Oral inoculation of neonatal Suffolk sheep with the agent of classical scrapie results in PrP Sc accumulation in sheep with the PRNP ARQ/ARQ but not the ARQ/ARR genotype

Justin J. Greenlee, United States Department of Agriculture
Jodi D. Smith, United States Department of Agriculture
Amir N. Hamir, United States Department of Agriculture

Available at: https://works.bepress.com/jodi-smith/2/
Short Communication

Oral inoculation of neonatal Suffolk sheep with the agent of classical scrapie results in PrPSc accumulation in sheep with the PRNP ARQ/ARQ but not the ARQ/ARR genotype

Justin J. Greenlee *, Jodi D. Smith 1, Amir N. Hamir 2

Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, 1920 Dayton Avenue, Ames, IA 50010, USA.

A R T I C L E  I N F O

Article history:
Received 28 August 2015
Received in revised form 10 February 2016
Accepted 21 February 2016

Keywords:
Prion protein
PRNP
Scrapie
Sheep
Transmissible spongiform encephalopathy

A B S T R A C T

Scrapie is a transmissible spongiform encephalopathy that can be transmitted amongst susceptible sheep. The prion protein gene (PRNP) profoundly influences the susceptibility of sheep to the scrapie agent. This study reports the failure to detect PrPSc in nervous or lymphoid tissues of Suffolk sheep of the PRNP ARQ/ARQ genotype after oral inoculation with a U.S. scrapie isolate. Lambs were inoculated within the first 24 h of birth with 1 ml of a 10% brain homogenate derived from a clinically affected ARQ/ARQ sheep. The inoculated sheep were observed daily throughout the experiment for clinical signs suggestive of scrapie until they were necropsied at 86 months post inoculation. Tissues were collected for examination by immunohistochemistry and enzyme immunoassay, but all failed to demonstrate evidence of scrapie infection. Neonatal sheep of the ARQ/ARQ genotype receiving the same inoculum developed scrapie with a prolonged incubation period and with abnormal prion present within the central nervous system, but not peripheral lymphoid tissues. Results of this study suggest that ARQ/ARR sheep are resistant to oral infection with the scrapie isolate used even during the neonatal period.

Transmissible spongiform encephalopathies (TSE) or prion diseases are fatal neurologic diseases of animals and humans. Susceptibility to scrapie, the natural TSE of sheep, appears to be age dependent (St Rose et al., 2007). Scrapie may be transmitted from infected ewes to their scrapie-susceptible fetus during gestation (Foster et al., 2013; Garza et al., 2011; Spiropoulos et al., 2014) or to susceptible lambs sharing the same environment during the perinatal period. Abnormal prion protein (PrPSc) accumulates at the fetal-maternal interface within the ovine placenta (Tuo et al., 2001) of scrapie infected ewes that are carrying lambs of susceptible genotypes (Alverson et al., 2006). Thus, exposure from placenta and placental fluids is thought to be one of the main routes of scrapie transmission, but milk has also been demonstrated to be a potential route of transmission (Konold et al., 2008). In a previous study, we demonstrated that 4-month-old lambs orally inoculated with scrapie inoculum succumbed to the disease in an average of 32 months post-inoculation (MPI) (Hamir et al., 2009). These lambs were inoculated with a large dose of inoculum (3 g of scrapie-infected brain tissue), yet only 5 of 9 recipients developed scrapie.

The susceptibility of sheep to scrapie is greatly influenced by host prion protein gene (PRNP). Amino-acid polymorphisms at codons 136, 154 and 171 are major determinants of relative susceptibility or resistance. The five common allelic variations, resulting from amino-acid substitutions are A136R154R171, A136R154H171, A136H154Q171, A136R154Q171 and V136R154Q171 (Belt et al., 1995). The V136 and Q171 haplotypes are linked to scrapie susceptibility, especially so in the homozygous (VRQ/VRQ) state (Diaz et al., 2005; Hunter et al., 1997a, 1997b), and the ARR/ARR genotype is strongly associated with resistance (Cosseddu et al., 2007; Diaz et al., 2005; Hunter et al., 1996). Therefore, PRNP polymorphisms found within a regional or national flock can influence selective breeding recommendations to reduce the incidence of scrapie (Dawson et al., 1998; Warner et al., 2006). Many scrapie eradication programs are based on using rams of resistant genotypes: arginine (R) at codon 171. This approach enhances the resistance to classical scrapie on the flock level. This breeding program can result in the birth of ARQ/ARR lambs that are moderately resistant to scrapie. The purpose of this experiment was to test the susceptibility of PRNP ARQ/ARR Suffolk sheep to a U.S. scrapie isolate after oral exposure as neonates.

This experiment was carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, DC) and the Guide for the Care and Use of Agricultural Animals in Research and
The protocol was approved by the Institutional Animal Care and Use Committee at the National Animal Disease Center (protocol number: 3283). Results of susceptible sheep (ARQ/ARQ) inoculated under the same protocol were previously published (Hamir et al., 2009). Briefly, four ARQ/ARR (see genotyping method below) lambs and their dams were obtained from the scrapie-free sheep flock at the National Animal Disease Center. Lambs were orally inoculated within the first 24 h with 1 ml of inoculum (no. 13–7) (Hamir et al., 2009) as a 1% brain homogenate (wt./vol.) prepared in PBS. The inoculum was derived from clinically ill sheep with PrPSc demonstrated by immunohistochemistry on brain sections (wt./vol.) prepared in PBS. The inoculum was derived from clinically ill sheep with PrPSc demonstrated by immunohistochemistry on brain sections. They were examined at necropsy and two sets of tissue samples were collected. One set of tissues included representative age matched neonates of ARQ/ARQ genotype that received the same inoculum (Hamir et al., 2009) and ARQ/ARQ and ARQ/ARR lambs that received the same inoculum but via the intracranial route (Greenlee et al., 2014).

Predicted PRNP amino acid sequences were determined as described previously (Greenlee et al., 2012). Primers specific for the functional gene in sheep (forward primer Sheep-F2: 5′-GGA GTG ACG TGG GCC TCT GC-3′ and reverse primer Sheep-R4: 5′-CTC CCT CCC CCA ACC TGG CA-3′) amplified a 775-bp region beginning at codon 19 in the prion protein-coding region in sheep. All PCR reactions were as follows: 95 °C for 5 min, followed by 30 cycles of denaturation (95 °C, 20 s), annealing (60 °C, 20 s) and extension (72 °C, 60 s) followed by an extension cycle (72 °C, 7 min). Following filtration, PCR products were sequenced using primers Sheep-F2, Sheep-F3 (5′-ATG GAG GTG GCT GGC GCC AA-3′), Sheep-R3 (5′-TCC CCC TTG GTG GTG GTG GT-3′) and Sheep-R4. Resulting sequences were analyzed using Geneious version 6, created by Biomatters. Available from (http://www.geneious.com/).

The sheep were observed twice daily for the development of clinical signs. They were examined at necropsy and two sets of tissue samples were collected. One set of tissues included representative sections of lymphoid tissues (spleen, pharyngeal and palatine tonsil, retropharyngeal and mesenteric lymph node, and ileal Peyer’s patches), liver, kidney, skin, striated muscles (heart, tongue, diaphragm, masseter), thyroid gland, nasal turbinate, lung, intestines (ileum), adrenergic gland, pituitary gland, trigeminal ganglion, brain (hemisections of cerebral cortex, cerebellum, superior colliculus and brainstem including obex) and eye (retina). These tissues were fixed in 10% buffered formalin, embedded in paraffin wax, sectioned (4 μm or 3 μm for lymphoid tissues), and stained with hematoxylin and eosin (HE) for light microscopy. The second set of tissues was frozen.

Enzyme immunoassays (EIA) were performed on brainstem, retropharyngeal lymph node, and tonsil as previously described (Smith and Greenlee, 2014) using a commercially available kit (HerdChek BSE-Scrapie Ag Test Kit, EIA, IDEXX Laboratories, Westbrook, ME).

All paraffin embedded tissues were stained by an automated immunohistochemical (IHC) method for detection of PrPSc as described previously (Hamir et al., 2005) using a cocktail of 2 primary monoclonal antibodies was used: F89/160.1.5 (O’Rourke et al., 1997, 1998, 2000) and F99/97.6.1 (O’Rourke et al., 1997, 1998, 2000), each at a concentration of 5 μg/ml.

Sheep genotypes were confirmed to be ARQ/ARR with all sheep shown to be homozygous at other potentially polymorphic sites: 112M, 127G, 137M, 138S, 141L, 151R, 154R, 157M, 176N, 180H, 189Q, 195T, 196T, 211R, 220Q, 223R. The orally inoculated sheep were observed daily for the duration of the experiment without any clinical signs suggestive of scrapie infection. Rectal biopsies were performed at approximately 81 MPI, but they failed to demonstrate PrPSc immunoreactivity. Due to recurring lameness in two of the sheep, the experiment was terminated at 86.5 MPI and all of the sheep were euthanized. No gross lesions were noted at necropsy. Microscopic examination of hematoxylin and eosin stained slides of multiple levels of cerebrum, thalamus, hippocampus, colliculus, pons, and brainstem at the level of the obex (Fig. 1) did not reveal any changes suggestive of spongiform encephalopathy nor did immunohistochemistry reveal immunoreactivity for PrPSc in any neural or lymphoid tissue examined (Fig. 2). Similarly, EIA performed on retropharyngeal lymph node, palatine tonsil, and brainstem samples collected at the level of the obex failed to reveal the presence of the misfolded protein associated with scrapie infection. All optical density readings fell well below the negative threshold of 0.258 established according to kit instructions. This is in contrast to positive control sheep that included orally inoculated ARQ/ARQ neonates (Hamir et al., 2009) or intracranially inoculated ARQ/ARR lambs (Greenlee et al.) with evidence of PrPSc accumulation by IHC and

Fig. 1. Lack of evidence of spongiform change in the brainstem of ARQ/ARR sheep after oral inoculation as neonates. Spongiform change and neuronal degeneration is evident in the parasympathetic nucleus of the vagus nerve of an ARQ/ARQ sheep inoculated as a neonate (A) (Hamir et al.), but was not present in any of the ARQ/ARR sheep of the present study (B). Hematoxylin and eosin. Original magnification is 100×.
western blot of central nervous and lymphoid tissues (ARQ/ARQ oral) or central nervous tissues only (ARQ/ARR intracranial).

The ARQ/ARR Suffolk sheep in the present study that were orally inoculated as neonates were allowed to incubate for 86 months without any detectible evidence of scrapie infection. Previous studies suggest that ARQ/ARR sheep are poorly susceptible to the agent of scrapie by the oral route. Many attempts to transmit scrapie to ARQ/ARR sheep by the oral route have failed (Espenes et al., 2006; O’Rourke et al., 1997, 1998, 2000). However, one recent experimental study demonstrated clinical scrapie in orally inoculated ARQ/ARR Cheviot sheep, but with a prolonged incubation period (approximately 74 months) relative to more susceptible genotypes (Gonzalez et al., 2014). The experimental protocol for the present study was different than the successful experimental transmissions in that we inoculated younger Suffolk lambs (within 24 h of birth vs. 10–15 days old Cheviot lambs), but with a smaller amount of inoculum (0.1 g vs. 1 g repeated on 5 consecutive days). Lack of PrPSc immunoreactivity in any of the sheep inoculated for the present study may indicate a difference in scrapie isolate characteristics from those used in successful oral transmissions to ARQ/ARR sheep or breed related differences in the sheep used for these experiments. It also is possible that the amount of inoculum given in this instance is below the threshold to infect sheep of this genotype. While we cannot definitively prove that the amount of inoculum was adequate without performing additional studies, inoculation of susceptible sheep (ARQ/ARQ) with the same inoculum did result in 100% occurrence of clinical scrapie with a mean survival of 24 MPI (Hamir et al., 2009) compared to 32 MPI when the same genotype of sheep was inoculated orally at 4 months of age with 1.5 g of the same isolate.

Fig. 2. Lack of PrPSc immunoreactivity in the brainstem or tonsil of ARQ/ARR sheep after oral inoculation as neonates. Abundant PrPSc immunoreactivity (red) is present in the brainstem at the level of obex (parasympathetic nucleus of the vagus nerve; A) and palatine tonsil (C) of ARQ/ARQ sheep after oral inoculation as neonates (Hamir et al.), but in ARQ/ARR sheep, brainstem at the level of the obex (parasympathetic nucleus of the vagus nerve; B) and tonsil (D) are devoid of PrPSc immunoreactivity at 86 months post inoculation. Immunoenzyme (Alkaline phosphatase) staining, monoclonal antibodies F89/160.1.5 and F99/97.6.1, Fast Red chromagen, hematoxylin counterstain. Original magnification is 100× (A, B) or 40× (C, D).
infectious material on 2 consecutive days (Hamir et al., 2009). Since ARQ/ARQ sheep developed scrapie more rapidly with less total inoculum in the neonatal challenge model when compared to our previous work in older lambs, we consider this model (as applied in the present study) an efficient way of testing scrapie susceptibility by the oral route. Since the challenge dose in this model is derived from the brain of clinically affected sheep, it represents a highly concentrated source of PrPSc. Failure of any of the sheep in this study to develop scrapie or evidence of PrPSc accumulation suggests a substantial resistance of ARQ/ARR sheep to infection with the U.S. scrapie isolate used (Hamir et al., 2009). Interestingly, when ARQ/ARR sheep received the same inoculum used in the present study by the intracranial route, they developed scrapie, but after a prolonged (56 MPI on average) incubation and without evidence of PrPSc in lymphoid tissues (Greenlee et al.). Future studies will address the connection, if any, between ARQ/ARR sheep failing to accumulate PrPSc in the lymphoid tissues after intracranial inoculation and their resistance to scrapie after oral inoculation within the first 24 h of life as demonstrated in the present study. Determining if scrapie isolate or sheep breed characteristics are responsible for differences between our results and those of successful oral transmissions to ARQ/ARR sheep in the UK (Gonzalez et al.) also would require additional studies beyond the scope of this manuscript.

Conflict of interest statement
Conflicts of interest: none.

Acknowledgements

The authors thank Martha Church, Joe Lesan, Leisa Mandell, and Trudy Tatum for providing technical support to this project. This research was funded in its entirety by congressionally appropriated funds to the United States Department of Agriculture, Agriculture Research Service. The funders of the work did not influence study design, data collection and analysis, decision to publish, or preparation of the manuscript. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References