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-M132)$1536^{+/-}$ and Tg(CerPrP-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-M132)$1536^{+/-}$ and Tg(CerPrP-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