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## Laboratory examinations of transmissible spongiform encephalopathies in Denmark during 2016

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## **Report**

# **Laboratory Examinations of Transmissible Spongiform Encephalopathies in Denmark during 2016**

March 2017

**DTU Vet**  
National Veterinary Institute

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## Laboratory examinations of transmissible spongiform encephalopathies in Denmark

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## **Introduction**

The aim of this report is to give detailed information on the diagnostic examination on transmissible spongiform encephalopathies (TSE) performed in Denmark during 2016. The present annual report is the 21<sup>st</sup> on this topic published by the National Veterinary Institute, Technical University of Denmark (DTU-VET).

The report is made to fulfil the demands given by the EU Commission (Regulation No 999/2001 of the European Parliament and the Council of 22. May 2001) and the Office International des Epizooties (OIE) (Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5<sup>th</sup> edition 2008, Chapter 2.4.6 and Chapter 2.7.13) regarding diagnostic examinations.

The DTU-VET is the national reference laboratory of bovine spongiform encephalopathy (BSE) and TSE/Scrapie, and therefore the results of all neuropathological examinations on BSE and Scrapie in Denmark are given in the present report as in previous years.

## **Assignments**

This year's report includes all examinations of cattle, sheep, and goats displaying behaviour disorders and/or neurological signs with suspicion of having TSE as well as animals with neurological signs but without suspicion of TSE. This report also includes all examinations of cattle, sheep and goats, where the TSE rapid test performed came out with an inconclusive or a positive result. Furthermore, the report includes examinations of pet animals, fur bearing animals, wild animals, and zoo mammals displaying behaviour disorders and/or neurological signs. The number of pet animals, wildlife, and zoo animals examined, however, do not necessarily include all examined animals in Denmark since neuropathological examination may have been performed at other laboratories without the knowledge of the DTU-VET. In no case, however, suspicion of TSE was made since this would mean that the laboratory had to inform the Danish Veterinary and Food Administration due to notifiability of TSE. Similar, neuropathological examination of adult ruminants without clinical signs or having diseases not compatible with BSE or Scrapie may have been performed at other institutions. However, the brainstems of these animals were examined according to the surveillance program described below.

The Danish BSE surveillance program included during 2016 the testing of the following bovine animals:

- Bovines with a clinical suspicion of BSE. All cases examined at DTU-VET
- Emergency slaughter bovines older than 48 months. Private, approved laboratories
- Bovines older than 48 months with remarks at the ante mortem inspection performed by the official veterinarian at the slaughterhouse. Private, approved laboratories.
- All fallen stock of bovines older than 48 months. Private, approved laboratory.

The Danish TSE surveillance program included during 2016 the testing of the following small ruminants:

- Small ruminants with a clinical suspicion of TSE. All cases examined at DTU-VET
- A random sample of fallen stock animals older than 18 months to fulfil the requirements of the TSE legislation, which with the Danish sheep and goat population in 2016 were annually 500 sheep tests and 100 goat tests. Private, approved laboratories.

DTU-VET carried all tests in agreement with Annex X in the Regulation (EC) No. 999/2001.

From November 2014 DTU Vet stopped the performance of Western Blotting and has made arrangements with the EU Community Reference laboratory for TSE (Weybridge), APHA, U.K. (signed contract) for this part of the confirmatory testing. In the future positive/suspected samples unsuitable for Histopath./IHC will be forwarded to APHA, U.K. for final diagnosis. Positive samples in general will be forwarded to APHA for strain typing.

DTU-VET controlled test performance in the private laboratories. This included bimonthly submission of control data for review. An annual inspection visit of the private laboratories was performed by DANAK. DANAK is the national accreditation body in Denmark appointed by The Danish Safety Technology Authority, under the Ministry of Business and Growth Denmark.

The laboratories' approval of daily set-up's are done according to specified criteria, incorporating results of positive and negative controls in IDEXX HerdCheck assays. Assignment of test-positivity, test-negativity of tested tissue samples is made in relation to prefixed criteria as approved by the national reference laboratory. Retesting and possible reclassification of single samples with a positive or doubtful result takes place according to a prefixed schedule, in the case of borderline reactions or by demonstrable technical errors, such as grossly differing readings of duplicates or appearance of "clusters" of positive microtiter wells. Proficiency trials were performed twice a year co-ordinated by APHA.

### **Staff**

One veterinary pathologist in the Division of Diagnostics and Scientific Advice trained in the diagnosis of TSE at the EU Community Reference laboratory for TSE, APHA, Weybridge, UK, performed all the neurohistopathological examinations and IHC.

Furthermore, a senior epidemiology advisor performed the supervision of private, rapid testing laboratories. Furthermore, the TSE genotyping is performed by a molecular biologist in the Division of Diagnostics and Scientific Advice.

## Methods

### **Methodology for examination of clinically suspected cases of BSE or TSE/Scrapie**

Clinically suspected cases of BSE or TSE/Scrapie were usually euthanized by intravenous injection of an overdose of barbiturate, and the heads were submitted - generally overnight - to DTU-VET. The whole brain was removed from the skull. A cross-sectional sample of 5-10 gram from the brainstem just caudal to the obex region was taken and kept at 5°C until the case was completed).

The rest of the brainstem was fixed in 10% neutral buffered formalin for 3 days. Brainstem areas were selected according to the OIE Manual (medulla at the obex, medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi), cut into 3-5 mm tissue blocks and post fixed for a few days with daily change of formalin.

One half of the cerebrum was kept at 5°C until the case was completed. The rest of the brain material was fixed in 10% neutral buffered formalin for two weeks with change of fixative after one week. Transverse sections of the cerebrum and longitudinal sections of the cerebellum and the pituitary were, cut into 3-5 mm tissue blocks and post fixed for a few days with daily change of formalin.

For histopathology, sections were stained with haematoxylin and eosin. Furthermore, immunohistochemistry (IHC) for demonstration of disease specific prion protein (PrP<sup>D</sup>) was applied on the obex section of all cases.

If a case is inconclusive material is forwarded to the EU Community Reference Laboratory for TSE, Weybridge, UK, for further examination.

In case of positive animals, fresh brainstem material is forwarded to APHA for PrP strain-typing.

### **Methodology for examination of animals-at-risk (fallen stock, etc.)**

At DTU-VET no testing was performed in 2016.

### **Methodology for confirmatory examination of animals TSE positive by monitoring testing (when the rapid testing is positive)**

In case of a positive or inconclusive result of the monitoring tests (rapid test) the confirmatory examinations were performed at DTU-VET according to the following procedure: A) cross-sectional sample of 5-10 gram from the brainstem just caudal to the obex region was taken and kept at 5°C until the case was completed).

B) For confirmatory examination the brainstem was fixed in 10% neutral buffered formalin for 3 days. Brainstem areas were selected according to the OIE Manual (medulla at the obex (and medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi if possible). Cut into 3-5 mm tissue blocks (obex), and post fixed for a few days with daily change of formalin. Sections were stained with haematoxylin and eosin as well as by immunohistochemistry (IHC) for demonstration of disease specific prion protein (PrP<sup>D</sup>).

The case was reported as BSE/Scrapie-positive if confirmatory testing revealed a positive result. In cases of severely autolysed / unsuitable material frozen tissue was forwarded to the EU Community Reference laboratory for TSE, APHA, Weybridge, UK, for further examination.

**Methodology for examination of wildlife animals, pet animals, fur animals, zoo, and other animals**

Mink older than seven months of age and other animals older than 1 year of age with clinical signs indicating a neurological disorder were examined for spongiform encephalopathies. Furthermore, fallen, adult animals with CNS lesions are reported. Only mammalian species were included.

The brain was divided into two equal parts by longitudinal section. One half of the brain was fixed in 10% neutral buffered formalin for two weeks. The fixative was changed after one week. The other half was stored at -18°C. The formalin fixed tissue was cut with 4 transversal sections into 5 equally large portions (ensuring that brain stem areas were selected according to the OIE Manual medulla at the obex, medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi) and embedded in paraffin in a routine manner. Transverse sections of cerebellum were always included. Sections were stained with haematoxylin and eosin and examined histopathologically including spongiform encephalopathies.

## **Results**

### **BSE in bovines born in Denmark**

During 2016 no case of indigenous BSE was diagnosed.

### **Scrapie in small ruminants born in Denmark**

During 2016 one case of indigenous TSE was diagnosed in a fallen sheep (atypical scrapie).

### **Examination of suspected BSE and Scrapie cases**

During 2016 one suspicion of clinical TSE in bovines was reported – which was PrP<sup>D</sup> negative. Table 1 and 2.

According to the Danish Veterinary and Food Administration one bovine animal was suspected of TSE due to clinical symptoms, but rejected without testing according to TSE Regulation 999/2001 as later amended, Article 12(1). Article 12(1) makes it possible in those cases where the result of the clinical examination (including treatment) reveals another disease, to reject the suspicion.

### **Examination of animals-at-risk (fallen stock, etc.)**

This year 19.367 bovines have been tested as fallen stock and 1307 as emergency slaughter animals – all negative and all at a private laboratory.

Moreover, 680 sheep and 132 goats, all analysed at a private laboratory, have been tested as fallen stock. One sheep was found positive and by confirmatory testing at APHA the case was categorized as atypical scrapie (Table 3). All the other animals were tested negative for TSE.

### **Examination of healthy slaughter animals**

The private, approved laboratory tested 21 slaughter animals during 2016. The laboratories found no animals positive.

### **Examination of CNS from wildlife animals, pet animals, fur animals, and zoo animals**

Lesions consistent with chronic wasting disease were not observed in 27 adult cervidae (Table 4) (26 roe deer (*Capreolus capreolus*) and 1 red deer (*Cervus elaphus*). One roe deer had mild perivascular infiltration in cerebrum whereas another roe deer showed increased thickness and mineralization of vessels in CNS. Of three adult cats examined one revealed multifocal, non-suppurative necrotizing encephalitis and four adult dogs revealed no CNS lesions.



**Table 1. Cause of submission for suspected cases of bovine spongiform encephalopathy and Scrapie during 2015.**

According to Order no. 800 of 13. July 2006 and 930 of 7 September 2006 on Bovine Spongiform Encephalopathy and Scrapie.

Cause	Numbers
Animals displaying behaviour disorders and/or neurological signs	1
Moribund animals without signs of infectious disease or traumatic signs	0
Other progressive diseases	0
No available information	0
<b>Non-TSE signs</b>	
Acute onset of neurological signs among several animals, suspicion of listeriosis	0

**Table 2. Summary of neuropathological findings in one bovine suspected of having transmissible spongiform encephalopathy 2016.**

Run. No.	Date	Lab. No.	CHR. No.	CKR.No.	Breed	Age (months)	Region	Histopathology	Additional results regarding BSE	Year of confirmation
1	18-02-2016	16-1825	123612	021335-00695	Cow	16	East	Perivascular edema in CNS	Negative by IHC	2016

**Table 3. Summary of findings in a fallen sheep having transmissible spongiform encephalopathy 2016.**

Run. No.	Date	Lab. No.	CHR. No.	CKR.No.	Breed	Age (months)	Region	Histopathology	Additional results regarding TSE	Year of confirmation
1	10-11-2016	16-17755	111054	111054-00023	sheep	196	East	Not done due autolysis	The Western immunoblotting profile of the samples shows molecular migration and banding ratios consistent with those seen in atypical scrapie. <b>This result provides sufficient evidence to classify this case as POSITIVE ATYPICAL SCRAPIE*. Genotype pending!</b>	2016

\*) All material analysed at the EU Community Reference laboratory for TSE, APHA, Weybridge, UK,

**Table 4. Laboratory examinations at the DTU-VET 2015.**

In accordance with the Danish surveillance programme and other tests.

<b>Bovines.</b> Group of animals	Samples	Negative	Positive
Emergency slaughter animals	0	0	0
Remarks at the ante mortem inspection	0	0	0
Fallen stock	0	0	0
Subtotal	0	0	0
Clinical suspects	1	1	0
Confirmatory testing of slaughter animals	0	0	0
Confirmatory testing of fallen stock	0	0	0
<b>Total, bovines</b>	<b>1</b>	<b>1</b>	<b>0</b>
<b>Small ruminants.</b> Group of animals			
Clinical suspects	0	0	0
Confirmatory testing of slaughter animals	0	0	0
Confirmatory testing of fallen stock	1	0	1
Fallen stock	0	0	0
<b>Total, small ruminants</b>	<b>1</b>	<b>0</b>	<b>1</b>
Wildlife (cervidae)	27	27	0
Cats and dogs	7	7	0