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The impact of vaccines and the future of genetically modified poxvirus vaccines for poultry

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Abstract

The regular use of live or killed vaccines against infectious agents has remarkably improved the efficiency of poultry production. In some cases eradication of disease has been possible when the pathogen is antigenically stable and confined to a certain geographical area. In other instances monovalent or polyvalent live or killed vaccines have been effective in reducing mortality and morbidity. Many conventional vaccines are developed by trial and error and basic information about their genetic make-up is not known. While the poultry industry has benefited from the regular use of conventional vaccines, there is need for a new generation of effective vaccines that require minimal handling of birds during administration. Using molecular techniques, it is possible to identify the genes associated with virulence and protection. In genetically engineered vaccines, genes that encode protective antigens can be expressed in bacterial or viral vectors. In this regard, avianpox virus vectors appear to be promising for the generation of polyvalent vaccines expressing antigens from multiple pathogens.

Keywords: poxvirus; vaccines; genetic modification; poultry

Introduction

Immunization with attenuated live or killed vaccines has proved to be highly effective in controlling many diseases in humans and animals. Effective disease control is vital for all phases of poultry production. In this regard, the highly competitive poultry industry is dependent upon the most efficient production methods. The poultry industry has made significant progress in production efficiency through innovative management practices, proper nutrition and the regular use of vaccines to reduce disease-related losses. Prevention of diseases by vaccination has been shown to be extremely beneficial, in decreasing not only mortality and morbidity but also the cost of animal production In addition to providing protection, vaccines also reduce the spread of infection. In recent years poultry production has changed from numerous small farms to relatively few large operations. Because of the intensive nature of production units, contagious diseases can spread rapidly and the economic consequences can be devastating. Despite regular vaccination of poultry to prevent diseases, disease outbreaks still occur frequently. Currently, a large number of live viral vaccines (e.g. Newcastle disease, infectious laryngotracheitis, fowlpox, infectious bursal disease, avian encephalomyelitis and infectious bronchitis) as well as several killed vaccines are used by the poultry industry. It is not uncommon for birds to receive several applications of the same or combined vaccines during their lifetime. The use of combined vaccines reduces the cost of production and administration. Without the protective effect of these vaccines, the industry would not be where it is today.

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Traditional approach to vaccine development

The traditional approach to the development of immunizing agents involves the use of both modified live (attenuated) and inactivated vaccines. Attenuated vaccines are generally preferred since they provide immunity of a longer duration and are more easily produced. However, they may pose the threat of reversion to virulence and often need to be maintained at the correct passage level. Attaining the balance between maximum immunogenicity and minimum virulence for the host may involve some risk, including the potential for the vaccine virus to revert to a more virulent form under field conditions. Furthermore, *in vivo* recombination of different attenuated strains may result in the generation of a strain that is more virulent than its parents. Although these concerns do not apply when using inactivated or subunit vaccines, frequent administration is usually required, and killed vaccines are more expensive than live ones. Even though traditional vaccines are usually efficacious in eliciting a protective immune response in the host, none can claim to be perfect.

Examples of impact of traditional vaccines

While there are many examples of the impact of live and killed vaccines on the production efficiency of poultry, the following two examples of conventional vaccines are from my own research. The first example is a long-term study with a live duck hepatitis virus vaccine; the second involves a killed *Riemerella anatipestifer* vaccine. Both of these vaccines affected operations at one of the largest duck farms in the USA, where about 10 million ducks are raised annually.

Duck hepatitis virus vaccine

Duck hepatitis is a highly contagious viral disease with high mortality in young ducklings (3 weeks of age or younger). Older ducks (4 weeks or older) develop resistance to the virus and do not develop clinical disease. Duck hepatitis was endemic in young ducklings at the Maple Leaf Duck Farms in Indiana. Breeder duck immunization was practiced at the farm with a view to protecting the offspring by the passive transfer of maternal antibody. This approach reduced some losses, although mortality (ranging from 10 to 20%) continued to occur in young ducklings. Attempts to minimize these losses by intramuscular vaccination of ducklings with attenuated duck hepatitis virus vaccine were not completely successful, as the disease remained endemic.

Initial experimental studies revealed that oral transmission was important in the epidemiology of duck hepatitis and that the virus continued to multiply in the gastrointestinal tract of older ducks without evidence of clinical disease. Fecal shedding by older birds maintained a continuous source of virus for infection of young ducklings. Since natural transmission of duck hepatitis virus occurred through the oral route and the virus was excreted in feces, it was postulated that oral exposure with an attenuated vaccine would result in the development of protective immunity within the gastrointestinal mucosa by direct contact of the vaccine virus with the local epithelium.

Experimental studies on oral vaccination of susceptible ducklings lacking passive immunity (from non-vaccinated parents) demonstrated that vaccination provided protection against challenge with virulent virus, while unvaccinated controls were susceptible (15–65% mortality) to similar challenge. Similarly, in passively immune ducklings, oral vaccination provided additional protection against oral challenge with virulent duck hepatitis virus, although the susceptibility of unvaccinated controls was variable (0–36% mortality). Based on these results, oral vaccination through drinking water was initiated at Maple Leaf Duck Farms. Given the success of the preliminary oral vaccination trials, vaccination through drinking water was continued for 10 years. Oral vaccination through drinking water led to the development of local immunity of the target tissue, the gastrointestinal tract, minimizing the chance of multiplication of the virulent virus on these farms due to lack of susceptible hosts (Tripathy and Hanson, 1986). Since the initiation of the oral vaccination program there have been no cases of clinical duck hepatitis. Vaccination of young ducklings was eventually discontinued and no evidence of disease has been observed during the following 20-year period. Eradication of duck hepatitis at these farms is an example of the impact of vaccination against a single agent.

As in the case of duck hepatitis, vaccines are most effective when the microbial agent involved in the disease has a stable antigenic phenotype. However, some pathogenic conditions are caused by closely related organisms with antigenic variations, and protection is limited to individual strains. Moreover, the virulence and antigenic composition of the pathogen may vary within the changing microbial population, limiting the value of any single or stable vaccine formulation over a large geographic area. In such cases combinations of related strains or even the use of autogenous vaccines may become necessary, as described below for a bacterial disease in ducks.

Killed Riemerella anatipestifer *vaccine*

One common highly contagious bacterial disease in ducks is caused by *Riemerella anatipestifer*. It is characterized by perihepatitis, pericarditis, torticollis, loss of weight and high mortality. The disease was endemic in ducks at Maple Leaf Farms, causing high mortality and condemnation with significant economic losses.

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Following initial isolation and characterization of the etiological agent, an inactivated vaccine was prepared, and vaccination under field conditions was very effective in reducing losses. In the first study over 100 000 birds were vaccinated with an *R. anatipestifer* bacterin and a similar number were kept unvaccinated. Vaccinated birds experienced 60% less depletion (mortality and condemnation) than unvaccinated birds. Similar encouraging results were obtained during numerous studies with *R. anatipestifer* bacterins containing single or multiple serotypes. In one field study 31 200 ducks, which received a single dose of autogenous bacterin containing three strains of *R. anatipestifer*, had 10.26% depletion (7.13% mortality and 3.13% condemnation) while another group of 38 360 ducks vaccinated twice with the same bacterin experienced a depletion of only 3.56% (mortality 2.2%, condemnation 1.36%). On a different farm, of 34 020 ducks, a single vaccination with an autogenous *R. anatipestifer* bacterin reduced mortality and condemnation to less than 1%. However, it soon became apparent that antigenically different serotypes of *R. anatipestifer* of variable pathogenicity were present in the duck population. Therefore, three or more serotypes of *R. anatipestifer* were included in a polyvalent autogenous vaccine to provide protection against the infecting serotypes.

New generation of genetically engineered vaccines

Realizing the impact and importance of vaccines for the prevention and control of diseases, research on all aspects of vaccination against human and animal diseases has increased considerably in recent years. In spite of the fact that vaccines are popular interventions for the prevention of diseases, limited efforts have been made towards producing a new generation of effective poultry vaccines. Nevertheless, research on vectored vaccines, subunit vaccines and DNA vaccines is ongoing. Ideally, the poultry industry would like to have effective vaccines that require less frequent handling of the birds and are inexpensive. Currently, administration of a large number of individual vaccines involves frequent handling, resulting in increased stress for the birds and increased labor cost. One way to reduce these problems would be the production of vaccines capable of providing protection against multiple pathogens. Such vaccines can be designed by incorporating genes that encode specific protective antigens of various pathogens into the genome of a live avirulent carrier. Since in a live vectored vaccine only the protective antigen(s) of a pathogen are expressed for presentation to the immune system of the host, the chance of reversion to virulence is eliminated and the beneficial properties of both live and killed vaccines are retained.

Using current molecular methods it is possible to determine the biological pathways needed by pathogens to survive in their respective hosts. Moreover, genes encoding protective antigens of pathogens as well as those associated with virulence can be easily identified. This information can be used to overcome the limitations of traditional approaches to vaccine development and assist in designing a new generation of effective vaccines. Although protective antigens can be expressed either by bacterial or viral vectors, the latter are preferred. In this regard, the genomes of several viral vectors, including poxviruses, baculoviruses, herpesviruses and adenoviruses, have been manipulated to enable the expression of foreign proteins. Larger viruses, such as herpes and pox, have an advantage in that they can accommodate a substantial amount of foreign genetic material. However, because herpes viruses have the potential for delayed persistence and oncogenesis, avipox viruses like fowlpox and pigeonpox virus appear more advantageous for the development of poultry vaccines.

A major advantage of viral-vectored vaccines is the ability to elicit a T-cell-mediated as well as a humoral immune response to the antigen delivered by the vector. The main safety concern is whether the vector itself is virulent and capable of producing clinical disease in the host. Attenuated live fowlpox virus vaccines of chicken embryo or cell culture origin have been used safely in commercial poultry for more than 60 years. Extensive experience with fowlpox virus as a live vaccine, its restricted host range and large genome, capable of accommodating substantial amounts of foreign DNA, are desirable features for its potential use as a vector for immunization against important pathogens of poultry (Tripathy and Reed, 2003).

Fowlpox virus-vectored vaccines

The first recombinant fowlpox virus expressing a specific protein from an avian pathogen, avian influenza virus, was generated in the late 1980s. Subsequently, fowlpox virus vaccines expressing antigens of Newcastle disease, Marek's disease, infectious laryngotracheitis and infectious bursal disease viruses were generated. In each instance, immunization of susceptible birds with recombinant virus resulted in the development of specific antibodies and protection against subsequent challenge with the respective pathogen (Tripathy, 1996). Further, because fowl pox virus has the capacity to accommodate a large amount of foreign DNA in its genome without loss of viability, it could simultaneously express antigens from several pathogens for the generation of a polyvalent vaccine (Tripathy, 2002).

In developing a new generation of vaccines, it is important to consider vaccine efficacy, safety and cost. Currently, two commercial fowlpox virus-vectored vaccines expressing either avian influenza or Newcastle disease virus genes are available. Results of experimental and field studies have revealed that they are safe and efficacious. The fowlpox virus-vectored avian influenza

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vaccine has been used extensively in Mexico. A similar vaccine designed to protect against Newcastle disease virus has been available in the USA for some time, but it has not been used routinely by the poultry industry. One of the reasons for its limited use has been its high cost compared with inexpensive conventional vaccines. Given the current prices of poultry meat and eggs, the industry cannot afford an expensive vaccine even if it is highly effective and superior to current conventional ones. Therefore, manufacturers must consider the cost-effectiveness of any new vaccine during its development. Another concern has been that immunity generated against fowlpox virus after initial vaccination may prevent the subsequent use of this recombinant virus containing gene(s) of different pathogens as an immunizing agent in the same animal. To circumvent this problem, other antigenically distinct avian pox viruses, such as quailpox, canarypox and condorpox, are being considered as vaccines for reimmunization.

Fowlpox virus-vectored vaccines can be developed at a reasonable price if genes from multiple pathogens can be incorporated into the virus genome. But such a vector must have several non-essential regions in its genome, and strong homologous virus-specific promoters are required. Until recently only a few non-essential regions had been identified in the fowlpox genome and a limited number of homologous promoters were available. Recently, we found that fowlpox virus contains many genes that are not essential for replication (Srinivasan *et al.*, 2001). Such loci could be used effectively for the insertion of various genes from multiple pathogens. Previously, in lieu of homologous fowlpox virus promoters, heterologous vaccinia virus regulatory elements were used in creating recombinant fowlpox virus vaccines. However, we also recently identified several fowlpox virus promoters and determined their efficacy (Srinivasan *et al.*, 2003). Use of these homologous promoters should allow the optimal expression of foreign proteins by the recombinant fowlpox virus.

It is very encouraging to survey the wealth of genetic information on various poultry pathogens that has become available in last 10 years. For example, the complete nucleotide sequence of a vaccine-like fowlpox virus has been determined (Afonso *et al.*, 2000). Moreover, several non-essential regions and transcriptional regulatory elements in the fowlpox virus genome have been evaluated. Additionally, several genes associated with virulence and prolonged persistence have been identified (Singh *et al.*, 2003). The concurrent discovery of more genes that encode specific protective antigens of other poultry pathogens means that it should now be possible to develop a polyvalent fowlpox virus-vectored vaccine incorporating genes of several pathogens. Since fowlpox virus infects the oral and upper respiratory tract of chickens, such vaccines may eventually be administered ocularly or orally. Similar recombinant vaccines expressing multiple antigens can be designed for subcutaneous, intramuscular or *in ovo* administration.

Although fowlpox virus currently appears to be the vector of choice, the avipoxviruses are a diverse group of viruses, infecting a variety of birds. Therefore, it is possible that in the near future monovalent or polyvalent recombinant vaccines using other avian poxviruses, such as canarypox, pigeonpox, quailpox, psittacinepox, sparrowpox and condorpox viruses, will be created for use in commercial poultry (Kim *et al.*, 2003). The complete nucleotide sequence of the genome of canarypox virus has been determined recently (Tulman *et al.*, 2004).

Conclusion

As the structure of the poultry industry has changed from small to large units and the efficiency of production has improved significantly, the need for cost-effective vaccines for the prevention of diseases has become increasingly important. In this regard, a new generation of vectored vaccines for which the basic technology has already been established holds great promise. Such vaccines appear to have a great future role in efficient poultry production.

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