

# Nationwide seroprevalence study of Tick-borne encephalitis virus in Danish roe deer (*C. capreolus*)

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## Objective

The objective of this study was to determine whether Tickborne Encephalitis virus (TBE-virus) foci could exist outside the known endemic areas in Denmark. To explore this, we performed a high intensity sero-prevalence study of TBE-virus throughout Denmark using roe deer (*C. capreolus*) as sentinel. Presented here are the preliminary results.

## Introduction

Tickborne encephalitis (TBE) is considered endemic on the Danish Island of Bornholm in the Baltic Sea, and was for many past decades thought to represent an isolated focus in Denmark (1-5). However, seroprevalence studies of TBE-virus in roe deer and dogs conducted in the years 2002 to 2009 indicated that TBE-virus existed in parts of Denmark from where no human cases had been reported (6-7). This was recently (2009-2011) further supported by two clinical cases of TBE in humans living and working in the forest "Tokkekøb Hegn" and in ticks from the same area (8-9). Figure 1, illustrates the known endemic TBE areas and possible TBE-virus foci based on sentinels prior to this study.



**Photo 1:** Blood collection when dressing freshly killed roe deer. Photo by hunter Thomas Rasmussen

## Method

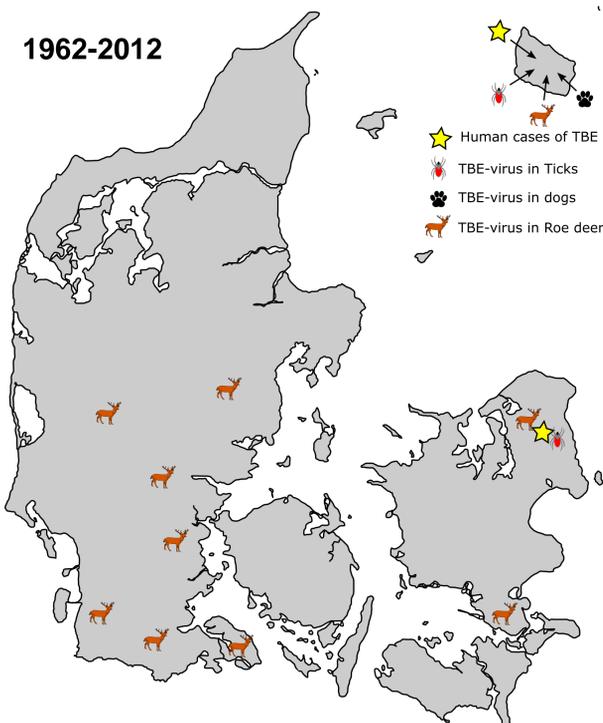
Danish hunters were invited to participate during the regular hunting season of 2013-14. They were asked to obtain blood samples from roe deer when dressing freshly killed animals in the field, Photo 1. For each animal, location, sex, age, and degree of tick infestation were noted. Blood collection kits were distributed to the hunters by mail, and blood samples subsequently returned by mail to The Research Unit of Clinical Microbiology, University of Southern Denmark. From here the serum or preferably buffycoat were sent to the Department of Virology, Medical University Vienna where they were examined for the existence of TBE-virus specific antibodies by Neutralisation-test (NT-test).

## Results

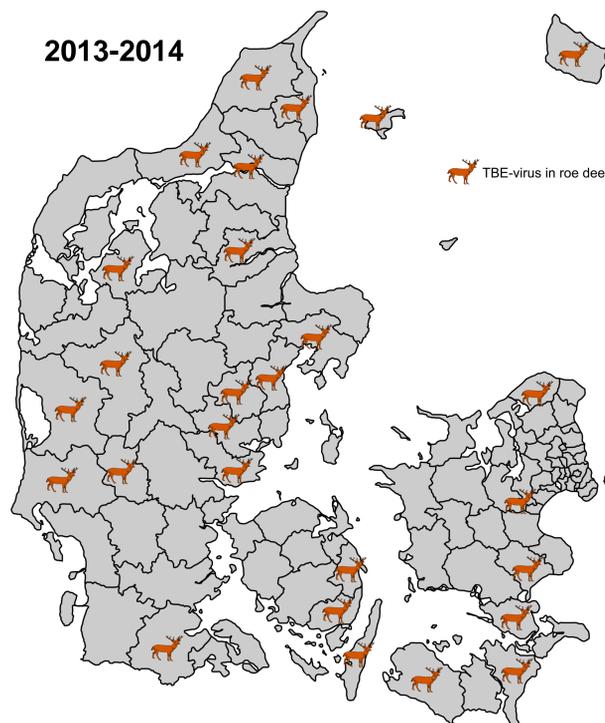
815 samples were collected. Analysis of 364 selected samples has so far yielded 51 NT-positive and 282 NT-negative roe deer\*, Table 1. 31 samples could not be analysed due to toxic effect of the serum on the cell.

The remaining 451 samples will undergo NT-testing at the Department of Virology, Medical University Vienna in the autumn of 2015.

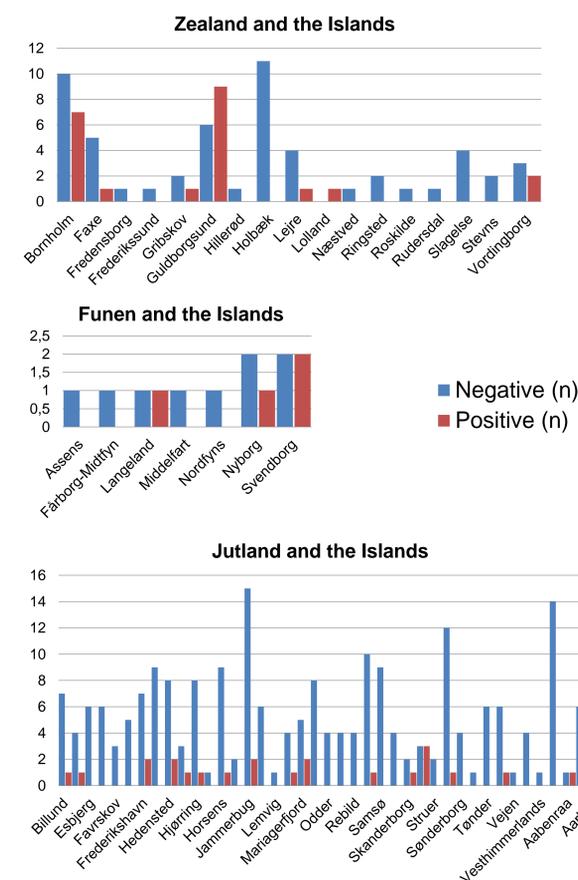
The positive samples span all of Denmark and include areas that have no past history of human TBE cases. Many positive samples originated from locations which also in previous sentinel studies have provided TBE-positive samples, but new possible TBE-virus foci have also appeared, Figure 2, shows the location of the TBE-positive roe deer (2013-2014) based on municipality.



**Figure 1:** Areas of Denmark with confirmed TBE in humans, TBE-virus in ticks and TBE-virus antibodies identified by NT-test in roe deer and dogs, before 2013. OBS: The location of roe deer is based on state forest districts



**Figure 2:** Areas of Denmark with TBE-virus antibodies in roe deer identified by NT-test, based on municipality. Hunting season of 2013-2014.



**Table 1:** Results of TBE-virus NT-test on Danish roe deer collected in the hunting season of 2013-2014, based on municipality.

## Conclusions

These preliminary results supports our hypothesis that TBE-virus exists in Denmark beyond the currently known foci on Bornholm and North Zealand, and that medical doctors in all of Denmark should keep TBE in mind when treating a patient with symptoms of encephalitis and a history of tick exposure.

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