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Pathogen variation across time and space: sequencing to characterize *Mannheimia haemolytica* diversity

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Abstract

Bovine respiratory disease complex (BRDC) is a major animal health and economic issue that affects cattle industries worldwide. Within the USA, the beef cattle industry loses up to an estimated 1 billion dollars a year due to BRDC. There are many contributors to BRDC, including environmental stressors and viral and/or bacterial infections. One species of bacteria in particular, *Mannheimia haemolytica*, is recognized as the major cause of severe fibrinonecrotic pneumonia in cattle. *M. haemolytica* is an opportunistic pathogen that normally populates the upper respiratory tract of cattle, and invades the lower respiratory tract in stressed and/or virally infected cattle by mechanisms that are not completely understood. However, not all *M. haemolytica* appear to be equally pathogenic to cattle. Thus, a test could be developed to distinguish *M. haemolytica* genetic subtypes by their propensity to cause respiratory disease, allowing isolation and/or treatment of cattle harboring strains with an increased propensity to cause disease. To that end, the genomes of over 300 *M. haemolytica* strains are being sequenced.

Keywords: bovine respiratory disease, *Mannheimia haemolytica*, pathogen, genomic variation

Bovine respiratory disease complex (BRDC)

BRDC manifests from multiple causes and predisposing factors (Taylor *et al.*, 2010; Caswell, 2014). Viruses, including bovine coronavirus (BCV), bovine herpesvirus 1 (BHV-1), bovine parainfluenza virus type 3 (BPIV-3), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhea virus (BVDV) cause or predispose cattle to BRDC (Caswell, 2014). Bacteria, including *Bibersteinia trehalosi, Histophilus somni, Mannheimia haemolytica, Mycoplasma bovis*, and *Pasteurella multocida*, many of which are normal flora of the bovine upper respiratory tract, cause BRDC (Caswell, 2014). Additionally, a myriad of environmental factors contribute to BRDC, including transportation, sale barn auctions, and comingling (Cusak *et al.*, 2003; Taylor *et al.*, 2010). Thus, BRDC manifests from complicated interactions between and within individuals of a population, the stressors they are subjected to, and the microbes that they harbor, including those that act as opportunistic pathogens.

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Mannheimia haemolytica

M. haemolytica is a primary agent of fibrinonecrotic pneumonia in cattle and a major bacterial component of BRDC (Rice *et al.*, 2008); the bacteria is classified within the Pasteurellaceae family and is represented by distinct Gram-negative, nonmotile bacteria that may be either rods or coccobacilli, and that share a number of metabolic and/or biochemical phenotypes (Angen *et al.*, 1999a, Mutters *et al.*, 2005). When grown on blood agar plates, *M. haemolytica* colonies may be surrounded by a distinct zone of β-hemolysis, due to production of leukotoxin (LKT), which is a major virulence factor (Mutters *et al.*, 2005, Singh *et al.*, 2011). Importantly, *M. haemolytica* is a commensal organism of the bovine nasopharynx and tonsilar crypts (Shoo *et al.*, 1990; Radostits *et al.*, 2000). However, when animals are environmentally stressed and/or immuncompromized by viral infections, *M. haemolytica* can invade the lungs, evade the host immune response, and cause pulmonary inflammation with leukocyte damage and apoptosis due to LKT, lipopolysaccharide, and other virulence factors, and eventual death of the bovine host (Radostits *et al.*, 2000).

Considerable evidence shows that *M. haemolytica* bacteria are genetically and phenotypically diverse, and that distinct subsets of *M. haemolytica* associate with disease, whereas others do not (Frank and Smith, 1983; Quirie *et al.*, 1986; Rice *et al.*, 2008; Klima *et al.*, 2014). *M. haemolytica* are typically encased in capsules, which are virulence factors that interfere with host cellular phagocytosis (Chae *et al.*, 1990). There are 12 capsular serotypes for *M. haemolytica* (A1, A2, A5, A6, A7, A8, A9, A12, A13, A14, A16, and A17) (Angen *et al.*, 1999b). Isolates of serotypes A2 and A4 are commonly found in the upper respiratory tract of healthy cattle, and rarely in the lungs of diseased animals. Conversely, isolates of serotype A1 in particular, and also those of serotype A6, are predominantly overrepresented in the lungs of diseased animals. See Rice *et al.* (2008) and Singh *et al.* (2011) for reviews of serotype associations.

In addition to capsular serotyping, *M. haemolytica* diversity has been characterized using pulsed-field gel electrophoresis (PFGE) (Klima *et al.*, 2011, 2014; Timsit *et al.*, 2013). This is a technique that detects insertions and/or deletions, genome rearrangements, and, to a smaller extent, nucleotide polymorphisms within microbial genomes through restriction enzyme digests and electrophoretic banding profiles (Kudva *et al.*, 2002; Foley *et al.*, 2009; Goering, 2010; Sabat *et al.*, 2013). *M. haemolytica* isolates originating from the lungs of diseased animals have been found to have PFGE banding profiles that suggest closer genetic similarity to one another than to isolates originating from the upper respiratory tract of non-diseased animals (Klima *et al.*, 2014). Consequently, two very different techniques that assess microbial diversity, capsular serotyping, and PFGE, both support the notion that *M. haemolytica* subtypes do not share an equal propensity for associating with, or causing, respiratory disease in cattle. This also indicates that a potential approach to managing or preventing bovine respiratory disease attributable to *M. haemolytica* could involve testing cattle for the subtypes they are harboring, and isolating and/or treating those that are harboring strains with an increased propensity to cause disease.

Goals and approach

The goals of this ongoing project are to (1) identify fundamental genetic variation within *M. haemolytica* strains of North America; (2) develop a set of nucleotide polymorphisms to detect that variation; and (3) use the set to identify genetic determinants that influence *M. haemolytica* pathogenicity in cattle. This is being accomplished through whole genome sequencing of *M. haemolytica* strains that originated from either the upper or lower respiratory tract of cattle that were either (1) clinically ill or deceased, or (2) disease free. Two collections of *M. haemolytica* strains have been assembled that are comprised of strains isolated from the lungs of clinically ill or deceased cattle throughout North America (Portis *et al.*, 2012). One collection is being used primarily for the discovery of genetic variation, including nucleotide polymorphisms, and consists of 158 epidemiologically unlinked strains originating from cattle within 35 US states, as well as the Canadian provinces of Alberta, British Columbia, Manitoba, Ontario, and Saskatchewan. The strains were isolated from beef or dairy cattle from 2002 to 2011, with a majority originating from beef animals. The second collection is being used primarily for validation of the discovery collection, and consists of 163 epidemiologically unlinked strains originating from 29 US states, as well as the Canadian provinces of Alberta, Ontario, and Saskatchewan. Strains of the validation collection were isolated from beef or dairy cattle from 2002 to 2011, with a higher representation of dairy cattle germplasm than the discovery population. These two collections represent a deep sampling of North American *M. haemolytica* genetic subtypes that cause or associate with respiratory disease. Additionally, a third *M. haemolytica* collection has just recently been assembled that is comprised of strains isolated from the nasopharnynx of US cattle that were not afflicted with respiratory disease.

Whole genome sequencing of the *M. haemolytica* strains from the collections described above will reveal genetic diversity across the entire genome that will serve as a roadmap for defining *M. haemolytica* subtypes, and for testing them for an association with respiratory disease. Tests will be developed that will identify *M. haemolytica* subtypes. The results from this study will be published and placed into the public domain for use without restriction.

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