



Allen D. Leman Swine Conference



Volume 39
2012

Published by: Veterinary Continuing Education

Sponsors

We thank the following sponsors:

Platinum

Bayer Animal Health
Pfizer Animal Health

Gold

Novartis Animal Health

Silver

Boehringer Ingelheim Vetmedica, Inc.
National Pork Board
Newport Laboratories

Bronze

Merck Animal Health

Copper

AgStar Financial Services
Elanco Animal Health
GlobalVetLINK
IDEXX
Novus International, Inc.
PIC USA
USDA PRRS CAP

University of Minnesota Institutional Partners

College of Veterinary Medicine
University of Minnesota Extension
College of Food, Agriculture and Natural Resources Sciences

Using antigenic cartography to quantify antigenic evolution in swine influenza A viruses and for vaccine strain selection

Nicola S. Lewis¹; Pravina Kitikoon²; Amy L. Vincent²; Marie R. Gramer³

¹Department of Zoology, University of Cambridge, Downing Street, Cambridge, UK; ²National Animal Disease Center, USDA-ARS, Ames, Iowa; ³Minnesota Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, Minnesota

Introduction

Swine are an important host for influenza A viruses and variants arising in pigs can be of zoonotic potential to other host species, with the potential to cause epidemics and pandemics. The haemagglutinin (HA) protein is of key importance in the control of swine influenza because HA is the primary target of the protective immune response and the main component of currently licensed influenza vaccines. However, the influenza virus HA protein changes over time via a process called antigenic drift, and vaccine strains must reflect the currently circulating strains to remain effective. Vaccinating pigs against influenza virus is a common practice in the U.S. swine industry, with fully licensed commercial vaccines or autogenous vaccines. Autogenous vaccine usage against influenza virus has continued to increase due to the diversity of viruses co-circulating in the North American pig population and the inability of the animal biologics industry to change the vaccine composition as rapidly as the viruses are changing.¹ Antigenic drift is assessed primarily by the haemagglutination inhibition (HI) assay. HI assay data can be more thoroughly analyzed by using more visual and computational methods such as antigenic cartography. Using antigenic cartography, we quantify and visualize the antigenic differences among viruses as an antigenic map. Integrating these measured antigenic differences with the genetic sequence data and experimental cross-protection studies in the host, we aim to understand the molecular basis of antigenic evolution and select vaccine strains which might better represent the antigenic variation seen in the field.

Materials and methods

The experimental work was carried out at USDA-ARS, University of Minnesota and University of Cambridge, UK. Four-week-old cross-bred pigs were obtained from a herd free of both influenza virus and porcine reproductive and respiratory syndrome virus (PRRSV) infections. Two pigs per virus were immunized with inactivated virus combined with commercial adjuvant by an intramuscular route. Two doses of vaccine were given approximately 2-3 weeks apart; pigs with HI titers < 1:80 after the second

dose were given a third dose of vaccine prior to final blood collection. At the end of the vaccination period, pigs were humanely euthanized with pentobarbital (Sleepaway, Fort Dodge Animal Health, Fort Dodge, IA) approximately 2 weeks after the final vaccination for blood collection. Sera were heat inactivated at 56°C and treated to remove non-specific agglutinators with a 20% suspension of kaolin followed by adsorption with 0.5% turkey red blood cells (RBC). The HI assays were performed with turkey RBC's with standard techniques (WHO Manual on Animal Influenza Diagnosis and Surveillance), (<http://www.wpro.who.int.NR/rdonlyres/EFD2B9A7-2265-4AD0-BC98-97937B4FA83C/0/manualonanimalaidiagnosisandsurveillance.pdf>). The reciprocal of the ratio between heterologous and homologous HI titers for individual serum samples were calculated to indicate the fold-change between heterologous and homologous reactions.

The quantitative analyses of the antigenic properties of swine influenza A (H1) viruses were performed using antigenic cartography, previously used for human and swine influenza A (H3N2) viruses^{4,14} and swine origin A (H1N1) influenza virus in humans,⁶ swine influenza A (H1) viruses^{11,12} and equine influenza A (H3N8) viruses.¹⁰

Viruses were deep sequenced using an Illumina Genome Analyzer (Illumina, Inc., San Diego, CA, USA). Samples for sequencing were prepared as described previously⁵ with Uni-12 and Uni-13 primers and gene specific primers⁸ to amplify all 8 segments in one reaction. Sequence assembly was performed as previously described.⁹ The prepared reads were assembled using Whole Genome Shotgun and EST Sequence Assembler, MIRA 3.4.0 (German Cancer Research Centre, Heidelberg, Germany). Manual editing for certain sequence was performed with GAP4 from the Staden package.² Manually edited sequences were exported back to MIRA for recalling of the consensus sequence prior to the final collection of the assembled sequences.

A maximum-likelihood (ML) phylogenetic tree for the HA1 domain nucleotide sequences was inferred using PhyML⁷ with HKY model, with the transition/transversion ratio estimated from the empirical data.

Results

The 58 swine influenza A (H1) viruses isolated from 1930-2012 grouped into four distinct antigenic clusters. The ML phylogenetic tree shows that the genetic evolution of the same swine influenza A (H1) viruses resulted in the alpha, beta, gamma and delta 1 and 2 phylogenetic clusters as previously reported.¹¹ When we color the ML phylogenetic tree according to the antigenic cluster to which the isolate belongs we find that the antigenic cluster cannot always be predicted from the phylogenetic clade. To investigate the molecular basis of the antigenic clusters, we aligned the amino acid sequences, grouped and color coded them based on antigenic cluster, and marked the cluster difference amino acid substitutions. We find that antigenic clusters are defined by just a few amino acid substitutions despite numerous nucleotide differences among the strains.

When we measure the antigenic distances among antigenic clusters, we find on average strains within a cluster are between 3 and 8 antigenic units (one antigenic unit being equivalent to a two-fold difference in HI assay titre), or at least a four-fold difference in HI assay titre away from strains in other antigenic clusters. When we measure from the serum raised to the first strain isolated in each cluster to all points within that cluster, we find evidence for antigenic drift over time within the clusters consisting of delta 1 and 2 phylogenetic strains and no evidence within the other two antigenic clusters.

Discussion

The antigenic relationships among the swine influenza A (H1) viruses show that the viruses group into four antigenic clusters. The clusters have spent extensive periods of time co-circulating with no apparent global replacement of one antigenic cluster with another.

Vaccination is the primary method of controlling swine influenza. Vaccine updates in other hosts are recommended after an assessment of antigenic, genetic, and field data. Wilson and Cox observed that vaccine updates occurred for human influenza viruses when there were 4 or 5 amino acid substitutions in at least 2 antigenic sites.¹⁵ The OIE expert surveillance panel for equine influenza also annually reviews the strains used in the equine influenza virus vaccine by considering antigenic, genetic, and epidemiological data on currently circulating strains.¹³ In equine influenza H3 we showed that just one amino acid substitution results in antigenic distances between the immunizing virus and the challenge virus that would result in a loss of vaccine protection and equine vaccine cross-protection studies have previously showed that vaccination with a strain from one cluster does not prevent infection with a strain from the other co-circulating cluster.^{3,10}

We can quantitatively relate the swine serum-derived antigenic data presented here back to the immunological response to vaccination in the pig and to the protection against different clusters that a particular vaccine strain might afford because commercial swine influenza virus vaccines undergo clinical trials.

By using swine cross-protection study data we can directly relate the antigenic differences among circulating swine influenza H1 viruses to the potential for vaccine breakdown and thus both monitor potentially significant antigenic drift and improve vaccine strain selection.

Acknowledgements

This work was funded by USDA-ARS and -APHIS.

References

1. Beaudoin, A., S. Johnson, P. Davies, J. Bender, M. Gramer. 2012. Characterization of influenza A outbreaks in Minnesota swine herds and measures taken to reduce the risk of zoonotic transmission. *Zoonoses and public health* 59:96–106.
2. Bonfield, J. K., K. Smith, R. Staden. 1995. A new DNA sequence assembly program. *Nucleic acids research* 23:4992–4999.
3. Daly, J. M., P. J. Yates, J. R. Newton, A. Park, W. Henley, J. L. Wood, N. Davis-Poynter, J. A. Mumford. 2004. Evidence supporting the inclusion of strains from each of the two co-circulating lineages of H3N8 equine influenza virus in vaccines. *Vaccine* 22:4101–4109.
4. de Jong, J. C., D. J. Smith, A. S. Lapedes, I. Donatelli, L. Campitelli, G. Barigazzi, K. Van Reeth, T. C. Jones, G. F. Rimmelzwaan, A. D. Osterhaus, R. A. Fouchier. 2007. Antigenic and genetic evolution of swine influenza A (H3N2) viruses in Europe. *Journal of Virology* 81:4315–4322.
5. Ducatez, M. F., B. Hause, E. Stigger-Rosser, D. Darnell, C. Corzo, K. Juleen, R. Simonson, C. Brockwell-Staats, A. Rubrum, D. Wang, A. Webb, J. C. Crumpton, J. Lowe, M. Gramer, R. J. Webby. 2011. Multiple reassortment between pandemic (H1N1) 2009 and endemic influenza viruses in pigs, United States. *Emerging infectious diseases* 17:1624–1629.
6. Garten, R. J., C. T. Davis, C. A. Russell, B. Shu, S. Lindstrom, A. Balish, W. M. Sessions, X. Xu, E. Skepner, V. Deyde, M. Okomo-Adhiambo, L. Gubareva, J. Barnes, C. B. Smith, S. L. Emery, M. J. Hillman, P. Rivailier, J. Smagala, M. de Graaf, D. F. Burke, R. A. Fouchier, C. Pappas, C. M. Alpuche-Aranda, H. Lopez-Gatell, H. Olivera, I. Lopez, C. A. Myers, D. Faix, P. J. Blair, C. Yu, K. M. Keene, P. D. Dotson, Jr., D. Boxrud, A. R. Sambol, S. H. Abid, K. St George, T. Bannerman, A. L. Moore, D. J. Stringer, P. Blevins, G. J. Demmler-Harrison, M. Ginsberg, P. Kriner, S. Waterman, S. Smole, H. F. Guevara, E. A. Belongia, P. A. Clark, S. T. Beatrice, R. Donis, J. Katz, L. Finelli, C. B. Bridges, M. Shaw, D. B. Jernigan, T. M. Uyeki, D. J. Smith, A. I. Klimov, N. J. Cox. 2009. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* 325:197–201.
7. Guindon, S., O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696–704.

Using antigenic cartography to quantify antigenic evolution in swine influenza A viruses

8. Hoffmann, E., J. Stech, Y. Guan, R. G. Webster, D. R. Perez. 2001. Universal primer set for the full-length amplification of all influenza A viruses. *Archives of Virology* 146:2275–2289.
9. Kitikoon, P., A. L. Vincent, P. C. Gauger, S. N. Schlink, D. O. Bayles, M. R. Gramer, D. Darnell, R. J. Webby, K. M. Lager, S. L. Swenson, A. Klimov. 2012. Pathogenicity and Transmission in Pigs of the Novel A(H3N2)v Influenza Virus Isolated from Humans and Characterization of Swine H3N2 Viruses Isolated in 2010–2011. *Journal of Virology* 86:6804–6814.
10. Lewis, N. S., J. M. Daly, C. A. Russell, D. L. Horton, E. Skepner, N. A. Bryant, D. F. Burke, A. S. Rash, J. L. Wood, T. M. Chambers, R. A. Fouchier, J. A. Mumford, D. M. Elton, D. J. Smith. 2011. Antigenic and genetic evolution of equine influenza A (H3N8) virus from 1968 to 2007. *Journal of Virology* 85:12742–12749.
11. Lorusso, A., A. L. Vincent, M. L. Harland, D. Alt, D. O. Bayles, S. L. Swenson, M. R. Gramer, C. A. Russell, D. J. Smith, K. M. Lager, N. S. Lewis. 2011. Genetic and antigenic characterization of H1 influenza viruses from United States swine from 2008. *The Journal of general virology* 92:919–930.
12. Nfon, C. K., Y. Berhane, T. Hisanaga, S. Zhang, K. Handel, H. Kehler, O. Labrecque, N. S. Lewis, A. L. Vincent, J. Copps, S. Alexandersen, J. Pasick. 2011. Characterization of H1N1 swine influenza viruses circulating in Canadian pigs in 2009. *Journal of Virology* 85:8667–8679.
13. OIE. 2010. Conclusions and recommendations from the Expert Surveillance Panel on Equine Influenza Vaccines. *Office International des Epizooties Bulletin* 2010–2:44–45.
14. Smith, D. J., A. S. Lapedes, J. C. de Jong, T. M. Bestebroer, G. F. Rimmelzwaan, A. D. Osterhaus, R. A. Fouchier. 2004. Mapping the antigenic and genetic evolution of influenza virus. *Science* 305:371–376.
15. Wilson, I. A., N. J. Cox. 1990. Structural basis of immune recognition of influenza virus hemagglutinin. *Annual review of immunology* 8:737–771.

