STREP
Thematic priority: Food quality and safety

FOOD-CT-2006-36353
goatBSE

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

Deliverable 1.5

In vitro assays to measure species barriers to be used in risk assessments.

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Dissemination Level		
PU	Public	PU
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Deliverable 1.5

In vitro assays to measure species barriers to be used in risk assessments.

While for bacteria growth in appropriate media and for viruses co-culture with suitable host cell lines is the choice to propagate the agent, for prions *in vitro* conversion has been recognized as a versatile tool for multiplication. The conversion event implies that the prion protein (PrP) substrate is converted from proteinase K (PK) susceptible PrP^{C} to the PK resistant disease associated form PrP^{Sc}. This process is accomplished by using as **inoculum** infected brain material and as **substrate** a proper TSE negative prion protein (PrP) source including a number of undefined cofactors. Furthermore, cell free *in vitro* conversion assays represent a system for investigating potential barriers for TSE transmission between species and genotype carriers. These methods are still in development in nearly all TSE laboratories. At the start of the project progress in the field did mainly occur with model rodent propagation systems (e.g. 263K scrapie in hamsters, and ME7 and RML scrapie in mice); ruminant TSE conversions were considered as less efficient which probably relates to low PrP substrate levels in the denaturing and radioactive cell-free systems used at that time.

The GoatBSE project aims at development of suitable *in vitro* conversion assays that could serve as tools for risk assessments with respect to goat and TSE issues. In previous developments PrP from transfected mammalian cells was used as substrate after immuno-precipitation and radioactive labelling, while the conversion process required partial denaturation of the inoculum with guanidine. Current systems of conversion are using inoculum without the need of denaturation, while the PrP substrate can be either in pure form from bacteria or in crude form as in mammalian brain homogenates and cell extracts. Using bacterial expressed PrP^{C}, cell-free conversion was achieved with murine (Eiden et al., 2006) or murine/bovine PrP^{C} chimeras (Kupfer et al., 2006). The results suggested that the effect of single amino acid substitutions and strain specificities observed in vivo are encoded by the intrinsic properties of PrP^{C} and PrP^{Sc}. This assay was also successful in the cell-free conversion of caprine PrP variants (Fig. D1.5, A). Another successful system using a sonication treatment, the protein misfolding cyclic assay (PMCA), could in principle be effective in generating PrP^{Sc} from mammalian PrP in all its glycoforms (Fig. D1.5, B). A more recent technique uses shaking, the quaking-induced conversion or QUIC. Thus, several combinations have been investigated and - as summarized in Table D1.5 - are either successful enough to expect broad PrP type applications including all goat genotypes as well as other species, or are uncertain with respect to their conversion efficiency and still need further improvement.

With the accomplished technological progress, it will be possible to estimate the susceptibility of the different PrP goat genotypes and species barriers as a possible *in vitro* tool for risk estimations. Furthermore, goatBSE isolates from the various goat inoculation experiments (BSE and scrapie; various goat PrP genotypes) from WP2 will be subjected to in vitro conversion. Several of these goatBSE isolates however are only available in year 4 and later. For this, the appropriate substrates will be the prokaryotic *E. coli* produced recombinant PrP substrates and the hamster cell culture produced eukaryotic PrP variants.

publications/presentations:

- **Kupfer L, Eiden M, Buschmann A, Groschup MH**. Amino acid sequence and prion strain specific effects on the in vitro and in vivo convertibility of ovine/murine and bovine/murine prion protein chimeras. Biochim Biophys Acta. 2007 1772(6):704-13.
- **Eiden M, Palm GJ, Hinrichs W, Matthey U, Zahn R, Groschup MH**. Synergistic and strain-specific effects of bovine spongiform encephalopathy and scrapie prions in the cell-free conversion of recombinant prion protein. J Gen Virol. 2006 87:3753-61.
- **Priem J, Langeveld J, VanKeulen L, VanZijderveld F, Bossers A.** Poster Prion 2008, Madrid, 8-10 Oct 2008. Spain: P8.25, Protein misfolding cyclic amplification (PMCA) using ruminant species.
- **Padilla D, Priem J, Miranda A, Torres JM, Bossers A.** Poster Prion2009, Porto Carras, 23-25 Sep 2009. Greece: P4.39, Transgenic mice brains can efficiently be used to measure species-barriers in vitro by PMCA.
- **Eiden M, Ortega-Soto E, Groschup M.** Poster Prion2009, Porto Carras, 23-25 Sep 2009. Greece: P4.5, The exchange of single aminoacids in ovine and caprine prion protein variants influences convertibility of the prion protein in vitro.
- **Eiden M, Franz M, Ortega Soto E, Groschup M.** Poster Prion 2008, Madrid, 8-10 Oct 2008. Spain:: P8.13. Influence of prion sequence in small ruminants on conversion of the prion protein *in vitro*.

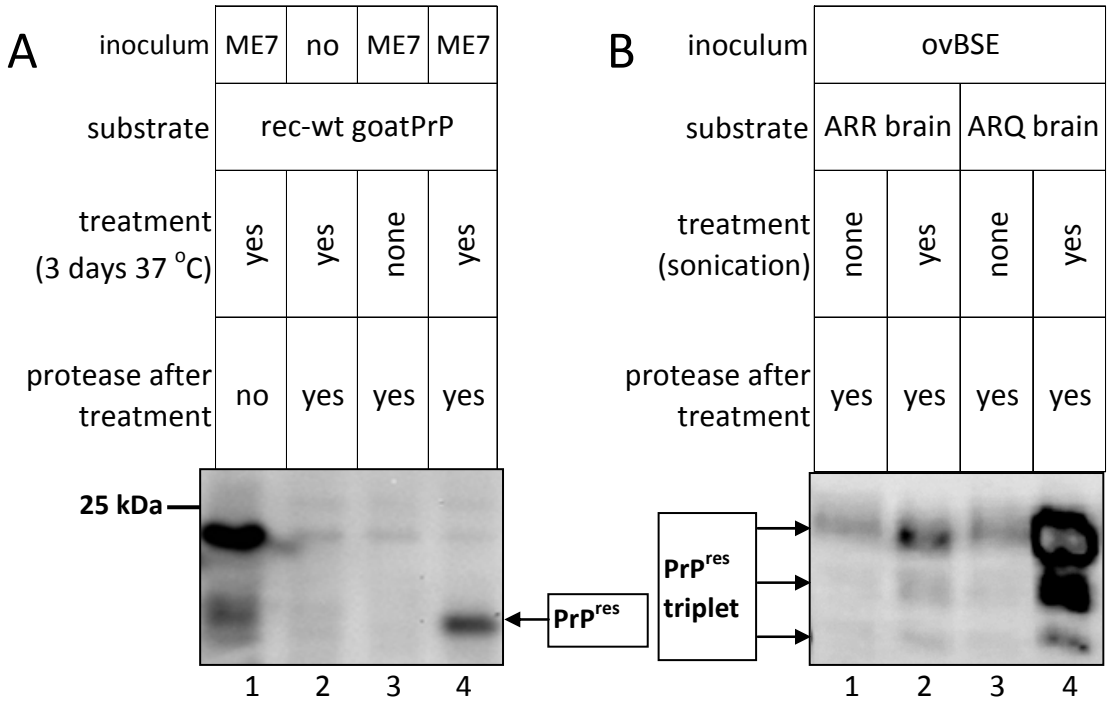

Figure D1.5: Cell free conversions using as substrate either rec wt-goatPrP from *E.* coli or mammalian PrP from healthy sheep brain. In A, newly converted PrP^{res} fragment could be detected in lane 4. No PrP^{res} was detected in control samples without inoculum (lane 2) nor the untreated sample (lane 3). Undigested control is shown in lane 1. Detection was carried with antibody P4. In B, PMCA set-up was used; aliquots of samples were not treated (directly frozen = none, negative control) or subjected to sonication; the inoculum was ovine ARQ/ARQ BSE brain while the substrate was healthy PrP homozygous brain material either from ARR or ARQ sheep. Detection was performed using antibody 9A2.

Table D1.5: *In vitro* PrP conversion combinations, substrate availability and conversion efficiency.

combination	TSE inoculum*	PrP substrate source		conversion	
		tissue	availability[†]	method[‡]	efficiency
1	ME7	rec PrP (*E. coli)*	unlimited[a]	37°C	acceptable
2	gt scrapie	rec PrP (*E. coli)*	unlimited[a]	37°C	uncertain
3	RML	rec PrP (*E. coli)*	unlimited[a]	QUIC	uncertain
4	scrapie	brain	limited[b]	PMCA	acceptable
5	scrapie	hamster cell culture	unlimited[c]	PMCA	acceptable
6	scrapie	transgenic brain	limited[d]	PMCA	good

* The scrapie sources investigated were both from sheep (several alleles) and from goat (wild type). ME7 and RML are mouse passaged scrapie materials that originally derived from a scrapie sheep.

[†] The genotype combinations available: [a]for bacterial PrP are available: wild type, 112T, 137I, 141F, 142M, 143R, 146D, 146S, 151R, 211Q, 215R and 222K, while 154H is pending; [b]for sheep brain are limited to a number of mostly homozygous individuals and for goat brain the number and genotype possibilities are also small; [c]for the cell produced PrP's there is ample choice of sheep alleles; for goat PrP's are available the alleles wildtype, 142M, 154H, and 211Q as well as bovine and human PrP; pending are production of goat alleles 143R, 146S, 146D and 222K; [d]for PrP from transgenic mice: availability of all major small ruminant genotypes is not expected.

[‡] Method of conversion is incubation without shaking (37 °C for 3 days), protein misfolding cyclic assay (PMCA = using sonication) or quaking-induced conversion (QUIC = using shaking).