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Short communication

Seroprevalence of tick-borne-encephalitis virus in wild game in Mecklenburg-Western Pomerania (north-eastern Germany)

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ABSTRACT

Mecklenburg-Western Pomerania, a federal state in the north east of Germany, has never been a risk area for TBEV infection, but a few autochthonous cases, along with TBEV-RNA detection in ticks, have shown a low level of activity in natural foci of the virus in the past. As wild game and domestic animals have been shown to be useful sentinels for TBEV we examined sera from wild game shot in Mecklenburg-Western Pomerania for the prevalence of TBEV antibodies.

A total of 359 sera from wild game were investigated. All animals were shot in Mecklenburg-Western Pomerania in 2012. Thirteen of 359 sera tested positive or borderline for anti-TBEV-IgG with ELISA and four samples tested positive using NT.

The four TBEV-positive sera confirmed by NT constitute the first detection of TBEV-antibodies in sera of wild game in Mecklenburg-Western Pomerania since 1986–1989. This underlines that the serological examination of wild game can be a useful tool in defining areas of possible TBEV infection, especially in areas of low TBEV-endemicity.

1. Introduction

Tick-borne encephalitis (TBE) is the most widespread tick-transmitted viral disease in central Europe. The TBE virus (TBEV) belongs to the genus Flavivirus (Fam. Flaviviridae), which has three different subtypes: the European subtype, transmitted mainly by *Ixodes ricinus*, the Siberian subtype and the Far Eastern Subtype, both of which are transmitted mainly by *Ixodes persulcatus* (Süss, 2011).

Wild game and domestic animals usually develop an antibody titer after infection with TBEV without showing specific clinical signs of illness (Nosek et al., 1967; Duscher et al., 2015). These animals are only viremic for a very short time, but as their antibodies persist over a longer period, they are useful sentinels for TBEV (Gerth et al., 1995; Klaus et al., 2012; Jaenson et al., 2012; Balling et al., 2014; Duscher et al., 2015; Imhoff et al., 2015). Furthermore, it has been shown for the Czech Republic that the size of the wild boar population correlates positively with the annual incidence of TBE. In the Czech Republic, Belgium, Croatia and in other European countries, surveys of wild animal species have successfully been performed to highlight possible risk areas of TBEV transmission (Balling et al., 2014; Kriz et al., 2014; Duscher et al., 2015; Imhoff et al., 2015).

Mecklenburg-Western Pomerania, a federal state in the north east of Germany, has never been declared a risk area according to the definition used by the German public health authority, but a few autochthonous cases, along with TBEV-RNA detection in ticks, have shown a low level of activity in natural foci of the virus in the past. Between 1992 and 2003, a total of 16,089 ticks tested negative for TBEV in Mecklenburg-Western Pomerania and it was thought that TBEV had disappeared from this area (Health Department of the State of Mecklenburg-Western Pomerania, unpublished data; Klaus et al., 2010). In 2004 the first autochthonous case of human TBEV infection for 19 years was reported, to be followed by other autochthonous human cases. Ticks collected from the areas where the human infections occurred were shown to harbour TBEV-RNA for the first time in 15 years (Süss et al., 1992; Hemmer et al., 2005; Robert Koch Institute, 2007; Frimmel et al., 2010, 2014).

From 1986–1989 on the Island of Usedom (in the eastern part of Western Pomerania), 5 of 500 samples of wild boar and deer were seropositive for TBEV (Süss et al., 1992). A recent seroprevalence

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Table 1
Results and sampling locations of sera tested positive by ELISA and SNT, sorted by positivity using SNT and district.

District	Georeference	Location	Species	ELISA (VIEU) result	ELISA interpretation	SNT result	SNT interpretation
VR	54,4277 12,7696	Zingst	Red deer	166	+	160	+
VR	54,4277 12,7696	Zingst	Fallow deer	166	+	10	+
VG	53,7089 13,9274	Meiersberg	Red deer	188	+	60	+
VG	53,6333 14,0333	Spechtberg	Roe deer	<5	–	20	+
VR	54,4277 12,7696	Zingst	Wild boar	171	+	<10	–
VG	53,4869 14,0334	Pasewalk	Wild boar	152	+	<10	–
VG	53,4869 14,0334	Pasewalk	Wild boar	106	(+)	<10	–
LUP	53,4514 11,1454	Granzin	Wild boar	215	+	<10	–
LUP	53,4738 10,7915	Greven	Wild boar	170	+	<10	–
LUP	53,4738 10,7915	Greven	Wild boar	85	(+)	<10	–
LRO	54,2389 12,2217	Torfbrücke	Red deer	234	+	<10	–
MSE	53,2606 12,5774	Kieve	Red deer	144	+	<10	–
MSE	53,3004 12,8916	Leussow	Fallow deer	70	(+)	<10	–
MSE	53,5000 12,7500	Kargow	Red deer	121	(+)	<10	–

Legend.
Vienna Units (VIEU).
+ positive.
(+) borderline.
– negative.
Administrative districts: Vorpommern-Ruegen (VR), Vorpommern-Greifswald (VG), Ludwigslust-Parchim (LUP), Rostock (LRO), Mecklenburgische Seenplatte (MSE).

study of wild game in Saxony, eastern Germany, showed that high seroprevalence of TBEV in wild game can be found in areas of low TBEV transmission risk (Balling et al., 2014).

We examined sera from wild game shot in Mecklenburg-Western Pomerania for the prevalence of TBEV antibodies in order to update existing data on autochthonous cases and TBEV prevalence in ticks (Hemmer et al., 2005; Frimmel et al., 2014).

2. Materials and methods

A total of 359 sera from wild game were investigated—229 sera from wild boar, 51 from red deer, 24 from roe deer, and 55 from fallow deer. All animals were shot without context to the study in Mecklenburg-Western Pomerania in 2012. The sera were processed and stored as prescribed by law at the State Institute for Agriculture, Food Safety and Fisheries Mecklenburg-Western Pomerania. Serum samples of between 200 and 400 μl in volume were stored at −80 °C in deepwell plates for further examination.

2.1. ELISA

All 359 samples were tested using the "Immunozym FSME IgG All Species ELISA kit®" (Progen, Heidelberg, Germany) according to the manufacturer's instructions. Results were expressed as Vienna units per ml (VIEU/ml) with <63 VIEU/ml considered negative, between 63 and 126 VIEU/ml as borderline and over 126 VIEU/ml as positive, according to the manufacturer's recommendations.

2.2. Virus neutralization test (NT)

The virus neutralization test (NT) was performed as described before (Holzmann et al., 1996). NT titers (NT 100) were expressed as the reciprocal of the serum dilution that was able to suppress virus infection to such an extent that no viral antigen could be detected by ELISA in the supernatant (OD at 450 nm <0.1). The test was repeated twice and the results were averaged. NT100 titers ≥10 were considered positive. Each test included titrations of virus as a control in the absence of antibodies, along with three positive and one negative serum controls.

3. Results

3.1. ELISA

Thirteen of 359 sera tested positive or borderline for anti-TBEV-IgG with ELISA (3.6%). Four of 51 serum samples from red deer, four of 229 samples from wild boar and one of 55 samples from fallow deer were ELISA positive. Two wild boar sera and one red and fallow deer serum respectively exhibited borderline reactions in the ELISA (Table 1, Fig. 1).

3.2. Virus neutralization test

As the gold-standard for minimizing false-positive results, NT was performed as a control on the nine ELISA-positive sera, the four ELISA-borderline sera and, additionally, on five randomly chosen sera which were negative according to ELISA. Four samples tested positive using NT. These included two red deer sera and one fallow deer serum, which were all ELISA-positive, and one roe deer serum which had tested negative using ELISA. Detailed results are referred in Table 1 and Fig. 1 (Table 1, Fig. 1).