MVP Laboratories, Inc.

Flu News

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Influenza Vaccines

In a group of immune competent pigs, most influenza infections are mild and recovery usually occurs within 7-10 days after infection. However, there can be sustained effects of the virus within the population. During infection, the pigs can experience high fevers, anorexia, coughing, difficult breathing, nasal discharge and poor weight gain. Particularly if there are secondary infections that delay recovery, these clinical signs can linger and have a significant effect on weight gain and feed efficiency.

Vaccines can be very effective at reducing economic losses and are usually part of the comprehensive plan to decrease influenza infection in a herd. If you are going to use a vaccine, it is important to understand how they provide protection.

Influenza vaccines are made using inactivated or "killed" viruses. Inactivated virus vaccines only elicit a response from the antibody producing part of the immune system *(humoral immunity)* and the protein or "antigen" that most of this immune response is directed toward is the hemagglutinin protein on the surface of the virus.

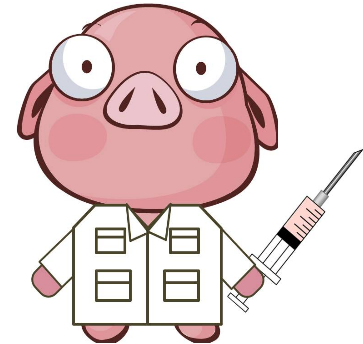
Infection with influenza A viruses is initiated by the binding of the hemagglutinin (HA) protein on the surface of the virus to sialic acid sugars on the surface of epithelial cells (Figure 1A).

The anti-HA antibodies created by the *humoral* immune system in response to vaccination bind to the HA and prevent the virus from binding to the sialic acid sugars on the surface of epithelial cells. The virus is then blocked from binding the sialic acid sugar and the virus is then unable to infect the cell (Figure 1B).

Ideally, vaccination will result in an antibody that will be a perfect match to the virus and provide complete protection, but influenza viruses are expert at evading the humoral immune response by changing their surface proteins. Even slight changes in the three-dimensional protein shape of hemagglutinin can result in less binding or even no binding at all (Figure 1C).

This antigen-antibody mismatch can mean partial protection or little to no protection, depending on how cross-reactive the antibodies are to other viruses. This is why a scientific approach to vaccine selection includes genetic and antigenic evaluations of the current strain of virus in the herd, as well as other viruses isolated from the herd.

The rapid turnover of the population in a swine herd creates a situation where young, susceptible animals are continually introduced to the population and influenza viruses can persist. The genetic and antigenic history of the viruses from a herd can provide insights into the evolution of the virus within the herd and aid in vaccine selection.



Did you get your flu vaccine?

Vaccination is the first step towards protecting yourself and your family.

The current flu vaccine contains both seasonal influenza and the 2009 pandemic strains.

More information is available at: www.cdc.gov/h1n1flu

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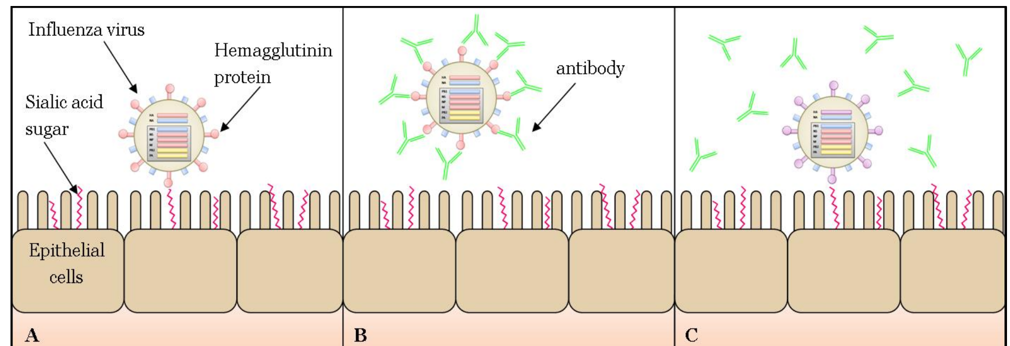

Figure 1. Diagrams of influenza virus showing: (A) binding to sialic acid sugar on the surface of an epithelial cell, (B) antibody binding preventing binding to sialic acid sugar, and (C) a different hemagglutinin protein on the virus (not drawn to scale).

Figure 1. (A) The roller culture bottles have had culture media added to them under sterile conditions. (B) An example of a culture flask (www.sigmaaldrich.com).

Figure 2. Roller culture bottles rotating inside the incubator shelves.

Figure 3. Checking the trypsinization of the cells.

Figure 4. Examining the cells using an inverted microscope for lysis or cytopathic effects (CPE) to determine if the influenza virus has grown.

The making of an influenza virus vaccine

The most common influenza A virus subtypes found in North American pigs are H1N1, H1N2 and H3N2. Among the H3N2 subtype there are four clusters (I, II, III, IV) with limited cross-reactivity between the clusters. However, there is good cross-reactivity within the clusters and cluster IV is currently dominant among the strains in circulation.

Among the SwH1 subtypes, it has been shown that there is cross-reactivity between the classic swine viruses (SwH1α) and the reassortant swine viruses (SwH1β and SwH1γ), but there is limited to no cross-reactivity between these viruses and the human-like swine viruses (SwH1δ)[4]. Additionally, it was found that the human-like swine viruses have two distinct clusters that have limited to no cross-reactivity between them (SwH1δ_1 and SwH1δ_2)[5]. What all of this boils down to is that a broad spectrum vaccine would have to include: an H3N2 virus (likely cluster IV), a SwH1 (α, β or γ), a SwH1δ_1 and a SwH1δ_2.

For a commercial vaccine, a mixture of 2-4 viruses has been selected to represent the viruses currently circulating in North America. These viruses are selected based on their ability to produce antibodies that cross-react with many viruses and provide a broad spectrum of protection.

Autogenous vaccines are herd specific, using viruses that were isolated within the last two years from the herd that the vaccine will be used on. Within two years, these strains are replaced by more recent isolates as they become available from the field. Therefore, these viruses represent what is currently circulating in the herd.

Autogenous vaccines can contain anywhere from 1 to 5 viruses, but they typically have 2-4 viruses and a mixture of subtypes. If historical sequencing data is available for viruses isolated from the farm, phylogenetic analysis can aid the selection process by looking at the evolution of the viruses from that herd.

Once the virus or viruses are selected for an autogenous vaccine, they are grown in large volumes. For swine influenza viruses, Madin Darby canine kidney (MDCK) cells have been shown to have the highest sensitivity of the available cells lines and are most commonly used to grow virus[3].

Before the virus can be grown, the MDCK cells have to be prepared. The MDCK cells are grown in plastic culture flasks or bottles forming a thin, confluent layer of cells that adhere to the walls of the bottle. Roller bottles (Figure 1A) are round and provide more surface area for the cells to adhere without taking up as much space as the culture flasks (Figure 1B).

The cells are incubated at 37°C (body temperature) with a small amount of nutrient rich liquid (culture media) on shelves that continuously rotate the bottles (Figure 2). Each time the cells are regrown from a previous growth it is referred to as a “passage” and the virus has to be grown on cells that are 20 passages or less from the USDA-approved master cell stock.

This restriction can make growing the virus difficult since some swine influenza viruses grow better on MDCK cells that have been passaged over a hundred times. If the isolate fails to grow well, meaning the final concentration of virus grown is too low to make a vaccine, then it may be necessary to collect another sample or select a different virus from the farm.

Once the cells have grown for a couple of days into a confluent monolayer, they are ready to infect with virus. Trypsin is added to improve the uptake of the virus by the cells (Figure 3). Then, a sample of the original viral isolate is added to the bottle containing the cells. The cultures are incubated at the optimal temperature for replication (35°C for swine influenza virus) and are checked daily for 2-3 days until the virus has grown.

Cell cultures are examined using an inverted microscope that looks through the bottom of the container (Figure 4). As an influenza virus grows, it causes lysis of the cells or cytopathic effects (CPE). When most of the cells have lysed (ruptured), the virus is ready to harvest.

The virus is harvested from the cell culture by pouring off the liquid media leaving the cellular debris material at the bottom of the container. The fluid that is removed from the bottle (supernatant) contains the virus, while the cellular debris is left behind.

Next the virus concentration is determined. This is done using a hemagglutination (HA) titer utilizing the natural ability of influenza viruses to agglutinate turkey or chicken red blood cells (RBCs). A small sample of the virus culture is diluted 2-fold several times in a series and is mixed with RBCs. The titer is determined as the highest dilution that agglutinates the RBCs and the HA units are calculated from the titer (Figure 5). Ideally, there are at least 250 HA units per isolate per dose of vaccine.

Once the virus is grown, it needs to be inactivated or "killed" so that it is no longer infectious. All of the commercial and autogenous influenza vaccines for pigs currently approved for use by the USDA contain inactivated influenza viruses.

Heat inactivation of the viruses doesn't work very well because it causes significant changes in the shape of the antigenic proteins (epitopes), which will reduce immunogenicity. Viruses can also be inactivated using ultraviolet radiation, but this technique has additional safety requirements and requires special facilities. Therefore, viruses are usually inactivated using chemicals.

While influenza viruses can be inactivated with formaldehyde for use in vaccines, it has been found that aziridine compounds like binary ethyleneimine (BEI) are superior for safety and antigenicity reasons. The published inactivation rates are consistent throughout the literature and the end-point is easily calculated, producing consistently inactivated and safe results. Changes in the proteins during the inactivation process can result in decreased antigenicity. The antigenic superiority of BEI has been reported in the literature[1]. To confirm inactivation, each vaccine batch (called a serial) that has been inactivated must pass an inactivation assurance test on cell culture prior to being released for use in a vaccine.

Since the virus is inactivated, something is needed to boost the immune response to the viral antigens. The viruses in "live" and in "attenuated" vaccines can still replicate within the host. This offers the immune system a prolonged exposure to the virus to produce both *innate* (cellular) and *humoral* immune responses. On the other hand, inactivated virus vaccines do not replicate in the host and thus produce mostly a *humoral* immune response to the hemagglutinin protein. This limited antibody response is why a second or "booster" dose is required to enhance the immune response to a protective titer.

Therefore, adjuvants are added to the vaccines to enhance the immune response to the virus. These are usually oil-based or oil-in-water emulsions that are specifically developed by vaccine companies in order to extend the immune response with minimal tissue damage. The adjuvant formulations vary among manufacturers and are usually considered to be proprietary information.

In a recent study, the adjuvant EMULSIGEN®-D (Figure 6), an oil-in-water emulsion containing dimethyldioctadecylammonium bromide, mixed with freeze-dried swine influenza vaccine viruses was found to be highly effective at producing detectable antibodies[2].

After the adjuvant is selected and the virus or viruses are inactivated, they are mixed together. Low doses of antibiotics, such as amphotericin B and gentamicin, can be added to preserve the finished product. Bottles of the finished product are then filled under sterile conditions (Figure 7A) and labeled (Figure 7B). At this point the vaccine serial must pass a 14 day sterility test as per 9 CFR113.26 before it is released for use.

Once the customer receives the product and vaccinates animals according to their veterinarian's instructions, it is a good idea to monitor the efficacy of the vaccine being used.

Autogenous vaccines are only made for use in the herd of origin or by herds that have an epidemiological link specific to the influenza virus in the herd of origin.

These serials are not tested for efficacy at the time of manufacture. Vaccine efficacy is usually best determined by vaccine-challenge experiments. Since this option is expensive and requires special facilities, the hemagglutination inhibition (HI assay) titer can be used to look for antibodies in a vaccinated pig against the virus isolated from a farm. Although the presence of antibodies does not guarantee protection in every pig, a titer of 1:40 or greater is considered to be protective.

Also, if the vaccine strategy depends on passive transfer of antibodies from dam to piglets, it is a good idea to periodically check the piglets to verify that there was not a failure of passive transfer.

Another method used to monitor the effectiveness the vaccine within the herd is to collect routine samples, such as nasal swabs pigs with respiratory signs and post-mortem tissues, such as lung, from pigs that die from respiratory disease. Viruses isolated during bouts of infection in vaccinated animals can be serologically and genetically compared to the viruses in the vaccine.

Molecular characterization of these outbreak viruses combined with the production data on the farm can provide a benchmark for evaluating the efficacy of the vaccine program. Regular assessments of the herd disease status and vaccination strategy over time should lead to the development of a robust influenza control program and help reduce the costs associated with influenza infection.

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1. Bahnemann HG. *Vaccine* 8: 299-303.
2. Lin BC, et al. *Proc. AASV* 221-228.
3. Regula G, et al. *JAVMA* 217:888-895 (2000).
4. Vincent AL, et al. (2006).
5. Vincent AL, et al. *J Gen Virol* Epub (2010).

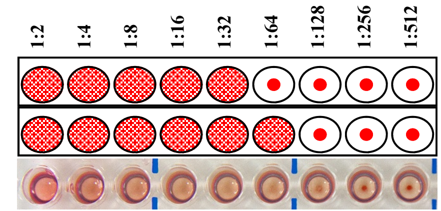

Figure 5. (A,B) Diagram and (C) photograph of a hemagglutination (HA) reaction of RBCs with an influenza virus. The titers would be read out as: A=1:32, B=1:64 and D=1:64-128.

Figure 6. Bulk container of oil-in-water adjuvant (EMULSIGEN®-D).

Figure 7. (A) A bottle being filled with an autogenous vaccine. (B) Finished vaccine product.

Photographs for this issue provided courtesy of:

Jack McGonigle

About the Author:

Dr. Susan Detmer is currently working with the Minnesota Center for Excellence for Influenza Research & Surveillance (MCEIRS) under her research advisors, Drs. Marie Gramer and Sagar Goyal. Her field of research is in control and characterization of swine influenza viruses, in particular the molecular epidemiology of swine influenza viruses.

In the next issue:

Interspecies transmission of influenza A viruses

Update on influenza A viruses circulating in North American pigs

The pandemic virus (pH1N1) was first reported in the human population in April, 2009 and was found in Canadian pigs shortly thereafter. During the pandemic, the first appearance of pH1N1 in American pigs was at the Minnesota State Fair in August 2009. Since then, pH1N1 has become the dominant strain of influenza circulating in American pigs. As of August 10, 2010, we have been in what is called the post-pandemic period and we continue to monitor pH1N1 for changes.

During the year before pH1N1 was found in American pigs (August 2008 to August 2009), the viruses currently circulating in North American pigs were: 60% H1N1, 13% H1N2 and 27% H3N2. During the pandemic period (August 2009 to August 2010) there was a noticeable drop in H3N2 viruses to 16% while H1N2 increased to 24% and H1N1 remained at 60%. During the post-pandemic period (August 2010 to present) the distribution of subtypes was: 37% H1N1, ***40% H1N2*** and 22% H3N2 (Figure 1*).

During this same time period, there was a dramatic shift in distribution within the H1 subtypes where the classic SwH1 alpha and reassortant beta viruses decreased: 5% to 1% to 0% and 36% to 6% to 4%, while the delta 2 (H1N1 subset related to human seasonal flu) viruses remained stable: 10% to 14% to 9%. The delta 1 (H1N2 subset related to human seasonal flu) expanded from 13% to 21% to ***41%*** during the same periods. The gamma viruses were the most frequently found in all 3 time periods: 37%, 57% and 46%. One gamma virus in particular, pH1N1, comprised 29% of all SwH1 viruses during the pandemic and 19% of all post-pandemic SwH1 viruses (Figure 2†).

*Based on unpublished data of positive influenza A RT-PCR tests where the subtype was definitively determined at UMVDL.
†Based on unpublished sequence data of hemagglutinin (HA) gene at UMVDL.

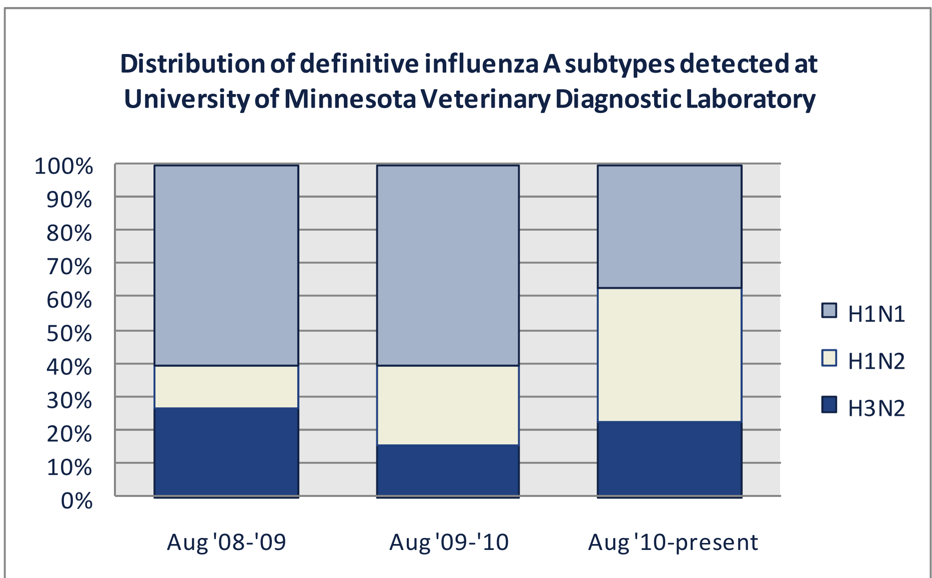

Figure 1. A graph of the distribution of influenza A viruses detected in swine samples at the University of Minnesota Veterinary Diagnostic Laboratory that were definitively subtyped*.

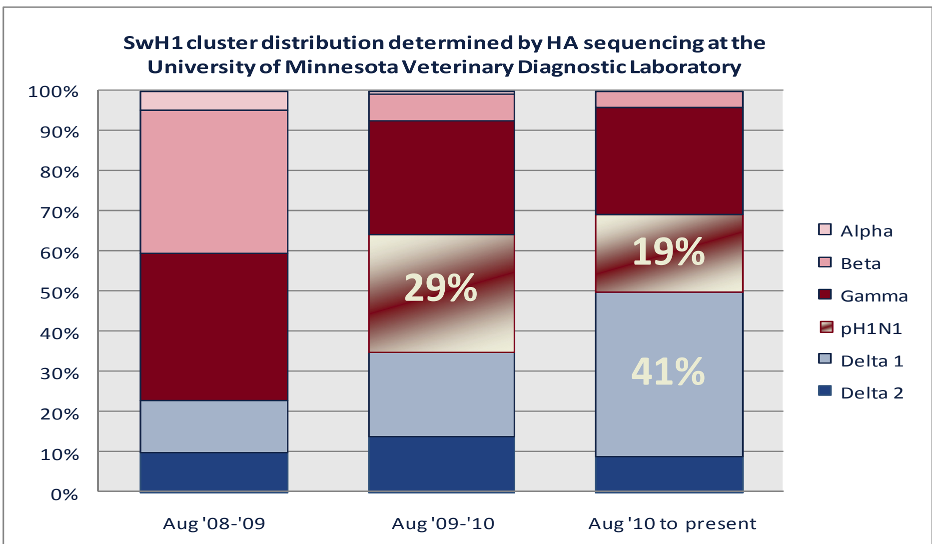

Figure 2. A graph showing the distribution of SwH1 clusters for viruses that had hemagglutinin gene sequencing performed at the University of Minnesota Veterinary Diagnostic Laboratory†.

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