Genomic-based identification of host and environmental *Listeria monocytogenes* strains associated with an abortion outbreak in beef heifers

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Introduction

Listeria monocytogenes (LM) is a bacterial pathogen that causes late-term abortions in cattle, and definitive diagnosis and point-source determination may be challenging. In 2014, a diagnostic work-up of listerial abortions was initiated by a novel sample collection protocol. To determine the source(s) of the outbreak, LM strains isolated from cases and their environment were subjected to whole-genome sequencing and compared.

Materials and Methods

Twenty crossbred heifers that aborted from late January into February were identified. Presumptive diagnosis of listerial abortion was based on 2 positive, late-term aborted fetuses. To confirm that LM was the cause of the outbreak, proximal vaginal/distal cervical swabs (VCS) or retained fetal membranes (RFM) from heifers were submitted for LM culture and PCR. Silage and water sources were also submitted for culture and PCR. Resulting strains were subjected to whole-genome sequencing and compared.

Results

Heifer submissions identified 16/20 LM positive samples. Genomic sequencing of fetal, heifer, and environmental isolates demonstrated that 10/18 animal samples were closely related to each other, and to strains isolated from silage and drinking water. The remaining case strains were similar to each other, but genetically distinct from all other case and environmental strains.

Significance

When fetal materials are unavailable, CVS and RFM samples may be an effective means to diagnose abortions caused by LM. In this investigation, 2 LM case strains were responsible for the listeriosis outbreak, 1 causing the majority of the outbreak and linked to contaminated silage and/or water. The source of the other strain remains unknown.

Assessment and comparison of the barriers to interspecies transmission of bovine spongiform encephalopathy and chronic wasting disease prions

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Introduction

Prion diseases are transmissible, neurodegenerative diseases caused by templated conversion of the normal cellular prion protein to a misfolded, neurotoxic form. After conversion, the misfolded protein aggregates into characteristic protease-resistant amyloid plaques, which are hallmarks of prion disease. Species barriers among prion diseases have been observed epidemiologically and experimentally, but the mechanism(s) mediating this phenomenon remains unknown, making it impossible to predict the risk of transmission to new species, including humans, and to formulate

therapies and prophylactics. Bovine spongiform encephalopathy (BSE), first identified in the UK and then throughout Europe, is the prototype trans-species transmissible prion disease, having affected cattle, humans, ungulates, and felids naturally, and multiple other species experimentally. Chronic wasting disease (CWD) is an endemic prion disease of deer and elk distinguished from BSE by its unprecedented horizontal transmissibility among cervid hosts. Thus far, CWD has not been shown to cross species barriers in nature, although several experimental inoculation studies have resulted in trans-species transmission. The CWD species barriers are fortunate, as the facile prion shedding and resultant environmental contamination would constitute a major economic and public health threat. Mounting evidence indicates that primary structure homology alone does not comprise the prion species barrier. First, the primary sequence of bovine and white tail deer prion protein is very well-conserved. Additionally, the secondary structure of the misfolded protein is suspected to be a β-sheet rich configuration in all species. Thus, tertiary structure and the quaternary arrangement of the amyloid fibrils are attractive molecular explanations for the species barrier. Functional demonstration of the mechanism(s) resulting in the species barriers is the principal question addressed in this research.

Materials and Methods

Most studies of the effects of primary or tertiary prion protein structures on trans-species prion transmission have relied upon animal bioassays, making the influence of prion protein structure vs host co-factors (e.g. trafficking and innate immune interactions) difficult to dissect. Here, we use real-time quaking-induced conversion (RT-QuIC), which relies on the conversion of recombinant PrPC (rPrPC) by an infectious seed and detection with an amyloid-binding dye, to investigate the propensity for and the kinetics of trans-species prion conversion. This system makes it possible to investigate the molecular mechanism of trans-species transmission, which may be able to be exploited for the development of therapeutics, prevention measures, and risk assessment paradigms. To assess trans-species conversion in the RT-QuIC system, we compared CWD and BSE prions, as well as feline CWD (fCWD) and feline spongiform encephalopathy (FSE). Each prion was seeded into each host recombinant PrP (full-length rPrP of white-tailed deer, bovine or feline).

Results

We demonstrated that fCWD is a more efficient seed for feline rPrP than for white-tailed deer rPrP, which suggests adaptation to the new host. Conversely, FSE maintained sufficient BSE characteristics to more efficiently convert bovine rPrP than feline rPrP. Additionally, human rPrP was competent for conversion by CWD and fCWD. This insinuates that, at the level of protein:protein interactions, the barrier preventing transmission of CWD to humans is more similar to BSE than previously estimated.

Significance

These studies will contribute uniquely to efforts to use simplified, in vitro assays to understand the mechanism of prion conversion and species barriers. Until the species barrier is defined, it is impossible to predict transmission, the risk to humans and other hosts, or the efficacy of potential therapeutics or prophylactics for prion disease.

Serum neutralizing antibody concentrations against viral bovine respiratory pathogens in nursing beef calves

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Introduction

Nursing calf bovine respiratory disease (BRD) is a problem for some herds, and the timing of maternal antibody decline may be related to risk for calves to develop BRD. Limited information is available on the distribution of serum maternal antibody titers among calves within and between cow-calf herds. Understanding how maternal antibody concentrations vary within and between herds may help us understand what puts nursing (preweaned) calves at risk for BRD. The objective of this study was to test the effect of calf age and farm on the magnitude of serum neutralizing antibody (SNA) titers