodships ranged from 0.8% in Dolnoslaskie to 4.3% in Podlaskie voivodship, and seroprevalence in non-endemic voivodships ranged from 1.9% in Wielkopolskie to 4.3% in Pomorskie. The geographic extent and distribution of seroprevalence in the studied region are presented in Fig. 3a. In endemic, compared to non-endemic provinces, the highest seroprevalence was detected in the age group >60 years (7% vs. 1%) and in inhabitants of villages (4% vs. 2%). In non-endemic, compared to endemic provinces, the highest seroprevalence was found in the age groups 30–39 years (5% vs. 2%) and

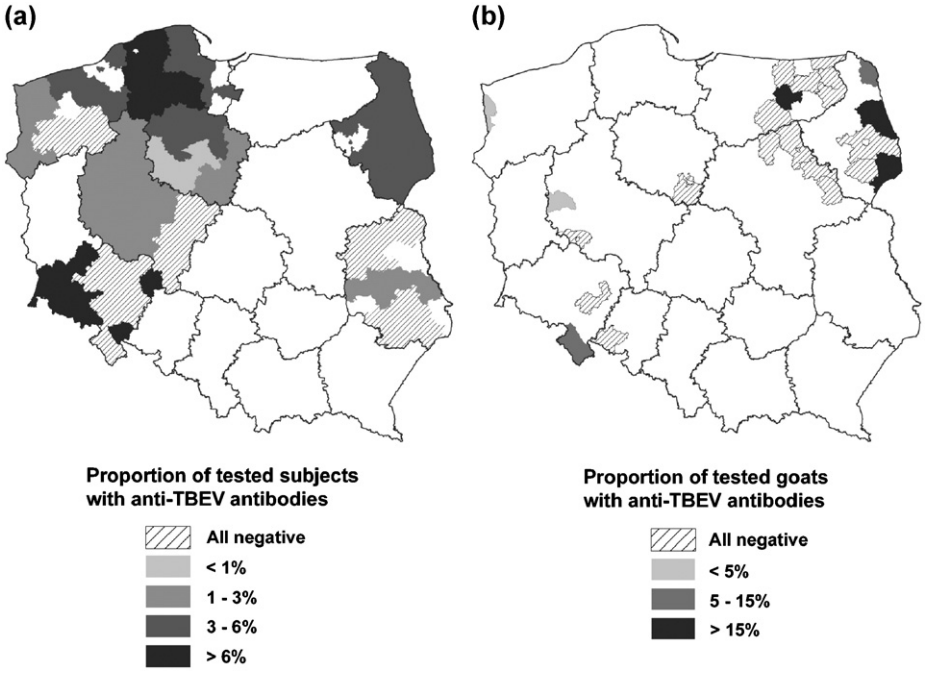

Fig. 3. Occurrence of tick-borne encephalitis virus in Poland based on the seroprevalence among humans in 7 voivodships (a) and among goats in 25 administrative districts (b), 1996–2006.

Table 1. Prevalence of human antibodies against tick-borne encephalitis virus by age group, gender, and residence town size, Poland, 1996–2005

Character	Non-endemic regions			Endemic regions		
	Samples tested	Samples positive	Percent positive (%)	Samples tested	Samples positive	Percent positive (%)
Total	1037	27	2.6	461	12	2.6
Age group						
0–9	236	3	1	113	3	3
10–19	148	4	3	99	3	3
20–29	224	5	2	69	1	1
30–39	138	7	5	50	1	2
40–49	111	4	4	48	0	0
50–59	101	3	3	37	1	3
60+	79	1	1	45	3	7
Gender						
Female	536	14	2.6	240	5	2.1
Male	501	13	2.6	221	7	3.2
Residence town size						
Village	275	5	2	129	5	4
Town <20,000	199	4	2	72	2	3
Town 20,000–100,000	196	3	1.5	99	1	1
Town >100,000	367	15	4.1	161	4	2.5

40–49 (4% vs. 0%) and in inhabitants of towns inhabited by >100,000 people (4.1% vs. 2.5%) (Table 1). To assess the possible effect of the year of serum collection, the distribution of the years of collection was assessed. Of 1498 serum samples, 72% were collected in 2004 (seroprevalence 35/1048, 3.3%), 16% were collected in 2002 (seroprevalence 4/234, 1.7%), and only 12% were collected in remaining years (no positive sera).

Out of 358 goat samples, 17 (4.7%) were positive for TBEV antibodies. Seroprevalence in goats was 14/151 (9.3%) in endemic provinces and 3/207 (1.4%) in non-endemic provinces. The geographic extent of the studied areas and the location of seropositive results are presented in Fig. 3b. In endemic regions of Podlaskie voivodship, a high seroprevalence was found with up to 75% in particular herds. In non-endemic regions, 3 goats in 2 districts were found to be TBE positive. An additional inquiry has indicated that the suspect goats were not transferred to or from other voivodships during their lifetime.

Discussion

The present study explored the possible existence of TBE endemic foci in regions of Poland considered free of disease based on information provided by the routine communicable disease surveillance system. The results of this pilot survey indicate that altogether as many inhabitants of non-endemic regions as inhabitants of endemic regions were exposed to TBE virus. In the 4 north-western non-endemic voivodships, where the TBEV antibody prevalence was $>1\%$, only sporadic cases were reported during 1970–2005. Their geographical extent is essentially compatible with the TBE seroprevalence spatial distribution found in the 1965–1972 studies of foresters and the general population (Wroblewska et al., 1968, 1973). When studying the prevalence of TBEV antibodies in regions considered non-endemic, high TBE seroprevalences indicate the existence of local endemic foci of TBE, exposure of positive subjects to TBEV while travelling to known endemic regions in Poland or other European countries, and/or TBE vaccination. Results obtained from goats support the hypothesis that undiscovered endemic areas may exist in Poland. Goats are less mobile and allow easier investigation of their travel history. Goats included in the present study were living on farms which allowed them free grazing on local pastures and can be therefore considered as sensitive indicators of local presence of TBEV in ticks. The two districts where TBEV antibody-positive goats were found did not report a single human case during the previous 15 years. Additionally, high seroprevalences were found in these districts (or neighbouring areas) in 1968–1971 studies (Fig. 2). The approach of using companion animals or livestock as indicators of TBEV local circulation was used by several authors in Germany and Russia (Korenberg et al., 1984; Leutloff et al., 2006).

The existence of TBE endemic foci can be validated by the detection of TBEV virus in ticks using standard methodology concerning tick collection. To date, only two studies investigated the presence of TBEV in ticks and/or rodents in Poland: one in the Podlaskie voivodship in 1981 (Bednarz et al., 1984) and the second one in Pomorskie voivodship in 1985 (Bednarz et al., 1985). In Podlaskie voivodship, 4 TBEV strains were isolated from 134 rodents. The study in Pomorskie voivodship identified 7 TBEV strains isolated from 294 feral rodents in the area of Gdansk, but no virus was isolated from ticks. Even if it could be considered as a direct evidence of the existence of local TBE foci, only 2 autochthonous human TBE cases were reported from Pomorskie voivodship during 1994–2005.

The socio-demographic profile of seropositive subjects in non-endemic regions suggests that some of them might have been exposed to TBEV during travels to known endemic regions. This would mean, however, that they were missed by the surveillance system, which should sensitively detect both locally acquired (autochthonous) and imported cases. Due to increasing national and international travel and increasing popularity of countryside visits, an increase in the number of travel-related infections would be expected. The most popular tourist destinations for Polish families include TBE-endemic areas in the Mazury Lakes region in Poland, the Baltic countries, the Czech Republic, and Austria (Laciak, 2006). Possible reasons for non-reporting of TBE cases by physicians in different regions of Poland are related to unawareness of local disease foci, and unwillingness to refer patients to diagnostic testing when no specific antiviral treatment is available. This is supported by findings of a previous study (Stefanoff et al., 2005), in which more specific laboratory confirmation of TBE was identified in regions considered free of disease, compared to endemic regions. High surveillance specificity indirectly implies low sensitivity in the detection of TBE cases since only more clinically overt and better diagnosed cases are reported to the system. A thorough review of diagnostic protocols in non-endemic regions and work towards improvement of TBE surveillance sensitivity is necessary.

The most important limitation of the present study has been the unavailability of data on the vaccination status and travel history of the studied populations. The unknown vaccination status may not lead to important misclassification, since according to official vaccination coverage data, a maximum of 18,000 persons was vaccinated each year during 1996–2005, which is less than 0.05% of the Polish population. The origin of human samples collected at the serum bank is unknown, which may limit representativeness. However, even if the studied sample does not fully represent the general population in the studied regions, the study provides sound evidence that a considerable proportion of the inhabitants of non-endemic regions exposed to TBE virus was not properly diagnosed, nor that the disease was notified to the public health surveillance.

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