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The development of avian influenza vaccines for emergency use

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Abstract

Costly outbreaks of mildly and highly pathogenic avian influenza (AI) have occurred in the commercial poultry industry in Europe and the United States in the past two years. The current approach is to control the disease by depopulation of infected flocks followed by cleaning and disinfection of the premises. The cost of eradication of influenza and the payments to the poultry producer continue to increase. The cost of the AI eradication in the Netherlands and the United States was more than 500 million USD. The use of vaccines to control AI is gaining acceptance by veterinary health agencies as a tool in eradication programmes. The choice of vaccines available includes purified subunit vaccines, genetically modified vaccines and the traditional whole-virus inactivated vaccines. The use of inactivated vaccines has been used successfully in many countries to stop the spread of avian influenza in the poultry industry. The fowlpox-vectored vaccine TROVAC AI H5™ has been used to vaccinate broiler chickens in Mexico for five years. The preparation of a supply of vaccine in advance of a disease outbreak has been used in the human health sector. A vaccine bank was created at Merial for foot-and-mouth disease more than 10 years ago. The idea of developing a vaccine bank for avian influenza is being discussed in the United States and in the European Union. Before a strategic plan for AI vaccines can be implemented, many questions about the AI strains needed, the amount of vaccine, the formulation, the priority of vaccination in the poultry industry and the cost to produce and maintain stored antigens or vaccines need to be addressed.
Keywords: avian influenza; vector vaccine; fowlpox; antigen or vaccine bank; TROVAC AI H5™; LPAI; HPAI

Introduction

For more than 30 years inactivated whole-virus avian influenza vaccines have been the only product available to control the spread of the disease from infected to susceptible birds. However, due to an international agreement, animal-health regulatory agencies relied on the destruction and removal of infected birds from susceptible ones. The number of outbreaks in the poultry-producing countries of the world has increased during the last 10-15 years. The United States had low-

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pathogenic AI infections in commercial layer complexes in the states of Connecticut and Texas. Turkeys and chickens were infected in the outbreak of low-pathogenic AI type H7 in the state of Virginia. In Europe the disease of both low and high pathotypes affected the commercial poultry industry of The Netherlands and Italy. The cost of eradicating the infected flocks runs in the millions of dollars. The magnitude of the outbreaks has increased over the years due to the increasing concentration of poultry production around the world.

Inactivated whole-virus AI vaccines

The role of and a justification for the use of inactivated AI vaccines for the control of mildly and highly pathogenic avian influenza has been described (Halvorson 2002). The state of Minnesota has used monovalent inactivated vaccines of several haemagglutinin subtypes in the turkey industry to avoid the destruction of infected flocks. An inactivated oil-emulsion vaccine was used to immunize 4 million layers in the state of Connecticut this year. Hong Kong started an experimental programme of vaccination using an inactivated H5 vaccine produced in Mexico. The experience with the use of inactivated H7 AI vaccine in Italy will be presented in this volume.

Inactivated AI vaccines are relatively simple to prepare and provide high levels of immunity when properly manufactured and administered. Merial produces five different inactivated AI vaccines (Table 1). Most of the AI vaccines have been requested by governments in the Middle East for endemic situations in the poultry industry. Merial has manufactured an inactivated H7N1 oil-emulsion vaccine for use in Italy.

One disadvantage of inactivated vaccines is that every bird must be inoculated individually using a syringe. The vaccination of birds at the farm is difficult and stressful on the birds. The time required to inject each bird depends on the age of the bird being vaccinated, the volume and viscosity of the vaccine and the number of people that are available to catch and vaccinate the flock. The cost of labour and the threat of spreading the disease with vaccination crews is a major concern. Attempts at replacing whole-virus vaccines with genetically modified vaccines have not occurred as quickly as expected.

Table 1. Inactivated AI vaccines

Inactivated AI vaccine name	Avian influenza type
GALLIMUNE FLU™	H7N1
BIOFLU/BIOENFLU	H6N2 & H9N2
FLUVAC	H7N3
GALLIMUNE FLU™	H9N2
GALLIMUNE 208™	H9N2 + Newcastle

New technologies for vaccines

Biotechnology has given us alternatives to the whole-virus vaccine and the potential for developing quantities of antigen or finished vaccines that can be stored for use in the event of a future outbreak. The feasibility of several different types of genetically engineered avian influenza vaccines has been demonstrated. However, only the fowlpox-virus-vectored vaccine has been used continuously in an AI-control effort.

Protein Sciences, Inc. developed a subunit vaccine of recombinant HA glycoprotein utilizing a baculovirus–insect cell expression system (Wilkinson 1997). The experimental vaccine with an adjuvant provided a good serological response and

protected vaccinated SPF chickens against challenge with a lethal strain of HPAI. The rHA without the addition of an adjuvant provided only partial protection. The subunit vaccine also requires individual injection of each bird. So far this approach has not been commercialized for veterinary use.

Kodihalli and Webster (1997) reported on the efficacy of DNA vaccines delivered with a gene gun at the Fourth International Symposium on Avian Influenza. Delivery of a single injection of DNA encoding the influenza HA provided immunity for the life of the chicken. The method for DNA injection depends on the use of the gold beads to carry the antigen into the tissue of the animal. The cost of the sophisticated injection system and the precision required to inject a chicken properly with DNA has prevented the mass application of the technology in the poultry industry.

The feasibility of using fowlpox as a vector to express the HA gene of AI was described in 1988 by researchers at Virogenetics and the New York Department of Health. The haemagglutinin gene from subtype H5 was successfully expressed in fowlpox (Taylor et al. 1988). The efficacy of a recombinant fowlpox–AI vaccine against a virulent H5N2 virus was decribed by Beard, Schnitzlein and Tripathy (1991).

Merial began registration of the GMO vaccine TROVAC AI H5 after a request from the US poultry industry in 1995. There was concern that the highly pathogenic AI H5 virus circulating in Mexico would enter the US. The vaccine uses the fowlpox virus from Merial's product named Diftosec, which has been used in Europe for many years. The pox virus was further modified with specific deletions in the genome to ensure safety and genetic stability. The HA gene from A/Turkey/Ireland/83 strain was inserted into a non-essential location in the pox-virus genome resulting in a virus that protect poultry from two infectious diseases, fowlpox and AI.

The vaccine provides excellent protection against a wide range of highly pathogenic subtype-H5 viruses (Swayne et al. 2000). It stimulated an antibody titre faster than inactivated oil-emulsion vaccines and it prevented mortality after exposure to lethal AI viruses for a minimum of 20 weeks. The GMO vaccine was designed to have a differential diagnostic test. The pox vector expresses only one gene, HA. When serum samples from vaccinated chickens are tested using the agar-gel precipitin test or with a commercial ELISA kit the samples will be negative compared to the positive control sample or infected birds. It is the nucleoprotein that is responsible for the positive reaction measured by the ELISA or AGP. The AGP is an inexpensive and simple test to use, however the results may require 24 to 48 hours to confirm. It has been reported that the pox-vectored vaccine was unable to stimulate measurable HI titres.

A recent experiment showed the critical importance of the choice of antigen used in the AI HI test. When the standard A/Turkey/Wisconsin/68 antigen was used in the HI test most of the serum sample titres were measured as 0 or low. However, when the homologous A/Turkey/Ireland/83 was substituted the measurable antibody titres increased. Chickens vaccinated subcutaneously at the day of hatch maintained a HI titre > 1:100 nine weeks after injection.

The TROVAC AI H5™ vaccine can be administered to birds at one-day age or older by wing-web stab or subcutaneous injection. The majority of the doses have been administered in the hatchery using automated injection machines vaccinating chicks with Marek's vaccine. There is a misconception that maternal antibody against the pox vector interferes with the development of immunity. In Mexico the vaccination programme for broiler breeders includes two vaccinations with live fowlpox vaccine. Millions of the progeny are successfully vaccinated every week with

TROVAC AI H5™. More than 700 million doses of fowlpox vectored AI H5 vaccine have been sold in Mexico since registration in 1998.

Previous studies indicated that TROVAC AI H5™ would not provide a consistent immunity when birds were previously vaccinated with live fowlpox vaccine or exposed to wildtype pox vaccine (Swayne, Beck and Kinney 2000). TROVAC AI H5 ™ did not prevent morbidity or provide the necessary 90% efficacy required for licensing when the birds were first immunized with fowlpox vaccine. The addition of an adjuvant may overcome this problem. SPF chickens vaccinated at one day of age with a live fowlpox vaccine and vaccinated twice with TROVAC AI H5™ vaccine and an adjuvant protected 85% of the birds against morbidity and mortality after a challenge with a highly pathogenic AI virus. Although this vaccination programme would not be feasible for broiler chickens it may be possible to design a vaccination programme using two injections of TROVAC AI H5™ for replacement pullets.

Merial has tried to develop a genetically engineered fowlpox-vectored virus containing an H7 gene. A construct identified as vFP1549 has been available for several years. The registration and development programme was delayed because of lack of interest in allowing GMO vaccines as part of an AI-control policy. The efficacy of the construct was proven using a highly pathogenic virus A/Chk/Pakistan/1369-CR2/95.

When the first outbreak of H7N1 was reported in Italy the efficacy of the construct vFP1549 was evaluated using the Italian virus. It was surprising to learn that the fowlpox-vectored AI vaccine failed to protect against the Italian virus A/Turkey/Italy/4580/99 (H7N1). An experiment was done to determine if the route of inoculation or the concentration of the construct would have an effect on the efficacy of the vaccine. The efficacy was slightly better when the chicks were inoculated by subcutaneous injection. However, at concentrations of $10^{2.5}$ $TCID_{50}$ or less the construct did not provide satisfactory immunity.

AI vaccine bank

The Southeastern Poultry and Egg Association formed a working group in 1995 to examine some major questions and develop an action plan for the occurrence of highly pathogenic AI in the United States. One of the recommendations from the task force was to allow for the manufacture of inactivated H5 Al vaccine by qualified vaccine manufacturers. The task force recommended that a financial plan be worked out to pay for the preparation of virus-laden allantoic fluids for storage if an emergency need for AI vaccine occurred. The concept of an antigen bank for avian-influenza vaccines in the United States never became a reality. As the threat of highly pathogenic influenza diminished the necessity for the preparation of a vaccine for emergency use disappeared, too.

The US Department of Agriculture/APHIS, the state veterinarians in poultry-producing states and representatives of the various sectors of the poultry industry began serious discussions concerning the control of both low- and highly pathogenic AI in 2002. The talks began after the outbreak of low-pathogenic AI H7 in the Shenandoah Valley of Virginia. A draft of a series of guidelines was discussed and a resolution for the use of AI vaccines was sent to APHIS by the Transmissible Diseases of Poultry committee at the US Animal Health Association meeting in St. Louis, Missouri in October of 2002. The guidelines included the possibility of using vaccines in conjunction with surveillance and a stamping out to control LPAI and HPAI.

The animal-health community around the world has made provisions for a vaccine bank for foot-and-mouth disease. The concepts that were successfully implemented could be used as a starting point for the creation of an AI bank. There are many factors that must be considered before an action plan can be developed.

Concerns of the biologics industry

The biologics manufacturers will need to accumulate a lot of information to prepare the proper antigens in the bank adequately. Which serotypes are required for storage? The serotypes H5 and H7 must be included because of the reoccurring outbreaks of low- and highly pathogenic viruses. Will the viruses held by the biologics companies be efficacious against the field strain? Some countries will insist that only homologous vaccines be prepared. Who will provide the actual virus for use in the antigen bank?

What type of vaccine should be prepared?

Inactivated oil-emulsion vaccines certainly seem necessary for breeders and layers that are placed in the field. The vectored vaccine could be used to vaccinate susceptible chicks that will continue to be placed in the country. What about the addition of other antigens? In Mexico the vaccine of choice was an inactivated Newcastle Disease-AI vaccine.

Which strains should be included in the vaccine bank?

The bank should include strains of H5 and H7 that are known to cause highly pathogenic avian influenza. Should the bank include MPAI strains? Which neuraminidase strains are needed? When avian influenza is manufactured for a specific country the vaccine is prepared using the strain provided. Would all countries accept a strain of virus with the same serotype even though it may not be the same isolate? Does the poultry industry need vaccines for subtypes H6 and H9?

What type of poultry will be vaccinated?

The target birds will have to be identified. Will the priority of vaccination be given to breeders and layers first? Then vaccine produced for chickens may not be as efficacious for turkeys. Will the formulation for commercial poultry be efficacious to exotic birds confined in zoos? How effective are the inactivated vaccines in ducks or waterfowl? These are important questions that will have to be addressed.

Formulation

Inactivated vaccines manufactured by different companies will have different formulations. The concentration of antigen, adjuvant, emulsion, raw materials will all vary from one source to another. The antigenicity and immunogenicity will vary from one virus to another. When preparing the H7N3 vaccine for Italy it was necessary to conduct nine animal vaccination tests to determine the proper volume of antigen, concentration and volume of the dose necessary for maximum efficacy. The proper formulation is essential if efficacy is going to guaranteed to the to reduce the spread of the disease. If multiple suppliers are going to deposit vaccines in the bank these must be similar enough to use any vaccine without altering the vaccination strategy.

Regulatory requirements

Regulatory-harmonization discussions for animal biologicals between the EU and the US have been going on for several years. Some progress has been made toward

mutual acceptance of products that can be classified as generic vaccines. However, acceptance of a vaccine for a potential zoonotic disease like AI will probably take much longer to resolve. Regulatory harmonization of all the requirements for the production of AI vaccines will be a very important factor. Any vaccine deposited in the bank must be acceptable to all animal-health regulatory agencies around the world. For instance, The Center for Veterinary Biologics in the US requires that antigens of chicken-embryo origin must be prepared in SPF eggs even for inactivated vaccines. This increases the cost of production and quality-control testing compared to Europe where SPF eggs are not required. Any multinational company would prefer to develop a single set of antigens requested by the poultry industry instead of regional antigen supplies based on local or national requirements.

Chain of control

The decision of whether inactivated antigen or final vaccine will dictate who will maintain control If the material stored is antigen then the control will remain with the manufacturer. The biologics company will be responsible for emulsification, filling and release. If final product is stored in the bank then a governmental laboratory could maintain the inventory and assume the responsibility for distributing the product to the end user.

Quality control

The level and exact requirements for quality-control testing will factor into the cost of goods. If the vaccine has to be fully controlled to USDA 9CFR or European Standards the cost of the vaccine will increase. Will the seed material be required to meet all the requirements of a true master-seed concept? The cost of conducting the full range of quality tests required in Europe for a live-virus master seed is approximately 100-150,000 US dollars. The cost will be much less if the requirements are relaxed during an emergency. In the US it has been proposed that in the event of a true epizootic the only requirement for release of final product could be a 24-48 hour sterility test. Will the EU accept the same standards?

Standardization of vaccines

The concentration of haemagglutinin was standardized in an inactivated influenza vaccine using the single-radial-immunodiffusion test produced by Wood et al. (1985). The SRD test provided a simple and reproducible method for standardizing the HA antigen. The SRD data correlated very well with protection against a lethal A/Chicken/Penn./1370/83 virus. A comparison of the efficacy of six inactivated H5N2 vaccines manufactured in Mexico was compared to a standardized oil-emulsion vaccine (Garcia et al. 1998). All of the vaccines prevented signs of AI infection but only one half of the vaccines prevented or reduced viral shedding.

Response time

Although the manufacturing of inactivated oil-emulsion vaccine is a simple technique it is time-consuming and can be labour-intensive. Antigens prepared in embryonated eggs usually require 72 hours of incubation after inoculation with the seed virus. If the AI virus is not well adapted to embryos it can prolong the incubation time. If the inactivation kinetics is already defined a minimum of two more days must be added to the preparation time. Emulsification, filling and bottling, labelling and packaging are additional steps involved for final product. The length of time necessary to prepare inactivated vaccine containing a known antigen could require

between 14 and28 days plus the control time. If a vaccine is to be produced with a new field isolate the time to market could be estimated in terms of months.

The fowlpox-vectored vaccine is produced in chicken-embryo-origin fibroblast cells. At Merial we have CEF cell culture available daily. The incubation time required for the fowlpox virus to reach peak virus titre is short. The yields in terms of doses of virus are high. The harvested viral fluids can be frozen and stored or dispensed into glass containers and lyophilized quickly. In a true emergency, millions of doses of TROVAC AI H5™ live vaccine can enter the market in less than seven days including a 24-hour sterility test.

Conclusion

The creation of a vaccine bank is possible but it will require co-operation from the poultry industry, animal-health agencies and the biologics manufacturers. The vaccine companies will strive to provide the products that their customers need to maintain the health of the poultry industry. However, investment in vaccine banks will only occur if there is a certainty that a return on investment for R&D and preparation of the antigens will be received. The preparation of inactivated whole-virus oil-emulsion vaccines requires more lead-time than the fowlpox-vectored vaccine for AI type H5. A best-case estimation for the preparation of an inactivated vaccine for emergency use is 4-6 months. Batches of 20 million doses each of TROVAC AI H5 can be prepared during a five-day work week. Discussions between the poultry scientific community, the poultry companies, regulators and the vaccine companies will need to continue regularly if the AI-vaccine banking system is to become a reality.

Acknowledgments

The authors wish to thank Ms. Joan Beck and Mr. Greg Hanes for their excellent technical assistance.

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