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Original article

# Prevalence of antibodies against tick-borne encephalitis virus in wild game from Saxony, Germany

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ABSTRACT

Tick-borne encephalitis (TBE) is the most important tick-transmitted viral disease in Europe and is caused by the flavivirus TBE-virus (TBEV). In Germany TBE is unevenly distributed with the vast majority of cases occurring in the south in so-called risk areas defined as regions with an incidence of at least 1 case in 100,000 inhabitants. However, in low endemic areas with lesser TBE cases the respective risk assessment is hard to achieve. We therefore intend to use the prevalence of antibodies against TBEV in wildlife to trace TBEV endemic areas as a surrogate marker for the notification of human cases. This study was conducted in Saxony, Germany, where 34 autochthonous cases were reported since 2001, thereby not allowing a geographic allocation within the state. A total of 1,851 sera from wild boar and 35 sera from roe deer from all Saxon districts shot between April 2011 and March 2013 were screened for the presence of antibodies against TBEV. The overall seropositivity for Saxony was 10.5%. Among the wild boar sera, most positive samples could be found in the districts Meißen (23%) and Vogtlandkreis (20%) followed by Dresden (18%), Erzgebirgskreis and Görlitz (both 10%). We conclude that seroprevalence studies in game animals represent a promising surrogate marker and should be considered for future determination of risk areas. Although we are currently unable to explain the discrepancy of the few human cases and the high seroprevalence in some districts, vaccination against TBE should be considered for people planning outdoor activities in Dresden, Meißen or Vogtlandkreis.



## Introduction

Tick-borne encephalitis (TBE) is the most important tick-transmitted viral disease in Europe (Süss, 2011). TBE is caused by TBE-virus (TBEV), a member of the family *Flaviviridae*, which consists of at least three subtypes, the European, the Siberian and the Far-Eastern subtype (Dobler et al., 2012). Until today, in Central Europe only the European subtype can be found with mortality rates of 0.5–2% (ECDC, 2012).

TBEV is mainly transmitted by the tick bite of *Ixodes ricinus*, the most common tick in Central Europe, but also cases of transmission by raw-milk consumption and its products are described (Holzmann et al., 2009; Hudopisk et al., 2013). The tick feeds on different hosts during its life span of 2–6 years depending on its current life stage (Růžek et al., 2013). The larvae mainly feed on rodents, while the nymphs prefer medium-sized animals such as squirrels, hedgehogs, hares and birds (Růžek et al., 2013). The adult ticks especially feed on large animals such as wild boars or deer serving as the meeting and mating site. Therefore, they play an important role as maintenance hosts for the tick population (Jaenson et al., 2012). In the transmission cycle of TBEV in nature, most animals do not develop sufficient virus titers in the blood to allow its transmission to other ticks via viremic blood (Dobler et al., 2012), because of a low-titred and short-lived viremia (Růžek et al., 2013). However, rodents seem to have a long-lasting viremia permitting efficient virus transmission to ticks (Achazi et al., 2011). Besides this the TBEV transfer to and between ticks is either possible through co-feeding (=contemporaneous feeding in close contact on a non-viremic host), or by horizontal (=feeding on a viremic host), transstadial or to a far lesser extent transovarial transmission (Růžek et al., 2013). Regardless of the mode of TBEV-transmission and the dynamics of TBEV-viremia, the respective animals react

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with an antibody response upon TBEV-infection (Jaenson et al., 2012), and these animals consequently may serve as indicator hosts for TBEV circulation in nature when tested for TBEV-specific antibodies as shown before for domestic small ruminants (Klaus et al., 2010, 2012).

In Germany, 4,169 human TBE cases were reported since 2001, of which 420 were notified in 2013 alone. Cases are reported according to the actual case definition of the Robert-Koch-Institute to the Local Health Authorities. If in average more than 1 case per 100,000 inhabitants in a 5-year-period is notified in a certain district, it is called a risk-area. Districts with an incidence of less than 1 per 100,000 are termed non-risk areas for TBE. Currently approximately 95% of the annual cases were reported from the South of Germany (Baden-Württemberg, Bavaria and South-Hesse). But also in the Free State of Saxony in the past 13 years 54 cases were reported, of which 34 were considered autochthonously acquired. Also in the bordering states Saxony-Anhalt and Brandenburg eleven and twenty so-called sporadic cases were observed in the same time period, respectively (RKI, 2014). 200–300 cases are annually reported in neighboring Poland and 450–750 cases in the Czech Republic, both TBEV-endemic countries meeting the German criteria of a risk area. Hence, Saxony is a non-risk TBE area in the triangle between high endemic areas like Bavaria and the Czech Republic in the South and non-endemic areas such as Brandenburg and Saxony-Anhalt in the North. Based on the latest TBE risk evaluation by the RKI, Vogtlandkreis for the first time and as the only district of Saxony meets the criteria of a risk area since 2014 (RKI, 2014).

Next to human cases, there are descriptions of clinical cases in animals, such as dogs (Bajer et al., 2013; Reiner and Fischer, 1998; Leschnik et al., 2002; Stadtbäumer et al., 2004; Weissenböck and Holzmann, 1996; Saenger et al., 2013), horses (Waldvogel et al., 1981; Klaus et al., 2013), a mouflon (Bago et al., 2002), monkeys (Klaus et al., 2010) and a goat (Zindel and Wyler, 1983). Seroprevalence studies in various animal species were conducted in Germany estimating the prevalence rates of antibodies against TBEV in different regions. Müller (1997) found 23% of 517 dogs seropositive from an area in southern Germany. In other studies, 7 of 240 blood samples from horses from Marburg-Biedenkopf district in Hesse (Müller et al., 2006) were positive in the serum-neutralization test (SNT) and Janitza-Futterer found 23.4% of 205 horse sera containing antibodies against TBEV in the area of Lake Constance (Janitza-Futterer, 2003). Also from risk-areas in Baden-Württemberg, 163 field collected goat sera were tested, of which 17.2% turned out to be positive by SNT (Klaus et al., 2011). Moreover, Klaus and colleagues screened more than 3500 sheep sera and more than 3,700 goat sera from districts all over Germany for the presence of antibodies against TBEV. In risk areas, they found seroprevalences of up to 33% and in non-risk areas of up to 13.9% (Klaus et al., 2012).

Furthermore, wildlife animals were used as sentinels in different investigations, showing that 8% of 304 trapped rodents from non-risk areas and 15% of 137 trapped rodents from a risk-area in Hesse were TBEV-positive (Achazi et al., 2011). Different authors found that game animals serve as good sentinels for antibody detection in epidemiological studies (Charrel et al., 2004; Gerth et al., 1995; Kiffner et al., 2011; Roelandt et al., 2010; van der Poel et al., 2005; Ytrehus et al., 2013). Surveys based on wild animal species were used in order to define TBEV-endemic and possible risk areas in Croatia (Jemeršić et al., 2014), Belgium (Linden et al., 2012), Denmark (Skarphédinsson et al., 2005), The Netherlands (van der Poel et al., 2005) or Norway (Ytrehus et al., 2013). In a current study in the Czech Republic, sera from zoo animals were screened for antibodies *against Borrelia burgdorferi s.l.* and TBE. A markhor and a reindeer from the same zoo located in a TBEV-endemic area showed a positive result (Sirmarova et al., 2014).

In Germany, the first seroprevalence-study on game animals was conducted on the Island Usedom by Süss between 1986 and 1989 showing 5 of 500 samples of deer and wild boar reactive (Süss et al., 1992). Gerth and colleagues found 50 antibody-positive sera out of 192 examined samples in ELISA in the area of Tübingen (Gerth et al., 1995) and Kahl et al. found TBEV-specific titers of 1:10–1:40 in a hemagglutination inhibition assay (HAI) in 5 out of 15 shot deer in West-Berlin (Kahl and Radda, 1988). In South-Hesse, 22.9% of 105 analyzed sera of roe deer had TBEV-neutralizing antibodies (Kiffner et al., 2011) and 1 out of 786 red foxes from North-Rhine-Westphalia (a non-risk area) was seropositive (Wurm et al., 2000). Investigations in small terrestrial mammals (rodents and shrews) in the TBE risk area of the southern Bavarian forest revealed 14% of 266 animals with neutralizing antibodies against TBEV (Kocianova et al., 1993).

In the 1960s–1980s, TBEV was known to be endemic in the eastern parts of Germany (Süss et al., 1992), but since then the disease seemed to decline without an apparent increase in vaccine coverage. In our study, we used the seroprevalence in wild game, especially wild boar, to evaluate the current situation in Saxony in terms of TBEV-endemicity thus providing evidence for the autochthonous occurrence of the reported sporadic TBE cases.

## Materials and methods

### *Wildlife sera*

A total of 1,851 sera from wild boar and 35 sera from roe deer were investigated. The animals from the eastern districts (Bautzen, Görlitz, Meißen, Dresden and Sächsische Schweiz-Osterzgebirge (=SSO), Fig. 1) were shot between April and October 2011 and the wild boar from the districts Chemnitz, Erzgebirgskreis, Leipzig, Leipziger Land, Mittelsachsen, Nordsachsen, Vogtlandkreis and Zwickau (see Fig. 1) were shot between June 2012 and March 2013. Hunters were asked to take blood from the thoracic cavity or by heart puncture and send it to the State Laboratories of Human and Veterinary Affairs. The blood samples were centrifuged and the serum was kept frozen until examination in our laboratory. The origin of the different sera is shown in Table 1.

**Table 1**
Serum samples of wild boar and roe deer according to the district within Saxony where they were hunted.

| Species | District | Number of samples | Positive[a] *n* (%) |
|---|---|---|---|
| Wild boar | Bautzen | 176 | 15 (8.52%) |
| Wild boar | Chemnitz | 4 | 0 (0%) |
| Roe deer | Dresden | 35 | 8 (22.86%) |
| Wild boar | Dresden | 34 | 6 (17.65%) |
| Wild boar | Erzgebirge | 86 | 9 (10.47%) |
| Wild boar | Görlitz | 344 | 36 (10.47%) |
| Wild boar | Leipziger Land | 143 | 8 (5.59%) |
| Wild boar | Leipzig | 7 | 0 (0%) |
| Wild boar | Meißen | 181 | 42 (23.2%) |
| Wild boar | Mittelsachsen | 194 | 10 (5.15%) |
| Wild boar | Nordsachsen | 191 | 3 (1.57%) |
| Wild boar | Sächsische Schweiz–Osterzgebirge (SSO) | 153 | 13 (8.5%) |
| Wild boar | Vogtland | 220 | 45 (20.45%) |
| Wild boar | Zwickau | 118 | 3 (2.54%) |
| Wild boar | All districts of Saxony | 1,851 | 190 (10.26%) |
| Wild boar and roe deer | All districts of Saxony | 1,886 | 198 (10.5%) |

[a] Samples were considered positive if SNT-Titer was ≥10.