Differentiation of *Mannheimia haemolytica* genotype 1 and 2 strains by visible phenotypic characteristics on solid media

Emily L. Wynn  
*USDA ARS. MARC*, emilywynn6@gmail.com

Gennie Schuller  
*USDA ARS. MARC*, gennie.schuller@usda.gov

John D. Loy  
*University of Nebraska-Lincoln*, jdloy@unl.edu

Aspen M. Workman  
*USDA ARS. MARC*, aspen.workman@ars.usda.gov

T. G. McDaneld  
*USDA ARS. MARC*, tara.mcdaneld@ars.usda.gov

See next page for additional authors
Follow this and additional works at: [https://digitalcommons.unl.edu/vetscipapers](https://digitalcommons.unl.edu/vetscipapers)

Part of the Biochemistry, Biophysics, and Structural Biology Commons, Cell and Developmental Biology Commons, Immunology and Infectious Disease Commons, Medical Sciences Commons, Veterinary Microbiology and Immunobiology Commons, and the Veterinary Pathology and Pathobiology Commons

Wynn, Emily L.; Schuller, Gennie; Loy, John D.; Workman, Aspen M.; McDaneld, T. G.; and Clawson, Michael L., “Differentiation of *Mannheimia haemolytica* genotype 1 and 2 strains by visible phenotypic characteristics on solid media” (2020). *Papers in Veterinary and Biomedical Science*. 362.  
[https://digitalcommons.unl.edu/vetscipapers/362](https://digitalcommons.unl.edu/vetscipapers/362)

This Article is brought to you for free and open access by the Veterinary and Biomedical Sciences, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Papers in Veterinary and Biomedical Science by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Note

Differentiation of *Mannheimia haemolytica* genotype 1 and 2 strains by visible phenotypic characteristics on solid media

Emily L. Wynn\(^a\), Gennie Schuller\(^b\), John D. Loy\(^b\), Aspen M. Workman\(^a\), Tara G. McDanel\(^d\), Michael L. Clawson\(^a,⁎\)

\(^a\) United States Department of Agriculture, Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, NE, USA
\(^b\) University of Nebraska-Lincoln, Institute of Agriculture and Natural Resources, School of Veterinary Medicine and Biomedical Sciences, Lincoln, NE, USA

**Ab**str**act**

Genotype 2 *Mannheimia haemolytica* associate with the lungs of cattle with bovine respiratory disease more frequently than genotype 1 strains. Different colony colors and morphologies were identified between genotype 1 and 2 solid media cultures. Genotype of strains, and frequency differences between them in mixed cultures are discernible by visual inspection.

*Mannheimia haemolytica* is a gram-negative bacterium often found in the upper respiratory tract of cattle (Rice et al., 2008). When cattle are stressed, *M. haemolytica* can invade their lungs and cause bovine respiratory disease (BRD) (Griffin et al., 2010). Not all strains associate equally with BRD (Klima et al., 2014). Two major genotypes of *M. haemolytica* have been identified in cattle (1 and 2). While both are found in the upper respiratory tract, genotype 2 strains are more frequently isolated from the lungs of cattle with BRD than genotype 1 strains (Clawson et al., 2016).

Accurate identification of *M. haemolytica* genotypes can facilitate investigations into their distribution, transmission, and pathogenicity. Recently, a matrix-assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF MS) assay was developed that distinguishes the two genotypes based on MS biomarkers (Loy and Clawson, 2017). The assay is typically performed on cultured bacterial strains. These color differences between genotype 1 and 2 strains were discernable on both chocolate and BHI blood agar plates under ambient light.

Two colony phenotypic characteristics were identified that could be used in combination to visually differentiate a genotype 1 *M. haemolytica* from a genotype 2; color and three-dimensional shape. Genotype 1 colonies were duller and grayish than genotype 2 colonies, which were a creamy white color (Fig. 1, Supplemental file 2). Two colony phenotypic characteristics were identified that could be used in combination to visually differentiate a genotype 1 *M. haemolytica* from a genotype 2; color and three-dimensional shape. Genotype 1 colonies were duller and grayish than genotype 2 colonies, which were a creamy white color (Fig. 1, Supplemental file 2). There was more phenotypic diversity within genotype 1 strains; some were very pale while others were brighter gray. However, all genotype 2 strains examined in this study were brighter and whiter than all genotype 1 strains. These color differences between genotype 1 and 2 strains were discernable on both chocolate and BHI blood agar plates under ambient light.

Genotype 1 colonies had a more complex three-dimensional shape than genotype 2 on both BHI blood and chocolate agar plates. The genotype 1 colonies had a raised outer edge, a depressed inner ring, and a raised center. In contrast, genotype 2 colonies predominantly had a smooth dome shape. As with color, there was more diversity in genotype 1 colony shape; some strains had a more exaggerated raised center and edge than others.

An easy method to quickly assess the three-dimensional shape of a *M. haemolytica* colony was to hold the agar plate at a 45-degree angle to a light source and observe the reflection of the light off of the colony (Fig. 2, Supplemental file 2). Due to their more complex three-dimensional shape, genotype 1 colonies reflected the light with a distortion near the edge of the colonies. For 11 of the 21 genotype 1 strains...

---

\(^⁎\) Corresponding author at: Genetics, Breeding, and Animal Health Research Unit, Agricultural Research Service, U.S. Meat Animal Research Center, USA.

E-mail address: mike.clawson@usda.gov (M.L. Clawson).

https://doi.org/10.1016/j.mimet.2020.105877

Received 2 November 2019; Received in revised form 11 February 2020; Accepted 19 February 2020

Available online 20 February 2020

0167-7012/ Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).
examined at 18 h, this distortion was large on either chocolate or blood agar plates (Fig. 2A, Supplemental files 1–2), while for the other 10 strains this distortion was a subtle bump on the edges of the reflection on both plate types (Fig. 2B, Supplemental files 1–2). Genotype 2 domed colonies predominantly reflected the light as a solid band with very little distortion in shape (Fig. 2C, Supplemental files 1–2). Fifteen out of the 17 genotype 2 strains had this reflection shape on either chocolate or blood agar plates (Supplemental files 1–2). The remaining two strains had a subtle bump on the edges of their reflection on both plate types but were still identifiable as a genotype 2 strain due to a creamy white colony color.

The exact morphology of a colony can vary over time, this was seen between the 18 and 24-h timepoints. As plates aged, the distorting effect of the reflection became more exaggerated. Due to this variation, during the course of this study some genotype 1 strains could have either bump or distorted reflections and some genotype 2 strains could have either smooth or bump reflections between plates. This indicates the ambiguity with the bump reflection as a genotype classifier in the absence additional information. It is important to note that the exact shape of the reflection will depend on the particular light source; in this study a long fluorescent light was used, about 3 ft above the plate. Regardless of the light source, the important feature to note is the distorting effect near the edges of the colony.

The colony color and morphology phenotypes of the collection were
most distinct when strains were streaked for isolation. When colonies were grown densely together, the differences become less distinct and harder to differentiate, especially on BHI blood agar. Using both colony colors and light reflections, the entire collection of *M. haemolytica* was accurately genotyped on chocolate agar by visual inspection (Supplemental files 1–2).

To further test the accuracy of these observations, 31 bovine nasal swab samples were plated on chocolate agar for *M. haemolytica* identification and genotyping by visual inspection from primary culture. The swabs had been stored at -80 °C in buffered peptone and 12% glycerol (McDaneld et al., 2018). Most nasal swab samples contained multiple species of bacteria and *M. haemolytica* DNA had been detected by qPCR (Loy et al., 2018), though the genotypes had never been determined. The samples were thawed to room temperature, and spread plates were grown overnight at 37 °C with 5% CO2. Two to four individual colonies that were suspected to be *M. haemolytica* were passaged to new chocolate agar plates. To confirm preliminary colony morphology observations, the *M. haemolytica* colonies were passaged one final time to new chocolate agar plates. Thirty-three colonies selected from 14 nasal swab sample cultures were visually genotyped by colony color alone, and 32 colonies from 17 nasal swab cultures were visually genotyped by both color and light reflection. All colonies were determined to be either genotype 1 or genotype 2, except for one colony that was called as ambiguous due to having the color of a genotype 2 and the reflection of a genotype 1 (Supplemental file 1). To determine the accuracy of these visual assessments, species and genotype identification was confirmed by MALDI-TOF MS (Loy and Clawson, 2017). Of the 33 colonies that were inspected solely by colony color, 30 were correctly genotyped (Cohen’s kappa, k = 0.79, substantial agreement). The three colonies that were misidentified were both genotype 2 strains that were visually identified as genotype 1. Of the 31 colonies that were determined to be genotype 1 or 2 by inspection of both colony color and light reflection, all were correctly genotyped (Cohen’s kappa, k = 1, perfect agreement). The one colony that was called as ambiguous by visual inspection was determined by the MALDI-TOF assay to be a cluster V *Mannheimia* sp. that has not yet been resolved to the species level (Angen et al., 1999).

When using both colony color and shape morphologies as criteria, *M. haemolytica* can be identified and genotyped with high accuracy (see Supplemental file 3 for a flow chart of colony color and shape morphology use). The ability to genotype *M. haemolytica* by visual inspection can be used as part of a diagnostic pipeline to ensure that the full diversity and frequencies of *M. haemolytica* genotypes is being captured from BRD cases and other samples of interest.

**Author statement**

ELW conceptualized the project, performed research, developed the reflection methodology, analyzed results, validated methods, and wrote the manuscript. GS performed research, developed the reflection method, validated methods, and edited the manuscript. JDL assisted in the project investigation and validation and edited the manuscript. AMW provided resources and assisted in the project investigation and edited the manuscript. TGM provided resources and assisted in the project investigation and edited the manuscript. MLC conceptualized and supervised the project, validated methods, analyzed results, and edited the manuscript.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgments**

We thank Stephanie Schmidt for secretarial support. The use of product and company names is necessary to accurately report the methods and results; however, the United States Department of Agriculture (USDA) neither guarantees nor warrants the standard of the products, and the use of names by the USDA implies no approval of the product to the exclusion of others that may also be suitable. The USDA is an equal opportunity provider and employer. This work was supported by the USDA, Agricultural Research Service of the United States and was partially supported by the Nebraska Experiment Station of the United States with funding from the Animal Health and Disease Research (section 1433) capacity funding program (accession 1017646) through the USDA National Institute of Food Agriculture of the United States.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2020.105877.

**References**

Angen, O., Mutters, R., Caugant, D.A., Olsen, J.E., Bisgaard, M., 1999. Taxonomic relationships of the *Pasteurella* haemolytica complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of Mannheimia haemolytica gen. nov., comb. nov., Mannheimia granulomatis comb. nov., Mannheimia glucosida sp. nov., Mannheimia ruderalis sp. nov. and Mannheimia varigena sp. nov. Int. J. Syst. Bacteriol. 49 (1), 67–68.

Clawson, M.L., Murray, R.W., Sweeney, M.T., Apley, M.D., DeDonder, K.D., Capik, S.P., Larson, R.L., Lubbers, B.V., White, B.J., Kalbfleisch, T.S., et al., 2016. Genomic signatures of *Mannheimia* haemolytica that associate with the lungs of cattle with respiratory disease, an integrative conjugative element, and antibiotic resistance genes. BMC Genomics 17 (1), 982.

Griffin, D., Chengappa, M.M., Kuszak, J., McVey, D.S., 2010. Bacterial pathogens of the bovine respiratory disease complex. Vet. Clin. North Am. Food Anim. Pract. 26, 381–394.

Klima, C.L., Alexander, T.W., Hendrick, S., McAllister, T.A., 2014. Characterization of *Mannheimia haemolytica* isolated from feedlot cattle that were healthy or treated for bovine respiratory disease. Can. J. Vet. Res. 78, 38–45.

Loy, J.D., Clawson, M.L., 2017. Rapid typing of *Mannheimia haemolytica* major genotypes 1 and 2 using MALDI-TOF mass spectrometry. J. Microbiol. Methods 136, 30–33.

Loy, J.D., Leger, L., Workman, A.M., Clawson, M.L., Bulut, E., Wang, B., 2018. Development of a multiplex real-time PCR assay using two thermocycling platforms for detection of major bacterial pathogens associated with bovine respiratory disease complex from clinical samples. J. Vet. Diagn. Invest. 30, 837–847.

McDaneld, T.G., Kuehn, L.A., Keele, J.W., 2018. Evaluating the microbiome of two sampling locations in the nasal cavity of cattle with bovine respiratory disease complex (BRDC). J. Anim. Sci. 96 (4), 1281–1287.

Rice, J.A., Carrasco-Medina, L., Hodgins, D.C., Shewen, P.E., 2008. *Mannheimia haemolytica* and bovine respiratory disease. Anim. Health Res. Rev. 8 (2), 117–128.