A Sialoreceptor Binding Motif in the *Mycoplasma synoviae* Adhesin VlhA

Meghan May1, Dylan W. Dunne2, Daniel R. Brown3

1 Department of Biomedical Sciences, College of Osteopathic Medicine, University of New England, Biddeford, Maine, United States of America, 2 Department of Biological Sciences, Jess and Mildred Fisher College of Science and Mathematics, Towson University, Towson, Maryland, United States of America, 3 Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, United States of America

**Abstract**

*Mycoplasma synoviae* depends on its adhesin VlhA to mediate cytadherence to sialylated host cell receptors. Allelic variants of VlhA arise through recombination between an assemblage of promoterless vlhA pseudogenes and a single transcription promoter site, creating lineages of *M. synoviae* that each express a different vlhA allele. The predicted full-length VlhA sequences adjacent to the promoter of nine lineages of *M. synoviae* varying in avidity of cytadherence were aligned with that of the reference strain MS53 and with a 60-a.a. hemagglutinating VlhA C-terminal fragment from a Tunisian lineage of strain WVU18533. Seven different sequence variants of an imperfectly conserved, single-copy, 12-a.a. candidate cytadherence motif were evident amid the flanking variable residues of the 11 total sequences examined. The motif was predicted to adopt a short hairpin structure in a low-complexity region near the C-terminus of VlhA. Biotinylated synthetic oligopeptides representing four selected variants of the 12-a.a. motif, with the whole synthesized 60-a.a. fragment as a positive control, differed (P < 0.01) in the extent they bound to chicken erythrocyte membranes. All bound to a greater extent (P < 0.01) than scrambled or irrelevant VlhA domain negative control peptides did. Experimentally introduced branched-chain amino acid (BCAA) substitutions Val31Le and Leu71Le did not significantly alter binding, whereas fold-destabilizing substitutions Thr4Gly and Ala9Gly tended to reduce it (P < 0.05). Binding was also reduced to background levels (P < 0.01) when the peptides were exposed to desialylated membranes, or were pre-saturated with free sialic acid before exposure to untreated membranes. From this evidence we conclude that the motif P-X-(BCAA)-X-F-X-(BCAA)-X-A-K-X-G binds sialic acid and likely mediates VlhA-dependent *M. synoviae* attachment to host cells. This conserved mechanism retains the potential for fine-scale rheostasis in binding avidity, which could be a general characteristic of pathogens that depend on analogous systems of antigenically variable adhesins. The motif may be useful to identify previously unrecognized adhesins.

Citation: May M, Dunne DW, Brown DR (2014) A Sialoreceptor Binding Motif in the *Mycoplasma synoviae* Adhesin VlhA. PLoS ONE 9(10): e110360. doi:10.1371/journal.pone.0110360

Editor: Mitchell F. Balish, Miami University, United States of America

Received June 30, 2014; Accepted September 15, 2014; Published October 22, 2014

Copyright: © 2014 May et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting information files.

Funding: This work was supported by the Robert M. Fisher Foundation (MM). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

**Introduction**

The bacterial pathogen *Mycoplasma synoviae* is associated with a broad spectrum of clinical manifestations ranging from inapparent infection to systemic disease of poultry. Infection is most commonly associated with inflammatory lesions of the joints, respiratory and/or reproductive tract and results in reduced feed conversion and poor egg quality. Less commonly, respiratory and/or reproductive tract and results in reduced feed conversion and poor egg quality. Less commonly, *M. synoviae* can be found infecting additional tissues in galliform birds (e.g. spleen, liver, central nervous system, skeletal muscle, and eye) [1–4] and respiratory tissues or sypoidal membranes of distantly related avian species such as ducks, geese, pigeons, and swallows [5].

Attachment to sialylated receptors on host cells is mediated by the *M. synoviae* variable lipoprotein hemagglutinin VlhA [6–7]. Previous analyses indicated that the vlhA gene family has been laterally transferred between *M. synoviae* and *Mycoplasma gallisepticum* possibly during coinfection of a shared avian host [8–9]. In *M. synoviae*, antigenic variants of this adhesin result from unidirectional recombination between a single expression site and a large reservoir of vlhA pseudogenes [10]. In contrast, altered expression in *M. gallisepticum* stems from the expansion and contraction of a poly-GAA repeat upstream of the promoters of each copy of vlhA [11]. The selective pressure of specific host immune responses to these antigens is thought to drive diversity in vlhA allele expression [10–13]. Despite the critical importance of cytadherence to the establishment and maintenance of infection, discrete VlhA types were demonstrated to have significantly different avidities for host cell binding, which can be quantified by agglutination of erythrocytes [14]. *M. synoviae*’s capacity for cytadherence maps surprisingly to a hypervariable C-terminal domain of VlhA called MSPA [15–16]. The precise means of attachment and how this capacity is retained despite such extensive sequence polymorphism and allele switching are not known. We sought to identify and characterize the specific motif that mediates adhesion of VlhA proteins to host cells.
Materials and Methods

Identification and Structural Modeling of the Putative Hemagglutination Motif (PHM)

The predicted full-length VlhA sequences adjacent to the single transcription promoter of nine lineages of *M. synoviae* varying in avidity of cytadherence (F10-2AS, FMT, K5016, K5395, MS53, MS117, MS173, MS178, and a 30X-passaged Florida lineage of strain WVU1853[^1]) were aligned with that of the reference strain MS53[^2] and with a 60-a.a. hemagglutinating VlhA C-terminal fragment from a ca. 12X-passaged Tunisian lineage of strain WVU1853[^3]. The multiple alignment was manually inspected for conserved motifs, evident as contiguous residues inferred to be under stabilizing selection (v[^4], 1) by using Bayesian models of sequence evolution in the Selecton v2.4 software suite[^5]. The secondary structures of full-length VlhA, MSPA and its C-terminal 60 residues, and of the putative hemagglutination motifs (PHMs) described were modeled using the Phyre2 suite of template-directed and *ab initio* protein structure prediction algorithms (http://www.sbg.bio.ic.ac.uk/phyre2)[^6]. The effects of individual amino acid substitutions on peptide structural stability were predicted by applying the Site Directed Mutator algorithm (http://mordred.bioc.cam.ac.uk, sdm/sdm.php)^[20] also to the pdb files generated by Phyre2.

Quantitative Binding of PHM Peptides

Twelve-a.a. peptides representing five variants of the PHM from strains FMT, K5016, K5395, MS53 and WVU1853[^7], plus the whole 60-a.a. hemagglutinating fragment of the Tunisian lineage of strain WVU1853[^7], were synthesized, biotinylated and lyophilized (Biomatik, Wilmington, DE). Purity of each lyophilized preparation was confirmed by HPLC to be 90–92% full-length peptide. Those strains were chosen because FMT, K5016, K5395 and the Florida lineage of WVU1853[^7] spanned a 20-fold range in quantitative hemagglutination phenotypes[^11], and the entire *vlhA* locus sequence of the reference strain MS53 has been published[^8,14]. Peptides having single directed mutations introduced at the conserved residues 3 or 4, or non-conserved residues 7 or 9, were also synthesized using the strain FMT motif PKVTFNLAAKEG as a parent. FMT was chosen as the parent motif because it had only one difference (Thr6Asn) from the most commonly observed amino acid at each residue (Figure 1). The functionally synonymous substitutions Val3Ile and Leu7Ile were predicted to be inconsequential, while Thr4Gly and Ala9Gly were predicted to affect PHM structure and/or function. Negative control peptides used in erythrocyte membrane-binding assays are also shown. Functionally non-synonymous substitutions relative to the most common amino acid at each residue are shaded in black, synonymous differences are shaded in gray, and identical residues are not shaded. (B) = branched chain amino acid.

doi:10.1371/journal.pone.0110360.g001

---

Figure 1. Aligned PHM and control peptide sequences. The putative hemagglutination motif (PHM) was deduced by aligning the adhesin protein VlhA allele present at the expression site of ten specimens of *M. synoviae* with a 60-a.a. hemagglutinating VlhA C-terminal fragment from the Tunisian lineage of strain WVU1853[^7], then inspecting the alignment for contiguous residues inferred to be under stabilizing selection. Peptides representing five variants of the PHM, including strains having a 20-fold range in quantitative hemagglutination phenotypes[^11], were synthesized. Directed mutations were introduced at selected residues relative to the PHM from strain FMT, which had only one difference (Thr6Asn) from the most common amino acid at each residue. The mutations Val3Ile and Leu7Ile were predicted to be inconsequential, while Thr4Gly and Ala9Gly were predicted to affect PHM structure and/or function. Negative control peptides used in erythrocyte membrane-binding assays are also shown. Functionally non-synonymous substitutions relative to the most common amino acid at each residue are shaded in black, synonymous differences are shaded in gray, and identical residues are not shaded. (B) = branched chain amino acid.
Figure 2. PHM structural predictions. (A) The putative hemagglutination motif (PHM; red) was predicted to adopt a hairpin structure of two anti-parallel β strands separated by a short disordered loop. (B) The motif (red, indicated by arrow) mapped to a low-complexity region near the carboxyterminal domain (CTD) of the M. synoviae adhesin protein VlhA cleavage product MSPA, shown here in the structure predicted for the Tunisian lineage of strain WVU1853. The N-terminal domain (NTD) of MSPA was predicted to have much greater 3-dimensional complexity. (C) The length of the disordered loop was predicted to be longer in PHM peptides that bound to avian erythrocyte membranes (representing Florida and
Peptide from strain FMT, and Thr4Gly tended to reduce binding, whereas Val3Ile and Leu7Ile did not significantly alter binding.

Tukey-Kramer Honestly Significant Difference test. As predicted, the directed substitution Ala9Gly significantly reduced binding versus the parent strain MS53.

After washing the membrane-coated and blocked wells 3× with 300 μL of PBS, 50 μg of biotinylated peptide solubilized in 50 μL of water was added to each of duplicate wells and allowed to bind for 1 hr at 37°C. After washing each well 3× with 300 μL of PBS, bound peptides were detected using horseradish peroxidase-conjugated streptavidin (2 μL/mL, Sigma-Aldrich, St. Louis, MO) for 1 hr at 37°C, cellular debris including hemoglobin was removed by washing each well 3× with 300 μL of PBS, pH 7.4, and sealed plates were blocked 1 hr at 37°C with 300 μL per well of 5% v/v fetal bovine serum in PBS.

After washing the membrane-coated and blocked wells 3× with 300 μL of PBS, 50 μg of biotinylated peptide solubilized in 50 μL of water was added to each of duplicate wells and allowed to bind for 1 hr at 37°C. After washing each well 3× with 300 μL of PBS, bound peptides were detected using horseradish peroxidase-conjugated streptavidin (2 μg/mL, Sigma-Aldrich, St. Louis, MO) and the chromogenic substrate 3,3′,5,5′-tetramethylbenzidine (Thermo Fisher Scientific, Waltham, MA) with an acid stop followed by spectrophotometric analysis (λ = 450 nm). The hemagglutinating 60-mer of the Tunisian lineage of strain WVU1853T served as the positive control peptide, and negative controls were a scrambled version of the PHM from strain FMT (LAFGAVKKT) and an irrelevant peptide (PNAPFVQMKGD) from a distant site in VlhA from the Florida lineage of strain WVU1853T (GenBank AEA01932.1). The effect of pre-saturation with ligand was tested by first incubating the peptides in 250 mg/ml N-acetylneuraminic acid (Sigma-Aldrich, St. Louis, MO) for 1 hr at 37°C, and sealed plates were blocked 1 hr at 37°C with 300 μL per well of 5% v/v fetal bovine serum in PBS.

The effect of peptide sequence on extent of adherence to membranes (n = 3 independent replications of each treatment combination, with duplicate measurements of each peptide within replicate) was analyzed by ANOVA, with Tukey-Kramer Honestly Significant Difference (HSD) post-hoc comparisons used to group the means when the main effect was significant (P<0.05 or less). The effects of membrane pre-treatment with sialidase and peptide pre-saturation with sialic acid were analyzed by ANOVA, with HSD or Dunnnett’s post-hoc comparisons to the corresponding native specimens when the main effect was significant. Statistical analyses were performed using Origin 9 (OriginLab, Northampton, MA) software.

Motif Distribution in M. synoviae and M. gallisepticum

M. synoviae strain MS53 vlhA pseudogene sequences and M. gallisepticum strains R, F, WI01, NY01, NC06, CA06, VA94, NC95, NC98, and NC96 were obtained from GenBank (accession numbers NC_007294.1, NC_004829.2, NC_017503.1, NC_018410.1, NC_018409.1, NC_018411.1, NC_018412.1, NC_018406.1, NC_018407.1, NC_018413.1, and NC_018408.1, respectively). Occurrences of PHM-encoding sequences were totaled and normalized to the total length of vlhA-encoding sequence in each strain. Each member of the vlhA pseudogene reservoir of M. synoviae strain MS53 was used to construct a neighbor-joining tree (bootstrap n = 100) using ClustalW2 [24]. The designated outgroup was vlhA 4.02 from M. gallisepticum.

Results

Identification of the PHM

When the full-length expressed VlhA protein MSPA sequences of nine strains of M. synoviae that vary in avidity of cytadherence were aligned with MSPA of the reference strain MS53 [8] and a 60-a.a. hemagglutinating peptide derived from the C-terminus of MSPA expressed by the Tunisian lineage of strain WVU1853T [15], an imperfectly conserved 12-a.a. motif was evident in all sequences (Figure 1). A total of seven different PHM sequence variants were evident among the 11 total sequences aligned.
invariably paired with Leu7, while Thr6 was invariably paired with a.a. hemagglutinating fragment and four strain variants of its binding of any specific ligand.  

The degree of structural complexity in the C-terminus of MSPA was otherwise too low for the algorithms to predict (99.4%). The degree of structural complexity in the C-terminus of MSPA (regional structure prediction confidence 70%) near the C-terminus of MSPA (Figure 2a, b). Fifty-three strains except MS53, on which sialic acid had no effect.  

Discussion  

One of the defining moments of many infections is the attachment of a disease-causing agent to its host. Understanding how the parasitic bacterial species M. synoviae colonizes a host cell’s surface is paramount to understanding how to prevent infection. It is known that the protein family VlhA is responsible for attachment by M. synoviae, but the functional motifs of the adhesin and the molecular basis for rheostasis in binding avidity have not been characterized. Proteins in this family from multiple strains of M. synoviae have been identified as having a role in the attachment to host blood cells [7,15]. Khiari et al. [15] mapped candidate 12-a.a. cytadherence motif (Figure 1) bound to chicken erythrocyte membranes in an ELISA format and could be detected by probing with horseradish peroxidase-conjugated streptavidin. Four of the peptides bound to membranes to a significantly greater extent (P<0.05) than scrambled or irrelevant control peptides did, but a peptide representing the corresponding motif from strain MS53 did not bind to membranes to any extent greater than background (Figure 3). Single neutral substitutions (predicted ΔAG = −0.25) experimentally introduced at conserved residue 3 (Val3Ile) or non-conserved residue 7 (Leu7Ile) did not alter binding to membranes with respect to the extent of binding by the parent motif of strain FMT, whereas the experimental destabilization substitution Thr4Gly (predicted ΔAG = −2.31) tended to reduce binding (Figure 3). The motif of strain MS53 differs naturally from all others by Ala9Pro (BLOSUM62 = −1; predicted ΔAG = −2.22), and the even more destabilizing substitution Ala9Gly (predicted ΔAG = −3.88) nearly abolished binding when introduced into the parent motif of strain FMT (P<0.05; Figure 3). These effects correlated with a predicted change in length of the disordered loop in the hairpin secondary structure of the motif (Figure 2c).  

Binding of the peptides to desialylated membranes was significantly reduced (P<0.01) relative to untreated membranes for all peptides except those representing strain MS53 and the scrambled and irrelevant controls (Figure 4a). When pre-incubated with free sialic acid, all peptides except the one representing strain MS53 and the scrambled and irrelevant controls had significantly diminished (P<0.01) capacity for membrane binding (Figure 4b). From this evidence we conclude that the composite amino acid motif P-X-(BCAA)-X-F-X-(BCAA)-X-A-K-X-G binds sialic acid and likely mediates VlhA-dependent M. synoviae attachment to sialylated receptors on the surface of avian erythrocytes.  

PHM Distribution among M. synoviae Strain MS53 vlhA Pseudogenes and Mycoplasma gallisepticum vlhA Homologs  

Candidate PHM sequences occurred in 45 of the 70 putative vlhA pseudogenes of M. synoviae strain MS53 [8], 39% of the time with no deviation from the consensus among the alleles expressed by the strains examined, 20% with a single deviation, and 17% with two deviations from consensus. Phylogenetic clustering of vlhA pseudogenes containing intact copies of the PHM did not correlate with their syntenic order in the strain MS53 genome (Figure S1). The PHM occurred at least 18-fold more frequently in strain MS53 (0.65 motifs/kb of vlhA sequence) than in the genomes of any of 10 strains of M. gallisepticum (0.014–0.037 motifs/kb of vlhA sequence), a species known to employ a different primary cytadherence mechanism [25] (Figure 5a). The rate of occurrence of imperfect PHMs was comparable between the two species (Figure 5b).
the capacity for attachment to the carboxyterminus of VlhA, and we utilized that finding to identify a specific motif sufficient to mediate VlhA binding to sialylated host cells.

Sequence conservation across adherent strains enabled the identification of a 12-residue putative hemagglutination motif that could be characterized further. This motif was predicted to have remarkably little structural complexity, in contrast to the complex topology of sialic acid ligand-binding domains of other microbes [26–27]. While residues at PHM positions 3 and 7 were conserved, substitution with similar residues having BLOSUM62 scores > 0 did not alter function. The conserved Thr residue at position 4 could be changed to the dissimilar residue Gly (BLOSUM62 = −3) without loss of function. It is thus likely that the binding mechanism will tolerate synonymous substitutions at positions 3 and 7, and nonsynonymous substitutions at position 4. Residue 9 was a conserved Ala in all adherent strains. Strain MS53, which has an unknown attachment phenotype but is an attenuated strain, had the nonsynonymous substitution Ala9Pro (BLOSUM62 = −1). Changing the strain FMT peptide to Gly9 (BLOSUM62 = 0) significantly diminished binding, and the strain MS53 peptide was non-adherent. Taken together, these results indicate that Ala9 is critical to PHM domain function. Our results indicate that the composite amino acid motif P-X-(BCAA)-X-F-X-(BCAA)-X-A-K-X-G mediates MSPA binding to avian erythrocytes. The potential

Figure 5. PHM distribution in *vlhA* genes and pseudogenes in *M. synoviae* and *M. gallisepticum*. (A) PHM-encoding sequence as a function of total kbp of *vlhA* sequence is elevated 18-fold in *M. synoviae* (MS) reference strain MS53, the only strain for which the entire *vlhA* locus sequence has been published, relative to 10 fully-sequenced strains of *M. gallisepticum* (MG), evidence that it is far more common in *M. synoviae*. (B) The relative proportions of perfect and imperfect PHM copies were comparable between strains of *M. synoviae* and *M. gallisepticum*. doi:10.1371/journal.pone.0110360.g005
to accommodate all amino acids with BLOSSUM26 scores >0 at PHM positions 3 and 7 (i.e., Ala, Met, Thr and Met, Phe, respectively) rather than restricting the parameters to branched-chain amino acids (Ile, Leu, Val) merits further analysis.

Previous studies indicated that whole M. synoviae cells interact with sialylated host cell receptors in order to facilitate attachment. Extrapolation from PHM-peptide-binding to whole cell attachment necessarily requires demonstration of peptide-sialic acid interactions. Desialylation of avian erythrocytes prior to antigen preparation resulted in significant losses of binding capacity for all PHM peptides except the scrambled and irrelevant controls and strain MS53, for which desialylation had no effect on binding. In a reciprocal experiment, pre-adsorption of peptides with free sialic acid prior to exposure to intact erythrocyte antigen similarly diminished binding capacity for all PHM peptides except the scrambled and irrelevant controls and strain MS53. These results indicate a specific interaction between sialic acid and the PHM and support the hypothesis that the PHM domain mediates attachment of whole M. synoviae cells to host sialic receptors.

The occurrence of PHM domains was not uniform among the pseudogenes of M. synoviae strain MS53, the only strain for which the entire pseudogene reservoir has been sequenced [8]. A majority (69%) of pseudogenes had perfect or near-perfect PHMs, while 31% had no discernible PHMs. To provide some context for the distribution of PHM domains in the sample of vlhA sequences existing within M. synoviae strain MS53, we examined the frequency and distribution in an alternative sample of vlhA sequences that exist distributed across multiple strains of M. gallisepticum. In contrast to the 45 copies in M. synoviae strain MS53, sequenced M. gallisepticum strains ranged from having just a single copy of vlhA encoding a PHM domain (strains W101 and F) up to a maximum of only 3 copies (strains R, NY01, NC06, CA06, VA94, NC95, and NC96). Normalization to the total amount of vlhA sequence within species confirmed that M. synoviae has a greatly elevated instance of PHM-encoding sequence relative to M. gallisepticum, and that the low frequency of PHM is consistent across strains of M. gallisepticum. The multiple independent cytidaradherence mechanisms of M. gallisepticum [28–32] may allow the decay of PHM domains within vlhA pseudogenes, while selective pressure to retain the functional motif in the homologous proteins in M. synoviae is substantially greater due to the absence of other mechanisms of cytidaradherence.

This work describes a novel functional motif associated with adherence to sialic acid, and its distribution across vlhA pseudogenes. This very specific protein fragment pattern may be a target to design novel drug therapies or vaccines to alleviate or prevent infection due to M. synoviae as well as other pathogens that use similar mechanisms to attach to their hosts, and allows for the identification of currently unrecognized microbial adhesins targeting sialic receptors.

Supporting Information

Figure S1 Distribution and relatedness of PHM-encoding pseudogenes. PHM-encoding pseudogenes (shaded) did not cluster together as a separate group from non-encoding pseudogenes. Relatedness of pseudogenes did not reflect gene synteny. (TIFF)

Acknowledgments

We thank Edan Tulman (University of Connecticut) for helpful discussions regarding vlhA loci in M. gallisepticum. This work was supported by the Robert M. Fisher Foundation (MM).

Author Contributions

Conceived and designed the experiments: MM DRB.Performed the experiments: DD MM. Analyzed the data: MM DRB. Contributed reagents/materials/analysis tools: MM. Wrote the paper: MM DRB.

References

1. Stikovski I, Kempf J (1996) Mycoplasmas in poultry. Rev Sci Tech. 15(4): 1495–525.
2. Sentis-Cué G, Shivasparad HS, Chin RP (2005) Systemic Mycoplasma synoviae infection in broiler chickens. Avian Pathol. 34(2): 157–62.
3. Chin RP, Meteyer CU, Yamamoto RM, Klein PN (1991) Isolation of Mycoplasma synoviae from the brains of commercial meat turkeys with meningitis vasculitis. Avian Dis. 35(3): 631–7.
4. Lockaby SB, Hoerr FJ, Lauerman LH, Kleven SH (1998) Pathogenicity of Mycoplasma synoviae in broiler chickens. Vet Pathol. 35(3): 178–90.
5. Brown DR, May M, Bradbury JM, Balish MF, Calcutt MJ, et al. (2010) Genus I. Mycoplasma. In: Krieg NR, Ludwig W, Brown DR, Whitman WB, Hedlund BP, editors. Bergey’s Manual of Systemic Bacteriology, Volume 4. Springer, Inc.: New York, NY.
6. Manchee R, Taylor-Robinson D (1969) Utilization of neuraminic acid receptors by mycoplasmas. J Bacteriol. 98(3): 914–9.
7. Noornohammadi A, Markham P, Whithekar K, Walker I, Gurevich V, et al. (1997) Mycoplasma synoviae has two distinct phase-variable major membrane antigens, one of which is a putative hemagglutinin. Infect Immun. 65(7): 3466–71.
8. Citti C, Browning GF, Rosengarten R (2005) Phenotypic diversity and cell attachment of whole M. synoviae cells to host sialic receptors.

This work describes a novel functional motif associated with adherence to sialic acid, and its distribution across vlhA pseudogenes. This very specific protein fragment pattern may be a target to design novel drug therapies or vaccines to alleviate or prevent infection due to M. synoviae as well as other pathogens that use similar mechanisms to attach to their hosts, and allows for the identification of currently unrecognized microbial adhesins targeting sialic receptors.

Supporting Information

Figure S1 Distribution and relatedness of PHM-encoding pseudogenes. PHM-encoding pseudogenes (shaded) did not cluster together as a separate group from non-encoding pseudogenes. Relatedness of pseudogenes did not reflect gene synteny. (TIFF)

Acknowledgments

We thank Edan Tulman (University of Connecticut) for helpful discussions regarding vlhA loci in M. gallisepticum. This work was supported by the Robert M. Fisher Foundation (MM).

Author Contributions

Conceived and designed the experiments: MM DRB. Performed the experiments: DD MM. Analyzed the data: MM DRB. Contributed reagents/materials/analysis tools: MM. Wrote the paper: MM DRB.
24. Larkin MA, Blackshields G, Brown NP, Chenna R, McGgettigan PA, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics. 23(21): 2947–8.
25. Papazisi L, Frasca S, Gladd M, Liao X, Yogev D, Geary SJ (2002) GapA and CmmA coexpression is essential for Mycoplasma gallisepticum cytadherence and virulence. Infect Immun. 70(12): 6839–43.
26. Tharakaraman K, Jayaraman A, Raman R, Viswanathan K, Stebbins NW, et al. (2013) Glycan receptor binding of the influenza A virus H7N9 hemagglutinin. Cell. 153(7): 1486–93.
27. Pang SS, Nguyen ST, Perry AJ, Day CJ, Panjikar S, et al. (2014) The three-dimensional structure of the extracellular adhesion domain of the sialic acid-binding adhesin SabA from Helicobacter pylori. J Biol Chem. 289(10): 6332–40.
28. Boguslavsky S, Menaker D, Lysnyansky I, Liu T, Levishon S, et al. (2000) Molecular characterization of the Mycoplasma gallisepticum pvpA gene which encodes a putative variable cytadhesin protein. Infect Immun. 68(7): 3956–64.
29. Forsyth MH, Tourtellotte ME, Geary SJ (1992) Localization of an immunodominant 64 kDa lipoprotein (LP 64) in the membrane of Mycoplasma gallisepticum and its role in cytadherence. Mol Microbiol. 6(15): 2999–106.
30. Goh MS, Gorton TS, Forsyth MH, Troy KE, Geary SJ (1998) Molecular and biochemical analysis of a 105 kDa Mycoplasma gallisepticum cytadhesin (GapA). Microbiology. 144 (11): 2971–8.
31. Jenkins C, Geary SJ, Gladd M, Djordjevic SP (2007) The Mycoplasma gallisepticum OsmC-like protein MG1142 resides on the cell surface and binds heparin. Microbiology. 153(5): 1455–63.
32. May M, Papazisi L, Gorton TS, Geary SJ (2006) Identification of fibronectin-binding proteins in Mycoplasma gallisepticum strain R. Infect Immun. 74(3): 1777–85.