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Evolving Importance of Biologics and Novel Delivery Systems in the Face of Microbial Resistance

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ABSTRACT Methods to control infectious diseases in livestock are growing in importance. As the size of the average farm increases-for poultry, dairy and beef cattle, swine, and fish-the risk of rapid spread of infectious diseases increases as well. This increases the need for alternative methods of control of infectious agents. Improvements in specific immunogens, adjuvants, and delivery systems are needed to meet the demand for vaccines to ensure a healthy and safe meat supply. This article explores the challenges, trends, and recent advances in the control of infectious diseases through the use of biologics.

KEYWORDS: biologics, vaccines, animal health, novel delivery systems.

METHODS OF CONTROL OF INFECTIOUS DISEASES

The control of infectious diseases is an enormously important economic issue to the animal industry. Infectious agents can devastate the health of livestock. Proper control and treatments are an integral part of raising livestock. Several methods are used to control infectious agents in large herds or flocks, including the following:

- Eradication. Although effective in eliminating affected animals and reducing the spread of disease, eradication of herds is costly because of the loss of genetic stock. This method also incurs the loss of short-term agricultural income and hence decreases the health of the industry in whole. Such outcomes could have major repercussions within a specific industry, or a country's economy.

- Management. Management practices to control infectious diseases include the maintenance of a closed herd, practicing an all in/all out handling of groups of animals, use of biosecurity methods to restrict the introduction of infectious agents to a farm, use of good animal hygiene, strategic use of disinfectants, and quarantine of newly purchased animals for a period prior to their introduction to a herd. Improvements in management have helped improve the health and well-being of livestock.

- Treatment. One alternative method for handling bacterial pathogens is the use of therapeutics to treat or prevent infections. However, as with any large population, it is impossible to expect that animals will never get sick. Therefore, the need for effective antibiotics will always exist. It has been estimated that 30% to 50% of all antimicrobial agents are used in agriculture [1-3]. Of these, a significant number are used subtherapeutically for growth promotion [3]. As with human medicine, prudent use of antibiotics is essential to preserve their efficacy and to minimize the emergence of resistant organisms.

- Vaccines. An economical method of controlling infectious diseases is immunization. As concerns for emergence of antimicrobial resistance increases, there is a growing interest in increasing the use of immunoprotection in the prevention of infectious diseases.

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Increasing demand for vaccines: Political and globalization pressures affecting infectious disease control and the need for vaccines

Besides the recognition of increasing antibiotic resistance as a concern in the control of bacterial infections in animals as well as humans, several other factors are driving the search for vaccines to control infectious diseases in animals.

- Change in pharmaceutical company efforts to develop new therapeutics. Most animal health companies develop antimicrobials based on discovery research conducted within the parent company's human pharmaceutical division. With the growing concern over using the same antimicrobials in animals and in humans, there is serious skepticism regarding the future approvability of new antimicrobial moieties for administration to animals.

- Consumer tastes. There is a growing consumer demand for wholesome meat products that are derived from animals raised without the use of hormones or antibiotics. In accordance with these demands, fast-food restaurants and other

retailers are now requiring that meat producers ensure that animals are not treated with certain antibiotics. It is estimated that in the next 10 years, 5 to 7 retailers could control as much as 75% of retail meat sales in the United States [4]. Accordingly, livestock producers are responding to these market demands by reducing the use of pharmaceutical agents

- Commerce. With the evolution of a more global economy, food and breeding stock are traded on a worldwide basis. There is a growing international risk of transferring infectious diseases. The effect of bovine spongioform encephalopathy and foot-and-mouth disease on commerce are recent examples of this principle. National regulatory programs protect parochial trade interests, and the corresponding changes in political expediency and consumer interests, have generated a greater need to control infectious diseases in more innovative ways. Ideally, these control methods will have a minimal impact on trade.

- The precautionary principle. In a global economy, political policies can dramatically affect the general practice of agriculture, and therefore trade. In a desire to reduce the emergence of antibiotic-resistant organisms that could be a threat to humans, the European Union has banned the use of nearly all growth-promoting antibiotics in food-producing animals. This concern is largely driven by the emergence of vancomycin-resistant enterococci (VRE), which is suspected to be linked to the use of a related growth-promoting antibiotic, avoparcin. It should be noted that there are contradictory reports regarding the overall impact of this ban. On the one hand, there is the suggestion of a reduction in the incidence of VREs in Denmark since avoparcin was removed from the market [5]. On the other hand, there is a report that more therapeutic antibiotics have been used to treat ill animals since the ban [6].

- Intensification of agriculture. More meat can be produced on less land than ever before. As the world continues to urbanize, this will become increasingly important as less land becomes available for food production. As more animals are raised in less space, the potential for infectious diseases increases.

SCOPE OF THE NEED FOR VACCINES—APPLICATIONS

The global interest in alternative methods for controlling infectious diseases is largely responsible for the extensive efforts to develop more effective biologics. Impetuses for these interests are described below.

- An alternative control for common infectious diseases. There is a need to develop better vaccines to eliminate problematic diseases where current vaccines are not sufficiently effective, or where antibiotics are still needed routinely to manage the disease. An example of the latter is bovine respiratory disease in North America, which costs producers an estimated $750 million annually [7].

- Control of infectious agents for which there is limited treatment. For some infectious microbes, as well as helminths and protozoa, either there is no treatment or there are concerns of growing resistance to therapeutics. Cryptosporidiosis affects many animals, especially young calves. However, there currently is no effective therapeutic agent. Systemic Escherichia coli infections in poultry have been a significant problem; treatment has always been difficult and is more so now with the decision to avoid the use of certain classes of antimicrobials. Therefore, alternative methods of controlling coliform infections in poultry are becoming even more important [8].

- Emerging infectious diseases. Emerging diseases in food and companion animals are an evolving area of significant concern. Examples include West Nile virus in horses, Neospora caninum abortion in cattle, and poor performance in swine due to circovirus infection. With these diseases, there are no pharmacological interventions approved to ameliorate the consequences.

- Zoonotic diseases. Although the pathogens of interest may not have any clinical impact on the animal species for which the vaccine is intended, the potential risk for infection by these pathogens in immunocompromised humans has heightened interest in the development of veterinary vaccines. Two examples of zoonotic diseases are toxoplasmosis and cat scratch disease, caused by the bacterium Bartonella felis. In the case of toxoplasmosis, there are few, if any, alternative therapeutic options.

- Control of foodborne diseases. There is a growing interest in developing vaccines to control foodborne diseases in livestock, even if the organisms do not cause disease in the animals themselves. There are several examples of this. Escherichia coli O157:H7 is carried primarily by cattle and has been associated with foodborne disease outbreaks attributed to the consumption both of ground beef in the United States and of vegetables worldwide [9,10]. This organism is carried by a very low percentage of animals. It is present on nearly all farms, but rarely causes disease in cattle. Many cattle, swine, and poultry carry salmonella and campylobacter, generally

without any clinical signs of infection. Yet, campylobacter has been found in a large number of retail market meat samples [11,12] and can cause disease in humans.

PROPERTIES OF AN IDEAL VACCINE

There are several characteristics of a useful and successful biologic. First and foremost, the product must be efficacious in preventing the targeted disease. Second, the product must be safe both for the target as well as nontarget species for which it is intended. Other characteristics include the following:

- The product should have minimal adverse side effects. Injection site reactions can adversely affect the carcass quality of cattle and swine. For companion animals, owners are less likely to allow follow-up injections or even use a product if a vaccine induces fever, swelling, or any pain in their pet. The association of injection site sarcomas in cats is also a concern for owners.

- The product should be stable, retaining its potency for a reasonable duration of time.

- Ideally, only one injection should be needed to reach the desired level of efficacy. This product trait is of growing importance because of difficulties associated with multiple administrations, in terms of both time and expense as well as the stress it can produce on livestock, poultry, and aquaculture species.

- The biologic should have a reasonable duration of immunity. This is important both in terms of labor costs and because of the risk of autoimmune responses associated with repeated exposure to excipients in companion animals.

- Economical considerations are important from the perspectives of both product manufacture costs and consumer expense. With regard to the latter, the cost of product will be measured against the economic gains associated with the lower incidence of disease.

- From the product manufacture perspective, the biologic must be reasonable to produce. If it is technologically complicated or requires intricate processing, the cost of goods could increase. If cost of goods is too high, pricing constraints will limit product profitability and, therefore, the likelihood of manufacture.

POTENTIAL IMPACT OF VACCINES AND DELIVERY SYSTEMS ON ANTIBIOTIC USAGE IN ANIMALS

One of the most pressing issues to stimulate the development of vaccines is food safety and the need to control antimicrobial resistance in animals. There has been a concerted worldwide effort to reduce antibiotic usage in both humans and animals. For example, in Norway, the antibiotic usage for animals declined by 37% from 1992 to 1996. The 96% reduction in antimicrobial usage in fish over this period significantly contributed to this overall decline [13]. A major contribution to the reduction in antibiotic usage in fish was the development of an effective vaccine to control farunculosis, a devastating bacterial disease of freshwater and marine fish. This antibiotic reduction is especially impressive as the biomass of fish actually doubled over this same time period. This is an important example of how vaccines can reduce antibiotic usage.

Another example is the control of enzootic Mycoplasma hyopneumoniae in swine. For many years, this disease was controlled by use of antibiotics in the feed because there were no vaccines available. The development of effective vaccines has been relatively recent. Currently, there is evidence suggesting that the use of vaccines may help improve overall health and swine feed efficiency while reducing the need for treatment. This is true not only for mycoplasma, but for other infections as well. In two different studies, vaccinated pigs gained weight faster, had fewer mortalities, had less pulmonary damage, and required fewer treatments than nonvaccinated pigs [14,15].

The successful control of Salmonella choleraesuis va. Kunzendorf in swine is another case in which a vaccine has reduced dependency on antibiotics. S. choleraesuis is an invasive form of salmonella that causes systemic disease in swine, including both pneumonia and diarrhea. Until the development of an effective vaccine, salmonellosis was a significant cause of loss to the swine industry. For many years, salmonellosis in swine was controlled by the administration of the antibiotic nitrofurantoin in feed. This practice was stopped when it was learned that nitrofurantoins are carcinogenic in humans, but this presented a challenge to the swine industry. A modified live vaccine administered intranasally or orally has been very effective in eliminating this disease that had previously ravaged swine operations [16]. S. choleraesuis is now a rare disease in swine herds in North America where the vaccine is used.

Poultry also have a significant incidence of salmonellosis, affecting both carcasses and shell eggs. Poultry vaccinated with salmonella vaccines have been shown to have less salmonellae present at slaughter [17,18].

These are a few examples where vaccines have significantly changed the management of bacteriologically important diseases. Future technologically advanced vaccines could have an even greater impact on the management and control of veterinary diseases.

THE ROLE OF THE BIOTECHNOLOGICAL REVOLUTION IN VACCINE DESIGN

The increased interest in the use of biologics to control infectious disease comes at the time of an exciting biotechnological revolution. Numerous advances have had an important impact on the development of biologics.

Advances in molecular biology have led to, and continue to result in, a better understanding of virulence mechanisms of infectious agents. This includes major breakthroughs in the sequencing of genes to entire genomes of viruses, bacteria, and protozoa, as well as helminthes. A better understanding of genomics has led to efficient ways to identify and manipulate specific genes. For example, in vivo expression technology [19] and signature tagged mutagenesis [20] have been used to identify virulence mechanisms or antibacterial targets. These methods can also be used to identify gene deletion vaccine candidates in bacteria. Without the ongoing revolution in genetic sequencing, these methods of developing gene-deleted organisms would not be possible. Auxotrophs of bacteria (mutants that have lost the ability to synthesize an essential amino acid or acids) have also been developed as safe, modified live vaccines [21]. All 3 methods are useful as vectors for expression of subunit antigens from other bacteria or viruses [22]. These are but a few examples of how advances in biotechnology are improving vaccinology.

IMPROVEMENTS IN ADJUVANT TECHNOLOGY

Adjuvants are substances that potentiate an immune response through any of a variety of mechanisms [23]. Accordingly, the delivery characteristics of the adjuvant in a formulation are a critical factor in determining product effectiveness [24]. Improvements in biotechnology have often resulted in improved immunogens that require better adjuvants to be most effective. Fortunately, in veterinary medicine, there have also been major advances in the understanding and development of safer, more effective, and less reactive adjuvants. These allow for the development of more potent immune responses that afford a long duration of protection. In some cases, newer adjuvants have the potential to induce very potent responses to subunit antigens that would not otherwise be effective without repeated inoculations. Adjuvants formulated as water in oil emulsions are replacing the more toxic aluminum hydroxide formulations for veterinary use. These new adjuvants allow for products that are highly effective with minimal adverse reactions. One example is the development of MF59 as an adjuvant [25]. Several other novel yet potent adjuvants are under investigation. For example, CpG DNA oligonucleotide motifs can be used to direct a faster onset and primarily B-cell or T-cell response [26]. Immune stimulating complexes (ISCOMs) and other saponin-containing adjuvants are highly useful for stimulating cell-mediated immune responses [27]. One of the more successful methods in this regard is the ISCOM-encapsulated influenza vaccine that is currently marketed for horses [28].

There is a growing understanding of the usefulness of mucosal immunity and of the ways to induce a mucosal response with vaccines. This is important because most infections begin at a mucosal site. The therapeutic utility of mucosal adjuvants has been limited because they are themselves potent toxins. Consequently, there is great interest in modifying these toxins to retain their adjuvanticity while reducing their toxicity. Recent advances have included genetically modified mucosal adjuvants—such as the exotoxin A component of Pseudomonas aeruginosa, the heat stable toxin of E coli, and the cholera toxin — for potency. In each case, the modified compounds are much safer than the natural parent compounds [29-31]. However, one major limitation confronting the development of new adjuvants in veterinary medicine is cost. The added manufacturing cost of including new adjuvants to the final market formulation will be a key factor restricting their implementation in veterinary medicine.

DELIVERY OF VACCINES, ROUTE OF ADMINISTRATION, AND EFFICACY

As novel vaccine antigens and adjuvants become available to improve immunogenicity, the next challenge is to determine more efficient methods of vaccine delivery to animals. Alternatives to parenteral administration are growing in importance to owners of both livestock and companion animals. Consumers are demanding products that need to be administered only once over the course of the livestock lifecycle, or at least less often than once a year. Equally important for consumers is a formulation—for example, oral or aerosol— that allows the product to be administered to hundreds or thousands of animals at a time.

Often the ideal time to vaccinate an animal is shortly after birth. However, maternal antibodies can interfere with the ability of newborns to respond to vaccines. Therefore, methods of delivery are needed that can induce a primary immune response in the very young, despite the presence of maternal antibodies. Ideally, there will be only a minimal need for booster vaccines over the remainder of that animal's life. Such demands necessitate the development of alternative delivery systems that can provide either a sustained release of antigen, from a single injection, for example, or a more potent and longer lasting (mucosal) immunity from administration by non-parenteral routes.

The desire for alternative methods of vaccination is increasing. Livestock producers would like to avoid injection site reactions that lead to carcass damage. With a large number of animals to vaccinate, it is also logistically difficult to handle each animal multiple times. Hence, sustained-release vaccines are of increasing interest to livestock producers. Vaccine delivery methods such as aerosolization, use of water or feed, or a single sustained-release injection would reduce handling, reduce labor costs to the producer, and eliminate injection site reactions.

ORAL DELIVERY SYSTEMS

Many different oral delivery systems have been tested for use with infectious agents in veterinary medicine, including liposomes, polymeric encapsulated vaccines as implants, oral baits, and microparticles for delivery of vaccines (23. Oral administration of bacterial antigens in a polymeric hydrogel containing antigens of M haemo-

lytica has been tested in cattle [32]. Antigens were encapsulated in poly (methacrylic acid) hydrogels and administered in a bolus. Vaccinated cattle had less mortality and less morbidity than nonvaccinated controls. Other polymeric delivery systems have been used to encapsulate antigens of swine respiratory bacterial pathogens, including Actinobacillus pleuropneumoniae and Mycoplasma hyopneumoniae. When these antigens were orally administered, the swine had less severe pneumonia than did their nonvaccinated counterparts [33,34]. A promising nonpolymeric delivery system is the bacterial ghost. Inactivated bacteria are loaded with antigens of other bacteria or viruses and are administered to animals either by injection or orally [35]. This unique delivery system has also been used to administer antigens of A pleuropneumoniae to swine. Not only were the swine less likely to develop pneumonia, but the immune response was so effective that no challenge organisms were recovered from the respiratory tract. These examples demonstrate methods to control major bacterial diseases in food animals. Commercial success of such vaccines could dramatically reduce the need for antibiotics to control these diseases.

EXPRESSION OF VACCINES IN TRANSGENIC PLANTS

The technology has advanced rapidly from the simple expression of peptides in tobacco leaves to the genetic expression of bacterial antigens by tuberous plants such as the potato. An experimental application of this technology is its use in the expression of a key bacterial antigen. The leukotoxin that functions as a primary virulence mechanism and an important immunogen of M haemolytica pneumonia in cattle has recently been expressed in clover [36]. The exciting aspect of this application is that clover is the primary diet of the targeted animal species. Another novel application is the expression of antigens in corn, the primary diet for livestock. The antigens are expressed in high amounts and are stable in the corn over time [37]. Thus, the ultimate goal is that normal foodstuffs can serve as mechanisms for vaccine delivery.

PARENTERAL AND INTRANASAL DELIVERY SYSTEMS

A variety of marketed one-shot injectable conventional vaccines have incorporated adjuvants into the product formulation in order to extend the duration of immunity. However, many of these newer products are not as effective as 2 injections of the same vaccine. Consequently, several methods are currently on the market, or are under investigation, for more sustained delivery of antigens. Of the several microparticles that have been investigated, one of the most well studied is poly lactide-co-glycolide [38]. A variation on the use of microparticles is the use of aliphatic polyester liquids to encapsulate antigens and to act as a repository, forming a more solid gel upon injection. One example of this technology is Atrigel®, which has been tested to deliver pseudorabies vaccine to swine [23]. A similar sustained-release liquid material using a different polymer, Saiber™, has been used to produce an intranasal vaccine to control Streptococcus equi, a highly contagious agent that causes strangles in horses [39]. Some formulations now incorporate an antigen with an adjuvant to improve the immune response. Microparticles have been used to target antigens with adjuvants to adhere to nasal mucosa [40]. Solid implants have also been studied as a mechanism for long-lasting antigen release over time [41].

NUCLEIC ACIDS

Progress continues to be made on this seemingly unlikely method of immunization. DNA is injected into muscle (IM) or skin (ID) of the animal, which subsequently produces the antigen encoded by the administered DNA [42]. Bovine herpes virus 1 is the most serious virus causing pneumonia in cattle. One of the important antigens of this virus is the glycoprotein gD, which can induce protective immune responses in cattle. The nucleic acids encoding this gD were in the first naked nucleic acid vaccine tested in cattle, and the vaccine was shown to induce a neutralizing immune response [43]. Nucleic acid vaccination has also been investigated in swine, poultry, and companion animals for a variety of infectious agents [44]. Nucleic acids can be administered by a variety of routes in addition to IM and ID, including transcutaneously and orally [45]. Although there is the potential for using this method of immunization to combat bacterial infections, most examples to date have concentrated on viral applications. Unfortunately, it appears that in many cases optimal immunity to the target antigen requires a follow-up booster with the actual peptide. This presents an interesting situation whereby a vaccine would require the use of a totally different formulation for the booster than for the initial immunization.

FUTURE CHALLENGES TO THE USE OF VACCINES TO CONTROL INFECTION

Progress is being made continually to improve vaccine technology. This, together with advances in delivery methods, could have an increasingly important role in controlling infectious disease. Most important, this progress can lead to less reliance on antibiotics and, accordingly, lessen the current concerns regarding the growing threat of antimicrobial resistance. Unfortunately, technology is not yet able to produce an effective vaccine to control every infectious disease in food animals. This is especially true for diseases in which multiple pathogens are involved, such as bovine respiratory disease complex, or diseases acquired at birth that cannot be eliminated by simply altering animal management procedures. A good example of the latter is Streptococcus suis infection in swine. Swine acquire the organism at birth from the dam. Young pigs are susceptible to both respiratory and neurological disease by this organism. To further complicate the control of this organism, there are over 30 known serotypes. There is currently no known common antigen that would be useful as a vaccine for all serotypes. In a co-infection model of S suis

and porcine respiratory and reproductive syndrome (a virus commonly found in conjunction with S suis), the best means of control was found to be the use of an antibiotic and not a vaccination [46].

CONCLUSION—THE CHALLENGE OF DEVELOPING NEW VACCINES TO SUPPLANT ANTIBIOTICS

Although it is desirable to control all infectious diseases by animal management, including vaccinations, effective methods are not as yet available to do so. Therefore, prudent use of therapeutic agents, including antibiotics, will remain an important component of medical management, not only to control the infection but also to reduce suffering and pain in affected animals. The increasing cost of identifying new antibiotics, public concern over the presence of drug residues in food, and the occurrence of antibiotic-resistant bacteria in foods has increased the veterinary community's interest in developing alternative methods for preventing infectious diseases. Successful vaccines will depend on the identification of more specific protective immunity and on a better characterization of the important immunogens. In some cases, it will be more practical to generate subunit recombinantly derived antigens or nucleic acid vaccines to control infectious diseases. Many of these newer subunit vaccines will require not only a method of adjuvanticity, but alternative methods of delivery as well.

For additional information on the use of vaccines in veterinary medicine, interested readers may wish to refer to general review articles on this subject [23,47-50].

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