P.93: Efficacy of the mass spectrometry-based N-TAAP method as a complementary test for discriminatory analysis of small ruminant TSEs

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Keywords: mass spectrometry, small ruminant TSEs, discriminatory analysis

Discriminatory western blot and ELISA tests are applied to brain stem samples from all cases found positive for small ruminant TSEs by active surveillance in the UK to screen for potential BSE-related abnormal prion protein (PrP^{BSE}). These tests are based on the observation that, following treatment with proteinase K (PK), misfolded PrP that accumulates during an infection with BSE prions has a lower molecular mass and different set of N-terminal epitopes compared to misfolded PrP^{Sc} induced by most classical scrapie prions.

Mass spectrometry (MS) based analysis of PK-treated PrP^{Sc}, developed at AHVLA, also allows differentiation between classical scrapie and ovine BSE.[1,2] MS based analysis allows identification and quantification of the relative contribution of each N-terminal PK cleavage site at the resolution of individual amino acid residues. This is referred to as the N-terminal amino acid profile (N-TAAP). This profile is characteristic of a given TSE and has allowed discrimination of classical scrapie from ovine BSE in all tests carried out so far.

Using N-TAAP, we have been able to show:

- the discrimination between BSE and classical scrapie using N-TAAP is robust: characteristic N-Terminal Amino Acid Profiles from classical scrapie and ovine BSE continue to be consistent even when different preparation methods and mass spectrometry platforms are used,
- the N-TAAP profiles from ovine BSE and classical scrapie vary with increasing intensity of PK treatment and so this needs to be carefully controlled,
- a 100% correct identification of BSE samples in a blinded trial of samples (ovine BSE, scrapie, control) provided by the AHVLA Biological Archive,
- a 100% correct identification of ovine and caprine BSE samples in a blind analysis of samples (ovine BSE, caprine BSE, bovine BSE, classical scrapie, mixed classical scrapie/BSE) provided by INRA.

These data support our view that the N-TAAP methodology provides robust differentiation between ovine BSE and classical scrapie at the molecular level.

Application of the N-TAAP method in small ruminant TSE surveillance may be used to differentiate scrapie cases with an unusual PrP profile from an ovine BSE case and enhance our ability to find unusual scrapie cases and track their epidemiology without recourse to expensive and time-consuming mouse bioassay and histopathology.

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P.94: Characterization of clinically suspect goat TSE cases from Cyprus

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Introduction. Scrapie of sheep and goats belongs to transmissible spongiform encephalopathies (TSEs), which are fatal neurodegenerative diseases in animals and humans. As in sheep, the susceptibility of goats to scrapie is influenced by the prion protein (PrP) genotype of the host, but goat PrP polymorphisms have a different range of variations. Among the over 40 polymorphisms resulting in amino acid changes, at least five seem to be associated with TSE susceptibility so far. However, only limited data are known concerning the pathogenesis of scrapie in goats. Therefore the aim of our study is to characterize the diversity of natural scrapie in clinically scrapie suspect goats from a scrapie eradication program on Cyprus by using biochemical and immunohistochemical methods.

Material and Methods. In total 42 goats from 21 flocks were necropsied, genotyped and further analyzed by BioRad TeSeE rapid test, immunohistochemistry and biochemical methods.

Results. Twenty five animals showed a clear positive result in the BioRad TeSeE rapid test and an accumulation of pathological prion protein (PrP^{Sc}) in the brain stem as shown by immunohistochemistry and PTA-immunoblot. Most TSE positive goats are wild type goats, only one goat revealed a polymorphism at codon 154. On the other hand none of the goats with the polymorphisms serine (S) or aspartic acid (D) at codon 146 were positive. PrP^{Sc} deposits were in most cases widely found in the enteric nervous system and in different tissues of the lymphoreticular system, even in one goat with a negative staining reaction at the obex region. However one goat showed a clear restriction to the retropharyngeal lymph node. Additional PrP^{Sc} accumulations

could be demonstrated in placental tissues. The discriminatory immunoblots showed for all TSE isolates clear scrapie-like properties, but some variations were demonstrable in the PrP^{Sc} glycosylation pattern of single isolates. Furthermore all scrapie samples examined showed a lower proteinase K long term resistance using a C-terminal antibody as compared to ovine and caprine BSE controls.

Conclusion. The differences between single animals concerning the spread of PrP^{Sc} in peripheral tissues as well as the deviations observed in the biochemical pattern, might indicate the existence of different scrapie strains and will further be characterized by mouse bioassay. The findings reported here are important for the understanding of classical scrapie pathogenesis in goats and have implications for surveillance strategies in particular concerning BSE/scrapie discrimination.

P.95: Evaluation of some PRNP polymorphisms in ARQ/ARQ animals from four Italian breeds

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Introduction. Susceptibility to classical scrapie is related to sheep PRNP genotype. Polymorphisms at codons 136, 154, 171 lead to five haplotypes associated with different degrees of susceptibility: ovines homozygous for the ARR allele (ARR/ARR) are resistant to the disease while VRQ/VRQ, ARQ/ARQ and VRQ/ARQ ones are at high risk to develop the disease. In Italy, as in other EU countries, PRNP genotyping was adopted for selective breeding programmes and for the management of disease outbreaks in which selective culling of susceptible genotypes is implemented in order to decrease the incidence of scrapie. Recently two new alleles associated to scrapie resistance ($AT_{137}RQ$ and $ARQK_{176}$) have been discovered in Sarda breed. Another study reported that, in infected Suffolk sheep, heterozygous $AM_{112}RQ$-$AT_{112}RQ$ showed lower attack rates and increased survival times in respect to methionine homozygotes. The possibility of exploiting these new polymorphism for genetic selection may impact to accelerate selection programmes while preserving genetic variability of breeds and decrease animal culls.

Here we report preliminary results of 112, 137, and 176 codons allelic frequencies in ARQ/ARQ sheep belonging to four ovine Italian breeds. To date, in Prp gene, almost 40 polymorphisms in different breeds have been discovered and other may exist that could confer resistance, we are also investigating all the known SNPs of the Prp gene in the same animals.

Materials and Methods. A total of 584 blood samples of healthy sheep were obtained from 5 flocks that belong to Sarda (n=275, 2 flocks), Comisana (n=100), Appenninica (n=100), Bergamasca (n=109). Genomic DNA was extracted using an automated magnetic-particle technology for rapid DNA purification. Animals were genotyped by an allelic discrimination assay in Real-Time PCR. PRNP CDS from ARQ/ARQ ovines has been bi-directionally sequenced and chromatograms were analysed.

Results and conclusion. Genotypic and haplotypic frequencies have been calcuted. A total of 131 ARQ/ARQ animals were sequenced: Sarda (n=51), Comisana (n=21), Appenninica (n=18) and Bergamasca (n=41). We observed: 3% overall frequency of N176K (range 0-15%) in two flocks of Sarda sheep; 2% M112T in Bergamasca, 17% in Appenninica and 67% in Comisana breeds. Other polymorphisms are still under evaluation.

Data analysis confirm the presence of N176K in Sarda sheep while M137T was not detected in any breed. Very interestingly, high frequencies of M112T were detected in many of the breeds analyzed suggesting a potential resistance of these animals to scrapie disease.

P.96: Estimate of the effectiveness of the Italian genetic classical scrapie breeding program in sheep of Umbria and Marche regions (2006–2012)

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Introduction. Resistance or susceptibility to scrapie in sheep is mainly influenced by the genotype of the animals and by the pathogen strain. The European Union has implemented a breeding program based on genetic selection (Reg.999/2001/CE) in order to eradicate scrapie. The aims of this study were to evaluate: (a) the performance of the genotyping process concerning the presence of susceptible and resistant genotypes, and (b) the possible correlation between the production attitude (milk, meat, mixed) and the susceptible genotype.

Material and methods. The trend of genetic selection, over the years 2006-2012, was assessed by *chi-square for trend.* The genotypes of 21326 animals were divided into susceptible and resistant, and separated for attitude on the basis of the information provided by the farmer or of the production traits of the breed. The association between attitude and genotype was considered statistically significant with a p-value <0.05. The strength of the association was assessed by odds ratios (OR; intervals confidence, 95% IC)

Result. Genotype trend (2006–2012) is not statistically significant, p-value=0.93. From data analysis, comparing in pairs the various attitudes, it results that animals with meat attitude have an almost double chance respect to the animals with milk attitude to have a susceptible genotype (OR=1.75, 95% IC 1.6-1.9).

Mixed attitude animals are more likely to have a susceptible genotype than those that only produce milk (p-value=0.0001; OR=1.5, 95% IC=1.4-1.7). There is no statistically significant difference between the meat and mixed attitudes.

Discussion. The genetic selection plan implemented in Umbria and Marche regions does not appear to have an impact