attiva, mediante metodiche innovative (Decisione 2000/374/CE e più recentemente Regolamento (CE) 1053/2003), diretto ad identificare la presenza della BSE in animali, al di sopra dei 30 mesi di età, regolarmente macellati, e a rischio, morti in azienda e con sintomatologia di tipo neurologico. Il limite di età è stato successivamente abbassato a 24 mesi. Va ricordato che le metodiche utilizzate consentono di ottenere la risposta del laboratorio entro tempi molto brevi, a differenza del passato, compatibili con le esigenze di conservazione e commercializzazione delle carni. Il piano di sorveglianza nazionale della scrapie è stato attivato nel 2002 a seguito dell'entrata in vigore del Regolamento (CE) 270/2002. Dal 2 gennaio 2001 all'1 marzo 2004, in Italia sono stati effettuati 2.136.320 test rapidi per BSE e dal 2002 ad oggi 84.074 per scrapie. Questo sistema di sorveglianza attiva ha permesso di rilevare 118 casi di BSE. A tutt'oggi sono stati invece diagnosticati 146 focolai di scrapie. Quale altra misura fondamentale è stata individuata la rimozione, in fase di macellazione, del materiale specifico a rischio (SRM): intesi come testa, inclusi occhi e cervello, tonsille, colonna vetebrale e midollo spinale, per bovini e ovicaprini di età superiore ai 12 mesi, nonchè intestini, dal duodeno al retto, per i bovini di tutte le età, e milza e ileo per gli ovicaprini di tutte le età. A tutt'oggi, si ritiene infatti che in questi organi sia concentrata l'infettività e quindi che una loro precoce eliminazione sia sufficiente a proteggere il consumatore. Ulteriori verifiche della contaminazione da parte di materiale specifico a rischio potranno essere effettuate in sede di macellazione secondo quanto previsto dal Regolamento (CE) 1139/2003. Ancora il divieto dell'utilizzo di proteine animali per l'alimentazione dei ruminanti e successivamente di tutti gli erbivori, nonché l'attivazione di un programma nazionale di controllo in questo settore ha permesso di approfondire le conoscenze in questo settore e di evidenziare l'utilizzo di componenti non più autorizzati fin dal 1994. Nel 2002 è iniziato anche il piano di sorveglianza nazionale della scrapie basato anch'esso sull'utilizzo dei test rapidi eseguiti su animali regolarmente macellati o morti, nonché sintomatici, di età superiore a 18 mesi. Accanto alla sorveglianza attiva della scrapie e sulla base delle esperienze già maturate in altri stati europei (Regno Unito, Germania, Francia), l'Unione Europea ha legiferato così da rendere possibile l'attivazione di piani di eradicazione della scrapie basati sulla selezione genetica degli ovini nei confronti della suscettibilità a questa malattia (Regolamento (CE) 260/2002, Decisione 2002/1003/CE, Decisione 2003/100/CE). Anche la normativa che regola il trattamento dei rifiuti derivanti dalla macellazione e dall'attività diagnostica veterinaria, nonché delle misure di sicurezza da adottare per garantire la sicurezza degli operatori, è stata riconsiderata alla luce degli eventi connessi alle TSE, in particolare attraverso il Regolamento (CE) 1774/2002 "Gestione dei sottoprodotti di origine animale non destinati al consumo umano" e più recentemente con il D.M. 16 ottobre 2003 "Misure sanitarie di protezione contro le encefalopatie spongiformi trasmissibili". Si sottolinea infine l'importanza di una corretta comunicazione delle strategie e delle misure adottate per il controllo delle encefalopatie spongiformi trasmissibili animali, considerata l'importanza che le stesse hanno acquisito negli ultimi anni sia come patologia specifica dei ruminanti sia per gli aspetti zoonosici della BSE.

## S10.4

## INFLUENCE OF POLYMORPHISMS OF THE PRP GENE ON THE PATHOGENESIS OF TSE IN SHEEP

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The dramatic epidemic of bovine spongiform encephalopathy (BSE) along with the description in humans of the variant Creutzfeldt-Jakob disease (vCJD) caused by the BSE agent, have demonstrated the importance of transmissible spongiform encephalopathies (TSE) for animal health and - for the first time - the zoonotic potential of some TSE agents. The experimentally-confirmed susceptibility of small ruminants to the BSE agent and the possibility of its unrecognised circulation within European sheep and goats populations, have further led health Authorities to reconsider the risks related to animal TSEs directing their the attention also to TSE of sheep and goats.

The new strategies of the EU for the control and prevention of small ruminants TSE are rooted on the implementation of breeding programs for the selection of genetically-resistant sheep populations. It is well known indeed, that polymorphisms of the prion protein (PrP) gene have great influence on the susceptibility of sheep to TSE; in particular, a specific genotype ($136^{AA}154^{RR}171^{RR}$) has been proved to be highly resistant to the disease and very few animals carrying this genotype have been reported to be affected to date. The aim of the program is to increase the frequency of the resistant genotype with the final objective of reducing scrapie cases and – eventually – eradicate the disease. Such an approach is ambitious and unprecedented in animal infectious disease control. Nevertheless, it appears the most realistic one. As a matter of fact, the traditional approaches successfully used for the prevention and control of "classical" animal infectious diseases are inefficient or unfeasible in the case of small ruminants TSE.

Although appearing as the most reasonable approach, the selection of resistant genotypes present some

uncertainties. These are related, in particular, 1) to our limited knowledge on the pathogenesis of TSE in semi-resistant or resistant genotypes, 2) to the actual meaning of "resistance" in relation to the possible existance of silent carrier states, 3) to the possible emergence, under the selective pressure of the breeding programs, of TSE strains which have no longer their genetic target in the susceptible genotypes, but in those now considered as resistant.
All the above uncertainties could affect the success of the program but has also great impact for diagnosis and surveillance. This underlines the need of a careful monitoring of the breeding programs which still represent only a component of the health strategies for the control and prevention of animal TSE.

## S10.5

## HIGH PRESSURE/TEMPERATURE INACTIVATION OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES INFECTIVITY

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Bovine spongiform encephalopathy (BSE) contamination of the human food chain most likely resulted from nervous system tissue in mechanically recovered meat used in the manufacture of processed meats. The widespread occurrence of BSE, coupled with uncertainties about the rigorous implementation of precautionary measures, underline the need for a processing step that would ensure the absence of infectivity in processed meat products.
We have previously shown that the application of several short pulses of high pressure (690-1200 MPa) to hot dog paste 'spiked' with 263K scrapie-infected brain can reduce the level of infectivity by $10^3$ to $10^6$ mean lethal doses ($LD_{50}$) per gram of tissue (Brown P. *et al.,* PNAS 100: 6093–6097, 2003). By using the same scrapie strain, we have recently explored a larger range of pressure/temperature/pulse combinations, and a variety of processed meat products (e.g., hamburger, pureed baby food, paté, meat broth, and pet food) as substrates. We have also studied high pressure inactivation of other strains of transmissible spongiform encephalopathies, (BSE; mouse adapted BSE, mBSE; mouse adapted vCJD, mvCJD).
Brain samples from BSE, mBSE, mvCJD and 263K affected animals were treated at 600MPa/130°C/5' and then assayed by western blotting to measure the amount of residual PrPres. In order to get an accurate estimate of PrPres concentration, each sample was analysed using a two-fold dilution series. This experimental setting allowed us to demonstrate a reduction in the content of PrPres in all treated samples in comparison with their untreated counterparts: mvCJD and 263K showed the highest reduction (32-fold, about 1.5 log), whereas BSE and mBSE showed only a 4 to 8 fold reduction respectively.
In the second part of this study, 263K-spiked hot-dog aliquots were subjected to a range of different combinations of pressure, temperature and length of treatment. Results from western blot analysis of these samples showed that an exposure as short as 1-minute yields 1 log reduction of PrPres, and that the maximal extent of PrPres clearance (1.5 log) is obtained after 3 minutes, with no increase after further exposure. Keeping the time and temperature constant, progressive destruction of PrPres was observed with pressure increases from 600 to 1000 MPa; keeping time and pressure constant, a similar outcome was obtained when the temperature was increased incrementally from 115 to 134°C.
The results of our study indicate that sensitivity to high pressure treatment is strain-specific, with the maximal effect observed in brain samples from animals infected with the 263K and mvCJD inocula. This is not surprising when considering that TSEs infectious strains can be easily distinguished on the basis of their pathological, biochemical, and physical properties, such as the resistance to inactivation following autoclaving or dry heat processing (Somerville R.A. *et al.,* J. Biol. Chem. 277: 11084-11089, 2002). The small reduction in PrPres concentration in cattle and mouse BSE is in accordance with data obtained by other authors using hydrated autoclaving and confirm that this strain is particularly resistant to inactivation procedures (Taylor D. *et al.,* J. Gen. Virol. 83: 3199-3204, 2002). These results, while awaiting support by ongoing infectivity bioassay studies, suggest that the BSE agent requires a different combination of conditions to achieve satisfactory decontamination. We have previously observed that pressures of 1200 MPa result in loss of $10^6$ mean lethal doses per gram of 263K scrapie-infected brain tissue, however, the use of harsh conditions of treatment in an industrial setting may encounter technical constraints and has to be weighed against the possible alteration of the food product. The definition of the process of PrPres clearance as the sum of pressure, temperature and time shows how to deal with this limitation: the same PrPres clearance can be obtained at two different pressures simply adjusting the peak temperature or, to a lesser extent, the time of treatment. These data (always subject to bioassay confirmation) will provide a useful background to develop inactivation protocols specifically designed for each food product yielding the desired combination of quality and safety.