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A new hot spot for tick-borne encephalitis (TBE): A marked increase of TBE cases in France in 2016

Aurélie Velay[a,b,*], Morgane Solis[a,b], Wallys Kack-Kack[a,b], Pierre Gantner[a,b], Marianne Maquart[c], Martin Martinot[d], Olivier Augereau[e], Dominique De Briel[e], Pierre Kieffer[f], Caroline Lohmann[g], Jean Dominique Poveda[h], Emmanuelle Cart-Tanneur[i], Xavier Argemi[j], Isabelle Leparc-Goffart[c], Sylvie de Martino[k,l], Benoit Jaulhac[k,l], Sophie Raguet[m], Marie-Josée Wendling[a], Yves Hansmann[j], Samira Fafi-Kremer[a,b]

[a] *Virology Laboratory, University Hospital of Strasbourg, F-67000 Strasbourg, France*
[b] *INSERM, IRM UMR_S 1109, F-67000 Strasbourg, France*
[c] *Centre National de Référence (CNR) des Arbovirus, Institut de Recherche Biomédicale des Armées (IRBA), Hôpital d'Instruction des Armées Laveran, F-13013 Marseille, France*
[d] *Service de Médecine Interne et de Rhumatologie, Hôpitaux Civils de Colmar, F-68000 Colmar, France*
[e] *Laboratoire de Microbiologie, Hôpitaux Civils de Colmar, F-68000 Colmar, France*
[f] *Service de Médecine interne-Maladies systémiques et auto-immunes rares, Groupe Hospitalier de la région de Mulhouse et Sud Alsace, F-68051 Mulhouse, France*
[g] *Laboratoire de Microbiologie, Groupe Hospitalier de la région de Mulhouse et Sud Alsace, F-68051 Mulhouse, France*
[h] *Département de Génétique et de Biologie Spécialisée, Laboratoire Cerba, F-95310 Saint-Ouen-L'aumône, France*
[i] *Département Immunologie, laboratoire Eurofins Biomnis, F-69007 Lyon, France*
[j] *Service des maladies infectieuses et tropicales, Hôpitaux Universitaires de Strasbourg, F-67000 Strasbourg, France*
[k] *EA7290 Early Bacterial Virulence, Lyme borreliosis Group, FMTS, Université de Strasbourg, F-67000 Strasbourg, France*
[l] *French National Reference Center for Borrelia, University Hospital, F-67000 Strasbourg, France*
[m] *Santé publique France, French national public health agency, Regional unit (Cire) Alsace Lorraine Champagne Ardenne, Saint-Maurice, France*

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ABSTRACT

Objectives: Tick-borne encephalitis virus (TBEV) is a zoonotic agent causing severe encephalitis. In 2016, in Northeastern France, we faced a TBEV infection increase, leading to a warning from the Regional Health Agency. Here, we report the confirmed TBE cases diagnosed between January 2013 and December 2016, with particular emphasis on the year 2016.

Methods: A total of 1643 blood and cerebrospinal fluid (CSF) samples from everywhere in France, corresponding to 1460 patients, were prospectively tested for anti-TBEV-specific IgM and IgG antibodies by ELISA. Additional 39 blood and CSF samples from patients with suspected Lyme neuroborreliosis were retrospectively investigated.

Results: The TBEV seropositivity rate was estimated to 5.89% and 54 patients were diagnosed as TBE-confirmed cases. A significant increase in TBE cases was observed during the year 2016 with 29 confirmed cases, instead of a mean of eight cases during the three previous years ($p = 0.0006$). Six imported cases and 48 autochthonous cases, located in the Alsace region ($n = 43$) and in the Alpine region ($n = 5$) were reported. Forty-six patients experienced neurological impairment. Nine patients showed an incomplete recovery at last follow-up (from 15 days to eight months post-infection). TBE diagnosis was performed earlier for patients taken in charge in the Alsace region than those hospitalized elsewhere in France ($p = 0.0087$). Among the 39 patients with suspected Lyme neuroborreliosis retrospectively investigated, one showed a TBEV recent infection.

Conclusion: The TBE increase that occurred in France in 2016 highlights the need to improve our knowledge about the true burden of TBEV infection and subsequent long-term outcomes.

1. Introduction

Tick-borne encephalitis (TBE) is a zoonotic disease caused by TBE virus (TBEV), member of the Flaviviridae family. Infections usually occur during recreational activities in endemic areas, through the saliva of infected tick bites. Transmission through consumption of

* Corresponding author at: Laboratoire de Virologie, Hôpitaux Universitaires de Strasbourg, F-67000 Strasbourg, France.
E-mail address: aurelie.velay@chru-strasbourg.fr (A. Velay).

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unpasteurized milk from infected animals (goats, sheep, and cows) is rare (Biernat et al., 2014; Gritsun et al., 2003; Süss, 2011). There are three TBEV subtypes, and the European one is transmitted by *Ixodes ricinus* ticks (Ecker et al., 1999). TBE prevalence is closely related to the ecology of ticks and depends on the level of exposure, and the vaccination rate of the population (Imhoff et al., 2015). In Europe, tick activity and virus transmission typically start in spring and persist until November (Biernat et al., 2014; Lindquist and Vapalahti, 2008).

TBEV infection has been a growing public health problem in Europe over the past 20 years, except for countries with intensive vaccination programs (Donoso Mantke et al., 2011; Heinz et al., 2013; Kunze, 2014). An expansion of risk areas into new regions (Denmark, Norway, and The Netherlands) has also been reported (Biernat et al., 2014; de Graaf et al., 2016; Frimmel et al., 2014; Mohareb et al., 2013; Süss, 2011).

This infection carries a potential risk of complicated course of disease, neurological sequelae and death (reported mortality rate from 0 to 3.9%) (Karelis et al., 2012; Mickiene et al., 2002). Infections caused by the European subtype have a typical biphasic course in symptomatic patients. In clinically apparent cases, after an incubation period from 7 to 14 days, patients undergo a first stage consisting of an influenza-like illness, lasting 2–4 days. A symptom-free interval of approximately one week typically ensues. The second stage affects approximately one third of infected patients and is characterized by a second febrile phase, associated with neurological disorders of varying severity: meningitis, meningoencephalitis, meningoencephalomyelitis, or meningoencephalo-radiculitis (Zambito Marsala et al., 2014).

Surveillance and notification schemes are not yet standardized among European countries. In 2011 a case definition for TBE was validated by the European Commission (Commission implementing decision of 8 August 2012 amending Decision 2002/253/EC).

In France, since 1968, about 10 cases are reported each year, mainly from the Alsace region (Northeastern France). Since 2003, one or two cases are reported each year from the French Alpine region, and in 2006 an autochthonous case was diagnosed in Southwestern France (Donoso Mantke et al., 2011; Hansmann et al., 2006; Herpe et al., 2007). These few cases raise the question of the presence of the virus over a larger area of France than the commonly expected Alsace region. Notification of TBEV infection is still not mandatory in France. In 2016, we faced a marked increase of TBEV infection cases, leading to a warning from the Regional Health Agency (ARS Grand Est) issued at the beginning of July 2016.

Here, we report the confirmed TBE cases diagnosed in France between January 2013 and December 2016, with particular emphasis on the year 2016. This study aimed to describe the epidemiology and the clinical findings observed among the TBE-confirmed cases over a four-year period. We comment on hospitalization duration, the delay within the serological diagnosis of TBEV infection was performed, and the occurrence of post-infection sequelae. Finally, we try to partially answer to the question of underdiagnosis of TBEV infection by analyzing sera and CSF samples collected elsewhere in France, from patients with an history of tick bite presenting with neurological symptoms and negative for Lyme borreliosis between April and September 2016.

2. Methods

2.1. Patients' samples

From January 2013 to December 2016, a total of 1643 samples (202 CSF and 1441 serum samples), corresponding to 1460 patients were sent from all across France to the Strasbourg University Hospital laboratory of Virology for TBEV-specific IgM and IgG antibodies screening (Table 1).

We retrospectively investigated four CSF and 35 serum samples collected between April and September 2016 by the *Borrelia* National Reference Center (NRC) from 39 patients presenting with neurologic impairment, history of tick bite and found negative for Lyme borreliosis.

2.2. Serological assays

All samples were tested for TBEV infection using commercially available enzyme-linked immunosorbent assay (ELISA): the SERION ELISA classic TBE Virus IgG/IgM (Institut Virion/Serion GmbH, Würzburg, Germany). The results were interpreted according to the manufacturers' instructions. All patients tested for anti-TBEV IgM and IgG in our laboratory, were questioned about the history of a tick bite, recent or past travel and the geographical area concerned, the history of vaccination for yellow fever, Japanese encephalitis and tick-borne encephalitis. If necessary, to exclude possible cross reactivity, IgM and IgG positive samples were sent to the national reference center of arboviruses in Marseille for confirmation testing (In-house IgM-capture enzyme immunoassays (MAC-ELISA) and IgG indirect ELISA and/or plaque reduction neutralization testing (PRNT)).

2.3. Classification of the confirmed cases

For 51 patients, corresponding to 68 IgM and IgG positive samples, biological, clinical, and follow-up data were obtained from the physicians in charge of each patient. Two additional TBE-confirmed cases were obtained from Cerba laboratory using the Enzygnost Anti-TBE/FSME/ETG Virus kit (Siemens, Marburg, Germany) and another one from the NRC of arboviruses (In-house IgM-capture enzyme immunoassays (MAC-ELISA) and IgG indirect ELISA). All positive cases were classified as confirmed cases, according to the 2012 European Union case definitions for TBE (ECDC, 2012): a case is classified as confirmed when a patient met the clinical criteria (symptoms of inflammation of the CNS (e.g. meningitis, meningo-encephalitis, encephalomyelitis, encephaloradiculitis)) and at least one of the at least one of the following laboratory criteria: (i) TBE specific IgM and IgG antibodies in blood, (ii) TBE specific IgM antibodies in CSF, (iii) seroconversion or four-fold increase of TBE-specific antibodies in paired serum samples, (iiii) detection of TBE viral nucleic acid in a clinical specimen, (iiiii) or isolation of TBE virus from clinical specimen Similarly to our center practice, this case definition states that serological results should be interpreted according to the vaccination status and previous exposure to other flaviviral infections, and that confirmed cases in such situations should be validated by serum neutralisation assay or other equivalent assays.

2.4. Statistical analyses

Yearly clinical and epidemiological data were compared using nonparametric tests for comparison of quantitative data (Mann-Whitney or Kruskal-Wallis test according to the number of datasets). Pairwise multiple comparisons between groups were analyzed using Dunn's multiple comparison test. Chi-square test was used for comparing frequency counts. All statistical analyses were performed using the GraphPad prism 6 software (San Diego, CA, USA).

3. Results

3.1. Serological data

In 2016, the number of samples tested for the presence of anti-TBEV IgM and IgG (laboratory of Virology Strasbourg University Hospital) increased from less than 400 between 2013 and 2015 to 537 (Table 1). The rate of samples detected as recent infection significantly increased in 2016 (7.1%) compared to the three previous years (0.8–4.0%) ($p < 0.0001$). When considering all patients positive for anti-TBEV IgG with or without IgM, the TBEV seropositivity rate was estimated to 5.9%.