Appendix II

Aspects concerning TSSE rapid screening tests intended for small ruminants

In 2005, the European Food Standards Agency (EFSA) carried out an evaluation of the performance of 9 diagnostic tests for detecting TSE in sheep. This comparative study, which is the only study available to date, was published in the form of two reports, on 17 May and 26 September 2005 respectively.

Firstly, test performance was evaluated in detecting different forms of TSE in the central nervous system (CNS) of sheep, and more specifically in brain stem fragments for classical scrapie and BSE isolates and in cortex fragments for atypical scrapie isolates. The brain stem represents the most suitable part of the CNS for sampling as trepanation is unnecessary.

- All the tests showed a specificity of almost 100% (1000 negative samples from New Zealand sheep considered healthy);
- All the tests except one (FujiRebio), are recommended by the EFSA for use in detecting classical scrapie. In fact, the 8 tests adopted showed diagnostic sensitivity of almost 100% (219 positive samples from sheep affected by scrapie in the clinical stage) for detecting classical forms of scrapie. In terms of analytical sensitivity (detection of strong dilutions of a positive sample, mimicking the detection of samples from animals in the incubation stage), the test performances in descending order are as follows: Bio-Rad TeSeE Sheep & Goat > IDEXX > Enfer = Institut Pourquier = InPro CDI > Bio-Rad TeSeE > FujiRebio > Prionics LIA (the analytic sensitivity of the Prionics Western Blot test was not evaluated).
- All the tests evaluated correctly identified the three brain stem samples from sheep experimentally and orally infected by the BSE agent, in the clinical stage of the disease, and are therefore recommended for this purpose by the EFSA.
- All the tests, except the Prionics LIA and FujiRebio tests identified the three cortex samples from sheep infected by the Nor-98 atypical scrapie strain, and are therefore recommended by the EFSA for analysing cortex or cerebellum samples. However only the Bio-Rad and the IDEXX texts presented sufficient analytical sensitivity with these isolates (all the other tests only detect pure samples), and are the only tests recommended for analysis on a brain stem sample. In fact, in the case of atypical

scrapie, the concentration of PrPres is higher in the cerebellum and the cortex and lower in the brain stem, compared to classical scrapie isolates.

In the absence of strong argument refuting the pathogenicity of atypical isolates in humans, the Committee of experts considers that, within a large scale screening programme, the tests used for detecting TSE in sheep must be capable of detecting all TSE isolates in these species. As a result, all the tests evaluated by the EFSA, not including the FujiRebio and the Prionics LIA, can be used, on the condition that they are used at the same time on the brain stem and on a cortex or cerebellum sample (the only regions allowing for the detection of atypical scrapie stains for these 4 tests). In practice, the only alternative to the brain stem is the cerebellum which can also be removed (in more or less satisfactory conditions) through the foramen magnum. Cortex sampling which requires trepanation is difficult and dangerous to implement. If the tests are only carried out on the brain stem (according to memo N2006-8079 of the DGAL dated 27 March 2006 recommending the use of the brain stem in first line sampling), which again is the only suitable sampling region given that trepanation is not required and sampling can be carried out safely, the Committee considers that only the Bio-Rad Sheep and Goat and IDEXX tests are recommended, as only these tests detect all strains of scrapie in this type of sample. Otherwise, using the cerebellum as the only sampling region, notwithstanding the technical problems arising in sampling on this region of the anatomy, would widely reduce surveillance sensitivity with regards to the BSE agent (due to the weak expression of PrPres in cerebellum in BSE cases) and must not be retained as an alternative.

Secondly, the EFSA evaluated test performance in lymphoid organs. Only the mesenteric ganglions and the spleen were tested, and only by certain tests (Bio-Rad TeSeE, Bio-Rad TeSeE Sheep and Goat and IDEXX on the mesenteric lymph nodes and on the spleen, the test from the Institut Pourquier and the Prionics WB test only on the mesenteric lymph nodes). The EFSA recommends that only these tests be used on the peripheral organs for which they have been evaluated.

Within a large scale post mortem screening programme for TSE in sheep, the use of lymphoid organs would be useful in detecting infected animals earlier, before the central nervous system becomes infected or presents a measurable quantity of PrPres. Studies undertaken at the National Veterinary School of Toulouse suggest that the use of these peripheral organs increases twofold the number of positive animals with regards to the sole use of tests on the

CNS (Andreoletti, personal communication). However, it must be emphasized that TSE screening programmes for small ruminants are carried out more in the aim of detecting and eliminating affected flocks (population logic) than in the aim of eliminating individual animals in view of consumer protection (individual logic). As a result the use of lymphoid organs only allows for the identification of affected flocks a few days or weeks earlier compared to tests carried out on the CNS. In this case, the same flocks will be identified anyway but later on, on the condition that the tests are carried out systematically.
In this way, in systematic screening all the flocks will be detected sooner or later by tests on the CNS. Only these tests could therefore be applied. On the other hand, in screening per sample, the combination of tests on the CNS and the lymphoid organs would allow for the improvement in screening network performance.

In practice, among those lymphoid organs tested within the EFSA evaluation, the mesenteric lymph nodes are the easiest to sample. It is fitting to note that the possibility of using other lymphoid organs, such as the retropharyngeal lymph nodes or the tonsils, deserves to be studied for traceability reasons, taking into account the fact that these organs remain confined in the head with the CNS.