

Use of Antigenic Cartography in Vaccine Seed Strain Selection

Author(s): Ron A. M. Fouchier and Derek J. Smith

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*Research Note***Use of Antigenic Cartography in Vaccine Seed Strain Selection**Ron A. M. Fouchier^{AD} and Derek J. Smith^{ABC}^ADepartment of Virology, Erasmus MC, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands^BDepartment of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom^CFogarty International Center, National Institutes of Health, Bethesda, MD 20892

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SUMMARY. Human influenza A viruses are classic examples of antigenically variable pathogens that have a seemingly endless capacity to evade the host's immune response. The viral hemagglutinin (HA) and neuraminidase (NA) proteins are the main targets of our antibody response to combat infections. HA and NA continuously change to escape from humoral immunity, a process known as antigenic drift. As a result of antigenic drift, the human influenza vaccine is updated frequently. The World Health Organization (WHO) coordinates a global influenza surveillance network that, by the hemagglutination inhibition (HI) assay, routinely characterizes the antigenic properties of circulating strains in order to select new seed viruses for such vaccine updates. To facilitate a quantitative interpretation and easy visualization of HI data, a new computational technique called "antigenic cartography" was developed. Since its development, antigenic cartography has been applied routinely to assist the WHO with influenza surveillance activities. Until recently, antigenic variation was not considered a serious issue with influenza vaccines for poultry. However, because of the diversification of the Asian H5N1 lineage since 1996 into multiple genetic clades and subclades, and because of the long-term use of poultry vaccines against H5 in some parts of the world, this issue needs to be re-addressed. The antigenic properties of panels of avian H5N1 viruses were characterized by HI assay, using mammalian or avian antisera, and analyzed using antigenic cartography methods. These analyses revealed antigenic differences between circulating H5N1 viruses and the H5 viruses used in poultry vaccines. Considerable antigenic variation was also observed within and between H5N1 clades. These observations have important implications for the efficacy and long-term use of poultry vaccines.

RESUMEN. *Nota de Investigación*—Uso de la cartografía antigénica en la selección de semillas para vacunas.

Los virus de la influenza humana tipo A son ejemplos clásicos de patógenos que desarrollan variación antigénica y que aparentemente tienen una capacidad ilimitada para evadir la respuesta inmune del hospedero. La proteínas virales hemagglutinina (HA) y neuraminidasa (NA) son los blancos principales de nuestro sistema inmune para combatir la infección. Estas dos proteínas están cambiando continuamente para evadir a la inmunidad humoral en un proceso conocido como desviación antigénica (antigenic drift). Como resultado de esta desviación antigénica, las vacunas contra la influenza humana se están actualizando frecuentemente. La Organización Mundial de la Salud (OMS) coordina una red global de vigilancia contra la influenza, que mediante la prueba de la inhibición de la hemagglutinación, está caracterizando rutinariamente las propiedades antigénicas de las cepas circulantes con la finalidad de seleccionar nuevas semillas y actualizar las vacunas. Para facilitar una interpretación cuantitativa y una fácil visualización de los datos de la prueba de la inhibición de la hemagglutinación, se ha desarrollado una nueva técnica computacional llamada "cartografía antigénica." Desde su desarrollo, la cartografía antigénica se ha aplicado de manera rutinaria para ayudar a la OMS con sus actividades de vigilancia contra la influenza. Hasta hace poco, la variación antigénica no se había considerado como un problema grave con las vacunas de la influenza aviar en la avicultura. Sin embargo, debido a la diversificación del linaje asiático H5N1 en varios clados genéticos y subclados detectada desde el año 1996 y debido a la utilización a largo plazo de las vacunas aviarias contra el virus H5 en algunas partes del mundo, esta cuestión debe ser analizada nuevamente. Se caracterizaron las propiedades antigénicas de diferentes paneles de virus aviarios H5N1 mediante el ensayo de inhibición de la hemagglutinación, utilizando antisueros de mamíferos o aviarios y se analizaron utilizando métodos de cartografía antigénica. Estos análisis revelaron diferencias antigénicas entre los virus H5N1 circulantes y los virus H5 empleados en las vacunas avícolas. Se observó una variación antigénica considerable de los virus H5N1 entre diferentes clados y entre miembros del mismo clado. Estas observaciones tienen importantes implicaciones para la eficacia y el uso a largo plazo de las vacunas en la avicultura.

Key words: influenza A virus, vaccine, HI antibodies, antigenic drift

Abbreviations: HA = hemagglutinin; HI = hemagglutination inhibition; NA = neuraminidase; WHO = World Health Organization

Annual influenza epidemics in humans affect 5–15% of the world population, causing an estimated half-million deaths worldwide per year (9) and an enormous disease burden and economic losses (5,11). Influenza viruses can infect this proportion of people every year because they have an extensive capacity to evolve and, thus, to escape from population immunity. This viral immune escape mechanism is known as antigenic drift (7,12). Vaccines are in use to protect people "at risk" for severe disease against influenza virus

infection. Due to antigenic drift, the influenza vaccine requires frequent updating; the H3N2 virus component of the influenza vaccine has been updated more than 20 times since the introduction of the virus in humans in 1968.

The World Health Organization (WHO) Global Influenza Surveillance Network tracks and analyzes the evolution and epidemiology of influenza viruses for the purpose of vaccine seed strain selection. The network currently consists of more than 120 national influenza centers in over 90 countries around the world. Each center collects samples of locally circulating viruses for analysis and for communication to one of the four WHO reference

^DCorresponding author. E-mail: r.fouchier@erasmusmc.nl

laboratories. Each virus is characterized antigenically using the hemagglutination inhibition (HI) assay (3). The HI assay is a binding assay based on the ability of hemagglutinin (HA) to agglutinate red blood cells and on the complimentary ability of antisera raised against the same or related strains to block this agglutination (3). Thus, an HI titer gives information about the affinity of an antiserum for a virus strain and can be used to compare the antigenic similarity of viruses.

ANTIGENIC CARTOGRAPHY

Antigenic cartography is a new computational technique which facilitates a quantification and visualization of virus phenotype data, much like phylogeny algorithms facilitate analysis of nucleotide sequence data (8). "Antigenic maps" are simple visualizations of HI data, in which distances represent antigenic similarities between viruses (Fig. 1). In an antigenic map, the distance between antiserum point S and antigen point A corresponds to the difference between the log-2 of the maximum titer observed for antiserum S against any antigen and the log-2 of the titer for antiserum S against antigen A. Each titer in an HI table can thus be thought of as specifying a target distance for the points in an antigenic map. Modified multidimensional scaling methods (8) are used to arrange the antigen and antiserum points in an antigenic map to best satisfy the target distances specified by the HI data. The result is a map in which the distance between points represents antigenic distance as measured by the HI assay. Because antisera are tested against multiple antigens, and antigens are tested against multiple antisera, many measurements can be used to determine the position of the antigen and antiserum points in an antigenic map. As a result, maps can be interpreted at increased resolution as compared to the raw HI data. Thus, these maps allow a quantitative interpretation of virus phenotype data at a high resolution and an easy visualization of HI tables.

In an initial publication, HI datasets collected by the Dutch National Influenza Centre were analyzed retrospectively (8). Antigenic cartography methods were used to merge numerous HI tables, and to quantify and visualize the antigenic evolution of influenza A (H3N2) virus from 1968 onwards, in antigenic maps. The maps revealed high-level features of the antigenic evolution of influenza A (H3N2) virus (Fig. 1). The strains tended to group in clusters rather than to form a continuous antigenic lineage, and the order of clusters in the map was mostly chronologic from the original Hong Kong 1968 cluster to the more recent Fujian 2002 cluster. Clusters were roughly similar in size and were similarly spaced. By using blind prediction tests, it was shown that the newly developed methods were, on average, accurate to 0.8 of a 2-fold dilution in HI assay titer on the Dutch data, substantially more accurate than was thought possible for HI datasets.

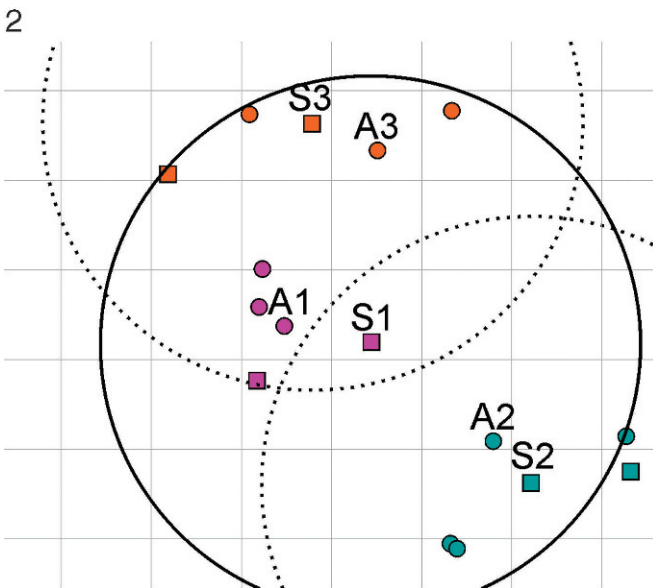
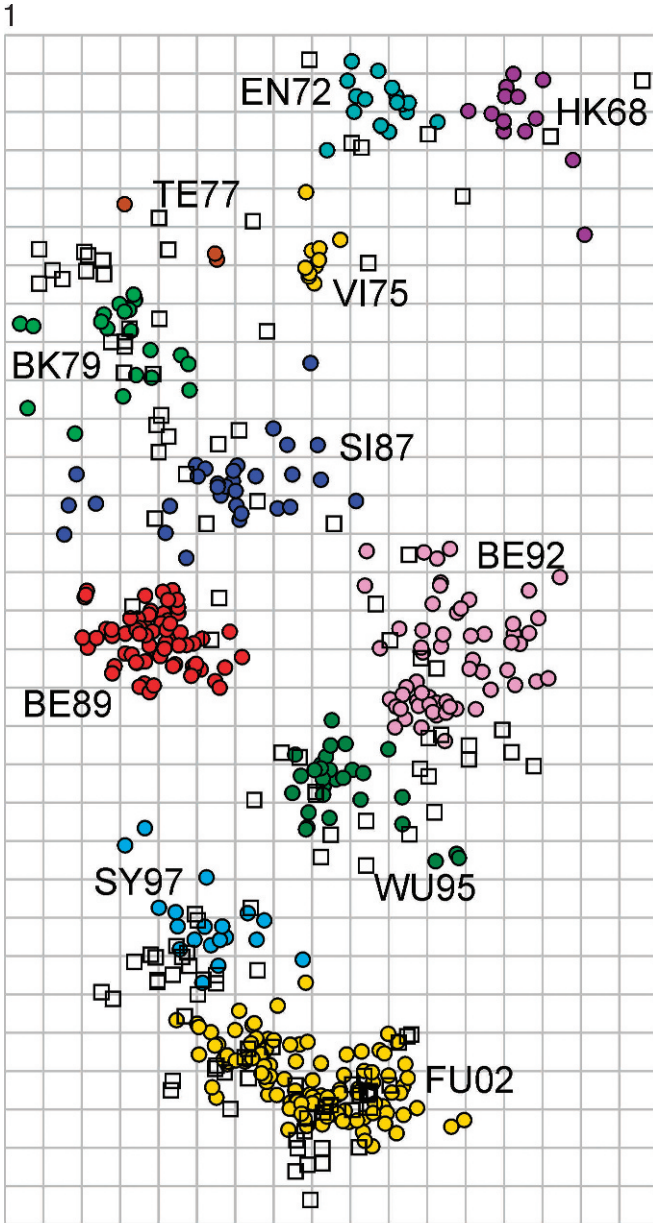
Since the initial publication, antigenic cartography has become a core component of the WHO surveillance activities. HI tables from the four WHO global reference laboratories are analyzed in real time to build maps that help in making decisions on possible changes in the influenza vaccine for the next season. Antigenic cartography is now also used for research and public health on other antigenically variable pathogens including influenza B virus, human influenza A (H1N1) virus, equine influenza (H3N8) virus, swine influenza (H3N2) virus (2), rabies virus, feline caliciviruses, HIV-1, Enterovirus 71, *Plasmodium* spp., and *Campylobacter jejuni*. The methods are generic and applicable to any type of binding assay; they have been applied successfully for virus neutralization data, ELISA data, and analyses of cytotoxic T-cell epitopes (1). More recently, antigenic cartography methods were used to study the global epidemiology of influenza A (H3N2) viruses (6).

ANTIGENIC CARTOGRAPHY AND AVIAN INFLUENZA

Vaccination of poultry is gaining acceptance as an option to assist in the control of avian influenza (10). Until recently, the development of poultry vaccines was straightforward, as vaccines based on any particular vaccine seed strain provided protective immunity to infection with virtually all other viruses of the same HA subtype (10). However, this view is changing, now that vaccines have been in use for longer time periods and on a larger scale. In Mexico, where large-scale vaccination has been used to control the LPAI and HPAI outbreaks that started in 1993, antigenic drift appears to have caused vaccine failure (4). Similarly, vaccination campaigns against HPAI H5N1 virus in China, Hong Kong SAR, Indonesia, and Egypt were quite effective initially, but failed to provide complete protection in poultry on the longer term, probably due to the emergence of antigenic drift virus variants (10). Thus, like in humans, it appears that poultry influenza vaccine seed strains should be reasonably matched, antigenically, to provide proper protection to the circulating field strains.

Because of the high variability of H5N1 influenza viruses in the eastern hemisphere, selecting the best vaccine candidates can be difficult. The primary criterion for the initial influenza vaccine seed strain selection is the antigenic data, which may be supported by serologic, genetic, and epidemiologic data. To provide ultimate proof of efficacy, the best vaccine seed strain(s) must be tested in vaccination-challenge experiments. Raw antigenic data are difficult to interpret, both within and between laboratories. Despite this difficulty, it is of great animal-health importance to quantify antigenic differences among avian influenza virus isolates in a reliable manner, both for vaccine seed strain selection purposes and for applied and basic research on virus evolution. Moreover, when vaccination strategies are employed to control avian influenza, a monitoring program should be implemented to detect the possible emergence of viruses with altered antigenic properties (antigenic drift). Because antigenic cartography methods have been useful in achieving these tasks for human influenza vaccine seed strain selection, use of these methods in the veterinary arena seems warranted.

Antigenic cartography methods have recently been used to study the antigenic relationships among animal influenza viruses isolated from a variety of hosts. For instance, the antigenic properties of representative sets of H5 viruses from around the world were tested in HI assays using panels of mammalian and avian antisera. Although HI assay protocols vary between different laboratories, variables such as different sources of red blood cells, different sources of virus isolates, and use of different species to generate antisera were found to have little effect on the antigenic maps. The maps generated with this data revealed a clustering of H5 strains, as previously seen for human and swine H3N2 strains (2,8). H5 influenza viruses from HPAI outbreaks prior to 1996, and LPAI H5 viruses from wild birds, formed a relatively small antigenic cluster, despite the large geographic and temporal variation of the strains in this cluster, which were isolated between 1959 and 2002 in Europe, Asia, and North America. In contrast, the HPAI H5N1 viruses that emerged in Southeast Asia after 1996 were antigenically distinct and formed several antigenic clusters representing strains from the different clades that are circulating on the eastern hemisphere (14); the antigenic differences between clades were at least as large as those observed for drift variants of seasonal human influenza virus associated with vaccine updates, more than a 4-fold difference in the HI assay (8). In agreement with the large antigenic differences between circulating H5N1 strains, the WHO network has generated numerous reassortant vaccine seed viruses for potential future use as



H5N1 vaccines in humans (13). Based on this information, it would seem logical that the antigenic differences between circulating H5N1 strains also need to be taken into account when vaccination is considered as a strategy to control avian influenza in avian species.

Beyond the identification of viruses with particular antigenic properties, e.g., to select the most appropriate vaccine seed strain(s), antigenic cartography may assist in the initial analyses of serologic responses of (vaccinated) animals. As indicated above, antigenic maps are based on the reaction of antisera with antigens, and the positions of antisera in the maps can be equally informative to the position of the antigens. When evaluating (postvaccination) antisera, at least three critical parameters need to be considered as surrogate markers for the potency of the vaccine: 1) the magnitude of the antibody response, 2) the focus of the antibody response, and 3) the breadth of the antibody response. Antigenic maps are not needed to evaluate the magnitude of the antibody response, as the maximum (homologous or heterologous) antibody titers are obtained directly from the serologic test of choice, e.g., the HI assay. For evaluation of focus and breadth, however, antigenic cartography methods facilitate the quantitative assessment and visualization of the antigenic data. This is illustrated in the exemplary map of Fig. 2. In this map, the “focus” of antiserum S1 is clearly better than for antisera S2 and S3. While antiserum S2 may have a high titer to virus A2 and its relatives, it will have relatively low responsiveness to A3-like viruses and, *vice versa*, serum S3 will react relatively poorly with A2-like viruses, although its titers to A3 may be normal. In contrast, antiserum S1, due to its central position in the map, has reasonable reactivity with A1, A2, and A3-like viruses. Thus, the focus of antiserum S1 is superior to that of S2 and S3, given the variation of the viruses analyzed. The position of this antiserum in the map

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Fig. 1. Antigenic map for influenza A (H3N2) virus from 1968 to 2008. The relative positions of strains (colored circles) and antisera (open squares) were adjusted such that the distances between strains and antisera in the map represent the corresponding HI measurements with the least error. The distances between antigens and antisera are inversely related to the HI titers. Strain color represents the antigenic cluster to which the strain belongs. Clusters were identified by a k-means clustering algorithm and named after the first vaccine seed strain in the cluster; two letters refer to the location of isolation (Hong Kong, England, Victoria, Texas, Bangkok, Sichuan, Beijing, Wuhan, Sydney, and Fujian) and two digits refer to year of isolation. The vertical and horizontal axes both represent antigenic distance and, because only the relative positions of antigens and antisera can be determined, the orientation of the map within these axes is free. The spacing between grid lines is one unit of antigenic distance, corresponding to a 2-fold dilution of antiserum in the HI assay. Two units correspond to 4-fold dilution, three units to 8-fold dilution, and so forth.

Fig. 2. Antigenic cartography to evaluate postvaccination antisera. For this hypothetical antigenic map, three clusters of viruses were evaluated. Antisera (colored squares; S1, S2, S3) were raised against different viruses (colored circles; A1, A2, A3) and tested in HI or a virus neutralization assay. The position of antiserum S1 in the map indicates that it is more cross-reactive than are antisera S2 and S3. The circle in the map indicates the position in the map where titers with a given antiserum drop off below 40; given a maximum titer of 320, the threshold value of 40 is reached when antigens are three units away from the antiserum. The favorable focus and breadth of reactivity of antiserum S1 is shown as a solid circle, and the less-favorable focus and breadth for antisera S2 and S3 are shown as dotted circles. The spacing between grid lines is one unit of antigenic distance, corresponding to a 2-fold dilution of antiserum in the HI assay.

predicts that it will react most favorably with the viruses under investigation, provided that the maximum titers of the sera are in the same range. When antibody responses are measured as a surrogate marker for vaccine immunogenicity or potency, predefined cut-off antibody levels are often used, e.g., titers >40 are considered to be protective. For any antiserum in antigenic maps, such cut-off levels can be easily visualized, particularly in light of the reactivity with each of the antigenic variants of influenza virus in the map. The “breadth” of the antibody response of antiserum S1 can be drawn in the map of Fig. 2 as a circle. The circle drawn for antiserum S1 indicates that this antiserum will have titers greater than or equal to the predefined threshold level of 40 against all the strains in the map. In contrast, the circles drawn for antisera S2 and S3 do not cover all viruses in the map, indicating that antibody titers to some of the viruses are too low. While information on both the focus and breadth of antisera might be available from the raw serologic data to the expert eye, antigenic cartography methods increase the resolution of the quantitative interpretation of the data, allow prediction of unmeasured titers, and provide a visualization that is easy to use.

Antigenic variation of avian influenza viruses represents a new challenge for the development and application of poultry vaccines. It has become clear that different vaccine seed strains may result in variable vaccine efficacy against infection with different viruses of the same subtype. The most optimal vaccine seed strain may even vary for different vaccination campaigns, as the antigenic properties of the circulating field strains have been shown to vary temporally, geographically, or even by host species and poultry production sector. Moreover, since vaccination generally represents only one strategy out of the many measures employed to prevent, manage, or eradicate avian influenza, appropriate monitoring programs for the effectiveness of the vaccine, and for the potential emergence of antigenic drift variants, need to be implemented.

Although dealing with the antigenic variation of avian influenza appears to be a serious challenge, a blueprint for a successful action plan is already available. The WHO-coordinated global influenza surveillance network has been effective in tracking the antigenic evolution of human influenza viruses for many years and has generated appropriate vaccine seed strains, when needed. In this network, national reference laboratories assemble virus collections for antigenic characterization from a variety of sources of virus isolates in the country. Strains of interest are shared with global reference laboratories to ensure a thorough, coordinated, and integrated analysis of the antigenic properties of the strains that circulate around the world. New vaccine seed strains are then generated by the network as the need emerges. For the avian influenza field, a similar strategy may be envisaged. Such a coordinated network would likely be more successful, efficient, and cost-effective than is an *ad hoc* or local monitoring program. This is particularly important as we rely on high quality serologic data for antigenic characterization and as we demand ultimate proof of effectiveness of vaccine seed strains in expensive and time-consuming vaccination-challenge experiments in poultry. As a welcome side effect, such a coordinated global avian influenza surveillance network would facilitate more close interactions—and integration—with the WHO-coordinated human influenza surveillance network, fitting in beautifully with the one world, one health initiative.

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