CHAPTER 9

# Conventional and Biotechnologically Engineered Bovine Vaccines

A.A. Potter and L.A. Babiuk

## 1. Introduction

Immunization against infectious agents has proven to be one of the most cost-effective methods of reducing economic loss in animals. However, even though immunization to prevent infections has been practiced for nearly 200 years, animals still continue to suffer from a large number of infectious diseases. Thus even though we have been able to prevent devastating disease outbreaks with vaccination, we have not eliminated the scourge of these diseases. The reasons for this are multifold and are related to management conditions, the age when animals get exposed to the pathogens, the patterns of pathogenesis of the respective organisms, as well as possible ineffectiveness of conventional vaccines. For example, many of the conventional vaccines are administered by the intramuscular route. These vaccines do not provide complete protection at the mucosal surface, the route of entry of the pathogen. Therefore, implementation of effective immunization regimes requires information regarding the specific immune responses involved in providing protection as well as the specific antigens that elicit the appropriate response. For example, if the virulence factors of a bacterium include an extracellular toxin that is crucial to pathogenesis it will be inappropriate to use bacterins to protect against this particular infection. In many cases a local immune response or a cellular immune response is crucial for both preventing infection as well as aiding in recovery. If we hope to elicit the correct immune response to the specific organism, a considerable amount of basic microbiological and immunological knowledge is required concerning the actual antigens and the types of immune responses involved. Unfortunately, many viral and bacterial infections encountered in veterinary medicine have not been adequately characterized at the molecular level nor have the host immune responses to these agents been characterized. If this was achieved then it may be possible to engineer better vaccines to control diseases for which we already have at least partially



R. Pandey et al. (eds.), *Veterinary Vaccines*

effective vaccines as well as for those where no effective immunization regimes are presently available. In the present review we will attempt to discuss the various types of vaccines used for controlling the major bovine diseases, and some of the practical problems regarding immunization that are most relevant to bovine medicine. We will also discuss the potential for improving present vaccines by modern biotechnological methods. However, even with all the tools of producing new vaccines, totally effective vaccines will not be forthcoming until we gain a considerable amount of fundamental information regarding the specific antigens involved in eliciting protective immunity and the host's response to these antigens. In veterinary medicine one must always take into consideration the economics of producing these vaccines. Thus even if it was possible to use some of the more sophisticated methods to produce effective vaccines, there is no guarantee that they can be produced economically to ensure widespread use. These factors will all be discussed in this review.

## A. Practical Problems of Implementing Effective Vaccination Programs in Cattle

For practical purposes we have divided this review into respiratory, enteric, systemic, and other infections. In cattle, there are unique problems in controlling respiratory and enteric infections. The most economically important enteric infections generally occur within the first few weeks of life. Examples of these infections include *Escherichia coli*, rotavirus, coronavirus, and a myriad of other viral, bacterial, and parasitic infections. The age of susceptibility as well as the site of infection provide major impediments to effective immunization. Unfortunately, it is impossible to induce immunity within 2–3 days after immunization, yet that is how rapidly some of these infections occur after birth. If one expects to induce active immunity the most effective vaccine should be one that induces local immunity to prevent infection of the gastrointestinal tract. However, the presence of passive antibody, acquired from the dam during suckling, may interfere with oral immunization. In the case of bovine respiratory disease, management systems differ around the world thereby making a universal vaccine very difficult to produce. In North America bovine respiratory disease occurs following weaning and movement of animals to large feedlots. The process of mixing, stress, and exposure to different pathogens upon entry to the feedlot ensures that animals are exposed to multiple pathogens within a very short time period. In many cases these pathogens can act synergistically to increase the severity of respiratory infections. It would be ideal to immunize animals prior to entry into feedlots (preconditioning) but unfortunately animal husbandry practices prevent this

type of approach from being implemented. As described for enteric infection, immunization upon entry into the feedlot does not provide sufficient time for immunity to develop to prevent infection. As a result animals suffer from what is called "shipping fever." Another consideration for vaccine efficacy is the route of vaccine administration. Differences in immune responses occur between vaccine administered intramuscularly, subcutaneously, or intradermally. In the development of vaccines, knowledge about the pathogenesis of the disease and immune responses involved in inducing protection must be considered for judicious designing of vaccination protocols. Thus, if the pathogen is strictly localized to mucosal surfaces, systemic immunity may be of limited value. However, if the virus is both local and systemic then parenteral administration will be effective in preventing systemic spread. Unfortunately, in vaccine design, this is not always the primary concern. Often the primary concern is ease of administration. If effective vaccines are to be developed all these factors must be taken into consideration.

## B. Types of Vaccines

At present the majority of veterinary vaccines are produced by conventional methods similar to those implemented by Jenner or Pasteur. These include conventional live vaccines or killed vaccines. Both of these types of vaccines have proven to be effective in at least partially reducing the clinical manifestations following exposure to virulent field strains of the pathogen. In the case of live vaccines one of the major impediments to their development is to ensure that the organism is attenuated sufficiently not to cause disease but still replicate to a sufficient level to induce the appropriate immune response. To date many of the vaccines produced by attenuation have been produced emperically with no true understanding of the genes that have been altered or the nature of the alterations. As a result of this serendipitous attenuation, live vaccines carry the remote risk that they may revert to a virulent state. As will be described later, a better understanding of virulence mechanisms is allowing us to directly alter the virulence genes or delete them completely to ensure complete attenuation. Similarly, with killed vaccines it is well known that only certain proteins of the pathogen are important in inducing protective immunity and that other proteins may suppress immunity to the protective ones. With techniques available to identify these proteins and produce the protective ones in large quantities, it is envisioned that more effective killed vaccines will be generated in the near future. It must be emphasized that developments in vaccine delivery and adjuvants will need to be conducted in parallel with vaccine development to make these new vaccines as efficacious as possible (see Section 6).

## 2. New Technologies for Vaccine Development

Improvements in conventional biochemistry, recombinant DNA technology, peptide synthesis, molecular genetics, and protein purification has laid the foundation for the development of new vaccines which should be more efficacious, cost effective and lead to fewer side effects. In this section we will briefly review what we perceive to be the vaccines of the future. In some instances, these new vaccines will be used to "spike" conventional vaccines to improve immunogenicity to selective components whereas in other cases there new vaccines will comprise the total vaccine.

### A. Subunit Vaccines

Subunit vaccines can be defined as those that contain one or more pure or semipure antigens. The potential advantages of using such a product are numerous, including increased safety, less antigenic competition due to the presence of less relevant components, ability to target the vaccine to the site where immunity is required (mucosal sites), and the ability to differentiate vaccinated from infected animals. This latter feature is extremely important for pathogens which persist for long periods of time (latent) in the animal and where countries are trying to eliminate the specific disease. Similar types of diagnostic methods can also be used in conjunction with "marked" live vaccines (see later). Although subunit vaccines can be produced by conventional technology, the economics of purification are generally not cost effective due to the low quantities of protective antigens produced by the organism. Also, since the organism is grown *in vitro*, some of the relevant antigens may not be present. An example of this would be bacterial proteins required for the scavenging of nutrients such as iron or carbohydrate. These impediments can be overcome by using recombinant DNA or synthetic peptide technology.

The development of recombinant DNA technology in the 1970s combined with advances in gene expression during the past decade has made it possible to produce large quantities of proteins in heterologous cells. Methods involved have been described elsewhere and will not be dealt with here except to state that they involve the following steps (12). First, it is imperative that the protective antigens be identified. Usually one or at most only a few proteins of the organism are involved in inducing protective immunity. To identify the protective antigens a considerable degree of knowledge concerning the pathogen itself, the pathogenesis of the organism, and the host responses to that organism is required. Once the protective antigens are known, the gene coding for the protein needs to be identified, cloned and expressed.

A number of expression systems are being used to produce large quantities of these subunit vaccines. They include: (a) prokaryotic

systems and (2) eukaryotic systems including yeasts, mammalian cells, insect cells, algae, and filamentous fungi. The expression system chosen will often depend on the source of the gene being expressed. For example, prokaryotic systems are very attractive for production of subunit vaccines from bacteria. Yields as high as 2–5 g/liter of the desired product can be easily achieved in these systems (57). In contrast, insect and mammalian cell expression systems only yield 100 mg/liter of culture. Unfortunately, it is not always possible to express genes from viruses in prokaryotic systems and have them function in the same way as they would if they were produced in eukaryotic systems. This is related to the post-translational processing and folding of viral proteins in bacterial systems.

For the production of bacterial vaccines, bacterial expression systems are ideal. Depending on the yield and downstream processing capabilities and requirements, it is possible to engineer the bacterium in such a way as to have the product targeted to the periplasm, outer membrane, secreted into the medium or retained intracellularly (83). If the product is retained, the protein is often present as an insoluble protein mass (inclusion bodies), which requires solubilization and refolding into the native state (163). If the product is secreted, it may be possible to harvest the product directly from the media and use the culture fluid as a vaccine without any further downstream processing.

While *E. coli* and other Gram-negative organisms have been engineered to secrete some proteins into the growth medium, Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* are potentially better systems (70,126). *Bacillus* sp. have been used for many years for industrial scale fermentations and, therefore, the logistics of scale-up from laboratory to commercial production levels are not as difficult to achieve as with other organisms. *B. subtilis* produces extracellular proteases that can affect the stability of secreted proteins but mutants lacking proteolytic activity are availabe (70,185). A number of secretion vectors have been constructed and can function as plasmids or integrated into the chromosome. While yields are not as high as with intracellular expression systems, the products are soluble and downstream processing costs are reduced since they do not have to be denatured and refolded. Effective secretion systems for *S. aureus* also have been developed based on the protein A gene. These have been shown to function in both *E. coli* and *S. aureus* and are suitable for peptides or small proteins (126).

In the case of viral subunit vaccines, prokaryotic expression systems are generally of limited use due to the fact that prokaryotic cells do not posttranslationally modify viral proteins correctly. As a result, a considerable amount of interest has been generated in the development of eukaryotic expression systems. These include yeast, mammalian cells, filamentous fungi, blue-green algae, and insect cells (16,68,85,97,182, 186). The primary advantage of yeast as an expression system is that extensive industrial experience is available with the yeast *Saccharomyces*

*cerevisiae*. This organism is not pathogenic for animals and, more importantly, the expense of removing all the yeast proteins from the vaccine does not appear to be required. This will greatly reduce the cost of the final product. Unfortunately in some cases yeast may overglycosylate proteins, which may influence immune responses to the specific subunit protein being produced. It is well known that the degree of glycosylation can influence immunogenicity of a variety of proteins. Although there are no vaccines licensed for use in veterinary medicine that have been produced in yeast, the first licensed human recombinant DNA vaccine, for hepatitis B, is still produced in *S. cerevisae* (186). Other varieties of yeast such as *Pichia pastoris* are also being developed for use as hosts for gene expression. Using these newer systems, the level of production of foreign protein has been increased to approximately 400 mg/liter.

A second expression system that has received considerable attention is the baculovirus, *Autographa californica* nuclear polyhydrous virus (AcNPV), grown in insect cells (85,106). The principle behind the use of baculovirus/insect cultures to produce foreign products is that some genes such as that coding for the polyhedron of the AcNPV virus are dispensable for virus replication. If one uses the high efficiency promoter of the polyhedron gene to drive the transcription of a foreign gene, which replaced the polyhedron gene, large quantities of protein can be produced. Furthermore depending upon the construction, the protein can either remain within the cell or be secreted into the culture medium. The development of synthetic media requiring no serum should dramatically reduce the cost of culturing cells as well as remove the potential problem of introducing extraneous protein and agents such as bovine virus diarrhea virus (BVD) into the vaccine (88,107). One possible disadvantage of the insect viral expression system is that insect cells do not posttranslationally modify the protein in a fashion identical to that occuring in mammalian cells. Thus, as with yeast, some loss of immunogenicity may occur for some proteins if they are not glycosylated appropriately.

The ideal subunit expression system would be to use mammalian cells for production of viral proteins or glycoproteins. To achieve this goal, one can use mammalian viruses such as vaccinia, herpes, adenoviruses, picornaviruses, and Sindbis virus for expression of the desired protein (45,66,68,119). Similarly it is possible to directly engineer a mammalian cell to express fully functional processed proteins (16,182). Unfortunately in many of these cases the yields of the expressed protein are still generally low for their potential production of vaccines for veterinary use.

In many cases it would be advantageous to immunize an animal with a vaccine containing a number of protective proteins from different organisms. This can be achieved by purifying individual proteins from different expression systems. An even more attractive approach is the possibility of developing chimeric proteins containing the protective epitopes from various organisms as one protein. This requires the iden-

tification of the protective components from two different organisms, constructing one chimeric gene containing the protective proteins from the different organisms and expressing them in the expression system of choice. This would dramatically reduce production costs. An attractive possibility might be to identify epitopes on rotavirus and coronavirus and express them on the surface of *E. coli*. Thus it might be possible to immunize calves against *E. coli* K99 expressing linear epitopes of rotavirus and coronavirus as one vaccine. This appears to be at least feasible for rotavirus since the important protective epitopes on this virus have been identified and are known to be linear epitopes (73). Unfortunately at present the majority of the important epitopes identified on coronavirus are conformational (40). As stated above, *E. coli* would not be the expression system of choice for these epitopes. Although there are only two recombinant products that have been approved for use in food animals, a number of relevant genes from pathogens of cattle have been cloned and expressed. It is anticipated that over the next 2–5 years field testing and commercialization of a number of these recombinant subunit products will occur.

## B. Peptides

Subunit vaccines can be produced by chemical synthesis of short polypeptides. Although the technology is not new, improvements made during the past decade in solid phase peptide synthesis have increased the efficiency of the process and lowered the cost. Developments in two complementary areas during the 1970s have made the application of synthetic peptides to vaccine production possible. These are the availability of monoclonal antibodies to define protective epitopes on antigens and the ability to accurately sequence DNA in order to localize these epitopes. While there are no commercially available peptides vaccine for the veterinary market, a number of experimental vaccines have been developed.

One of the first viruses used to investigate the feasibility of using synthetic peptides as a vaccine was foot-and-mouth disease virus (FMDV) (17,20). Unfortunately, in many viruses, including FMDV, antigenic variation can occur. Thus, one needs to find crucial conserved epitopes on the virus before a single broad spectrum synthetic peptide vaccine can be developed. Conserved regions on a number of viruses have been identified. For example, in the case of bovine rotavirus, VP4 contains a proteolytic cleavage site that is conserved on all rotavirus serotypes (44). This protein must be cleaved for virus infectivity. Animals immunized with a peptide corresponding to the cleavage site are protected from infection by a number of rotavirus serotypes (73). Based on these findings we feel that peptide vaccines may be part of the armamentarium of the

vaccinologist in the future. However, once again effective delivery of these peptides, in combination with carriers, adjuvants or appropriate vectors will need to be considered before a truly effective peptide vaccine can be developed.

## C. Live Vaccines

One of the most exciting areas of vaccine research over the past 5 years has been the development of genetically attenuated viral and bacterial vaccines. In principle, a properly delivered live vaccine should be more effective than the corresponding subunit product since all of the relevant antigens are present to stimulate both cellular and humoral immunity, at the appropriate site (e.g., mucosal). The main problem with live vaccines in the past has been the methods used to reduce virulence of the organism. These have included passage *in vitro*, passage through alternate host cells (in the case of viruses), chemical modification, heat inactivation, and the use of conditional mutations (e.g., streptomycin dependence, temperature-sensitive mutants, etc.). In many cases the genetic basis for attenuation was not clear and, therefore, reversion to virulence during commercial scale production or following administration into the animal remained an ever present threat. Perhaps a larger problem is that exposure of an animal to live, albeit attenuated, organism can result in immunosuppression and predisposition to other infections. These problems highlight the need for more rational and defined methods of attenuation and delivery of live organisms as vaccines.

The establishment of a successful bacterial infection requires the expression of a large number of genes specifying classical virulence determinants such as fimbriae and toxins, and components needed for physiological functions of the cell. Strains can be attenuated by creating defined mutations in genes involved in either class of function. For example, *Bacillus anthracis* mutants, which do not produce capsule, are avirulent, although they are still capable of replication in the host (76). Anthrax strains that are defective in aromatic amino acid biosynthesis are less virulent than the nonencapsulated strains and yet provide protection in experimental disease models (75). Thus, the impairment of specific physiological functions that cannot be complemented by the host can be effective in the reduction of virulence. Although we have used *B. anthracis* as an example, above, most of the work has been carried out with enteric pathogens, mainly *Salmonella* sp. (28). Examples of some bacterial attenuating mutations are listed in Table 9.1. Each of these mutations can result in different levels of attenuation as measured by the ability of the organism to persist in the host. For example, *pur* mutants of *Salmonella typhimurium* are able to establish only a transient infection whereas *aro* mutants of the same organism establish infections that are

**Table 9.1.** Bacterial mutations which reduce virulence.[a]

| Gene | Function | Species |
|---|---|---|
| *aroA, C, D* | Aromatic amino acid biosynthesis | *Salmonella, Bordetella, Bacillus, Yersinia* |
| *cya, crp* | Adenylate cyclase | *Salmonella, Bordetella* |
| *purA, E* | Purine metabolism | *Salmonella, Yersinia* |
| *ompR* | Porin regulation | *Salmonella* |
| *phoP, Q* | Acid phosphatase | *Salmonella* |
| *galE* | Galactose epimerase | *Salmonella* |

[a] In addition to the above, mutations to rifampicin-resistance, streptomycin-dependence, and various temperature-sensitive mutations have been shown to reduce bacterial virulence, but these have not been well characterized. Mutations in classical virulence determinants are not included.

more persistent albeit subclinical (28). Therefore, it is in theory possible to attenuate organisms to varying degrees depending upon the mutations that are introduced. This technology can be applied to virtually any species of bacteria although it is easier to accomplish in organisms that are genetically characterized. Ideally, at least 2 deletion mutations should be introduced into a host organism in order to ensure safety. For example, Munson et al. (120) developed *cya/crp* double mutants of *Salmonella typhimurium* that when used as a vaccine resulted in protection against challenge by both *Salmonella* and *E. coli* infection in poultry (120). Similar mutations could be introduced into bacterial pathogens of cattle.

Once a vaccine strain has been selected, it is possible to introduce foreign genes into the strain in order to produce a multivalent vaccine. In this case the attenuated host strain would act as a delivery vehicle for heterologous antigens. Table 9.2 lists some representative examples of heterologous genes that have been delivered in attenuated *Salmonella* strains. Generally speaking, high-level expression of the heterologous

**Table 9.2.** List of representative antigens delivered in attenuated *Salmonella* strains.

| Source | Antigen | Attenuating mutation |
|---|---|---|
| *E. coli* | LT, B-subunit | *galE, aroA* |
| *E. coli* | K88 pilin genes | *aroA, galE* |
| *E. coli* | K1 capsular polysaccharide | aroA |
| *C. tetani* | tetanus toxin, c-fragment | aroA, *aroC* |
| *Str. sobrinus* | spaA | *cya crp* |
| *Str.* pyogenes | m5 protein | *aroA* |
| S. *sonnei* | form 1 antigen | *galE* |

gene product is not required since large numbers of bacteria colonize the host. Therefore, strong promoters used for subunit vaccine production such as *tac* or Lamda-pL are not required. Rather, natural systems using outer-membrane protein promoters and signal sequences, or similar sequences from other surface structures are the most useful. For example, oligonucleotides coding for protective epitopes can be placed in the gene coding for the flagellin protein and expressed on the surface of *S. typhimurium* (95). In cases where recombinant protein production interferes with the growth of attenuated bacteria, it is possible to construct regulated expression systems that synthesize protein only in response to signals encountered in the target animal. For example, promoters that are activated in the absence of free iron or the presence of specific carbohydrates could be used (123). Thus, the bacteria could be easily grown in the laboratory or production facility by limiting the production of otherwise lethal components while maintaining the efficacy of the product.

Organisms attenuated by specific genetic manipulation are not the only potentially useful bacterial systems for the delivery of foreign antigens. Recently, *Mycobacterium bovis* BCG has been adapted for use as a delivery vehicle (77,112). *M. bovis* has been used extensively for vaccination and has excellent adjuvant properties capable of stimulating both cell-mediated and humoral immune responses. While this system will likely find application mainly in human medicine, it is conceivable that it could be adapted for use in the veterinary market if companion diagnostic tests were developed to distinguish vaccinated from infected animals.

Viruses can be used to produce proteins from other viruses. These proteins can then be used either as subunit vaccines or as a live vectored vaccine. Vaccinia virus was one of the first viruses into which foreign viral genes were inserted and shown to be capable of producing the foreign protein and inducing immunity to that foreign antigen (47,119). Since those early experiments, vaccinia has been used extensively to express genes from almost every conceivable virus. In addition, cytokine genes have also been incorporated into vaccinia virus to reduce its virulence and possibly modulate immunity to any coexpressed proteins (48). Vaccinia appears to be extremely attractive for foreign gene insertion, since its genome is very large and it is possible to delete large quantities of DNA and still maintain a viable virus. Based on this observation it should theoretically be possible to insert multiple genes into the vaccinia genome and thereby produce a multivalent vaccine. With increased knowledge of vaccinia virus genetics and promotors that are capable of producing high quantities of proteins, it is proving to be extremely attractive as a carrier for cattle vaccines. The recent introduction of genes coding for Rinderpest proteins into vaccinia and the demonstration of its efficacy in preventing Rinderpest virus infections make it an attractive vehicle for

producing vaccines for Third World countries where thermal stability and cost of the vaccine are primary concerns (198).

The discovery that other viruses, such as herpesvirus and adenoviruses, also have regions within their genome that are nonessential for either *in vitro* or *in vivo* replication has prompted investigators to test these viruses as potential viral vectors. For example, bovine herpesviruses have a number of genes that can be deleted (TK,gIII) without significantly altering replication *in vitro* (90). Such deletions can dramatically change the virulence characteristics of the virus *in vivo* as well as the ability of virus to induce latency (89). Since animals immunized with these herpesviruses deletion mutants will not produce antibody against the protein produced by the deleted gene, these vaccines can be used in conjunction with a diagnostic test to differentiate between animals that are potential carriers of latent field strains of virus and those that have been immunized with the vaccine. This approach is very attractive for immunizing animals in countries or regions where elimination of a specific virus infection is a target (108). European countries that are moving toward bovine herpesvirus and pseudorabies virus eradication programs appear to be embarking on a course to eliminate conventional herpesvirus vaccines. The "marked" vaccines can either be used as live vaccines or killed conventional vaccines.

Adenovirus has also been shown to be an acceptable vector for producing immunity to a variety of antigens inserted into deletable regions of the human and canine adenovirus genome (66,140). Similar studies are now proceeding to identify homologous regions of the bovine adenovirus that can be deleted and used as insertion sites for foreign genes. Since both herpes and adenoviruses are considerably smaller than vaccinia, there is less potential for inserting as many genes into them. However, these viruses may offer practical advantages such as delivery of the virus into the oral or respiratory tract as well as political advantages since countries are still reluctant to reintroduce vaccinia immunization. The introduction of vaccinia recombinants into wildlife populations to control wildlife rabies obviously is overcoming some of the concerns about using vaccinia as a vehicle for controlling infections (132).

In addition to using DNA viruses as vectors recent studies have shown that RNA viruses such as Sindbis and polio can also serve as vectors for vaccine antigens. In the case of polio virus, sequences coding for epitopes from a variety of viral and bacterial pathogens have been incorporated into the virus (21,45). Exposure of animals to the engineered virus resulted in development of immunity not only to polio virus but also to the foreign epitope. Since cattle can be infected with a number of picornaviruses, the ease of constructing the chimeric viruses and efficiency of replication *in vitro* makes this approach easy to test in cattle. One impediment to this approach is the restriction on the size of the genetic material that can be incorporated into picornaviruses.

## 3. Bovine Respiratory Disease

Bovine respiratory disease is the leading cause of morbidity and mortality in cattle around the world. This syndrome can be divided into two major classes depending on the age of the animal and management practices that lead to this disease complex. For example, enzootic pneumonia normally occurs in calves reared under a variety of different management systems. In contrast, "shipping fever" occurs in calves that have been weaned and transported to feedlots for final finishing. Other management conditions will obviously result in manifestations of different types of respiratory disease. In spite of many years of investigation into the pathogenesis and etiology of the various types of bovine respiratory diseases we do not fully understand all the interactions between the host and the pathogen that lead to respiratory disease. What does appear clear is that within this complex, whether it be enzootic pneumonia, shipping fever, or other manifestations of this disease syndrome, no one factor is solely responsible for precipitating the disease. Most people will agree that management (inclement weather, stress, mixing of animals, weaning, crowding, poor nutrition, or immunity) and viral infections (infectious bovine rhinotracheitis, bovine respiratory syncytical virus, parainfluenza 3, bovine viral diarrhea virus, and adenovirus) interact to create an environment that favors colonization and growth of the bacterial agents (*P. haemolytica*, *P. multocida*, *H. somnus*, *Mycoplasma* spp., and *Chlamydia* spp.) in the lung. This does not mean that an animal needs to be exposed to all of these infectious agents and environmental insults at the same time. However, it appears that as the number of insults accumulate the animal has a greater chance of succumbing to infection and severe respiratory disease. In some cases one specific predisposing factor is the major culprit in precipitating the disease, where as in other cases another factor is more important. As a result of these multifactorial interactions an animal suffers from infection and must be treated. In spite of treatment and vaccination, some animals die or develop chronic respiratory disease. Treatment costs, death losses, and reduced performance all lead to tremendous economical losses to the cattle producer and indirectly to the consumer and the economy of each country involved in raising cattle. Unfortunately, even with the best vaccines these losses would not be eliminated without altering management practices. Furthermore, since there are such a large number of pathogens involved in inducing or precipitating the disease it is probably unrealistic to imagine that even with the best vaccines for the most common causes of bovine respiratory disease that this syndrome will be eliminated. In this section we will describe the vaccines available for some of those pathogens perceived to be the most important in this disease complex and how we envisage that these products could be improved through modern vaccine technology.

## A. Bacterial Infections

The principal bacteria associated with bovine respiratory disease are *Pasteurella haemolytica*, *Haemophilus somnus*, *Pasteurella multocida*, *Mycoplasma* spp., and *Chlamydia* spp. However, other bacteria can, under appropriate conditions, cause respiratory infections in cattle. Vaccines for at least some of these organisms have been commercially available for a number of years. These vaccines include bacterins, extracts, and live cells.

### i. Pasteurella haemolytica

*Pasteurella haemolytica* has traditionally been the organism most commonly isolated from the lungs of pneumonic animals in North American feedlots. There are at least 15 serotypes and 2 biotypes of *P. haemolytica*, with biotype A, serotype 1 being the most common isolate found in respiratory disease. However, in many cases it is very difficult to experimentally reproduce the disease by exposure of animals to an aerosol of *P. haemolytica* itself. Second, it is often possible to isolate *P. haemolytica* from the upper respiratory tract and tonsillar crypts of healthy animals. These factors all support the contention that *P. haemolytica* is an opportunistic pathogen. Although the mechanism of pathogenesis by *P. haemolytica* is not completely clear, a number of potential virulence determinants have been identified. These include a secreted leukotoxin, proteases, neuraminidase, capsular polysaccharide, endotoxin, outer-membrane components, and fimbriae (4,13,39,117, 129,137,167). While each of these antigens may provide some level of immunity it appears likely that a combination of secreted and cellular components will be necessary to provide maximal resistance to *P. haemolytica* infections (170).

In experimental trials, vaccination with several products including extracts, live bacteria, and bacterins have provided some degree of protection (25,33,111,169,197). Unfortunately, data obtained from field trials were not as convincing (15,34,110). This is not surprising, since vaccination often occurs at entry into feedlots and there is insufficient time for protective immunity to develop. Second, many of the extracts or bacterins do not contain sufficient quantities of the extracellular leukotoxin, a component recognized to be crucial in preventing damage to the leukocytes that are responsible for aiding in clearing the Pasteurella from the lungs (54,168). Third, although extract vaccines are probably more efficacious than bacterins, there are problems associated with antigenic competition between protective and nonprotective components and immunosuppression due to some as yet undefined bacterial components (34).

The live *P. haemolytica* vaccines include streptomycin-dependent, chemically altered, and attenuated live strains of *P. haemolytica* (18,

84,148). Intradermal vaccination of calves with the modified live vaccine has given mixed results. In some conditions it appeared to be protective where as in other cases there was no decrease in morbidity or mortality (148,173). One can only speculate as to the reasons for these inconsistent results but they could be related to the route of administration and the level of replication of the bacterium in the animal. Many producers treat animals with antibiotics upon entry into feedlots to reduce bovine respiratory disease. Obviously, if animals are treated either through medicated feed or direct injection of antibiotics this will have a impact on the degree of replication of the bacterium and as a result the level of immunity induced by the vaccine. Regardless of the reasons for mixed responses, under field conditions, this type of vaccine has not made a significant impact on reducing bovine respiratory disease.

As a result of the problems encountered with live and killed bacterial cell products, a number of investigators have focused on identifying the important protective components of *P. haemolytica* and producing vaccines containing predominantly those components (34). At present an extract vaccine supplemented with culture supernatant containing leukotoxin is showing some benefit in high risk calves under field conditions (80). With a greater understanding of the protective components present in the bacterins and the extracts as well as the ability to produce these components economically by recombinant DNA techniques it is envisaged that the new and improved future vaccines will be subunit products containing only a few protective components. Candidate antigens for these vaccines include leukotoxin and one or more outer surface proteins. The gene coding for leukotoxin has been cloned and expressed in *E. coli* and experimental plus field data indicate that it is protective on its own but increased protection can be attained by combining it with other *P. haemolytica* antigens (101,138). The leukotoxin has been shown to be structurally similar to the *E. coli* $\alpha$-hemolysin (102), as well as several other toxins produced by Gram-negative pathogens (93,179,193). Therefore, the potential exists to develop cross protective vaccines based on leukotoxin by constructing chimeric genes which code for neutralizing epitopes specific for each protein. Genes for a serotype 1 specific antigen plus other outer membrane proteins have been cloned and expressed (60). Preliminary data indicate that at least some of these outer membrane proteins, when combined with leukotoxin, provide enhanced protection against *P. haemolytica* infection (Potter et al., unpublished results) and we anticipate that fully recombinant subunit vaccines will be available in the very near future.

### ii. Haemophilus somnus

*H. somnus* is associated with a number of disease syndromes of cattle, including ITEME, pneumonia, myocarditis, arthritis, and reproductive

disorders (67). The incidence of *H. somnus* in bovine respiratory disease has increased in recent years, perhaps due to more reliable detection of the organism. Both upper and lower respiratory tract infections are encountered in the field, including laryngitis, tracheitis, and suppurative bronchopneumonia. There are reports that under experimental conditions, exposure of calves to respiratory viruses results in increased susceptibility to *H. somnus* pneumonia (136). All commercially available vaccines are killed bacterins. Both experimental and field efficacy has been demonstrated for only one of these products (62,150,162,178), and two vaccinations are required for adequate protection, a practise not often followed in the field. An experimental acellular vaccine consisting of the anionic fraction of a surface component extract was shown to be effective in the prevention of ITEME, but this has not been field tested and is not commercially available (177). However, the results demonstrate that vaccination with the appropriate subunit antigens can be effective. Gogolewski et al. (56) demonstrated that antibody directed against a 40,000 MW outer membrane protein (OMP) was capable of providing passive immunity, while similar experiments with a 78,000 MW OMP did not result in protection. Therefore, the 40,000 MW OMP appears to be a good candidate antigen for a subunit vaccine. Other potential virulence determinants include fimbriae, Fc receptor, and surface components necessary for nutrient scavenging and transport (Pontarollo and Potter, unpublished observations, 194,196). Czuprinski and Hamilton (36) have shown that *H. somnus* is able to survive in phagocytic cells and also to impair neutrophil function (96). The latter is due to secreted adenine, guanine, and guanidine monophosphate plus high-molecular-weight components (29). It would be a great advantage for any subunit vaccine to block both neutrophil suppression and the ability of the organism to survive in phagocytic cells, although more work has to be done to identify the mechanism involved before such a vaccine can be developed. As with *P. haemolytica*, it is likely that at least two subunit antigens will be necessary for protection, and the choice of antigens may vary depending upon the particular disease syndrome being targeted. Combination *H. somnus–P. haemolytica* subunit vaccines might also include shared antigens such as the 78,000 MW antigen recently described by Kania et al. (86). This *H. somnus* outer membrane protein was present on all strains examined, reacted strongly with convalescent serum, and cross-reacted with proteins of similar molecular weights from *P. multocida*, *P. haemolytica*, *Actinobacillus lignieresii*, *A. equuli*, *Enterobacter cloacae*, *H. influenzae*, and *H. agni* (86).

### iii. Mycoplasma

The principal mycoplasmas involved with bovine respiratory disease are *M. bovis*, *M. dispar*, and *M. mycoides*. The latter is the causative agent of contagious bovine pleuropneumonia (CBPP). Attenuated vaccines

have been available for a number of years and are generally effective, but vaccination is controlled by local legislation in parts of the world. Vaccination with inactivated *M. bovis* has been shown to prevent pneumonia and mastitis, but killed *M. dispar* vaccines are not effective. When compared to the other bacterial agents associated with bovine respiratory disease, mycoplasma virulence is poorly understood. Adhesion to the respiratory epithelium is an important step in pathogensis, but the nature of the adhesin has not yet been shown, although ruthenium red-staining material may be involved (153). *M. bovis* and *M. dispar* both impair the phagocytic capacity of bovine neutrophils and *M. mycoides* can induce leukopenia (153). This may be one mechanism by which mycoplasma can predispose high risk cattle to infection with other bacterial agents. Prior to the development of new vaccines it is clear that we must have a greater understanding of these virulence mechanisms of the organisms and their interaction with the host.

## B. Viruses Causing Bovine Respiratory Disease

As stated above, bovine respiratory disease is often a complex where in any one of a number of viruses can either cause infections individually or collectively. The viruses incriminated in bovine respiratory disease include bovine herpesvirus-1 (BHV-1) also referred to as infectious bovine rhinotracheitis, parainfluenza-3 (PI-3), bovine respiratory syncytial virus (BRSV), and adenoviruses (154). Although bovine viral diarrhea (BVD) virus has also been incriminated in bovine respiratory disease it will be discussed in Section 5B. Of these viruses, bovine adenoviruses are probably of the least significance in causing severe clinical infections. In addition to causing respiratory infections, adenoviruses of cattle can spread systemically and cause a variety of other often self-limiting diseases, with the severity possibly increasing following specific stressors. Although a number of vaccines have been tested to control bovine adenovirus infections, their general mild nature and the large number of serotypes (nine) present, it seems unlikely that vaccination will ever play a predominant role in the control of adenovirus infections globally. Vaccines against bovine adenovirus containing various combinations of serotype 1, 3, and 5 have shown some ability to prevent infection against the homologous serotype in Europe. At least 2–4 doses are required to confer protection. Such vaccines are not licensed in North America (22). The observation that it is possible to insert genes into a number of nonessential regions of adenoviruses has prompted a number of investigators to speculate that adenoviruses may be a useful vector as a live delivery method for delivering vaccines to mucosal areas. If this proves to be correct then one could immunize against the foreign antigen being produced by adenovirus as well as against adenovirus itself. These possibilities are highly likely in the future.

The most widely used virus vaccines to control bovine respiratory disease include a combination of BHV-1, PI-3, and BRSV. Many vaccine companies combine all 3 or a minimum of 2 of these pathogens either as live attenuated vaccines or killed vaccines. Although all of these vaccines can induce some immunity following either single or double vaccinations the actual value of the vaccines for controlling respiratory diseases in cattle has been questioned. There are a number of reasons for this possible lack of efficacy. (a) To ensure that the viruses themselves do not cause infections they need to be attenuated sufficiently to reduce the level of replication *in vivo*. If this is achieved they may not replicate sufficiently in the animal to produce a sufficient antigenic mass to stimulate a high enough level of immunity. (b) In the case of killed virus vaccines, the quantity of the individual protective components may not be sufficient to stimulate the immune response. (c) Probably the most important reason for the lack of efficacy is in how they are used. Animals are often weaned and transported to sales barns, where they are mixed with other animals and their pathogens before being transported to feedlots where they are immunized. Thus some animals are probably infected even before vaccination and adequate immunity develops. If immunization with good vaccine occurred prior to weaning and transportation they should have a much better performance record.

Another possible reason why these vaccines do not significantly reduce bovine respiratory disease is that the disease is a complex, and that etiological agents other than the ones present in the vaccine could be inducing the disease. Thus, the development of vaccines for all of the agents, as well as immunization prior to movement and mixing of cattle should greatly reduce disease incidence. Whether this will ever become a reality remains to be determined. Recent evidence indicates that some of the live virus vaccines in the bovine respiratory disease complex may actually be immunosuppressive (72,155). Thus combining a myriad of viral and bacterial antigens in one vaccine may be contraindicated. To overcome this immune interference, considerable progress has been made at identifying the important protective proteins of all of the viruses involved in the respiratory disease complex. These include the gI, gIII, and gIV glycoproteins of BHV-1, the G and F proteins of BRSV, and the HN and F proteins of PI-3. In the case of BRSV it is possible that the F and G proteins from the two serotypes will be required for maximal protection. These proteins are being cloned in various expression systems and will hopefully provide excellent immunity against the viruses involved in this complex.

At present excellent models exist to test the efficacy of BHV-1 and PI-3 vaccines, therefore, it should be easy to prove the efficacy of the subunit vaccines for these two viruses (10). Unfortunately, there is not a good model available for testing efficacy of BRSV vaccines. This will probably delay the speed with which effective vaccines can be developed and

verified for BRSV. A second approach to developing new vaccines to the bovine respiratory disease complex is to use either adenovirus or bovine herpesvirus as a vehicle for delivering protective antigens from a number of the respiratory disease pathogens (see Section 2C). Bovine herpesvirus has at least 5 different genes that are considered to be nonessential for virus replication *in vitro*. These include TK, gIII, and gX. A number of these genes have been deleted from BHV-1 virus and substituted with genes coding for the protective proteins of other viruses involved in the disease complex (98). Although none of these chimeric virus vaccines has been licensed yet, it is envisaged that within the next 5 years we will witness a number of vaccines based on this technology on the market. An advantage of these chimeric viruses is that one can deliver the vaccine intranasally to provide protection at the site of initial infection. The economics of producing one vaccine that will protect against 3 or 4 different viruses should also be very attractive. However, regardless of how effective these future vaccines are in inducing immunity, it will be important to incorporate alterations in management systems to provide an adequate opportunity for the development of immunity to the pathogens prior to movement of animals into high risk environments.

## 4. Enteric Pathogens

As in the case of the bovine respiratory disease complex, calf diarrhea is also a disease complex involving interactions between different viral and bacterial infectious agents, environmental factors, and the immunological status of the animal. The major viral causes of neonatal diarrhea include rotavirus and coronavirus. However, bovine viral diarrhea, bredavirus, and a few minor viruses such as calicivirus and astroviruses have also been incriminated as pathogens in calfhood diarrhea. Bacteria involved in inducing diarrhea include enterotoxigenic *E. coli, Clostridium perfringens* Type C, *Salmonella* spp., and *Campylobacter* spp. In each case the severity of diarrhea is related not only to the virulence of the specific pathogen but to the age of the animal at the time of infection as well as to the presence of other pathogens. It has been shown in a number of studies that only a minority of cases of diarrhea in cattle are caused by a single pathogen (11,160,175). Therefore, if two pathogens can coinfect an animal their combined effect may be much more severe than if they infected the animal individually. In addition to the interactions of various pathogens, a number of other factors such as climatic conditions, standard of housing, hygiene, population density, and nutritional and immunological status of the animal all influence the severity of diarrhea. These cofactors are outside the scope of this review and therefore will not be discussed further. However, one should not overlook the importance of these cofactors in determining the severity of diarrhea in calves. Since

most cases of diarrhea in newborn calves are clinically characterized by an acute perfuse watery diarrhea leading to progressive dehydration and acidosis, it is impossible to differentiate the causes of diarrhea based on clinical observations. If animals are not treated quickly, death can occur. As a consequence, clinicians must institute therapeutic and control strategies to ensure survival of the animal. In many cases this treatment involves the use of intensive fluid and electrolyte therapy to replace the water and electrolyte deficits and alkalizing agents such as sodium bicarbonate to reverse acidosis. Oral therapy is effective only if used before the animal becomes overly dehydrated and diarrheic. Additional treatments usually involve restriction of milk intake to prevent the osmotic movement of fluids into the lumen. If these management systems are implemented quickly mortality is often quite low.

## A. Bacterial Vaccines

### i. *E. coli*

Although enterotoxigenic *E. coli* is most often associated with bovine diarrhea during the first 3–5 days of life, it is not uncommon to see occurrences of the disease for an additional 2 weeks. Many serotypes have been found to cause disease, including O8, O9, O20, O64, and O101 (3,64). The main determinants of virulence associated with these strains are colonization factors, capsular polysaccharide, and toxins (3). Effective vaccines for the prevention of neonatal *E. coli* infections have been available for a number of years. These include formalin and heat-killed bacterins, live vaccines, fimbrial capsular extracts, and toxoids. Acres et al. (2) showed that protection correlated with anti-K99 fimbriae antibody levels, but not with antibody to capsular polysaccharide, although the latter component has been shown to induce protective immunity (65,121). All of these vaccines are administered to the dam 3 weeks prior to parturition and antibodies are transferred by colostrum and milk to the newborn. Since most infections in calves occur during the first 3–5 days postpartum, this method of vaccination is usually effective. In addition to vaccinating the dam, feeding calves colostrum with high levels of antibody or monoclonal antibody to K99 has proven to be very effective in controlling *E. coli* K99 induced diarrhea (166).

The initial stage in colonization of the intestine is mediated by fimbriae (52,116). K99 and/or F41 fimbriae are associated with virulence and both are effective immunogens, forming the basis of many effective vaccines presently available (1,2,64). Since type-1 fimbriae do not play a major role in colonization of the intestine they do not induce protection in cattle or humans. Capsular polysaccharide may play a role in colonization, perhaps as a secondary event to fimbrial-mediated binding. Antibody to

capsular polysaccharide has been shown to correlate with protection in some studies (65,121).

Enterotoxins have been shown to be virulence determinants. These toxins are members of either heat-labile (LT) or heat-stable (ST) families. Heat-labile toxins are antigenically related to cholera toxin and exert their effect by interfering with the regulation of adenylate cyclase activity (46,64). This is mediated by the A-1 subunit of the toxin. Heat-stable toxins can belong to one of two families, STa or STb. STa is unrelated to LT and exerts its effects by the stimulation of intestinal guanylate cyclase (46). STb, which is structurally distinct from STa, does not effect guanylate or adenylate cyclase.

The genes coding for fimbrial antigens plus LT, STa, and STb have all been cloned in *E. coli*, raising the possibility of using either recombinant subunits, or more likely, live attenuated vaccine stains producing these antigens. The principal advantage of using attenuated bacteria to deliver recombinant antigens is their ability to stimulate an effective mucosal immune reponse. The ability to manipulate these toxin genes has resulted in the production of a toxoid that retains its antigenic properties (64). Therefore, it is possible to construct attenuated *Salmonella* strains, for example, which carry K99 and/or F41 fimbrial genes and produce toxoids, which are specifically targetted to the gut (see above). Licensed swine vaccines based on recombinant strains that produce fimbriae are currently available in the United States and Europe.

### ii. *Salmonella*

*Salmonella* infections in animals can cause enteritis, abortion, septicemia, or a combinations of the above three diseases. The bovine enteric form is often caused by *Salmonella typhimurium* and *Salmonella dublin*. Both adult animals and calves are susceptible to the disease, but unlike *E. coli* diarrhea, it is usually not seen in calves less that 2–3 weeks of age. *Salmonella* strains produce a number of virulence determinants similar to those described for *E. coli*. Colonization and invasive factors have been described and several different toxins have been implicated in stimulating intestinal fluid production. The pathogenesis of *Salmonella* infections is different from enterotoxigenic *E. coli* in that the organism can replicate intracellularly in macrophages (30,32). Therefore, it is inaccessible to antibiotic therapy and this can lead to the development of chronic carriers. Although humoral antibody is likely important in resistance to disease, cell-mediated immunity is more important, as one would expect with an intracellular pathogen (100,152). Vaccination with attenuated strains can lead to protection without a humoral response, whereas vaccination with bacterins which elicit a good antibody response are not always effective (100,152,174). Attenuated vaccine strains (*aroA*) have been shown to elicit antibody, delayed-type hypersensitivity and cytotoxic T-lymphocyte

responses in mice (49,135,158). In a recent study, 7 out of 8 calves which were immunized orally with an *aroA aroD S. typhimurium* strain 7 days after birth were protected against experimental challenge 7 weeks after vaccination. These studies demonstrate the feasibility of using live *Salmonella* vaccines (81).

## B. Viruses

As stated above, rotavirus and coronavirus appear to be the most common viral pathogens involved in gastroenteritis of neonatal calves. It is for these two viruses that vaccines have been developed. Since no vaccines are presently available for bredaviruses, astroviruses, caliciviruses, and other viruses seen periodically in diarrheaic calves they will not be discussed further. However, the principle of vaccination of neonatal calves described for rota and coronaviruses could be applied to controlling or developing vaccines against these latter viruses. Vaccination against rotavirus and coronaviruses has been directed at two basic modes of immunization: (a) active immunization of the calf and (b) passive immunization of the calf via hyperimmunizing the dam to transmit antibody to the calf during suckling. In both approaches vaccination appears to have limited effectiveness (180,190). The possible reason that active immunization is of limited value is directly related to the epidemiology of this infection. To provide adequate protection from infection, local immunity in the gastrointestinal tract is required (160,180). To provide local immunity a oral vaccine has been developed for calves. Since almost all cattle have antibody to rotavirus and coronavirus in their milk, antibodies in the milk quickly neutralize the vaccine virus and thereby prevent induction of immunity. Thus the vaccine must be administered at a time prior to suckling. This is often difficult to achieve and a delay in suckling may influence antibody transfer to the calf. A second reason for lack of possible activity in the field is that the present vaccines contain only one serotype (serotype 6). Recent studies indicate that calves can be infected with more than one serotype and immunization of calves with one serotype does not always protect against challenge with a heterologous serotype (180). A third reason why oral vaccines are of limited value is that calves are susceptible to infection early in life, very shortly after colostral antibodies decline. This occurs within 5–7 days postcalving. Therefore, the time interval between oral immunization and exposure to field strains of virus is insufficient to develop protective levels of immunity required to prevent infection in the neonatal calf. Thus, the epidemiology of this disease makes it very difficult to implement effective active immunization methods.

The impediments to active immunization of the calf has led to the recent trend of hyperimmunizing the dam at mid-gestation and boosting at late gestation. This procedure results in much higher initial levels of

colostrum and milk antibodies. More importantly, even though milk antibodies drop after parturition they remain above a threshold level for the first few weeks after parturition. Under experimental conditions such an approach has resulted in protection from diarrhea. Unfortunately, under field conditions, the efficacy of the presently licensed vaccines has been questioned (159,180,190). The reason for the low level of effectiveness of these vaccines is probably related to the low levels of virus in the vaccine. Unfortunately, both of these viruses are relatively difficult to culture *in vitro* to high levels. However, improvements in growing these viruses during the past few years has increased the antigenic mass in the vaccines. To further increase the efficacy of these vaccines will require the use of genetic engineering techniques. Considerable progress has been made recently in this regard. For example, in rotaviruses, VP4 contains a proteolytic cleavage site that is conserved among all rotavirus serotypes. A synthetic peptide vaccine directed against the VP4 cleavage site has been shown to induce immunity against not only the bovine rotavirus serotype 6, from which the peptide was derived, but also against a variety of other serotypes from various species (73). Neutralizing antibodies have also been produced against a baculovirus produced VP4 protein (106). Since both synthetic peptides and recombinant proteins have shown to induce high levels of neutralizing antibody, it is envisaged that this protein or a portion of it could be produced in *E. coli* at sufficient levels that would make the vaccine economical. The ability to produce rotavirus proteins in *E. coli* makes it very attractive to insert the rotavirus gene into a *E. coli* K99 producing strain; thus one vaccine production system could result in a vaccine against both the *E. coli* and rotavirus. In the case of bovine coronaviruses, the proteins involved in inducing neutralizing antibodies have also been identified and cloned. Unfortunately, in this case the majority of the protective epitopes are conformational, thereby requiring recombinant subunit production in eukaryotic systems.

The final method of providing high levels of antibody in the lumen of the calf is by feeding monoclonal antibodies to the animal. Although this has proven to be very effective in preventing *E. coli* induced diarrhea (see above), it is not envisaged to be practical for viral induced neonatal diarrheas. The reason for this is that diarrhea in young calves can occur over an extended period of time. Thus it would not be economical to feed monoclonal antibodies to calves for a 3-week period. However, in a very severe outbreak it may be possible to prevent infection until management conditions are altered.

The final method of reducing enteric infections is by proper management. Since it is assumed that infection occurs as a result of virus shedding from adults in the environment, animals should not be crowded into contaminated areas. Movement of young calves into clean environments, away from other animals, will greatly reduce the rate of infection and economic loss (1,3).

## 5. Systemic Diseases

## A. Bacterial Infections

### i. Hemorrhagic Septicemia

*Pasteurella multocida* causes not only respiratory disease in cattle but also hemorrhagic septicemia in cattle and water buffalo. The disease can result in severe morbidity and mortality, primarily in tropical regions such as Asia, Africa, and South America. Two serotypes, B:2 and E:2, are associated with the disease, with the latter occurring primarily in Africa. Virulence determinants and protective antigens have not been studied in detail although the B:2 strains produce hyaluronidase, neuraminidase, and cell-associated protein antigens (27,42,69,141,142,143). Capsular polysaccharide is also produced and vaccines based on capsule have been shown to be protective in experimental challenge models (26,122,134). While *P. multocida* strains associated with other disease syndromes produce a dermonecrotic toxin, no relationship between toxin production and hemorrhagic septicemia has been observed.

Following exposure of animals to the organism, clinically healthy animals often carry the bacteria in the nasopharynx and tonsils. Shedding of the bacteria by such carriers can be induced by environmental stress. It is possible to passively protect animals from experimental challenge with serum from hyperimmune animals, indicating that a humoral response to the bacteria should be sufficient for protection in the field (26). In fact, oil-adjuvanted bacterins are generally effective in preventing disease. As with the pneumonic strains, live vaccines including streptomycin-dependent mutants have been used successfully in experimental vaccine challenge trials (192). Also subcutaneous vaccination with live B:3,4 strains, could protect against experimental challenge with B:2 strain. Since the disease occurs principally in isolated areas, the ideal vaccine would have to be stable, inexpensive, and easy to administer. The most appropriate type of vaccine would be a live attenuated strain that has defects in one or more "housekeeping" genes (see above) and could be delivered in drinking water or intranasally. While other products (bacterins and subunits) may be as stable as attenuated organisms and inexpensive to produce, intramuscular injection may not be as practical in rural areas of developing countries where animal density is low.

### ii. Anthrax

*Bacillus anthracis*, the causative agent of anthrax, is a Gram-positive, spore-forming organism found throughout the world. Spores can be found in environmental and tissue samples that have been exposed to the

atmosphere. Such spores can enter the host by ingestion, inhalation, or through skin lesions and the organism quickly enters the bloodstream where it establishes secondary sites of infection. Death is usually rapid, due to bacteremia and toxemia.

Two virulence determinants have been well characterized—the poly-D-glutamate capsule and the anthrax toxin (87,99,139). Genes coding for these virulence determinants are carried on two plasmids, pXO1 and pXO2 (115,184). Antibody against toxin is necessary for protection while antibody to capsule is not required (76). The currently used veterinary vaccine is based on this principle and contains spores from a toxigenic, noncapsulated strain. Vaccination results in protective immunity within 10 days, with annual boosters required. Current research is focussing on the development of attenuated *B. anthracis* strains and also subunit vaccines based on anthrax toxin. Vaccination with transposon-induced mutants defective in aromatic amino acid biosynthesis have been shown to be protective in a mouse model, as has recombinant *B. subtilis* carrying the genes for the protective antigen (PA) toxin component (75). Well-characterized, attenuated strains and delivery systems such as these will likely be the focus of a new generation of veterinary vaccines. Potential recombinant subunit vaccines are also being developed, based on the protective antigen. The protective antigen, one of the three components of anthrax toxin, binds to host receptors and is then proteolytically cleaved (172,188). This cleavage is necessary for interaction with the other toxin components. The gene coding for PA has been altered by site-directed mutagenesis to remove the cleavage site and the modified gene expressed in *B. subtilis* (172). Administration of this modified PA to mice blocked the lethal action of authentic PA, presumably by competition for receptors. The altered protein may be an effective subunit vaccine for humans, replacing the subunit PA vaccine now used. It may also form the basis of an effective subunit vaccine in cattle if the downstream processing of the antigen is minimized to reduce production costs to an economical level.

### iii. Brucellosis

Brucellosis is a disease of global importance affecting most species of livestock. *Brucella abortus* infection often occurs in pregnant cattle as a result of contact with aborted fetuses or placental tissue. The bacteria are then able to colonize and penetrate the mucosal epithelium. The organism is able to replicate intracellularly in phagocytic cells and this virulence trait may enable it to reach other tissues, especially regional lymph nodes, although this is certainly not a requirement for secondary infection. This process is slow, taking weeks to months and chronicity of the disease is aided by its survival in phagocytic cells. Attenuated *B. abortus* strain 19 has been used extensively as a veterinary vaccine and it

has been shown to protect 65–85% of calves vaccinated in the field (181). Strain 19 delivered orally also protects against experimental challenge (125). Inactivated adjuvanted virulent strains have also been used with similar efficacy rates. Since most countries still slaughter *Brucella* infected animals, it is important for one to be able to distinguish vaccinated from infected animals. Thus, either a subunit vaccine or subunit component delivered on a live attenuated carrier (e.g., *Salmonella*) is attractive. It is clear that any vaccine should stimulate not only a humoral response, but also cell-mediated immunity. In this respect, the appropriate antigen delivered with attenuated carriers such as *S. typhimurium*, *Francisella tularensis*, or BCG may form a very effective vaccine.

### iv. Vibriosis

Vibriosis is a bovine venereal disease caused by *Campylobacter fetus*. The organism is spread by sexual contact or contaminated semen resulting in infertility, delayed conception, and abortion. The disease is characterized by inflammation of the epithelial surfaces of the female genital tract. Virulence determinants of the organism have not been studied in detail, but it is clear that *C. fetus* is resistant to phagocytosis, possibly mediated by capsular polysaccharide. In the presence of opsonizing IgG, the organism is efficiently killed (35). Thus, commercially available bacterins appear to be effective due to their ability to stimulate a systemic IgG response. The predominant antibody isotype found in the genital tract is IgA, which cannot opsonize *C. fetus*, but will compete with IgG (35). Thus, stimulation of an efficient mucosal response to vaccination may not be desirable in this case. Since commercially available vaccines are effective in the prevention of vibriosis, it is unlikely that new products will appear in the near future. However, improved diagnostics are needed as companion products to vaccines for the screening of contaminated semen. Thus, species-specific reagents, including monoclonal antibodies to surface components and gene probes, would be useful companion products with traditional vaccines.

### v. Moraxella bovis

*Moraxella bovis* is the agent responsible for infectious bovine keratoconjunctivitis (IBK), or pinkeye. Once established in a herd it can spread rapidly, infecting a large number of animals. Symptoms of the disease appear quickly and the infection is generally cleared within 1–2 months. A less severe form of IBK can also be caused by Mycoplasma infection and this can result in an increased susceptibility to colonization by *M. bovis* (147). However, this is not a prerequisite for *M. bovis* infection. The primary economic problem is weight loss in beef cattle or decreased milk production in dairy cows. *M. bovis* is a relatively well-characterized veterinary pathogen and a number of virulence determinants have been

identified. These include well-characterized fimbriae, thought to be involved in colonization, heat-labile hemolysin, hyaluronidase, and collagenase (8,133,161). Nonpiliated, nonhemolytic strains are encountered frequently in the field as part of the normal bovine flora. These can also occur spontaneously upon laboratory passage of virulent strains. Such variants are avirulent, and nonhemolytic strains have been shown to be effective live vaccines, probably due to the induction of localized humoral or cellular immunity (146). Conventional bacterins and extracts are not always effective as vaccines, whereas subunits composed of pili have been shown to be protective in experimental models (145). The gene coding for the fimbrial subunit has been cloned and expressed in *E. coli* and this may make the production of a subunit vaccine economically feasible for the veterinary market (109). The assembly of fimbrial subunits on the surface of a live delivery vehicle (see above) is also an attractive alternative since such a product should potentially stimulate a cell mediated immune response.

### vi. Clostridia and Leptospira

At least 7 different clostridial species are economically important pathogens of livestock. These include *Clostridium novyi*, *C. chauvoei*, *C. haemolyticum*, *C. septicum*, *C. perfringens*, *C. sordellii*, and *C. tetani*. All of these species produce one or more potent toxins that are central to the disease process eventhough their modes of action are different. A summary of the *Clostridial* species, disease syndromes, and virulence determinants is shown in Table 9.3.

Conventional vaccines against *Clostridial* diseases are composed of chemically inactivated, aluminium hydroxide-absorbed cultures with or

**Table 9.3.** Disease syndromes and toxins produced by clostridial species.

| Species | Disease syndrome | Toxin produced |
|---|---|---|
| *C. botulinum* | Botulism | Strains produce one of eight different neurotoxins ($C_2$ = inhibitor of protein synthesis); Types A, B, C, D are associated with cattle |
| *C. tetani* | Tetanus | Tetanus toxin; hemolysin (tetanolysin) |
| *C. chauvoei* | Blackleg, wound infection | $\alpha$-Toxin (necrotizing hemolysin); $\beta$-toxin (deoxyribonuclease); $\gamma$-toxin (hyaluronidase); $\delta$-toxin (hemolysin) |
| *C. septicum* | Wound infection | $\alpha$-, $\beta$-, $\gamma$-, $\delta$-Toxins ($\alpha$, $\beta$ serologically related to *C. chauvoei*), neuraminidase |
| *C. novyi* | Gas gangrene | Type A produces $\alpha$-, $\gamma$-, $\delta$-, $\varepsilon$-toxins; Type B produces above plus $\beta$-, $\zeta$-, $\varepsilon$-, $\theta$-toxins; Type C is nontoxigenic |
| *C. haemolyticum* | Bacillary hemoglobinuria | Produces *C. novyi* $\beta$- (phospholipase), $\varepsilon$-, and $\theta$-toxins |

without detoxified supernatants. In addition, vaccines composed of the toxoid alone are effective products and are available for some species. Vaccines are usually sold as 2-way, 7-way, or 8-way products, containing *C. chauvoei* and *C. septicum* (2-way), *C. chauvoei*, *C. septicum*, *C. novyi*, *C. perfringens* Type C and Type D, *C. sordellii* or *C. tetani* (7-way), and the 7-way product plus *C. haemolyticum* (8-way). Due to the effectiveness of these products, it is unlikely that they will be replaced in the near future. The toxins produced by *Clostridial* species have generated considerable interest for the human vaccine market. For example, genes coding for tetanus toxin, which are localized on a bacteriophage, have been cloned and expressed in *E. coli*, raising the possibility of producing a recombinant DNA subunit vaccine. It is clear from Table 9.3 that the production of recombinant subunit vaccines for all *Clostridial* species in cattle will be a major undertaking. Due to the number of antigens that would have to be produced the costs will be excessive when compared to conventional products. However, it may be feasible to supplement chemically inactivated cells with recombinant toxoids rather than the detoxified culture supernatants currently in use.

Leptospirosis in cattle can be caused by several serovars, including *L. canicula*, *L. hardjo*, *L. pomona*, *L. icterohaemorrhagiae*, and *L. grippotyphosa*. Upon infection, these organisms colonize the liver and then spread to other tissues especially the kidney. The most common clinical signs of disease include fever, depression, anorexia, abortion, stillbirth, and decreased milk production in dairy cattle. Frequently, the mode of transmission is via organisms that are shed in the urine. Leptospiras are also capable of passing to the placenta causing fetal infection and abortion. Although very little is known about the basic physiology or pathogenesis of these organisms, this has not hindered development of effective vaccines. Inactivated bacterins have been used extensively for vaccination and this has met with a high degree of success in the field. Due to minimal cross-protection between serovars, five-way bacterins containing each serovar are needed. These conventional products are safe and effective and it is therefore unlikely that a focused effort will be made to apply the tools of modern biotechnology to improve their efficacy.

### vii. Mastitis

Bovine mastitis can be caused by infection with a wide range of Gram-negative and Gram-positive organisms, the most common being *E. coli*, *S. aureus*, and several environmental streptococcal species (*Streptococcus agalactiae*, *Str. dsygalactiae* and *Str. uberis* for example). It is the largest single factor contributing to economic losses caused by infectious disease to the dairy producers world wide. The mammary gland provides a rich environment for the growth and multiplication of bacteria that

enter through the teat canal. Chemotactic metabolites produced by the organisms result in an influx of PMNs, causing a severe inflammatory response (63). If the infection persists for an extended period, then the mammary gland exhibits clinical mastitis. The control of mastitis by immunization against specific pathogens, mostly *S. aureus* and *E. coli*, has been practised for a number of years. However, vaccination against one or two pathogens in a multifactorial disease syndrome cannot be expected to reduce the overall incidence of the disease.

The *E. coli* strains associated with mastitis are indistinguishable from fecal isolates and likely originate from contaminated bedding. These strains produce typical *E. coli* virulence determinants, such as capsule, fimbriae, endotoxin, and cytotoxin, with the latter two being the most important in eliciting a severe inflammatory response and damaging the teat end and mammary parenchymal tissue. Blockage of iron acquisition has been shown to be bacteriostatic and thus iron-regulated outer-membrane proteins produced for the transport of iron may be useful antigens for a subunit vaccine (183). Immunization with the *E. coli* mutant J5 has been shown to reduce the severity of experimental mastitis and field studies have shown that vaccination could increase annual income by $32.00/cow (58,59). Immunization with a commercially available *E. coli* scours vaccine also reduced the severity of the experimentally induced coliform mastitis (187). However, neither vaccine is efficacious in reducing the rate of new *E. coli* infections.

The principal Gram-positive organism associated with mastitis is *S. aureus*, a common inhabitant of the skin. The organism produces a wide range of enzymes and virulence determinants including coagulase, hyaluronidase, nucleases, lipases, proteases, and a number of toxins ($\alpha$-, $\beta$-, $\gamma$-, and $\delta$-leukocidin) (9). $\alpha$-Toxin and leukocidin affect PMNs and can lyse target cells before or after phagocytosis (i.e., from both extra- and intracellular environments) (189). However, the organism is often resistant to phagocytosis and this is possibly mediated by capsule, protein A or coagulase-mediated aggregation of cells (6). Not all strains produce capsule or protein A and the role of the former in virulence is unclear. Vaccination with $\alpha$-toxin or coagulase does not result in protection against experimental challenge, whereas vaccination with protein A can offer minimal protection (131). However, since not all strains produce each virulence determinant, a single subunit is unlikely to work in the field. A virulent isolate which was attenuated by *in vitro* passage until it became nonhemolytic was shown to be an effective experimental live vaccine (191). Inactivated bacterins which are currently lisenced for use are only partially effective. A mixed lysate of *S. aureus* strains containing polyvalent antigens is also commercially available in North America and can provide immunity to experimental challenge for up to one year.

The three streptococci, *Str. agalactiae* (Group B), *Str. dysgalactiae* (Group C), and *Str. uberis*, are not as well characterized as *S. aureus* and

*E. coli*. Streptococcal virulence determinants include hyaluronic acid capsule, produced largely by Groups A and C, fimbriae used for attachment, M protein, streptolysin O, streptolysin S, hyaluronidase, and streptokinase (14). Other extracellular products such as proteases and nucleases are also produced. Not all groups produce each virulence determinant and there can be considerable variation between strains within a group. The capsule produced by some strains of *Str. dysgalactiae* has antiphagocytic properties but is produced only by exponentially growing cells. Hyaluronidase, which is synthesized later in the growth phase, effectively removes the capsule. At the present time there are no effective vaccines for streptococcal mastitis.

Anderson (7) has pointed out one fundamental problem with the development of vaccines for mastitis. That is, the colonization of the mammary gland by Gram-negative or Gram-positive bacteria invariably results in an inflammatory response, which is in itself a definition of mastitis. Since the inflammatory response is the primary defense mechanism against bacterial colonization in the mammary gland, immunization is likely to enhance this mechanism and therefore the reaction of an immunized gland to infection will be mastitis. It is therefore desirable for a vaccine to induce essentially a subclinical case of mastitis, which is eliminated quickly. It is likely that one of the easiest way to achieve this will be vaccination with live attenuated strains of *E. coli*, *S. aureus*, and streptococcal species, with or without recombinant antigens. Development of such vaccines will likely take several years.

## B. Systemic Viral Infections

### i. Foot-and-Mouth Disease (FMD)

The most dreaded bovine virus disease in many countries is FMD. Although the disease in cattle rarely leads to death, production losses can be high. For countries that have FMDV, losses due to trade embargoes on export of farm products are the major economic loss. Thus in addition to being a economically important disease in its own right it is also a politically important disease. In epidemic areas such as Africa, Asia, and South America, vaccination is the method of control. Although both live attenuated and inactivated vaccines have been used, the majority of the vaccines are prepared by inactivation of tissue culture grown virus. It is estimated that over 1.5 billion doses of virus are administered annually. This makes FMDV vaccines the most frequently used products to control any animal disease in the world. In North America, Australia, and Europe, where the disease does not normally occur, vaccination is not used. The method of control in these areas is an embargo on cattle and unprocessed cattle products. These embargoes are supported by

legislation and local veterinarians are required to notify the appropriate authorities upon the initial observation of vesicular disease.

Due to the economic importance of FMD, extensive research has been conducted on methods of immunization and a better understanding of the molecular biology of this virus in particular. As a result of these activities the molecular structure and antigenic components involved in inducing protective immunity have been dissected. It has clearly been shown that there are a number of serotypes: O, A, C, SAT1, SAT2, SAT3, and ASIA 1. In addition to these 7 serotypes, a considerable amount of antigenic variability occurs within these serotypes. As a result of this antigenic variability, it is important to ensure that vaccination occurs with the specific serotypes that are circulating within the country where control is being attempted.

Molecular studies on the antigenic structure of FMDV has indicated that the major immunogenic site is located on the VP1 protein of the virus. As a result of this localization, FMDV VP1 was one of the first proteins expressed in *E. coli* and tested as a vaccine in the early 1980s (91). In addition to serving as a model for understanding the problems of protein folding it also demonstrated that a vaccine produced by genetic engineering can protect animals from a disease caused by a virus. Molecular analysis of the VP1 protein indicated the location of the protective epitopes as well as the basis for antigenic variation responsible for evasion of immune responses. This virus protein has served as a model for synthetic peptide vaccines and has helped elucidate how a change in a single amino acid may influence the immunogenicity of proteins (17,20). Thus by substituting one specific amino acid within a 20 amino acid peptide one could broaden the neutralizing capacity such that the peptide would now induce protective immunity to heterologous virus within the same serotype as well as the homologous virus strain. Based on these observations, Dr. Brown concluded that it should be possible to tailor peptide vaccines that have a broad antigenic range of protection. Whether these vaccines will ever replace the current cheaper vaccines remains to be determined.

### ii. Bovine Viral Diarrhea

Since animals infected with bovine virus diarrhea (BVD) can manifest a variety of clinical signs ranging from enteric to respiratory and systemic infection, BVD virus is considered in this section of generalized infections rather than being allocated to any one of the specific entities. Originally it was thought that BVD and mucosal disease were two different viral infections. However, it is now clear that both of these are just different manifestations of the same virus. This disease occurs worldwide and can cause morbidity and mortality in its own right but as a result of its ability to cause immunosuppression it also plays an important role

in predisposing animals to secondary infections (155). In most cases, infection of seronegative animals results in a transient subclinical infection. However, there are reports that the virus can cause clinical disease in healthy seronegative animals. The most important feature of bovine virus diarrhea is its ability to cause fetal infections. Depending on the virus strains, the time of gestation that a pregnant animal is infected and its serological status will determine the eventual outcome of the disease. *In utero* infection with noncytopathic BVD virus occurring prior to 120 days of gestation leads to immunotolerance and persistence of the virus, possibly for the life of the animal. If the immunotolerant animal is later exposed to an antigenically related cytopathic BVD virus, it cannot mount an effective immune response and the result is uncontrolled replication and severe mucosal disease (19). Thus, to control severe mucosal disease caused by BVD it is important to immunize cattle prior to breeding. This can be achieved by using either live or inactivated vaccines. However, there is no assurance that immunization with any vaccine will prevent fetal infection. Whether this is due to the multiple variants of the BVD virus or its ability to effect leukocytes and spread to the fetus, is not fully understood but it is clear that there is a need for vaccines that are safe and efficacious against all field strains of BVD virus. Whether it will be possible to identify conserved epitopes within the various strains of BVD virus, produce them by genetic engineering methods, and provide fetal protection remains to be determined. Due to the immunosuppressive nature of live BVD vaccines it is not recommended that animals entering the feedlots or other high risk areas be immunized (155).

Monoclonal antibodies that neutralize virus *in vitro* have been developed (41). Whether these antibodies can recognize all variants of BVD remains to be determined. Recently, considerable progress has been made at localizing the genes coding for the specific proteins recognized by neutralizing monoclonal antibodies (31). As a result of these developments it is hoped that large quantities of the proteins will be produced and tested for their ability to reduce or prevent BVD infections. Whether any of these vaccines will be able to prevent fetal (*in utero*) infection remains to be determined.

### iii. Rinderpest

Rinderpest is a member of the morbillivirus genus of the Paramyxovirus family. This virus can cause acute systemic disease in ruminants resulting in erosion of the mucosal epithelium in the respiratory and digestive tract. If introduced into seronegative herds, the disease can be extremely explosive and result in large economic losses. Fortunately this virus has been eliminated from a number of countries and now occurs only in Africa and Asia. In countries free of rinderpest, control measures are

designed to prevent introduction of the virus. These control measures are similar to those described above for foot-and-mouth disease virus. In countries where rinderpest is enzootic, or where the disease has a high probability of being introduced, vaccination is the method of control (144,164,165). Although inactivated viral vaccines have been used, immunity induced by these vaccines was often temporary, requiring repeated annual revaccination. The development of live attenuated vaccines has dramatically improved the level and duration of immunity. In fact, it has been stated that the live attenuated tissue culture vaccines are among the best available for any bovine disease. The basis for this statement is that the vaccines induce life long immunity and are cheap. One of the problems with this vaccine is its thermal stability. To maintain vaccine efficacy it is mandatory to maintain an adequate cold chain from manufacturing to administration. Unfortunately in many of the countries in Africa and Asia, where rinderpest is a problem, maintenance of the cold chain may not always be as effective as one desires. In an attempt to overcome this problem a heat-resistant strain has been developed. In addition other methods of producing the vaccine are being investigated. The most recent approach, using recombinant DNA technology, involves incorporation of the F and HN protein of rinderpest into vaccinia virus (198). Animals immunized with the recombinant vaccinia virus, carrying the genes coding for rinderpest proteins, developed immunity to rinderpest and were resistant to challenge with virulent virus. Whether this vaccine will be licensed for use in controlling rinderpest and will eventually replace the highly effective live attenuated rinderpest vaccine remains to be determined.

### iv. Minor Bovine Viruses

A number of other viruses that can cause infections of cattle include Akabane disease, caused by a mosquito-borne virus in the Bunyavirus family. This virus is restricted to areas of Japan, Australia, some countries in the Pacific Southwest, East and South Africa, and some Middle Eastern countries. Inactivated viral vaccines have been shown to be effective at preventing abortions and congenital abnormalities in cattle infected with the virus (92). Annual boosters are required. Recently a live attenuated vaccine has been licensed in Japan (74).

Although bluetongue is generally considered to be a major problem in sheep it can cause infection in cattle as well (23). In general cattle suffer milder infections than sheep and have a low mortality rate. Attenuated vaccines have shown to be successful in preventing clinical bluetongue or congenital abnormalities. However, it must be emphasized that immunization of pregnant cattle should not be practised since the vaccine virus is not sufficiently attenuated to prevent in from causing congenital abnormalities. Since there are 24 different immunological serotypes, it is

important to design the vaccines to contain the constellation of serotypes endemic in the specific area. Recently a number of the bluetongue virus genes involved in inducing neutralizing antibody have been cloned and expressed in a variety of expression systems. One of the more interesting developments is the observation that coexpression of a number of individual genes in baculovirus can result in assembly of virus-like particles (50,103). It may be possible that this totally recombinant self assembled virus-like particle may prove to be an excellent vaccine against bluetongue virus. Whether this will indeed prove to be a new approach to immunization remains to be determined (156).

Rift valley fever virus can infect cattle as well as sheep, goats, and humans. In areas where the virus in endemic an attenuated vaccine is used (171). As is the case with many viruses that can cause abortions or congenital infections it is not recommended that pregnant animals be immunized with the live attenuated vaccines. Whether the new minute plaque or highly mutagenized candidate vaccines could be used in pregnant animals awaits further investigation. Formalin-inactivated vaccines are safe but need to be given at least twice before effective immunity develops. These vaccines are not very stable since they are provided in a liquid form. They are also more expensive than the live attenuated vaccines. In all cases annual booster immunization is recommended to maintain protection (195).

### v. Viruses Causing Skin Infections

Several viruses have the ability to produce either localized or systemic infections of the skin. These include members of the Poxvirus, Herpesviruses, and Papilloma virus families. Within the poxviruses a number of members can cause skin infections in cattle. These include vaccinia virus, cowpox, and pseudocowpox (130). These infections are generally rare in North America but are more common in other parts of the world. Variants of vaccinia virus can also cause infections in water buffalo in various areas of India and Indonesia. All of the members of the poxvirus family induce similar types of lesions, which initially start out as small papules eventually developing into larger lesions. In the case of lumpy skin disease, lesions may develop as plaques and then ulcerate. In the majority of pox infections mortality is generally very low but economic losses may be high due to loss of milk production and in the case of lumpy skin disease damage to hides. Since these viruses do not cause severe economic losses, vaccines are generally not in use. However, based on the observation that these viruses induce excellent immunity it should be possible to develop vaccines against them if a vaccine was needed.

Bovine herpesvirus-2 can induce either generalized skin lesions throughout the body or more localized lesions of the mammary gland

(55). For this reason the virus is often called bovine mammalitis virus. In contrast to the lesions caused by the poxviruses, BHV-2 produces much more obvious ulcerative lesions. Upon introduction of the virus into a totally susceptible herd the frequency of infection in very high with virus being spread from one cow to another by mechanical methods during milking. However, the mortality rate is very low. The major economic losses are due to loss of milk production or complications resulting from mastitis. No commercial vaccines are presently available for BHV-2 and it is unlikely that vaccines will be developed for this disease even though experimental vaccines have been shown to be effective; the infection occurs worldwide and only one serotype of the virus is responsible for infection (157).

Warts are a common infection of cattle. Although animals of all ages can be affected, the incidence is highest in calves and yearlings, especially if they are held in close proximity. This indicates the infectious nature of the disease. The disease is often self-limiting, thus it is often unnecessary to implement control measures. However, autongenous vaccines produced by formalin inactivation of homogenous warts are often used to expedite the regression of warts (176). These vaccines are given either intradermally or subcutaneously. The value of such a vaccine is often questioned, since many warts regress spontaneously even without such treatment. However, surgical removal of the wart and reintroduction of antigens in the form of formalized autongenous vaccines can ensure cure. An experimental recombinant vaccinia papilloma virus vaccine has been developed (114). However, since protection appears to be serotype specific, immunization will need to be carried out with the appropriate serotype (78,79).

## 6. Delivery/Adjuvants

In many instances where killed vaccines are used to elicit immune responses, it is important to include adjuvants to nonspecifically stimulate the immune response toward the injected antigens. This is becoming even more crucial with the subunit vaccines produced by recombinant DNA technology or synthetic peptides since the purified proteins are poorly immunogenic on their own. In the present review we will not discuss adjuvants in detail. However, we feel that a brief introduction is warranted since the efficacy of the presently licensed conventional vaccines and the future new generation of vaccines can be influenced by the type of adjuvant used. For more detail the reader is referred to an excellent review by Allison and Byers (5) regarding the different classes of adjuvants. The requirements for good adjuvants include the ability to enhance cell-mediated immunity, an adequate level of humoral immunity of the correct isotype, as well as the ability to elicit both B and T cell

memory. In addition, the adjuvant should not result in tissue damage (granulomas) at the site of injection and it should not induce pyrexia or autoimmune responses. Unfortunately, many of the agents that stimulate high levels of immunity also elicit some of the undesired side effects. One of the best adjuvants, Freund's complete adjuvant, is not suitable for use in food-producing animals (51). Furthermore, its adverse side effects are so dramatic that they should not be used in any animal. However, it has laid the foundation for our understanding of the requirements of effective adjuvants. Some of this work was pioneered by Ribi Immunochemical Research Incorporation which resulted in switching from a water in oil mixture to an oil in water mixture (151). The concentration of oil has been reduced to 1–2% of the vaccine with minimal occurrence of granulomas and abscesses. Other organizations are developing a wide range of adjuvants, which undoubtedly will be added to their repertoire of vaccines. These include vehicles or slow release formulations, cytokines (interleukins and interferons), immunostimulatory complexes (ISCOMS), liposomes or variations thereof (virosomes, immunosomes), purified bacterial components, surface active components (saponin), and quaternary amines (avridine and dimethyl dioctadecyl ammonium bromide) (5,37,43,53,61,71,82,94,104,113,118). One common feature of all these agents is that they enhance some aspect of the immune response. Since it is well known that protection for some organisms is mediated by one or the other arm of the immune response (humoral or cellular), it is important to choose the adjuvant that will stimulate the most appropriate immune response. Furthermore, the route of administration may influence whether cellular or humoral immunity is preferentially enhanced. Thus in vaccine design all of these factors must be considered. Finally the ease of administration in the field must not be overlooked.

In addition to improving adjuvants it is often important to link subunit antigens, especially peptides, to larger carrier molecules to improve immunogenicity or to target the antigen to antigen-presenting cells. The recent development of viral-based particles (hepatitis B, tobacco mosaic virus, and yeast TY-VLPs) has increased the immunogenicity of subunit vaccines (38,68,124). Another recent development, which may further improve the immunogenicity of these subunit vaccines, is to actually target the antigen to antigen-presenting cells. Such targeting has recently been demonstrated using antigens linked to anti-MHC Class 2 antibodies or solid matrix particles (24,149). Finally the increase in our knowledge of immune regulation and the interactions and roles of cytokines in immune responses should provide us with very effective methods to enhance immunity to many of the newer vaccines. However, parallel advances will need to be made with regard to slowed delivery and targeting of specific cytokines and the antigens for maximal immune responses (127,128).

**Table 9.4.** Summary of organisms where potential future vaccines could be developed by recombinant DNA technology.

| | Bacterial | Viral |
|---|---|---|
| Respiratory | *Pasteurella haemolytica* | Bovine herpesvirus-1 (BHV) |
| | *Haemophilus somnus* | Parainfluenza-3 (PI-3) |
| | *Mycoplasma* sp. | Bovine respiratory syncytial virus (BRSV) |
| | *Pasteurella multocida* | Adenovirus |
| Enteric | *Escherichia coli* | Rotavirus |
| | *Salmonella* sp. | Coronavirus |
| Systemic and other | *Pasteurella multocida* | Foot-and-mouth disease virus (FMDV) |
| | *Bacillus anthracis* | Bovine virus diarrhea (BVD) |
| | *Brucella abortus* | Rinderpest |
| | *Moraxella bovis* | Bluetongue |
| | *Staphylococcus aureus* | Papilloma |
| | Streptococcal sp. | |

## 7. Summary and Future

As is evident from the material presented in this review there are a number of viral and bacterial diseases of cattle for which improved vaccines would make a significant impact on the economics of livestock production. However, it is also evident that the recent technology available for identifying important antigens involved in inducing protective immunity combined with methodologies to characterize the specific immunological responses involved in recovery from infections provides a great potential for improving vaccines used in veterinary medicine. It is envisaged that within the next decade many of the conventionally produced vaccines will either be supplemented with recombinant components or be totally produced by recombinant DNA technology either as live or subunit vaccines (Table 9.4). However, even with these new vaccines, factors other than the antigen itself must be considered in immunization strategies to improve the animals resistance to infection. These include more appropriate delivery systems and their combination with immunomodulators or adjuvants to increase the immune response to these vaccines. There are, however, a number of conventionally produced vaccines that are safe, effective, and economical and therefore will continue to be used. Many academic institutions as well as companies are devoting a considerable amount of effort toward these areas of investigation. However, regardless of all the scientific knowledge that is available, vaccine strategies must also consider the practical problems of animal husbandry in various parts of the world. In some instances the best scientific approach is not always practical under field conditions and a compromise often has to be reached. Fortunately, many of the vaccine companies recognize these diverse animal husbandry practices and, therefore, design their vaccines to accommodate them. Unfortunately, such compromises may result in reduced efficacy of vaccines.

## References


1. Acres SD: Epidemiology of neonatal diarrhea. Ph.D. Thesis, University of Saskatchewan, 1976.
2. Acres SD, Isaacson RE, Babiuk LA, Kapitany RA: Immunization of calves against enterotoxigenic colibacillosis by vaccinating dams with purified K99 antigen and whole cell bacterins. Infect Immun 1979; 25:121–126.
3. Acres SD: Enterotoxigenic *Escherichia coli* infections in newborn calves: A review. J Dairy Sci 1985; 68:229–256.
4. Adlam C, Knight JM, Mugridge A, et al: Purification characterization and immunological properties of the serotype-specific capsular polysaccharide of *Pasteurella haemolytica* (serotype A1) organisms. J Gen Microbiol 1984; 130:2415–2426.
5. Allison AC, Byars NE: Adjuvants for a New Generation of Vaccines In: Woodrow GC, Levine MM (eds): New Generation Vaccines. New York: Marcel Dekker, 1990, pp 129–140.
6. Anderson JC. Staphylococcus. In: Gyles GL, Thoen CO (eds): Pathogenesis of Bacterial Infections in Animals. Ames: Iowa State University Press, 1986, pp 14–20.
7. Anderson JC: The problem of immunization against Staphylococcal mastitis. Br Vet J 1978; 134:412–420.
8. Arora AK: Toxic effects of *Moraxella bovis* and their relationship to the pathogenesis of infectious bovine kerato-conjunctivitis. Vet Arch 1982; 52:175–182.
9. Arvidson SO: Extracellular enzymes from *Staphylococcus aureus*. In: Adlam, C, Easmon CSF (eds): Staphylocci and Staphylococcal Infections, Vol. 2. New York: Academic Press, 1983, pp 745–808.
10. Babiuk LA, Acres SD: Experimental models for bovine respiratory diseases. In: Loan RW (ed): Bovine Respiratory Disease: A Symposium. Texas A&M University Press, 1984, pp 287–325.
11. Babiuk LA, Sabara M, Hudson G: Viral induced enteritis in animals. Prog Vet Microbiol 1984; 1:80–120.
12. Bachrach HL: New approaches to vaccines. Adv Vet Sci Comp Med 1985; 30:1–38.
13. Baluyut CS, Simonson RR, Bemrich WJ, Maheswaran SK: Interaction of *Pasteurella haemolytica* with bovine neutrophils: Identification and partial characterization of a cytotoxin. Am J Vet Res 1981; 42:1920–1926.
14. Barnum DA: Streptococcus. In: Gyles CL, Thoen CO (eds): Pathogenesis of Bacterial Infections in Animals. Ames: Iowa State University Press, 1986, pp 3–13.
15. Bennet BW: Efficacy of *Pasteurella* bacterins for yearling feedlot heifers. Bovine Practice 1982; 3:26–30.
16. Berman PW, Gregory T, Dowbenko D, Lasky L: Production of viral glycoproteins in genetically engineered mammalian cell lines for use as vaccines against immune deficiency retrovirus. Appl Virol Res 1988; 1:17–24.
17. Bittle JL, Houghten RA, Alexander H, Shinnick TM, Sutcliffe JG, Lerner RA, Rowlands DJ, Brown F: Protection against foot-and-mouth disease by immunization with a chemically synthesized peptide predicted from the viral nucleotide sequence Nature (London) 1982; 298:30–33.

18. Blanchard-Channell MT, Ashfag MK, Kadel WL: Efficacy of a streptomycin-dependent, live *Pasteurella haemolytica* vaccine against challenge exposure to *Pasteurella haemolytica* in cattle. Am J Vet Res 1987; 48:637–642.
19. Bolin SR, McClurkin AW, Cutlip RC, Coria MF: Severe clinical disease induced in cattle persistently infected with noncytopathic bovine virus diarrhea virus by superinfection with cytopathic bovine viral diarrhea virus. Am J Vet Res 1985; 46:573–576.
20. Brown F, Bomford RH: Synthetic peptides in animal health. In: Babiuk LA, Philips JP (eds): Animal Biotechnology, Oxford, Pergammon Press, 1990, pp 1–19.
21. Burke KL, Dunn G, Ferguson M, Minor PD, Almond JW: Antigen chimaeras of poliovirus as potential new vaccines. Nature (London) 1988; 332:81–82.
22. Burki F: Bovine adenoviruses. In: Dinter Z, Morein B (eds): Virus Infections of Vertebrates, Vol. 3. Amsterdam, Elsevier, 1990, pp 161–169.
23. Campbell CH, Grubman MJ: Current knowledge on the biochemistry and immunology of blue tongue. Prog Vet Microbiol Immunol 1985; 1:58–79.
24. Carayanniotic G, Vizi E, Parker JM, Hodges RS, et al: Delivery of synthetic peptides by anti-class II MHC monoclonal antibodies induces specific adjuvant-free IgG responses *in vivo*. Mol Immunol 1988; 25:907–911.
25. Cardella MA, Adviento MA, Nervig RM: Vaccination studies against bovine *Pasteurella* pneumonia. Can J Vet Res 1987; 51:204–211.
26. Carter GR, de Alwis MCL: Haemorrhagic septicaemia. In: Adlam C, Rutter JM (eds): Pasteurella and Pasteurellosis. London: Academic Press, 1989, pp 131–160.
27. Carter GR, Chengappa MM: Hyaluronidase production by Type B *Pasteurella multocida* from cases of hemorrhagic septicaemia. J Clin Microbiol 1980; 11:94–96.
28. Charles I, Dougan G: Gene expression and the development of live enteric vaccines. Trends Biotechnol 1990; 8:117–121.
29. Chiang Y-W, Kaeberle ML, Roth JA: Identification of suppressive components in *Haemophilus somnus* fractions which inhibit bovine polymorphonuclear leukocyte function. Infect Immun 1986; 52:792–797.
30. Clarke RC, Gyles CL: Salmonella. In: Gyles CL, Thoen CO (eds): Pathogenesis of Bacterial Infections in Animals. Ames: University of Iowa Press, 1986, pp 95–109.
31. Collett MS, Larson R, Belzer SK, Retzel E: Proteins encoded by bovine viral diarrhea virus: the genomic organization of a pestivirus. Virology 1988; 165:200–208.
32. Collins FM, Campbell SG: Immunity to intracellular bacteria. Vet Immunol Immunopathol 1982; 3:5–66.
33. Confer AW, Panciera RJ, Fulton RW, et al: Effect of vaccination with live or killed *Pasteurella haemolytica* on resistance to experimental bovine pneumonic pasteurellosis. Am J Vet Res 1985; 46:342–347.
34. Confer AW, Panciera RJ, Mosier DA: Bovine pneumonic pasteurellosis: Immunity to *Pasteurella haemolytica*. J Am Vet Med Assoc 1988; 193: 1308–1316.
35. Corbeil LB, Schurig GG, Duncan JZ, Wilkie BN, Winter AJ: Immunity in the female bovine reproductive tract based on the response to *Campylobacter*

*fetus*. In: Butler JE, Duncan JR, Nielson K (eds): The Ruminant Immune System. New York: Plenum, 1981, pp 729–743.
36. Czuprynski CJ, Hamilton HL: Bovine neutrophils ingest but do not kill *Haemophilus somnus in vitro*. Infect Immun 1985; 50:431–436.
37. Dalsgaard K, Jensen MH, Sorenson KJ: Saponin adjuvants IV. Evaluation of the adjuvant Quil A in the vaccination of cattle against foot-and-mouth disease. Acta Vet Scand 1977; 18:349–360.
38. Delpeyroux F, Chenciner N, Lim A, Malpiece Y, Blondel B, Crainic R, van der Werf S, Streeck RE: A poliovirus neutralization epitope expressed on hybrid hepatitis B surface antigen particles. Science 1986; 233:472–475.
39. Deneer HG, Potter AA: Iron-repressible outer-membrane proteins of *Pasteurella haemolytica*. J Gen Microbiol 1989; 135:435–443.
40. Deregt D, Babiuk LA: Monoclonal antibodies to bovine coronavirus: Characterization and topographical mapping of neutralizing epitopes of the E2 and E3 glycoproteins. Virology 1987; 161:410–420.
41. Deregt D, Masri SA, Cho HJ, Bielefeldt Ohmann H: Monoclonal antibodies to the p80/p125 and gp53 proteins of bovine viral diarrhea virus. Their potential use as diagnostic reagents. Can J Vet Res 1990; 54:343–348.
42. Drzeniek R, Scharmann W, Balke E: Neuraminidase and N-acetylneuraminate pyruvate-lyase of *Pasteurella multocida*. J Gen Microbiol 1972; 72:357–368.
43. Ellouz F, Adam A, Ciorabu R: Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. Biochem Biophys Res Commun 1975; 59:1317–1325.
44. Estes MK, Cohen J: Rotavirus gene structure and function. Microbiol Rev 1989; 53:410–449.
45. Evans DJ, McKeating J, Meredith JM, Burke KL: An engineered poliovirus chimera elicits broadly reactive HIV-1 neutralizing antibodies. Nature 1989; 339:385–389.
46. Field M: Modes of action of enterotoxins from *Vibrio cholerae* and *Escherichia coli*. Rev Infect Dis 1979; 1:918–925.
47. Flexner C, Moss B: Vaccinia as a live vector carrying cloned genes. In: Woodrow GC, Levine MM (eds): New Generation Vaccines. New York: Marcel Dekker, 1990, pp 189–206.
48. Flexner C, Hugin A, Moss B: Prevention of vaccinia virus infection in immunodeficient mice by vector-directed IL-2 expression. Nature (London) 1987; 330:259–262.
49. Flynn JL, Weiss WR, Norris KA, et al: Generation of a cytotoxic T-lymphocyte response using a *Salmonella* antigen-delivery system. Mol Microbiol 1990; 4:2111–2118.
50. French TJ, Roy P: Synthesis of bluetongue (BTV) corelike particles by a recombinant baculovirus expressing the two major structural core proteins of BTV. J Virol 1990; 64:1530–1536.
51. Freund J: The mode of action of immunological adjuvants. Adv Tuberc Res 1956; 7:130–148.
52. Gaastra W, de Graaf FK: Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol Rev 1982; 46:129–161.
53. Gall D: The adjuvant activity of aliphatic nitrogenous bases. Immunology 1966; 11:369–386.

54. Gentry MJ, Confer AW, Panciera RJ: Serum neutralization of cytotoxin from *Pasteurella haemolytica* A1 and resistance to experimental bovine pneumonic pasteurellosis. Vet Immunol Immunopathol 1985; 9:239–250.
55. Gibbs EPL, Rweyemamu MM: Bovine herpesvirus. Vet Bull 1977; 47: 411–425.
56. Gogolewski RP, Kania SA, Liggitt HD, Corbeil LB: Protective ability of monospecific sera against 78KDa and 40-KDa outer membrane antigens of "*Haemophilus somnus*." Infect Immun 1988; 56:2301–2316.
57. Gold L: Expression of heterologous proteins in *Escherichia coli*. Methods Enzymol 1990; 185:11–13.
58. Gonzalez RN, Cullor JS, Jasper DE, et al: Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. Can J Vet Res 1989; 53:301–305.
59. Gonzalez RN, Mohammed HO, Cullor JC, et al: Efficacy and financial benefits of preventing clinical coliform mastitis in dairy cows by a mutant (J5) *Escherichia coli* vaccine. Proceedings of the International Symposium on Bovine Mastitis, Indianapolis, 1990, pp 205–209.
60. Gonzalez-Rayos C, Lo TYC, Shewen PE, Beveridge TJ: Cloning of a serotype-specific antigen from *Pasteurella haemolytica* A1. Infect Immun 1986; 53:505–510.
61. Gregoriadis G, Davis D, Davies A: Liposomes as immunological adjuvants: antigen incorporation studies. Vaccine 1987; 5:141–151.
62. Groom SC, Little PB: Vaccination of cattle against experimentally induced *Haemophilus somnus* pneumonia. Am J Vet Res 1988; 49:793–800.
63. Guidry AJ: Mastitis and the immune system of the mammary gland. In: Larson BL (ed): Lactation. Ames: Iowa State University Press, 1985, pp 229–262.
64. Gyles CL, Maas WK: Recombinant DNA technology and enterotoxigenic *Escherichia coli* vaccines. Prog Vet Microbiol Immun 1987; 3:139–158.
65. Hadad JJ, Gyles CL: The role of K antigens of enteropathogenic *Escherichia coli* in colonization of the small intestine of calves. Can J Comp Med 1982; 46:21–26.
66. Haj-Adhmed Y, Graham FL: Development of a helper independent human adenovirus vector and its use in the transfer of the herpes simplex thymidine kinase gene. J Virol 1986; 57:267–274.
67. Harris FW, Janzen ED: The *Haemophilus somnus* disease complex (Haemophilosis): A review. Can Vet J 1989; 30:816–822.
68. Haynes JR, Cunningham J, von Seefried A, Lennick M: Development of genetically-engineered candidate polio vaccine employing the self assembly properties of the tobacco mosaic virus coat protein. Bio/Technol 1986; 4:637–641.
69. Heddleston KL, Rebers PA: Properties of free endotoxin from *Pasteurella multocida*. Am J Vet Res 1975; 36:573–574.
70. Henner DJ: Expression of heterologous genes in *Bacillus subtilis*. Methods Enzymol 1990; 185:199–201.
71. Hunter RL, Bennet B: Structural basis of the activity of surface-active adjuvants. In: Nervig RM, Gough PM, Kaeberle ML, Whetsone CA (eds): Advances in Carriers and Adjuvants for Veterinary Biologics. Ames: Iowa State University Press, 1989, pp 61–70.

72. Hutchings DL, Campos M, Qualtiere L, Babiuk LA: Inhibition of antigen-induced and interleukin-2 induced proliferation of bovine peripheral blood leukocytes by inactivated bovine herpesvirus-1. J Virol 1990; 64:4146–4151.
73. Ijaz MK, Attah-Poku SK, Redmond M, Parker MD, et al: Heterotypic passive protection induced by synthetic peptides corresponding to VP7 and VP4 of bovine rotavirus. J Virol 1991; 65:3106–3113.
74. Inaba Y, Matumoto M: Akabane virus. In: Dinter Z, Morein B (ed): Virus Infections of Vertebrates, Vol. 3. Amsterdam: Elsevier, 1990, pp 467–480.
75. Ivins BE, Welkos SL, Knudson GB, Little SF: Immunization against anthrax with aromatic compound-dependent (Aro-) mutants of *Bacillus anthracis* and with recombinant straints of *Bacillus subtilis* that produce anthrax protective antigen. Infect Immun 1990; 58:303–308.
76. Ivins BE, Ezzell JW Jr, Jemski J, et al: Immunization studies with attenuated strains of *Bacillus anthracis*. Infect Immun 1986; 52:454–458.
77. Jacobs WR, Snapper SB, Lugosi L, Bloom BR: Development of BCG as a recombinant vaccine vehicle. Curr Top Microbiol Immunol 1990; 155: 153–160.
78. Jarrett WF, O'Neil BW, Gaukrogen JM, et al: Studies on vaccination against papillomaviruses: A comparison of purified virus, tumor extract and transformed cells in prophylactic vaccination. Vet Rec 1990; 126:449–452.
79. Jarrett WF, O'Neil BW, Gaukrogen JM, et al: Studies on vaccination against papillomaviruses: The immunity after infection and vaccination with bovine papillomaviruses of different types. Vet Rec 1990; 126:473–475.
80. Jim K, Guichon T, Shaw G: Protecting calves from pneumonic pasteurellosis. Vet Med 1988; 83:1084–1087.
81. Jones PW, Dougan G, Hayward C, et al: Oral vaccination of calves against experimental salmonellosis using a double *aro* mutant of *Salmonella typhimurium*. Vaccine 1991; 9:29–34.
82. Joo I, Emod J: Adjuvant effect of DEAE-dextran on cholera vaccines. Vaccine 1988; 6:233–237.
83. Josephson S, Bishop R: Secretion of peptides from *E. coli*: A production system for the pharmaceutical industry. Trends Biotechnol 1988; 6:218–224.
84. Kadel WL, Chengappa MM, Herren CE: Field trial evaluation of a *Pasteurella* vaccine in preconditioned and non-preconditioned lightweight calves. Am J Vet Res 1985; 46:1944–1948.
85. Kang CY: Baculovirus vectors for expression of foreign genes. Adv Virus Res 1988; 35:117–192.
86. Kania SA, Gogolewski RP, Corbeil LB: Characterization of a 78-kilodalton outer membrane protein of *Haemophilus somnus*. Infect Immun 1990; 58:237–244.
87. Keppie J, Harris-Smith PW, Smith H: The chemical basis of the virulence of *Bacillus anthracis*. IX. Its agressins and their mode of action. Br J Exp Pathol 1963; 44:446–453.
88. King AA, Harkness JW: Viral contamination of foetal bovine serum. Vet Rec 1975; 97:16.
89. Kit S, Sheppard M, Ichimura H, Kit M: Second generation pseudorabies virus vaccine with deletions in thymidine kinase and glycoprotein genes. Am J Vet Res 1987; 48:780–793.

90. Kit S, Qavi H, Gaines JD, Billinglsey P, et al: Thymidine kinase negative bovine herpesvirus type 1 mutant is stable and highly attenuated in calves. Arch Virol 1985; 86:53–83.
91. Kleid DG, Yansura D, Small B, Dowbenko D: Cloned viral protein for foot and mouth disease: Response in cattle and swine. Science 1981; 214: 1125–1129.
92. Konno S, Nakagawa M: Akabane disease in cattle: Congential abnormalities caused by viral infection. Experimental disease. Vet Pathol 1982; 19: 267–279.
93. Koronakis V, Cross M, Senior B, et al: The secreted hemolysins of *Proteus mirabilis*, *Proteus vulgaris* and *Morganella morganii* are genetically related to each other and to the alpha-hemolysin of *Eschericia coli*. J Bacteriol 1987; 169:1509–1515.
94. Kreuter J, Liehl E, Berg U, Soliva J, Speiser PP: Influence of hydrophobicity on the adjuvant effect on particulate polymeric adjuvants. Vaccine 1988; 6:253–256.
95. Kuwajima G, Asaka J-I, Fujiwara T, et al: Presentation of an antigenic determinant from hen egg-white lysozyme on the flagellar filament of *Escherichia coli*. Bio/Technol 1988; 6:1080–1083.
96. Lederer JA, Brown JF, Czuprynski CJ: *Haemophilus somnus*, a facultative intracellular pathogen of bovine mononuclear phagocytes. Infect Immun 1987; 55:381–387.
97. Leung WC, Manavathu EK, Zwaagstra J, Surunaraynan K: Development of fungal and algal cells for expression of foreign genes. In: Kurstak E, Marusyk R, Murphy F, van Regenmortel M (eds): Applied Virology. New York: Academic Press, 1986, pp 25–30.
98. Liang X, Babiuk LA, van Drunen Littel-van den Hurk S, Fitzpatrick D, et al: Bovine herpesvirus-1 attachment to permissive cells is mediated by its major glycoproteins gI, gIII and gIV. J Virol 1991; 65:1124–1132.
99. Lincoln RE, Fish DC: Anthrax toxin. In: Monte T, Kadis S, Ajl S (eds): Microbial Toxins. New York: Academic Press, 1970, pp 361–414.
100. Lindberg AA, Robertson, JA: *Salmonella typhimurium* infection in calves: Cell-mediated and humoral immune reactions before and after challenge with live virulent bacteria in calves given live or inactivated vaccines. Infect Immun 1983; 41:751–757.
101. Lo RYC, Shewen PE, Strathdee CA, Greer CN: Cloning and expression of the leukotoxin gene of *Pasteurella haemolytica* A1 in *Escherichia coli* K-12. Infect Immun 1985; 50:667–671.
102. Lo RYC, Strathdee CA, Shewen PE: Nucleotide sequence of the leukotoxin genes of *Pasteurella haemolytica* A1. Infect Immun 1987; 55:1987–1996.
103. Loudon PT, Roy P: Assembly of five bluetongue virus proteins expressed by recombinant baculoviruses: Inclusion of the large protein VP1 in the core and virus-like particles. Virology 1991; 180:798–802.
104. Lowell GH, Ballou WR, Smith LF, Wirtz RA, et al: Proteosome-lipopeptide vaccines: Enhancement of immunogenicity for malaria CS peptides. Science 1988; 240:800–802.
105. Lucknow VA, Summers MD: Trends in the development of baculovirus expression vectors. Bio/Technol 1988; 6:47–551.

106. Mackow ER, Vo PT, Broome R, Bass D, et al: Immunization with baculovirus-expressed VP4 protein passively protects against simian and murine rotavirus challenge. J Virol 1990; 64:1698–1703.
107. Maiorella B, Inlow D, Shauger A, Harano D: Large-scale insert cell-culture for recombinant protein production. Bio/Technol 1988; 6:1406–1410.
108. Marchioli CC, Yancey RJ, Wardley RC, Thomsen DR, et al: A vaccine strain of pseudorabies virus with deletions in the thymidine kinase and glycoprotein X genes. Am J Vet Res 1987; 11:1577–1583.
109. Marrs CF, Schoolnik G, Koomey JM, et al: Cloning and sequencing of a *Moraxella bovis* piliu gene. J Bacteriol 1985; 163:132–139.
110. Martin SW: Vaccination: Is it effective in preventing respiratory disease or influencing weight gains in feedlot calves. Can Vet J 1983; 24:10–19.
111. Matsumoto M, Schmitz JA, Syuto B, et al: Immunogenicity of a soluble antigen against *Pasteurella haemolytica*-associated pneumonia in calves. Vet Res Commun 1984; 8:117–130.
112. Matsuo K, Yamaguchi R, Yamazaki A, et al: Establishment of a foreign antigen secretion system in mycobacteria. Infect Immun 1990; 58:4049–4054.
113. McKercher PD: Oil adjuvants: Their use in veterinary biologics. In: Nervig RM, Gough PM, Kaeberle ML, Whetstone CA (eds): Advances in Carriers and Adjuvants for Veterinary Biologics. Ames: Iowa State University Press, 1984, pp 115–119.
114. Meneguizze G, Kieny MP, Lecocq JP, et al: Vaccinia recombinants expressing early bovine papilloma (BPV1) proteins: retardation of BPV1 tumor development. Vaccine 1990; 8:199–204.
115. Mikesell P, Ivins BE, Ristroph JD, Dreier TM: Evidence for plasmid-mediated toxin production in *Bacillus anthracis*. Infect Immun 1983; 39: 371–376.
116. Mooi FR, Roosendaal B, Oudega B, de Graaf FK: Genetics and biogenesis of the K88ab and K99 fimbrial adhesins. In: Lark DL (ed): Protein-Carbohydrate Interactions in Biological Systems. London: Academic Press, 1986, pp 19–26.
117. Morck DW, Raybould TJ, Acres SD, et al: Electron microscopic description of glycocalyx and fimbriae on the surface of *Pasteurella haemolytica* A1. Can J Vet Res 1987; 51:83–88.
118. Morein B, Sundquist B, Hoglund S, Dalsgaard K, Osterhaus ADME: ISCOM, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. Nature 1984; 308:457–460.
119. Moss B, Flexner C: Vaccinia virus expression vectors. Annu Rev Immunol 1987; 5:305–324.
120. Munson M, Kelly SM, Curtiss R: Oral immunization with a virulent *Salmonella typhimurium* to induce cross-protective immunity to *Escherichia coli*-induced air sacculitis and septicemia in chickens. Conf Res Workers in Dis, Chicago, 1989, p 61.
121. Myers LL: Enteric colibacillosis in calves: Immunogenicity and antigenicity of *E. coli* antigens. Am J Vet Res 1978; 39:761–765.
122. Nagy LK, Hartaningsih N, Sudana IG: Vaccination experiments with capsular antigen of *Pasteurella multocia*. Proc FAO/APHCA Workshop on Haemorrhagic Septicaemia, Columbo, Sri Lanka, 1979.

123. Neilands JD, Nakamura K: Regulation of iron assimilation in microorganisms. Nutr Rev 1985; 43:193–197.
124. Neurath AR, Strick N, Girard M: Hepatitis B virus surface antigen (HBsAg) as a carrier for synthetic peptides having an attached hydrophobic tail. Mol Immunol 1989; 26:53–62.
125. Nicoletti P, Milward FW: Protection by oral administration of *Brucella abortus* strain 19 against oral challenge with a pathogenic strain of *Brucella*. Am J Vet Res 1983; 44:1641–1643.
126. Nilsson B, Holmgren E, Josephson S, et al: Efficient secretion and purification of human insulin-like growth factor 1 with a gene fusion vector in *Staphylococci*. Nucl Acids Res 1985; 13:1151–1162.
127. Nunberg JH, Doyle MV, Newell AD, Anderson GA, York CJ: Interleukin-2 as an adjuvant to vaccination. In: Ginsberg H, Brown F, Lerner RA, Chanock RM (eds): Vaccines 88. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1988, pp 247–252.
128. O'Hagan DT, Palin K, Davis SS, Artursoon A, Sjoholm I: Microparticles as potential orally active immunological adjuvants. Vaccine 1989; 7:421–424.
129. Otulakowski GL, Shewen PE, Udoh AE, et al: Proteolysis of sialoglycoproteins by *Pasteurella haemolytica* cytotoxic culture supernatant. Infect Immun 1983; 42:64–70.
130. Pandey R, Kaushik AK, Grover YP: Biology of orthopoxvirus infections of domestic ruminants. Prog Vet Microbiol Immunol 1985; 1:199–228.
131. Pankey JW, Boddie NT, Watts JL, Nickerson SC: Evaluation of Protein A and a commercial bacterin as vaccines against *Staphylococcus aureus* mastitis by experimental challenge. J Dairy Sci 1985; 68:726–731.
132. Pastoret PP, Brochier B, Languet B, Thomas I, et al: First field trial of fox vaccination against rabies using a vaccinia-rabies recombinant virus. Vet Rec 1988; 123:481–483.
133. Pederson KB, Froholm LO, Bovre K: Fimbriation and colony type of *Moraxella bovis* in relation to conjunctival colonization and development of keratoconjunctivitis in cattle. Acta Pathol Microbiol Scand 1972; 80: 911–918.
134. Penn CW, Nagy LK: Isolation of a protective, non-toxic capsular material from *Pasteurella multocida*, types B and E. Res Vet Sci 1976; 20:90–96.
135. Poirier TP, Kehoe MA, Beachey EH: Protective immunity evoked by oral administration of attenuated *aroA Salmonella typhimurium* expressing cloned streptococcal M protein. J Exp Med 1989; 168:25–32.
136. Potgieter LND, Helman RG, Greene W, et al: Experimental bovine respiratory tract disease with *Haemophilus somnus*. Vet Pathol 1988; 25: 124–130.
137. Potter AA, Ready K, Gilchrist J: Purification of fimbriae from *Pasteurella haemolytica* A1. Microb Pathog 1988; 4:311–316.
138. Potter AA, Harland R: Development of a subunit vaccine for *Pasteurella haemolytica*. ASM Conference on Biotechnology, Chicago, 1990 (abstract).
139. Preisz H: Experimentelle studien über virulenz, enpfänglichkeit und immunität beim milzbrand. Zentralblatt für Bacteriologie, Parasitenkunde und Infektionshrankheiten, Abteil Orig 1909; 49:341–452.

140. Prevec L, Schneider M, Rosenthal KL, Belbeck LW, et al: Use of human adenovirus-based vectors for antigen expression in animals. J Gen Virol 1989; 70:429–434.
141. Prince GH, Smith JE: Antigenic studies on *Pasteurella multocida* using immunodiffusion techniques. I. Identification and nomenclature of the soluble antigens of a bovine haemorrhagic strain. J Comp Pathol 1966; 76:303–314.
142. Prince GH, Smith JE: Antigenic studies on *Pasteurella multocida* using immunodiffusion techniques. II. Relationships with other gram-negative species. J Comp Pathol 1966; 76:315–320.
143. Prince GH, Smith JE: Antigenic studies on *Pasteurella multocida* using immunodiffusion techniques III. Relationship between strains of *Pasteurella multocida*. J Comp Pathol 1966; 76:321–332.
144. Provost A: Scientific and technical basis for the eradication of rinderpest in intertropical Africa. Rev Sci Tech Off Int Epizoot 1982; 1:619–631.
145. Pugh GW, Kopecky KE, McDonald TJ: Infections bovine keratoconjunctivitis: Subconjunctival administration of a *Moraxella bovis* plus preparation enhances immunogenicity. Am J Vet Res 1985; 46:811–815.
146. Pugh GW, McDonald TJ, Kopecky KE: Experimental infectious bovine keratoconjunctivitis: Efficacy of a vaccine prepared from nonhemolytic strains of *Moraxella bovis*. Am J Vet Res 1982; 43:1081–1084.
147. Punch PL, Slatter DH: Review of infectious bovine keratoconjunctivitis. Vet Bull 1984; 54:193–207.
148. Purdy CW, Livingston CW, Frank GH: A live *Pasteurella haemolytica* vaccine efficacy trial. J Am Vet Med Assoc 1986; 188:589–591.
149. Randall R: Solid matrix-antibody-antigen (SMAA) complexes for constructing multivalent subunit vaccines. Immunol Today 1989; 10:336–339.
150. Ribble CR, Jim GK, Janzen ED: Efficacy of immunization of feedlot calves with a commercial *Haemophilus somnus* bacterin. Can J Vet Res 1988; 52:191–198.
151. Ribi E: Structure-function relationship of bacterial adjuvants. In: Nervig RM, Gough PM, Kaeberle ML, Whetstone CA (eds): Advances in Carriers and Adjuvants for Veterinary Biologics. Ames: Iowa State University Press, 1984, pp 35–49.
152. Robertsson JA, Lindberg AA, Hoiseth S, Stocker BAD: *Salmonella typhimurium* infection in calves: Protection and survival of virulent challenge bacteria after immunization with live or inactivated vaccines. Infect Immun 1983; 41:742–750.
153. Rosendal, S: Mycoplasma. In: Gyles CI, Thoen CO (eds): Pathogenesis of Bacterial Infections in Animals. Ames: Iowa State University Press, 1986, pp 205–215.
154. Rosenquist BD: Viruses as etiological agents of bovine respiratory disease. In: Loan RW (ed): Bovine Respiratory Disease: A Symposium. College Station: Texas A&M University Press, 1983, pp 363–376.
155. Roth JA: Immunosuppression and immunomodulation in bovine respiratory disease. In: Loan RW (ed): Bovine Respiratory Disease: A Symposium. College Station: Texas A&M University Press, 1983, pp 143–192.
156. Roy P, Urakawa T, van Kijk AA, Erasmus BJ: Recombinant virus vaccine for bluetongue in sheep. J Virol 1990; 64:1998–2003.

157. Rweyemamu MM, Johnson RH: The development of a vaccine for bovine herpesvirus mammalitis. Res Vet Sci 1969; 10:419–427.
158. Sadoff JC, Ballou WR, Baron LS, et al: Oral *Salmonella typhimurium* vaccine expressing circumsporozoite protein protects against malaria. Science 1988; 240:336–338.
159. Saif LJ, Jackwood DJ: Enteric virus vaccines. In: Saif JL, Theil KW (ed): Viral Diarrheas of Man and Animals. Boca Raton, FL: CRC Press, 1990, pp 313–329.
160. Saif LJ, Smith KL, Landmeier KL, Bohl BJ, Theil KW, et al: Immune response of pregnant cows to bovine rotavirus immunization. Am J Vet Res 1984; 45:49–57.
161. Sandhu TS, White FH: Production and characterization of *Moraxella bovis* hemolysin. Am J Vet Res 1977; 38:883–885.
162. Saunders JR, Janzen ED: *Haemophilus somnus* infections II: A Canadian field trial of a commercial bacterin. Can Vet J 1980; 21:219–224.
163. Schein CH: Production of soluble recombinant proteins in bacteria. Bio/Technol 1989; 7:1141–1149.
164. Scott GR: Rinderpest in the 1980's. Prog Vet Microbiol Immunol 1985; 1:145–174.
165. Scott GR: Rinderpest virus. In: Dinter Z, Morein B (ed): Viral Infections of Vertebrates, Vol. 3. Amsterdam: Elsevier, 1990, pp 341–354.
166. Sherman DM, Acres SD, Sadowski PL, et al: Protection of calves against fatal enterotoxigenic colibacillosis by orally administered *Escherichia coli* K99-specific monoclonal antibody. Infect Immun 1983; 42:653–662.
167. Shewen PE, Wilkie BN: Cytotoxin of *Pasteurella haemolytica* acting on bovine leukocytes. Infect Immun 1982; 35:91–94.
168. Shewen PE, Wilkie BN: *Pasteurella haemolytica* cytotoxin neutralizing activity in sera from Ontario beef cattle. Can J Comp Med 1983; 47: 497–498.
169. Shewen PE, Wilkie BN: Vaccination of calves with leukotoxic culture supernatant from *Pasteurella haemolytica*. Can J Vet Res 1988; 52:30–36.
170. Shewen PE, Wilkie BW: Antibody titer to *Pasteurella haemolytica* A1 in Ontario beef cattle. Can J Comp Med 1982; 46:354–356.
171. Shimshony A, Barzilai R: Rift valley fever. Adv Vet Sci Comp Med 1983; 27:347–362.
172. Singh Y, Chaudhary VK, Leppla SH: A deleted variant of *Bacillus anthracis* protective antigen is non-toxic and blocks anthrax toxin action *in vivo*. J Biol Chem 1989; 264:19103–19107.
173. Smith CK, Davidson JN, Henry CW: Evaluating a live vaccine for *Pasteurella haemolytica* in dairy calves. Vet Med 1985; 80:78–88.
174. Smith BP, Reina-Guerra M, Hoiseth SU, et al: Aromatic-dependent *Salmonella typhimurium* as modified live vaccines for calves. AM J Vet Res 1984; 45:59–66.
175. Snodgrass DR: Mixed infections of the intestinal tract. In: Saif LJ, Theil KW (eds): Viral Diarrheas of Man and Animals. Boca Raton, FL: CRC Press, 1990, pp 279–286.
176. Ssenyanga GS, Onapito JS, Nakasala-Situma J, Omara-Opyene AL: Therapeutic value of partial excision of lesions combined with administration

of an autogenous vaccine during an episode of cutaneous papillomatosis in cattle of Uganda. JAVMA 1991; 197:739–740.
177. Stephens LR, Little PB, Wilkie BN, Barnum DA: Isolation of *Haemophilus somnus* antigens and their use as vaccines for prevention of bovine thromboembolic meningoencephalitis. Am J Vet Res 1984; 45:234–239.
178. Stephens LR, Little PB, Humphrey JD, et al: Vaccination of cattle against experimentally induced thromboembolic meningoencephalitis with a *Haemophilus somnus* bacterin. Am J Vet Res 1982; 43:1339–1342.
179. Strathdee CA, Lo RYC: Regulation of expression of the *Pasteurella haemolytica* leukotoxin determinant. J Bacteriol 1989; 171:5955–5962.
180. Theil KW: Group A Rotaviruses. In: Saif LJ, Theil KW (eds): Viral Diarrheas of Man and Animals. Boca Raton, FL, CRC Press, 1990, pp 35–72.
181. Thoen CO, Enright F: Brucella. In: Gyles CL, Thoen CO (eds): Pathogenesis of Bacterial Infections in Animals. Ames: Iowa State University Press, 1986, pp 160–171.
182. Tikoo SK, Fitzpatrick DR, Babiuk LA, Zamb TJ: Molecular cloning, sequencing, and expression of functional bovine herpesviruses-1 glycoprotein IV in transfected bovine cells. J Virol 1990; 64:5132–5142.
183. Todhunter DA, Smith KL, Hogan JS, Schoenberger PS: Iron regulated outer membrane proteins of coliform bacteria isolated from bovine intramammary infections. Proceedings of the International Symposium on Bovine Mastitis, Indianapolis, 1990, pp 64–68.
184. Uchida I, Sekizaki T, Hashimoto K, Terakado N: Association of the encapsulation of *Bacillus anthracis* with a 60 megadalton plasmid. J Gen Microbiol 1985; 131:363–367.
185. Ulmanen I, Lundstrom K, Lehtovaara P, et al: Transcription and translation of foreign genes in *Bacillus subtilis* by the aid of a secretion vector. J Bacteriol 1985; 162:176–182.
186. Valenzuela P, Coit O, Medina-Selky MA, Kuo CH, et al: Antigen engineering in yeast: Synthesis and assembly of hybrid hepatitis B surface antigen-herpes simplex 1 gD particles. Biotechnology 1985; 3:323–326.
187. Van Roekel D, Clark P: Reduction of clinical symptoms associated with *E. coli* mastitis following vaccination with a commercial *E. coli* vaccine. Proceedings of the International Symposium on Bovine Mastitis, Indianapolis, 1990, pp 416–417.
188. Vodkin MH, Leppla SH: Cloning of the protective antigen gene of *Bacillus anthracis*. Cell 1983; 34:693–697.
189. Wadstrom T: Biological effects of cell damaging toxins. In: Adlam C, Easmon CSF (eds): Staphylococci and Staphylococcal Infections, Vol. 2. New York: Academic Press, 1983, pp 671–704.
190. Waltner-Toews D, Martin SW, Meek AH, McMillan I, Crouch CF: A field trial to evaluate the efficacy of a combined rotavirus-coronavirus/E. coli vaccine in dairy cattle. Can J Comp Med 1985; 49:1–9.
191. Watson DL: Evaluation of attenuated, live staphylococcal mastitis vaccine in lactating heifers. J Dairy Sci 1984; 67:2608–2613.
192. Wei BD, Carter GR: Live streptomycin-dependent *Pasteurella multocida* vaccine for the prevention of hemorrhagic septicemia. Am J Vet Res 1978; 39:1534–1537.

193. Welch RA: Identification of two different hemolysin determinants in uropathogenic *Proteus* isolates. Infect Immun 1987; 55:2183–2190.
194. Widders PR, Dorrance L, Yarnall M, Corbeil LB: Immunoglobin binding activity among pathogenic and carrier isolates of *Haemophilus somnus*. Infect Immun 1989; 57:639–642.
195. Wood OL, Meegan JM, Morrill JC, Stephenson EH: Rift valley fever virus. In: Dinter Z, Morein B (eds): Viruses of Vertebrates, Vol. 3. Amsterdam: Elsevier, 1990, pp 481–494.
196. Yarnall M, Gogoleuski RP, Corbeil LB: Characterization of two *Haemophilus somnus* Fc receptors. J Gen Microbiol 1988; 134:1993–1999.
197. Yates WDG, Stockdale PGH, Babiuk LA, et al: Prevention of experimental bovine pneumonic pasteurellosis with an extract of *Pasteurella haemolytica*. Can J Comp Med 1983; 47:250–256.
198. Yilma T, Hsu D, Jones L, Owens S, et al: Protection of cattle against rinderpest and vaccinia virus recombinants expressing the HA and F genes. Science 1988; 242:1058–1061.