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## Sampling quality in bovine spongiform encephalopathy routine monitoring in Belgium - short communication

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### ABSTRACT

The bovine spongiform encephalopathy (BSE) epidemics have led to active surveillance in Europe. Detection methods are focused on the obex, where prions first accumulate in the central nervous system (CNS). Sampling is made with a calibrated syringe (from Bio-Rad or Idexx) that must sample a target area in the obex area. This study checked the precision of the sampling in Belgian routine laboratories in 2010 and 2011 (analysed obex: N = 184 and N = 368). For that purpose, the obex was divided into 130 identical square zones with 50 in the target area. The success of sampling the target area was identical when comparing the years (72.8%). The different laboratories obtained similar results. However, in 2011, one of the four laboratories had a lower number of zones inside the target area than the best laboratory. As a conclusion, obex sampling in Belgium is adequate, provided the technicians are well trained. This is especially true when confronted with atypical BSE, for which the obex is not the primary region of prion accumulation.

**Key words:** sampling, syringe, obex, bovine, prion

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Bovine spongiform encephalopathy (BSE), also known as mad cow disease, is one of the prion diseases, which are infectious neurodegenerative diseases, with slow development and lethal outcome. They are unique as a normal host cellular protein, prion protein (PrP<sup>c</sup>), is typically affected by conformational change and aggregation, which leads to the accumulation of PrP<sup>d</sup> (associated to the disease), usually in the nervous system (BRUCE et al., 1997). There is in fact no foreign nucleic acid involved (GRIFFITH, 1967). PrP<sup>d</sup> is partly resistant to digestion by proteases and the resultant product of such

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digestion (PrP<sup>res</sup>) is used as a disease marker (BENNION and DAGGETT, 2002). Generally, the brain shows microscopic symmetrical spongiosis. Prion diseases are therefore also called transmissible spongiform encephalopathies (TSE). These diseases in animals are mainly transmitted by the dietary route and have already been described for centuries in sheep, *Ovis aries*, as scrapie. In humans, the main group of TSE diseases is known as Creutzfeldt-Jakob disease (CJD). Evidence for transmission of BSE to humans has been found, causing a variant form of CJD (vCJD, BROWN and MASTRIANNI, 2010).

Since 2001, an active surveillance program has been in place in all European Union (EU) member states, using validated rapid tests. The principal aim of this surveillance is to detect TSE in ruminants and eliminate prions from the food chain. As in the preclinical stage of BSE, PrP<sup>res</sup> usually accumulates first in the obex, the detection methods are focused on the brainstem (OIE 2010). For bovines (*Bos taurus*), the sampling method proposed for the Bio-Rad ELISA implies the use of a calibrated syringe (Bio-Rad 355-1175 and Idexx 98-14074-00). This syringe has to be inserted into the obex from the caudal part of the brainstem.

Optimal sampling is essential to perform efficient routine monitoring for active BSE surveillance. In fact, prions first accumulate in a restricted zone of the obex (Fig. 1). The efficiency of the detection tests depends directly on the obex part sampled. Therefore, the quality of obex sampling was evaluated for all Belgian routine laboratories included in this active surveillance in 2010 and 2011. Initially 16 laboratories were present and 184 sampled obex were investigated. However, in 2011 only four laboratories continued to have a comparable number of samples. As the workload of these remaining laboratories drastically increased, the sampling effort was increased and 368 obex were analysed.

Table 1. Percentage of obex samples inside the target area during two successive years in routine laboratories.

	2010	2011				
	16 labs	4 labs <sup>1</sup>	Lab 1	Lab 2	Lab 3	Lab 4
# correct samples	134	268	72	62	66	68
# investigated samples	184	368	100	94	81	93
Success	72.8%	72.8%	72.0%	66.0%	81.5%	73.1%

<sup>1</sup> The only laboratories still active in 2011.

For this evaluation, the obex cross-section was divided into 130 identical square zones, with 50 considered as the target zone for BSE sampling (inside the two thick orange limits, see Fig. 1). This limit surrounds the first sites of prion accumulation (OIE, 2010). Each observed hole made by the sampling syringe was localised according to anatomical structures by histopathological examination of a cross section under a microscope. Each

hole was attributed to a square composed of four zones. The sampling was considered successful if at least one of these four zones was inside the target area. The sampling was further evaluated by the number of zones contained in the target area (from 0 to 4).

The success rate was not significantly different between the laboratories (Kruskal-Wallis one-way ANOVA; 2010:  $N = 184$ ,  $df = 15$ ,  $K = 23.4$ ,  $P = 0.10$ ; 2011:  $N = 368$ ,  $df = 3$ ,  $K = 23.4$ ,  $P = 0.15$ , see Table 1 for 2011). However, in 2011, the number of zones inside the target area was lower in laboratory 2 than in laboratory 4, with the two other laboratories having average results (Kruskal-Wallis one-way ANOVA;  $N = 368$ ,  $df = 3$ ,

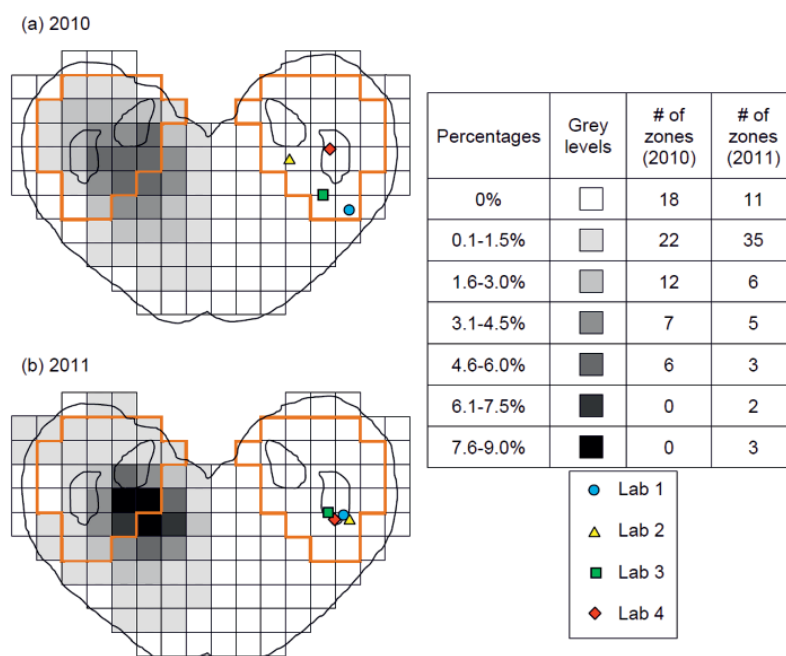


Fig. 1. Two schematics of an obex section divided into 130 zones. The 50 zones considered as the target for BSE sampling are indicated inside the two thick orange limits (each comprises 25 zones). On the left side of the obex, the grey level of each zone gives the frequency of its sampling by routine laboratories. The table on the right mentions the frequency ranges (for the seven grey levels) and the total number of zones for each category during the two study years (based on a half obex of 65 zones). The right side of the obex shows the average localisation of sampling for the four laboratories still active in 2011. The nuclei inside the obex represent the nucleus of the solitary tract and the nucleus of the spinal tract of the trigeminal nerve, as well as the dorsal motor nucleus of the vagus nerve.

$K = 11.6$ ,  $P = 0.009$ ; Mann-Whitney U test as post-hoc test,  $N = 94 \times 93$ ,  $U = 3465$ ,  $P = 0.011$ ).

Even if the global proportion of successful sampling (i.e. with at least one zone in the target area) remained extremely stable between 2010 and 2011 (72.8%, see Table 1), the number of zones inside the target area diminished almost significantly from 2010 to 2011 ( $3.1 \pm 1.2$  vs.  $2.8 \pm 1.2$ : Mann-Whitney U test,  $N = 134 \times 268$ ,  $U = 19.9$ ,  $P = 0.056$ ). This could be linked to the lower number of labs in 2011. Additionally, from 2010 to 2011, the sample localisations were more concentrated: the three more sampled zones accumulated 18.2% vs. 25.7% of all sampling (6.5%, 6.3%, 5.4% vs. 8.9%, 8.6%, 8.2%, see also Fig. 1). This could be due to the larger dataset. Despite the tendency of more concentrated samples, the overall proportion of successful sampling could still be improved. In 2011, this could be linked to the fact that one of the more sampled zones was just outside the target area (see Fig. 1).

In the majority of cases at least part of the sample was in the right place. This means that obex sampling in Belgium routine laboratories is adequate to perform efficient routine monitoring for BSE. As the target area was rather strict and focused on the zones with the highest concentrations of neurons, sampling just outside this area will probably not lead to missed cases. Nevertheless, frequent and good training of BSE laboratory technicians is essential to maintain and optimize sampling localization.

As large dietary-related BSE epidemics seem to be a thing of the past, future BSE cases may be expected to be mainly sporadic cases. During the epidemic, BSE showed strain homogeneity (SAFAR et al., 1998) because of the probable contamination of the food chain by a single strain in the United Kingdom. However, more efficient diagnostic techniques have recently allowed some countries to report rare variants of BSE, which can usually be classified into H- or L-type (JACOBS et al., 2007). These types may have an unusual location of the PrP<sup>res</sup> accumulation in the brain since L-types at least have a preferential PrP<sup>res</sup> distribution in the forebrain region (CASALONE et al., 2004).

The current rapid tests seem suitable to detect those atypical cases, even on the basis of standard obex sampling (POLAK and ZMUDZINSKI, 2011). However, as the brainstem in atypical types might not be the first site for PrP<sup>res</sup> accumulation, sampling quality has to be maximised to detect atypical cases, even at the very early stages of the disease. As the new strains seem more virulent than classical types, at least in mice models (BERINGUE et al., 2008) and because they have a very low occurrence, they represent one of the next challenges in the field of prions.

To our knowledge this study is the first report evaluating the sampling quality for BSE routine monitoring in Europe using calibrated syringes. From the results in Belgium, it may be concluded that sampling of the obex using the sampling syringes is adequate.

Good diagnostics in the future require good training of technicians and awareness of the benefit of additional sample sites to detect atypical cases.

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**DOBLY, A., S. VAN DER HEYDEN, F. DI SILVESTRO, S. ROELS: Kvaliteta uzorkovanja kod sustavnog praćenja govede spongiformne encefalopatije u Belgiji. Vet. arhiv 83, 341-346, 2013.**

**SAŽETAK**

Epidemije govede spongiformne encefalopatije nužno su dovele do donošenja mjera za njezin aktivni nadzor u Europi. Metode dokazivanja usmjerene su na obex u središnjem živčanom sustavu gdje se prioni najprije nakupljaju. Uzorci se uzimaju kalibriranom štrcaljkom (Bio-Rad ili Idexx) na ciljnom mjestu na području obeksa. U ovom istraživanju provjerena je točnost uzimanja uzoraka u rutinskim dijagnostičkim laboratorijima u Belgiji u 2010. i 2011. kada je bilo analizirano 184 odnosno 368 uzoraka tkiva obeksa. U tu je svrhu obex bio podijeljen na 130 identičnih kvadratičnih područja s 50 ciljnih mjesta. Uspješnost uzimanja uzoraka s ciljnog mjesta bila je identična na godišnjoj razini (72,8%). Različiti laboratoriji postigli su slične rezultate. Ipak, u 2011. jedan od četiri laboratorija imao je manji broj zona unutar ciljnog područja u usporedbi s rezultatima najboljeg laboratorija. Zaključuje se da je uzimanje uzoraka tkiva obeksa u Belgiji zadovoljavajuće i da je tehničko osoblje dobro educirano. To je osobito važno kad je riječ u atipičnoj govedoj spongiformnoj encefalopatiji kod koje obex nije primarno područje nakupljanja priona.

**Ključne riječi:** uzorkovanje, štrcaljka, obex, govedo, prion

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