

An evaluation of techniques for the discrimination of natural scrapie and experimental bovine spongiform encephalopathy in sheep

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Introduction

Bovine spongiform encephalopathy (BSE) may have transmitted to European sheep via contaminated feed before the introduction of bans to reduce the risk of further exposure via this route. Several ovine TSE agents (or prions strains) have been characterised by the relative incubation times they take to produce clinical signs of disease in a panel of laboratory mouse strains (C57Bl, VM, C57Bl x VM F1, RIII) and the brain lesion profiles of these mice (Fraser et al., 1992; Bruce et al., 2002). The BSE agent has similarly been characterised from cattle, sheep and several exotic species and may be more quickly identifiable because of the uniformity of its transmission characteristics at primary passage in some strains of mice (RIIIIs) (Bruce et al., 2002). Attempts to accelerate this process by introducing transgenic mice over-expressing alleles of the prion protein of various species have yet to be validated and, in general, this bioassay approach is time-consuming (2-3 years) and costly, and so unsuitable, for the purpose of discriminating BSE from the endemic ovine TSE, scrapie, in the sheep population.

Enzyme-linked immunosorbent assays (ELISAs), SDS-PAGE/Western blot (WB) and immuno-histochemical (IHC) methods for the rapid screening (in days or weeks) of TSEs in sheep (and goats) have been developed based on the detection of the abnormal prion protein (PrP^D) in the brain and other tissues of ruminants (Deslys et al., 2001; Van Keulen et al., 2000; Jeffrey et al., 2001); and these techniques have been refined to detect differences in the physico-chemical properties of PrP^D from animals with an experimental infection with the BSE agent and PrP^D from animals with natural or experimental scrapie (Stack et al., 2002; Thuring et al., 2004; Thuring et al., 2005).

The detection of the first naturally-occurring case of BSE in a small ruminant was confirmed by a combination of these biological and biochemical typing techniques (Eloit et al., 2005), and this combination of tests is the basis of the

discriminatory testing part of the protocol for laboratory screening for the presence of TSEs in ovine and caprine detailed in Annex 10 to Regulation (EC) No 999/2001. Before its introduction in January 2005, the EU CRL organised a blinded ring trial to assess the suitability of the differential ELISA and WB immunochemical methods to be used as part of the differential TSE testing protocol for ovine and caprine animals.¹ The design of this study, the number and types of samples used, the laboratories involved and a summary of the results are provided below :

1. Laboratories and Methods

In 2004, French (AFFSA, dWB; CEA-Fontenay-aux-Roses, dWB; CEA/SACLAY, dELISA) US (UCSF, dTR-FIA/WB) and UK (VLA, dWB) laboratories submitted tests to a ring trial evaluation of their ability to discriminate a sample of tissue from a BSE-affected sheep from a sample of tissue from a sheep affected by scrapie using either an discriminatory ELISA (dELISA), a Western blot (dWB) or a combination of time-resolved fluoroimmunoassay and Western blotting (dTR-FIA/WB). A third technique, discriminatory immunohistochemistry (dIHC), was trialed by the VLA and CIDC-Lelystad, Netherlands. Subsequently groups from the CIDC-Lelystad, Netherlands (WB, dIHC), Italy (ISS, dWB) and Germany (FLI, dWB) completed a more limited evaluation of their discriminatory methods. The CRL STEG was formed mainly of members from the participating laboratories.

All ELISA and WB methods, each with their particular innovation, were based on the fact that sheep scrapie PrP^D and sheep BSE PrP^D have a differential susceptibility to proteases : the hydrolases remove an epitope from sheep BSE PrP^D which is retained by sheep scrapie PrP^D. The dIHC methods do

¹ Note samples submitted for primary molecular testing with a discriminatory WB will be “ ..from clinical suspect cases and from animals ...regarded as positive scrapie cases [as a result of primary screening] or which display characteristics which are deemed by the testing laboratory to merit investigation ..” Regulation 999/2001 section 3.2 c i.

not use exogenous proteases but rely on differential binding of several mAbs and the spatial resolution in tissue sections typical of the histo-technique.

2. Materials

2.1 Ring Trial

There were forty-one ring trial samples, prepared as macerates of brain stem tissue and frozen prior to coding, randomisation and dispatch: clinical, experimental BSE in sheep (14), clinical, confirmed scrapie sheep signs (12), sheep-scrapie suspects showing neurological clinical signs but which were negative by statutory tests for TSEs (9), sheep without clinical signs or known exposure to scrapie (2), confirmed BSE cattle cases (2), and NZ cattle negative controls (2). For all 41 samples, medulla at the obex, fixed in formaldehyde, was also collected and coded for discriminatory IHC². More details of the samples are described given in Table 1.

Table 1

Sample type	Number	Source	Breed / Genotype
Ovine BSE	4	Oral primary challenge with bovine BSE brain	Suffolk ARQ/ARQ
Ovine BSE	10	Intracerebral challenge with Cheviot AHQ/AHQ ovine BSE (1 st sheep to sheep challenge)	Romney/ ARQ/ARQ
Sheep scrapie, positive by statutory tests	12	Routine field GB surveillance cases submitted to VLA	3 VRQ/ARH 2 VRQ/ARQ 2 VRQ/VRQ 2 VRQ/ARR 2 ARQ/AHQ 1 ARQ/ARQ
Scrapie clinical suspects, negative by statutory tests	9	Routine field GB surveillance cases submitted to VLA	2 VRQ/ARQ 2 VRQ/ARR 1 VRQ/VRQ 2 ARQ/AHQ 2 ARQ/ARQ
Control sheep –ve	2	Imported from New Zealand	1 ARR/ARR, 1 ARQ/ARQ

² The sample blocks supplied to VLA and CIDC for dIHC were identical with the exception of two wax blocks from sheep from the primary oral challenge of BSE. Two other wax blocks were substituted which were obtained from two other sheep in this primary challenge group for the evaluation of the CIDC method

Confirmed Bovine BSE positives	2	Routine field GB surveillance cases submitted to VLA	
Bovine negative controls	2	Non-exposed, from New Zealand	

2.2 Evaluation in additional laboratories

A more limited sample set was also sent to Germany, Italy and the Netherlands for evaluation of their particular dWBs (see Table 2).

Table 2

FLI, Germany	ISS, Rome, Italy	CIDC Lelystad, Netherlands
1 Scrapie +ve VRQ/VRQ	1 Scrapie +ve VRQ/ARR	1 Scrapie +ve VRQ/ARH
1 Scrapie +ve VRQ/ARH	1 Scrapie +ve VRQ/ARQ	1 Scrapie +ve VRQ/ARQ
1 Scrapie +ve ARQ/ARQ	1 Scrapie +ve AHQ/ARQ	1 Scrapie +ve VRQ/VRQ
1 Scrapie – ve VRQ/ARQ	1 Scrapie – ve AHQ/ARQ	1 Scrapie –ve ARQ/ARQ
1 Scrapie -ve ARQ/ARQ	1 Scrapie – ve VRQ/ARR	1 Scrapie –ve AHQ/ARQ
2 BSE in sheep ic pass 2 ARQ/ARQ	2 BSE into sheep ic pass 2 ARQ/ARQ	2 BSE in sheep ic pass 2 ARQ/ARQ
1 BSE in sheep oral primary ARQ/ARQ	1 BSE in sheep oral primary ARQ/ARQ	1 BSE in sheep oral primary ARQ/ARQ
2 BSE (bovine) +ve	2 BSE (bovine) +ve	2 BSE (bovine) +ve
2 BSE (bovine) non-exposed	2 BSE (bovine) non-exposed	2 BSE (bovine) Non-exposed

3. Results

The qualitative data from each laboratory is given in Tables 3 and 4. Essentially all tests were able to discriminate between natural scrapie in sheep and the types of BSE in sheep available from experimental animals.

4. Discussion

These tests were approved for use to support the requirements of discriminatory testing protocol detailed in the EC Regulation (999/2001) and its amendments based on this set of data.

Currently, 21 laboratories in EU MSs are approved for the use of these tests or their modifications and in 2006 and 2007 each lab successfully underwent a proficiency testing QA round that used 5 test samples (cattle BSE[2 cases], sheep scrapie [1 case], cattle negative [1 case], sheep negative [1 case]).

In 2007, the number of laboratories (in parentheses) using each approved test was as follows :

VLA dWB (9), Modified VLA dWB (1)

CEA dWB (4), Modified CEA dWB (3)

AFSSA dWB (2), and one laboratory each is using the FLI dWB (Gretzschel et al., 2005) and the ISS dWB.

Table 3. Evaluation of initial discriminatory tests in ring trial

Sample Type	VLA ¹ dIHC	VLA dWB	AFSSA dWB	CEA dELISA	CEA dWB	UCSF dWB/TF- FIA	UCSF dTF-FIA/ WB	Test Result
Scrapie (Ov) +ve VRQ/ARR	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve VRQ/ARQ	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve ARQ/AHQ	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve VRQ/VRQ	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve VRQ/ARH	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve VRQ/ARH	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) -ve ARQ/AHQ	■	■	■	■	■	■	■	Ovine negative
Scrapie (Ov) -ve VRQ/ARR	■	■	■	■	■	■	■	Ovine negative
Scrapie (Ov) -ve VRQ/ARQ	■	■	■	■	■	■	■	Ovine negative
Scrapie (Ov) -ve ARQ/ARQ	■	■	■	■	■	■	■	Ovine negative
Scrapie (Ov) -ve VRQ/VRQ	■	■	■	■	■	■	■	Ovine negative
Scrapie (Ov) +ve VRQ/ARH	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve VRQ/ARQ	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve VRQ/VRQ	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve ARQ/ARQ	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve ARQ/AHQ	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) +ve VRQ/ARR	■	■	■	■	■	■	■	Scrapie +ve
Scrapie (Ov) -ve ARQ/ARQ	■	■	■	■	■	■	■	Ovine negative
Scrapie (Ov) -ve ARQ/AHQ	■	■	■	■	■	■	■	Ovine negative
Scrapie (Ov) -ve VRQ/ARR	■	■	■	■	■	■	■	Ovine negative
Scrapie (Ov) -ve VRQ/ARQ	■	■	■	■	■	■	■	Ovine negative
New Zealand -ve ARR/ARR	■	■	■	■	■	■	■	Ovine negative
New Zealand -veARQ/ARQ	■	■	■	■	■	■	■	Ovine negative
BSE (Bovine) +ve	■	■	■	■	■	■	■	BSE +ve
BSE (Bovine) +ve	■	■	■	■	■	■	■	BSE +ve
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
BSE in sheep ic (2nd pass)	■	■	■	■	■	■	■	BSE in sheep
NZ 5g BSE oral primary	■	■	■	■	■	■	■	BSE in sheep
NZ 5g BSE oral primary	■	■	■	■	■	■	■	BSE in sheep
NZ 5g BSE oral primary	■	■	■	■	■	■	■	BSE in sheep
NZ 5g BSE oral primary	■	■	■	■	■	■	■	BSE in sheep
BSE (Bovine) Non-exposed	■	■	■	■	■	S20 (NT) ²	S20 (NT) ²	BSE -ve
BSE (Bovine) Non-exposed	■	■	■	■	■	S11 (NT) ²	S11 (NT) ²	BSE -ve

¹ CIDC-dIHC gave identical results but is not included in this table because it was not part of the initial trial
² NT = Not tested by UCSF due to dehydration of the sample

Samples FLI Germany	Result	Samples ISS, Rome Italy	Result	Samples CIDC Lelystad	Result
Scrapie +ve VRQ/VRQ	Scrapie	Scrapie +ve VRQ/ARR	Scrapie	Scrapie +ve VRQ/ARH	Scrapie
Scrapie +ve VRQ/ARH	Scrapie	Scrapie +ve VRQ/ARQ	Scrapie	Scrapie +ve VRQ/ARQ	Scrapie
Scrapie +ve ARQ/ARQ	Scrapie	Scrapie +ve AHQ/ARQ	Scrapie	Scrapie +ve VRQ/VRQ	Scrapie
Scrapie – ve VRQ/ARQ	Negative	Scrapie – ve AHQ/ARQ	Negative	Scrapie –ve ARQ/ARQ	Negative
Scrapie -ve ARQ/ARQ	Negative	Scrapie – ve VRQ/ARR	Negative	Scrapie –ve AHQ/ARQ	Negative
BSE sheep ic pass 2	BSE	BSE sheep ic pass 2	BSE	BSE sheep ic pass 2	BSE
BSE sheep ic pass 2	BSE	BSE sheep ic pass 2	BSE	BSE in sheep ic pass 2	BSE
BSE sheep oral primary	BSE	BSE sheep oral primary	BSE	BSE in sheep oral primary	BSE
BSE (bovine) +ve	BSE	BSE (bovine) +ve	BSE	BSE (bovine) +ve	BSE
BSE (bovine) +ve	BSE	BSE (bovine) +ve	BSE	BSE (bovine) +ve	BSE
BSE (bovine) non-exposed	Negative	BSE (bovine) non-exposed	Negative	BSE (bovine) Non-exposed	Negative
BSE (bovine) non-exposed	Negative	BSE (bovine) non-exposed	Negative	BSE (bovine) non-exposed	Negative

Table 4 . Evaluation of the three additional discriminatory Western immunoblotting techniques

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