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Prion Diseases in Animals

P.75: Biodegradation of infectious prions in compost

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Keywords: bovine spongiform encephalopathy, BSE, chronic wasting disease, CWD, specified risk materials

Introduction. Composting may serve as a practical and economical means of disposing of specified risk materials (SRM) or carcasses potentially infected with prion disease. Economic losses as a result of the occurrence of bovine spongiform encephalopathy (PrP^{BSE}) in farmed cattle are estimated at over $11 billion in Canada and the United States. Our study investigated the degradation of prions associated with scrapie (PrP^{263K}), chronic waste disease (PrP^{CWD}), and PrP^{BSE} in laboratory-scale composters and PrP^{263K} in field-scale compost piles.

Materials and Methods. Laboratory-scale composting was conducted over 28 days in constructed 110L vessels. Infectious prions, i.e., PrP^{263K}, PrP^{CWD} and PrP^{BSE}, were composted in a cattle manure-wood shavings matrix with or without poultry feathers. Compost was mixed at day 14 to generate a second heating cycle, with temperature exceeding ≥55°C for 2 days in the first cycle and for 1 day in the second cycle. Degradation of PrP^{263K}, PrP^{CWD}, and PrP^{BSE} over time was monitored using Western blotting (WB), and with additional analysis on PrP^{263K} using protein misfolding cyclic amplification (PMCA). For field scale compost experiments, duplicate mortality compost structures containing ~100 tonnes of material including PrP^{263K} adhered to stainless steel beads were used. Degradation of PrP^{263K} was assessed at 0, 14, 56, 112, and 230 days via bioassay by intracranial implantation of composted beads in Syrian golden hamsters.

Results. Western blotting analysis indicated that PrP^{263K} was reduced by 2 $\log_{10}$, PrP^{CWD} by 1-2 $\log_{10}$, and PrP^{BSE} by 1 $\log_{10}$ after two cycles of lab-scale composting. Further analysis using PMCA confirmed a 2 $\log_{10}$ reduction in PrP^{263K}. Enrichment for proteolytic microorganisms through the addition of feather keratin to compost enhanced degradation of PrP^{263K} during the first composting cycle. Temperatures remained >55°C for 39 days in field-scale compost. Field-scale ccomposting effectively reduced the development of TSE in the hamsters intracranially implanted with beads composted for 14, 56, and 112 days. After 230 days of composting, only one in five hamsters succumbed to TSE disease.

Conclusion. A calculation using four-parameter logistic regression model suggests that composting achieved at least a 4.8 $\log_{10}$ reduction in PrP^{263K} infectivity. Our findings demonstrate that under field conditions, composting dramatically reduces PrP^{TSE} and when overall risk is considered, may be a viable method for the disposal of SRM.

P.76: Evaluating the effectiveness of genetic screening for the identification and classification of increasing sheep resistance in Romania

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Keywords: haplotypes, scrapie resistance, sheep, breeding program

Introduction. Following Regulation (EC) no. 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies, that include intensive genetic selection for scrapie resistance, our pilot study is focused on evaluating the effectiveness of such screening for the identification and classification of sheep resistance to scrapie in Romania.

Material and Methods. Study was conducted in two selected farms for a period of two years, without significantly reducing the number of animals and consequently avoids economic losses. A total number of 2515 sheep (1280 sheep from the first farm and 1235 sheep from the second) were examined for codons 136, 154 and 171 polymorphisms in order to identify a convenient and applicable method to improve the sheep population resistance to scrapie. **Results.** Five haplotypes (ARR, AHQ, ARH, ARQ and VRQ) were observed in this study. Of the total number sheep being analyzed, 421 were classified as category 1 (resistant to scrapie homozygous form), 1045 in category 2 (resistant to scrapie heterozygous form), 884 in category 3 (medium susceptibility

to scrapie), 65 in category 4 (genetically resistant, but with VRQ haplotype) and 100 in category 5 (highly susceptible – at least one VRQ haplotype).

Conclusion. For the following breeding season, our recommendation is to remove the scrapie susceptible categories 3, 4 and 5 from the breeding program. This study provided valuable information on future actions to improve genetic resistance scrapie in sheep.

P.77: Molecular interaction analysis of prions and potential peripheral receptors

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Background. Former research illustrates Complement, a branch of the innate immune system, as a critical mediator and promoter of prion disease pathogenesis. Specifically, mice deficient in certain Complement components either entirely resist disease or exhibit delayed disease onset.[1-4] The work in this study aims to identify Complement-prion interactions with the ultimate goal to elucidate the precise molecular players involved in binding prions prior to propagation in lymphoid tissues and subsequent neuroinvasion and neurodegeneration.

Methods. We employed Surface Plasmon Resonance (SPR) technology to observe whether Complement proteins C3b, C3d, Factor H, C1q, or Complement receptors CD21/35 bind high density recombinant PrP^C, thought to mimic prion amyloid, as well as bona fide infectious prion rods enriched from the brain of an elk with Chronic Wasting Disease (CWD).

Results. Our studies reveal a strong interaction between CD21/35 or C1q with high density PrP^C or prion rods, whereas C3b and Factor H bind high density PrP^C minimally and prion rods negligibly. Interestingly, however, C3d, a cleavage product of C3b and a ligand of CD21, binds both high density PrP^C and prion rods.

Conclusions. These data support in vivo results revealing CD21/35 deficiencies as more protective than deficiencies in their ligands,[1-3] although we cannot rule out the possibility of preferential binding to soluble prions, i.e. oligomers, over insoluble prion amyloid. Additionally, these data provide additional insight into molecular mechanisms of extraneural prion trafficking and propagation, and may provide therapeutic target(s) for preventing disease spreading. Future directions include elucidating the role of Factor H in a mouse adapted Scrapie model, as well as observing differences, if any, between the interactions of prions and CD21 or CD35.

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P.78: Lack of correlation between PrP^{Sc} and infectivity in a murine model of P102L GSS

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The hallmark pathology of the Transmissible Spongiform Encephalopathy group of diseases has been described by vacuolation of the neurophil, gliosis and deposition of a misfolded host protein termed the prion protein (PrP). Current dogma cites the abnormal protease resistant form of this protein (PrP^d) termed prions, as the sole infectious agent in this group of diseases affecting both humans and animals. Evidence from several studies apparently challenges this central role of the prion, with data demonstrating negligible detectable PrP^d in the presence of high infectivity. A recent study carried out in our laboratory detected high levels of infectivity in spleen and brain of a transgenic murine model of Gerstmann-Sträussler-Scheinker (GSS/101LL) disease without a corresponding high level of PrP^d deposition. Using a range of techniques such as subcellular fractionation studies, bioassay, proteomics, PrP detection by western blotting and immunohistochemistry including PET blots we have investigated the relationship between abnormal protein and infectivity in TSE disease processes.

We utilised a subcellular fractionation technique to establish which fractions of a GSS/101LL infected brain homogenate contained the most infectious particles and if these correlated with the presence of PrP^d. Crude fractionation of a GSS/101LL murine brain homogenate determined that PrP^d was found mainly in the P2 synaptosomal fraction. Further purification by differential sucrose fractionation of this P2 fraction was carried out and each fraction inoculated into groups of 101LL mice to determine infectivity levels by bioassay. These data established that there was a high level of infectivity present with no significant difference between subcellular fractions. Groups of mice injected with inoculum pre-treated with detergent did not significantly differ in titre from non-treated. Subsequent western blotting of each fraction failed to detect any level of PrP present. Differential protein expression profiling highlighted a protein peak at 10858Da which was up-regulated in infected fractions. Identification of this protein is ongoing.

Having established infectivity in the absence of detectable PrP^d we sought to determine whether material from this model could convert PrP^C to PrP-res(proteinase K resistant) by in vitro amplification. In a RT-QuIC conversion assay, brain homogenate

from GSS/101LL transgenic mice displaying low levels of PrP^d by ICC and western analysis, seeded the conversion of recombinant PrP to similar levels to that observed with models of TSE disease displaying large depositions of PrP^d.

These data question the assumption that detectable/deposition of PrP^d is necessarily the best biomarker of all TSE diseases and further questions the nature of the agent involved.

P.79: Bioassay of plasma from prion-infected sheep in ovine PrP transgenic *Drosophila*

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It is important to determine the level of infectious prions in blood to ensure the safety of blood-products for human medicine. To begin to develop a sensitive diagnostic prion disease blood test we have assessed the response of ovine PrP transgenic *Drosophila* to plasma from scrapie-affected sheep. We have measured the locomotor activity of adult VRQ ovine PrP transgenic flies after exposure at the larval stage to plasma isolated from VRQ/VRQ sheep with prion disease. Ovine PrP transgenic flies exposed to plasma from sheep with experimental or natural classical scrapie showed an accelerated decline in locomotor activity compared to the response seen after exposure to scrapie-free plasma. This toxic phenotype in the fly, evident after exposure to plasma isolated from natural scrapie-infected sheep ≥6 months of age, was more pronounced after exposure to plasma obtained during the clinical rather than pre-clinical phase of sheep scrapie. Titration analysis in PrP transgenic *Drosophila* showed that the level of toxicity in the plasma of clinically-affected sheep was comparable to that found in sheep brain material at terminal scrapie disease. Plasma samples from scrapie-affected sheep did not affect the locomotor activity of non-transgenic 51D control flies. These data show that PrP transgenic *Drosophila* can act as a novel animal model for the successful bioassay of blood samples from prion-infected individuals.

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P.80: Pharmacodynamics of a PrP^C siRNA therapeutic that can cross the blood brain barrier

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The emergence of prion diseases in wildlife populations and the increasing impact of prion diseases on human health has led to an increase in the study of antiprion compounds. Recent studies have found antiprion compounds that can inhibit the infectious prion isomer (PrP^{Res}) or down regulate the normal cellular prion protein (PrP^C). These compounds are often found through the screening of drug or chemical compound libraries. However, most of these chemicals cannot cross the blood brain barrier to effectively inhibit PrP^{Res} formation in brain tissue or to specifically target neuronal PrP^C. Also, these compounds tend to have multiple off target effects, and are often too toxic to use in animal or human subjects. Therefore, we have proposed using siRNA that is targeted towards PrP^C, and complexed to the RVG-9r peptide, which will target the siRNA to nicotinic acetylcholine receptors within the CNS. Our siRNA therapeutic has proven effective in eliminating prion disease from several neuronal cell lines. We are now testing the PrP^C siRNA in vivo through an intravascular route, and evaluating the pharmacokinetics using live imaging and flow cytometry. To avoid serum degradation and facilitate passage through the blood brain barrier in vivo, we have complexed our PrP^C siRNA - RVG-9r peptide to liposomes. Wild type mice treated with the siRNA therapeutic through an intravascular route have detectable siRNA and peptide signals in the brain using the IVIS live imaging system within 15 minutes after injection. There is minimal to no peripheral detection of siRNA and peptide using live imaging, except in the kidneys/bladder which also contain nicotinic acetylcholine receptors. Using flow cytometry, we can detect a 25-90% decrease in neuronal PrP^C and a 30-80% decrease of PrP^C within the kidneys 24 hours after treatment. We are now trying to optimize the PrP^C siRNA therapeutic for the maximum decrease in neuronal PrP^C expression.

P.81: Biological characteristic comparison of various species chronic wasting disease case in cervidized transgenic mice

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Keywords: chronic wasting disease, strain characterization, transgenic mice

Introduction. Chronic wasting disease (CWD) has been recognized as an important prion disease in North American deer and Rocky mountain elk.[1] This disease was confirmed only in elk in the Republic of Korea in 2001, 2004 and 2005.[2,3] Additional CWD cases were observed in red deer, sika deer, and crossbred sika and red deer in 2010.[4] We investigated to assess the difference of biological characterization for other species, which is elk, red deer, sika deer, and crossbred.

Materials and Methods. A total of 6-week-old 48 female TgElk mice, which is overexpressed elk prion protein, were inoculated intracranially with 20 µl of a 10% brain homogenates of the elk, red deer, sika deer and crossbred CWD for the 1st passage and with 1% brain homogenates of CWD affected TgElk mice for 2nd passage respectively. The inoculums were set up approximately 1.8 O.D value of AgELISA test (TeSeE ELISA kit) for similar quantitative dose. We measured survival time from inoculated time to dead and analyzed western blot pattern. The significance of survival time analyzed the log rank test using Prism 5.04 between groups.

Results. When transmitted the CWD infected cervid to TgElk mice (1st passage), the mean survival time of each groups was 146±34 days in elk, 154±20 days in red deer, 158±17 days in sika deer, and 139±17 days in crossbred. Homogenates of CWD affected TgElk mice were injected into naïve TgElk mice (2nd passage), resulting in a mean incubation period of 128±25 days, 124±28 days, 123±14 days, and 114±10 days respectively. Analyzing the log rank test of each group, they were not significant. Western blot analysis of disease associated prion proteins in brain homogenates from the infected TgElk mice of each groups detected the protease resistant PrP^{Sc}. These western blot patterns showed that the strongest signal was observed for diglycosylated band and the weakest signal was unglycosylated form. The glycosylation pattern between groups was not discriminated.

Conclusions. Findings from the present study indicated that the biological characterization of different species cervid observed to be similar figures, which were like to survival times and western blot pattern, although it used transgenic mice to overexpress elk prion protein gene. Therefore, these results showed that there were no species-specific characterization differences in elk, red deer, sika deer and crossbred in Korea CWD cases.

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P.82: Emergence of a new TSE strain following passage of classical BSE in VRQ/VRQ sheep

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Keywords: classical BSE, TSE, strain, VRQ/VRQ, mouse bioassay

The natural host of classical BSE (C-BSE) is cattle. However, it can transmit to other species, including humans, whilst the properties of the agent remain unchanged. This has led to the widespread belief that C-BSE is attributable to a single strain. Transmission of C-BSE to sheep depends on the ovine PrP sequence: susceptibility is associated with the ARQ and AHQ alleles while the VRQ allele is believed to confer resistance. However, this resistance is relative as VRQ/VRQ sheep can succumb to C-BSE albeit with prolonged incubation periods.[1] As the properties of TSE agents are more likely to change when they cross transmission barriers we analysed the biological properties of the agent isolated from a VRQ/VRQ and ARQ/ARQ sheep challenged orally with bovine C-BSE. Each source was used to inoculate RIII, tg338 (VRQ ovine transgene), tgshpXI (ARQ ovine transgene) and tg110 (bovine transgene) mice. The disease phenotype in the mice showed that the strain isolated in all four mouse lines from the ARQ/ARQ source and the strain isolated from the VRQ/VRQ source in three the mouse lines (RIII, tgshpXI and tg110) were indistinguishable from each other, and comparable with cattle classical C-BSE isolates studied previously in our laboratory. Conversely, 8 out of 10 tg338 mice challenged with the VRQ/VRQ isolate showed a short survival rate (SR) (189-372 days post inoculation – dpi); the remaining 2 mice showed a prolonged SR (748 &818 dpi) which was comparable with the SR produced by the ARQ/ARQ ovine and bovine sources (610-817 dpi). Strain typing analysis suggested that the strain associated with prolonged SR was compatible with the one derived from ARQ/ARQ ovine and bovine C-BSE

sources. Strain characterisation of the agent isolated in the short SR mice excluded C-, L- or H- type BSE as well as CH1641or any other known scrapie strains, classical or atypical. Therefore, we conclude that this is a new C-BSE derived strain. This strain maintained its phenotype in tg338 mice after a serial transmission, with a further reduction in the SR by 50%. However, upon transmission from tg338 to tg110 mice the phenotype of the agent reverted to the C-BSE phenotype usually isolated from cattle or ARQ/ARQ passaged C-BSE. We propose that this strain, which is maintained only through the tg338 mouse, was generated in the VRQ/VRQ sheep as the transmission barrier between VRQ/VRQ sheep and tg338 mice is expected to be minimal.

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P.83: Early atypical cases of scrapie in UK sheep

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Atypical scrapie was first discovered in clinically affected sheep in Norway in 1998. It is distinguishable from classical natural scrapie by brain pathology in cerebellum rather than medulla, and by the biochemistry of PrP^{Sc} which in atypical scrapie is much less proteinase K resistant but which also has, on Western blots, a characteristic low molecular weight band of varying size estimate of ~8 to 12kD which is absent in classical scrapie. Since 1998, cases similar to Nor98 and with a tendency to occur in older animals, have been identified throughout Europe, including the UK, mainly through the process of active surveillance of asymptomatic sheep. Atypical scrapie occurs in sheep with *PRNP* genotypes considered to be resistant to classical scrapie such as those with AHQ and ARR alleles, and also with the influence of codon 141 such that genotypes including the $AF_{141}RQ$ allele are more susceptible than those with $AL_{141}RQ$. Incidence of atypical scrapie is low but persistent and as a result of the measures taken to control classical scrapie in UK and EU, the relative incidence of atypical scrapie has increased to 95% and 27% respectively of all scrapie notifications (European Commission annual report, 2010).

It was originally not certain whether atypical scrapie was a newly emerging TSE disease or whether this form of scrapie had been prevalent in flocks for much longer and only being identified because of increased and improved surveillance methods. As a result of tissue archive searches, we have previously reported that atypical scrapie had been occurring as early as 1989 and the UK Veterinary Laboratories Agency reported a case from 1987. However we sought to take advantage of the Neuropathogensis Unit (NPU) sheep tissue archive (now stored at The Roslin Institute) which has samples dating back to the 1960s to try to find even earlier examples of atypical scrapie to establish whether there had been any change in its phenotype over the decades of its known existence in UK sheep. Immunohistochemistry and Western blotting studies revealed a small number of candidate atypical cases from the 1970s and 1980s which were further strain typed in tg338 mice. Most were confirmed as similar to Nor98, however one case dating from 1972 shows evidence for a very unusual and possibly a mixed classical/atypical infection.

P.84: Caesarean derivation and hand-rearing does not prevent scrapie in the offspring of infected ewes

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Natural transmission of scrapie between sheep is not fully understood. Although scrapie has been demonstrated to transmit between adult sheep, the available evidence suggests the very young lamb is at high risk of picking up infection from their scrapie affected mothers. Early literature claimed evidence of *in utero* transmission but the studies were compromised by lack of knowledge of the control of disease susceptibility by the *PRNP* gene. More recently it has been thought likely that lambs become infected after birth by ingestion of contaminated birth fluids, including blood, or from contact with voided placental tissues. It is possible that multiple infection routes function in natural transmission of sheep scrapie so it is difficult to conduct clean experiments to differentiate pre- and post-natal factors. However as highly sensitive methods have now shown that PrP^{Sc} is present in infected ewe amniotic fluid (which is ingested repeatedly by developing fetuses) this should be seriously considered as a possible route by which oral infection could be achieved.

In this complex study we experimentally infected pregnant ewes of known *PRNP* genotype with a distinctive scrapie strain (SSBP/1) and looked for evidence of transmission of SSBP/1 to the offspring. The sheep were from the NPU Cheviot flock which has endemic natural scrapie from which SSBP/1 can be differentiated on the basis of histology, genetics of disease incidence and strain typing bioassay in mice. This presentation describes one segment of the study in which, for a proportion of the infected ewes, lambs were removed by midline laparotomy just prior to the estimated birth date and then hand reared in complete isolation from the adult sheep. The lambs were fed by human handlers and given colostrum and reconstituted milk powder from sources in New Zealand prior to weaning. This treatment however did not prevent scrapie appearing in some of the offspring of fully susceptible mothers. Following mouse strain typing, in tg338 mice, we demonstrate clear evidence of the appearance of SSBP/1 scrapie in the lambs. Taking into account all the stringent disease security measures that we had in place it is difficult for us to understand how this strain could have infected the lambs in any other way than pre-natally from their SSBP/1 infected mothers. Our results strongly suggest that in utero transmission of scrapie may have occurred in these sheep.

P.85: Transcriptome analysis by microarray and RT-qPCR of blood from BSE-infected cattle

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Background. Transmissible Spongiform Encephalopathies (TSEs) are fatal neurodegenerative disorders which affect humans and a wide variety of animals. The molecular events at the basis of TSEs are still poorly defined, and at present there are no sensitive pre-clinical diagnostic tests and no effective treatments. Prompt detection of prion diseases is particularly desirable given their proven transmissibility compared to other neurodegenerative pathologies such as Alzheimer's and Parkinson's diseases. In an attempt to identify novel biomarkers and targets for TSEs, we conducted the first wide-spectrum microarray analysis in whole blood of BSE-infected cattle during the progression of the pathology.

Materials and methods. Total RNA was isolated from whole blood of presymptomatic, symptomatic and control cattle using the Ribopure blood kit (Ambion). The integrity of the RNA was assessed using an Agilent 2100 Bioanalyzer and 120 ng of each RNA sample was subjected to the standard one-cycle amplification protocol developed by Affymetrix. The cRNA was hybridized on the GeneChip® Bovine Genome Arrays containing over 23,000 bovine transcripts. Raw data were quality checked and analyzed using Limma package.

Results. The Bioinformatics analysis revealed approximately 250 and 210 differentially expressed genes (DEGs) in presymptomatic and symptomatic animals, respectively. 40 DEGs were in common between the two groups, and most intriguingly they all followed the same pattern of expression in both groups. At present we are validating the most interesting candidate genes using RT-qPCR.

Conclusions. A substantial modification in gene expression pattern was observed in blood samples from BSE-infected cattle, especially in pathways related to immune response and signalling. Remarkably, this study showed that DEGs are detected in blood since the early presymptomatic stage of the disease and therefore they could be evaluated as biomarkers to be used for the development of preclinical diagnostic tests. Additional efforts should be made in the future to further dissect the identified pathways and understand their role in prion diseases.

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P.86: Neurotropism property of classical versus atypical TSE agents studied using comparative stereotaxic injections within four different sites of entry

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Introduction. In natural prion diseases such as scrapie and bovine spongiform encephalopathy (BSE), classical and atypical forms can be distinguished on their specific neuroanatomical targeting in term of accumulation sites of the diseased-related form of the prion protein (PrPd). This suggests that atypical strains may have different neurotropism property compared to classical strains. In experimental studies based on direct brain exposure, the exact site of injection of this classical and atypical infectious agent could have an impact on the PrPd cerebral distribution in the host. Because this represents a critical parameter used to identify the strain of agents responsible for these diseases, the aim of this study was to characterize the possible effect of the precise site of entry of these infectious agents on their dissemination, identified by the study of the cerebral distribution of PrPd.

Material and Methods. Classical (C506M3 and BSE) and atypical (Nor 98 and L-BSE) sources of infectious agents were analyzed in two mouse lines, C57Bl/6 and TgOvPrP4, a transgenic line expressing ovine prion protein solely, using stereotaxic injections in the brain. Four sites of injection were studied: cortex, striatum, cerebellum and ventricles. At the terminal stage of the disease, brains were removed and prepared for histological studies. The PrPd distribution and type of deposits were analyzed using immunohistochemistry, Paraffin Embedded Tissue Blot, completed with Congo red staining.

Results. Among interesting observations is a poor effect of injection site on PrPd brain mapping for the classical scrapie strain, notably in the C57Bl/6 line. Remarkably, an asymmetry of PrPd distributions was observed in both mouse lines for C-BSE agent after injection into the striatum, a feature not being or far from visible for C506M3 strain. This asymmetry was not related to the distribution of the vacuolar lesions which remains symmetrical. The data of atypical Nor 98 and L-BSE transmissions studies in TgOvPrP4 under final analysis will be also reported.

Conclusion. Our results suggest already a distinction between classical scrapie and BSE agents in response to a different site of entry in the brain, the C-BSE agent appearing to be more sensitive to the initial site of entry in the brain than the scrapie agent used in this study.

P.87: *PRNP* polymorphisms in Greek goats affected with natural scrapie

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Introduction. As a part of an EU EMIDA ERA-NET project entitled "Towards breeding of goats for genetically determined TSE resistance" we analyzed 295 brain tissue samples from scrapie affected Greek goats for *PRNP* gene variability and disease association.

Material and Methods. In total, 295 brain tissue samples from naturally affected goats were analyzed from 71 herds. qPCR was used for genotyping the *PRNP* coding region positions 110, 146, 211 and 222 and in a selected number of animals direct sequencing was carried out for verification of polymorphisms and *PRNP* gene promoter region analysis. The *SPRN* gene encoding Shadoo protein was partly sequenced for animals with genotype 222QK.

Results. All goats were *PRNP* genotypes 110TT and 146NN equivalent to wildtype PrP. In contrast, the polymorphism R211Q was detected in 11 animals (3.7%) from 4 herds. Surprisingly, the polymorphism Q222K was detected in 13 samples (4.4%) from 5 herds with a total population of 1138 animals. Negative controls were not collected at the time, but our analysis of 25 healthy herds with over 800 animals resulted in an average K222 carrier frequency of 7.5% (3-16%). Based on this data, we estimate the frequency of scrapie cases amongst K222 carriers being 7-15% in these 5 herds. We reasoned that the unexpectedly high occurrence of the K222 variant in scrapie-affected goats could be linked to an unusual *PRNP* promoter haplotype linked to different expression control; this was not supported by our sequence analysis. A polymorphism in the SPRN gene previously associated with scrapie susceptibility in Italian goats was found in 33% of the 222QK affected Greek goats.

Conclusion. The absence of 146S/D polymorphisms in scrapie positive Greek goats supports the hypothesis that they are associated with resistance. The observation of scrapie-positive K222 carriers in 4 different geographical areas and in 5 out of 71 infected flocks was surprising based on previous studies that had shown strong association of K222 with resistance.[1] Our study appears to indicate a much higher incidence of scrapie in 222K carriers than reported for French goats. There was no evidence from the *PRNP* gene sequences that the K222 allele in these Greek goats contains additional mutations associated with susceptibility nor that there is a particular *SPRN* allele. This leaves us to propose that either especially strong infection pressure in these herds or a different scrapie strain may be responsible. Investigations to test this are currently performed.

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P.88: Allelic variants at codon 146 in the *PRNP* gene show significant differences in the risk for natural scrapie in Cypriot goats

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Introduction. Scrapie is highly prevalent in the goat population of Cyprus. Previous studies have shown the association between the polymorphisms serine (S) or aspartic acid (D) at codon 146 of the *PRNP* gene and resistance to scrapie. The objective of this study was to investigate the possibility that putatively resistant animals were sub-clinically infected and to further elucidate the resistance to scrapie in natural conditions of animals carrying S_{146} or D_{146} alleles.

Material and Methods. A systematic assessment of the detectability of PrP^{Sc} in clinically healthy and scrapie affected animals from long-term infected herds was conducted on all individual animals from four infected herds from among those with the highest prevalence of scrapie. All of the goats over twelve months of age (a total of 1,075 animals) were culled, genotyped and tested.

Results. A total of 234 goats (21.7%) were positive to either the rapid test or immunohistochemistry (IHC) at the obex or the lymphoreticular tissue (LRT). The odds of scrapie infection occurring in NN_{146} goats was 101 (95% credible interval: 19-2938) times higher than for non-NN_{146} or unknown genotypes adjusted by herd, age and test. There were significant differences in the within herd prevalences of infection and increasing age is significantly associated with an increase in the likelihood of individual goats being positive. IHC applied to lymphoreticular tissues produced the highest sensitivity (94%. 95% CI: 90-97%), and there was no evidence of subclinical infection in any of the non-NN_{146} genotypes.

Conclusions. All the cases detected were NN_{146} except one case of undeterminable genotype. Being NN_{146} is a statistically significant risk factor for the presence of detectable scrapie infection. This is the first study where resistance to natural exposure during the entire productive life of goats with allelic variants at codon 146 has been demonstrated. Although represented at low frequency at the time of conducting this study, namely 4.9% for D_{146} and 5.9% for S_{146}, the presence of putatively resistant non-N_{146} alleles in the Cypriot goat population provides a potential tool to reduce/eradicate scrapie provided that co-ordinated

nationwide breeding programs are implemented and maintained over time. This study contributes to the growing body of evidence on the genetic resistance to scrapie in goats.

P.89: Prions survive long-term burial in soil with some groundwater dissemination

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An intrinsic property of prions is their extreme resistance to degradation. When they are deposited within the environment, whether from inappropriate disposal by man or from fallen diseased livestock, there is the potential to further propagate cases of disease for many years. It is evidenced that the spread of scrapie in sheep and chronic wasting disease in deer have occurred in this manner.

We mimicked such scenarios under large-scale field conditions to determine the extent to which TSE infectivity survives or disseminates in soil and soil water over five years. The mouse passaged BSE strain, 301V, was used to spike buried bovine heads, or was buried as an uncontained bolus in large soil-filled lysimeters. Two soils were examined, a free-draining sandy loam and a water-retentive clay loam.

Infectivity, determined by bioassay in mice, was recovered from all heads exhumed annually for 5 years from both soil types, with little reduction in the amount of infectivity over time. Small amounts of infectivity were found in soil samples immediately surrounding the heads but not in samples remote from them. Commensurate with this there was no evidence of significant lateral movement of infectivity from the bolus buried in a large soil mass. However large amounts of infectivity were recovered at the original bolus burial site in both soils. There was limited vertical upward movement of infectivity from the bolus buried in clay and downward movement from the bolus buried in sand perhaps reflecting the clay soils propensity to flood.

Throughout the course of the experiment rainwater particulate from several lysimeters was trapped on glass-fibre filters. Extracts from these filters were subject to serial PMCA (protein misfolding cyclic amplification) which was optimised using 301V-spiked samples and blinded controls. All positive and negative control samples were correctly determined. We have tested 44 samples from rainwater passed through the clay lysimeter filters, and found 9 positive samples, mainly from the initial 8 months of the experiment.

We conclude that TSE infectivity is likely to survive burial for long time periods with minimal loss of infectivity and limited movement from the original burial site. However PMCA results have shown that there is the potential for rainwater to elute TSE-related material from soil which could lead to the contamination of a wider area. These experiments reinforce the importance of risk assessment when disposing of TSE risk materials.

P.90: Genetic variability in the gene for PRNP in goat breeds of Sicily, Italy

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The prion protein (PrP) gene polymorphism strongly influences the susceptibility of small ruminants to Transmissible Spongiform Encephalopathies (TSEs); genetic selection to enrich the sheep flocks for TSE resistant animals, is being implemented with success in the EU. In contrast the genetic basis of scrapie susceptibility in goats is not well understood. The analysis on polymorphism in the *prp* gene associated to disease resistance in goat is still ongoing and no genetic selection can be planned until gene polymorphisms related to disease resistance are confirmed. Epidemiological and experimental studies provided some association between determinate PrP polymorphisms and resistance to TSEs in goats. Among them, the Q/K polymorphism at the codon 222 (Q222K) yielded the most promising results.

The PrP polymorphysm in Sicilian goats breed are almost unknown. To study PrP gene polymorphism, the coding sequence of the caprine PrP gene was determined in 312 goats of three breeds commonly reared in Sicily: Girgentana (n=64), Red Mediterranean (n=68) and Maltese (n=136). We also considered a Sicilian cross-breed group (n=44).

Blood samples were collected from several flocks(>20). The samples were collected in Sicilian provinces with more than 150 animals for breed and particularly in male animals.

Genomic DNA isolated from blood was amplified for the coding region of the PRNP gene by PCR and then sequenced in Abi 3130 genetic analyzer. Sequence alignment was carried out using the SeqScape software v2.5 (Applied Biosystems).

In total, 10 polymorphic sites were identified, G37V, M137I, I142M, I142T, H143R, R154H, P168Q, R211Q, Q222K and S240P. As in Italian goats, two main PRNP alleles were present, characterized by the presence of Ser/Pro at codon 240. The P240 was the most widespread while the S240, homologous to the wild-type allele in sheep was less frequent than the P240. Surprisingly we found 222K in all breeds and in Sicilian cross-breed group and the *Girgentana* breed showed the highest frequency of 222K (0,30).

This is the first study on caprine PRNP polymorphisms distribution in Sicily. The finding of significant differences among allele distributions in Sicilian goat breeds, especially if involved in modulating resistance/susceptibility, need to be carefully considered for the feasibility of selection plans for resistance to scrapie. This preliminary result seems promising for the realization

of a plan based on the selection of 222K allele in the Girgentana breed.

P.91: Ancient DNA polymorphisms analysis to investigate scrapie susceptibility in Sicilian sheep remains from medieval archaeological sites

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Encephalopathy in sheep was at first described in Ireland in 1732 and was called "scrapie" because the animals tend to tear their hair. Historically it seems to be the result of an incestuous union as breeding practice in old farms. In Sicily the animal bones found in association with the human skeletal remains from the tombs or city-sites, comprised a broad range of domestic. Usually, species included in the collected bones are domestic animals commonly eaten as sheep or goats, cattle, pigs, chickens and a small partridge. In this contest, the assemblage and the species identification is often difficult. Based on DNA barcoding, all the investigated bones were confirmed as belonging to the Ovine specie. Most of the elements were not whitened or brittle, suggesting none exposure period to weathering and the soft tissues were decomposed slowly. The aim of this study was to investigate the SNP variants of the ovine PrP gene (PrnP) that is associated with the risk of scrapie disease. Sheep susceptibility to classic scrapie is associated with mutational points that leads to the recognized variants: codon 136 (Alanine/Valine), codon 141 (Leucine/Phenylalanine), codon 154 (Arginine/Histidine) and codon 171 (Arginine/Glutamine/Histidine). Ancient DNA was isolated directly from 200 animal skeletal remains and collected on four Sicilian medieval archaeological sites (10^{th} – 12^{th} century). aDNA extraction was performed from the epiphysis of the skeletal remains containing dry bone marrow cells and used as template to perform real time PCR and sequencing. The obtained data were analyzed for the assignment of the PrnP genotype at the 136, 141, 154 and 171 codons. Results showed that the 90% of the animals (n=180) had a resistant homozygote genotype at each codon (ALRR/ALRR), while only the 10% had a low sensitivity to scrapie disease (ALRR/ALRQ); no animals were found with the most well-known sensitive genotypes containing Q, F, V and H codons. The high incidence of animals resistant genotype in various independent districts, suggest a positive trend towards scrapie free herds for the animals bred in Sicily in old age. In conclusion, the genotype obtained for the medieval bone sheep differ strongly at the scrapie hot spot respect those of the modern ovine so that the polymorphisms that leads to susceptibility probably occurred in Sicily after the Middle Age.

P.92: Differentiation of TSE reference strains in mice by immunohistochemical PrP^{Sc} profiles

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Introduction. TSE strains are routinely identified by their incubation period and vacuolation profile in the brain after intracerebral inoculation and passaging in inbred mouse lines. There are some major drawbacks to this method that are related to the variation in vacuolation that exists in the brains of mice infected with the same TSE strain and to variation between observers and laboratories in scoring vacuolation and determining the final incubation period. We investigated the potential of PrP^{Sc} immunohistochemistry as a possible alternative method to differentiate between TSE strains in mice.

Materials and Methods. TSE reference strains ME7, 87A/87V, 22A/22C, 79A/79V and 301C/301V were intracerebrally inoculated in RIII ($sinc^{s7}$ / $L_{108}T_{189}$) or VM ($sinc^{p7}$ / $F_{108}V_{189}$) inbred mice. At terminal disease, the brain was collected and fixed in 10% phosphate buffered formalin, cut into coronal or sagittal sections and processed routinely into paraffin. Sections were pretreated with formic acid and citrate autoclaving and immunostained with Mab 6C2 (mouse PrP epitope $_{110}$HVAGAAA$_{116}$) or 2G11 (mouse epitope $_{149}$YRENMY$_{154}$). Immunohistochemical PrP^{Sc} profiles were drawn up by scanning light microscopy with a BX51 microscope equipped with a motorised stage and Cell D® imaging software (Olympus, Germany). True color images were phase color coded according to the intensity of the DAB immunolabelling with light brown pixels being displayed as yellow, middle brown pixels as red and dark brown pixels as dark red (intensity cut-offs at 60 and 130 on a scale of 255).

Results. Differences in the PrP^{Sc} profiles were most clearly visible in the cerebral cortex and the cornu ammonis and dentate gyrus regions of the hippocampus. A further differentiation could be made by the differences in the immunostaining in the cerebellar cortex and the overall PrP^{Sc} staining pattern. Based on these 4 immunohistochemical features, a decision tree could be drawn up to discriminate the TSE reference strains in RIII and VM mice.

Conclusion. A practical decision tree was designed that is useful for future strain typing work in RIII and VM mice. These data will be used as the basis for further studies to examine whether TSE strains in ruminants can be identified already after primary passage in mice and whether mixtures of strains can be recognized within a single mouse brain. In addition, we will be able to determine to what extent TSE strains adapt after passage from the natural host into mice.

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

on resistant genotype increment. This phenomenon has been described also in other Italian regions. This is probably due to the poor information of farmers resulting in a consequent reluctance towards the implementation of a genetic selection program while preferring to select animals on the basis of their productive performances or phenotypic characteristics.

Regarding to the correlation between the production purpose of the animals and the genotype, it appears that there is a greater attention to dairy sheep probably due to the fact that often this activity is predominant. Furthermore meat animals are slaughtered very young, under the age prescribed for scrapie control (18 months), resulting in less motivation of the farmer to implement a plan of genetic selection.

P.97: Comparative approach between two different Real Time PCR tests to select the most rapid and efficient method: the identification of single nucleotide polymorphisms in codons 136, 154, 171 and 141 to evaluate the susceptibility to SCRAPIE

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Keywords: genotype, Scrapie, RT-PCR

Prion diseases are transmissible spongiform encephalopathies in humans and animals, including Scrapie in sheep. Although Scrapie is an infectious disease, susceptibility/resistance in sheep is influenced by polymorphism of the Prp gene, which encodes the PrP^{C}. The RT-PCR is now being seen as a 'gold standard' for comparative purposes across a number of disciplines inclusive of regulation and legislation. For a given RT PCR assay, measurement uncertainty estimation helps identify components of variability and make reasonable estimates of these components' effects upon the end result. Current quality regulations dictate that any result from an analytical laboratory should be given with an associated uncertainty estimation, and this is now included under the remit of ISO 17025. The objective of this study was to compare the field performances between two different tests in Real Time PCR, to select the most rapid and efficient method to identify single nucleotide polymorphisms in codons 136, 154 and 171, and then to evaluate the susceptibility to SCRAPIE, in the regional sheep population. Fourty samples with known genotype have been tested with both Kits. The kits of this study were: Scrapie Genotype Plus Kit for Classic and Atipical scrapie, which allows the study of codons A136V, R154H, R171H, R171Q and L141F, involved in atypical scrapie, kit is produced by the Real Gene. These kits were compared with LightMix 480HT Scrapie Susceptibility Mutation Detection Kit, for the detection of ovine prion protein (PRNP) gene mutations in the codons 136, 141, 154 and 171 in a single reaction. DNA extraction samples were carried out with the kit " Illustrates blood genomicPrep mini spin kit" manufactured by the company GE Healthcare. The validation indexes obtained with different diagnostic methods show a sensitivity of 100%, a specificity of 100%, an accuracy of 100% and a concordance (Kappa) 1.00 (95% IC:0.98-1.00). The main difference between the kits used in this study is the different time necessary for to obtain genotype whole. With the LightMix 480HT Scrapie Kit the detection of ovine prion protein (PRNP) gene mutations in the codons 136, 141, 154 and 171 is possible in a single reaction wells, while with the Scrapie Genotype Plus Kit is possible to program the instrument just once to be able to use protocols relative to more polymorphisms, and consequently it will be possible to perform directly on the same plate, in the same run, the analysis of more polymorphisms but in different reaction wells.

P.98: Gliosis in natural Scrapie

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Gliosis has been widely described in Transmissible Spongiform Encephalopathies (TSEs) as a constant finding always present with vacuolation and PrP^{Sc} deposits, but few efforts have been made to exhaustively deal with the role of these cells in this group of diseases.

In the present study a model of ovine natural Scrapie was used in order to determine the role of glia in TSEs. To determine the glial role in the spread of the disease through the assessment of those changes in relation to glial populations regarding different clinical stages of the disease has been intended by using this animal model. With this aim, sheep at different stages of Scrapie (pre-clinical, clinical and terminal) were included in the study. Histological and immunohistochemical techniques have been carried out on sagittal sections corresponding to cerebella from affected and non affected cases.

The most relevant results concerning this TSE model associated with distribution and morphology of astroglial and microglial cells in relation with neurons are described in the present study. The involvement of glial population in the propagation of the disease has been evidenced here.

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P.99: Transgenic mice expressing the CJD^{178} and FFI mutations do not develop spontaneous prion infectivity

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Fatal familial insomnia (FFI) and a subtype of genetic Creutzfeldt-Jakob disease (CJD^{178}) are clinically and neuropathologically distinct diseases linked to the D178N mutation in the gene encoding PrP^{C}. The disease phenotype is determined by the M/V polymorphism at codon 129 of the mutant allele: D178N/M129 segregates with FFI, while D178N/V129 is associated with CJD^{178}. We have generated transgenic Tg(FFI) and Tg(CJD) mice that express the mouse PrP homologues of these mutations (moPrP D177N/M128 and D177N/V128) with or without the epitope for antibody 3F4. These mice synthesize misfolded forms of mutant PrP in their brains that are detergent insoluble and mildly PK resistant, and develop fatal neurological syndromes that recapitulate key features of CJD^{178} and FFI.

To test whether prions were generated de novo in the brains of the mutant mice, 10% brain homogenates were inoculated intracerebrally into C57BL/6J, Tg*a*20, Tg(WT-E1) mice overexpressing wild-type moPrP with the 3F4 epitope,[1] and Tg(CJD-G1) mice, which express low levels of 3F4-tagged D177N/V128 PrP and do not get sick spontaneously.[2] None of the animals inoculated with brain homogenates from Tg(FFI) and Tg(CJD) mice developed neurological disease. Moreover, none of the brains from inoculated mice contained PrP that was detergent insoluble or that yielded typical or atypical (i.e. 1E4- or SAF84-immunoreactive)[3,4] PK resistant fragments.

To test the possibility that the brains of the mutant mice contained prions below the threshold of detection by bioassay in mice, we subjected the brain homogenates to serial protein misfolding cyclic amplification (PMCA) in the absence or in the presence of an RML seed. Seeded PMCA produced forms of D177N/M128 and D177N/V128 PrPs that were highly PK resistant, and that induced formation of PrP^{Sc} upon inoculation in Tg*a*20 mice, demonstrating that these molecules are not intrinsically resistant to PrP^{Sc} conversion. In contrast, there was no amplification of PK resistant PrP in the unseeded samples, and they did not induce formation of PrP^{Sc} when inoculated in Tg*a*20 mice. Thus, D177N/M128 and D177N/V128 PrPs in the brains of Tg mice do not undergo spontaneous conversion to PrP^{Sc}. This, in conjunction with the observation that D177N/M128 and D177N/V128 PrPs are structurally different,[5] indicates that the Tg(FFI) and Tg(CJD) phenotypes are encoded by misfolded variants of mutant PrP that are toxic but not infectious.

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P.100: Neuropathological and ultrastractural alterations in transgenic mice expressing the fatal familial insomnia mutation

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Fatal familial insomnia is a genetic prion disorder associated with D178N/M129 PrP mutation. The disease is characterized clinically by loss of sleep, dysautonomia, impairment of attention, learning and memory deficits, and motor signs, such as ataxia and myoclonic jerks. Neuropathologically there is predominant thalamic degeneration, with variable involvement of the cerebral cortex, inferior olivary nuclei, cerebellum and brainstem. We have generated transgenic mice expressing the mouse PrP homolog of the FFI mutation on a PrP-null background, and found that these mice synthesize a detergent-insoluble and mildly protease resistant form of the mutant protein in their brains. Tg(FFI) mice expressing the mutant PrP at ~2X the endogenous PrP level develop a progressive neurological illness characterized by sleep abnormalities, ataxia and alterations of recognition and spatial working memory.

To investigate the effects of the FFI mutation on brain structure we used magnetic resonance imaging. In Tg(FFI) mice at the terminal stage of the illness (~430 days of age) the thalamic and cerebellar volumes were significantly reduced compared to nontransgenic littermates, whereas there were no differences in the hippocampus, frontal cortex and caudatum/putamen.

Tg(FFI) mice showed several neuropathological abnormalities on histological analysis. Immunohistochemistry with the

anti-GFAP antibody revealed astrogliosis mainly in the external layer of the cerebral cortex, in the hippocampus and cerebellum. Prominent microgliosis revealed by the anti-IBA-1 and anti-CD11b antibodies was seen in the hippocampus, cerebral cortex and thalamus. There was moderate PrP deposition in the form of diffuse "synaptic-type" immunoreactivity in the stratum moleculare of the hippocampus, and focal deposits in several subcortical structures including the basal ganglia and fiber tracts.

Examination of Tg(FFI) brains by transmission electron microscopy detected neuronal ultrastructural abnormalities in several brain regions, including the neocortex, hippocampus, thalamus and cerebellum. These included spongiform vacuoles, autophagosomes, autophagolysosomes, multivesicular bodies and increased amounts of lipofuscin. The most consistent finding, however, was a marked alteration of the Golgi, whose cisternae appeared swirled, forming an onion-like structure. These abnormalities were never seen in age-matched nontransgenic controls or Tg(WT) mice overexpressing wild-type PrP.

In conclusion our data indicate that Tg(FFI) mice develop several neuropathological alterations typical of prion diseases. The abnormal Golgi morphology suggests that alterations of the secretory pathway could play a role in the pathogenesis.

P.101: Effect of ARK haplotype on PrP^{Sc} accumulation in placentas from natural scrapie infected sheep

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Susceptibility of sheep to scrapie is influenced by the host PRNP genotype. Large-scale genotyping surveys in different countries have identified rare haplotypes, such as ARK, but their effect on scrapie susceptibility has not been clarified. In natural cases, scrapie has been described in an ARK/ARH sheep from Italy with a weak PrP^{Sc} positivity only in the brainstem[1] and in an ARK/ARQ sheep from Spain with PrP^{Sc} immunolabeling in lymphoreticular and central nervous systems.[2] In experimental scrapie, PrP^{Sc} has also been detected in 2 ARK/ARQ sheep in the lymphoreticular and central nervous systems.[3] PRNP genotype also influences PrP^{Sc} deposition in placenta. Scrapie infected sheep may accumulate PrP^{Sc} in placenta only when the fetus presents a susceptible genotype (VRQ/VRQ, ARQ/VRQ or ARQ/ARQ).

The aim of the present study is to assess the effect of fetal genotype ARK/ARQ on the PrP^{Sc} deposition in placentas from natural scrapie infected sheep and compare this effect with that of fetal genotype ARQ/ARQ.

Five ARQ/ARQ ewes presenting PrP^{Sc} deposits in lymphoid tissue biopsies and clinical signs of scrapie were mated with an ARK/ARK ram. Except for one, all of the infected ewes were euthanized during gestation because they were in a terminal clinical stage of the disease. A total of 9 placentas were collected: 2 placentas at 2, 2.5, 3, and 3.5 months of gestation and 1 placenta at parturition. In addition, 7 placentas from 7 naturally scrapie infected ARQ/ARQ sheep presenting clinical signs and carrying fetuses of ARQ/ARQ genotype were collected at 2.5 (2 placentas), 3 (2 placentas) and 3.5 months (1 placenta) of gestation and at parturition (2 placentas). For each placenta, at least 4 placentomes/cotyledons were sampled for PrP^{Sc} detection by immunohistochemistry (using monoclonal antibody L42) and Western Blot (Prionics-Check Western Small Ruminant test modified).

PrP^{Sc} deposits were not detected in any placenta from fetuses carrying an ARK/ARQ genotype. In placentas from fetuses presenting an ARQ/ARQ genotype, PrP^{Sc} deposits were detected in those collected since 3rd month of gestation and after (3 placentas). At 3rd month of gestation, only 1 of the 2 placentas assessed was positive. These results suggest that placentas from fetuses of ARK/ARQ genotypes are resistant to PrP^{Sc} accumulation or at least, more resistant than those from fetuses of ARQ/ARQ genotypes.

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P.102: Scrapie control in Iceland—past and present

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Scrapie in sheep has been endemic in Iceland for over 130 years and has in the past caused considerable losses to the sheep industry, which is the main farming activity in the country. In 1978 a rigorous scrapie control program was established and since 1986 the strategy has been to cull all scrapie flocks in order to eradicate the disease. In 1993 further enhancements of the program were made, mainly in the practical aspects of handling scrapie cases. After disinfection of premises and a three-year waiting period, farmers can restock with healthy sheep from scrapie-free zones. That plan is still in effect for classical scrapie, but in 2012 different measures for atypical or Nor98 cases were adapted. These include restricted culling of older sheep, testing culled sheep for scrapie and increased surveillance of the affected farm for five years. In the past the fight against other diseases in sheep has undoubtedly affected the control as well as epidemiology of scrapie in Iceland. In the 1930′s the country was divided into 36 movement restriction zones, in an effort to stop the spread of the so called Karakul diseases (maedi/visna and paratuberculosis). A few of these zones, marked by man-made fences or

natural boundaries such as rivers and glaciers, are still scrapie-free. Marketing with live sheep is very limited, mostly from zones considered free of scrapie and import of live sheep from abroad has been banned since the middle of last century. Active surveillance for scrapie has been in practice since 1978, but no cases were detected among healthy slaughter until 2004, when rapid testing was implemented. Most classical scrapie cases in Iceland are still detected through passive surveillance, but majority of atypical cases have been detected through active surveillance. Since 1986, the goal of the Icelandic government has been to eradicate the disease by the means of culling and cleaning. The goal of complete eradication has not yet been reached, but yearly incidence has lowered considerably and is down to a few cases per year. This is a drastic decrease from over one hundred infected farms at the height of the epidemic a few decades ago. On some farms scrapie has been detected in a repeated manner, i.e. the disease is reoccurring despite extensive cleanup and restocking. In the last twenty years, 40% of detected cases came from a farm with a previous history of scrapie.

P.103: Experimental prion disease in mice compromised in repair of oxidative DNA damage

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The role of DNA damage caused by reactive oxygen species (ROS) in prion disease is poorly understood. Here, we report the development and characteristics of experimental prion disease (RML prions) in mice with combined knock-out of base excision repair (BER) pathway enzymes Mutyh and Ogg1 in comparison with isogenic wild-type mice. Base excision repair is the main pathway for removal of DNA bases damaged by reactive oxygen species. Ogg 1 initiates removal of the major oxidation product 8-oxoguanine (8-oxoG) in DNA, and Mutyh initiates removal of adenine that has been misincorporated opposite 8-oxoG. The clinical signs of prion disease appeared unaffected by Mutyh and Ogg1 expression. The prion-induced pathology and lesion profile were similar between the knock-out mice and the controls. Western blot analysis of partially proteinase-resistant PrP revealed that the banding pattern (PrP^{Sc} type) was identical between the two groups of mice. However, the *ogg1* $^{-/-}$ and *mutyh* $^{-/-}$ mice had a significantly shorter clinical phase of the disease.

This shows that the fundamentals of prion propagation and pathological manifestation are not influenced by the BER pathway enzymes studies here, but that progressive accumulation of oxidative lesions may accelerate the final toxic phase of prion disease.

P.104: Association of *AGER* gene polymorphisms with susceptibility to scrapie in goats

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Introduction. Genetic selection towards resistance is a promising approach to control scrapie in goats. Besides known associations of *PRNP* polymorphisms and scrapie, it is desirable to investigate possible correlations between the disease and other genes useful for selective breeding. Previously, we found a linkage between scrapie positivity and an indel polymorphism of the *SPRN* gene, coding for the prion-like Shadoo protein. Now we have investigated the genetic variability of the Receptor for the Advanced Glycation End Product (*AGER*) gene and its implication in susceptibility/resistance to caprine scrapie. We selected this gene since it plays a role in Alzheimer's disease and because interaction between AGER and prion peptides was demonstrated to activate the microglia amplifying neuronal damage.

Material and Methods. Analyses were carried out on 92 goats (29 scrapie positive and 63 negative controls) from different scrapie outbreaks. Two overlapping PCRs were set up based on homologous bovine sequences to amplify the entire *AGER* gene (~ 3 Kb).

Allele and genotype frequencies were calculated for each polymorphism. Their associations with the scrapie status were assessed performing the Chi-Square test and were considered statistically significant for $p < 0.05$.

Results. Analysis of *AGER* genetic variability showed the occurrence of 10 SNPs and one indel polymorphism. Two of these mutations were associated with susceptibility to scrapie. The SNP at position 416 (A→G) in the gene ORF showed a higher frequency of the A allele in cases than in controls ($p=0.029$). This result was confirmed also by genotype analysis: grouped A/G and A/A genotypes were significantly associated with scrapie positivity ($p=0.011$). Moreover, the deletion of a GTGTGT motif at position 989 was significantly associated with scrapie when genotypes were considered in the analysis ($p=0.020$). Allele analysis revealed only a tendency ($p=0.080$), even though consistent with the genotype result.

Conclusions. This study showed that the allele 416-A and the A/G and A/A genotypes of the caprine *AGER* gene are related to susceptibility to scrapie. Consequently, goats carrying the 416-G allele seem to be at lower risk to develop the disease. Furthermore, the allele carrying the GTGTGT deletion of the 989-indel polymorphism was mostly present in scrapie positive goats. Our results demonstrate a correlation between the *AGER* gene and susceptibility to scrapie in goats thus providing an

ancillary target for scrapie selection. Moreover, our findings support the idea that AGER is involved in the mechanism of neuronal dysfunction associated with prion diseases.

P.105: Transmissible Spongiform Encephalopathies rapid post mortem tests in goats: Strategies to improve the efficiency of the active surveillance statutory programme

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Annex X to Regulation (EC) No 999/2001 lists the approved rapid post mortem tests which may be used within the framework of the EU monitoring programmes. Little is known regarding the efficiency of different rapid methods on goats. The authors compared the performance of the *IDEXX HerdChek*® BSE-scrapie, the *Bio-Rad*® *TeSeE*™ *SAP* and the *Bio-Rad*® *TeSeE*™ *Sheep/Goat* tests over 96 central nervous system goat tissues including 20 experimentally obtained BSE positives, 41 scrapie positives and 35 negative samples prepared following the European reference laboratory standard method of homogenate preparation (50% w/v protocol). The relation between age, prion type, PNRP genotype and test performance were further investigated. Clear differences in relative diagnostic sensitivity were shown, being the *IDEXX HerdChek*® BSE-scrapie the best performing system setting its 95% IC sensitivity at 100%. Interestingly, *BioRad*® *TeSeE*™ *Sheep/Goat* test showed a 95% IC sensitivity of 85% on BSE positive samples and an optimistic 82.9% on scrapie ones (considering the only "suspect" result as positive), while *BioRad*®*TeSeE*™ *SAP* reached only a 75% sensitivity on BSE samples but a 90.2% on scrapie positives. The reproducibility was not absolute as some discrepant results in any combination of tests was shown, nevertheless, the lower limit of the 95% CI was always above 0.78 considering the samples as a whole, and always above 0.74 when the stratification by PrP^{Sc} profile was applied, values consistent with a substantial-good-fair to good agreement. All the tests correctly identified the 35 negative samples, showing a 100% specificity. Our findings on BSE samples do not suggest any contribution of the original inocula (bovine or goat BSE) on the test results whereas the mean age of the natural scrapie positive animals set at 70 months, confirming the hypothesis that the sensitivity of detection using brainstem appeared to be dependent on the age of tested individuals. The studies on the correlation between rapid test performance and genotype is ongoing. Considering the current lack of genetic selection for eradicating/controlling classical goat scrapie at population level, the efficiency of the surveillance in place would be crucially dependent on the system used to identify infected flocks.

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P.106: In vitro generated mouse prion protein causes brain neurodegeneration in FVB/N female mice

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Recently we reported that lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 was able to instantly convert mouse prion protein into a beta-rich isoform ($moPrP^{\beta}$) resistant to proteinase K digestion under normal physiological conditions. In this study we tested whether subcutaneously (sc) administered $moPrP^{\beta}$ (29-232) is able to cause prion-like pathology in vivo. Six groups of 15 FVB/N female mice each were treated sc with: (1) Saline, (2) LPS (0.1 µg/g of body weight), (3) $moPrP^{\beta}$ (45 µg/mice), (4) LPS+$moPrP^{\beta}$, (5) RML (Rocky Mountain Laboratory scrapie prions at 10^7 ID units), and (6) LPS+RML. Saline and LPS were administered over a period of 6wk using ALZET® osmotic minipumps (ALZET, Cupertino, CA) implanted sc, whereas $moPrP^{\beta}$ and RML were administered as one time sc injection at the beginning of LPS infusion. Five animals from each treatment group were euthanized at 11wk post-inoculation (pi), with no clinical signs of prion disease. The rest of the mice were left to develop clinical signs associated with prion disease until terminal sickness. Hematoxilin & eosin (H&E) and PrP^{Sc}-stainings were conducted from brain tissues to determine presence of vacuolation and PrP^{Sc} accumulation, respectively. Western blot (WB) analysis and scrapie cell assay (SCA) using L929 cells were also conducted to evaluate presence of resistant PrP (PrP-res). All treatment groups, except for saline, showed mild brain vacuolation at 11wk pi in the cerebral cortex (Cc), thalamus (Th), midbrain (Mb), and cerebellum (Cr) and mild PrP^{Sc} accumulation only in the LPS+RML treatment. Terminally sick mice exhibiting clinical signs of prion disease from the LPS, $moPrP^{\beta}$, and LPS+$moPrP^{\beta}$ treated groups showed widespread brain neurodegeneration in the Cc, Th, Mb, and Cr comparable to positive controls. Computer evaluated vacuolation of terminally sick mice showed numerically larger size vacuoles in the Cr and Mb brain regions of LPS, $moPrP^{\beta}$, LPS+PrP^{β}, and LPS+RML treated mice

vs RML group. The PrP^{Sc}-staining showed intense PrP^{Sc} accumulation in the LPS+RML treated versus RML group. Meanwhile the LPS, $moPrP^{\beta}$, and LPS+$moPrP^{\beta}$ treated animals had mild PrP^{Sc} accumulation compared to saline group. Only the RML-treated mice exhibited PrP-res bands at both 11wk and terminal sickness. The SCA showed PrP^{Sc}-positive signals only in the terminally sick RML-treated mice. In conclusion $moPrP^{\beta}$, LPS, and LPS+PrP^{β} treated mice developed brain neurodegeneration at 11wk and at terminal sickness. In addition, treatment with LPS seems to aggravate PrP^{Sc} accumulation in the RML treated mice. Further research is underway to determine whether $moPrP^{\beta}$ causes an atypical prion disease.

P.107: Metabolomics approach reveals lipid and carbohydrate alterations in the urine of FVB/N female mice subcutaneously infected with RML and RML plus lipopolysaccharide

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The objective of this study was to search for predictive biomarkers of prion disease (PD) in the urine of scrapie infected mice. Two groups of 15 FVB/N wild type female mice were inoculated subcutaneously (sc) either with RML (Rocky Mountain Lab scrapie prion strain) or RML+ lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4. A one time injection of RML (10^7 ID 50 units) was given to the animals with LPS (0.1 μg/g of body weight) infused sc for 6wk using ALZET® osmotic minipumps (ALZET, Cupertino, CA). Uninfected control mice (n = 15) were infused for 6wk with saline only. Five animals from each group were euthanized at 11 wk post inoculation (pi) with no clinical signs of disease, and the rest were left to develop clinical signs of prion disease. All the remaining mice treated with RML+LPS were euthanized prior to 250 days pi after observing typical clinical signs of PD while 20% of the RML-treated mice survived over 600 days. Urine samples were collected at 11wk, one month prior to exhibition of clinical signs (D-1), and before euthanasia (D). A minimum of 3 samples per time point per treatment group were collected for analysis using a combination of direct injection mass spectrometry (Absolute*IDQ* Kit) with a reverse-phase LC-MS/MS Kit. Data were analyzed using MetaboAnalyst 2.0. Comparisons were made in a longitudinal and horizontal manner using the different time points to study the metabolite trend over time from 11wk to D, and comparing both RML-treated groups at each time point, respectively, versus the controls with $P<0.05$ set as cut off value. Results showed various lipid alterations in the urine of RML and RML+LPS infected mice including several sphingolipids, glycerophospholipids, and acylcarnitines. In addition, sugars were also found to be fluctuating in these treatment groups from 11wk to D. Comparisons between the two RML-inoculated treatment groups showed various similarities in the pattern of lipid and sugar fluctuations from 11 wk to D. More research is warranted to validate the identified metabolites in future studies.

P.108: Subcutaneous injection of LPS alone or its combination with mouse recombinant prion PrP or the RML isolates of scrapie prions alters the expression of hepatic genes related to innate and adaptive immunity in FVB/N female mice

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Keywords: liver, recombinant prion protein, genes, innate and adaptive immunity, LPS, RML

The liver is continuously exposed to antigenic loads from gastrointestinal tract via portal vein and blood-borne antigens via hepatic artery. Therefore, it is essential for the liver immune system to be suitably equipped in order to protect itself from pathogens. Previously we reported that lipopolysaccharide converts mouse recombinant PrP into a beta-rich isoform resistant to proteinase K digestion.[1] The objective of this study was to assess alterations in the expression of hepatic genes related to innate and adaptive immunity in response to different treatments. A total of 60 FVB/N female mice were randomly assigned to 6 treatment groups and subcutaneously (sc) administered with: (1) saline, (2) lipolysaccharide (LPS) from *Escherichia coli* 0111:B4, (3) mouse recombinant prion protein (moPrP), (4) moPrP + LPS, (5) Rocky Mountain Laboratory (RML) at 10^7 ID 50 units of RML scrapie prions, and 6) RML + LPS. Mouse PrP (PrP 29-231; 45 μg/mouse) and RML (10^7 ID 50 units of RML scrapie prions) were administered once at the start of the experiment by sc injection, whereas LPS (0.1 μg/g of BW) and saline were infused sc by ALZET® osmotic mini pumps at 0.11μL/h for 6 wk at the beginning of the experiment. We used the mouse innate and adaptive immune responses RT² profiler™ PCR array and comparisons were made between the treatment groups and saline treated negative control. Fold change > 1.5 and $P < 0.1$ were taken as cut-off values to identify differentially regulated genes. The results indicated that LPS altered the expression of nine hepatic genes *(Ccr5, Cxcl10, Irf3, Irf7, Lyz2, Mbl2, Myd88, Nfkbia, Stat1)* related to innate and adaptive immune responses, whereas moPrP modulated the expression of *Cxcl10, Il6, Mbl2 and Tlr5* only. Interestingly, when LPS was combined with RML a greater number of genes were differentially expressed *(Casp1,*

Ccr5, Cd80, Cd86, Cxcl10, Il1a, Il1b, Lyz2, Nlrp3, Tlr2, Tlr5, and Tlr7). On the other hand, the combination of moPrP with LPS altered the expression of *Casp1, Crp, Cxcl10, Tlr5,* and *Tlr7.* RML alone affected the expression of only the *Nlrp3* gene. In conclusion, moPrP or RML alone or their combination with LPS altered the expression of hepatic genes related to innate and adaptive immune responses in FVB/N mice. More research is warranted to understand the reason for the aberrant expression of these genes in the liver.

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P.109: Altered expression of genes involved in insulin signaling in the liver of FVB/N female mice injected subcutaneously with lipopolysaccharide and mouse recombinant prion protein

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Keywords: mice, recombinant mouse prion protein, lipopolysaccharide, liver, insulin signaling, gene expression

Perturbation of insulin contributes to pathogenesis of various neurodegenerative disorders like Alzheimer's, Parkinson's, and Huntington's disease. Given the implication of insulin signaling pathway in various neurodegenerative diseases, we hypothesized that there might be aberrant expression of hepatic genes involved in insulin signaling pathway in liver of terminally sick mice following different treatments. To compare the gene expression differences between the treatments and the negative control group, 60 FVB/N female mice were randomly assigned to 6 treatment groups and subcutaneously (sc) administered with: (1) saline (negative controls), (2) lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4, (3) mouse recombinant prion protein (moPrP) converted to β-rich isoform by LPS, (4) moPrP + LPS, (5) Rocky Mountain Lab (RML at 10^7 ID 50 units), and (6) RML + LPS. All treatments were conducted at the start of the experiment where saline and LPS (0.1 μg/g of BW) were infused sc by ALZET® osmotic mini pumps at 0.11 μL/h for 6 wk, whereas, moPrP (29-231; 45 μg/mouse) and RML were administered once sc at the beginning of the experiment. The mRNA expression of 84 genes that constitute insulin signaling pathway was analysed by the Mouse Insulin Signaling Pathway RT² Profiler™ PCR Array (SABiosciences, Frederick, USA). In the analysis, comparisons were made between the treatment groups and saline treated negative control where, $P < 0.1$ and fold change > 1.5 were taken as cut-off values to identify differentially regulated genes. Results indicated altered expression of genes in the LPS- and moPr-injected groups. Interestingly, in the LPS treated group *Pparg*, *Frs3*, *Gsk3b*, *Hk2*, *Kras*, *Pik3r2*, *Raf1*, and *Rras2* were up-regulated whereas *Ptprf* and *Acaca* were down-regulated. In this comparison group, *Pparg* was the most up-regulated gene with > 4.0-fold regulation and *Rras2* showed the lowest magnitude of up-regulation (1.66 fold regulation). In moPrP group, a total of 11 genes were differentially regulated where *Bcl2l1, Cap1, Cbl, Grb2, Jun, Kras*, and *Slc2a1* were up-regulated and *Cebpa*, *Ldlr*, *Pklr*, and *Srebf1* were down-regulated. The expression of *Cebpb* was up-regulated in moPrP + LPS group, whereas the expression of 6 genes (*Acaca*, *Dok2*, *Grb2*, *Igflr*, *Prkci* and *Slc2a1*) were differentially regulated in RML + LPS group. The expression of genes in the RML group remained unchanged except for *Pparg*, *Shc1*, and *Slc2a1.* In conclusion moPrP, RML, and LPS treatments and combination of RML with LPS altered the expression of hepatic genes related to insulin signaling pathway.

P.110: Investigation of genetic susceptibility of goats to scrapie using PMCA

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Breeding programs of sheep for the selection of genetically-resistant populations have been adopted in the EU as the main strategy against scrapie. However, a similar approach for the control of scrapie in goats is painfully lacking. Studies in goats evidenced a plethora of PrP polymorphisms, some of which in particular Q222K and N146D/S, have been associated with resistance to scrapie. Still open questions are related to the resistance contribution of each allele when in homozigosity or with respect to different circulating scrapie strains. Here we studied the genetic susceptibility of goats to scrapie by PMCA, a technique able to reproduce several aspects of prion biology, including the in vivo genetic susceptibility of sheep to scrapie.[1] For this purpose, we used brain homogenates from transgenic mice expressing wt PrP^C (Tg501), 222K PrP^C (Tg516), or a combination of the two to mimic the heterozygosis, as substrates for PMCA. Scrapie seeds were selected among scrapie isolates from different EU countries which have been previously studied by bioassay. For each seed/substrate combination, an amplification factor (AF) was calculated as already reported.[1] For each inoculum, the protective effect of K222 was calculated as AF^{wt}/AF^{222K}.

Preliminary data were obtained from three biologically different sources of scrapie, i.e. Italian sheep and goat isolates and

a Greek goat isolate. We found that the AF observed with wt substrates was ~10 times higher for Italian isolates compared to the Greek one. With both sources, K222 PrP exerted an obvious protective effect, either in homozygosis and heterozygosis, giving in all cases very low or null AFs. However, by comparing AFs obtained in wt and K222 substrates, the protective effect of K222 for Italian isolates was higher than for the Greek isolate, being ~100 and ~4, respectively.

Overall, our preliminary data confirm that K222 PrP is protective against goat scrapie and suggest that this effect might be similar in homozygous and heterozygous goats. However, our findings also emphasize that PrP polymorphisms might actually exert variable protective effects depending on the scrapie strain. Interestingly, the lower protective effect of K222 observed with the Greek isolate is consistent with the detection of scrapie in K222 goats from heavily affected flocks in Greece,[2] but not in Italy.[3]

Further studies using additional isolates and PrP substrates will help to better clarify the genetic susceptibility of goats to scrapie.

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P.111: Detection of classical scrapie in a sheep with blood chimerism

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Chimerism is the presence of two or more genetically distinct cell populations within an individual animal. Thought to result from the in utero transfer of stem cells via placental anastomosis, chimerism is known to occur in multiparous small ruminant pregnancies. Scrapie is an invariably fatal transmissible spongiform encephalopathy (TSE) of sheep and goats, and susceptibility to disease is heavily influenced by polymorphisms in the host prion protein gene (PRNP). In sheep, genetic resistance is associated with particular amino acid combinations at codons 136 and 171 such that AA at 136 (136AA) and RR or QR at 171 (171RR or 171QR) are associated with high resistance to classical scrapie while 136AA 171QQ animals are considered susceptible. Here we describe the diagnosis of classical scrapie in an animal found to possess circulating cells expressing two disparate PRNP genotypes.

Through the course of disease control actions on a classical scrapie infected sheep farm, PRNP genotyping was conducted on blood samples from the flock using a real-time PCR assay. This allowed the establishment of a genetically resistant cohort (136AA 171QR and 171RR) which would remain on site following cleaning and disinfection of the premises. Several months later, a mature ewe was found dead on the farm and PrP^{Sc} was detected in the brainstem and lymph nodes by ELISA, western blot and immunohistochemistry in a manner consistent with classical scrapie. Repeat PRNP analysis of DNA extracted from the initial blood sample was compared with DNA extracted from brainstem, lymph node and hair follicles. Blood and lymph nodes produced a 136AA 171QR genotype while the brainstem and hair follicle were found to be 136AA 171QQ. Consistent with chimerism, the male-specific SRY gene was detected in blood and lymphoid tissues but not in the brain and hair follicles of this ewe. Further, microsatellite analysis of tissues demonstrated the existence of greater than 2 alleles at several sites, and an overabundance of one allele over another at other sites supportive of blood chimerism. To our knowledge this represents the first report of classical scrapie in a sheep with blood chimerism and suggests consideration of this phenomenon when interpreting PRNP genotype results.

P.112: Safety, specificity and immunogenicity of a PrP^{Sc}-specific prion vaccine based on the YYR disease specific epitope

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Prion diseases represent a unique class of infectious disease where the induced or spontaneous misfolding of a self-protein, PrP^{C}, results in the generation of an infectious species, PrP^{Sc}. PrP^{Sc} then acts as a template or seed to promote the misfolding of additional PrP^{C} in an auto-catalytic and self-propagating manner. Alterations in PrP protein structure during this conversion process result in selective exposure of antigenic determinants in the misfolded species that are concealed in non-pathogenic PrP, referred to as disease specific epitopes (DSEs). Our priority is to design vaccines based on these regions that are capable of generating strong antibody responses that are highly specific for the misfolded conformation, thus circumventing potential adverse consequences of PrP autoreactivity. Here, we describe translation of the YYR prion DSE into a PrP^{Sc}-specific peptide vaccine focusing on our investigations

of vaccine immunogenicity, specificity, and safety. Importantly, in order to ensure real-world applicability of candidate vaccines, all reagents and approaches are consistent with safety, financial, and regulatory constraints applicable to commercial veterinary vaccines. Induction of robust IgG antibody responses was achieved through optimization of the YYR epitope sequence and length, coupled with fusion to the highly immunogenic carrier protein Leukotoxin. Specificity for the misfolded conformation was verified through DSE antibody reactivity with PrP^{Sc}, and not PrP^{C}, in immunoprecipitation assays with infectious and non-infectious brain homogenate. In addition, the DSE-vaccines exhibited strong safety profiles, as DSE-antibodies were incapable of facilitating template-directed misfolding in both in vitro and in vivo systems. Although the DSE antibodies remain non-reactive with wild-type PrP^{C}, we have demonstrated that mutations in PrP associated with familial prion disease induce structural changes enabling DSE antibody binding, thus complicating the potential translation of conformation specific DSE vaccines into human therapies for familial prion disease. Currently, our DSE-based peptide vaccines are being assessed for in vitro neutralization of PrP^{C}-PrP^{Sc} conversion using cell-based assays, as well as in vivo efficacy through challenge trials in several target species of interest.

P.113: Identification of ante-mortem prion disease biomarkers

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Keywords: Creutzfeldt-Jakob disease, ante-mortem, biomarkers, 14-3-3, neuron-specific enolase

Ante-mortem testing of Creutzfeldt-Jakob disease (CJD) is currently performed once clinical symptoms are present, during the terminal stage of disease. Surrogate markers are used in combination with other methods for differential diagnosis of CJD; however, post-mortem analysis of the diseased brain is the only definitive diagnosis. The identification of pre-clinical disease markers would offer efficacy in diagnosis, as well as provide further insight into mechanisms involved with neurodegeneration and facilitate development of drug efficacy regimes. To identify pre-clinical ante-mortem markers of prion disease, we adapted prion disease to rats, facilitating a comparative analysis between species. The rat prion infection allows us to identify and assess the abundance of prion disease biomarkers at multiple time-points during the course of the disease. This contrasts with human samples, which are generally only available at clinical stage. We are analyzing a readily available bio-fluid, cerebrospinal fluid (CSF), as the composition has been found to reflect the pathological processes of the brain. Proteomics were assayed using mass spectrometry, comparing clinically affected rats with uninfected rats. Results revealed a number of proteins up-regulated and/or specific to prion disease. These proteins included CJD biomarkers 14-3-3s and neuron-specific enolase (NSE). Biomarkers from prion-infected rat CSF are similar to those detected in CJD, demonstrating the utility of using rat prion disease for biomarker identification. By tracking the progression of prion infection in rats, we will be able to further define and compare the pathophysiological changes associated with prion infection to better understand mechanisms of neurodegeneration. Identifying molecular biomarkers of prion disease during pre-clinical phase and clinical phase of disease may lead to the development of a pre-clinical ante-mortem screening test for prion diseases.

P.114: Adaptation of prion-infected brain organotypic cultures to other species and brain regions

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Keywords: prion, organotypic culture, CWD, cortex, hippocampus, cerebellum

Introduction. The development of the prion organotypic cerebellar slice culture assay (POSCA)[1] has generated a powerful tool for the study of prion disease pathogenesis, as the model itself undergoes aspects of prion pathology, including neuronal loss[2] and a reduction in Purkinje cell spine density.[3] Because this technique is based on a mouse model, we theorized that it should be possible to substitute a transgenic mouse into the system. Here we show that the POSCA can be adapted to a cervidized mouse model and infected with chronic wasting disease (CWD). In addition, we are able to infect non-cerebellar brain regions with prion strains.

Materials and Methods. Ten to twelve day old tga20 or tg33 mouse pups were sacrificed for the experiments. Tga20 mice are on a C57Bl6 background and overexpress mouse PrP^{C} by six times. Tg33 mice express deer PrP^{C} (wildtype Q95G96 polymorphism) on a mouse *Prnp* knockout background. Cerebellar slice cultures were prepared as described previously.[1] For coronal sections through the frontal cortex and hippocampus, whole brains were fixed in low melting point agarose and cut into 250µM sections using a vibratome. Slices were then plated on a 30mm diameter Millicell insert with 0.4µM pore size and infected with rodent-adapted scrapie. For rodent-adapted scrapie infections, slices were treated with 10ug/mL brain homogenate from uninfected control mice or infected terminally ill mice. For CWD infections, slices were treated with 100ug/ml or 1mg/ml of CWD from terminally ill wild-type deer (homozygous for Q95G96 polymorphism). Infection was confirmed by Western blotting for

Proteinase K-resistant PrP using SAF83 (rodent-adapted scrapie) or Bar224 (CWD).

Results. PK-resistant PrP is detectable at day 42 in coronal slices infected with rodent-adapted scrapie. In the CWD model system, PK-resistant PrP is detectable at day 35 (1mg/ml) and day 43 (100ug/ml) and increases over the duration of study (70 days). Interestingly, the banding profile of the replicated CWD PrP is distinct from the input PrP^{CWD}.

Conclusion. Our results demonstrate that the POSCA is a versatile technique that it is not only suitable for rodent-adapted scrapie studies, but also for prion strains from other species. Additionally, we show that an adapted version of the POSCA technique can be used to study other brain regions to investigate the susceptibility of different brain regions to prion infection, removing the confounder of potentially different trafficking efficiency of prions to these brain regions in the brain in vivo.

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P.115: Impact of humic acids on PrP^{CWD} fate in soil environment

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The geographic range of chronic wasting disease (CWD) continues to expand among cervids in North America. In Canada, expanding of CWD in Alberta in Saskatchewan will threaten northern caribou herds. Soils can serve as an environmental reservoir for infectious PrP^{CWD}; prions bound to soil compounds remain infectious in the soils for many years. Humic acids (HA) are principal component of soil system, part of soil organic matter, and the complex mixtures of polyphenol–polycarboxilic acids with colloidal and supramolecular characteristics. HA are natural polyanions that result among the most reactive compounds in the soil and possess the largest specific positive-charged surface area. HA content and composition depend on soil type and vary from 0.1 to 5% in surface soil mineral horizons. Soils of southern regions of Canadian prairie provinces, where CWD is endemic, have higher HA content compared to northern soils. HA along with other soil constituencies bind prions but, due to the large variability of the micro-chemical environments in soils, the PrP sorption in soils is very difficult to predict. Analysis of HA-prion interactions and their impact on PrP^{CWD} infectivity is one of key issue to study fate of PrP^{CWD} in soil environment.

PrP^{CWD} was incubated with different concentration solutions of purified HA and detected by western blot. Reduction in PrP^{CWD} molecular weight as well as decreased PrP^{CWD} recovery were observed with increases of HA concentration. Low recovery of PrP^{CWD} suggests that prions are either irreversible bound (encapsulate) to soil organic matter or that partial degradation and/or structural changes of PrP^{CWD} occur so that only a portion of PrP^{CWD} remains detectable. The interactions between prions and HA were analyzed using standard purified HA; however, as HA are never present in soils as pure component in solution, the next step in studying HA impact on PrP^{CWD} is testing natural HA extracted from soils of CWD-endemic regions. We are also testing whether PrP^{CWD} in the presence of HA remains infectious or if these interactions could reduce infectivity in contaminated territories.

P.116: Antibodies against PrP^{Sc} in CWD infected deer and a rapid diagnostic test for chronic wasting disease

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Monoclonal antibodies (mAbs) specific for epitopes of the abnormal prion protein isoform (PrP^{Sc}) are required for diagnosis of chronic wasting disease (CWD) and investigation of the disease pathology and epidemiology. In this study, three sets of hybridomas that stably secrete mAbs against PrP^{CWD} were established by immunizing PrP knockout mice with CWD-associated fibril protein (CWDAF) derived from deer infected with wt/wt CWD sample, synthetic mouse PrP peptide and mouse recombinant PrP. The reactivity of these antibodies to PrP^{C} of different species, as well as PK-resistant PrP^{Sc} of four CWD strains infected deer and four prion strains of infected mice and hamsters were investigated.

We report, in addition, a reliable, rapid and sensitive diagnostic test for detection of CWD. The immunoassay employed novel anti-PrP C- and N-termini mAb. We hypothesize that the preservation and stability of N-terminus retained PrP in the CWD-associated aggregate makes it a specific indicator for CWD. The assay involves extraction of insoluble PrP from soluble PrP in tissue homogenates, the solubilization of insoluble PrP fraction in 8M guanidine hydrochloride, capture of PrP^{CWD} using an anti-PrP C-terminus mAb followed by detection with the HRP-labeled anti-PrP N-terminus (aa: 95-104) antibody. The assay does not require protease. The assay highly specific discriminates between normal and CWD-associated PrP in brain homogenates and is potentially applicable for detection of PrP^{Sc} in the cases of other prion diseases.

P.117: Evaluating the standard scrapie cell assay as a tool for screening anti-prion compounds

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The standard scrapie cell assay (SSCA) facilitates quantitative in vitro analysis of prion titres. Given its potential for high-throughput and its robust nature, the SSCA may be useful to examine potential anti-prion compounds. Here we demonstrate the effectiveness of the SSCA in evaluating the inhibition of murine prion strains and CWD isolates using known anti-prion compounds.

Methods. L929 (mouse fibroblast) and C2C12 (mouse myotube) cells were exposed to RML, 22L or ME7 prions (10^2 to 10^7 dilutions of 10% whole brain homogenates, w/v) in 96 well culture dishes. Elk21- (RK13 cells expressing Elk PrP^C) cells were exposed to elk and white-tailed deer CWD (10^2 to 10^7 dilutions of 10% whole brain homogenates, w/v). Inhibition of prion replication was examined using five known anti-prion compounds: Pentosan polysulphate, dextran sulphate 500, iota-carageenan, congo red and amphotericin B. Cells were passaged when confluent (within 5 days), except for the C2C12 myotubes that were maintained as a confluent layer. After the third passage, 20 000 cells were collected and loaded onto ELISPOT plates. Controls included L929, C2C12 and Elk21- cells exposed to uninfected brain homogenates. RK13 cells were exposed to 0.1% (w/v) prion agent to monitor residual inoculum.

Results. The SSCA demonstrated that all 5 anti-prion compounds inhibited prion replication. In the untreated controls, PrP^{Sc} accumulated in both the L929 and C2C12 cell lines. L929 cells responded to all three mouse strains (RML, 22L and ME7), with 22L and ME7 generating the highest signal. In C2C12 cells, RML generated the largest response. Interestingly, the different murine strains (RML, 22L and ME7) displayed different sensitivities to the five anti-prion compounds. The accumulation of PrP^{Sc} in Elk21- cells varied with 0.1% (w/v) elk CWD generating the largest response and CWD from white-tailed deer (0.1% w/v) generating a response almost 5-fold less.

Conclusions and Future Directions. The L929, C2C12 and Elk21- cell lines provide a solid platform for high-throughput analysis of infectious prion titre. All three cell lines respond to either the murine strains (in L929 and C2C12 cells) or CWD isolates (in Elk21- cells). Furthermore, we have demonstrated the added utility of the SSCA in evaluating various anti-prion compounds. Therefore, the SSCA will be effective when examining libraries of potential anti-prion compounds.

P.118: Divergence of chronic wasting disease strains: The effect of single amino acid polymorphisms in the host PrP^C molecule and agent adaptation

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Chronic wasting disease (CWD) is a prion disease responsible for epizootics in free-ranging and captive members of the *Cervidae* family. Susceptibility of cervids to CWD is influenced by the primary amino acid sequence of the host cellular prion protein (PrP^C). In white-tailed deer, *Prnp* alleles including Q95G96 (*wild type* allele), Q95S96 (*S96*) and H95G96 (*H95*) encode PrP^C isoforms that differentially impact susceptibility to CWD infection. Deer with at least one allele copy of *S96* or *H95* exhibit longer survival times following oral CWD infection.[1] Biochemical analysis of CWD allotypes (*wt*/*wt*, *wt*/*S96*, *wt*/*H95*, *H95*/*S96*) revealed differences in disease-related PrP^{CWD} suggesting that changes in the primary sequence of host PrP^C can alter CWD agent properties. Existence of prion agents with distinct biological properties, "strains", has been documented in various susceptible mammalian species. Prion strains impact disease duration, neuropathological features, clinical symptoms, diagnosis and differences in overcoming the species barrier upon transmission to new hosts. Prion strains have been hypothesized to exist as quasi-species of different prion conformations that, upon passage, can be selected depending on the host. We hypothesized that, during CWD infection of deer with different *Prnp* genotypes, a mismatch between the prion protein sequence of the invading CWD agent and the host PrP^C would impact disease characteristics as well as the properties of the adapted CWD agent. We tested this by evaluating the transmissibility of experimentally-derived deer CWD allotypes in transgenic mice expressing *wt* and *S96-Prnp* alleles.[2] *Tg wt-Prnp* mice were susceptible to all CWD allotypes tested and presented with similar disease symptoms, neuropathology and PrP^{CWD} type. *Tg S96-Prnp* mice, previously shown to be resistant to CWD infection,[2,3] succumbed with prion disease only when inoculated with CWD allotypes containing H95-PrP. Clinically affected *S96-Prnp* mice presented symptoms, neuropathology and PrP^{CWD} type that contrasted with the *wt* transgenic line. Our data suggest that amino acid changes in the sequence of deer PrP altered the conformational quasi-species, leading to emergence of conformational variants that, upon transmission, can behave as agents with distinct properties. Our findings indicate that structural modifications associated with the deer *H95-Prnp* allele facilitated the adaptation of a novel CWD strain into a resistant genotype, implicating *Prnp* genotype heterogeneity in the emergence of CWD agents with novel transmission properties.

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P.119: Characterization mesenchymal stem cells obtained from peripheral blood and bone marrow of naturally infected sheep with classical scrapie

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Keywords: Mesenchymal stem cell, prion, scrapie, sheep

Mesenchymal stem cells (MSCs) are a type of adult stem cells with self-renewal and differentiation capacities. MSCs can be infected with prions and have been proposed as in vitro cell-based models for prion replication. In addition, MSC properties related to differentiation and immunomodulation are of particular interest for cell therapy of neurodegenerative diseases. Although autologous-MSCs are widely used for cell therapy, to our knowledge, the effect of the disease in the characteristics of these cells has never been investigated. We present here the isolation and study of MSCs obtained from bone marrow (BM) and peripheral blood (PB) of sheep naturally infected with classical scrapie.

MSCs were isolated from Rasa Aragonesa sheep showing clinical signs of scrapie (ScMSCs) and controls (cMSCs). All the animals displayed the ARQ/ARQ genotype for the *PRNP* gene. Cell characteristics were analysed after three passages of expansion.

ScMSCs obtained from BM displayed similar proliferation and differentiation characteristics to control cells. Only exceptional markers like CD29 were significantly regulated, compared to controls. A slight overexpression of PrP^{C} was detected in BM-ScMSCs. This characteristic could be useful for the development of in vitro model for prion replication.

Besides the infectivity reported in scrapie-infected sheep bone marrow and the expression of PrP^{C} in ovine MSCs, PMCA did not detect PrP^{Sc} in BM-ScMSCs. Ovine MSCs can be infected by prions in vitro but lose the infectivity in a few passages. Further studies are necessaries to clarify if these cells are infected in their niche and lose infection in culture or they were never infected.

Additionally, MSCs can be mobilized and migrate to prion lesions in brain by blood in response to chemoattractive factors. Since autologous PB-MSCs from patients may also be an ideal alternative stem cell source, the characteristics of PB-MSCs obtained from scrapie affected animals are being investigated.

P.120: Development of an *antemortem* test for chronic wasting disease based on RT-QuIC of rectal biopsy and nasal brushings

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Chronic wasting disease (CWD) is an infectious prion disease that affects cervids. Given migratory behavior of elk herds and ease in horizontal transmission of CWD, surveillance on new cases is becoming increasingly relevant. In this scenario, we propose real-time quaking induced conversion (RT-QuIC) analysis of rectal biopsies and nasal brush samples collected from free-ranging elk as novel approach to *antemortem* diagnosis of CWD.

A total of 172 rectal biopsies and 49 matching nasal brushes were collected. Out of 172, 6 rectal biopsies were positive by RT-QuIC, and later confirmed positive by IHC. Only preliminary results on nasal brushes are presented. In summary, our RT-QuIC results showed 100% sensitivity and 100% specificity for rectal biopsies while sensitivity and specificity for nasal brushes are yet to be established.

This application of the RT-QuIC should improve prospects for practical detection of minimal levels of prion seeding activity in easily collectable and diagnostically relevant biological tissues.

P.121: Efficient transmission of prion disease through environmental contamination

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Chronic wasting disease (CWD) is a prion disorder effecting captive and free-ranging deer and elk. The efficient propagation suggests that horizontal transmission through contaminated environment may play an important role. It has been shown that infectious prions enter the environment through saliva, feces, urine, blood or placenta tissue from infected animals, as well as by carcasses from diseased animals and can stay infectious inside soil over several years.

We hypothesize that environmental components getting in contact with infectious prions can also play a role for the horizontal transmission of prion diseases. To study this issue, surfaces composed of various environmentally relevant materials were exposed to infectious prions and the attachment and retention of infectious material was studied in vitro and in vivo. We analyzed polypropylene, glass, stainless steel, wood, stone, aluminum, concrete and brass surfaces exposed to 263K-infected brain homogenate. For in vitro analyses, the material was incubated in serial dilutions of 263K-brain homogenate, washed thoroughly and analyzed for the presence of PrP^{Sc} by PMCA. The results show that even highly diluted PrP^{Sc} can bind efficiently to polypropylene, stainless steel, glass, wood and stone and propagate the conversion of normal prion protein. For in vivo experiments, hamsters were ic injected with implants incubated in 1% 263K-infected brain homogenate. Hamsters, inoculated with 263K-contaminated implants of all groups, developed typical signs of prion disease, whereas control animals inoculated with non-contaminated materials did not.

In addition, in order to study the transmission in a more natural setting, we exposed a group of hamster to habit in the presence of spheres composed of various materials that were pretreated with 263K prions. Many of the hamsters exposed to these contaminated materials developed typical signs of the disease that were confirmed by immunohistological and biochemical analyses.

These findings suggest that various surfaces can efficiently bind infectious prions and act as carriers of infectivity, suggesting that diverse elements in the environment may play an important role in horizontal prion transmission.

P.122: Effect of humic substances on scrapie infectivity experimentally evaluated in lambs: Preliminary results

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Scrapie in sheep and chronic wasting disease in cervids are examples of environmental transmissible spongiform encephalopathies (TSEs). Increasing evidence suggests that soil may serve as a natural reservoir for prion infectivity. Prions may enter the environment via oral, nasal and skin secretions, urine, feces and parturient materials from both preclinical and diseased animals. Several in vitro studies suggest that after ingestion by ruminants, prions in contaminated soil seem resistant to rumen digestion and thus enter the lower gastrointestinal tracts. From here the infectious agents are up-taken across the epithelium and initiate infection along the lymphoid tissues and reach the CNS. It has been hypothesized that the odds of prion infection in free-ranging animals might be increased by high clay content in soil, but the contribution of soil humic substances (HS) (i.e. supramolecules able to polymerize and aggregate, forming micelles and supramolecular ensembles) to environmental TSEs has been so far neglected. Recent results have shown that prions may be strongly retained in soil rich in organic matter, which would thus reduce the odds of infectivity among grazing lands. The antiprion activity of HS might contribute to reduce prion accessibility in the intestinal tract of animals, as well as prevent the environmental transmission of TSEs among ruminants. To this purpose, we tested in vivo the ability of HS to reduce prion accessibility in lambs. The animals were divided into four groups: scrapie-infected brain homogenates administered per os (n=8, positive control), scrapie-infected brain homogenates and HS per os (n = 8), scrapie-infected brain homogenates and HS via intragastric gavage n = 8) and HS per os (n = 4, negative control). The animals were kept for 8 months; during this period they were fed hey and feed. One animal per group was sacrificed on a monthly basis (negative controls every two months) to investigate PrP^{Sc} diffusion to the target organs. Tonsils, thymus, viscera, SNC, nervous ganglia, lymph node samples were collected from each lamb and tested through immunohistochemistry (IHC) and ELISA. Through investigation into prion levels in different tissues of these animals, this project aims at providing preliminary results on the correlation between soil organic matter and the infectivity of prions released in soil.

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P.123: Experimental transmission of classical and atypical BSE in goats

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Background. In small ruminants, natural transmission of classical BSE (C-BSE) has been described only in goats,[1,2] but there are no data about the transmission of atypical BSE in this species.

Objectives: To investigate the transmissibility of L-type BSE (BASE) in Saanen goats and to characterize disease phenotype in comparison to C-BSE.

Materials and methods. Twenty goats were divided into groups and they were challenged with C-BSE and BASE by intracerebral and oral route. Histopathological examination, immunohistochemistry and Western Blot analysis were performed on samples collected from all the animals.

Results. C-BSE was successfully transmitted via the intracerebral route: vacuolar changes were mainly detected at the level of basal ganglia, thalamus and brainstem and PrP^{Sc} deposition was characterized by granular and glial patterns. Molecular features of C-BSE agent in goats were undistinguishable from those of the original inoculum. At 29 months post inoculation, scratching, broken hair and alopecia were the only clinical signs shown by BASE goats challenged by intracerebral or oral route.

Conclusions. These data confirm that goat is susceptible to C-BSE agent; however longer incubation period and mild clinical signs let hypothesize a major resistance of this species to BASE agent.

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P.124: Prion protein interaction with and adsorption by soil humic substances

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Scrapie and chronic wasting disease are the only transmissible spongiform encephalopathies (TSEs) that appear to be environmentally transmissible. Increasing evidence suggests that soil may serve as a natural reservoir of prion infectivity. Attachment to soil particles is likely to influence the persistence and infectivity of prions in the environment. Soil with high clay content but poor in organic matter may enhance prion transmission. The potential of natural soil organic matter in affecting prion replication and infectivity has not been fully clarified. Here, we present evidence of the interaction between humic substances (HS) with recombinant mouse (Mo) PrP (used as surrogate for PrP^{Sc}). Natural HS interact with MoPrP forming insoluble adducts. Biophysical and atomic force microscopy imaging experiments showed that MoPrP retains its native folding when it is encapsulated in ordered structures made of HS. The PrP-HS complex is protected from protease-K. Interestingly, HS induced elimination of prions from chronically scrapie-infected mouse hypothalamic cells, ScGT1, in a dose-dependent manner. Prion encapsulation in HS may be highly environmentally relevant for soil rich in organic matter. Prions should be strongly retained in soils with a higher organic matter content, which would thus reduce the odds of infectivity among grazing lands. The anti-prion activity of HS might contribute to reduce prion bioavailability in the intestinal tract of free-ranging animals and prevent the environmental transmission of TSEs among ruminants.

Acknowledgments. This study was financed by the Italian Ministry of Agricultural, Food and Forestry Policies (SCRASU Project).

P.125: Cell substrates used to produce biologics failed to propagate the agent of atypical bovine spongiform encephalopathy (L-BSE)

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Keywords: cell culture, biologics, prion, atypical BSE

Background. TSE agents have contaminated human-tissue-derived therapeutics and animal vaccines. Many biologics are prepared in cell cultures. Although most cultures have resisted infection with TSE agents, a few are susceptible.

We are investigating susceptibility of several cell lines to infection with the agents causing classical (C-BSE) and atypical BSE (L-BSE, BASE).

Methods. We inoculated brain suspensions from cattle with C- or L-BSE into several cell lines used to manufacture biologicals. As positive control, NIH-3T3 and L929 fibroblasts were exposed to 22L scrapie agent. Cells exposed to TSE agents were serially propagated for 30 passages and samples tested for TSE-associated PrP (PrP^{TSE}).

Tissue homogenate from frontal cortex of a naturally infected cow, used as L-BSE inoculum, was biochemically characterized and infectivity assessed by experimental interspecies transmission studies.

Results. No PrP^{TSE} has been detected in any C-BSE- or L-BSE-exposed cell line under study so far, and infectivity bioassays have been negative for C-BSE to date. Known susceptible murine cells exposed to mouse-adapted 22L scrapie agent as positive controls accumulated PrP^{TSE}, and infectivity was detected by bioassay in mice.

Conclusions. To date, no candidate cell substrate exposed to C-BSE or L-BSE-derived agents has accumulated PrP^{TSE} or propagated a TSE agent.

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The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Administration determination or policy.

P.126: Successful transmission of chronic wasting disease (CWD) into mice over-expressing bovine prion protein (TgSB3985)

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Keywords: chronic wasting disease, transmission, transgenic mouse, bovine prion protein

Background. CWD is a disease affecting wild and farm-raised cervids in North America. Epidemiological studies provide no evidence of CWD transmission to humans. Multiple attempts have failed to infect transgenic mice expressing human PRNP gene with CWD. The extremely low efficiency of PrP^{CWD} to convert normal human PrP^{C} in vitro provides additional evidence that transmission of CWD to humans cannot be easily achieved. However, a concern about the risk of CWD transmission to humans still exists. This study aimed to establish and characterize an experimental model of CWD in TgSB3985 mice with the following attempt of transmission to TgHu mice.

Materials and Methods. TgSB3985 mice and wild-type FVB/NCrl mice were intracranially injected with 1% brain homogenate from a CWD-infected Tga20 mouse (CWD/Tga20). TgSB3985 and TgRM (over-expressing human PrP) were similarly injected with 5% brain homogenates from CWD-infected white-tailed deer (CWD/WTD) or elk (CWD/Elk). Animals were observed for clinical signs of neurological disease and were euthanized when moribund. Brains and spleens were removed from all mice for PrP^{CWD} detection by Western blotting (WB). A histological analysis of brains from selected animals was performed: brains were scored for the severity of spongiform change, astrogliosis, and PrP^{CWD} deposition in ten brain regions.

Results. Clinical presentation was consistent with TSE. More than 90% of TgSB3985 and wild-type mice infected with CWD/Tga20, tested positive for PrPres in the brain but only mice in the latter group carried PrP^{CWD} in their spleens. We found evidence for co-existence or divergence of two CWD/Tga20 strains based on biochemical and histological profiles. In TgSB3985 mice infected with CWD-elk or CWD-WTD, no animals tested positive for PrP^{CWD} in the brain or in the spleen by WB. However, on neuropathological examination we found presence of amyloid plaques that stained positive for PrP^{CWD} in three CWD/WTD- and two CWD/Elk-infected TgSB3985 mice. The neuropathologic profiles in CWD/WTD- and CWD/Elk-infected mice were similar but unique as compared to profiles of BSE, BSE-H or CWD/Tg20 agents propagated in TgSB3985 mice. None of CWD-infected TgRM mice tested positive for PrP^{CWD} by WB or by immunohistochemical detection.

Conclusions. To our knowledge, this is the first established experimental model of CWD in TgSB3985. We found evidence for co-existence or divergence of two CWD strains adapted to Tga20 mice and their replication in TgSB3985 mice. Finally, we observed phenotypic differences between cervid-derived CWD and CWD/Tg20 strains upon propagation in TgSB3985 mice. Further studies are underway to characterize these strains.

P.127: Carotid body involvement in terminal experimental prion disease

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Introduction. Blood-borne transmission of prions has been demonstrated in animals, including humans. Therefore innervated tissues intimately associated with blood may serve as routes for neuroinvasion following exposure to prions. Chemosensitive cells in the carotid body (CB) monitor arterial levels of oxygen, carbon dioxide and pH to influence respiration. The central projections of the sensory fibers that innervate the chemosensitive cells of the CB terminate in the solitary nucleus in the medulla, reported to be an early site of accumulation of the infectious isoform of the prion protein (PrP^{Sc}) in infected animals. To determine if the CB supports accumulation of PrP^{Sc} we collected the CB of uninfected animals and animals in the terminal stages of prion disease and processed tissue sections for the presence of PrP^{Sc}.

Methods. Blocks of tissue surrounding the bifurcation of the common carotid artery were taken from uninfected (n = 4) or clinically-ill male golden Syrian hamsters intracerebrally (ic) inoculated with 22AH-, 22CH- or 139H-infected brain homogenate (n = 4–5 in each group). The block of tissue with the CB, also contained the superior cervical ganglia (SCG), nodose ganglia (NG), and anterior cervical lymph nodes (LN). The tissue block was embedded in paraffin and serial sections were cut using a microtome. A series of 4 adjacent tissue sections spaced not further than 250 µm apart were stained with periodic acid-Schiff or immunohistochemically processed for the presence of the prion protein. In addition, select tissue sections were immunohistochemically processed for tyrosine hydroxylase, a marker of catecholaminergic neurons, to assist in differentiating sympathetic ganglia from parasympathetic sensory ganglia.

Results. PrP^{Sc} was not detected in any of the chemosensitive cells of the CB, or in neurons of the SCG or NG, or in the LNs of uninfected animals. PrP^{Sc} was detected in the chemosensory cells of 11/12 CB of infected animals. PrP^{Sc} was also detected in the germinal centers of 13/14 LNs, and in neurons in the SCG and NG of all infected animals.

Conclusions and significance. Chemosensitive cells of the CB support prion replication. The chemosensitive cells of the CB have the potential to be involved in the centripetal and/or centrifugal spread of prions in infected animals.

P.128: Prion strain targeting independent of strain-specific extraneural transport

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Prion strains are characterized by strain-specific differences in neuropathology, incubation period, clinical disease, host-range and tissue tropism. Prion strains can have distinct PrP^{Sc} fibril structure, aggregate sizes and conformational stability consistent with the hypothesis that the conformation of PrP^{Sc} encodes prion strain diversity. The relationship between these strain specific properties of PrP^{Sc} and prion pathogenesis is poorly understood. The hyper (HY) and drowsy (DY) strains of hamster-adapted transmissible mink encephalopathy (TME) have distinct PrP^{Sc} conformations and differ in tissue tropism and susceptibility to infection by extraneural routes of infection. While it is known that DY TME is not detected in secondary lymphoreticular system tissues of infected hosts, the underlying mechanisms are not known. To explore the strain-specific differences in tissue tropism, we systematically compared the pathogenesis of these two strains by utilizing several extraneural routes of infection. Inoculation of hamsters with HY TME agent by extranasal (e.n), intraperitoneal (i.p) and intranerve (i.n.) inoculation resulted in the development of clinical disease. Hamsters inoculated by the same routes with the DY TME agent resulted in clinical disease following i.n. inoculation but not following e.n. or i.p. routes of inoculation. To determine where the restriction in DY TME pathogenesis occurs prior to neuroinvasion we examined the spread of PrP^{Sc} at early time points post extraneural inoculation. Following e.n. inoculation we found DY PrP^{Sc} crossing the nasal epithelia via paracellular transport and within lymphatic vessels of the lamina propria of the nasal cavity within minutes of inhalation. Blood from these animals has quaking-induced conversion seeding activity that was not observed in mock-inoculated controls. These results indicate that the failure of DY TME agent to cause disease is not due to deficiencies in transepithelial transport or transport to blood. Next we asked if DY PrP^{Sc} transport to secondary lymhoreticular (LRS) system tissues was restricted following i.p. infection. DY PrP^{Sc} was detected in LRS tissues at early time points post infection, similar to HY TME positive control animals. Overall, differences in tropism are not due to strain-specific differences in transport, similar to what we have observed in the CNS.

P.129: Early prion trafficking in deer exposed to chronic wasting diseases

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The presumed natural route of prion exposure in chronic wasting disease (CWD) is through environmental aerosol exposure and/or oral ingestion. In animal studies that have mimicked these environmental exposures through intranasal (IN) or oral inoculation (PO), detection of prion trafficking relies on replication of the misfolded prion isoform, PrP^{CWD}, in tissues to reach a threshold of detection using traditional assays such as western blot and immunohistochemistry. The earliest PrP^{CWD} has been detected in tissues using these methods is 3 months post inoculation by biopsy of tonsil of live deer or 1.5 months in pharyngeal lymph nodes of necropsied deer. However, the locations of uptake after PrP^{CWD} exposure and routes of trafficking prior to this time point remain unknown. Here we study Reeve's muntjac deer and white-tailed deer inoculated with CWD prions (brain homogenate) by a combined PO/IN route. Animals were sacrificed and tissues collected at 24 hours, 72 hours, and 8 weeks after exposure. At these early time points of infection PrP^{CWD} is below the threshold of detection by traditional western blot and immunohistochemistry techniques. To remedy this limitation, tissues were assayed for PrP^{CWD} using several amplification techniques: real-time quaking induced conversion assay (RT-QuIC), protein misfolding cyclic amplification (PMCA), and tyramide signal amplification (TSA) immunohistochemistry. Using these techniques we have been able to detect PrP^{CWD} reaching the obex region of the medulla as early as 8 weeks after mucosal inoculation. Additionally, we are able to compare the sensitivities of these amplification assays in detection of small amounts of PrP^{CWD} during early CWD infection.

P.130: Analysis of prion infection in primary cortical neurons

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Introduction. Prion diseases are fatal neurodegenerative disorders that are characterized by the accumulation of an abnormal isoform of prion protein (PrP^{Sc}), reactive astrogliosis, microglial activation and the vacuolation of neurons and neuropils in the

central nervous system. Although multiple events, including ER stress caused by the accumulation of PrP^{Sc} aggregates, activated astrocytes and/or microglia or synaptic and dendritic alterations, have been suggested to be involved in neurodegeneration, its molecular mechanism is not fully understood yet. Neuronal cell lines used for analyses of the cellular mechanism of prion propagation have so far shown little cytopathic effect. Thus, a novel ex vivo experiment system, in which the generation of PrP^{Sc} in neurons and neurodegeneration can be reproduced, is required. Thus we analyzed prion infection in primary cortical neurons.

Materials and Methods. Mouse primary cortical neurons were obtained from 15-day mouse embryo. Four different prion strains, 22L, Chandler, Obihiro, and BSE-KUS, were used. PrP^{Sc}-specific staining was carried out using mAb132.

Results and Discussion. All the four prion strains could effectively produce PrP^{Sc} in primary cortical neurons, confirming the prion infection. Interestingly, the shape of PrP^{Sc}-staining observed confocal microscopy differed with strains; string shape staining was pronounced in cortical neurons infected with 22L or Chandler strains, whereas granular staining was mainly observed in Obihiro and BSE-KUS strain infection. A slight decrease in cell viability and in the expression of synaptic proteins such as PSD95 and N-cadherin was observed; however, double staining of PrP^{Sc} with tunnel-staining and cleaved caspase-3 did not reveal apoptosis of primary cortical neurons infected with prions. Efficient PrP^{Sc} generation in cortical neurons without neuronal cell death suggests that certain causes other than neurons, such as factors produced from activated astrocytes and/or microglia play a critical role in the neurodegeneration caused by prion infection. Analyses of neuron-glia interaction using prion-infected primary cortical neurons co-cultured with astrocytes or microglia may provide a clue to elucidate the neurodegenerative mechanisms of prion diseases.

P.131: Transmission of sheep-bovine spongiform encephalopathy in pigs

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Introduction. The transmissible spongiform encephalopathies (TSE) don´t occur in swine in natural conditions. However, the bovine spongiform encephalopathy (BSE) agent, inoculated by 3 simultaneous routes in pigs, is able to reproduce a neurological disease in these animals. On the other hand, the BSE agent after passage in sheep under experimental conditions (sheep-BSE) exhibits altered pathobiologic properties. This new agent is able to cross the cattle-pig transmission barrier more efficiently than BSE. The potential propagation of TSE in animals from the human food chain, including pigs, needs to be assessed regarding the risk for human infection by animals other than TSE-infected ruminants. The aim of this work was to determine the susceptibility of pigs to the Sheep-BSE agent and describe the pathological findings and PrP^{Sc} deposition in different tissues.

Material and Methods. Seven minipigs were challenged intracerebrally with sheep-BSE agent. Clinical observation and postmortem histopathology, immunohistochemistry (antibody 2G11) and Western blotting were performed on central nervous system (CNS), peripheral nervous system (PNS) and other tissues.

Results. One pig was culled in an early incubation stage, and remaining six were culled at the presence of clinical sings. Pigs developed a clinical disease with locomotor disorders in an average time of 23 months post inoculation, showing clinical findings in most of them earlier than those described in the BSE in pigs experimental infection. TSE wasn´t confirmed in the preclinical pig. In clinical pigs, the entire cerebral cortex showed severe neuropil vacuolation, extensive and severe vacuolar changes affecting the thalamus, hippocampus and cerebellum. PrP^{Sc} was found in CNS of all clinical pigs (6/6). Intracellular (intraneuronal and intraglial) and neuropil-associated PrP^{Sc} deposition was consistently observed in the brainstem, thalamus, and deeper layers of the cerebral cortex. Also, PrP^{Sc} was observed in PNS, mainly in the myenteric plexus and also in nerves belonging to the skeleton muscle. Moreover, the glycosylation profile showed a 3 band pattern with a predominant monoglycosylated band in positive pig samples. This features concern on the potential risk of utilization of meat and bound meal of small ruminants in feeding pigs.

P.132: Full-length PrP^{C} but not PrP-C1 is depleted in autolytic brainstem samples of cattle

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Introduction. In summers 2011 to 2013 in Switzerland, several brainstem samples from cattle in a severely autolytic stage were reactive with some of the BSE screening tests, but remained unconfirmed. In the Western immunoblot (WB), a truncated form of PrP^{res} type was detected after proteinase K (PK) digestion, with a profile distinct from either H-, L- or C-BSE. In order to investigate whether this particular PrP profile was related to the effects of severe autolysis, we compared the PrP species present in these autolytic samples to those in non-autolytic BSE negative samples and BSE positive samples.

Material and methods. Fallen stock autolytic brainstem samples that were initially reactive in the screening laboratory were selected. Freshly prepared control brains were collected at the slaughterhouse. All samples were analyzed with and without PK digestion and deglycosylation respectively using standard

methods. They were then submitted to WB for epitope mapping. Bovine PrP specific antibodies that bind to epitopes spanning from the N-terminal to the C-terminal regions were used: BG4, 9A2, 6H4, Sha31, 94B4 and ROS-JB10.

Results. Three PrP species each with distinct molecular masses were identified after deglycosylation of the BSE positive and negative samples: full-length PrP (25KDa), PrP-C1 (18KDa) and PrP-C2 (20KDa). Full-length PrP was found together with PrP-C1 in non PK-digested fresh BSE negative and BSE positive samples. In autolytic samples, only PrP-C1 was present, and full-length PrP was faint or not detectable. Both PrP species were sensitive to PK digestion in these samples under the conditions applied. As expected, PrP-C2 was found in high amounts in BSE positive samples and was resistant to PK treatment.

Conclusions. The main difference between the autolytic samples and the non-autolytic negative samples was the absence of full-length PrP in autolytic samples, suggesting that full-length PrP is more susceptible to degradation than PrP-C1. We assume that the PrP-C1 fragment plays a role in the inconsistent reactivity of autolytic samples in the screening tests.

P.133: Establishment of transgenic mice integrated with human prion gene

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Prion diseases are neurodegenerative diseases characterized by the accumulation of abnormal prion protein (PrP). Experimental transmission of human PrP to animals is considered a useful tool in the investigations on human prion diseases because one of the most critical features of prion is that abnormal PrP cannot be evaluated precisely in vitro. Therefore, we recently produced transgenic mice integrated with human prion genes for etiological study on susceptibility of human prion diseases. In this study, we constructed the transgenic mice expressing human PrP with codon 129M and 129V, respectively, and analyzed the expression of integrated exogenous human PrP in the mice. The exogenous human PrP was detected in brain and spleen of the offspring by using RT-PCR, western blotting, and immunohistochemistry. For a test for prion disease susceptibility, we inoculated the brain homogenates of vCJD and sCJD into the established transgenic lines. As a result, in early time of infection (dpi 50), no detectable PrP^{Sc} was observed in the brain and spleen in these transgenic mice. Further changes of deposition of prion in the middle and late time of infection (dpi 100, 150, 200 or more) are checked up in these mice. For the further study, we will investigate correlation between vCJD and MM type at codon 129 using these mice as the MM type is known to a susceptible factor in the prion infection.

P.134: Fibrinogen β chain as potential biomarker for an intra-vitam scrapie diagnosis

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Introduction. Different techniques with high diagnostic sensitivity (eg. PMCA, RT-QUIC, Exosomes) have been developed in recent years in order to make possible an ante-mortem diagnosis of prion diseases, currently based on the post-mortem detection of the pathological prion protein in the nervous system. At the same time, proteomic studies have been performed to identify alternative biomarkers in accessible tissues or body fluids of affected animals. With this purpose we studied plasma of sheep with natural scrapie by two-dimensional gel electrophoresis (2D-PAGE).

Materials and methods. Ovine plasma samples were collected from four different Italian naturally scrapie-affected flocks. Twenty animals were chosen, ten positive and ten negative sheep, coupled by age, sex and breed. To increase the visibility of low abundant proteins, plasma samples were subjected to ProteoMiner™ (Biorad) before 2D-PAGE. Spots were detected by colloidal Comassie blue staining; image analysis and gel comparison were carried out by BioNumerics® software; proteins were identified by nanoLC-ESI-LIT-MS/MS (Thermo) analysis and database search by MASCOT software package (Matrix Sciex).

Results. Two spots, with isoelectric point between 6.5 and 7 and molecular weight between 65 and 70 kDa, have shown a statistically significant increased expression in the positive scrapie samples. NanoLC-ESI-LIT-MS/MS analysis has been carried out on these spots, which have been identified as isoforms of fibrinogen β chain.

Conclusion. Fibrinogen is a multimeric protein, synthesized by liver, which undergoes several post-translational modification, such as hydroxylation, glycosylation, phosphorylation and sulphation. Anyway plasma fibrinogen concentration is conditioned by a great number of physiological and pathological stimuli, so in this study we investigated the possible presence on fibrinogen β chain of specific post-translational modifications in scrapie affected animals. To assess the specificity of this potential marker, further analyses are undergoing in our laboratory on plasma samples from sheep with different pathologies.

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P.135: Comparative transcriptomics of rodent adapted scrapie

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While extensive analysis of the transcriptional response to scrapie have been completed in mice, the direct relevance and applicability of these findings to human disease remains obscure. To better frame the molecular pathogenesis of prion diseases, identify novel biomarkers, and understand the variability associated with existing surrogate biomarkers of CJD, in both preclinical and clinical phases of disease, we have generated a rat prion disease by adapting mouse RML scrapie agent into the rat by serial passage. We examined changes in gene expression between infected and uninfected rats during adaptation of RML prions to rats as well as at three time points during disease. Gene expression profiles were validated by quantitative PCR and ontological analysis performed.

Gene expression profiling determined that rats respond to prion infection by inducing a neuro-inflammatory response, consistent with prion disease responses from mice, sheep, cattle and humans. At the level of individual genes, however, rat gene expression changes diverged substantially from mice and a significant fraction (~70%) of transcripts found to be deregulated greater than two fold in response to mouse scrapie were not changed in rats and vice versa. While the biological significance of this finding remains obscure, the approach has highlighted the value of comparative analysis to identify those gene expression changes that are conserved, and thus more pertinent to the underlying pathology induced by prion infection and more applicable to human prion diseases. We assemble, analyze and present those genes whose expression is conserved in response to rodent adapted scrapie infection along the course of disease.

P.136: Glia-derived brain inflammation and enhanced susceptibility to kainate seizures in a transgenic mouse model of genetic Creutzfeldt-Jakob disease

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Prion diseases are neurodegenerative disorders caused by a misfolded form of the cellular prion protein (PrP). Creutzfeldt-Jakob disease (CJD) is the most common human prion disease, recognized clinically by global cortical dementia, motor abnormalities and myoclonus, and neuropathologically by PrP deposition, gliosis and neuron loss. There is evidence of seizure-like activity in CJD.[1] We generated Tg(CJD) mice expressing the mouse PrP homolog of the D178N/V129 linked to a genetic CJD. These mice synthesize a misfolded form of mutant PrP in their brains, and develop clinical and pathological features reminiscent of CJD, including cognitive and motor abnormalities, and altered EEG activity.[2] In the present study, we investigated whether Tg(CJD) mice had increased susceptibility to seizures.

Focal-onset acute seizures were induced by unilateral intrahippocampal injection of kainic acid (KA) in presymptomatic Tg(CJD), PrP knockout (PrPko) mice and wild-type controls. EEG recordings showed an increased number of seizures, and longer time in ictal activity in Tg(CJD) compared to PrPko and wild-type mice. Immunohistochemical analysis showed intrinsic glial activation and increased expression of IL-1β in the hippocampus. The hippocampal levels of Tyr1472 phosphorylated NR2B subunit were increased in PSD-enriched fraction of Tg(CJD) mice; at the same time, the total levels of NR2B were significantly reduced.

These results show proinflammatory processes in activated astrocytes in the brain of pre-symptomatic mice that may alter their seizure threshold,[3] and suggest a possible involvement of IL-1β-induced activation of NR2B receptor subunits in neuronal hyperexcitability in Tg(CJD) mice as reported in mouse models of temporal lobe epilepsy.[4,5]

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P.137: Intracerebral and oral challenge studies indicate a protective role for lichens against prion infection

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Few biological systems have been identified that degrade the transmissible spongiform encephalopathy (TSE)-associated form of the prion protein (PrP^{TSE}) and reduce TSE infectivity. Stability

of the TSE agent allows scrapie and chronic wasting disease agents to persist in the environment for years and cause disease in naïve hosts. Critical to controlling disease transmission is the identification of naturally occurring or engineered agents that can inactivate prions in the environment or in TSE-infected hosts. We previously found that extracts of at least 40 species of lichens, ubiquitous, symbiotic organisms composed of a fungus and a photosynthetic partner, are capable of degrading PrP^{TSE} under mild conditions. The observed degradation is dependent upon the proteolytic activity of a serine protease present in many lichen species. We tested the hypothesis that extracts of protease-positive lichen species can reduce TSE infectivity by treating infected brain homogenate with lichen extracts and examining infectivity in mice. We found that extracts of the protease-positive species *Parmelia sulcata*, *Cladonia rangiferina,* and *Lobaria pulmonaria* diminished TSE infectious titer by approximately three log units. In the case of *C. rangiferina,* the extent of PrP^{TSE} degradation observed by immunoblotting underestimated the observed reduction in infectious titer. Furthermore, when mice were fed *P. sulcata* and orally challenged with mouse-adapted RML scrapie, there was a delayed onset of clinical signs and reduced disease attack rate compared to a protease-negative lichen species or control. Surprisingly, this protective effect was not observed in mice fed *C. rangiferina*. In vitro, degradation of PrP^{TSE} was observed upon extraction of *P. sulcata,* but not *C. rangiferina,* in simulated intestinal fluid, while no degradation was observed for either lichen in simulated gastric fluid, suggesting that the *P. sulcata* protease is extractable and proteolytically active within the mouse intestinal tract. Efforts are currently underway to both better understand the mechanism by which *P. sulcata* can protect mice from prion disease as well as to isolate and identify the anti-prion protease from lichens.

P.138: Phenotypic diversity in meadow vole (*Microtus pennsylvanicus*) prion diseases following challenge with chronic wasting disease isolates

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Chronic wasting disease (CWD), a prion disease of cervids (deer, elk and moose), is spreading unchecked through large sections of North America. Transmission of CWD among cervids is especially facile and can occur through direct animal-to-animal contact and indirectly through contact with prions shed from infected animals. The disease transmission threat posed by CWD to other wildlife species remains unknown, but other species are inevitably exposed to CWD by consumption of infectious materials and through contact with environmental CWD contamination. In this study, we investigated the transmission and adaptation of various white-tailed deer CWD isolates in the meadow vole (*Microtus pennsylvanicus*), a native North American rodent that is sympatric with current CWD epizootics that we have previously established is susceptible to CWD. We found that serial subpassage of CWD from white-tailed deer homozygous for glycine at position 96 (96GG) of the prion protein in meadow voles resulted in the selection of a single prion strain that was characterized by homogeneity in incubation period, abnormal prion protein (PrP^{TSE}) glycoform ratio, lesion profile and PrP^{TSE} deposition pattern. In contrast, passage of CWD from heterozygous 96GS genotype deer produced four unique disease phenotypes upon first passage. Subpassage of these types ultimately resulted in selection of a single strain by third passage that was distinct from the 96GG genotype CWD-derived strain. We also establish that meadow voles are susceptible to CWD via peripheral challenge, albeit with lower attack rates and longer incubation periods. Interestingly, oral challenge of meadow voles with CWD resulted in subclinical infection in primary passage animals, but manifested as clinical prion disease upon subpassage. Our data establish that meadow voles are permissive to CWD via peripheral exposure route, suggesting they could serve as an environmental reservoir for CWD. Additionally, our data are consistent with the hypothesis that at least two strains of CWD circulate in naturally-infected cervid populations and provide evidence that meadow voles are a useful tool for CWD strain typing.

P.139: Inactivation of disease-associated prion protein by peroxymonosulfate

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Prions are notoriously resistant to inactivation by most methods that are effective against conventional pathogens. This has prompted investigation of alternative inactivation methods that can be applied in medical, agricultural, and environmental settings. Peroxymonosulfate is an oxidant under investigation for in situ chemical oxidation of recalcitrant organic chemicals. Activation of peroxymonosulfate by transition metals leads to the production of radical species. Here, we investigate the ability of peroxymonosulfate to degrade pathogenic prion protein. Initial results indicate that peroxymonosulfate rapidly degrades disease-associated prion protein. Oxidation of pathogenic prion protein can proceed directly without the activation of peroxymonosulfate. Activation of peroxymonosulfate by cobalt to produce mainly sulfate radicals appears to enhance degradation of pathogenic prion protein. We found that activated peroxymonosulfate substantially reduced template-directed misfolding ability of prions as measured by protein misfolding cyclic amplification. This study indicates that peroxymonosulfate holds promise as a prion decontaminant agent and warrants further research.

P.140: BSE monitoring in the Russian Federation in 2011-2013

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Keywords: BSE, monitoring, BSE risk status of the cattle population

Background. The goal of the study was to improve monitoring of bovine spongiform encephalopathy (BSE) in the Russian Federation (RF) aimed at the solution of the main tasks:

- confirmation of efficacy of imposed in 1989 restrictions on import of feeds, live cattle and beef from some countries, where the level of BSE spread was significant;
- confirmation of efficacy of the introduced in 1990 ban on feeding of ruminant protein to ruminants, as well as brought into force in 1996-2001 measures focused on the improvement of import control and feed production for ruminants.

The solution of these tasks is the area of responsibility of the Federal Service for Veterinary and Phytosanitary Surveillance (FSVPS).

Material and methods. BSE monitoring in 2011-2012 was carried out in 23 RF regions where more than a thousand of bovine animals from controlled BSE risk countries had been imported. In 2013 fifty six RF Subjects were involved in the implementation of the monitoring program; in those Subjects the adult cattle population amounted to 8.35 million or 94.6% of the whole adult cattle population in RF. Bio-Rad diagnostic kits and equipment were used for brain sample testing.

Results. Until 2011 the BSE monitoring was carried out only in several regions of the European part of RF by virtue of the fact that animals were imported into those regions from EU countries, USA and Canada as from 2004. About 8,600 cattle brain samples were tested for BSE in RF before 2011.

Cattle brain samples at the rate of 5,258 and 2,598 were collected within monitoring program in 2011 and 2012, respectively. In 2013 11,687 cattle brain tissue samples were tested for BSE. The age of 90% of animals was 3–8 years.

Conclusion. As a result of conducted in 2011-2013 studies the disease agent was detected in none of 19.5 thousand tested samples. The obtained result shows that applied measures aimed at BSE control are effective. It is necessary to examine 3.33% of cattle adult population from all Subjects of RF in proportion to their quantity in order to prove with 95% confidence the absence of BSE in RF. The chief problems in the arrangement of such activities are associated with insufficient number of laboratories certified for carrying out BSE studies as well as need for collection and delivery of samples from huge territories of RF.

P.141: Abundant prion shedding in CWD-infected deer revealed by Realtime conversion

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Background/Introduction. Chronic wasting disease (CWD) is unique among prion diseases in its efficient lateral transmission in nature. While the presence of infectious prions in body fluids and excreta of infected cervids has been demonstrated by bioassay, the dynamics, magnitude, and consequences of prion shedding remain unknown. The present studies were undertaken to determine the kinetics, duration, and magnitude of prion shedding in infected white-tailed deer.

Materials and Methods. Longitudinal samples were collected from white-tailed deer over a 2-year span after either oral (n=11)] aerosol (n = 6) CWD exposure. The assay protocol employed phosphotungstic acid precipitation of either whole saliva or the pelleted fraction of urine to seed recombinant Syrian hamster prion PrP substrate in RT-QuIC reactions. Prion seeding activity was assayed in 8 replicates of each sample employing thioflavin T detection in a 96-well plate-based fluorometer. Prion seeding reaction rate was determined by taking the inverse of the time at which samples exceeded a threshold of 5 standard deviations above the mean fluorescence of negative controls (1/time to threshold). Seeding activity was quantitated by comparing the realtime conversion reaction rate to a standard curve derived from a reference bioassayed brain pool homogenate from deer with terminal CWD.

Results. We analyzed >200 longitudinally collected, blinded, then randomized saliva and urine samples from 17 CWD-infected and 3 uninfected white-tailed deer. We detected prion shedding as early as 3 months post exposure and sustained thereafter throughout the disease course in both aerosol and orally exposed deer. The incidence of non-specific false positive results from >500 saliva and urine samples from negative control deer was 0.8%. By comparing real-time reaction rates for these body fluids to a bioassayed serially diluted brain control, we estimated that ≤1 ml of saliva or urine from pre-symptomatic infected deer constitutes a lethal infectious prion dose.

Conclusion. CWD prions are shed in saliva and urine of infected deer as early as 3 months post infection and throughout the subsequent >1.5 year course of infection. In current work we are examining the relationship of prionemia to excretion and the impact of excreted prion binding to surfaces and particulates in the environment.

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P.142: Stereotaxic delivery of M1000 prions to the CA1 hippocampal region of wild type mice induces acute transient ethological and memory disturbance

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Keywords: acute prion toxicity, animal model, memory

Defining the toxic species underpinning pathogenesis in neurodegenerative diseases remains a major research goal. In Alzheimer disease (AD), good evidence exists for direct toxic effects of newly formed amyloid plaques rapidly inducing dysmorphic changes in juxtaposed neurites[1] and for hippocampally injected pre-fibrillar oligomers of Aβ1-42 causing significant acute disturbances of synaptic function, dendritic spine ultrastructural changes and memory impairment.[2] With respect to prion diseases, size fractionation and sedimentation velocity fractionation approaches have shown that transmission efficiency (when normalized per total PrP) correlates with oligomeric forms of misfolded aberrant conformers (PrP^{Sc}) of the normal prion protein (PrP^{C}).[3,4] There is limited in vitro evidence to support direct toxic effects of recombinant soluble, oligomeric PrP enriched in β-sheet content,[5,6] as well as "purified" PrP^{Sc} and proteinase-treated PrP^{Sc} extracted from the brains of terminally sick rodents.[7,8] However, there has been no previous report of studies demonstrating direct, acute toxicity of PrP^{Sc} in vivo. For this project, brain homogenates derived from either terminally-sick mice infected with mouse-adapted M1000 prions or age-matched controls inoculated with normal brain homogenates, were stereotaxically injected above the CA1 region of the hippocampus (coordinates -2.5 mm from bregma, +/- 2.5 mm laterally and -1.7 mm depth) of 10 week old female WT C57BL/6 mice (n = 10 per group). Five days following stereotaxic injection, the mice commenced a series of motoric, behavioural/ethological and cognitive testing, which included Rotarod, Y-maze, Open Field, Burrowing and the Barnes Maze. All testing was performed within 16 days of inoculation to minimise pathogenic contributions from de-novo M1000 prion propagation.[9] No difference was observed in the Rotarod gross motor performance of the two groups. A significant increase in activity was observed in the Open Field test (unpaired t-test p-value < 0.0001) at 7 days, as well as a significant increase in pellets displaced with the burrowing test at the 2 hour time-point (unpaired t-test p-value < 0.05) 9 days after injection. Interestingly, these changes reverted to the level of control animals at 16 days post-injection. Although no inter-group differences were observed in the Y-maze, an increase in total errors was observed in M1000 exposed mice in the Barnes Maze (ANOVA general linear model p-value = 0.031), appearing most notable on days 9 and 10 following stereotaxic injection. Brain morphological and biochemical analyses at different time-points after stereotaxic injection are ongoing. Taken together, these data support an acute, transient, in vivo toxicity of pre-formed M1000 prions in relation to behaviour and memory performance.

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P.143: Prions in plants: Assaying grasses from rocky mountain national park for PrP^{Res}

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Chronic wasting disease (CWD) affects cervids such as elk, deer, and moose and has become endemic over the last decade. The disease is one of many transmissible spongiform encephalopathies which occur due to the accumulation of an abnormally folded, proteinase K resistant, form of the normal cellular prion protein PrP^{C}. This abnormally folded form, PrP^{res}, seeds conversion of PrP^{C} into PrP^{res} and eventually forms amyloid fibrils. The exact mechanisms behind transmission and spread of CWD are unknown but research has shown that it can be spread through direct animal to animal contact or via indirect exposure to contaminated feed and water sources. We want to further explore the latter and determine whether prions can be detected in grasses and other plants by use of the protein misfolding cyclic amplification assay (PMCA). Here we describe the optimization and

ability of PMCA to detect PrP^{res} in rice grass samples spiked with known concentrations of prions. Additionally, we have sampled plants from Rocky Mountain National Park and assayed them via PMCA.

P.144: Measuring cytokine profiles longitudinally during prion infection

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Keywords: prion immunology, cytokines

Introduction. Many studies have been performed analyzing the effects of inflammation during acute and chronic transmissible spongiform encephalopathies. It is suggested that both peripheral and central immune responses play a key role in prion-associated neurodegenerative disease due to persistent release of inflammatory cytokines from microglia cells, astrocytes, and other immune cells. It is important to identify the cytokines present throughout prion disease to determine if the observed spongiosis and neuronal death are a result of immunopathology. The purpose of this study is to measure cytokine profiles longitudinally in transgenic mice infected with prions as compared to control animals inoculated with normal brain homogenate. This study represents the first longitudinal experiment analyzing systemic and neuro-inflammation in the same prion-infected animals throughout their entire disease course.

Materials and Methods. In a preliminary study, serum cytokine levels were measured in cervidPrP-expressing mice infected with CWD prions, and control mice inoculated with normal brain homogenate using the BioPlex suspension array system. We have analyzed IL-1β, TNF-α, IFN-γ, GM-CSF, IL-2, IL-6, IL-10, IL-4, and IL-5 at baseline levels, day one post inoculation, and at two-week intervals through terminal disease. At the time of sacrifice, mouse serum and brain homogenate cytokine levels were also analyzed.

As a distinct continuation of this study, TGA20 mice infected with RML mouse-adapted prions will be analyzed in a similar longitudinal fashion, with additional sacrifices at 40, 60 and 80 days post inoculation for extended comparison of central cytokine profiles. Age and sex related cytokine variations will be taken into account and in addition to the cytokines listed above, IL-12p70, IL-13, and IL-18 will also be analyzed.

P.145: Assessing the risk of transmission of prion infectivity by blood transfusion in a non-human primate model: The Baxter study

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There have been four reported cases of secondary transmission of variant CJD infectivity associated with the transfusion of blood or blood components collected from variant CJD patients during the asymptomatic phase of the disease. Establishing efficient experimental models for assessing the risk of further transmission of variant CJD infectivity via blood transfusion is of paramount importance in view of the most recent study of archived appendix samples in which the prevalence of subclinical variant CJD infection in the UK was estimated to be approximately 500 per million of the population. The squirrel monkey (*Saimiri sciureus*) has been shown to be highly susceptible to experimental challenge with human prion disease. Neuropathological and biochemical analyses of CNS tissue have shown that sporadic CJD and variant CJD can be distinguished in the squirrel monkey and that many of the characteristics that define these disease subtypes are conserved on transmission. We investigated the ability of blood from variant CJD and sporadic CJD-infected squirrel monkeys to transmit infectivity by the intravenous route. Recipient animals were sacrificed eight years after their first blood transfusion: none had any clinical signs of prion disease, and microscopic examination of the brain showed no evidence of spongiform vacuolation in either the variant CJD or sporadic-CJD transfused animals. Immunohistochemistry and biochemical investigations failed to show any evidence of PrP^{TSE} in CNS or lymphoreticular tissues examined from the blood recipients. Therefore, we have been unable to detect evidence for the transmission of prion disease via blood transfusion in this established non-human primate model. The implications of these findings for assessing the risk of secondary transmission of prion disease via blood in humans will be discussed.

P.146: Kinetics and cell association of chronic wasting disease prions shed in saliva and urine of white-tailed deer

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Chronic wasting disease, a transmissible spongiform encephalopathy (TSE) of deer, elk, and moose, is unique among prion diseases in its relatively efficient horizontal transmissibility. Recent studies have shown that excreta—saliva, urine, and feces—from CWD-positive cervids may play an important role in horizontal transmission of CWD, and although the precise onset of shedding in these excreta is unknown, it is thought to occur long before the onset of clinical symptoms. High levels of prion seeding activity have been demonstrated in excretory tissues of deer, including tongue, salivary glands, kidney, and urinary bladder, though the origin(s) and cellular nature of infectious prions in excreta is unknown. We hypothesized that excretory shedding of CWD prions in saliva and urine would coincide with the appearance of PrP^{d} appearance in peripheral lymphatic tissues, and that infectivity would associate with cellular preparations of these excreta. Following intracerebral inoculation of susceptible Tg[CerPrP] mice, we observed efficient transmission in saliva collected as early as 12 months post-exposure, coinciding with peripheral PrP^{d} appearance in tonsil biopsies; while urine collected at terminal disease was only minimally infectious in transgenic mice. We also found that acellular preparations of saliva, and cellular preparations of urine, were capable of transmitting CWD infection to transgenic Tg[CerPrP] mice with incubation periods similar to that of whole saliva or urine; saliva and urine from CWD-negative deer failed to induce prion disease in these mice. Infectious titers were determined for obex and bodily fluids, and were similar to those previously described. These findings extend our understanding of CWD shedding in white-tailed deer, and offer insight into the source and cellular associations of infectious CWD prions in excreta.

P.147: Gene expression profiling of brains from bovine spongiform encephalopathy (BSE)-infected cynomolgus macaques

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Background. Prion diseases are fatal neurodegenerative disorders whose pathogenesis mechanisms are not fully understood. In this context, the analysis of gene expression alterations occurring in prion-infected animals represents a powerful tool that may contribute to unravel the molecular basis of prion diseases and therefore discover novel potential targets for diagnosis and therapeutics. Here we present the first large-scale gene expression profiling of brains from BSE-infected cynomolgus macaques, which are an excellent model for human prion disorders.

Results. The study was conducted using the GeneChip® Rhesus Macaque Genome Array and revealed 301 transcripts with expression changes greater than twofold. Among these, the bioinformatics analysis identified 86 genes with known functions, most of which are involved in cellular development, cell death and survival, lipid homeostasis, and acute phase response signaling. RT-qPCR was performed on selected gene transcripts in order to validate the differential expression in infected animals versus controls. The results obtained with the microarray technology were confirmed and five genes were found to be highly regulated. In brief, *HBB* and *HBA2* were down-regulated in infected macaques, whereas *TTR*, *APOC1* and *SERPINA3* were up-regulated.

Conclusions. Some genes involved in oxygen or lipid transport and in innate immunity were found to be dysregulated in prion infected macaques. These genes are known to be involved in other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Their protein products may become potential targets for both general diagnosis and therapeutic purposes of many neurodegenerative diseases.

P.148: L-BSE in genetically susceptible and resistant sheep: Changes in prion strain or phenotypic plasticity of the disease-associated prion protein?

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Sheep with prion protein (PrP) gene polymorphisms QQ171 and RQ171 were shown to be susceptible to the prion causing L-type bovine spongiform encephalopathy (L-BSE), although RQ171 sheep specifically propagated a distinctive prion molecular phenotype in their brains, characterized by a high molecular mass protease-resistant PrP (PrPres) fragment, distinct from L-BSE in QQ171 sheep.

Transmission studies were performed from QQ171 and RQ171 ovine L-BSE in transgenic mice expressing either bovine or ovine PrP and in C57Bl/6 wild type mice.

In both transgenic lines, ovine L-BSE transmitted similarly to cattle-derived L-BSE, with respect to survival periods, histopathology, biochemical features of PrPres in the brain, as well as splenotropism, clearly differing from ovine classical BSE or from scrapie strain CH1641. At the first passage, PrPres was found in the spleen of ovine PrP transgenic mice infected with RQ171 ovine L-BSE, the molecular phenotype is similar to the brain PrPres signature of RQ171 sheep used as inoculum. Both QQ171 and RQ171 transmitted a prion disease in wild-type mice, whereas we failed to transmit any disease from L-BSE in cattle or from L-BSE first passaged in hamsters or microcebes. Remarkably PrPres molecular features also showed a high apparent molecular mass in C57Bl/6, clearly distinct from BSE.

After passage in sheep, with either QQ171 or RQ171 genotype, the L-BSE agent maintained its specific strain properties in bovine and ovine transgenic mice, although striking PrPres molecular changes could be found in RQ171 sheep and in the spleen of ovine PrP transgenic mice.[1] In addition passage in sheep also allowed the L-BSE agent to propagate in wild type mice, with unexpected molecular features. These observations suggest either selection of prion strain or phenotypic plasticity of the disease-associated prion protein.

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P.149: Highly infectious prions generated by a single round of microplate-based protein misfolding cyclic amplification

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Introduction. Prions are transmissible agents responsible for incurable neurodegenerative diseases affecting both human and animals. The causal agent is of proteinaceous nature, resulting from the host normal prion protein PrP^{C} conformational transition from an alpha helix rich soluble state to beta sheet-enriched and aggregated structures termed PrP^{Sc}.

PMCA is a technique that emerged in the last decade as a valuable tool to amplify prions in a test tube. Here we report a number of improvements allowing efficient amplification of several prion strains in a single 48h round and in a microplate format. We then determined by bioassay in reporter mice the infectious titer of the PMCA-derived prions of one of the amplified scrapie strain.

Material and Methods. The detailed PCR microplate based mb-PMCA (miniaturized bead-PMCA) protocol was described.[1] 10% brain lysates of tg338, tg650, tg20 and tg7 transgenic mice over-expressing ovine, human, mouse and hamster PrP were prepared in PMCA buffer. Ovine prions (127S), mouse prions (139A, 22L, RML, Chandler, ME7, mouse-adapted BSE), hamster prions (263K, Sc237, HY and DY) and human prions (vCJD, sCJD VV1, VV2 and MV2 types) were used as seed for mb-PMCA reactions.

Results. We first report significant simplification and improvements of the PMCA technique, leading to high throughput and highly efficient amplification of PrP^{Sc} from several prion strains of different species (ovine, mice, hamster, human) in a single 48h round. We then demonstrate by end-point titration in reporter mice that this method restores an infectivity titer of 127S scrapie prion strain comparable to that of in vivo brain-derived prions, whatever the input dilution seeding the PMCA reaction.

Conclusion. The method developed here allows for the first time large-scale, fast, and reliable cell-free amplification of sub-infectious levels of prions from different species. The mb-PMCA format should help developing high-throughput prion assays for cognitive, diagnostic, and therapeutic applications. This simplified assay could be adapted to automated purposes and serve for urgently needed ante mortem diagnostic tests, by using bodily fluids containing small amounts of prion infectivity. Such assay is of paramount importance to reduce the transfusion risk in the human population, to identify asymptomatic carriers of variant

Creutzfeldt-Jakob disease and to search for prion decontamination reagents.

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P.150: Zoonotic potential of L-type BSE prions: A new prion disease in humans?

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Two novel prion strains, referred to as BSE-L and BSE-H, have been recognized in bovines through active prion surveillance programs, both being distinct from the epizootic, 'classical', BSE strain (C-BSE). Both H and L-types have been detected worldwide as rare cases occurring in aged animals. Like C-BSE prions, H- and L-types prions can propagate with relative ease in foreign species or in transgenic mouse lines expressing heterologous PrP sequences. A prion exhibiting biological properties similar to C-BSE agent sometimes emerged from these cross-species transmissions. Previously, L-type prions were shown to transmit to transgenic mice expressing human PrP with methionine at codon 129 with higher efficacy than C-BSE prions. Here, we examined whether L-type prions propagate without any apparent transmission barrier in these mice and whether such 'humanised' L-type prions share biological properties with CJD prions. L-type prions and a panel of human CJD cases with various genotypes at codon 129 and electrophoretic PrP^{res} signatures were serially transmitted by intracerebral route to human PrP mice. The biological phenotypes induced by these agents were compared by all the standard methods currently used to distinguish between prion strains. At each passage, L-type prions were also transmitted back to bovine PrP mice to assess whether the agent has evolved upon passaging on the human PrP sequence. L-type prions transmitted to human PrP mice at 100% attack rate, without notable alteration in the mean incubation times over 5 passages. At each passage, 'humanized' L-type prions were able to transmit back to bovine PrP transgenic mice without apparent transmission barrier, as based on the survival time and the restoration of a L-type BSE phenotype. Comparison of mean incubation times on primary and subsequent passages in human PrP mice showed no overlap between L-type and sporadic CJD agents. While the electrophoretic signature and regional distribution of PrP^{res} in L-type diseased mouse brains resembled that seen after transmission of MM2 CJD strain type, both agents exhibited distinct resistance of the associated PrP^{res} molecules to protease denaturation. In summary, L-type prions can be passaged on the human PrP sequence without any obvious transmission barrier. The phenotype obtained differs from the classical CJD prion types known so far. Careful extrapolation would suggest that the zoonotic transmission of this agent could establish a new prion disease type in humans.

P.151: Reduction of PrP^C and shadoo during prion infection

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Keywords: prion replication, PrP^C substrate, Shadoo, subclinical disease

During prion infections, the cellular prion protein, PrP^C, is conformationally converted into a misfolded form, PrP^{Sc}. The accumulation of PrP^{Sc} parallels a significant preclinical reduction in PrP-like Shadoo (Sho) glycoprotein levels, which has not been observed in any other type of neurodegenerative disorder. Since PrP^C and Sho share similar cell membrane environments, we hypothesized that the slowly evolving pathogenesis of preclinical prion disease is caused by depletion of the PrP^C substrate that is required for PrP^{Sc} formation and toxic signaling. By analyzing brain material from different rodent models with the conformation dependent immunoassay, we were able to specifically monitor PrP^C and discover net reductions ranging from 31% ± 3.9% to 68% ± 8.4% in a variety of prion diseases that include sheep scrapie, human Creutzfeldt-Jakob disease, and cervid chronic wasting disease. The reduced PrP^C levels observed in vivo were determined sufficient for compromising prion replication in protein misfolding cyclic amplification. Time course studies demonstrated that PrP^C downregulation begins preclinically in wild type and hemizygous *Prnp*$^{0/+}$ mice, occurring less than halfway through the disease incubation. Moreover, PrP^C downregulation was recapitulated in in vitro cell culture models chronically propagating mouse-adapted scrapie or chronic wasting disease prions, where levels were respectively reduced by approximately 35% and 55% compared to noninfected controls. In parallel, de novo

infection of another cell culture model resulted in PrP^C reduction until 21 dpi compared to mock-inoculated cells. Assuming a common underlying mechanism for reductions in PrP^C and Sho, we have applied inhibitors of protein quality control systems to recently developed in vitro and ex vivo models for Sho downregulation. Drugs that enhance this natural protective PrP^C downregulatory response may have a potent beneficial effect in treating prion disease.

P.152: QuIC amplification of BSE infected sheep blood identifies prion aggregates in peripheral blood cells and plasma

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To date there have been 4 cases of vCJD infection in humans which have likely arisen as the result of blood transfusion. However, the direct measurement of the infectious agent is technically challenging due to the low amounts of blood borne prions in preclinical and clinical animals. The development of in vitro amplification procedures, such as the Real-Time Quaking Induced Conversion assay (RT-QuIC)[1,2] and enhanced QuIC (eQuIC)[3,4] is enabling more rapid and sensitive methods for the ante-mortem detection of prions in blood.

We have used an established in vivo blood transfusion model in sheep, in which BSE is transmitted by the transfusion of blood between donors and naïve recipients, and shown that all types of blood components contain sufficient levels of prion-associated infectivity to cause disease.[5] As part of this study, we are accumulating a unique source of thousands of blood samples (consisting of whole blood, plasma, buffy coat and red cell concentrates) collected at serial time points from BSE-infected animals and negative controls from the initiation of infection through to clinical endpoint. Critically, we know when during the sampling regime blood was infectious as demonstrated by the transmission of BSE following transfusion. We aim to understand better the relationship between infectivity seen in vivo and the in vitro detection of prion aggregates in blood.

We report here for the first time, the use of QuIC methodologies to successfully amplify prions from BSE-infected blood components. Using a new mAb, BC6,[6] for immunocapture of PrP prior to amplification, we detected prion protein aggregation from 1 million peripheral blood mononuclear cells (PBMCs) from BSE-infected sheep; 6 out of 7 sheep (including 2 donors whose transfusion of buffy coats lead to disease in recipient sheep) were known to have pre-clinically transmitted infectivity to transfused recipients. RT-QuIC in conjunction with phosphtungstic acid precipitation was also able to amplify prion aggregates from PBMCs in addition to plasma from clinical stage, BSE-infected sheep. These plasma samples were from BSE-positive donors some of which 14/18 were known to have given rise to positive transmission of BSE following transfusion to recipients. We are extending our current analyses to blinded blood samples from the same animals collected at pre-clinical time points.

These results suggest that QuIC assays are robust and may provide a reliable method for the consistent detection of the abnormal prion protein in ante-mortem blood samples.

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P.153: Understanding the relationship between abnormal prion protein in lymphoid tissues and infectivity in blood: the outcome of eight years of blood transfusion studies in sheep

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We have shown that clinically-relevant components from asymptomatic blood donors transmit BSE, in sheep, following a single transfusion.[1] We report the ongoing cycle of infection that is maintained when those blood recipients donate blood to others.

Units of blood were collected from BSE infected donor sheep before the onset of clinical signs and prepared to multiple clinically-relevant components (including leucoreduced equivalents) and transfused to primary recipients. Before these recipients showed clinical signs, units of blood were collected and

transfused to secondary recipients. A range of lymphoid tissue and brain were collected post mortem for examination of disease-associated protein using western blotting and immunohistochemical approaches with a panel of antibodies.

We examined the relationship between the presence of abnormal prion (PrP^d) protein in lymphoid & neuronal tissues and the presence of infectivity in blood. 44% of secondary recipients receiving either whole blood or buffy coat from asymptomatic donors (the primary transfusion recipients) developed clinical signs of BSE. PrP^d was evident in brain in all paired primary and secondary recipients (n=8 for both); however the same was not true of the lymphoid tissues examined. In the primary recipients, PrP^d was evident in spleen (8/8), tonsil (8/8), pre-scapular lymph node (7/8), mesenteric lymph node (7/8) and distal ileal Peyer's patch (DiPP, 7/8). For the secondary transfusion recipients, less deposition of PrP^d in lymphoid tissue was evident, whereby only spleen (8/8) and DiPP (7/8) were consistently positive. PrP^d was only detected in only 3/8 tonsils, 4/8 pre-scapular lymph nodes and 1/8 mesenteric lymph nodes examined. The remaining secondary recipients are currently healthy so the infections status in brain and lymphoid tissue is unknown.

Our data highlight the importance of multiple control measures to safeguard blood supplies; that careful targeting of lymphoid tissues and use of different assay methods and antibodies is critical for a definitive confirmation of infection; the urgency to establish the association of sub-clinical vCJD and the presence of infectivity in blood of those individuals.

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P.154: Urinary shedding of prions in Chronic Wasting Disease infected white-tailed deer

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Background/Introduction. Chronic wasting disease (CWD) is unique among prion diseases in its efficient lateral transmission in nature, yet the dynamics and magnitude of shedding and its immediate and long term consequences remain unknown. The present study was designed to determine the frequency and time span in which CWD prions are shed in urine from infected white-tailed deer using adapted real-time quaking-induced conversion (RT-QuIC) methodology.

Materials and Methods. Longitudinal urine samples were collected by free catch or catheterization over a 2-year period from oral-route infected [CWD+ (n = 11)] and aerosol-route-infected [CWD+ (n = 6); CWD- (n = 3)] white-tailed deer. High speed centrifugation pelleted material from 500 µl of urine was treated with sodium phosphotungstic acid (Na-PTA), resuspended in 0.05% SDS buffer, and used as seed in RT-QuIC assays employing recombinant Syrian hamster prion PrP substrate. Eight (8) replicates of each sample were run and prion seeding activity was recorded as thioflavin T binding fluorescence (480 nm emission) using a fluorimeter-shaker. Samples were considered positive if they crossed an established threshold (5 standard deviations above the negative mean fluorescence).

Results. In our oral-route inoculation studies, prion seeding activity has been demonstrated in urine collected at 6 months post-inoculation in 6 of 10 deer (11 of 80 replicates; 14%), and intermittently at later time points in all 11 CWD+ exposed deer. Our aerosol-route inoculation studies also showed prion seeding activity in urine collected at 6 months post-inoculation in 1 of 2 deer (3 of 16 replicates; 19%), and intermittently at later time points in 4 of 6 CWD+ exposed deer. Urine from sham-inoculated control deer and all baseline samples yielded 3 false-positive prion seeding activities (3 of 352 replicates; 0.8%).

Conclusion. CWD prions (as inferred by prion seeding activity by RT-QuIC) are shed in urine of infected deer as early as 6 months post inoculation and throughout the subsequent disease course. Further studies are in progress refining the real-time urinary prion assay sensitivity and we are examining more closely the excretion time frame, magnitude, and sample variables in relationship to inoculation route and prionemia in naturally and experimentally CWD-infected cervids.

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P.155: Overexpression of BAT3 alleviates prion protein fragment PrP106-126-induced neuronal apoptosis

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Keywords: prion diseases, neuronal apoptosis, PrP106-126, BAT3, neurodegenerative diseases.

Introduction. Prion diseases are a group of infectious neurodegenerative diseases characterized by neuronal death and degeneration.[1] The neurotoxic prion protein fragment 106-126 (PrP106-126) possesses similar physicochemical and pathogenic properties to PrP^{Sc}, shows partial proteinase K resistance, and is neurotoxic in vitro including the ability to cause apoptosis in hippocampal neurons and induce proliferation of astrocytes.[2,3] Human leukocyte antigen-B-associated transcript 3 (BAT3) is an important apoptosis regulator. [4,5] We therefore investigated the

interactions between BAT3 and prion protein and the potential role of BAT3 in PrP106-126-induced apoptosis.

Material and Methods. BAT3 and Prion protein were overexpressed in Hela, Neuro2A or primary neuronal cells by transfection with BAT3-HA or PRNP-EGFP expression plasmids, and their relationship studied by immunofluorescence and western blotting. The effect of BAT3 on PrP106-126-induced cytotoxicity and apoptosis were detected by the CCK-8 assay and Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL) assay. The expression of cytochrome *c* and Bcl-2 were examined by western blotting.

Results. BAT3 interacted with prion protein and enhanced PrP expression. After PrP106-126 peptide treated, BAT3 was transported from the nucleus to cytoplasm, increased cell viability and protected neurons from PrP106-126-induced apoptosis through stabilizing the level of Bcl-2 protein and inhibiting the release of cytochrome *c* to cytoplasm.

Conclusion. Our present data showed a novel molecular mechanism of overexpression of BAT3 alleviates PrP106-126-induced Neuronal Apoptosis, which may be important for neuron survival in prion diseases in vivo, providing new insights to the basic regulatory mechanism of neuronal death in neurodegenerative diseases.

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P.156: Infectivity of BSE prions treated by thermophilic anaerobic digestion process in bovinized Tg mice

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Background. Although BSE prevalence has declined, treatment of specified risk materials (SRM) is still a challenge. Previous studies demonstrated thermophilic anaerobic digestion (TAD) as a promising technology for on-farm SRM treatment, reducing protease-resistant PrP of BSE below the detection threshold of Western blotting (> 3.5 log at 21 days of treatment). Here we assessed TAD treatment on BSE infectivity in vivo.

Materials and Methods. Infectious BSE brain homogenate was spiked in an active TAD digester (8% of final concentration) and digested in batch module at 55 °C for 90 days. Study groups included: BSE in TAD ("E1"), BSE in enhanced TAD ("E2") with 4 grams of cellulose added at each sampling time, and BSE in saline at 55 °C ("E3"). Samples were taken at day 0, 7, 14, 21, 28, 35, 42, 60 and 90. Selected samples were diluted 1:10, heat-inactivated at 80 °C for 20 minutes and used for intracranial injection into transgenic (Tg) mice expressing bovine PrP^{C} (TgBovXV mice).

Results. The longest survival time was observed for mice inoculated with E2 samples at day 35 (313 ±22.22 days), followed by E1 at day 35 (303±32.8 days) and E2 at day 21 (293±27.72 days), which were longer than samples from E2 at day 0 (267±32.99 days) and E3 at day 60 (269 ±16.5 days). Average survival time for mice inoculated with 1:10 dilution of BSE was 282±12.12 days. There was a significant difference of survival time between the mice in E2 at day 35 and E2 at day 0 ($p < 0.02$, t-test), E3 at day 60 ($p < 0.01$) as well as the infectious dose-titration group at 1:10($p<0.02$), respectively. There was also a statistical difference between the E1 at day 35 and E2 at day 0 samples.

Conclusions. TAD's disinfection effect on BSE was influenced by carbon source and digestion time, implying a reliance on the microbial species in the bioreactor. We anticipate that the effect of the TAD process on BSE would approximate to a 2-log reduction for a 5-week digestion period; however a better estimate will emerge from our infectious dose titration study.

P.157: Post-Golgi trafficking of membrane proteins impaired by prion infection

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Keywords: Neurodegeneration, prion, post-Golgi trafficking

Conformational conversion of the normal cellular prion protein, PrP^{C}, into the abnormally folded, relatively proteinase K (PK)-resistant PrP^{Sc}, plays a pivotal role in prion diseases such as Creutzfeldt-Jakob disease and scrapie. However, the pathogenic mechanism of the diseases is unknown. The Golgi apparatus is a specialized cytoplasmic organelle near the nucleus which sorts membrane and luminal proteins via vesicle transport from the endoplasmic reticulum (ER) to lysosomes, secretory vesicles and the cell surface. In neurons, it is involved in the axonal transport of proteins and functional damage to it could have significant implications. Indeed, the Golgi apparatus might play a role in various neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. We show here that post-Golgi vesicular trafficking is delayed in

mouse neuroblastoma N2a cells persistently infected with scrapie prions.[1] PrP^{C} cell surface expression diminishes and instead accumulates in the Golgi apparatus.[1] Most PrP^{Sc} is in endosomal compartments, being particularly abundant in recycling endosomes.[1] We also show that cell surface IR is reduced in infected cells, and downstream signaling of the IR is disturbed.[1] Furthermore, cell surface PrP^{C} and IR are reduced in prion-infected mouse brains well before the onset of clinical symptoms.[1] These results suggest that prion infection could impair post-Golgi vesicle transportation of some membrane proteins, including PrP^{C} and IR, to the cell surface by accumulating PrP^{Sc} in recycling endosomes eventually causing neuronal dysfunction.

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P.158: Structurally and phenotypically different prions in CWD-infected white-tailed deer

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Prions can exist as multiple strains within mammals. We could detect, for the first time, two distinct chronic wasting disease (CWD) isolates in white-tailed deer (WTD). WTD had been challenged with CWD from either mule deer (MD) or WTD. Brain-derived prions from MD-infected WTD and WTD-infected WTD could be distinguished by biochemical, biophysical and biological methods. PK-mediated limited proteolysis at different pH-values indicated conformational differences between pathological prion proteins (PrP^{TSE}) from MD-infected WTD and WTD-infected WTD. More specifically, Fourier-transform infrared microspectroscopy revealed secondary structure differences between highly purified PrP^{TSE} extracts from MD-infected WTD and WTD-infected WTD. Different sedimentation velocities of PrP^{TSE} in gradient centrifugations provided additional evidence for structure differences between prions from MD-infected WTD and WTD-infected WTD. Brain homogenate from WTD-infected WTD showed a substantially lower seeding activity on cellular prion protein (PrP^{C}) of Syrian hamsters in protein misfolding cyclic amplification (PMCA) than its conformationally distinct counterpart from MD-infected WTD. When hamsters were intracerebrally inoculated with brain tissue from MD-infected WTD disease could be transmitted, which was not observed after similar inoculation with brain homogenate from WTD-infected WTD. In an ongoing macaque-study both CWD-isolates are currently being further tested for their transmissibility to primates.

P.159: Transgenic mice overexpressing rabbit prion protein are susceptible to BSE, BASE and scrapie prion strains but resistant to CWD and atypical scrapie

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Interspecies transmission of prions is a well established phenomenon, both experimentally and in field conditions. Upon passage through new hosts prion strains have proven their capacity to change their properties. It is, in fact, a source of strain diversity which needs to be considered when assessing the potential risks associated with consumption of prion contaminated protein sources.

Rabbits were considered for decades a prion resistant species until proven recently otherwise. To determine the extent of rabbit susceptibility to prions and to assess their effects on the passage of different prion strains through this species, a transgenic mouse model overexpressing rabbit PrP^{C} was developed (TgRab). Intracerebral challenges with prion strains originating from a variety of species including field isolates (SSBP1 scrapie, Nor98-like scrapie, BSE, BASE and CWD), experimental murine strains (ME7 and RML), experimentally obtained strains (sheepBSE) and strains obtained by in vitro crossing of the species barrier using saPMCA (BSE-$RabPrP^{res}$, SSBP1-$RabPrP^{res}$ and CWD-$RabPrP^{res}$) have been performed.

Interestingly, on first passage, TgRab were susceptible to the majority of prions tested with the exception of SSBP1 scrapie, CWD and Nor98 scrapie. Furthermore TgRab were capable of propagating strain-specific features such as differences in incubation periods, brain lesion and PrP^{d} deposition profiles and PK resistant western blotting band patterns. Our results confirm previous studies shattering the myth that rabbits are resistant to prion infection and this should be taken into account when choosing protein sources to feed rabbits.

P.160: Transgenic fatal familial insomnia mice indicate prion-independent mechanisms of pathogenesis and phenotypic expression of disease

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Fatal familial insomnia (FFI) and a genetic form of Creutzfeldt-Jakob disease (CJD^{178}) are linked, respectively, to the D178N/M129 and D178N/V129 prion protein (PrP) mutations. Tg(FFI) mice expressing the mouse PrP homolog of D178N/M129 on a PrP-null background develop a neurological illness highly reminiscent of FFI, with severe sleep alterations and thalamic atrophy. Re-introduction of wild-type PrP mitigates the sleep abnormalities but has no effect on other neurological deficits. Tg(FFI) neurons show morphological alterations of the Golgi associated with accumulation of misfolded mutant PrP. These are very different from the endoplasmic reticulum abnormalities seen in Tg(CJD) mice modeling CJD^{178},[1] suggesting that mutation-specific alterations of the secretory pathway may play a role in the phenotypic heterogeneity. The brains of Tg(FFI) and Tg(CJD) mice do not contain prions detectable by bioassay in mice or protein misfolding cyclic amplification, indicating that mutant PrP does not need to acquire an infectious conformation to encode disease-specific phenotypes. These results establish a new mouse model of FFI and support the emerging concept that the pathogenic properties of mutant PrP are enciphered in misfolded forms of the protein that are not infectious.

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P.161: Adaptation of RT-QuIC for the detection of BSE in cattle

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Transmissible spongiform encephalopathies (TSEs) are infectious neurodegenerative diseases that affect a variety of mammals including cervids, cattle and humans. Among other methods, real-time quaking-induced conversion (RT-QuIC) has been used successfully to detect prions in tissues and body fluids derived from infected hamsters, deer, and CJD patients. It has been shown that the sensitivity and specificity of the assay is dependent on different parameters, such as the origin of the recombinant PrP substrate and the used salt conditions. To our knowledge, RT-QuiC has not yet been adapted for the detection of BSE in cattle. To do so, we first tested recombinant PrP substrates from different species (human, macaque, saimiri), which all led to the amplification of macaque-adapted BSE. To our surprise, cattle-derived BSE, even in amounts of 1 µg per reaction, did not result in RT-QuiC reactivity. Thus, we produced two different recombinant bovine PrP substrates, one full length rbovPrP(23-231) and a shorter variant rbovPrP(90-231). Using these substrates, we found RT-QuiC seeding activity in BSE-brain homogenates, indicating that the reaction is strictly dependent on the primary PrP sequence. Comparison of the substrates revealed a markedly different seeding efficiency depending on both substrate and NaCl concentration. Using 900 mM NaCl per well we reproducibly found seeding activity for cattle-derived BSE, but the overall sensitivity was comparably low with a limit of detection > 10 ng brain tissue. However, using these conditions we were able to detect BSE with 100% specificity. We are currently evaluating the influence of different detergents, which can lead to dramatic changes in the performance of RT-QuiC. Further optimization of the assay conditions is necessary to apply RT-QuiC for routine testing of BSE brain samples and its application to other diagnostic specimen from cattle. Due to the known sensitivity of RT-QuiC that is comparable to bioassays in other species, this might be a first step to establish an ante mortem test for BSE in cattle.

P.162: The role of the misfolded prion protein in neurodegeneration

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The disease-associated prion is thought to arise from the conversion of the host protein, PrP^C, to a misfolded isoform – whereby it typically rearranges into a protease-resistant conformation, although other protease-sensitive misfolded prion species have been identified. However, the role that misfolded prion protein plays within neurodegeneration remains unclear. Previous studies have indicated that the misfolded prion protein is not directly toxic to neurons in the absence of PrP^C. Whether misfolded prion protein is toxic to neurons in the presence of PrP^C, or if a different toxic species exists, remains critically unanswered.

Typically, accumulation and spread of disease-associated prions is slow and progressive, and correlates tightly with TSE-associated pathology. Contrasting experiments show that animals may die of prion disease with characteristic hallmarks of prion pathology (vacuolation, neuronal loss, gliosis) in the absence of detectable misfolded protein. We have used this model to examine whether misfolded prion protein or "prion seeds", capable of sustaining the development of a Thioflavin-T product in a QuIC assay, are present at sites of pathology.

We show that a disease-associated prion seed is widespread throughout the brain from an early stage within the incubation period. In contrast to current perspectives of a slow, progressive spread of disease-associated prion to specific targets, the spread of prion seeds is extremely rapid and not restricted to particular brain regions. Specifically, we detect prion seeds in PrP^C-rich, "healthy" regions of the brain throughout the progression of disease. Moreover, we show that 'healthy' regions are exposed to prion seeds for the same time-period as regions of the brain which eventually succumb to neurodegeneration—ruling out a temporal exposure hypothesis of toxicity. Finally, we demonstrate that the prion seed used to initiate the QuIC assay is resistant to proteinase K digestion in tissue from both "healthy" and affected regions.

Our results indicate a clear dissociation between prion seeds and neurotoxicity. They highlight the need to understand the response of different populations of neurons to the presence of prion seeds that may evoke neurodegeneration in some populations but resilience in others.

P.163: Bayesian hierarchical modeling of chronic wasting disease in free-ranging white-tailed deer in the eastern U.S.

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Introduction. Chronic wasting disease (CWD) is a prion disease that affects both free-ranging and captive cervid populations. In the past 45 years, CWD has spread from a single region in Colorado to all bordering states, as well as Canada, the Midwest and the northeastern United States. In 2005, CWD was detected in the eastern U.S. in a free-ranging white-tailed deer (*Odocoileus virginianus*) killed by a vehicle in West Virginia followed by positives from Virginia, Maryland, and Pennsylvania. Although considerable information has been learned about CWD in wildlife from several areas of the U.S. and Canada, little information is available on spatial epidemiology of disease in the eastern U.S.

Materials and Methods. In order to develop a CWD surveillance plan for the region, we determined covariates and the best scale for analysis by exploring habitat use and estimating the mean size of home range for deer in the central Appalachian region (6 km^2). We conducted Bayesian hierarchical modeling in WinBUGS on 24 a priori models using 11,320 free-ranging white-tailed deer (69 positive, 11,251 negative) that have been tested for CWD since 2005. Testing for CWD was conducted using standard protocols on a variety of tissues extracted from hunter-harvested deer that included retropharyngeal lymph nodes, tonsil lymph nodes, and the medulla oblongata sectioned at the obex.

Results. We found 94% of models weights were accounted for in our top model that identified habitats such as developed and open as covariates that increased the odds of infection for CWD in this region. Contrary to research in the endemic area of Colorado, we did not identify clay soil as a significant predictor of disease even though clay soil ranged from 9% to 19% in our study samples. Furthermore, contrary to results from the recent expansion of CWD into the agricultural Midwestern U.S. (Wisconsin, Illinois), we identified developed and open habitats were better predictors of disease occurrence compared to forest habitat considered more critical to deer population dynamics in the U.S.

Conclusions. Our results suggested that the odds of infection for CWD is likely controlled by areas that congregate deer thus increasing direct transmission (deer-to-deer interactions) or indirect transmission (deer-to-environment) by sharing or depositing infectious prion proteins in these preferred habitats. Epidemiology of CWD in the eastern U.S. is likely controlled by separate factors than found in the Midwestern and endemic areas for CWD and can assist in performing more efficient surveillance efforts for the region.

P.164: Comparative susceptibility of New Zealand sheep with a range of *PRNP* genotypes to challenge with bovine spongiform encephalopathy and scrapie

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Polymorphisms of the sheep *PRNP* gene have been linked to susceptibility and resistance to classical and atypical scrapie and bovine spongiform encephalopathy (BSE). The three most significant polymorphisms originally identified occur at codons A136V, R154H and Q171R, and there is now evidence that additional *PRNP* polymorphisms have an influence on survival times (e.g. M112T). Following the emergence of variant Creutzfeldt-Jakob disease (vCJD) in humans as a result of exposure to BSE, there were heightened concerns about the possible presence of BSE in the sheep population. To assist with research into the pathogenesis of BSE and scrapie, sheep were imported to the UK from New Zealand to establish a scrapie-free breeding flock. Since it was not known whether other genetic factors, apart from *PRNP* genotype, would influence the susceptibility of New Zealand sheep to prion infection, the aim of this study was to determine their response to experimental challenge with BSE and the scrapie isolate SSBP/1.

Groups of 5-10 sheep of three different breeds and six *PRNP* genotypes (VRQ/VRQ, VRQ/ARQ, VRQ/ARR, ARQ/ARQ, ARQ/ARR, ARR/ARR) were challenged either by intracerebral inoculation of BSE or by subcutaneous inoculation of SSBP/1. The sheep were monitored over a period of >10 years for the development of clinical signs. Brain and lymphoid tissues samples from each sheep were analysed for detection of disease-associated PrP (PrP^{d} or PrP^{Sc}). Additional genetic analysis was carried out as the effect of other *PRNP* codons became known. Attack rates and incubation periods were compared with those observed following similar challenges of sheep from the Roslin Scrapie Flock.

Preliminary results gave the first indication that ARR/ARR sheep are susceptible to BSE infection.[1] The complete results show that differing proportions of sheep of all *PRNP* genotypes tested were susceptible to BSE. There were no major differences in susceptibility between the New Zealand sheep and the Roslin Scrapie Flock, with the exception of ARQ/ARQ sheep challenged with SSBP/1, which were completely susceptible in the former and resistant in the latter. The 141 codon genotype was shown to significantly influence incubation times in both BSE- and scrapie-infected sheep, although with more marked effects in the former. This study is one of the only large scale comparative studies of susceptibility to BSE across a wide range of genotypes, and also suggests non-*PRNP* genetic effects on susceptibility to peripheral challenge with scrapie.

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P.165: The flexible tail of the prion protein mediates the toxicity of anti-prion antibodies

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Introduction. The cellular prion protein (PrP^{C}) is an extracellular, membrane-anchored protein that is highly abundant in the nervous system, and consists of two domains, an N-terminal flexible tail (FT) and a C-terminal globular domain (GD). Widespread deposition of an aggregated and misfolded isoform of PrP^{C}, denoted as PrP^{Sc}, is a distinctive hallmark of fatal infectious prion diseases and is accompanied by dramatic neuronal loss. While it is well established that PrP^{C} is required for PrP^{Sc}-mediated toxicity in prion infections, which suggests a crucial interaction between PrP^{Sc} and PrP^{C}, the mechanisms by which aggregated prions are lethal to neurons remain a mystery.

Results. Recently, we established the first ex vivo model of prion disease using cerebellar organotypic cultured slices (COCS), which recapitulates all of the major pathological features of a genuine prion infection. Moreover, we mimicked prion-induced neuronal death ex vivo and in vivo with antibodies, and monovalent Fab and single chain Fv derivatives directed against certain epitopes of the GD. The latter approach ("the antibody model") eliminates the need to work with infectious prions and shortens the timeframe of neurotoxicity. The antibody model and prion infections were characterized by calpain activation and an ROS burst. Furthermore, neuronal death was blocked by pharmacological ROS scavengers and calpain inhibitors, indicating that neuronal death is mediated by similar signaling pathways in both models. GD antibody-mediated neurotoxicity was also prevented by pretreatment with antibodies against the FT and by shortening of the FT, thus designating the FT as the effector module of neuronal death. Congruently, blockade of the FT with specific antibodies was beneficial in genuine prion infections and in mice expressing a truncated toxic PrP variant.

Conclusions. We have shown that GD antibody-induced neurotoxicity is a good model to study PrP-dependent neurotoxicity that is uncoupled from PrP^{Sc} replication/infectivity. Our results uncovered two PrP modules, the GD as the receptor module of toxic ligands and the FT as the effector module of toxicity. We believe that therapeutic agents interfering with these domains

may be a novel option for future drug development to treat prion-mediated neurodegeneration.

P.166: PrP^{TSE} association with blood-circulating exosomes during the pre-symptomatic and clinical phases of TSE

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Background. Presence of disease-associated misfolded prion protein (PrP^{TSE}) has been demonstrated in the blood of vCJD patients. However, its origin and distribution in this fluid are still unknown. Various studies have identified PrP^{C} among the protein cargo in human blood-circulating microvesicles, including exosomes; and infectivity was shown to be present in exosomes isolated from the conditioned media of TSE-infected cells upon injection into mice. These findings raised the possibility that exosomes may serve as vehicles for the transfusion transmission of TSEs. In this study we isolated exosomes from plasma samples of experimental models infected with human TSE agents and demonstrated the presence of PrP^{TSE} by biochemical methods.

Materials and Methods. Exosomes were isolated with ExoQuick from plasma collected from animals infected with human TSEs at various time points during the incubation period and at clinical stage; and from appropriate uninfected controls. Exosome pellets were mixed with uninfected mouse brain homogenates and amplified by saPMCA. PrP^{TSE} was detected by Western blot, after proteinase K digestion.

Results. saPMCA allowed the specific detection of PrP^{TSE} in plasma exosomal samples from TSE-infected animals during the pre-clinical and clinical phases of the disease, as early as 6 weeks post infection. PrP^{TSE}-containing exosomal preparations were positive for the exosomal marker Hsp70 and negative for Golgi and nuclear markers by Western blotting. Nanoparticle tracking analysis showed 114 nm particles consistent with the expected size of exosomes; demonstrating that a fraction of blood-circulating PrP^{TSE} is exosome bound. Serial amplification of exosomal PrP^{TSE} by PMCA followed by intracerebral inoculation into Tga20 mice resulted in a clinical phenotype indistinguishable from that caused by the inoculation of brain-derived material. Western blot analysis demonstrated presence of PrP^{res} in the brains of Tga20 mice injected with exosomal preparations. Comparative histological brain profiling of inoculated Tga20 is currently under way.

Conclusions. We report the biochemical detection of PrP^{TSE} in plasma exosomal fractions, obtained from pre-clinical and clinically sick animals, for the first time. Biochemical identification of PrP^{TSE} in blood-derived exosomes from TSE-infected models provides an invaluable foundation for the development of new diagnostics and potential targets for TSEs treatment and the design of appropriate removal technologies to ensure blood safety. Further characterization of PrP^{TSE}-containing microvesicles will allow identification of the cellular origin of prion-containing exosomes and the site(s) of prion replication in blood.

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P.167: Prion fate in lymphoid structures devoid of follicular dendritic cells

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Keywords: prions, follicular dendritic cells, granuloma

Background. The conversion of the cellular prion protein (PrP^{C}) into an abnormal, aggregated isoform (PrP^{Sc}) is central to prion pathogenesis. While PrP^{Sc} accumulation and the resulting damages are essentially confined to the central nervous system, PrP^{Sc} can also accumulate silently in lymphoid organs. In addition, spleen appears much less selective than the brain during prion cross-species transmission events.[1] In the spleen, follicular dendritic cells (FDCs) are considered essential for prion replication.[2] Recently, mouse granulomas, which are lymphoid structures associated with chronic inflammation, were shown to accumulate prions.[3] We addressed whether prion uptake and replication dynamics are similar between spleens and granulomas, despite the reported absence of typical FDCs in these ectopic structures.

Objective and Methods. We compared prion replication dynamics in spleen and granulomas from ovine PrP transgenic mice (tg338 line) infected with the lymphotropic 127S scrapie strain. Two inoculum sources with differing PrP^{Sc} oligomeric sizes were used, owing that trapping and subsequent addressing of antigens to lymphoid tissues is size dependent.[4] Mice were injected twice with complete Freund adjuvant to induce granuloma formation before intra-peritoneal inoculation of the two prion preparations. Mice were euthanized at defined time points for immunohistochemical and biochemical analyses of brains, spleens and granulomas.

Results. A assessed with FDC-specific immunostaining, the granulomas induced in tg338 mice were devoid of FDCs. By this method, PrP^{Sc} was detected in all the spleens and granulomas collected as soon as 30 days post inoculation, whatever the source of inoculum. At that stage, the amounts of PrP^{res} and the PMCA templating activity of both spleens and granulomas extracts appeared superimposable. In contrast, PrP^{res} was detected in the brain only at later stage of disease (90 days). At this stage, PMCA reactions seeded with 107-108-fold diluted spleen and brain material were positive. Granuloma templating activity was 10-fold lower. Thermolysin digestion studies revealed that, whatever the inoculum, granuloma PrP^{Sc} was truncated endogenously,

as in the spleen. Finally, spleen and granuloma PrP^{Sc} aggregates exhibited similar size distribution, as assessed by sedimentation velocity gradients.

Conclusions. These data suggest that both 127S prions fate and biochemical nature of the PrP^{Sc} species generated appear conserved between spleens and granulomas. Prions could thus replicate in lymphoid structures devoid of FDCs.

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P.168: Evolution of the biological properties of L-BSE after passage in sheep with susceptible and resistant PrP genotypes

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Background. Cattle L-BSE was efficiently transmitted to sheep with susceptible (QQ^{171}) and resistant (QR^{171}) PrP genotypes.[1] Notably, the PrP^{Sc} signature of L-BSE was preserved in QQ^{171} sheep but not in QR^{171} sheep.[2] Notwithstanding, bioassay in transgenic mice expressing bovine or ovine (ARQ) PrP^{C} showed that L-BSE strain was preserved in both, QQ^{171} and QR^{171} sheep-passaged L-BSE.[3]

Here we studied the biological properties of sheep-passaged L-BSE by bioassay in bank voles and transgenic mice expressing the ovine VRQ PrP (tg338), both characterized by a comparatively low susceptibility to cattle L-BSE.

Material and Methods. Voles and tg338 mice were intracerebrally inoculated with cattle L-BSE and sheep-passaged (QQ^{171} and QR^{171}) L-BSE isolates. Survival time, lesion profiles, Pet-blot and WB analysis were used for strain typing.

Results. Cattle L-BSE transmitted quite inefficiently to tg338 mice, with survival time >400 days post-infection (d.p.i.), while sheep-passaged inocula were much more efficient and all gave terminal disease by ~140 d.p.i. However, after sub-passage all inocula converged to a survival time of ~145 d.p.i.. and showed overlapping pathological phenotypes.

In voles, cattle L-BSE transmitted with very long survival times (~800 d.p.i.) and was accompanied by an upward shift of the PrP^{Sc} type. Again, all sheep-passaged L-BSE isolates transmitted much more efficiently, with similar survival times of ~360 d.p.i.. Upon second passage, three different strains were isolated in vole, characterized by distinct pathological phenotypes. This divergence is epitomized by the different survival times of vole-adapted L-BSE strains, which were ~400 d.p.i. for cattle L-BSE, ~130 d.p.i. for QQ^{171}-passaged L-BSE and ~225 d.p.i. for QR^{171}-passaged L-BSE.

Conclusions. These findings, along with previously published data,[3] show that the original L-BSE strain was recovered after passage in sheep when bioassay was performed in animal models expressing bovine or ovine PrP^{C}. In contrast, strain changes were observed in both, QQ^{171}- and QR^{171}-passaged L-BSE by bioassay in vole, a species with divergent PrP sequence compared to ruminants. Importantly, QQ^{171}- and QR^{171}-passaged L-BSE were characterised by different PrP^{Sc} types and, accordingly, showed different biological properties when transmitted to voles, but not when transmitted to other animal models. Overall, our work support the hypothesis that prion isolates are likely composed of multiple prion components, emphasizes the role of host PrP polymorphisms on strain selection and mutation, and highlights the risk for new potentially zoonotic strains that could emerge from prion evolution in animal reservoirs.

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P.169: PrP^{Sc} distribution in brain areas of a natural German H-type BSE case

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Keywords: BSE H-type, brain, muscle

Ten years after the initial description of atypical BSE cases of the H-type and L-type, the distribution of PrP^{Sc} in different brain areas and peripheral tissues of natural cases of these BSE forms is still not fully understood. Intracerebral challenge experiments have been performed with both atypical BSE forms in cattle, and the distribution of the abnormal prion protein and infectivity has been analysed in a variety of tissues, confirming the general restriction to the central nervous system as it was already generally acknowledged for classical BSE, but showing a slightly earlier and stronger involvement of the peripheral nervous system and the skeletal muscle.

However, data from cattle orally challenged with atypical BSE, which might mimic the natural situation, are not yet available. Unfortunately, for most natural cases of atypical BSE, only the obex region is available for further analysis. The PrP^{Sc} distribution in the brains of natural L-type BSE cases in Italy has been described in some detail, but comparably few such data are yet available for natural H-type cases. Here we describe the analysis of different brain areas and muscle samples of a natural H-type BSE case diagnosed in Germany in 2014, and compare these data with those obtained from the respective samples collected from cattle challenged intracerebrally with H-type BSE.

P.170: Potential detection of oral transmission of H type atypical BSE in cattle using in vitro conversion

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Keywords: Atypical BSE, oral transmission, RT-QuIC

The detection of bovine spongiform encephalopathy (BSE) has had a significant negative impact on the cattle industry worldwide. In response, governments took actions to prevent transmission and additional threats to animal health and food safety. While these measures seem to be effective for controlling classical BSE, the more recently discovered atypical BSE has presented a new challenge. To generate data for risk assessment and control measures, we have challenged cattle orally with atypical BSE to determine transmissibility and mis-folded prion (PrP^{Sc}) tissue distribution. Upon presentation of clinical symptoms, animals were euthanized and tested for characteristic histopathological changes as well as PrP^{Sc} deposition.

The H-type challenged animal displayed vacuolation exclusively in rostral brain areas but the L-type challenged animal showed no evidence thereof. To our surprise, neither of the animals euthanized, which were displaying clinical signs indicative of BSE, showed conclusive mis-folded prion accumulation in the brain or gut using standard molecular or immunohistochemical assays. To confirm presence or absence of prion infectivity, we employed an optimized real-time quaking induced conversion (RT-QuIC) assay developed at the Rocky Mountain Laboratory, Hamilton, USA.

Detection of PrP^{Sc} was unsuccessful for brain samples tests from the orally inoculated L type animal using the RT-QuIC. It is possible that these negative results were related to the tissue sampling locations or that type specific optimization is needed to detect PrP^{Sc} in this animal. We were however able to consistently detect the presence of mis-folded prions in the brain of the H-type inoculated animal. Considering the negative and inconclusive results with other PrP^{Sc} detection methods, positive results using the optimized RT-QuIC suggests the method is extremely sensitive for H-type BSE detection. This may be evidence of the first successful oral transmission of H type atypical BSE in cattle and additional investigation of samples from these animals are ongoing.

P.171: Towards validating RT-QuIC versus standard TSE test platforms and bioassay

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Introduction. Chronic wasting disease (CWD) is a well-established prion disease affecting farmed and wild cervids in North America. Infected animals propagate and shed the CWD prion, greatly exacerbating its presence, posing great risks for wildlife ecology, aboriginal lifestyle, and control measures. In vitro PrP conversion assays have been developed as an ultrasensitive approach for ante-mortem prion detection. However, there are limited comprehensive data regarding their performance in a diagnostic setting, particularly how in vitro PrP conversion assays rank against conventional test platforms and bioassay models.

Materials and Methods. Using recombinant full-length elk PrP^{C}, we compared the analytical performance of Real-Time Quaking-Induced Conversion (RT-QuIC) assay versus conventional test platforms: an in-house western-blot, the TeSeE ELISA (BioRad) and HerdChek CWD EIA (IDEXX) using dilution series of CWD brain homogenates. RT-QuIC sensitivity was also evaluated on an end-point titred elk CWD brain homogenate by $Tg(CerPrP\text{-}M132)1536^{+/-}$ and $Tg(CerPrP\text{-}E226)5037^{+/-}$ bioassay models.[1] By using analyses of signal distribution, we outlined diagnostic criteria for RT-QuIC to achieve 100% sensitivity and 100% specificity, and evaluated assay reproducibility by multifactorial univariate ANOVAs. Penultimate detectable dilutions for the RT-QuIC and the ELISAs were determined by LD_{50}-like criteria pertaining to test cut-offs. Detection limits for the western-blot were determined by band signal appearance.[2]

Results. The RT-QuIC exhibited ~4-fold greater sensitivity for elk CWD in brain homogenate than the most sensitive ELISA results (HerdChek CWD EIA). In addition, RT-QuIC performed equally well on tonsil as on brain, demonstrating its potential for use on RAMALT samples. RT-QuIC showed ~16 fold greater sensitivity for elk CWD infectivity than the $Tg(CerPrP\text{-}M132)1536^{+/-}$ and $Tg(CerPrP\text{-}E226)5037^{+/-}$ mouse bioassay models using the elk CWD homogenate in Bian et al.[1] Kinetic profiles of all elk CWD-seeded reactions are consistent in resemblance, independent of tissue sample type or animal tested.

Conclusion. This study provides a comprehensive illustration regarding relative performance between conventional TSE test platforms, the RT-QuIC, and bioassay. We show the RT-QuIC is slightly more sensitive than the best obtained ELISA result for elk CWD, and approximately 1 $\log_{10}$ more sensitive for elk CWD infectivity than the aforementioned bioassay models, consistent

with other recently published data.[3-5] In addition, our kinetic data suggest seeded reactions are an ordered, on-pathway amyloid formation mechanism, as previously described.[6] The consistencies in our reaction behaviour bestow the confidence required to satisfy rigorous diagnostic demands. Future research is aimed toward qualitative analysis of our elk CWD-seeded RT-QuIC reaction products.

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P.172: BSE exposure risk from bovine intestine and mesentery

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Keywords: Bovine Spongiform Encephalopathy (BSE), cattle, intestine, mesentery, specified risk material (SRM), quantitative risk assessment (QRA)

Bovine intestines and mesenteries in the European Union (EU) are considered among the tissues potentially containing the highest level of BSE infectivity and have to be removed from the food and feed chain. A quantitative assessment of the BSE infectious load potentially entering the food and feed chain yearly in the European Union (EU) was developed. The evolution of the BSE infectious titre and of the weight of the structures accumulating infectivity was considered. The number of BSE infected cattle entering undetected in the food and feed chain yearly was estimated. A model (TSEi) was developed to estimates the evolution of the BSE infectious load in animals and the total yearly infectious load that could enter the food and feed chain. In a BSE infected bovine, the distribution of infectivity in intestines and mesentery varies with the age. Up to 36 months of age the infectivity is mainly associated (on average more than 90%) with the last 4 metres of small intestine and the caecum, over 36 and under 60 months of age, there is an inter-individual variability, from 60 months of age the infectivity is mainly associated (on average more than 90%) with the mesenteric nerves and the celiac and mesenteric ganglion complex. The total amount of infectivity peaks, about 15 $BoID_{50}$, in animals younger than 18 months, it declines to 8-9 $BoID_{50}$ (24–48 months of age) and it drops to 0.7 $BoID_{50}$ in animals older than 60 months. The ileo-caecal plate is the most infectious part of the intestine and it can be used to estimate the potential maximum level of exposure for an individual consumer. In the EU, between 2007 and 2012, the yearly amount of BSE infectivity associated with intestine and mesentery from animals entering the food and feed chain was reduced by a factor of 10 (from about 23,000 to about 2,000 $BoID_{50}$). However, the maximum level of exposure to the BSE agent from intestine remained stable (on average about 1.5-1.6 $BoID_{50}$ per meter). In case of re-emergence of BSE in the EU there would be an increase of the potential maximum level of exposure to BSE from intestine. According to the TSEi model the removal of the last four metres of the small intestine and of the caecum from the food and feed chain would result in a major reduction of the BSE exposure risk associated with intestine and mesentery in cattle.

P.173: Evaluation of immunogenicity of prion vaccine administered together with vaccine enhancing agent

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Transmissible spongiform encephalopathy (TSE) is a neurodegenerative disorder characterized by pathologic accumulation of a misfolded form of a normal cellular protein in neurons. Emergence of TSEs in wildlife populations and the ability of some TSEs to cross species barriers have prompted concern regarding the lack of treatment options or prevention strategies. Efforts at vaccine development have been hampered by the difficulty of overcoming self-tolerance. Studies in our lab have demonstrated that vaccine induced immunity is often diminished due to the recruitment of anti-inflammatory myeloid cells. We hypothesized that utilizing an effective antigen while simultaneously inhibiting monocyte migration could elicit a more effective anti-prion response.

The vaccine was formulated using a peptide fragment of the human prion protein (PrP106-126). This peptide spontaneously forms fibrillar aggregates and is thought to mediate the

conversion from the normal cellular prion protein (PrP^{C}) to the pathogenic form (PrP^{Sc}). To enhance vaccine efficacy, a monocyte migration inhibitor was administered (RS102895). To further target the pathogenic PrP^{Sc} , the peptide was reconstituted in an acidic solution and incubated at 37°C to increase fibrillization. Wild type mice were divided into three groups consisting of a control group, a vaccine group and a group receiving the vaccine plus RS102895.

Antibody responses were assessed using ELISA and Western Blot. Both groups of vaccinated mice exhibited significantly increased vaccine titers when compared to vaccinated mice that did not receive this compound. In addition, the vaccine group that received RS102895 displayed a dramatic cell mediated immune response as evidenced by production of interferon gamma when splenic lymphocytes were stimulated with the vaccine antigen. This group also exhibited increased concentrations of antibodies against both PrP 106-126 and PrP^{C}.

This vaccination regime shows great promise in eliciting an immune response, thus overcoming self-tolerance. Our results suggest that this strategy could overcome the limitations that have thus far prevented successful development of a prion vaccine.

P.174: TSE strain differences in conventional mice by triplex western blotting

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TSE strains are conventionally identified by their incubation period and vacuolation profile in the brain after intracerebral passaging in inbred mouse lines differing in their PrP genotype: RIII ($sinc^{s7}/L_{108}T_{189}$) or VM ($sinc^{p7}/F_{108}V_{189}$). Analysis of biochemical characteristics of PrP^{Sc} can be an additional, unequivocal and sensitive means to differentiate between TSE strains. We investigated the potential of the proteinase K (PK) resistant PrP^{Sc} profiles in our triplex Western blotting method (triplex-WB)[1] as possible tool to differentiate between strains.

TSE reference strains ME7, 87A/87V, 22A/22C, 79A/79V and 301C/301V were inoculated in RIII or VM inbred mice which differ in their PrP genotype at codons 108 and 189. Sagitally taken half brains were used for homogenisation and subsequent PK digestion. For triplex-WB, antibodies 12B2 (mouse epitope $_{88}WGQGG_{92}$), Sha31 ($_{144}YEDRYYRE_{151}$) and SAF84 ($_{162}YRPVDQY_{168}$) served to analyse PrP^{Sc} on a single lane simultaneously for molecular mass, 12B2 Nterminal epitope content, glycoprofile, and presence of a dual PrP^{res} population.[1]

The results obtained by triplex-WB analyses showed that the combination of glycosylation profile and 12B2 epitope content of PrP^{Sc} allowed to distinguish between all reference strains within each mouse line except for ME7 and 22A in VM mice.

These data will serve as basis for studies to identify TSE strains in ruminants already after primary passage in mice either as single or as mixed strains within a single mouse brain.

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P.175: Effects of a naturally occurring amino acid substitution in bovine PrP: A novel model for inherited prion disease in a natural host species

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The most common hereditary prion disease is a genetic form of Creutzfeldt-Jakob disease (CJD) in humans where a mutation in the prion gene (*PRNP*) results in a glutamic acid to lysine substitution at position 200 in the prion protein. Models of E200K CJD in transgenic mice have proven interesting but have significant limitations including inconsistencies with regard to disease phenotype, use of mixed species chimeric protein constructs, and relatively short life span and long time to disease onset in these animals. These factors limit the opportunity to effectively study the proposed role of the polymorphism in accelerating disease. Here, we present the first model of E200K disease in a natural host—cattle carrying the homologous mutation (E211K). This amino acid substitution was associated with a case of bovine spongiform encephalopathy in 2006. We assessed this bovine protein variant as a model for genetic prion disease by evaluating the changes in protein stability for the disease associated variants relative to the wild-type and compare those to the homologous human proteins, characterizing the expression of the cattle E211K carriers in relation to findings in humans, and providing evidence that the impacts of copper-induced oxidative stress are different in cattle as compared to observations in the mouse model.

P.176: Comparison of dynamic perturbed networks in mouse models of Huntington's disease and prion infection

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We applied our strategy that previously identified preclinical network changes induced by prion infection[1] to the protein-misfolding (prion) disease caused by CAG repeat expansions in the huntingtin gene (*Htt*).[2] Analysis of phenotypically diverse mice that differ in genetic background and the disease-inducing entity (prion strain or CAG repeat length) is key to this approach. To identify the earliest perturbations in dynamic gene expression networks in the striatum of *Htt* CAG knock-in heterozygous mice, we generated time course transcriptomic data using Agilent Mouse 8×60K arrays for global gene expression profiling on 719 mice from 10 lines of 4- to 20-week-old mice. C57BL/6J mice with three different disease-associated CAG repeat lengths (Q50, Q92, Q111) or the wild-type (wt) *Htt* allele were analyzed along with CD1, 129S2, and FVB/N mice carrying the Q111 *Htt* allele or the corresponding wt allele. We collected at least 3 mice per week and, in addition to expression profiling, tracked nuclear localization of HTT and repeat length stability in the striatum, traits known to vary depending on genetic background and repeat length. Linear modeling revealed a total of 809 genes (935 probes) that were differentially expressed between Q111 and wt mice in a combined analysis of all four strains. The onset of differential gene expression at 10 to 12 weeks coincided with onset of HTT nuclear localization in C57BL/6J and CD1 mice, but not in FVB/N mice, which showed similar mislocalization of HTT. The magnitude of differential expression between Q111 and wt mice increased as the mice aged. Differentially expressed genes (DEGs) include many genes that are dysregulated in striatum of humans with late-stage Huntington's disease. Gene co-expression network analysis revealed that many of the DEGs are part of a single, tightly co-expressed module enriched for synaptic functions. This contrasts with the multiple modules perturbed early in prion disease, even within the striatum. Ongoing analyses are attempting to glean further biological insights from these data and link network variance to "tipping points" from healthy to disease states for Huntington's disease, prion infection and other neurodegenerative disorders.

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P.177: Elements modulating the prion species barrier and its passage consequences

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The phenotypic features of Transmissible Spongiform Encephalopathy (TSE) strains may be modified during passage across a species barrier. In this study we investigated the biochemical and biological characteristics of Bovine Spongiform Encephalopathy (BSE) infectious agent after transmission in both natural host species (cattle, sheep, pigs, and mice) and in transgenic mice overexpressing the corresponding cellular prion protein (PrP^{C}) in comparison with other non-BSE related prions from the same species. After these passages, most characteristics of the BSE agent remained unchanged. BSE-derived agents only showed slight modifications in the biochemical properties of the accumulated PrP^{Sc}, which were demonstrated to be reversible upon re-inoculation into transgenic mice expressing bovine-PrP^{C}. Transmission experiments in transgenic mice expressing bovine, porcine or human-PrP revealed that all BSE-derived agents were transmitted with no or a weak transmission barrier. In contrast, a high species barrier was observed for the non-BSE related prions that harboured an identical PrP amino acid sequence such as sheep-scrapie, mouse RML or human sCJD isolates, supporting the theory that the prion transmission barrier is modulated by strain properties (presumably conformation-dependent) rather than by PrP amino acid sequence differences between host and donor.

As identical results were observed with prions propagated either in natural hosts or in transgenic mouse models, we postulate that the species barrier and its passage consequences are uniquely governed by the host PrP^{C} sequence and not influenced by the PrP^{C} expression level or genetic factors other than the PrP^{C} amino acid sequence. All these findings unequivocally demonstrate that the species barrier and its passage consequences are uniquely driven by the PrP^{C} sequence, and not by other host genetic factors, demonstrating the validity of transgenic PrP animals as models for studies of the species barrier.

The results presented herein reinforce the idea that the BSE agent is highly promiscuous, infecting other species, maintaining its properties in the new species, and even increasing its capabilities to jump to other species including humans. These data are essential for the development of an accurate risk assessment for BSE.

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P.178: Longitudinal quantitative analysis of CWD prions shed in saliva of deer

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Background/Introduction. Chronic Wasting Disease (CWD) is an emergent rapidly spreading fatal prion disease of cervids (deer, elk and moose). CWD has now been identified in 22 States (including two new states within the last year), 2 Canadian provinces, and South Korea. Shedding of infectious prions in excreta (saliva, urine, feces) may be an important factor in CWD transmission. Here we apply an adapted version of a rapid in vitro assay [real-time quaking-induced conversion (RT-QuIC)] to determine the time of onset, length, pattern, and magnitude of prion shedding in saliva of infected deer.

Materials and Methods. The RT-QuIC assay was performed as previously described in Henderson et al. PLoS-One (2013). Saliva samples were quantitated by comparison to a RT-QuIC reaction rate standard curve of a bioassayed obex sample from a terminally ill cervid.

Results. To better understand the onset and length of CWD prion shedding we analyzed >150 longitudinally collected, blinded, then randomized saliva samples from 17 CWD-infected and 3 uninfected white-tailed deer. We observed prion shedding, as detected by the RT-QuIC assay, as early as 3 months from inoculation and sustained shedding throughout the disease course in both aerosol and orally exposed deer. We estimated the infectious lethal dose of prions shed in saliva from infected deer by comparing real-time reaction rates of saliva samples to a bioassayed serially diluted brain control. Our results indicate that as little as 1 ml of saliva from pre-symptomatic infected deer constitutes a lethal CWD prion dose.

Conclusions. During the pre-symptomatic stage of CWD infection and throughout the course of disease deer may be shedding multiple LD50 doses per day in their saliva. CWD prion shedding through saliva and excreta may account for the unprecedented spread of this prion disease in nature.

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