STREP
Thematic priority: Food quality and safety

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goatBSE

Proposal for improvement of goat TSE discriminative diagnosis and susceptibility based assessment of BSE infectivity in goat milk and meat.

Deliverable 3.6
An overview of currently available diagnostic assays validated for goat scrapie and BSE in goats

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Deliverable 3.6

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
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An overview of currently available diagnostic assays validated for goat scrapie and BSE in goats.

The capacity to discriminate accurately BSE cases within a background of a variety of scrapie isolates appears crucial in terms of control measures and human health protection. Active surveillance has been possible by the emergence of relatively quick methods:

1. microscopic methods on tissue section by immunohistochemistry, duration 4-6 days.
2. even more rapid biochemical techniques, duration a few hours.

These techniques are able to confirm a TSE case with high sensitivity and specificity.

Therefore, the first tool to confirm the presence of cases are biochemical methods, and these have been developed and applied with great success to monitor the BSE epidemic in cattle since 2001, and have been applied also to follow the eventual spread of BSE to small ruminants (sheep and goats). In time, methods for cattle were further adapted to also test sheep and goats. However, most tests for these small ruminants had been developed with sheep TSE infected brain mainly because this was the most frequently TSE affected species in the regions where the BSE epidemic had spread. In the more mediterranean regions goat production is considerable, and scrapie is a frequently occurring problem, while often holdings are consisting of a mix of sheep and goats. Due to the BSE epidemic, it has become necessary to follow in small ruminants the scrapie status and to monitor whether BSE was hiding. In addition, export of sheep and goats is dependent of scrapie free status of holdings.

While most current diagnostic and discriminatory TSE tests have been developed for brain from sheep, it has to be awaited whether goat TSEs behave the same in such tests. In cases where BSE is suspected in small ruminants, bio-assays with very long durations (1-3 years) remain indispensable where rapid PrP-specific antibody dependent assays (immunohistochemistry and biochemical assays) yield dubious or inadequate results[1]. It is evident from current knowledge that TSEs in small ruminants do occur in various forms of which a number have been defined due to their histological and biochemical properties. These forms are: classical scrapie, CH1641 scrapie, Nor98/atypical scrapie, BSE (until now two cases have been diagnosed in farmed goats but never in sheep[2]), and in Italy a special form of classcial scrapie was recognized which has special biochemical and transmission features in rodent models, which for simplicity will be called here Italian scrapie[3].

This document overviews the tests investigated and found suitable for general application on goat brain tissues and where possible on immunohistochemical use with respect to recognition of the above mentioned variants. The basic study materials in these analyses were the 32 cases in the geographical selection panel (see in deliverable D3.2 table D3.2-2) and some additional ones from the inital set of 70 samples (as discussed in deliverable D3.4).

Each of the partners involved (CVI NL, INRA FR, UEDIN UK, UNIZAR SP, FLI GE, IZSTO IT, ISS IT, CERTH-INA GR, CEA FR) has used and further developed in-house their techniques. The most general conclusion about these analyses was that goat field TSEs behave as scrapie, and that the techniques developed before to discriminate between scrapie and BSE in sheep also apply for the large set of goat isolates (Table 3.6-1). Though this was suggested in the literature (see e.g. footnote 2), a broad study like this was necessary due to the large and different polymorphism palets of sheep and goat PrP. PrP-specific antibodies play an important role, and many different monoclonal antibodies are available in the prion field thanks to the generation of $PrP^{o/o}$ knock-out mice that are not tolerant for

[1] Aspects of bioassays of goat brain tissues in rodent models have been amply discussed in the GoatBSE deliverables D3.1 through D3.5.

[2] Eloit et al., Vet. Rec. 2005, April 16, 523-524; Jeffrey et al., J. Comp. Path. 2006, 134, 171–181; Spiropoulos et al., Emerging Infect. Dis. 2011, 17:2253-2261.

[3] See GoatBSE deliverables D3.1 and D3.5.

immunisation with PrP derived products. Some frequently used antibodies and their PrP-epitope location have been assembled in a diagram (Figure D3.6-1). It has been shown that monoclonal F99 was indeed insufficient for detection of the 222K PrP protein (see Mazza et al., 2012. Lysine at position 222 of the goat prion protein destroys the binding of monoclonal antibody F99/97.6.1, J Vet Diagn Invest **24**: 971).

Previous analysis of the intracellular size of PrP^{Sc} (in short PrP^{Sc} fragment mapping) has shown a difference between scrapie infected sheep and sheep infected with BSE in the efficiency of intracellular enzymatic degradation of PrP^{Sc}[4]. In scrapie infected sheep, intracellular PrP^{Sc} in both neural and lymphoid tissues is truncated to a fragment size of approximately a.a. ~93-230. In contrast in BSE infected sheep, intracellular PrP^{Sc} size depended on the type of cell involved: intraneuronal PrP^{Sc} showed a truncayed size of ~100-230 whereas PrP^{Sc} fragment size within tingible body macrophages in the lymphoid tissues was ~114-230.

A separate action on 47 CYPRIOTIC CASES has been carried out by one of the partners (see next page, below Fig.D3.6-1).

	Western blotting approaches on goat TSE isolates	tests on homogenates (Wblot or ELISA)								
	lab	CVI	INRA	UEDIN	UNIZAR	FLI	IZSTO	ISS	CERTH	CEA
	type of test (and potential for discriminatory use)									
PrP^{res} properties (fixed PK)	N-terminal epitope P4 & 12B2 (BSE&CH1641 vs. cl.scrapie)	x	x			x		x		
	MW unglycosylated PrP^{res} (cl.scrapie vs BSE & C1641)	x						x		
	glycoprofile & banding pattern (BSE vs classical scrapie-Nor98-CH1641)	x				x		x		
	double PrP^{res} population (CH1641 vs BSE vs cl.scrapie)	x								
PrP^{res} (PK variable)	PK-sensitivity N-terminus (BSE vs CH1641 vs lt. scrapie vs cl. scrapie)		x					x		x
	PK-senstivity core	x								
	PK-sensitivity non-glycosylated PrP^{res}					x				
EU validated routine tests	Bio-Rad TeSeE SAP						x			
	Bio-Rad TeSeE Sheep/Goat						x			
	Idexx HerdChek Antigen Test Kit EIA						x		x	
	CEDI-tect (out of use); conformation dependent assay	x								
	lectin								x	
	differential ELISA (BSE vs intermediate scrapie vs cl.scrapie vs Nor98)		x							x

	Immuno-histochemistry on goat TSE CNS and LRS sections	tests on tissue sections (IHC)								
	lab	CVI	INRA	UEDIN	UNIZAR	FLI	IZSTO	ISS	CERTH	CEA
	tissue target location in obex or lymph node									
CNS	single antibody applications	x	x	x	x	x	x	x		
	epitope search (PrP-processing)	x			x					
	epitope mapping in neuropil				x					
	epitope mapping intraneuronal staining	x			x					
	intracellular and membrane staining				x					
	perineuronal staining (obex)				x					
LRS (LN)	multi- and unigranular staining TBM's in lymph nodes	x				?				

Table D3.6-1. Overview of TSE testing in the ten partners' laboratories. The tests have been divided in those to be applied on homogenates (biochemical assays) and on tissue sections by microscopy (immuno-histochemical assays, IHC).

[4] Jeffrey et al. Differential Diagnosis of Infections with the Bovine Spongiform Encephalopathy (BSE) and Scrapie Agents in SheepJ. Comp. Path. 2001, Vol. 125, 271–284; Thuring CMA, et al. 2005. Immuno-histochemical differentiation of (pre)-clinical BSE and scrapie infection in sheep. J Comp Pathol. 132:59-69.

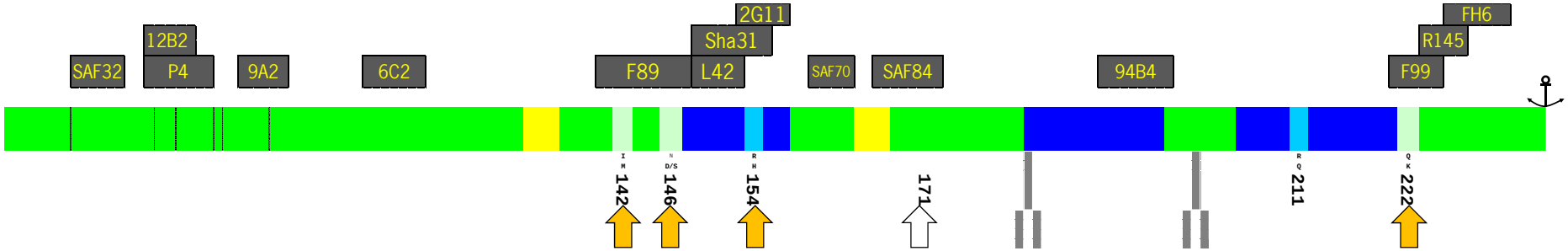

Figure 3.6-1. Epitope PrP^{res}-distribution of different PrP-specific antibodies of interest in goat and sheep TSE testing, with respect of detecting polymorphic variants. Above The orange arrows point to sites where a PrP-specific antibodies would not bind the polymorphically altered PrP expression product linked to TSE susceptibility variation like 142M, 146S, 146D, 154H, 211Q and 222K. The goatBSE project has shown that the 222K allelic expression product will not be detected by antibody F99 if it were present inthe prion material of a TSE carrier. Likewise, the 146S and 146D allelic variants might not be detected by the antibody 12F10, though within epitopes amino acid substitutions are frequently allowed being not essential for the binding. It is crucial - before allowing routine tests for small ruminants on the market - to know whether tests are dependent of such antibodies, otherwise this would hamper the reliability of monitoring programs using these tests for active surveillance. The open arrow: while SAF84 has been in use for sheep confirmatory testing, it would not have been able to detect the 171R allele if it had been used for detecting scrapie or BSE in sheep carrying this TSE resistance codon that is nowadays in use for resistance breeding in sheep.

Cyprus case study by partner 6 (FLI GE, together with partner 10 CERTH-INA GR and Cyprus Veterinary Services).
Fourty seven goats (Damascus breed) originating from 24 Cypriot flocks and showing signs compatible with scrapie were culled and submitted to sampling several tissues. Genotyping was performed by partner 10, yielding several polymorphisms at codons 42, 102, 138, 142, 146, 151, 154, 179, 211, 222 and 240. CNS and LRS have been studied by IHC and discriminatory western blots. IHC and rapid testing 27 goats were identified as positive. On CNS a complete agreement of results was obtained between the two tests.
By IHC on LRS using the antibodies 6C2, F99 and L42 most of the animals, including one goat with a negative result in the brain stem, revealed a wide distribution of PrP^{Sc} in the LRS. In particular the retropharyngeal LN, tonsil and spleen were highly affected. However one clear scrapie positive goat showed an only sparse distribution of PrP^{Sc} in the LRS confined to a weak accumulation in the retropharyngeal lymph nodes. Information on the genotypes of these cases in relation to the codon polymorphisms is highly interesting and can be asked to partner 6.

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