NeuroPrion Workshop: New developments in TSEs of domestic and wild animals
Chalkidiki, Greece, 22nd September 2009
Programme & Abstracts

Organized by EU funded projects NeuroPrion (TSEgoat group and Cervids group) and goatBSE
Photography credits:
Deer: Candace Mathiason Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA
Goats: Eleni Hadjikiriakou, www.redbubble.com
Sheep: Peter Bates and Nicky Commander, Veterinary Laboratories Agency UK
Programme

NeuroPrion Workshop: New developments in TSEs of domestic and wild animals. Chalkidiki, Greece, 22nd September 2009

Organized by EU funded projects NeuroPrion (TSEgoat group and Cervids group) and goatBSE.

It is a pleasure to again run a workshop just prior to the Prion2009 conference on TSEs. The occurrence of TSEs in the field and farms carries potential risks of the agents in the environment and food. The ease of CWD spread and the shedding of CWD prions among cervids as well as the examples of prion infections in goat herds, and the transmission of scrapie through milk all argue for better containment and eventual eradication of these diseases. As a follow-up to previous workshops, participants of NeuroPrion TSEgoat and cervid groups, and goatBSE have again organized a workshop to bring researchers together to discuss these veterinary and public health issues. This year we are also pleased to add a CWD research group leader from the United States to the organising committee. Apt presentations have been actively sought by the organisers including some from interesting abstracts sent to the Prion2009 conference.

Mick Stack, Veterinary Laboratories Agencies, Addlestone, Surrey, United Kingdom
m.j.stack@vla.defra.gsi.gov.uk (contact person)

Jan Langeveld, Central Veterinary Institute of WageningenUR, Lelystad, The Netherlands
jan.langeveld@wur.nl

Edward Hoover, Colorado State University, Fort Collins, CO, USA
edward.hoover@colostate.edu

Venue: Meliton Hotel, Porto Carras
Meeting room number will be posted in the hotel lobby.

Timetable at a glance

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30 - 9:30</td>
<td>Reception/registration</td>
</tr>
<tr>
<td>9:30 - 11:30</td>
<td>Goat and sheep presentations</td>
</tr>
<tr>
<td>11:30 - 11:50</td>
<td>Coffee break</td>
</tr>
<tr>
<td>11:50 - 14:20</td>
<td>CWD presentations</td>
</tr>
<tr>
<td>14:20 - 15:20</td>
<td>Lunch</td>
</tr>
</tbody>
</table>
New developments in TSEs of domestic and wild animals

9:25  Welcome from Mick Stack

SESSION I - Session leader: Jan Langeveld

Thierry Baron

Cecilia Bucalossi

10.10 - 10.30  P3. Ovine and bovine PRNP transgenic mice allows discrimination between Scrapie and BSE in co-infected mice and sheep.
Frederick Lantier

10.30 - 10.50  P4. Genetic, immunohistochemical and biochemical studies on goat TSE cases from Cyprus.
Martin Eiden and Christine Hoffmann

10.50 - 11.10  P5. PrPsc deposition in placenta from natural infected ewes.
Maria Carmen Garza

Oliver Bannach

11.30 - 11.50  Coffee

SESSION II - Session leader: Edward Hoover

11.50 - 12.10  P7. Detection of CWD by RAMALT biopsy in two white-tailed deer farms.
Aru Balachandran

12.10 - 12.30  P8. Infectious prions in pre-clinical deer and transmission of CWD solely by environmental exposure.
Candace Mathiason

12.30 - 12.50  P9. Detection of low level CWD infection in deer after oral exposure to urine and feces.
Nicholas Haley

12.50 - 13.10  P10. CWD transmission via aerosol and oral lesions.
Nathanial Denkers

Jason Bartz

13.30 - 13.50  P12. Trafficking of CWD prions via the enteric autonomic nervous system.
Davis Seelig

Mick Stack

14.20 - 15.20  Lunch
P1. Unusual TSE isolates: an update on “CH1641-like” scrapie and atypical BSEs

Thierry Baron, Simon Nicot, Johann Vulin, Anne-Gaëlle Biacabe, and Anna Bencsik.
E-mail t.baron@afssa.fr
Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Lyon, France.

Background: Molecular studies of the CH1641 experimental scrapie isolate revealed that some scrapie cases could show some molecular similarities with BSE, although the TSE agent involved in such cases obviously differed from BSE as shown by transmission studies in mice. Such scrapie isolates have now been identified in natural conditions in both sheep and goats in several European countries, including France, UK and Cyprus. Such isolates also require further studies to clarify their possible relationship with recently recognized atypical forms of BSEs (H-type and L-type BSE (or BASE)) and recent molecular data showing the possible presence of previously unrecognized C-terminal fragments of PrP could help in this task.

Objectives: Unusual TSE isolates are studied for understanding the strain(s) involved in these cases and how they could behave after cross-species transmissions, should they occur.

Methods: Unusual isolates of TSEs in small ruminants ("CH1641-like") and cattle (H-type and L-type BSEs) are being studied by 1) molecular analysis by Western blot analysis of the protease-resistant PrP (PrPres) in the tissues of small ruminants and 2) bioassays in ovine transgenic (TgOvPrP4) and wild-type (C57Bl/6) mice.

Results and Discussion: From the molecular point of view, the “CH1641-like” isolates show very consistent features, in both sheep and goats, and differ from both classical BSE and L-type BSE by several molecular features which will be described. Among these, the most striking one is the presence of a C-terminally cleaved PrP product (PrP\textsuperscript{\#2})(unglycosylated form at ~ 14 kDa), detected by antibodies against the C-terminal part of the protein (SAF84), beside the usually described protease-resistant protein (PrP\textsuperscript{\#1})(unglycosylated form at ~ 19-20 kDa). This additional PrP\textsuperscript{\#2} product is resistant to both proteinase K and thermolysin digestion. After transmission in ovine transgenic mice (TgOvPrP4), “CH1641-like” scrapie also differs from both classical BSE and L-type BSE, not only by these PrP\textsuperscript{\#} molecular features, but also by the pattern of PrP\textsuperscript{\#} deposits as shown by immunohistochemistry. Overall these data show the usefulness of the analysis of C-terminal PrP fragments to better understand the diversity of TSE strains.

The two known forms of atypical BSEs have now been recognized in a number of European and non-European countries, with a total of about 50 cases reported. We will show preliminary results from the analysis of a panel of French atypical BSE cases regarding their behaviour after serial passages in C57Bl/6 wild-type mice.
**P2. Assessment of genetic susceptibility of sheep to scrapie: comparison between PMCA and in vivo studies**

**Cecilia Bucalossi¹, GianMario Cosseddu¹, Claudia D'Agostino¹, Michele Angelo Di Bari¹, Chiappini¹, Barbara¹, Michela Conte¹, Francesca Rosone², Francesco Giordani², Luigi De Grossi², Romolo Nonno¹, Umberto Agrimi¹, Gabriele Vaccari¹**

*Email: cecilia.bucalossi@iss.it*

¹ Istituto Superiore di Sanità, Italy
² Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Italy

**Background:** The susceptibility of sheep to scrapie is mainly influenced by the prion protein polymorphisms A136V, R154H, and Q171R/H. In a previous study, we challenged sheep carrying different genotypes (ARQ/ARQ, ARQ/ARR, ARR/ARR, ARQ/AHQ, AHQ/ARH, ARQ/ARQK₁₇₆ and ARQ/AT₁₃₇RQ) with classical scrapie. The survival times observed indicated different levels of susceptibility to scrapie, with the following ranking order: ARQ/ARQ > ARQ/AHQ > AHQ/ARH. The presence of the ARR allele (ARQ/ARR, ARR/ARR) conferred protection from disease. In the same experiment evidences of the protective effect of the AT₁₃₇RQ and ARQK₁₇₆ alleles were first reported (Vaccari, G. et al. 2007).

**Objectives:** 1. To analyze in vitro, by the Protein Misfolding Cyclic Amplification (PMCA), the conversion efficiency of the above-mentioned sheep genotypes triggered by sheep scrapie; 2. To compare the results of PMCA with those from in vivo studies.

**Methods:** Brain homogenates (10% w/v) from healthy sheep carrying the same genotypes tested in vivo were prepared as sources of PrP<sup>C</sup> (substrates). Brain homogenate (10% w/v) from a ARQ/ARQ sheep with natural scrapie was used as infectious seed. Dilutions from 10⁻¹ to 10⁻³ of the seed into the various substrates were submitted to PMCA. The conversion efficiency was assessed by calculating an amplification factor for each genotype.

**Results:** The ARQ/ARQ substrate showed the highest level of conversion efficiency. All the others genotypes showed lower amplification factors. In particular, the ARQ/AHQ substrate converted more efficiently than the AHQ/ARH. All genotypes which showed to be protective in vivo (ARQ/ARR, ARR/ARR, ARQ/ARQK₁₇₆ and ARQ/AT₁₃₇RQ) showed similar and weak amplification by PMCA.

**Discussion:** The conversion efficiency of PMCA mimicked the ranking order of genotype related susceptibility observed in vivo. Our results suggest that PMCA represents a high-throughput in vitro alternative to transmission studies for testing the susceptibility of the numerous sheep PrP genotypes to a variety of TSE sources.
Bioassays using ovine and bovine PRNP transgenic mice allow discrimination between scrapie and BSE in co-infected mice and sheep

I. Lantier¹, P. Berthon¹, H. Leroux¹, C. Rossignol¹, C. Lacroux², P. Bernardet³, H. Simmons⁴, J.M. Torres⁵, S. Simon⁶, O. Andreoletti² and F. Lantier¹

E-mail: Frederic.Lantier@tours.inra.fr
¹INRA, IASP, Centre of Tours, Nouzilly, France
²INRA-ENVT, IHAP, ENV Toulouse, France
³INRA, PFIE, Centre of Tours, Nouzilly, France
⁴VLA, WHEYBRIDGE, Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, UK
⁵CEA, IBitec-S, F-91191 Gif sur Yvette, France
⁶INIA, Madrid, Spain

Background: Differentiation between Scrapie and BSE infection in small ruminant flocks is currently based on discriminating biochemical tests performed on obex samples. We have shown through Scrapie-BSE co-infection experiments in sheep that BSE prion protein is not detected in nervous tissues on the contrary of Scrapie PrPsc. This was true with the 3 Scrapie strains tested. In lymphoid tissues, however, BSE PrPres was easily detected, pointing out the capacity of BSE to disseminate in lymphoid tissues.

Objective: Confirm BSE and Scrapie strains immunochemical identification through the inoculation of mouse lines transgenic for the bovine (Tg110) or ovine (Tg338) PRNP gene and investigate for silent carriage of BSE strain in nervous tissues of co-infected sheep.

Method: Groups of 6-7 mice were IC inoculated either with control strains, a mixture of ovine BSE and Scrapie (0.5:0.5 vol/vol) or with spleen and obex tissues from BSE/Scrapie co-infected sheep sampled at terminal stage of the disease.

Results: Clinical signs appeared with shorter incubation period in mice transgenic for the PrP gene corresponding to the origin of the TSE strain inoculated, i.e. bovine Tg110 in about 230 days after inoculation of BSE containing tissues and ovine Tg338 mice in 200-350 days, according to the Scrapie strain. Tg338 mice were highly resistant to BSE and reciprocally bovine Tg110 mice were highly resistant to Scrapie. Re-inoculation in these two mouse lines of tissues from co-infected sheep allowed confirming development of Scrapie in nervous tissues and BSE in lymphoid tissues of co-infected sheep. It also evidenced, at a lower level, BSE into their obex.

Discussion: Inoculation of transgenic Tg110 and Tg338 mice with BSE-Scrapie mixtures demonstrated their capacities to discriminate between these strains. These lines were used to investigate the carrier status of BSE-Scrapie co-infected sheep and allowed to evidence previously immunochemically undetected BSE in the brain of these animals, thus confirming that their diagnostic was impaired by the development of Scrapie.

Supported by EU contract QLRT-01309; animals were maintained by INRA-PFIE.
P4. Genetic, immunohistochemical and biochemical studies on goat TSE cases from Cyprus

Martin Eiden1, Christine Hoffmann1, Elizabeth Ortega Soto1, Susanne Freyse1, Pavlos Toumazos2, Penelope Papasavva-Stylianou3, Martin H. Groschup1

E-mail: Martin.Eiden@fli.bund.de

1Institute for Novel and Emerging Infectious Diseases at the Friedrich-Loeffler-Institut, Isle of Riems, Germany,
2Animal Health and Welfare Division, Veterinary Services, Nicosia, Cyprus,
3TSE's Laboratory, Veterinary Services, Nicosia, Cyprus

Introduction: Scrapie of sheep and goats belongs to transmissible spongiform encephalopathies (TSEs) or prion diseases, which are fatal neurodegenerative diseases in animals and humans. The susceptibility to scrapie is influenced by the prion genotype of the host, which has been studied particularly in sheep. Similar to sheep, goats harbor several PrP genotypes, however, the correlation with scrapie susceptibility remains unsolved up to now due to the limited number of individual caprine genotypes.

To further analyze scrapie in goats, caprine samples from a scrapie eradication program on Cyprus were analyzed by different methods. In order to determine molecular basis of resistance to scrapie, substitution of single amino acids at specific positions throughout the whole prion protein were generated and subsequently analyzed in a cell-free conversion assay.

Methods: Samples from cypriot goats were genotyped and further examined by BioRad TeSeE rapid test, Immunohistochemistry and biochemical methods (so-called FLI-Test as discriminatory immunoblot). Additionally ovine and caprine prion alleles were expressed in E. coli and purified according to standard procedures. Cell-free conversions were carried out by incubating recombinant prion protein with purified pathological prion protein (PrPSc) seeds in an appropriate buffer. Detection of newly converted PK-resistant PrPres fragments was carried out by immunoblotting and incubation with monoclonal antibody P4.

Results and discussion: In total 42 goats from 21 flocks were necropsied as clinical suspect cases. In 25 goats which showed a clear positive result in the BioRad TeSeE rapid test an accumulation of PrPSc in the obex region was demonstrated by immunohistochemistry. PrPSc deposits were also found in different tissues of the lymphoreticular system, even in animals with a negative staining reaction at the obex region. The discriminatory immunoblots showed for all TSE cases clear scrapie-like properties.

Furthermore, in-vitro-studies demonstrate that several prion alleles show a significantly reduced convertibility or are even inconvertible by PrPSc seeds. This highlights that specific amino acid residues induce a resistance of the cellular prion protein with regard to conversion and prion replication. Animals, which are heterozygous for susceptible and resistant prion alleles, had a reduced susceptibility to scrapie, indicating dominance of resistant over susceptible alleles. This dominant-negative effect can be imitated in vitro by co-incubating recombinant resistant and susceptible prion alleles in the cell-free conversion assay. In summary, our data indicate that in vitro analysis data of prion conversion experiments are consistent with the situation in vivo.
P5. PrP\textsuperscript{Sc} deposition in placentas from naturally infected ewes

Garza MC\textsuperscript{1}, Acín C\textsuperscript{1}, Álvarez J\textsuperscript{2}, Sanz A\textsuperscript{2}, Monzón M\textsuperscript{1}, Sarasa R\textsuperscript{1}, Badiola JJ\textsuperscript{1}, Morleón E\textsuperscript{1}

\textsuperscript{1}Centro de Referencia de Encefalopatías Espongiformes Transmisibles y Enfermedades Animales Emergentes. Universidad de Zaragoza. E-mail mcgarza@unizar.es.

\textsuperscript{2}Centro de Investigación y Tecnología Agroalimentaria de Aragón.

**Background:** Sheep scrapie is endemic in many countries, and it is widely accepted that one important way of horizontal transmission is through direct or indirect contact with infected placentas. PrP\textsuperscript{Sc} deposition in placentas depends mainly on the PRNP genotypes of the ewe and the foetus, so that it occurs in the placenta of scrapie infected sheep carrying a foetus of susceptible genotype, but it doesn’t seem to occur in fetuses carrying of resistant genotype (ARR haplotype).

**Objective:** The aim of the present study was to assess the PrP\textsuperscript{Sc} deposition in placentas from sheep with natural scrapie carrying fetuses of different PRNP genotypes.

**Materials and Methods:** A total of 22 ARQ/ARQ ewes positive for PrP\textsuperscript{Sc} in lymphoid tissue biopsies were included in the study. Infected ewes were mated with ARQ/ARQ or ARR/ARR rams. In addition, 4 already pregnant infected ewes of different genotypes and from different flocks. A total of 41 placentas from 32 gestations were collected and were analyzed for PrP\textsuperscript{Sc} detection by immunohistochemistry, Western blot analysis and ELISA.

**Results and Discussion:** PrP\textsuperscript{Sc} deposits were detected in 16 of 22 placentas from fetuses of ARQ/ARQ genotype. In placentas from fetuses with other haplotypes PrP\textsuperscript{Sc} was detected in 9 cases. Immunohistochemical results suggest that PrP\textsuperscript{Sc} can accumulate in placentas from fetuses carrying the ARR haplotype, when PrP\textsuperscript{Sc} is widespread at terminal stages of the disease.
P6. Ante Mortem Diagnosis of Sheep Scrapie by Single Particle Counting

Oliver Bannach, Franziska Henke, Eva Birkmann, Detlev Riesner

Heinrich-Heine-Universität Düsseldorf, Institut für Physikalische Biologie, Germany
Email: bannach@biophys.uni-duesseldorf.de

Background: Prion diseases can be transmitted via body fluids. Four cases of variant CJD had recently been reported, that were most likely caused by transfusion of contaminated blood. This raised serious concerns about blood product safety, and emphasizes the need of a robust test system to detect prions in living humans or animals.

Objectives: Our present goal is to detect prions in blood of Scrapie-afflicted sheep. We present the application of an ultra-sensitive diagnostic assay based on surface-FIDA that allows us to count single prion particles.

Methods: Prion aggregates are immobilized via a capture antibody to a chip surface, are labeled with fluorescent antibodies, and detected by confocal laser scanning. To optimize the surface-FIDA assay in respect to characteristic parameters of prion aggregates, we employ a Dual-Color Fluorescence Correlation Spectroscope (FCS) equipped with an XY-scanning piezo unit. The fluorescence intensities were recorded dependent on focus position and can be translated into image data. Using antibodies with different fluorescent labels and evaluating the crosscorrelation increases the specificity of detection.

Results: In order to estimate the applicability of our assay to blood plasma we added in a first step recombinant prion protein aggregates to ovine plasma. PrP epitopes are masked by plasma components such as LDL and thus antibody detection is impaired. To address this issue we developed a purification method employing Sarkosyl and a mixture of lipases. This treatment leads to a recovery of signal intensity suggesting that PrP epitopes become accessible for both capture and detection. A serial measurement of blinded plasma samples from clinical sheeps was conducted. Preliminary data showed that 6 out of 10 positive samples could be clearly differentiated from 5 negative control samples.

Discussion: Next we will analyze samples from preclinical stages. Moreover, we aim to apply our cell-extract-free amplification system in order to increase the sensitivity of the assay.
P7. Diagnosis of preclinical CWD in farmed white-tailed deer in Canada by the immunohistochemical examination of recto-anal mucosa-associated lymphoid tissue (RAMALT)

Aru Balachandran1, Bruce Thomsen2, Thomas Gidlewski3, Terry Spraker4, Gordon Mitchell1, Andre Soutyrine1, Noel Harrington1, Randy Munger3, David Schneider5 and Katherine O’Rourke5

E-mail: Aru.Balachandran@inspection.gc.ca
1Canadian Food Inspection Agency, Ottawa, Ontario, CANADA
2USDA-National Veterinary Services Laboratory, Ames, Iowa, USA
3USDA-APHIS-Veterinary Services, Fort Collins, Colorado, USA
4Colorado State University, Fort Collins, Colorado, USA
5USDA-Agricultural Research Service, Pullman, Washington, USA

Background: Approved testing for diagnosis of chronic wasting disease (CWD) in cervids includes postmortem detection of disease-associated prion protein (PrP\textsuperscript{CWD}) in lymph nodes or brain. Detection of PrP\textsuperscript{CWD} in recto-anal mucosa-associated lymphoid tissue (RAMALT) offers the possibility of live animal, preclinical diagnosis; however, recent reports suggest certain factors affect such detection in cervids. The present observational study reports preliminary analysis of two white-tailed deer farm depopulations in Canada.

Methods: For this analysis deer were considered CWD-positive if PrP\textsuperscript{CWD} was detected in the brain, retropharyngeal lymph node (RPLN) or tonsil by immunohistochemistry, ELISA or western blot.

Results: Farm A included 122 deer with an overall disease prevalence of 31%; Farm B included 385 deer with an overall disease prevalence of 21%. Approximate overall immunohistochemistry test sensitivities were: RPLN = 0.90, tonsil = 0.87, RAMALT = 0.72 and brain = 0.60. In CWD-positive deer from Farm A, PrP\textsuperscript{CWD} was detected in 55% of RAMALT biopsies from obex grade 0 deer, and in all RAMALT biopsies from deer with obex grades greater than 0. In CWD-positive deer from Farm B, PrP\textsuperscript{CWD} was detected in 33%, 77%, 96% of RAMALT biopsies respectively from obex grades 0-2 deer, and in all RAMALT biopsies from obex grades 3 and 4 deer. RAMALT follicle count data was available for only a subset of samples and was highly variable.

Discussion and Conclusions: This preliminary analysis suggests diagnostic evaluation of RAMALT in captive white-tailed deer has an intermediate sensitivity compared to the range associated with currently approved tissue sites. False-negative RAMALT results were most common in deer early in disease progression. Heterozygosity at PRNP codon 96 may be associated with more limited detection of PrP\textsuperscript{CWD}. 
P8. Infectious prions in pre-clinical deer and transmission of CWD solely by environmental exposure

Candace K. Mathiason¹, Sheila A. Hays¹, Jenny Powers², Jeanette Hayes-Klug¹, Julia Langenberg³, Sallie J. Dahmes⁴, David A. Osborn⁵, Karl V. Miller⁶, Robert J. Warren⁶, Gary L. Mason¹, and Edward A. Hoover¹

¹Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA, E-mail: ckm@lamar.colostate.edu
²National Park Service, Fort Collins, CO, USA,
³Wisconsin Department of Natural Resources, Madison, WI, USA,
⁴WASCO Inc., Monroe, GA, USA,
⁵Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, USA.

Introduction: The potential presence of prions in body fluids is perhaps most relevant to chronic wasting disease (CWD) of cervids, owing to its facile transmission, geographic expansion, and the relatively large amount of aberrant prion protein in peripheral lymphoid tissues. Nevertheless the exact mode by which the CWD prions are trafficked, shed and transmitted remains unknown.

Methods: Bioassay studies comprised of 4 cohorts of naïve deer were exposed either orally to (1) saliva, or (2) urine and feces, or via intravenous transfusion of (3) whole blood from pre-symptomatic or clinically affected CWD+ deer, or by contact with (4) environmental fomites (bedding, water and feed buckets) from CWD+ deer transitioning from pre-symptomatic to clinical disease. Two additional cohorts of naïve deer served as study controls including positive control deer exposed to brain from CWD+ deer and a negative control cohort consisting of deer receiving inocula from CWD- deer. The recipient animals were maintained under rigorous indoor isolation conditions to exclude potential adventitious prion exposure and monitored for CWD infection for a minimum of 18 months post infection by serial tonsil biopsy and terminal necropsy.

Results: Infectious prions capable of transmitting CWD were detected in saliva (by the oral route), in blood (by transfusion) and in the environment (by contact exposure) of CWD+ pre-symptomatic and clinically affected deer. PrP\textsuperscript{CWD} was first detected in tonsils between 3 and 15 months post inoculation. To our surprise, no deer fed urine and feces from CWD+ donors developed indicators of CWD infection after 18 months, despite multiple exposures.

Conclusion: We have previously reported that saliva and blood from CWD-infected deer contain sufficient infectious prions to transmit disease upon passage into naïve deer. Here we again use bioassays in deer to show that blood and saliva of pre-symptomatic deer contain infectious prions capable of infecting naïve deer and that naïve deer exposed only to environmental fomites from the suites of CWD-infected deer acquired CWD infection after a period of 15 months post initial exposure. These results: (a) help to further explain the basis for the facile transmission of CWD, (b) highlight the complexities associated with CWD transmission among cervids in their natural environment, (c) emphasize the potential utility of blood-based testing to detect pre-clinical CWD infection, and (d) could augur similar transmission dynamics in other prion infections.
P9. Detection of sub-clinical CWD infection in conventional test-negative deer long after oral exposure to urine and feces from CWD+ deer

Nicholas J. Haley1, Candace K. Mathiason1, Mark D. Zabel1, Glenn C. Telling2 and Edward A. Hoover1

1Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523, USA. E-mail: njhaley@lamar.colostate.edu
2Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky, Lexington, KY 40506 USA

Background: Chronic wasting disease (CWD) of cervids is a prion disease distinguished by its high level of transmissibility, wherein bodily fluids and excretions are thought to play an important role. Using cervid bioassay and established CWD detection methods, we have previously identified infectious prions in saliva and blood but not urine or feces of CWD+ donors. More recently, we were able to identify very low concentrations of CWD prions in urine of deer by cervid PrP transgenic (Tg[CerPrP]) mouse bioassay and serial protein misfolding cyclic amplification (sPMCA), leading us to reconsider the findings of our initial cervid bioassay experiments.

Objectives: In the current study, we sought to determine whether deer previously exposed orally to urine and feces from CWD+ sources, while conventional test-negative, may actually be harboring very low level CWD infection, not evident in the 19 month observation period in the previous studies. We further attempted to map the centripetal spread of PrPcWD in these animals from the gastrointestinal tract to the central nervous system in these animals.

Methods: Obex, vagal nerve, intermediolateral spinal cord segments, sections of ileum, and a wide range of lymphoreticular system (LRS) tissues from conventional test-negative deer were reanalyzed for CWD prions by sPMCA and cervid transgenic mouse bioassay in parallel with appropriate tissue-matched positive and negative controls.

Results: PrPcWD was detected in the tissues of orally exposed deer by both sPMCA and Tg[CerPrP] mouse bioassay; each assay revealed very low levels of CWD prions previously undetectable by western blot, ELISA, or IHC. Serial PMCA analysis of individual tissues identified that obex alone was positive in urine and feces exposed deer. Both LRS and neural tissues of positive control deer successfully amplified PrPcWD, while negative control tissues showed no comparable amplification.

Discussion: The finding of subclinical infection in deer orally exposed to urine and feces (1) suggests that a prolonged subclinical state can exist such that observation periods in excess of two years may be needed to detect CWD infection and (2) illustrates the sensitive and specific application of sPMCA in the diagnosis of low level prion infection. Based on our data, it appears that low doses of prions following oral exposure to urine and saliva bypass significant amplification in the LRS, likely using the neural routes as a conduit between the gut and central nervous system as has been hypothesized in other cases of naturally occurring prion diseases.
P10. Aerosol, Nasal, and Oral Transmission of Chronic Wasting Disease

Nathaniel D. Denkers¹, Glenn C. Telling² and Edward A. Hoover¹

¹Department of Microbiology, Immunology, and Pathology Colorado State University, Fort Collins, Colorado 80523. E-mail: nddenk@lamar.colostate.edu
²Department of Microbiology, Immunology and Molecular Genetics, and Department of Neurology, University of Kentucky, Lexington, Kentucky 40536

Purpose: While the exact mechanisms of chronic wasting disease (CWD) prion transmission, entry, and trafficking remain incompletely elucidated, transmission by exposure of the oral and/or nasal mucous membranes seems certain. Little is known regarding the potential risk posed by aerosolized prions. In addition, as part of foraging, cervids likely experience minor lesions in the oral mucous membranes. We explored whether CWD may be transmissible by aerosol or nasal mucosal exposure and whether or not micro-abrasions to the lingual mucosa may enhance susceptibility to oral CWD infection in mice transgenetically expressing cervid PrP.

Methods: FVB mice transgenically expressing the normal cervid PrP⁰ protein [Tg(cerPrP) mice] were exposed to CWD prions by either nose-only exposure to an aerosol (0.5 ml of a 5% w/v brain homogenate), by drop-wise instillation into the nostrils (10µl of a 10% w/v brain homogenate), or inoculated orally (same inoculum) with or without micro-abrasions on the lingual mucosa. Cohorts were sacrificed at 1, 2, 12, 52, 78, and 104 weeks post inoculation (pi) or when signs of neurologic disease were observed. Mice were assessed for PrP⁰ by western blotting and immunohistochemistry, with particular scrutiny directed to the nasal mucosa, vomeronasal organ, tongue, lymphoid tissue, and brain.

Results: Six of 7 aerosol-exposed and 2 of 9 IN-inoculated Tg(cerPrP) mice developed clinical signs of neurologic dysfunction mandating euthanasia between 411 and 755 dpi. Between 296 and 515 dpi, 9 of 9 CWD-inoculated mice with lingual lesions developed clinical signs of neurologic dysfunction mandating euthanasia. Conversely, all mice without oral lesions remain asymptomatic at >575 dpi. All of the symptomatic mice were positive for PrP⁰ by western blot and immunohistochemistry. No evidence of PrP⁰ could be detected in any Tg(cerPrP) mice sacrificed and examined at any of the pre-terminal time points.

Conclusions: CWD can be transmitted by aerosol and nasal exposure—potentially implicating exposure via the respiratory mucosa in CWD. Micro-abrasions to the lingual surface substantially facilitated CWD transmission, suggesting a co-factor that may be significant in foraging cervids or other species. These findings could have implications for the mucosal transmission of other prion diseases.
P11. Prion Protein Adsorption to Soil in a Competitive Matrix

Jason C. Bartz1*, Samuel E. Saunders2, and Shannon L. Bartelt-Hunt2

1Department of Medical Microbiology and Immunology, Creighton University, Omaha, Nebraska, United States of America. E-mail: jbartz@creighton.edu
2Department of Civil Engineering, University of Nebraska-Lincoln, Peter Kiewit Institute, Omaha, Nebraska, United States of America

Background: It is likely that the soil environment serves as a stable reservoir of infectious CWD and scrapie prions, facilitating a sustained incidence of CWD in free-ranging cervid populations and complicating efforts to eliminate disease in captive livestock herds. Prion adsorption to soil could play an important role in prion transport and in situ proteolysis and may induce conformational changes that enhance or decrease infectivity.

Objective: We sought to evaluate prion protein adsorption to soil and soil minerals as a function of time and aqueous concentration and compare these results to previous adsorption studies which used recombinant PrP or purified PrPSc. We hypothesized that the competitive matrix in which prions enter the environment (e.g. tissue, excreta) could significantly affect prion interactions with soil, and that these interactions may vary with prion strain and species.

Methods: We combine two previously published methods to quantify adsorbed prions via direct detection. Prion adsorption to two whole soils, sand, and clay was investigated in both kinetic and isothermal studies for up to 60 days. Prion-infected brain homogenates were used as complex, relevant prion sources.

Results: We determined that maximum PrP adsorption requires days or weeks, depending on the soil or mineral, and is two to five orders of magnitude lower than previous studies using purified PrPSc or recPrP. Our data also provide evidence that the N-terminal enhances adsorption of PrPSc to clay but may hinder adsorption to sand. PrP adsorption was maximal at an intermediate aqueous concentration, most likely due to the competitive brain homogenate matrix in which it enters the soil environment.

Discussion: Because PrP adsorption to soil is slow and less avid in tissue homogenate, the possibility of prion transport in soil environments cannot be excluded and requires further investigation. Our results suggest that the brain homogenate matrix can greatly affect PrP interactions with soil.
P12. Trafficking of CWD prions via the autonomic nervous system

Davis Seelig\textsuperscript{1}, Gary Mason\textsuperscript{1}, Glen Telling\textsuperscript{2}, Edward Hoover\textsuperscript{1}

\textsuperscript{1}Colorado State University, United States
\textit{Email: davis.seelig@colostate.edu}
\textsuperscript{2}University of Kentucky, United States

\textbf{Background:} The pathway by which CWD prions efficiently transit from the periphery to the central nervous system remains incompletely understood. Here we provide evidence that the autonomic nervous system (ANS) serves as a significant pathway for centripetal and centrifugal CWD spread in cervid PrP transgenic mice (Tg[CerPrP]) infected via oral and parenteral routes of exposure.

\textbf{Objectives:} The chief objective of this work was to determine the role of the ANS in the trafficking of CWD prions by longitudinally mapping the PrP\textsuperscript{\textsc{res}} tropism patterns in neural and non-neural tissues of multi-route inoculated Tg[CerPrP] mice.

\textbf{Methods:} Five groups of n=10 Tg[CerPrP] mice were inoculated with CWD prions via either the intracerebral (IC), intraperitoneal (IP), intravenous (IV), or oral (PO) route. Mice were sacrificed at specified time points post inoculation and at the onset of clinical disease. CWD infection was documented and prion trafficking patterns determined by detection of PrP\textsuperscript{\textsc{res}} using sensitive amplified immunohistochemistry methods.

\textbf{Results:} Early and progressive PrP\textsuperscript{\textsc{res}} depositions were detected in the parasympathetic, sympathetic, and enteric nervous systems, in particular within the myenteric plexus, vagus nerve, dorsal motor nucleus of the vagus, solitary tract, lateral ventricular nuclei of the hypothalamus, periqueductal gray matter, and spinal cord. Moreover, PrP\textsuperscript{\textsc{res}} was detected in intimate association with fibers and cells of the enteric nervous system, namely enteroglial cells, and sympathetically-innervated lymphoid organs, including the spleen, Peyer’s patches, and mesenteric lymph nodes.

\textbf{Discussion:} We present evidence for a temporal-based pattern of PrP\textsuperscript{\textsc{res}} accumulation in the parasympathetic and sympathetic elements of the autonomic and enteric nervous systems, implicating these elements as major pathways for CWD prion neuroinvasion and gastrointestinal prion shedding. These patterns of spread, including the accumulations in the enteric nervous system, may explain the ease by which CWD prions are taken up from and shed into the environment, and thereby its high degree of horizontal transmission.
P13. NeuroPrion cervid group activities and surveillance for TSEs in European cervids

Michael Stack

Veterinary Laboratories Agency, Weybridge, Surrey, United Kingdom, KT15 3NB
E-mail: m.j.stack@vla.defra.gsi.gov.uk

Background: In 2004, The European Food Safety Authority (EFSA) published an opinion on a surveillance programme for TSEs in deer within the European Union (EU). As an information gathering exercise, Commission officials and a scientist attended the 2nd International CWD Symposium held in Madison Wisconsin July 12 -14, 2005. Pre-conference tours of sampling outposts in the hunting area and the USDA diagnostic laboratory were arranged and a meeting with CWD experts from USA and Canada also took place. The objectives were to obtain a situation report on CWD in the USA and Canada and to highlight the best scientific approaches for surveillance in Europe based on the knowledge gained. Also, in 2005, the NeuroPrion initiative in Europe funded a cervids working group. In March 2007, the EU adopted Commission decision 182/2007/EC, which provided the legal basis for a time-limited survey for TSEs in deer. In January 2007 several EU countries started to monitor farmed and wild deer for TSEs. The survey comes to an end in Summer 2009 when it is hoped that EU Member States will meet their targets designed to detect disease at 0.5% prevalence with 95% confidence.

Questions: The NeuroPrion cervid group has 14 members and are attempting to answer the following questions:
● Are European cervids susceptible to CWD or other TSEs?
● Are diagnostic tests developed for CWD hosts suitable for European cervids?
● What is the likelihood/risk of CWD occurring in Europe?
● Will CWD/BSE transmit experimentally to European deer?
● Could we discriminate between CWD/scrapie or BSE infection if a positive were found?

Methods: With the help of a continued supply of proficiency samples from Canada the screening methods, confirmatory and discriminatory tests are all in place and are working.

Results: No positive European cervid TSE cases have been found.

Conclusions: Although transmission experiments with oral doses of BSE and CWD have shown susceptibility in the European red deer it would appear that European cervids have not been exposed to TSEs, neither in a captive nor wild environment.
Other Selected Abstracts of interest for Workshop Attendees (listed by submission number) which will be presented as posters or talks at Prion2009

Yutzy et al Detection of cellular prion protein (PrPc) in plasma from healthy cynomolgus monkeys (Macaca fascicularis) and changes observed after BSE infection
Topic: Basic Mechanisms of Neurodegeneration and Pathology
ID 11

McGovern et al Scrapie alters immune complex trapping and the maturation cycle of follicular dendritic cells
Topic: Transmission and Pathogenesis
ID 17

Larska et al Differences in the expression levels of selected genes in the brain tissue of cattle naturally infected with classical and atypical BSE.
Topic: Basic Mechanisms of Neurodegeneration and Pathology
ID 30

Martin Eiden et al The exchange of single aminoacids in ovine and caprine prion variants influences convertibility of the prion protein in vitro
Topic: Transmission and Pathogenesis
ID 42

Cancellotti et al Different contribution of PrP glycoforms in modulating TSE infection within and between different hosts
Topic: Transmission and Pathogenesis
ID 46

Rybakov et al Monitoring of bovine spongiform encephalopathy in the Russian Federation using a kit TeSeE (Bio-Rad) and immunohistochemical method
Topic: Epidemiology and Risk Assessment
ID 47

Toppets et al Distribution and frequency of nerve fibres in ovine palatine and pharyngeal tonsils
Topic: Transmission and Pathogenesis
ID 49

Hoffmann et al BSE/Scrapie phenotype are maintained following co-infection of ovine PrP overexpressing transgenic mice (Tgshp IX)
Topic: Transmission and Pathogenesis
ID 59

Everest et al Detection and localisation of abnormal prion protein in the liver of TSE-affected sheep
Topic: Transmission and Pathogenesis
ID 78

Bradford et al Removal of PrP expression from neurones prior to scrapie infection prevents synaptic loss and neuronal death but not disease.
Topic: Basic Mechanisms of Neurodegeneration and Pathology
ID 89

Konold et al Electrophysiological studies in TSE-affected cattle
Topic: Basic Mechanisms of Neurodegeneration and Pathology
ID 96

Julini et al Atypical scrapie cases in Italy: neuroanatomical distribution of pathological prion protein with different antibodies
Topic: Natural and Experimental Strains
ID 101

Mathiason et al Tracking Prion Infectivity in the Blood of Deer with Chronic Wasting Disease
Topic: Transmission and Pathogenesis
ID 112

Langeveld et al Limited presence of arginine-171 containing PrP in PK resistant fraction of naturally scrapie infected ARR/VRQ sheep.
Topic: Transmission and Pathogenesis
ID 118
Khan, & Chakrabartty et al
Correlating prion disease susceptibility in animals with the tendency of their prion proteins to adopt \(-\_\)sheet rich structures in vitro

**Topic: Transmission and Pathogenesis**
**ID 145**

Dobly et al
PRNP gene sequencing in Belgian goats: codon frequency

**Topic: Genetics**
**ID 147**

Márquez et al
Titration and characterisation of a Catalan BSE isolate in a transgenic murine model of BSE

**Topic: Natural and Experimental Strains**
**ID 149**

Beck et al
Investigating the influence of ovine PrP genotype on lesion profile: Beyond the 3 codon genotype

**Topic: Genetics**
**ID 153**

Stack et al
An in-depth investigation of PrPd distribution in the different brain regions of BSE cases in Great Britain.

**Topic: Diagnostics, Therapeutics and Decontamination**
**ID 155**

Cali et al
Co-existence of scrapie prion protein type 1 and 2 in sporadic Creutzfeldt-Jakob disease: its effect on the phenotype and prion type characteristics

**Topic: Basic Mechanisms of Neurodegeneration and Pathology**
**ID 159**

Darshan et al
Comparing BSE Risk Factors and Management of the Three Largest Cattle Producers: India, Brazil and China

**Topic: Epidemiology and Risk Assessment**
**ID 160**

Maestrale et al
A prevalence study in QQ171 sheep investigating the role of individual and flock-level risk factors for scrapie

**Topic: Epidemiology and Risk Assessment**
**ID 169**

Van den Broeke et al
Genetic analysis of the ribosomal protein SA family in sheep

**Topic: Genetics**
**ID 170**

Ligios et al
Prion infectivity in milk from arq/arq sheep experimentally Infected with scrapie and maedi-visna virus

**Topic: Transmission and Pathogenesis**
**ID 185**

Yokoyama et al
BSE in hamsters: An animal model lacking PrPSc accumulation in lymphoid tissues

**Topic: Transmission and Pathogenesis**
**ID 192**

Priem et al
Specific detection of prions in sheep blood fractions by modified PMCA

**Topic: Diagnostics, Therapeutics and Decontamination**
**ID 194**

Wemheuer et al
Parallels between different forms of sheep scrapie and types of Creutzfeldt-Jakob Disease (CJD)

**Topic: Basic Mechanisms of Neurodegeneration and Pathology**
**ID 198**

Melchior et al
Scrapie surveillance in the Netherlands; the effect of selection for scrapie resistance in sheep.

**Topic: Epidemiology and Risk Assessment**
**ID 203**

Greenlee et al
Prolonged incubation time in sheep with QK171 Genotype

**Topic: Transmission and Pathogenesis**
**ID 205**
Aline de Koeijer: Quantifying the effect of the EU control measures in 2001 on the decline of the BSE epidemic in Europe

**Topic: Epidemiology and Risk Assessment**

**ID 221**

Acín et al: Spanish goat prion protein gene variability and implications for susceptibility to classical scrapie disease

**Topic: Genetics**

**ID 225**

Bucalossi et al: Assessment of genetic susceptibility of sheep to scrapie: comparison between PMCA and in vitro studies

**Topic: Genetics**

**ID 240**

Filali et al: Gene expression variations in Spanish natural sheep scrapie disease determined by sheep oligo DNA microarrays

**Topic: Genetics**

**ID 241**

Huélamo et al: Modulated conversion of PrPc into PrPSc in sheep by PMCA based on PRNP genotype.

**Topic: Genetics**

**ID 243**

Griffiths et al: Transmission of TSEs to a highly susceptible transgenic mouse model overexpressing ovine PrP (A136H154Q171)

**Topic: Transmission and Pathogenesis**

**ID 250**

Suardi et al: Infectivity in skeletal muscle of BASE-infected cattle

**Topic: Transmission and Pathogenesis**

**ID 256**

Oraby et al: Estimation of the size of the Bovine Spongiform Encephalopathy Epidemic in Canada

**Topic: Epidemiology and Risk Assessment**

**ID 262**

Knox et al: A comparative analysis of urine biomarkers in two cohorts of BSE infected cattle

**Topic: Diagnostics, Therapeutics and Decontamination**

**ID 271**

Schmädicke et al: Estimation of Chronic Wasting Disease (CWD) infectivity in cell culture

**Topic: Transmission and Pathogenesis**

**ID 286**

McCulloch et al: Determining the role of follicular dendritic cells in scrapie pathogenesis

**Topic: Transmission and Pathogenesis**

**ID 287**

Gossner et al: Candidate gene analysis in distinct regions of the central nervous system during the development of SSBP/1 sheep scrapie

**Topic: Basic Mechanisms of Neurodegeneration and Pathology**

**ID 294**

Lee et al: Polymorphisms of the prion protein gene (PRNP) in Hanwoo (Bos Taurus coreanae)

**Topic: Genetics**

**ID 302**

Murdoch et al: Genome-wide association study of BSE in European Holstein cattle

**Topic: Genetics**

**ID 315**

Johnson, & McKenzie: Adaptation of chronic wasting disease (CWD) into hamsters: evidence of a novel strain of CWD

**Topic: Natural and Experimental Strains**

**ID 317**

Webb et al: A retrospective immunohistochemical study reveals atypical scrapie has existed in the United Kingdom since at least 1987

**Topic: Epidemiology and Risk Assessment**

**ID 323**
Useful contacts:

EU project NeuroPrion (FOOD-CT-2004-506579)

NeuroPrion communication: Benjamin Scherr. Commissariat a l’Energie Atomique DRM/DSV/GIDTIP.
18 route du Panorama, 92265 Fontenay aux Roses, France.
Tel: +33 1 46 54 78 71  E-mail: benjamin.scherr@cea.fr

NeuroPrion Task 2 - Theme leader of Animal Control: Jan Langeveld Central Veterinary Institute of Wageningen UR (CVI), P.O. Box 65, 8200 AB, Lelystad, The Netherlands.
Tel: +31 320 237217  E-mail: jan.langeveld@wur.nl

NeuroPrion Cervid group Coordinator: Mick Stack, Molecular, Pathogenesis and Genetics Department, Veterinary Laboratories Agency (VLA), Woodham Lane, Addlestone, Surrey, KT15 3NB United Kingdom.
Tel: (+44) (0)1932 357320  E-mail: m.j.stack@vla.defra.gsi.gov.uk

NeuroPrion TSE-Goat Group Coordinator: Jan Langeveld (see above)

NeuroPrion Scrapie and BSE Epidemiology Group Coordinator: Thomas Hagenaars, Central Veterinary Institute of Wageningen UR (CVI), P.O. Box 65, 8200 AB, Lelystad, The Netherlands.
Tel: +31 320 238398  E-mail: thomas.hagenaars@wur.nl

EU project GoatBSE (FOOD-CT-2006-36353):

GoatBSE coordinator: Alex Bossers, Central Veterinary Institute of Wageningen UR (CVI), P.O. Box 65, 8200 AB, Lelystad, The Netherlands.
Tel: +31 320 238921  E-mail: alex.bossers@wur.nl
Notes