



Original article

Detection of tick-borne encephalitis virus antibodies in sera of sheep and goats in Mecklenburg-Western Pomerania (north-eastern Germany)

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1. Introduction

Tick-borne encephalitis (TBE) is the most widespread arthropod-borne viral disease in central Europe. The TBE virus (TBEV) belongs to the genus *Flavivirus* (family *Flaviviridae*), and has three different subtypes: the European subtype, transmitted mainly by *Ixodes ricinus*, the Siberian subtype and the Far Eastern subtype, which are mainly transmitted by *Ixodes persulcatus* (Süß, 2011). In nature, the virus circulates between tick vectors and their vertebrate hosts, within in geographically defined foci (Dobler et al., 2011). TBEV also can be transmitted by ingestion of unpasteurized milk or milk products from infected domestic animals, such as goats, sheep or cows. Alimentary TBEV infections and outbreaks have been linked in the past to unpasteurized dairy products, and are still reported today in many European countries (Apitzsch, 1967; Holzmann et al., 2009; Hudopisk et al., 2013; Markovinović et al., 2016; Brockmann et al., 2018; Kerlik et al., 2018).

None of the districts in Mecklenburg-Western Pomerania, one of the most popular holiday regions in Germany, has ever been declared a risk area for TBE, according to the definition used by the German public health authority. However, a handful of autochthonous cases, coupled with TBEV-RNA detection in ticks, have shown activity in natural foci of the virus. In the 1960 s and 1970 s, foci of TBE virus were well known to be active in Mecklenburg-Western Pomerania and human cases were reported regularly (Apitzsch, 1965,1967; Süß et al., 1992). In 2004, the first autochthonous case of human TBEV infection for 19 years was reported, and was followed by several further autochthonous human cases (Hemmer et al., 2005; Frimmel et al., 2010). In 2007, TBEV-RNA in questing ticks in Mecklenburg-Western Pomerania was detected for the first time since 1992 and four serum samples from wild game shot in 2012 tested positive in a virus neutralization test, thus proving activity of natural foci of TBEV in Mecklenburg-Western Pomerania (Frimmel et al., 2014,2016).

In regions of low TBE prevalence, sentinel animals are an excellent

way of assessing the risk of TBEV infection. In contrast to the established method of detecting natural foci by collecting and analyzing ticks, which is time consuming, expensive and often ineffective (Klaus et al., 2012; Balling et al., 2014; Imhoff et al., 2015; Frimmel et al., 2016), serological survey of sheep and goats is easier and may be more effective. TBE infections result in long-term immunity but are rarely clinically apparent in *Bovidae*. However, it has been proven that these animals carry specific antibodies following a TBEV infection and, using ELISA and NT, TBEV-specific antibodies can be reliably detected in serum for many years post-infection (Klaus et al., 2012,2014; Imhoff et al., 2015). Moreover, because herds of sheep and goat are sedentary and stay only in their designated fields, they are likely optimal sentinels for TBE risk areas. In summary, assessing seroprevalence in free-ranging animals, especially in sheep and goats, is a valuable and informative technique to identify patterns in TBEV foci, to describe the epidemiological situation and to identify areas of risk, even in regions of low TBE prevalence, such as Mecklenburg-Western Pomerania (Klaus et al., 2012,2014).

We examined sera from livestock (goats and sheep) from Mecklenburg-Western Pomerania for the presence of TBEV-specific antibodies to complement the data obtained in previous studies regarding ticks and wild game, concentrating on regions with former proof of TBEV activity (Frimmel et al., 2010,2016).

2. Material and methods

2.1. Serum samples

A total of 479 sera from livestock were investigated: 375 from sheep and 104 sera from goats. All sera were obtained for routine examination, with no reference to this current study, from 18 farms in Mecklenburg-Western Pomerania between 2014 and 2017. Samples were selected from sites with previous evidence of human cases of TBE, seropositive wild game or known former natural foci. Between two and

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60 serum samples were collected from each farm. Blood specimens were drawn by local veterinarians and were sent to the State Institute for Agriculture, Food Safety and Fisheries Mecklenburg-Western Pomerania for surveillance of epizootic diseases. The sera were processed and stored at the State Institute. Serum samples of between 200 and 400 µl in volume were stored at –20 °C in deep well plates and transferred in 2-ml Eppendorf tubes for further examination in our study.

2.2. ELISA

All 479 samples were tested using the “Immunozytm 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 above 126 VIEU/ml as positive, as per the manufacturer’s recommendations.

2.3. Virus Neutralization Test (NT)

As the gold-standard for minimizing false-positive results and to detect positive sera that tested borderline with ELISA, a virus NT was performed as a control on the eleven ELISA-borderline sera and, additionally, on ten randomly chosen sera which were negative according to ELISA. The virus neutralization test was performed using baby hamster kidney (BHK) cells and TBEV (strain Neudoerfl) as described before (Stiasny et al., 2009). Heat-inactivated serum samples were incubated with TBEV for 1 h at 37 °C. Cells were added and incubation was continued for three days. The presence of virus in the cell culture supernatant was assessed by a sandwich ELISA as described previously (Heinz et al., 1986), using guinea pig anti-TBE IgG as capturing antibody and rabbit anti-TBE IgG in combination with horseradish peroxidase-labeled anti-rabbit IgG from donkey (GE healthcare) as the detection system. The virus neutralization titer was defined as the reciprocal of the sample dilution that showed a 90% reduction in the absorbance readout compared to the control without antibody. NT titers ≥ 10 were considered positive.

3. Results

3.1. ELISA

In the ELISA tests, eleven of 479 sera tested borderline for anti-TBEV-IgG (2.3%). Nine of 375 serum samples from sheep (2.4%), and two of 104 samples from goats (1.9%) were borderline. None of the sera tested positive (Table 1, Fig. 1).

Table 1

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

District	Location/community	Species	ELISA (VIEU) result	ELISA interpretation	NT result	NT interpretation	Year
MSE	Blumenholz	Sheep	82	Borderline	30	+	2014
VG	Torgelow	Sheep	117	Borderline	10	+	2016
VR	Putbus	Sheep	69	Borderline	< 10	–	2014
VR	Putbus	Sheep	63	Borderline	< 10	–	2014
VR	Putbus	Sheep	78	Borderline	< 10	–	2014
VG	Stolpe	Sheep	79	Borderline	< 10	–	2014
VG	Stolpe	Sheep	71	Borderline	< 10	–	2014
VG	Ueckermünde	Goat	108	Borderline	< 10	–	2014
VG	Ueckermünde	Sheep	76	Borderline	< 10	–	2016
VG	Stolpe	Sheep	67	Borderline	< 10	–	2015
MSE	Neustrelitz	Goat	96	Borderline	< 10	–	2016

Vienna Units (VIEU).

+ positive.

– negative.

Administrative districts: Mecklenburgische Seenplatte (MSE), Vorpommern-Greifswald (VG), Vorpommern-Rügen (VR).

3.2. Virus Neutralization Test

Two samples of sheep sera tested positive using NT. One sample from the year 2014 from a flock in the administrative district Mecklenburgische Seenplatte, and one sample from the district Vorpommern-Greifswald from the year 2016. Detailed results are shown in Table 1 and Fig. 1.

4. Discussion

To the best of our knowledge, the two TBEV-positive sera confirmed by NT constitute the first detection of TBEV-specific antibodies in sera of livestock in Mecklenburg-Western Pomerania since 1965, when antibodies detected in cow’s milk revealed the possible source of an outbreak of TBE in a natural focus of TBE (Apitzsch, 1965,1967; Süß et al., 1992). We found one reactive sheep serum from this area near Torgelow in the Vorpommern-Greifswald district (Table 1, Fig. 1). Interestingly, in our previous study regarding wild game, two reactive sera were found in this region, illustrating stable activity of TBEV in this region (Frimmel et al., 2016, Fig. 1).

The other serum that tested positive by NT was obtained from the community Blumenholz/Weisdin, near Neustrelitz in the Mecklenburgische Seenplatte district (Table 1, Fig. 1). Between 1960 and 1985, several human cases have of TBE were reported in this area (Süss et al., 1992). The first autochthonous human case in Mecklenburg-Western Pomerania for 19 years occurred in 2004, about 20 km away from Blumenholz (Hemmer et al., 2005). In 2007, TBEV-RNA was detected in ticks at the where the infection was acquired (Frimmel et al., 2010).

Sheep and goats are valuable sentinels for TBE (Klaus et al., 2012). The number of seropositive animals seems to correlate with the number of positive ticks and human cases of TBE in regions of relatively high TBE-prevalence (Juceviciene et al., 2005). The larger and defined area of activity in comparison with small mammals or wild game may represent an advantage for being sentinels in areas of low TBEV-endemism (Klaus et al., 2012; Balling, et al., 2014; Imhoff et al., 2015). The relatively low rate of positive sera in our study can be explained by the observation, that seroprevalence in sheep and goats varies greatly between individual flocks, with the highest rates measured in or close to recognized foci. Small rodents, which are considered to be amplifier and bridge hosts for TBEV may have higher Seroprevalence rates, even in non-risk areas (Imhoff et al., 2015; Rieille et al., 2017)

In Mecklenburg-Western Pomerania, the risk of TBEV-infection is low but singular autochthonous cases do occur. The existence of active foci was proven by detection of TBEV-RNA in host-seeking ticks from the areas where the infections were acquired (Frimmel et al., 2010).

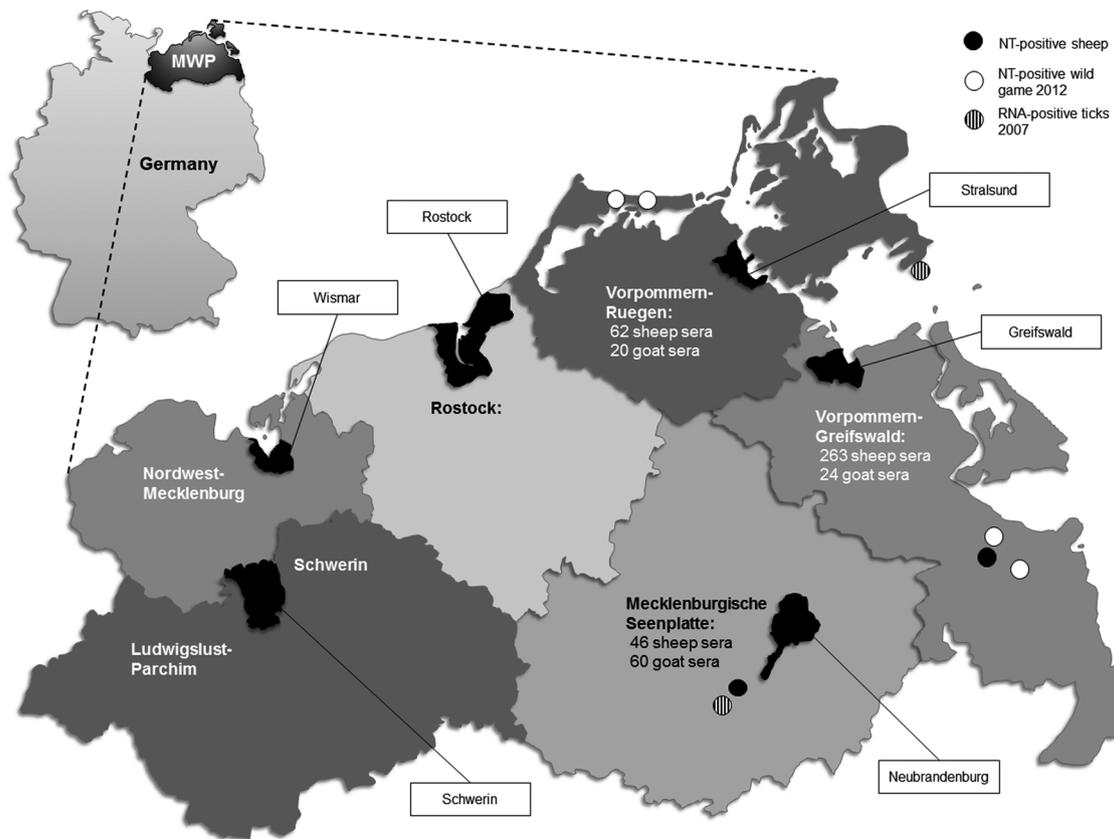


Fig. 1. Map of Mecklenburg-Western Pomerania showing NT-positive serum samples of sheep from the present study, NT-positive serum samples from wild game from 2012 and TBEV-RNA-positive ticks from 2007.

The presence of TBEV-specific antibodies in livestock animals underlines that the serological examination of domestic animals like sheep and goats can be a useful tool in defining areas of possible TBEV infection within low-risk regions. A similar conclusion was drawn by Salat et al. from Romania, where a relatively high number of TBEV-positive sheep was found, but only a few human cases are reported annually (Salat et al., 2017). We are aware that the number of animals tested in our study is relatively small. However, the positive data are highly encouraging and provide a convincing argument that further investigations of livestock, using a larger sample base and covering more western regions of Mecklenburg-Western Pomerania should be carried out. These data are of significant epidemiological interest, not least when we consider that Mecklenburg-Western Pomerania is one of the most popular tourist destinations in Germany, with about 30 million overnight stays per year (Federal Statistical Office, 2018).

The serological survey of livestock, wild game or smaller animals, such as rodents, is a promising technique for detecting new areas where TBEV may be endemic; the animals in question are valuable sentinels for regions with low prevalence (Achazi et al., 2011; Klaus et al., 2012; Balling et al., 2014; Imhoff et al., 2015; Zöldi et al., 2015; Frimmel et al., 2016). Returning to explore the natural foci described in 1992 may generate results that would serve as a useful addition to the present data, through the selective flagging and examination of ticks nearby the positive tested herds.

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