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A multiplex PCR assay for molecular capsular serotyping of *Mannheimia haemolytica* serotypes 1, 2, and 6

Cassidy L. Klima[a], Rahat Zaheer[a], Robert E. Briggs[b], Tim A. McAllister[a,*]

[a] Agriculture and Agri-Food Canada Research Centre, Lethbridge, Canada
[b] USDA, ARS, National Animal Disease Center, Ames, IA, USA

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ABSTRACT

Mannheimia haemolytica is an important respiratory pathogen of ruminants. Of the 12 capsular serovars identified, 1 and 6 are most frequently associated with disease in cattle, while 2 is largely a commensal. Comparative analysis of 24 *M. haemolytica* genomes was used to identify unique genes associated with capsular polysaccharide synthesis as amplification targets in a multiplex PCR assay to discriminate between serotype 1, 2, and 6 strains. The specificity of serotype specific gene targets was evaluated against 47 reference strains representing 12 known serovars of *M. haemolytica* and 101 field isolates identified through antisera agglutination as serotypes 1, 2, or 6. The results suggest this simple and cost-effective serotype specific PCR assay can be used as an alternative to agglutination based techniques to serotype the majority of *M. haemolytica* collected from bovines, thus averting the need to use animals and invest in expensive sera development for agglutination assays. In addition, the gene targets identified in this study can be used *in silico* to identify serotype 1, 2, and 6 strains in sequenced *M. haemolytica* isolates without the need for culture based analysis.

1. Introduction

Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in North American beef production systems (Ives and Richeson, 2015). Despite being one of the most extensively studied diseases in cattle (Taylor et al., 2010), the rate of mortality due to BRD has increased over the last decade (Engler et al., 2014). *Mannheimia haemolytica* is a primary bacterial agent associated with BRD, but also contributes to pneumonia in domestic sheep and wild ruminants (McRae et al., 2016). It is of particular concern in bighorn sheep populations where *Mannheimia* associated pneumonia incidences have decimated herds, once numbering in the millions now totaling around 70,000 (Batra et al., 2015). Originally part of the *Pasteurella haemolytica* complex, *Mannheimia haemolytica* was reclassified as its own species in 1999 (Angen et al., 1999) with 12 of the 17 original *P. haemolytica* capsular serovars renamed as *Mannheimia haemolytica* (1, 2, 5, 6, 7, 8, 9, 12, 13, 14, 16, and 17), serotype 11 reclassified as *Mannheimia glucosida*, and the remaining serovars (3, 4, 10, and 15) classified as *Pasteurella trehalosi. Pasteurella trehalosi* was later reclassified as *Bibersteinia trehalosi* (Blackall et al., 2007).

Mannheimia haemolytica serotypes 1, 2, and 6 are the most prevalent worldwide (Al-Ghamdi et al., 2000; Hauglund et al., 2015) with a link observed between serotype and host specificity. In cattle, serotypes 1, 2, and 6 are frequently recovered from the nasopharynx of healthy animals, but serotypes 1 and 6 are commonly recovered from the lungs of cattle that have died of BRD (Klima et al., 2011; Klima et al., 2014a; Klima et al., 2014b). In domestic sheep, disease is primarily associated with serotype 2 strains, with a broader range of serotypes being recovered from small ruminants than cattle (Fodor et al., 1984). Bighorn sheep appear susceptible to both serotype 1 and serotype 2 strains (Batra et al., 2015). *Mannheimia haemolytica* serotype 1 expresses a capsular polysaccharide consisting of *N*-acetylmannosaminuronic acid and *N*-acetylmannosamine repeats (Lo et al., 2001) that is believed to facilitate adherence, inhibit phagocytosis by neutrophils, and play a role in immune evasion by this pathogen (Hounsome et al., 2011; Gioia et al., 2006). The capsule of serotype 2 is composed of a linear polymer of *N*-acetylneuraminic acid (Neu5Ac) with α(2–8) linkages (Barrallo et al., 1999) whereas to our knowledge, the composition of the capsule of *M. haemolytica* serotype 6 has not been reported.

In addition to contributing to disease, *M. haemolytica* collected from BRD mortalities have been reported to harbour antimicrobial resistance (AMR) genes in association with integrative conjugative elements (Klima et al., 2014b; Eidam et al., 2014; Clawson et al., 2016), making this pathogen a potentially significant contributor to the environmental resistome within agricultural settings. A relationship between AMR and *Mannheimia haemolytica* genotype has recently been reported (Clawson

* Corresponding author at: Agriculture and Agri-Food Canada Research Centre, 5403 1-st Ave. South, Lethbridge, Alberta T1J 4B1, Canada.
E-mail address: Tim.McAllister@agr.gc.ca (T.A. McAllister).

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Table 1
Mannheimia haemolytica strains used in comparative genomics analysis and corresponding sequence homology for gene targets selected for the PCR assay.

Strain	NCBI accession no.	Animal status	Serotype[a]	Origin[b]	Percent nucleotide alignment between gene target and reference gene		
					M. haemolytica USDA-ARS-USMARC-185 TupA-like ATPgrasp Locus ID: D648_40 (837 bp)	*M. haemolytica* USDA-ARS-USMARC-183 hypothetical protein Locus ID: D650_690 (807 bp)	*M. haemolytica* D171 core-2/I-branching enzyme Locus ID: J450_08930 (777 bp)[c]
D174	NC_021739.1	Diseased - lung	6	Iowa	100	–	–
L038A	LFYA00000000	Diseased - lung	6	Alberta	100	–	–
USDA-ARS-USMARC-185	NC_020834.1	Diseased	6	Kansas		–	–
D38	AUNL00000000.1	Diseased - lung	6	Iowa	100	–	–
T14	LFXV00000000	Morbid	6	France	100	–	–
3927A	LFYE00000000	Healthy	6	Alberta	99	–	–
H23	AOGP00000000	Morbid	6	Alberta	100	–	–
MhSwine2000	ATTA00000000.1	Diseased - lung (pig)	1	Iowa	–	100	–
M42548	NC_021082.1	Diseased	n/a	Pennsylvania	–	100	–
535A	LFYB00000000	Morbid	1	Alberta	–	100	–
L024A	LFXX00000000	Diseased - lung	1	Texas	–	100	–
D193	ATSY00000000.1	Diseased - lung	1	Iowa	–	100	–
USDA-ARS-USMARC-183	NC_020833.1	Clinical BRD case	1	Kansas	–		–
D153	NC_021743.1	Diseased - lung	1	Iowa	–	99	–
L044A	LFXY00000000	Diseased - lung	1	Nebraska	–	100	–
PHL213	NZ_AASA00000000.1	Diseased - lung	1	n/a	–	100	–
157-4-1	LFYD00000000	Healthy	1	Alberta	–	100	–
MhBrain2012	ATSZ00000000.1	Diseased-cerebellum	1	Georgia	–	100	–
USMARC_2286	NC_021883.1	Healthy	n/a	n/a	–	100	–
USDA-ARS-USMARC-184	NZ_CP006957	n/a	n/a	n/a	–	–	100
587A	LFYC00000000	Healthy	2	Alberta	–	–	100
D35	AUNK00000000.1	Diseased - lung	2	Iowa	–	–	99
T2	LFXW00000000	Morbid	2	France	–	–	99*
Serotype A2 str. BOVINE	NZ_ACZY00000000.1	Diseased - lung	2	n/a	–	–	100
D171	NC_021738.1	Diseased - lung	2	Iowa	–	–	
Serotype A2 str. OVINE	NZ_ACZX00000000.1	Diseased - lung	2	n/a	–	–	100
L033A	LFXZ00000000	Diseased - lung	2	Nebraska	–	–	100

[a] n/a, serotype data not available.
[b] n/a, origin data not available.
c* alignment region (685 bp) cut off at the end of a contig.