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CHAPTER 16

Special Issues around Veterinary Vaccines

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OUTLINE

Maximizing Strength and Duration of Protective Immunity
   Novel vaccine and delivery systems

Enhancing Food Safety with Improved Vaccines
   Vaccines for food borne disease protection

Optimizing Vaccination Protocols
   Prenatal vaccination
   Neonatal and juvenile vaccination
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Strategizing the Use of Animal Vaccines for Protecting the Public
   Health, modeling, and national economies
   Use of models for preemptive vaccination and identification of emerging disease vaccine targets
   Reduction and control of zoonoses through strategic application of animal vaccines

ABSTRACT

The majority of vaccines licensed for controlling infectious disease of veterinary species today are based on technology that was introduced by Jenner using live vaccines and Pasteur using killed whole organism vaccines 200 and 100 years ago, respectively, yet this former technology has not stopped several successful vaccination programs from being developed. Much of veterinary vaccinology is driven by the realities that exist in raising production animals or working in veterinary practice, where making a living depends on keeping the animals healthy, because...
it is an industry where vaccines are like insurance policies—protection from events that one hopes never happen. For example, the USDA recognizes these varying levels of protection in the way that they allow label claims: (1) “aids in disease control,” (2) “for the prevention of disease,” and (3) “for the prevention of infection.” Additionally there may be indirect protection, or herd immunity, that results from vaccination of sufficient numbers of animals in a given population resulting in the reduction of the ability of a disease to transmit through the vaccinated individuals. The perception that vaccines provide sterilizing immunity, where the disease agent does not establish an infection, while widely held, is generally unfounded and largely unrealistic. Recent advances, especially in the last 15 years in genomics, proteomics, biotechnology, immunology, pathogenesis, and vaccine formulation and delivery have dramatically changed our approach to vaccine development. When used optimally, vaccines have been shown to prevent disease, reduce the need for pharmaceutical intervention, and improve the health and welfare of animals, and indirectly people as well. The challenge in developing an optimal vaccination program is in dealing with the great diversity that exists within the animal world, and as such there probably is no single optimal program for all occasions. While there is no magic solution to optimizing vaccination programs for animals, nonetheless, a solid understanding of the animal’s innate and environmental risk factors as well as the variables such as stress will enable the development of tailored vaccination schedules that best meets the needs of the animal. The use of vaccines in animal health is not restricted to the protection of morbidity and mortality of the animal hosts themselves, but they are regularly employed as key elements in public health programs. When appropriate biopreparedness, management modeling strategies, and contingency plans of the future are linked with (1) protective DIVA vaccines against zoonoses, (2) effective predictive modeling, and (3) deployable implementation policies, control, and prevention of serious zoonotic diseases of man and animals will become more achievable at local, state, and national levels.

MAXIMIZING STRENGTH AND DURATION OF PROTECTIVE IMMUNITY

Novel Vaccine and Delivery Systems

Novel Vaccines

The majority of vaccines licensed for controlling infectious disease of veterinary species today are based on technology that was introduced by Jenner using live vaccines and Pasteur using whole killed vaccines 200 and 100 years ago, respectively. Although these vaccines have been very useful in reducing economic losses to the industry, they were primarily produced by empirical processes with little knowledge of animal physiology, host responses to vaccines including the importance or relevance of Th1 versus Th2-like immune responses in controlling infection or the importance of specific virulence factors of the pathogen. However, recent advances, especially in the last 15 years in genomics, proteomics, biotechnology, immunology, pathogenesis, and vaccine formulation and delivery have dramatically changed our approach to vaccine development. As a result of these advances, we believe that the next generation of vaccines should be safer and more cost-effective.

Live Attenuated Vaccines

Historically, live attenuated vaccines were generated by repeated passage of the pathogen in vitro under different conditions that randomly generate mutants that have reduced virulence. Indeed, the majority of the live attenuated vaccines on the market today have been generated by this approach (van Oirschot, 1997). The selection of mutants can be further enhanced by exposing the pathogen to mutagenic agents or growing the pathogen at low temperatures. Under any of these conditions there is no control of which specific genes are mutated, nor is there control over the extent of attenuation. Thus, it requires testing each of these individual passaged agents to finally arrive at a level of attenuation that is acceptable.

Finally, since many mutations can be point mutations, there is a high probability of back mutation and reversion to virulence once introduced into the animals. Possibly the best example of this ability to revert to virulence is with polio virus where the oral polio virus given to humans contains a single-point mutation which can rapidly revert to a virulent form upon replication in vivo (Greensfelder, 2000). However, even with these potential problems, many very effective live vaccines are licensed and have had a major role in disease reduction even though the safety of these vaccines is of concern.

Fortunately, with more knowledge of each pathogen and the genes involved in inducing protection and virulence, it is possible to target specific genes involved in attenuation. By deleting an entire gene from the pathogen to attenuate the organism using genetic engineering techniques, the safety profile of the vaccines can be dramatically improved. This approach is not only safer, because it is more difficult to acquire a whole gene which would lead to reversion than it is to modify a point mutation, but it is also possible to
modulate the degree of attenuation. This is important to ensure that adequate replication of the attenuated vaccine occurs, to induce optimal levels of protective immunity but not cause any pathology. A final advantage of using live vaccines is that they can be delivered by the natural route of infection to provide protection at the natural site of entry (i.e., mucosal protection). However, even with all these advantages, these approaches are under much greater scrutiny by regulatory agencies than the less safe conventional methodology of attenuation, thereby, adding significantly more expense to the licensing of many potentially valuable vaccines needed by the industry.

Currently, the more popular method of attenuation is to delete or incapacitate an entire gene that is not required for replication. However, it is also possible to introduce temperature-sensitive (TS) mutations which would reduce the pathogens ability to replicate at temperatures present in the lower respiratory tract. Thus, the vaccine could infect the upper respiratory tract and induce immunity, but have limited replication in the lower respiratory tract where damage could lead to complications such as pneumonia and possibly death. In addition to introducing this single TS mutation, one could introduce multiple TS mutations, thereby providing a much safer and stable attenuation than is currently possible with conventional TS mutants. This approach is very attractive for many respiratory infections, but especially for influenza viruses where TS mutants are currently in use for control of equine influenza (Townsend et al., 2001).

By deleting nonessential genes, a vaccine can be designed to reduce the virulence of a pathogen. However, it is also possible to make vaccines by deleting essential genes, which will completely prevent the replication of the vaccine in vivo. This type of mutation results in replication defective organisms, making the vaccine totally safe. Such an approach has been used for a variety of different prototype vaccines. The principle behind this approach is that it is possible to culture the vaccine in vitro by supplying the elements required for replication. Once the agent is introduced in vivo, where the element is absent, the agent undergoes an abortive infection. However, during this abortive infection, sufficient antigens are produced to induce immunity. These types of defective vaccines can be produced against a number of different organisms. A variation on the replication defective vaccines approach is one where a specific gene is introduced requiring a specific metabolite or compound to replicate (Donnenberg and Kaper, 1991). This allows controlled replication in vitro as well as in vivo as long as the required compounds are introduced with the vaccine or individuals are fed the specific compound for a period of time to allow the vaccine to replicate in vivo. On removal of the required compound, the agent dies. These types of approaches are easy to introduce into bacteria for making bacterial vaccines. These types of vaccines should remove the concern about shedding of organisms in environments as they would rapidly die in the environment because of lack of complementary growth components.

Possibly the greatest advantage of live genetically engineered vaccines is the fact that these vaccines can be used as vectors to carry protective proteins from other pathogens. Thus, it is possible to immunize an individual against not only the organism that has been attenuated, but also against antigens which are being carried by the vector. Thus, a single immunization can protect individuals from a variety of diseases. This approach is gaining considerable interest from the veterinary biopharmaceutical industry since it would make vaccine production and delivery much more economical with a single vector carrying multiple vaccines manufactured in a single step. Furthermore, there should be no interference between the antigens as it is often seen with multiple agents at the time of vaccination. Currently, over a dozen viral vaccines based on a pox virus vector are licensed in veterinary medicine (Gerdts et al., 2006). Similarly, a number of companies are developing adenovirus-based vectors (Reddy et al., 1999a, 1999b). In the case of poxvirus-based vectors, one can either use mammalian or avian poxviruses as vectors. Since avian poxviruses undergo abortive replication in mammalian cells, they are gaining significant market share where they have been licensed. Thus, the safety concerns outweigh any genetic engineering concerns. The most recent report of developing solid immunity of monkeys to Ebola/Marburg and lassa virus following a single immunization with a VSV recombinant has created significant excitement using VSV as a potential vector (Jones et al., 2005). However, in all these instances of vectored vaccines, one is always concerned about the impact of preexisting immunity to the vector on the potential efficacy of the vaccine. The possible reason that the monkeys were totally protected from Ebola following VSVvectored immunization versus adenovirusvectored immunization is the fact that the monkeys were naïve to VSV.

Currently, it is possible to develop live vectored vaccines using bacteria or viruses as carriers, which then can be delivered by natural routes of infection, and by mass administration by aerosol or in feed or water, which is critical for the poultry industry. This approach overcomes a need to handle individual animals and also overcomes the disadvantage of
needle delivery. In addition, these vaccines can be used to induce immunity in ovo (Rautenschlein et al., 1999).

Currently, in ovo vaccination has gained significant popularity due to the ease of handling and providing immunity very early in life of poultry. In addition to carrying genes from other pathogens, these vaccines can also carry immunomodulatory genes to act as adjuvants to enhance the efficacy of the vaccine (Raggo et al., 1996; Giavedoni et al., 1997). Although these vectored vaccines have many advantages, consideration must be given to the antigen being delivered. For example, one would not choose a viral vector when immune responses to carbohydrate or lipopolysaccharide (LPS) antigens are needed. In contrast, a bacterial vector would be ideal for such a vaccine. Similarly, one would not choose a bacterial vector to deliver a viral protein whose confirmation is very sensitive to posttranslational modification. Thus, the appropriate vector is as important as the antigens being delivered by that vector.

### Killed and Subunit Vaccines

Conventional killed vaccines are produced by inactivating the infectious agents so it cannot replicate in the host, but without altering immunogenicity of the protective proteins. Thus, all the proteins of the infectious agent are present in the vaccine, but the nucleic acid has been damaged to the extent that the agent cannot replicate. Unfortunately, many inactivating agents do have an impact on immunogenicity by damaging or altering specific proteins, therefore, it is important to achieve a balance between full inactivation and reduction of immunogenicity (Ferguson et al., 1993). Unfortunately, with some infectious agents, especially those that may aggregate, it is not possible to have inactivating agents penetrate the aggregate and, as a result, some outbreaks of disease have occurred due to improper inactivation (Brown, 1993). Thus, there continues to be a search for new inactivating agents. For all inactivated vaccines it is critical that the protective antigens are present at the concentration sufficient to induce an immune response. In bacteria, many virulence factors may be extracellular secreted proteins, therefore, they are not present in sufficient quantities in whole inactivated organisms to induce protective immunity. Possibly, the best examples are the RTX toxins in the *Mannheimia*, *Actinobacillus*, and *Pasteurella* species (Shewen and Wilkie, 1982). These antigens are critical for effective vaccination, but are secreted and, therefore, are not present in whole, killed inactivated vaccines. To overcome this problem, companies have “spiked” the whole killed inactivated vaccines with culture supernatant containing the RTX toxins. Second, some virulence factors are only expressed under specific physiological conditions, i.e., iron-regulated proteins, thus, care must be taken to ensure that bacteria are cultured under the appropriate conditions to express the putative protective antigens (Deneer and Potter, 1989b, 1989a).

Since most inactivated vaccines are poorly immunogenic, they often require adjuvants to enhance their immunity. Many of these adjuvants and formulations cause tissue reactions (see below). Furthermore, they generally induce a good humoral immune response with minimal cell-mediated or mucosal immunity. In many cases, these later responses are critical for optimal protection. In addition, many of these vaccines are delivered by needles that may break and remain in the animal for long periods of time. This is both painful for the animal and is of concern to the consumer who might encounter such a foreign object in the meat. However, many very successful vaccines have used these technologies and continue to be used by the industry.

A variation on the theme of killed vaccines is that of subunit vaccines. Instead of developing vaccines containing all the antigens of the pathogen, it is possible to identify the few critical proteins that are involved in inducing protection and use them as a vaccine. Theoretically, it is possible to purify the proteins from any conventionally produced vaccine. However, this is very expensive, therefore, it is much more economical to clone the gene and produce it as a subunit vaccine using biotechnology approaches. Before embarking on a subunit vaccine program, it is critical to identify the critical antigens for incorporation into a vaccine and then produce these antigens in a commercial setting. Fortunately, our knowledge regarding the critical proteins and ability to isolate the genes coding for these proteins has become relatively routine in the last decade. Thus, the protective antigens for most important infectious disease agents in veterinary medicine are now known. For those agents where we still need to identify the putative protective antigen, we can use comparative biology and genomics to identify them relatively quickly. However, identification is only the first step in a vaccine production program. Once the antigen is identified, it must be produced in a commercially viable system.

For bacterial antigens, production in bacterial systems is relatively straightforward. These proteins can be produced either as secreted proteins or as inclusion bodies. However, for viral or parasitic antigens, the extent of posttranslational modification required to retain the three-dimensional structures dictates that they can be produced in eukaryotic
systems to ensure their immunogenicity. Although these are often more expensive and difficult to scale-up, they are required especially for viral glycoproteins. Since viral glycoproteins are generally anchored in cell membranes, this requires destruction of cells to purify the glycoprotein. To overcome this impediment, it is possible to remove the transmembrane anchor and allow secretion of the protein (Kowalski et al., 1993). This not only increases the yield, but also reduces the purification cost, thereby making the vaccines affordable for livestock application. Significant advances in production of subunit vaccines are being made by expressing antigens in plants, which confer most of the posttranslational events present in mammalian cells. Further advantages are that it is possible to target the vaccine antigen to oil bodies in the plant (van Rooijen and Moloney, 1995). This makes the vaccine very thermostable, allowing ease of purification and, more importantly, the vaccine is already incorporated in the metabolizable oil adjuvant. Although there are currently no licensed vaccines produced in plants, it is envisaged that this will occur shortly as a number of companies are currently testing a number of such vaccines.

**DNA Vaccines**

The newest addition to our armamentarium in vaccination is genetic vaccination or polynucleotide vaccines, commonly referred to as DNA vaccines. The concept of DNA vaccination is extremely simple in that a plasmid expressing a gene encoding the antigen of interest is injected into an animal, which then expresses the protein (vaccine) of interest. Thus, the animal acts as the bioreactor to produce the vaccine and, consequently, the animal is immunized. This simple process does not require any downstream protein purification, although it does require plasmid purification and abolishes the need for adjuvants. Another advantage, depending on the route of administration, is that these vaccines generally induce both humoral and cellular immunity. This is primarily because the antigen is produced endogenously and all posttranslational modifications are similar to those seen following regular viral infections. Furthermore, improvements in both enhancing immunity as well as driving the cell-mediated arm of the immune system has been achieved by coadministration of these DNA-based vaccines with plasmids encoding IL-12 and IL-15 (Hanlon et al., 2001).

Although the concept is simple, the actual events that must occur to make DNA vaccination possible are many. First, efficiency of transfection is very low. Thus, few plasmids actually get into the nucleus and express antigen. To overcome this problem various methods have been used to increase transfection including: (1) electroporation, (2) formulation in different polymers or lipid-based vesicles, and (3) mechanical delivery using air or gene guns (reviewed by van Drunen Littel-van den Hurk et al. (2004)). Second, in the case of RNA viruses or bacterial genes, it is often necessary to introduce introns and remove cryptic splice sites to ensure expression occurs even after entry of the plasmid into the nucleus. Altering codon bias has also proven in some cases to enhance expression. The effects made to enhance the efficacy of DNA vaccines in livestock have been reviewed on a number of occasions (Babiuk et al., 2000, 2002). Possibly the most efficient method of delivery of genetic vaccines is the use of alphavirus replicons (Schultz-Cherry et al., 2000). Since alphaviruses naturally target cells for infection, they are ideal delivery vehicles. Using the alphavirus replicons system, the envelope can be engineered in such a way that it more efficiently targets the antigen-presenting cells (dendritic cells) for active targeting of vaccines. Since the replicons are not able to reproduce they are deemed to be safe.

Although there are still many hurdles to overcome to make DNA vaccination routine in many species, the first DNA-based vaccine has already been licensed to control West Nile virus in horses. With the regulatory path having been set, it is highly likely others will be licensed shortly.

**Differentiating Infected from Vaccinated Animals (DIVA) Vaccines**

Previously, agricultural disease surveillance and eradication programs were largely based on the serological confirmation of infection and the destruction or quarantine of herds to limit the spread of disease. Due to the reliance on serological confirmation, most countries banned vaccination against exotic diseases. Possibly, the best example of such an approach is with foot-and-mouth disease. The introduction of foot-and-mouth diseases into countries that are normally free of this disease results in quarantine and slaughter to eradicate disease rather than to conduct ring vaccination. Since ring vaccination would result in serological positive animals the country would continue to be considered “infected” as long as there were serologically positive animals in the country. However, the application of a serological test to differentiate between vaccinated and infected animals has a potential to change this practice. As countries move to eradication of endemic diseases or controlling novel introduced diseases through vaccination programs

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it is critical to develop robust detection systems which differentiate vaccinated (immune) animals from naturally infected animals which might be carriers of the disease. This aspiration has led to the development of DIVA vaccines where vaccines lack one or more highly immunogenic proteins, but still contain the critical proteins needed for inducing protection. Since the vaccine lacks a specific protein, a companion diagnostic test is developed which can serologically differentiate infected from vaccinated animals (DIVA). Although DIVA vaccines/diagnostic tests can be used with conventional killed vaccines, for example, the killed FMD vaccines do not induce antibodies to the viral nonstructural proteins (NSPs) (Clavijo et al., 2004), the trend is to use gene deleted or subunit DIVA vaccines.

The first gene deleted vaccine was used to eradicate pseudorabies virus. This vaccine, was the result of a natural deletion of the glycoprotein (gE) gene from the virus (van Oirschot et al., 1986). This vaccine was used for decades to control pseudorabies virus in pigs and was known as the Barta strain. Coincidentally, it also has a gE deletion, which then could be used as a marker for differentiating vaccinated animals from latent carriers of the disease. As a result of the first DIVA vaccine, many areas are now free of pseudorabies virus. Although the pseudorabies virus was a naturally occurring virus, the availability of recombinant DNA technology, combined with our knowledge of gene sequences, and the ability to identify nonessential genes in many pathogens and delete them provides us with a great opportunity to develop DIVA vaccines for almost any infectious disease. As a result of these initial successes, several marker vaccines are already commercially available and their role and contribution to disease eradication appears promising. However, for DIVA vaccines to be embraced by the industry there will need for support by various levels of governments including the livestock industry to ensure that the biopharmacy industry develops these vaccines. For example, there are opportunities to develop DIVA vaccines for foot-and-mouth disease, but the international community does not yet appear to be willing to embrace vaccination against foot-and-mouth disease in countries which are normally foot-and-mouth disease free (Clavijo et al., 2004). In the case of foot-and-mouth disease, a DIVA test is available based on the detection of antibodies to NSPs that is the preferred diagnostic method to distinguish viral infected carrier animals from vaccinated animals, but is not yet embraced by the international trade community. Thus, even though the better technology is available, this will not guarantee that the technology will be embraced by the industry or the global community. Thus, some of these vaccines and their acceptance will not only be driven by economics to the individual producer, but it is also often politically motivated by trade interests and macroeconomic issues. Thus, once a country is declared disease-free that country can use its disease-free status as an effective trade barrier to keep out livestock from countries that are not disease-free. As more confidence is developed in DIVA vaccines and the diagnostic tests are simpler to perform, it is hoped that the entire globe would embrace these types of vaccines and reduce the accidental introduction of exotic diseases into countries or if introduced, will reduce the number of animals that are needlessly slaughtered to return the country to disease-free status.

**Formulation and Delivery**

Regardless of the type of vaccine developed, if it is not formulated or delivered properly, the full benefit of the vaccine will not be achieved. This is important because how it is formulated and delivered will dramatically influence the quality as well as the magnitude of the immune response generated. For example, a parenterally administered nonreplicating vaccine will induce only a systemic immune response, but have very little ability to induce mucosal immunity. Currently, there is extensive evidence to suggest that delivering the vaccine to mucosal sites is the most effective way to induce mucosal immunity. Another advantage of mucosal delivery is that the animal also develops systemic immunity, in addition to mucosal immunity. In addition to broadening the immune response, mucosal immunity has other advantages such as avoiding the pain and injection site reactions, and broken needles, etc. associated with parenteral injection. This route of administration can also be less expensive, but depending on management systems, may be more difficult to perform. For example, wild range cattle are not very receptive to intranasal delivery of vaccines; therefore, oral delivery may be more attractive in these circumstances. Unfortunately, despite many experimental reports few oral vaccines are licensed for livestock. The reason for this is the lack of effective delivery systems that target the gut-associated lymphoid tissue (GALT). The difficulty of delivering vaccines to the GALT is a result of a combination of many factors including degradation of the antigen and various physiological barriers. For example, in the ruminant, traversing the rumen to the intestine where the GALT is provides a major challenge. However, despite these challenges a number of promising developments are being pursued.

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Since many viruses and bacteria can survive the gastrointestinal tract they are being used as vectors for vaccine development. For oral vaccines, in the case of rabies, an advantage of using vaccinia as the vector is that one can exploit the host's natural protective mechanisms. The rabies glycoprotein binds to the CD205 receptor, which is associated with the uptake of antigens by M cells. Studies suggest that uptake of antigens by M cells may be a way to specifically target the immune system to the mucosal lining of the intestines. Furthermore, antigens can be delivered to specific areas of the immune system, such as the lymph nodes, by targeting them with specific ligands. This approach has been shown to be effective in eliciting a Th1-type immune response, which can be associated with a strong immune response against pathogens. However, it is important to note that the quality of the immune response can vary depending on the specific antigen and the delivery method used. Overall, the use of microparticles as delivery systems for vaccines has shown promise in enhancing the strength and duration of protective immunity.
producing alginate microparticles, it is also possible to encapsulate live microorganisms, thereby protecting them as they transit the stomach to the gastrointestinal tract. This was clearly demonstrated by the ability to encapsulate bovine adenovirus in alginate microspheres and develop immune responses following oral administration of the virus (Mittal et al., 2000).

A most recent class of synthetic biodegradable polymers for vaccine delivery as well as acting as an adjuvant in itself is the polyphosphazenes. Numerous reports have documented that polyphosphazenes perform better than conventional adjuvants when incorporated with vaccines (Andrianov et al., 1998; Payne and Andrianov, 1998). Furthermore, it is possible to formulate polyphosphazenes into microparticles with no organic solvents. Thus, the procedure is extremely mild and one captures the benefit of microencapsulation as well as adjuvanticity all in one type of particle. Although these polyphosphazene microparticles have proven to be very effective in preliminary studies in mice, they require further investigation for application to livestock and companion animals. Although we have identified the areas of current interest and research in microencapsulation and delivery of vaccines in microparticles, it must be emphasized that there are very few studies in large animals demonstrating the application of microparticle formulated antigens for oral immunization. Clearly, much more work is required in large animals but the knowledge that is gained regarding the needs to stimulate the GALT, the size of microparticles, and charges to enhance immune responses combined with preliminary studies in a number of veterinary species demonstrating their potential utility is encouraging and needs to continue to be pursued.

Due to the rather poor immunogenicity of most inactivated and subunit vaccines, they almost always require combination of the vaccine antigen with adjuvants to be effective. The current adjuvants can be classified into: (1) mineral salts, (2) synthetic adjuvants, (3) oil emulsions, (4) plant products, (5) lipid-based vehicles, (6) bacterial products, and, finally (7) molecular adjuvants or cytokines or any combination of the above (Cox and Coulter, 1997). In humans, only aluminum salts or MF-59 are licensed, whereas in veterinary medicine many different adjuvants are in current use. Unfortunately, the exact mechanism by which most adjuvants work is not fully understood and different adjuvants probably have different mechanisms of action. These range from creating a depot of the antigen to attract antigen-presenting cells to the site of vaccination to creating a cytokine environment at the vaccination site to help expand the immune reactive cells. Regardless of the mechanism of action, most licensed adjuvants do not possess all the required features of an ideal adjuvant, therefore, the quest continues for better adjuvants, which not only induce a balanced immune response, but also are safe and do not induce any injection site reactions.

Since many of the adjuvants attract cells of the immune system to the site of injection, one requires a fine balance between attracting the appropriate cells required for induction of immunity, but not cause overstimulation resulting in unwarranted injection site reactions. Indeed, it has been estimated that in cattle, these injection site reactions cost approximately $8/injection site as a result of the need for trimming (van Donkersgoed et al., 1999). It is for this reason that most injections are now given subcutaneously in the neck where meat cuts are of less quality. In the case of cats, aluminum adjuvants have been implicated in fibrosarcomas (Burton and Mason, 1997) and, therefore, the trend is to move to nonadjuvanted vaccines for cats. The major problem with most adjuvants is they generally induce a strong Th2-like immune response, but very little Th1-like immune responses. Because of these concerns, the quest continues for novel adjuvants that do not cause any adverse reactions, but still induce the desired response.

Currently, the most often used adjuvants include aluminum salts, which adhere antigen to their surface and act as a depot for the antigen which is then presented to the antigen-presenting cells infiltrating the injection site. Since the antigen needs to be absorbed to the aluminum salts, it is not surprising that all antigens do not absorb efficiently and require precise pH's for their absorption (Claesson et al., 1988). Another disadvantage of aluminum salts is that they generally induce only Th2-like responses with little cell-mediated immune responses.

Oil emulsions are another very commonly used family of adjuvants in veterinary vaccines. These oil emulsions vary from mineral to plant oils and can be oil in water or water in oil emulsions. The major problem with mineral oils is that they are nonmetabolizable, leading to residues and injection site reactions. To overcome these problems, metabolizable oils are replacing mineral oils. Many of these metabolizable oils are plant-based. In addition to using plant-derived oils, other plant components with immunomodulatory properties are also used, either in combination with oils or alone (Kensil et al., 1991; Wu et al., 1992). Possibly the best known plant-derived adjuvant is saponin (Quil A) or various fractions of Quil A, best known as QS21. One of the mechanisms of action of QS21 is its ability to stimulate cytokines, which help create the environment required to drive the immune response. To further enhance the activity

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of QS21, it is possible to combine them with other lipid components to form ISCOMs or cage-like structures containing the antigen of interest (Cox et al., 1998; Morein et al., 2004). Thus, ISCOMs act both as delivery vehicles and enhance immunity to the antigen incorporated into the ISCOMs. Currently, most ISCOM-based vaccines incorporate viral glycoproteins into the ISCOM. These vaccines can be delivered either parenterally or mucosally, and can induce both antibody and cellular responses (Takahashi et al., 1990; Morein et al., 2004). One of the disadvantages of ISCOMs is the poor loading capacity and the difficulty of incorporating nonmembranous antigens into ISCOMs (Reid, 1992). Similar to ISCOMs, liposomes have been used experimentally with excellent results. In addition to use with subunit vaccines, lipid-based delivery systems are also efficient in delivery of DNA-based vaccines (Zelphati and Szoka, 1996).

Early vaccines containing whole killed bacteria demonstrated that these vaccines induced significant reactions, which led to the suggestion that many of these components could be good adjuvants. Indeed, this was proven to be a correct assumption. Many of these bacterial products, whether they are LPS or various toxins (cholera toxin, Escherichia coli LT, etc.) or bacterial DNA generally induce cell signaling, primarily through Toll-like receptors (TLRs), and production of cytokines or chemokines. It is because of this activity, excessive stimulation can lead to adverse reactions. To overcome these excessive reactions, less toxic analogs of each of these bacterial components are being developed (Ulrich and Myers, 1995).

One of the least reactogenic bacterial components currently showing significant promise as an adjuvant is CpG (Krieg et al., 1999). These are specific synthetic oligonucleotide sequences that signal through TLR-9 receptors. Significant work in the last decade has identified specific CpG motifs that can stimulate both Th1 and Th2-like immune responses (Weeratna et al., 2000). Furthermore, they are active both with recombinant and conventional vaccines (Ioannou et al., 2002). In addition, they are active following in ovo delivery (Gomis et al., 2004).

Since many of the above adjuvants act through their ability to induce cytokines at the injection site, and since cytokines are critical for induction of immunity, numerous investigators have attempted to incorporate cytokines into vaccine formulation with limited success (Heath, 1995). The greatest concern with cytokines as adjuvants is the difficulty of maintaining the current concentration of the cytokine that will drive the immune response without overstimulation with toxic consequences. A recent report of using killed pseudomonas bacteria containing interferon gamma as an adjuvant demonstrated excellent adjuvanticity with no adverse effects (Gaertner et al., 2005). Thus, there is a high probability of eventually using such molecules as adjuvants.

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The use of vaccines in animal health is not restricted to the protection of morbidity and mortality of the hosts themselves, but they are regularly employed as key elements in public health programs. Among the first widely and successfully applied applications of vaccines in the animal health were for rabies, a principle zoonotic disease for a variety of domestic and wildlife hosts (see Chapter 33). Classical inactivated rabies vaccines have been used to control this devastating disease in domestic animals and wildlife targets have been addressed with attenuated live approaches and most recently and successfully with a recombinant vectored vaccine. Of perhaps even more profound potential impact on public health is in the control of H5N1 avian influenza, where the use of conventional and recombinant vaccines have been demonstrated to significantly decrease the spread and virus within infected flocks, thereby reducing the spread of the virus vertically into the human population. Other zoonotic diseases for which vaccines have real and potential impact on incidence in the human population are brucellosis in ruminants, Nipah virus and Norwalk Agent in swine.

Focus will be on those diseases for which the exposure to the zoonotic is typically through the food chain. These vaccines carry their own special issues in that the agent may not always be a principal disease in the host, rather a commensal, or part of the host natural flora. Campylobacter in poultry and E. coli O157 are typical examples of these, with the disease agent residing as part of the host normal flora and only becomes of concern when humans are exposed to contaminated food.

Safety of the water and food supply has clearly led to greatest improvements in public safety in the last century and a half. Statistics from the British home office indicate that in 1900, 140 per 100,000 in the population of the UK died annually from food-related illnesses. Improvements along the food chain reduced that number to approximately 0.4 per 100,000 by 1980, where it has remained constant since then.

Before moving on to animal diseases and the vaccines used to control them, it must be noted that the very vaccines used in food producing animals must...
in themselves not negatively affect the quality of the food. In the United States, production animals typically include beef, pork and poultry with minor inputs from the sheep and goat industry. The advent of modern vaccine technologies has provided tremendous benefits to large-scale production capabilities for these species and resulted in a positive economic impact with regard to cost of production for the primary producer and cost of product to the consumer. The use of vaccines in production animal medicine has translated into one aspect of the overall production scenario under the concept of “herd health management.” The herd health management concept transcends prevention of disease, animal housing and environment, nutrition, and management issues; all of which have contributed to the low cost, bountiful, high quality food supply that this nation has enjoyed for many decades.

There are issues routinely faced by producers with regard to the application of vaccine technologies to production animals. These include the efficiency of mass vaccination, the impact on food quality due to local reactions at the vaccination site, and the rare but serious issue of broken needles at the site of injection. The application of mass vaccination technologies has been most successful in the commercial poultry industry with products that are typically administered by aerosol exposure or via drinking water systems. These technologies have been more difficult to apply to swine and beef primarily because of the design of those production facilities and the formulations currently required to achieve effective vaccine response.

The issue of injection site reactions is primarily associated with swine and beef production because most vaccines are still administered with a traditional syringe and needle approach. These issues are being addressed by targeting specific “low meat value” sites for injections, particularly the lateral neck region. This preserves the integrity and value of the high quality meat cuts associated with the leg and loin regions. In addition, vaccine formulations have improved with better selection of less reactive adjuvants, lower dose volume and targeting efficacy with subcutaneous injections instead of requiring intramuscular deposition of the antigen.

The issue of broken needles is more difficult to address, and the potential for a break always exists during herd vaccination procedures. Though rare, the discovery of a needle in a meat product typically makes headlines and results in damage to the reputation and marketing potential of the processor. Some export customers, specifically the Japan market, set a zero tolerance for broken needles that result in the use of metal detectors to screen meat shipments. If a positive signal is detected in a single sample at the port of entry, the entire shipment is subject to rejection. This is a major expense to the processor. One relatively new technology that holds promise to address the broken needle issue as well as the injection site meat quality issue is the administration of vaccines via needle-free devices.

Several companies are now producing and distributing needle-free devices for the administration of vaccines to swine and cattle. These devices either utilize existing vaccine formulations or more preferably utilize formulations specifically designed to take advantage of the needle-free route of administration. One advantage exploited by needle-free technology is the ability to target the most efficient population of antigen-presenting cells (plasmacytoid dendritic cells) that are concentrated in the dermis. These cells are typically bypassed with a needle injection. Targeting dendritic cells has the potential to reduce antigen quantity, reduce or eliminate the need for adjuvants, and significantly reduce the volume of injection. All of these factors address both the injection site meat quality and broken needle issues.

Other vaccine technologies that have emerged in recent years include the use of recombinant virus vectors and nucleic acid-based vaccines. These technologies offer several potential advantages. They allow for the use of a vaccine that allows differentiation of infection from vaccine antigen exposure. This is critical in disease control situations, particularly when dealing with the introduction of exotic disease agents. In some instances, a recombinant vaccine will achieve a better immunologic response than can be obtained with the native antigen and the use of adjuvants may be reduced or eliminated. Nucleic acid vaccines offer a major advantage with regard to purity of the vaccine, elimination of adjuvants, the ability to overcome interference from maternal antibodies in young animals, and the opportunity to prime the immune system for a subsequent boost with a recombinant or native antigen formulation.

Prevention and control of infectious diseases in food animals will continue to be an issue faced by farmers and producers. Some agents are endemic in the respective species and others are highly contagious requiring fast diagnosis and rapid response to achieve control in the absence of vaccination. The other aspect of food animal vaccination that has come to the forefront in recent years is the potential for exotic agents to enter the United States either through natural or incidental routes or via intentional, terrorist introduction into the domestic herds or flocks.

One example of such a threat is influenza virus. The close proximity of people to swine and poultry in some Southeast Asia countries will continue to provide

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the opportunity for coinfection of hosts with multiple influenza viruses. The potential for reassortment of the virus genome and emergence of strains highly virulent to livestock and human populations will always exist. Vaccination is a leading defense against such a threat.

Vaccines for Food Borne Disease Protection

Salmonella and Campylobacter in Poultry

Among the most successfully and broadly applied commercial vaccines used in animal health are targeted to the poultry industry. An entire range of viral and bacterial vaccines are applied throughout the course of production and have allowed diseases such as Newcastle Virus and Marek’s disease, which would normally decimate a flock, to be strictly controlled. These vaccines may be applied in the drinking water in the poultry house, in aerosolized spray cabinets in the hatchery, and recently in mass administration in ovo devices. Poultry is also where we find the most consistently applied programs for food safety vaccines in animal health.

According to the Food-Net; bacterial pathogens with the highest relative incidence for causing bacterial food-borne illness in humans during the period 1996–2003 were campylobacter, salmonella, and shigella (Anonymous, 2005c). Most of the campylobacter and salmonella infections are acquired through the handling and consumption of contaminated poultry products. Although more than 2400 different serotypes of salmonella exist, only Salmonella enterica subspecies enterica (S. enteritidis, S. orien­enburg, S. Monteve­deo, S. New­port, S. typhimurium, S. An­atum, S. Der­by, and S. bredeny­) are responsible for gastrointes­tinal diseases in humans, and they are grouped as paratyphoid (PT) Salmonellae. S. enteritidis, S. typhimurium, and S. Monte­ve­deo are the most frequently isolated in poultry.

Usually an acute, self-limited gastrointestinal illness, characterized by diarrhea, fever, and abdominal cramps is most common presentation of campylobacter and salmonella infection in humans. Poultry infected with salmonella present minimal clinical signs and there are no clinical signs attributable to campylobacter in chickens of any ages. Both salmonella and campylobacter colonize chicken intestinal tract and large numbers of bacteria shed through feces.

Intestinal contamination is the main source of contamination in the processing plant. Campylobacter colonization (detectable levels) occurs at approximately 2–3 weeks of age and once established in a flock it spreads rapidly. The horizontal transmission between chickens has been well documented, the recent reports suggest that vertical transmission may play a role in spread of salmonella and campylobacter in poultry flocks as well (Gast, 2003; Shane and Stern, 2003).

Preharvest, harvest, and postharvest measures are used in an effort to reduce salmonella and campylobacter incidence in poultry. Preharvest measures include use of feed additives (antibiotics, prebiotics, probiotics, synbiotics, and short-chain organic acids), competitive exclusion, and vaccination. Several vaccines are available for S. typhimurium and S. enteritidis whereas there are no vaccines for campylobacter for chickens.

Salmonella infection is known to elicit strong humoral responses in chickens whereas, little is known about cellular immune responses. However, it is widely accepted that cell-mediated immunity plays a more important role than humoral immunity for tissue clearance of virulent strains while mucosal antibody responses and promyeolcytes seem to be important in intestinal clearance (Gast, 2003). Indeed, recent studies indicate oral inoculation of chickens with Salmonella typhimurium results in early expression of chemokines in spleen and liver, followed by increased expression of gamma interferon and increased number of CD4 and CD8 cells (Withanage et al., 2005). These responses correlate with Th-1 responses to systemic infections in other species. Responses to ST infection in the gut are not as clear, while in a one study an increase in proinflammatory cytokine expression in another there was no substantial increase in cytokine responses (Gast, 2003; Withanage et al., 2005). Vaccination has been widely used to control salmonella infections in poultry. Both live and inactivated vaccines have been utilized though the former have been shown to induce stronger and long-lasting immune responses (Gast, 2003; van Immerseel et al., 2005). However, neither vaccine type can induce complete protection against colonization of the intestines and therefore control programs remain and integrated approach of vaccination, proper preventative measures in the processing plant, handling in the transportation chain, and finally by the end consumer.

Currently no vaccines are available for the control of campylobacter in chickens though several experimental approaches have been shown to induce humoral immune responses and some degree of protection against challenge (Stern et al., 1990; Cawthraw et al., 1994; Khoury and Meinersmann, 1995; Noor et al., 1995; Widders et al., 1996, 1998; Rice et al., 1997; Shane and Stern, 2003). However, there is no evidence of complete protection against intestinal colonization in these studies (Stern et al., 1990; Cawthraw et al., 1994; Khoury and Meinersmann, 1995; Noor et al., 1995; Widders et al., 1996, 1998; Rice et al., 1997). One important
aspect of campylobacter colonization is that campylobacter is not usually detected in chickens less than 2–3 weeks of age under commercial broiler production conditions (Jacobs-Reitsma et al., 1995; Evans and Sayers, 2000). However, experimentally it has been shown that chickens less than 2–3 weeks of age are susceptible to campylobacter infection resulting in colonization of the gut suggesting that there is no genetic factors of age-related resistance to infection (Hald et al., 2001; Shane and Stern, 2003). The observed age-related resistance to colonization by campylobacter has recently been attributed to presence of high levels of maternal antibodies (Sahin et al., 2003) in which the decline of maternal antibodies associated with an increase in colonization rate. In addition, maternal antibodies were shown to opsonize campylobacter in in vitro experiments. However, it is well documented that chickens with maternal antibodies can be infected and colonized with campylobacter if higher doses of challenge inoculum were used (Noor et al., 1995; Hald et al., 2001).

Immune intervention for nondisease causing gut colonizing bacteria is indeed a challenge. One has to consider accessibility of specific immune components into gut content where the bacteria are located. This immediately excludes activity of immune cells in the content though there may be some activity at submucosal level. Although soluble-specific components such as immunoglobulins can be plausible to access to bacteria, their activity in the absence of other components such as complement and phagocytic cells, may not be sufficient to kill or eliminate the bacteria from the gut. One option is to prevent colonization in the first place using immune intervention, and in order to accomplish this, a better understanding of the factors leading to colonization may provide venues to explore vaccine development.

**E. coli in Cattle**

*E. coli* is an ubiquitous pathogen of both humans and animals. It is responsible for a wide range of diseases including enteric, septicemic, and urinary track infections. In recent years a significant increase of bloody diarrhea or hemorrhagic colitis caused by a particular strain of *E. coli* (*E. coli* O157:H7) has been observed in most industrialized countries and especially the United States. It has made headlines as hemolytic uremic syndrome, causing death, often in clusters, after consumption of undercooked ground meat, often referred to as “hamburger disease.”

The disease manifests in its most common form as severe bloody diarrhea accompanied by abdominal cramps with little or no fever which may resolve in 5–10 days (Anonymous, 2006). In a complication affecting mostly children and the elderly, 2–7% of infections will go to acute renal failure (hemolytic uremic syndrome) and may lead to death (3–5% overall death rate) or long-term complications.

*E. coli* O157 has an animal reservoir, the primary host being ruminants and specifically cattle. The great majority of cases can be linked back through the handling or consumption of contaminated meat, plants, vegetables, or water. Direct contamination on the farm or in other settings such as petting zoos is responsible for a significant number of cases. The incidence in the United States is estimated at 73,000 cases per annum, including 61 deaths (Anonymous, 2005b, 2006). The strain is highly pathogenic with as little as 1000 bacteria capable of causing the infection.

This makes infection by *E. coli* O157 one of the most frequently reported and severe food-borne illnesses in developed countries. The human disease, its etiology and management has been reviewed recently (Tarr et al., 2005).

While the genus and species *E. coli* is a very important pathogen of all animal species causing mainly enteric diseases such as neonatal diarrhea caused by enterotoxigenic *E. coli* (ETEC) in calves and piglets, *E. coli* O157 is not pathogenic for cattle. The bacterium colonizes the intestine of the ruminant very efficiently, causing a nonsymptomatic infection. The bacterium is then shed, often intermittently but in high numbers in the feces. Fecal contamination of the meat in the packing plant, bedding, water, fruits, or vegetables on the farm leads to human exposure. The asymptomatic infection is very widespread in many herds across the country. Within a herd, the individual rate is highly heterogeneous from less than 10% to the majority of animals shedding in the environment. Importantly some particular animals shed at very high levels (Matthews et al., 2006).

The genus and species *E. coli* is a very diverse group that comprises approximately 175 O antigen serotypes (e.g., O157). The O antigen is carried by the polysaccharide side chain of the LPS, a component of the bacterial outer membrane. Significant variation is also noted at the chromosomal level (Anonymous, 2005a). More importantly *E. coli*, for the purpose of this discussion are characterized by their virulence factors. Attachment: *E. coli* O157 is part of a group of strains called enteropathogenic *E. coli* (EPEC). Their attachment to the enterocyte causes a lesion that is characterized as effacing because they destroy the cytoskeleton (attaching/effacing *E. coli*). The molecular mechanism has been well described and necessitates at least four major proteins: Esp A, Esp B, Tir, and Intimin. Intimin (a bacterial outer membrane protein) and its receptor

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Tir are the attaching molecules. Over 33 virulence factors have been described in this complex process (Deng et al., 2004). An important finding for the understanding of disease transmission, epidemiology, and vaccine studies was the description of preferential adhesion sites in the rectum of the cattle (Naylor et al., 2003). Attachment is a key component of colonization in cattle and the first step of the disease in humans. Toxins: the toxic manifestation of the disease is caused by the secretion of two toxins, called Shiga toxin 1 and 2. The genes for these toxins are carried by bacteriophages. These toxins are also called verocytotoxins and the E. coli that secrete them verotoxigenic E. coli (STEC or VTEC). They are implicated in the apparition of the hemolytic uremic syndrome in humans (Karch et al., 2005). The resistance of cattle to the effect of these toxins provides an explanation for the absence of disease manifestation in these animals.

Many approaches have been suggested for the development of vaccines using many of the newer technologies as well as more traditional approaches. The target of the vaccination of cattle is the reduction or elimination of infection and excretion, and not disease prevention (as there are no symptoms). This is a particularly difficult target as it must reach a colonization site on the mucosal surface and eliminate or prevent colonization by bacteria that have developed exquisite solutions to parasitize this ecological niche. The most advanced approach is based on the use of E. coli secreted antigens that contain several of the proteins involved in the attachment (e.g., Esp and Tir). This approach uses traditional technology but is built on the very precise understanding of the pathogenic mechanisms. It is administered three times by the subcutaneous route. It has led to significant decreases in shedding levels both in challenge models and field trials (Potlier et al., 2004). In a more recent test, however, performed under field conditions, the vaccine failed to induce a reduction in prevalence of infection (van Donkersgoed et al., 2005).

Plant (tobacco cell) produced intimin caused a decreased duration of shedding when the vaccine was administered parenterally followed by a booster by the oral route (Judge et al., 2004). Antibodies against intimin passively administered to piglets provided significant protection against a challenge (Dean-Nystrom et al., 2002). A live modified strain of E. coli containing a truncated intimin molecule also provided protection against clinical disease in an A/E E. coli model in rabbits (Agin et al., 2005). Finally genetically modified toxoids of the shiga toxins will provide both neutralizing antibodies and protection against a toxin challenge in an homologous fashion leading to the conclusions that Stx1 and Stx2 are not cross protective (Wen et al., 2006).

It should also be noted that several other, nonvaccinal approaches have also been proposed to reduce infection at the farm level including nutrition and probiotics. The principal challenges are twofold: first, increase the intrinsic efficacy of the vaccination protocol through optimized formulation or administration regimes, and second, demonstrate the overall efficiency of on farm interventions strategies including infection in reducing the risk for humans. While this has not yet been demonstrated there are many reasons to believe that reduction at the source would provide significant benefits (Matthews et al., 2006).

Considering the highly sporadic as well as the food-borne nature of the disease there is little interest in developing a vaccine for humans. In humans, especially in the light of the increasing levels of antibiotic resistance observed in these strains, improving the treatment tools available, including immunological tools, will be a priority.

**Streptococcus suis**

Many streptococcal infections have been described in humans. Infection by S. suis is a rare zoonotic disease, usually involving specific risk groups consisting of individuals in close occupational contact with infected swine: farmers, slaughterhouse workers, and hunters (of feral swine). Recently, a rare occurrence of a large cluster of patients with a high level of mortality was observed in China (Huang et al., 2005; Normile, 2005). A small number of patients were exposed to the infectious meat (as opposed to the animals or their carcasses). The investigation did not point to a new variant of the bacteria. Thus, the human disease caused by S. suis remains a relatively rare but severe zoonotic disease and is not properly speaking a food-borne infection (as opposed to E. coli O157, salmonella or listeria). Clinical manifestations are most frequently meningitis (3/4 cases). Sepsis and other very diverse manifestations, including endocarditis, may be observed. Deafness is a very common long-term complication of this streptococcal meningitis (Maclnnes and Desrosiers, 1999). Death can occur but the disease is rarely fatal.

The S. suis infection in animals is widespread in pig populations across the world. It most frequently colonizes the upper respiratory tract of the swine without causing disease. It can, however, induce diseases either as a primary pathogen (perhaps because of increased virulence of certain strains) or become pathogenic secondarily to another factor: stress, immunodepression, concurrent infections (e.g., viral infections). The disease manifests in many different forms: death, fever, lameness, septicemia, and endocarditis (Martinez et al., 2002).
The disease occurs primarily in swine after they enter the fattening barn (beyond 12 weeks of age). The disease has a very significant impact on the economic performances of the herd. The agent *S. suis* is a gram-positive cocci and part of the broad genus *Streptococcus*. A very important feature of the organism is its significant diversity. At the serotype level, 35 different capsular types have been described. Serotype 2 is the most common isolate but its frequency varies significantly from country to country and between North America and Europe. Serotype 1/2 is the next most frequent isolate (Martinez et al., 2002). At the genetic level, significant diversity is observed between herds and even within herds. Interestingly, the pathogenic strains emerging within a herd seem to separate more distinctly from the isolates obtained in healthy swine (Berthelot-Herault et al., 2005). At the molecular level, differences are also observed in expression of putative virulence factors (Smith et al., 1996).

Despite best efforts to understand, the exact mechanisms of pathogenicity of the virulent strains as well as the ability to predict the virulence of isolate strains based on molecular models remain elusive. Various proteins, such as muramidase-released protein (MRP), extracellular protein factor (EPF), and a hemolysin (suilysine) have been suggested (Martinez et al., 2002). Mutants lacking these genes did not demonstrate attenuation (de Greeff et al., 2002). It has been suggested that the simultaneous presence of the three factors could make strains more virulent. More recently additional proteins have been proposed as virulence factors: fibronectin-binding protein (Gruening et al., 2006), and arginine deiminase system (ADS) (Okwumabua and Chinnapapakkagari, 2005). A 38 kDa protein of unknown function was found reactive with serum from infected animals and demonstrated protection (Llull et al., 2001). The capsular polysaccharides of *S. suis* as with other streptococci may also play an important role (Busque et al., 1997).

It follows that the molecular basis of virulence remains unknown and that antigenic variation exists within the putative virulence molecules identified that vaccines for this disease have not been able to use modern technologies, such as recombinant expression, which require a defined antigen. Essentially two types of vaccines have been made available, live attenuated strains (Wisselink et al., 2002; Haesebrouck et al., 2004) or inactivated whole cell bacterins. Neither has met with significant success. The majority of the market remains occupied by autogenous bacterins. Autogenous bacterins could provide a means to avoid the significant antigenic variation of the strain by providing a vaccine derived from the particular strain infecting the herd. Considering the variability of strains within herds, one should expect to change strains regularly to maintain efficacy. *S. suis* induced disease remains a very significant and costly disease for the swine industry. In addition, it is responsible for an infrequent but severe and potentially deadly zoonosis in humans. The lack of a detailed understanding of the virulence has precluded the development of efficacious modern vaccines.

**Cryptosporidiosis in Cattle**

Cryptosporidiosis is a disease of humans, livestock, companion animals, wildlife, birds, reptiles, and fish infected with one or more species of the protozoan parasite *Cryptosporidium*. The clinical outcome of the disease varies greatly from species to species and is also dependent on virulence factors on the parasite and the host immune response to them. In immunocompetent humans, the infection can be asymptomatic even though the parasite replicates and is excreted. When an illness develops the outcome ranges from mild enteric symptoms without diarrhea to a profuse watery diarrhea. Additional symptoms can include abdominal cramps, vomiting, low-grade fever, fatigue, anorexia (Ungar, 1990; Mac Kenzie et al., 1994). The disease is self-limiting and typically resolves within 2 weeks. Organisms usually infect the lower small intestine, but in immunosuppressed patients, it can extend from the esophagus, throughout the intestinal tract to the rectum. In some cases the appendix, gall bladder, bile ducts, pancreas, and occasionally even the respiratory tract have been infected (Ungar, 1990). In humans, the most frequently found organism, designated *Cryptosporidium parvum* genotype 1 (or human genotype), appears host-specific, infecting, and spreading only among humans. This organism is now recognized as a separate species, *Cryptosporidium hominis* (Morgan-Ryan et al., 2002). Another organism, designated *C. parvum* genotype 2 (or cattle genotype), is zoonotic, capable of infecting and spreading among many mammalian hosts. The main reservoir of *C. parvum* genotype 2 is cattle. Virtually all cattle are exposed to the parasite during the first few months of life. Calves will shed large number of oocysts (up to $10^7$/g of feces) for approximately 10 days. An active immune response will develop and control the infection. However, evidence suggests that most infected animals will carry *C. parvum* for life, shedding few oocysts intermittently. Collectively, data suggest that vast numbers of oocysts are passed into the environment and that cattle contribute significantly to these large numbers. Zoonotic transmission may be important in cases of direct exposure to cattle feces (farms, undeveloped countries) or in waterborne outbreaks (developed countries).

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Immunocompromised individuals frequently are observed with the cattle genotypes of Cryptosporidium (Pieniazek et al., 1999; Morgan et al., 2000). In the United States and Australia, most cases appear to be of human origin: of 14 food and waterborne outbreaks, 10 were of human genotype, 3 of bovine genotype, and 1 with both organisms. However, in Europe the bovine genotype is responsible for more infections than the human genotype (McLauchlin et al., 2000; Alves et al., 2000; Guyot et al., 2001). In the United Kingdom, zoonotic transmission has been considered to be the major route of infections in humans (Hunter et al., 2003).

As the sole mechanism for transmission, oocysts have evolved to be dispersed and survive in harsh environments for long periods of time. The oocysts are unusually resistant to natural stresses and many man-made disinfectants. To control infection in animal populations the current best strategy is to move animals to clean areas. For human populations, disinfection procedures are used.

Because all infections with Cryptosporidium are initiated by ingestion or inhalation of the oocysts, measures to prevent or limit the spread of infection must be targeted to eliminate or reduce oocysts in the environment. Despite the fact that more than 200 drugs have been tested for activity against C. parvum, in vitro or in vivo, there is still no effective treatment (Rehg, 1993; Fayer and Fetterer, 1995; Woods et al., 1996; Armson et al., 1999). A cattle vaccine against C. parvum might reduce the environmental contamination and consequently the frequency of waterborne cryptosporidiosis outbreaks in humans. Since cattle may be exposed to C. parvum from their day of birth, before their immune system is mature, an active vaccination is not likely to mediate any significant protection. Thus, vaccination against C. parvum is being attempted for the purpose of generating hyper-immune colostrum-containing antibodies that may be effective in passive immunotherapy against cryptosporidiosis in calves. Calves fed hyperimmune colostrums, prepared against oocysts, and challenged had significantly reduced patent periods, oocyst shedding and days of diarrhea compared to calves fed normal colostrums (Fayer and Fetterer, 1995). A number of recombinant C. parvum surface or internal antigens have been expressed by DNA-cloning technology. Immune colostrum specific for several recombinant C. parvum proteins have demonstrated efficacy in murine and ruminant models against cryptosporidiosis. The most promising candidate proteins for vaccine development are surface (CP15/60, CP15, and P23) and micronemal proteins (TRAP-C1, GP900) of sporozoites. Both CP15/60 and P23 are detected by Western blot analyses of cryptosporidium proteins using fecal antibodies and serum from cryptosporidium-infected humans and animals (Lumb et al., 1988; Mead et al., 1988; Hill et al., 1990; Reperant et al., 1994). CP15/60 is defined by the monoclonal antibody (MAb) 5C3. CP15/60 IgA MAb provided protection against infection when administered orally to suckling mice (Tilley et al., 1991). The comparison of two recombinant forms of CP15/60, one produced in a prokaryotic expression system and the other in a eukaryotic expression system, indicated that the most potent immune response was obtained using the eukaryotic form, possibly due to posttranslational modifications (Sagodira et al., 1999). It has been reported that preparturient cows immunized with DNA encoding CP15/60 in the mammary gland produced sporozoite-specific antibodies in the colostrums (Jenkins et al., 1999). Immune colostrum induced a 50% reduction against C. parvum development in mice.

P23 is defined by the MAb C6B6 (Mead et al., 1988). IgG1 (Perryman et al., 1996) and IgA (Enriquez and Riggs, 1998) MAbS reactive with P23 were shown to have significant anticyryptosporidial activity in mice. Perryman et al. (1999) reported that late gestation Holstein cows immunized with rC7, an immunodominant epitope of P23, developed specific antibodies. Calves receiving immune colostrum were significantly protected against diarrhea and were shedding significantly fewer oocysts than control calves. Antibodies directed against GP900 were shown to inhibit sporozoite in vitro, suggesting a direct role of the molecule in host–parasite interaction (Barnes et al., 1998). The reported involvement of TRAP-C1 homologs in substrate-dependent locomotion of sporozoite (Sultan et al., 1997), as well as in host cell attachment and invasion (Muller et al., 1993), suggest that TRAP-C1 should be considered as a promising vaccine candidate against cryptosporidiosis.

In summary, use of vaccines as a public health tool in the control of food-borne illnesses is a complex and important field. Reducing bio burdens in food producing species, where the target species normally is carrying, or shedding at an already very low rate, or where the human pathogen is part of the animal’s normal host flora, is a significant challenge for current vaccine technologies. In the case of developed market places, the threat of food-borne illness is already at impressively low levels, though public expectations are that they will drop still further. Therefore, it must be understood that for successful control, these programs must be components of fully integrated programs, including husbandry, meat processing, and handling at the retail and customer levels.
OPTIMIZING VACCINATION PROTOCOLS

Over the last decade there have been tremendous advances in our understanding of the immune systems of our major domestic animal species. Nonetheless, we still lag behind the depth of knowledge that exists for the immune systems of mice and men. This has not stopped successful vaccination programs from being developed. Much of vaccinology that is practiced in veterinary medicine, and the animal health industry in general, is empirical. It is driven by the realities that exist in raising production animals or working in veterinary practice, where making a living depends on keeping the animals healthy. It is an industry where vaccines are like insurance policies—protection from events that one hopes never happen, and where tenths of pennies can make or break the economics of a business. When used optimally, vaccines have been shown to prevent disease, reduce the need for pharmaceutical intervention (e.g., reduced antibiotic usage) and improve the health and welfare of animals (Knott et al., 1985; McIlroy et al., 1992).

Then there is the definition of protection. The perception that vaccines provide sterilizing immunity, where the disease agent does not establish an infection, while widely held, is generally unfounded and largely unrealistic. It is debatable if such immunity is even in the animal’s best interest as exposure to, and transient infection with, pathogens helps keep the immune response active. Vaccines usually provide protection on one of three levels. First, at the lowest level, protection is simply a reduction of clinical signs associated with the infection. The reduction may be decreased disease severity and/or duration, or simply a delay in the onset of the disease. The infection still becomes established and in most cases the animal will be contagious to other in-contact animals, although there may be a reduction in the level of shedding of the infectious agent. Such immunity is not usually capable of stopping an outbreak from progressing.

Second, vaccines may induce immunity that can prevent clinical signs associated with the infection, where the animal shows no overt signs of being infected. This level of protection may actually be associated with a reduction of shedding of the challenge organism, which can reduce spread during a disease outbreak. Unfortunately, there is no general rule to rely upon in this regard, as reduction in shedding tends to be specific for the strains involved—both of the vaccine and the infection. It must be recognized that vaccine protection that prevents clinical signs of disease being identified can lead to a false assumption that the pathogen is not present in a vaccinated population. In such circumstances, especially where economic pressures exist, there may be a tendency to decrease the use of vaccines with disastrous results.

Third, vaccine protection may prevent the infection from becoming established. While this is what many people think all vaccines do, it is a claim that rarely will be found on product labels. The United States Department of Agriculture (USDA), which regulates vaccines in the US, requires that for this claim to be granted the vaccine must be demonstrated to be able to “prevent all colonization or replication of the challenge organism in vaccinated and challenged animals” and “this must be supported with a very high degree of confidence by convincing data” (DeHaven, 2002).

The USDA recognizes these varying levels of protection in the way they allow label claims. The above three categories are often written on labels as “aids in disease control,” “for the prevention of disease,” and “for the prevention of infection.” The vaccine manufacturer has generated a great deal of data, which has been independently reviewed by a government agency, that become the basis for the label claims. Expecting a vaccine to do something that is unrealistic given the data and a label indication is perhaps the greatest cause of reported “vaccine failures.” To paraphrase the old adage, if in doubt always read the label.

While on the topic of the level of protection, it should be noted that vaccines can provide efficacy, or protection, that may come from both direct and indirect effects. The direct effect can be relied upon to provide individual immunity—that is the animal that receives the protective dose of vaccine benefits directly. Additionally there may be indirect protection, or herd immunity, that results from vaccination of sufficient numbers of animals in a given population. Herd immunity protects nonvaccinated animals within a population, whether a flock, herd, or group. It is the result of the reduction of the ability of a disease to transmit through the vaccinated individuals. For such immunity to occur, the vaccine should be capable of reducing the shed of the disease causing agent following infection of a vaccinated animal.

The challenge in developing an optimal vaccination program is in dealing with the great diversity that exists within the animal world, and as such there probably is no single optimal program for all occasions. The multitude of factors that need to be considered in choosing a vaccination program are complex—species/breed differences, and within these the genetic selection that has occurred, some of it for resistance to select diseases; the diverse husbandry practices employed, some dating back centuries; some mandated by governments; and the disparate

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prevalence of diseases has resulted in the adoption of tailored vaccination programs. Such programs generally are designed to consider the risks (e.g., disease prevalence and exposure likelihood), husbandry (e.g., animal-to-animal contact, nutrition), and environmental factors (e.g., wildlife vectors, weather) and of course the animals (e.g., age, previous disease experience).

The conditions that influence the tailored vaccination program change rapidly over the life of the animal. Indeed the time period over which protection is required varies greatly. Chickens, unless they are breeding animals, normally go to market by 6 weeks of age, pigs become pork when just 6 months old, and cattle and sheep frequently become someone’s dinner by 18 months of age. Even our beloved pets rarely live beyond their teen years. As we touch upon some of the more unique challenges that exist in optimizing vaccination protocols and practical veterinary vaccinology, the following pages are divided up by life-stage.

Prenatal Vaccination

Strengths

The protection that is provided by the mother to the unborn animal is critical in ensuring the delivery of healthy offspring. In livestock, producing new animals is what generates the income for the farm and as a result the use of vaccines during pregnancy has been widely adopted to enhance this protection. In utero, the fetus is protected from the environment and its pathogens. This protection is provided both physically and immunologically by the dam. The physical part is easy to see—fetal membranes and fluids shield the fetus behind a muscular uterine wall inside a multilayered abdomen. The immunological protection likewise is on several levels—innate and acquired immunity of both the dam and fetus—but unlike physical protection, immunological protection may continue beyond the in utero period into early life.

The immunity of the mother may prevent infections from reaching the fetus or may enable the dam to provide immune cells or antibodies to the fetus. Vaccination of the mother is commonly practiced to accomplish both. In species where litters are common, for example, pigs, vaccination of the dam is an effective way to protect the entire litter with a single vaccine dose. Even in single offspring dams, for example, cattle, vaccination is recognized as providing added insurance to getting a healthy newborn. It can also be an effective way for neonatal animals to gain early immunity against the plethora of infectious challenges they face in the first weeks of life. There is evidence that active vaccination of the fetus may occur with some antigens crossing the placenta and stimulating fetal immunity.

Sows, with a gestation period of 113 days, are often vaccinated near the time of breeding and again 2 weeks prior to farrowing. Such vaccinations have proven effective in increasing litter size and growth performance (Descamps et al., 1990). The vaccination in early pregnancy is aimed at protecting the litter by stimulating active immunity in the sow against common pathogens that cause abortion, metritis, or stillbirths, for example, Leptospira spp., parvovirus, Erysipelothrix rhusiopathiae. These vaccines, typically given by injection, induce an active immune response in the sow, preventing the viremia or bacteremia from occurring and thus protecting the unborn piglets.

Vaccinations in late pregnancy are aimed at boosting immunity to maximize the level of passive immunity (transplacental and colostral antibodies) that a dam passes on to her offspring. Typically these vaccinations are targeted at neonatal diseases causing enteric (e.g., rotavirus, E. coli) or respiratory problems (e.g., Pasteurella multocida, Bordetella bronchiseptica). Piglets born of vaccinated sows generally have performed significantly better than those of nonvaccinated sows when evaluated on a variety of parameters such as growth and average daily weight gain (Rüsing et al., 2002).

Similar vaccination protocols are used in dairy cattle. As with the sows, vaccination during pregnancy prevents abortion and helps ensure the birth of a healthy calf. In turn, the successful birth of a calf also ensures another lactation cycle, which after all is what a dairy is all about.

The late pregnancy vaccinations are expected to generate high levels of circulating antibodies that may be passed onto the newborn animal via colostrum. Colostrum is the first milk that is produced by mammals for their babies. Typically this is produced for 2–4 days and its consumption by the newborn is seen as very important for a variety of reasons. Research has shown that maternal antibodies may be concentrated in the colostrum to levels higher than that found in the dam’s circulation (Pfeffer et al., 2005). Through the use of dam vaccination, the specificity and level of these antibodies can be manipulated to tailor protection that is most appropriate for the geographical region or environment where the birth occurs. Maternal antibodies can be directly absorbed by the newborn and enter into circulation providing passive immunity against otherwise fatal infections. There is now good evidence that cells and cytokines of the dam’s immune system also pass via colostrum to the newborn. These components of colostrum significantly aid the development of the neonatal immune system.
result of the transient febrile reaction decreasing implantation of the embryo. Vaccination in late pregnancy, close to parturition, tends to be less effective. Around the time of parturition, there is a well-described phenomenon of reduced immune system activity (Houdijk et al., 2000). Vaccination may not deliver the expected efficacy if given under these suboptimal conditions.

Even when vaccines work well, inducing strong immune responses in the dam, there may be a downside. Passive antibodies, whether transplacental or colostral, can inhibit active immunization of the newborn. In some cases this blocking effect can last months. Unfortunately, the level of passive immunity that blocks vaccine strains may not be sufficient to protect against virulent pathogens. This “gap” between lack of protection and earliest ability to vaccinate has been well described for canine parvovirus (McGavin, 1985). Vaccination of the dam does not eliminate the problem and can actually shift the problem to a time period when the risk of exposure to disease is greater, for example, young animals taken to market.

Opportunities

As Albert Einstein said “In the middle of difficulty lies opportunity.” So it is with prenatal vaccination where every challenge becomes an opportunity and vaccine producers are continually seeking safer, more effective vaccines.

The diversity of approaches to improved safety is enormous but ever increasing regulatory hurdles, rather than true safety issues, make more and more vaccines carry the warning “Not for Use in Pregnant Animals.” The true benefits of prenatal vaccination are to enable consistent active immunity to develop in the unborn animal. Many neonatal diseases would disappear, if active immunity was present at birth. The resulting increase in health benefits would be significant. Such vaccines are not readily available at present. In ovo vaccination of chickens being perhaps the exception, although even there not all pathogens can be covered by safe and effective in ovo vaccines.

Challenges

Of course, there are downsides to prenatal vaccination. Not all vaccines are safe to be given to pregnant animals. The administration of live or adjuvanted vaccines during pregnancy carries the risk of inducing a transient febrile reaction and subsequent abortions or stillbirths that may follow a fever. Typically, live bovine herpesvirus vaccines in naïve cows have been associated with abortions due to fetal infection with the vaccine strain and similarly live porcine reproductive and respiratory virus vaccine has been found to reduce litter size and cause abortions in sows. The choice of which vaccines to use in pregnant animals should, as always, come after reading the label and understanding the risks involved.

The timing of administration can play a large part in influencing the safety and the effectiveness of the vaccine. Vaccination soon after breeding has been shown to reduce the conception rate. This is probably a direct result of the transient febrile reaction decreasing implantation of the embryo. Vaccination in late pregnancy, close to parturition, tends to be less effective. Around the time of parturition, there is a well-described phenomenon of reduced immune system activity (Houdijk et al., 2000). Vaccination may not deliver the expected efficacy if given under these suboptimal conditions.

Some maternal antibody crosses the placenta to protect the unborn animal. The level of this transfer depends on the nature of the placenta. Typically of our domestic species, dogs and cats provide the greatest in utero transplacental transfer of antibodies, while pigs, horses, and cattle provide the greatest colostral antibody transfer (Tizard, 2004) (Table 16.1). Even poultry get the benefits of prenatal vaccination. In ovo vaccination is now common. Vaccines of various types are injected directly into the egg at between 9 and 14 days of age, depending on the organism being used as a vaccine. The whole process is automated enabling millions of eggs to be vaccinated in a day. The end result is that at hatching the young birds are protected from select pathogens. Research has shown that in ovo vaccination may be more advantageous than posthatch vaccination (Rautenschlein and Haase, 2005).

### Table 16.1 In utero transplacental transfer of antibodies

| Species               | Type of placentation | Number of layers | Ig via placenta | Ig via colostrum |
|-----------------------|----------------------|------------------|----------------|-----------------|
| Pig, horse, donkey    | Epitheliochorial     | 6                | 0              | +++             |
| Ruminants             | Syndesmochorial      | 5                | 0              | +++             |
| Dog, cat              | Endotheliochorial    | 4                | +              | +++             |
| Primates              | Hemochorial          | 3                | ++             | +               |
| Rodents               | Hemendothelial       | 1                | +++            | +               |

Source: Tizard (2004).
Likewise improved efficacy would be highly desirable to help overcome the periparturient relaxation of immunity that occurs in dams. Such increased efficacy would result in greater consistency of transplacental and colostral immunity transfer. The development of products that could enhance the transfer of passive immunity to the neonates would be greatly welcomed.

Neonatal and Juvenile Vaccination

**Strengths**

After leaving the protection of the uterus, the neonate begins dealing with a plethora of new antigens, both environmental and infectious. Unfortunately, most of our domestic species are born with relatively immature immune systems inclined toward a Th2-mediated response (Morein et al., 2002). It is during these early weeks of life after birth that newborn animals are the most vulnerable and this is where vaccines can be most beneficially used. The husbandry practices that we use with many of our animals species often entails mixing of animals from a variety of sources, for example, the combining of 3 week old pigs from perhaps hundreds of litters when they move to the nursery, or mixing of hundreds of puppies at 6 weeks of age when they are transported to the pet shop. The combining of very young animals from diverse disease backgrounds when their immune systems are not yet fully developed and not yet primed by vaccination is a huge challenge for maintaining good health. The need for vaccination before, during, and after such commingling of animals is essential to reduce the suffering that disease outbreaks can cause.

No longer are we trying to vaccinate litters, or trying to vaccinate through the dam. The animal is now an individual, albeit perhaps still part of a larger group. It is possible to directly administer vaccines to the animal in accordance with an assessment of its individual needs. As most animal breeders expect to make money from selling the offspring, each individual animal has a monetary value. This enables a fairly objective view to be taken of risk benefit analysis—the benefits arising from the use of vaccines versus the cost of disease. Most animal breeders have tailored vaccination programs to the needs of their animals, with multiple injections of different vaccines being common. Typically neonatal animals receive multiple vaccinations covering the major diseases that their owners expect they may become exposed to during their lives. A kitten could receive eight different vaccines on two or three separate occasions, although many are given in combination—feline herpesvirus, feline calicivirus, feline panleukopenia, feline leukemia virus, rabies virus, and Chlamydia are common; with feline immunodeficiency virus, *B. bronchiseptica* and *Toxoplasma gondii* also available for cats. Puppy vaccinations often involve combinations containing protection against 11 different disease agents, for example, canine parvovirus, distemper, canine adenovirus types 1 and 2, canine parainfluenza, rabies virus, four different *Leptospira* species and *B. bronchiseptica*. These vaccines are usually given 2 or 3 times at intervals of 3–4 weeks starting at 8 weeks of life.

One consequence of now having to vaccinate the individual animals is that in large numbers the task can prove to be daunting. The labor and logistics of vaccinating 20,000 chickens or 10,000 piglets are quite involved. Fortunately, vaccines are available for mass administrations that are optimal for dealing with large flocks or herds. Several options exist with aerosol spray and drinking water being the most common. The vaccines typically are live agents that are diluted in water containing a stabilizer to prolong the in-use viability of the agent. Aerosol vaccination can be tailored to the particular vaccine by adjusting the droplet size that in turn alters the location within the respiratory tract where the droplet eventually lodges (Gough and Allan, 1973; Gomez and Correa, 1978). While originally developed for poultry vaccination, aerosol vaccination has been used in swine (Nielsen et al., 1990) and cattle (Kita et al., 1982) with the same factors as for poultry needing to be considered—droplet size, type of diluent, environmental conditions (e.g., wind and temperature) and strain, and dose of the vaccine organism.

Drinking water, as a vehicle to mass vaccinate animals, while pioneered for poultry, has been used in other species. The addition of the vaccine to regular tap water can be an effective way to vaccinate provided the chlorine is neutralized by the addition of 0.25% skim milk (Kim and Spradbrow, 1978). Like aerosol vaccination the number of doses required for adequate protection will be contingent on the type of agent in the vaccine and the age of the animal.

**Challenges**

Vaccination of very young animals poses numerous challenges but none as difficult as that of overcoming the interference of maternal immunity. The maternally derived antibodies that most young are born with, or acquire through colostrum consumption, can be protective but as the level wanes there is a period where it is no longer protective. What proves to be worse for the health of the young animal is that these residual antibodies may block effective immunization.
The traditional solution is to administer multiple doses around the period where the maternally derived protection is waning. As the timing cannot be accurately known even this does not guarantee that there is not a susceptible period. As numerous papers testify to, during the canine parvovirus epidemic of the 1980s, many puppies failed to be protected despite receiving vaccinations at weekly intervals (McGavin, 1985). Similar situations exist in other domesticated species, with the result that many doses of vaccine given to young animals are ineffective, not because of a problem with the vaccine, but with the timing of the delivery.

The use of injections to administer vaccines has proven a very effective delivery method but this too has caused problems. Transmission of disease through the use of contaminated needles or vaccine vials has been documented (Witter and Fadly, 2001; Niskanen and Lindberg, 2003). While good hygiene should prevent such problems, the field conditions under which animal vaccinations frequently occur are often not conducive to maintaining sanitary conditions. The presence of blood, mud, feces, and flies is not unexpected. In meat-producing animals the use of needles to deliver products can lead to another problem—broken needles in meat products. The inability to find a broken needle quite often results in the condemnation of the carcass to keep it out of the food chain. While this is a problem with any product, including medicines, delivered by injection, vaccines represent the most common reason for sticking a needle into a meat-producing animal.

The relative immaturity of the newborn's immune system is another challenge that must be factored into vaccination of the neonatal or juvenile animal. The bias for a Th2 response in the neonate (Morein et al., 2002) is not always the preferred type of immune response for protection against disease. The use of adjuvants that drive Th1 immune responses may be required to confirm effective protection. It has been demonstrated that neonatal calves may be able to develop a cell-mediated response comparable to adults, although antibody responses are markedly lower than seen in adult heifers (Nonnecke et al., 2005). This is in normal, healthy, young animals but there are a variety of situations where the young immune system is even less ready to respond to vaccination.

In colostrum-deprived animals the maturation of the immune system may be delayed because of a lack of exposure to specific components in this first milk. Vaccination of colostrum-deprived animals should be undertaken with care to recognize that their responsiveness may be compromised compared to that of animals receiving colostrum. Likewise, stress has been associated with poor immune responsiveness and there is little doubt that the newborn goes through many stresses in the first few weeks of life. There, of course, are the routine environmental stressors such as temperature, air quality, etc., but often more critical for animals is the dietary change including weaning at an early age that is routine in most of our domestic species. Pigs, for example, may be weaned at 2-3 weeks of age, and it has been shown that the composition of their starter diets, in particular the levels of protein, lysine, methionine, and threonine, may be critical in the development of their immune system (YongQing et al., 2001; Nonnecke et al., 2005). The simple event of handling the young animals to enable vaccination has been shown to be stressful and potentially reduces the response to the vaccine. Even a car trip to the veterinarian's office may have the same stress-related effect on the immune system. Unfortunately, when simply looking at a young animal it is not possible to determine its responsiveness to vaccination. It is nearly always a guess, which often results in the administration of multiple doses of vaccine to cover the poor responders.

**Opportunities**

The largest opportunity to improve vaccination of neonatal and juvenile animals is undoubtedly for vaccines that would consistently work in the face of maternal immunity. The health and financial benefits would be immense if we could remove the need to revaccinate young animals because active immunity was blocked by residual but waning passive immunity. The convenience of knowing that you could take your pup or kitten to the veterinarian once to have them immunized, or the need to only vaccinate a herd of 10,000 piglets once, are immense. Likewise sustained release, or long-acting vaccines could facilitate less handling of the animals and hence less stress. Such long-lasting vaccines would increase the likelihood that the vaccine antigens are present when the animal is able to respond to them.

The ability to test for the responsiveness of an animal to a vaccine before actually administering the vaccine could have huge practical benefits for animal owners, especially livestock producers who have large numbers of animals to vaccinate. Currently laboratory testing for antibody levels is possible, but the cost and time make this relatively impractical. It has proven cheaper to vaccinate and hope for a response than to bleed and test. The ability to determine, perhaps via a simple animal-side test, whether the animal has responded to vaccination would be beneficial.

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Considerable progress has been made in avoiding needle delivery of vaccines, and not just through alternative routes such as aerosol and drinking water. In the last 10 years, the development of needleless injectors has been an active area of research. These devices use a variety of approaches but most commonly involve high pressures to force the liquid vaccine directly through the skin without a needle. While most of these injectors are effective to some extent, the challenges of dealing with superficial skin contamination and the problem posed by the presence of a dense hair coat to obtain good skin contact for the injection, have hampered their adoption. It is likely that reliable and highly effective needle-less injection devices will be available within the next few years.

**Adult Vaccination**

**Strengths**

Many of the issues that surround vaccination of neonatal and juvenile animals tend to resolve with age. Adulthood generally brings a mature immune system including fully functioning innate immunity. The time of this varies by species but, in general, by 1 year of age most or our domestic species are nearing breeding age and can be considered young adults. As stated earlier, chickens and pigs may well have gone to slaughter before 1 year of age but breeding animals of these species are kept beyond 1 year of age. The value of an adult, having been reared and cared for, perhaps for years, means that the economics of vaccination is generally less of a concern. Multiple vaccinations are often given, considering specific disease risks and environmental factors, as part of a tailored vaccination program.

Adult animals, with their mature immune systems, absence of maternally derived immunity, and adjustment to their environmental stressors, are normally the best candidates for vaccination. Even in these near ideal candidates for vaccination, the number of doses that are required for efficacy will depend on the nature of the vaccine. Vaccines fall broadly into one of two categories—live or killed.

Live vaccines typically replicate in the host and hence deliver a large antigenic mass to the immune system resulting in rapid and strong immunity. Live vaccines have several advantages. Formulated without specific immune system stimulants, they generally cause less injection site reactions. Even when not being injected they have the advantage that most retain the infectivity of the wild-type pathogen enabling effective delivery by routes such as aerosol or orally. One potential negative is that, because they replicate, there is the risk that in an immunocompromised individual they may induce some signs of the disease they were meant to prevent.

Killed vaccines might be better termed nonreplicating to cover vaccines that never were alive, for example, subunit-containing vaccines. Most vaccines in this category are made from the culture of live organisms that are inactivated, or killed, using one of a variety of chemicals, with formaldehyde the commonest. The inactivated organisms on their own will often only stimulate a weak or transient immune response. While there are numerous causes of this phenomenon, the common explanations are that inactivation alters the conformation and hence presentation of some antigen epitopes and the inactivated antigens do not signal quite the same danger to the animal’s immune system. The “danger signal” model of immunity moves away from the self versus nonself based view and considers the idea that the immune system is more concerned about organisms that damage the animal rather than just being foreign (Gallucci and Matzinger, 2001). To overcome this shortcoming, the addition of an adjuvant is classically done to stimulate a greater immune response, or to drive a particular immune response, for example, Th2 versus Th1. Killed vaccines are safe, in that, without live agents in the final product, they cannot be the source of an infection. However, the presence of the adjuvant often results in greater injection site reactions than is seen with live vaccines. Injection site reactions can range from merely discomforting to quite painful, but in livestock they often end up as imperfections in the meat resulting in trim losses on the slaughter line. The route of vaccination is often dictated by the nature of the injection site reaction. In beef cattle most vaccines are given subcutaneously to avoid reactions damaging the meat. In show animals, for example, horses, intramuscular injections are preferred to avoid any blemishes that subcutaneous injections may cause.

The typical adult will fall into one of two categories with respect to vaccination—naive or preexposed. The true naïve adult will not have a problem with maternally derived antibodies blocking effective vaccination and normally responds well to vaccinations. In fact the responsiveness to vaccination is much more predictable (strength of protection engendered) and reliable (percentage of animals responding) in these naïve adult animals. This is critical as the adult animal tends to be more adventurous, often traveling and having greater contact with other animals. This is particularly true of the companion animals’ species where dogs, cats, and horses may go to shows, boarding facilities, and sporting events. At these venues their opportunity to interact with animals from a

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II. FUNDAMENTAL ASPECTS OF VACCINOLOGY

diverse range of geographies, and consequently disease backgrounds, is enormous. Even as our livestock species grow older they will have greater exposure risks—stock movement is common and introduced animals whether for breeding or to add production capacity frequently come from markets where mixing of animals is commonplace and disease transmission is prevalent. The onset and duration of immunity that occurs after vaccination of naïve animals will generally be representative of a primary immune response. Such responses tend to be slower in reaching protective levels and the memory may be shorter following a single vaccine dose.

The preexposed adult may have already experienced the disease or have a prior vaccination history. Often these two events will be indistinguishable without an accurate history of the animal. Fortunately when it comes to vaccination it does not matter, both recovery from infection and effective previous vaccination will have engendered an immune memory provided the exposure was not too long ago. The duration of immunity that follows exposure of the immune system to a disease agent, even a vaccine strain, depends on the translation of at least part of the initial response into a memory response. The presence of specific memory T cells enables an amnestic response regardless of the source of the agent inducing the original response. This phenomenon is leveraged in adult vaccination programs to decrease the frequency of revaccination. Traditionally, dog and cats were revaccinated on an annual basis throughout their lifetimes, although it was anticipated that routine vaccines were likely to provide far greater than 1 year of protection. Recent work has proven that under field conditions there is evidence of immunity that can persist for 48 months in some animals (Mouzin et al., 2004a, 2004b). Evidence in horses and cattle would indicate that the duration of immunity following vaccination may be only as long as 1 year but varies with the disease agent (Hannant et al., 1988; Peters et al., 2004). The vaccine manufacturer normally provides guidance as to the duration of immunity that may be anticipated following successful vaccination of animals. Depending on the individual circumstances the duration of immunity may be longer or shorter than the label indicates, especially in environments where exposure to the disease agent occurs. This results in natural boosting of immunity and essentially lifelong immunity.

Challenges

Like their younger counterparts, there are a variety of problems that may be associated with vaccination, for example, injection site reactions and mild transient signs of disease, which are a concern to owners. There are some issues however that tend to be seen more commonly in adults. The reason may be easily understood by remembering that the longer an animal lives the more vaccination events it is likely to have lived through. While this is good with respect to developing immunity, there may be downsides.

Transient lameness may occur following vaccination when the dose is administered into a leg or neck muscle and may be more noticeable in adults simply because of the greater weight of the animals compared to their neonatal or juvenile counterparts. Prior exposure to vaccines may induce a hypersensitivity to components, such as bovine serum albumin, used in manufacturing the product with the result that larger injection site reactions may occur. Other reactions such as arthropathy, vasculitis, neurological dysfunction, and thrombocytopenia have been associated with repeated vaccine exposure (Schattner, 2005). Fibrosarcomas in cats have been linked to vaccine reactions and usually have fatal outcomes (Hershey et al., 2000). Nonetheless the incidence of postvaccinal reactions is relatively rare. In one study, the reaction rate in over 1.2 million dogs receiving 3.4 million doses of vaccine was found to be less than 1 in 250 dogs (Moore et al., 2005).

The use of vaccines during periods of lactation has been found occasionally to cause transient decreases in milk production (Scott et al., 2001). The milk drop appears to be associated with a febrile response that in particular follows the administration of live viral vaccines. In cattle, the milk production loss typically is small but readily noticeable by diligent dairy staff.

Adult animals are often subject to stressful conditions that younger animals are spared. Whether it is the stress of lactation, or the stress of performance in racing or pulling wagons, the result is the same—potential down-regulation of the immune system and reduced responsiveness to vaccination.

Opportunities

The move in companion animals toward longer duration of immunity is a trend that is likely to expand into vaccines for livestock. The labor costs of handling mature livestock are considerable and yet the cost of a disease outbreak can financially devastate a producer. Several promising areas of vaccine research raise the likelihood of longer duration of immunity for all animal species.

Vaccine researchers are tapping into the trends in human health to improve the effectiveness of animal vaccines. The use of DNA vaccines with specific cytotoxic lymphocyte antigens and CpG motifs has
been found to induce more potent memory responses (Kennedy et al., 2005). Another promising area of research involves TLRs. These receptors are a family of transmembrane proteins that can interact with a variety of antigens from microbial organisms. Once activated by the interaction, the TLRs can induce an intracellular cascade resulting in select gene expression associated with the immune system. The discovery of TLR agonists or ligands may result in new classes of adjuvants that are more specific and yet safer.

The incorporation in vaccines of interleukins, or genes coding for them, has been a popular area of veterinary research. The intent is that coadministration of antigens with cytokines will result in enhanced immunity. Such products, should they come to market, are likely to also benefit neonatal animals as cytokines may help the maturation of the immune system.

In summary, there is no magic solution to optimizing vaccination programs for animals. A solid understanding of the animal’s innate and environmental risk factors as well as the variables such as stress will enable the development of tailored vaccination schedules that best meets the needs of the animal. While no vaccine will be 100% effective under all circumstances, careful consideration of what and when to use, can certainly enhance the chances of successfully protecting animals. Finally, if all else fails, read the label and follow the manufacturer’s recommendations. They are based on extensive research and are provided to help optimize the effectiveness of vaccines.

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Use of Models for Preemptive Vaccination and Identification of Emerging Disease Vaccine Targets

In response to the growing global crises associated with known, emerging, and unknown infectious diseases of man and animals, more robust disease models and surveillance systems are now being developed to assess the risks and identify potential targets for vaccine research and development; however, more comprehensive interoperable datasets, middleware, and improved micro- and macrolevel models will be required to make the system more efficient and effective (Gust et al., 2001). Several models indicate that vaccination strategies for FMD and respiratory pathogens are justified (Bates et al., 2003a, 2003b, 2003c; Pourbohloul et al., 2005). Even with more sophisticated FMD predictive modeling methods (Perez et al., 2005, 2006; Branscum et al., 2007; Shilegdamba et al., 2008), the accompanying implementable governmental policies will need to be in place to take tactical and
strategic advantage of newer FMD surveillance and vaccine technologies (Marshall and Roger, 2004; Kobayashi et al., 2007a, 2007b), or the control and eradication programs may be prolonged (Whiting, 2003; Perez et al., 2004). Additionally, for purposes of biopreparedness, predicting which vaccines and what quantity of vaccine should be stockpiled becomes a daunting task (Rossides, 2004). Additionally, for purposes of biopreparedness, predicting which vaccines and what quantity of vaccine should be stockpiled becomes a daunting task (Rossides, 2004); however, modeling approaches have been developed to facilitate the decision-making process given the uncertainties and complexities of naturally occurring much less intentional infectious disease outbreaks (Medema et al., 2004).

Reduction and Control of Zoonoses through Strategic Application of Animal Vaccines

In reviewing data from several sources, Lutticken et al. (2007) found that with the increasing demand for food, the enlarging scale of world food production, the augmented transportation of animals and food products, and simultaneous contact of animals with the environment, several microorganisms have established themselves in farmed animals, which although may be relatively harmless to animals are pathogenic to man. These investigators propose options for reducing the risk of transferring zoonotic agents from animals (particularly farm animals) to man by specifically applying veterinary vaccines against viral and bacterial diseases (Lutticken et al., 2007). Other investigations documented that avian influenza, West Nile virus, Bartonella henselae, rabies and anthrax vaccines which protect animals reduce or prevent transmission of zoonoses to humans (Capua, 2007; Lutticken et al., 2007; Marano et al., 2007; Miguens, 2007). Vaccination of vector/reservoir species, when efficacious vaccines are available, offers will offer significant advantages to combating zoonotic human disease (Zinsstag and Weiss, 2001; Marano et al., 2007). In summary, when appropriate biopreparedness, management strategies and contingency plans of the future (Westergaard, 2008) are linked with (1) protective DIVA vaccines (Clavijo et al., 2004; Selke et al., 2007) against zoonoses, (2) effective predictive modeling (Branscum et al., 2007), and (3) deployable implementation policies (Kobayashi et al., 2007b), control and prevention of serious zoonotic diseases of man and animals will become more achievable at local, state, and national levels.

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