SAFETY AND EFFICACY OF BOVINE RESPIRATORY DISEASE VACCINES

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Abstract
Achieving optimal safety and efficacy from vaccination programs to prevent bovine viral respiratory infections presents a complex challenge to researchers developing vaccines and to those using the vaccines.

Development of improved vaccines and designing the most effective vaccination programs requires an understanding of the protective antigens associated with the virus and of the protective components of the immune response to that virus. It is important to understand the role of circulating IgM and IgG, of mucosal IgA, and of the major subsets of T lymphocytes ($CD4^+$, $CD8^+$ and gamma delta T cells) in preventing or resolving viral infection. Vaccines that have been approved by government regulatory authorities must have been tested and proven to be safe and effective when used according to the label recommendations. However, there are many management conditions which may limit the safety and efficacy of vaccines when used under field conditions.

No vaccine is perfectly safe and completely effective in all potential conditions under which it may be used in the field. Veterinarians and owners must administer vaccines under optimal conditions to ensure the greatest possible safety and efficacy of the vaccine. Modified live vaccines have some important advantages as compared to killed vaccines, especially in terms of efficacy, however, killed vaccines have some safety advantages as compared to modified live vaccines. Vaccine research is focusing on developing new generation vaccines that combine the efficacy advantages of MLV vaccines and the safety advantages of killed vaccines. The optimal vaccine to select for a particular herd of cattle depends on the management practices of the herd and the relative risks of infection by the various respiratory pathogens.

Introduction
The factors affecting vaccine safety and efficacy were recently reviewed in a manuscript entitled "Mechanistic bases for adverse vaccine reactions and vaccine failures" (by JA Roth) in Advances in Veterinary Medicine 41: 681 - 700, 1999. In addition, immunosuppression by, and protective immunity to, bovine respiratory disease viral pathogens were recently reviewed in a manuscript entitled "Immunology and prevention of infection in feedlot cattle" (by JA Roth and L Perino) in Veterinary Clinics of North America; Food Animal Practice 14:233-256, 1998. Sections of these two manuscripts which deal with safety and efficacy of bovine respiratory disease viral vaccines are excerpted here.

Adverse Vaccine Reactions
When animals develop adverse clinical signs within a few days to weeks after vaccination it is important to determine whether those clinical signs were vaccine induced or were not due to vaccination and only coincidentally occurred after the vaccine was administered. Animals commonly experience adverse clinical signs from a wide variety of causes and animals are commonly vaccinated. Therefore, it is to be expected that occasionally adverse clinical signs will occur after animals have been vaccinated for reasons unrelated to vaccine administration. There are also many reasons why vaccines may induce adverse reactions in the animal. It is important to differentiate true adverse vaccine reactions from false adverse vaccine reactions. Some of the causes of true adverse vaccine reactions are summarized in Table 1 and are discussed in a recent review article (1).

Table 1: Potential mechanisms responsible for adverse vaccine reactions
! Contamination with extraneous agents
! Failure to inactivate agent in killed vaccine
! Residual virulence of vaccine organisms
! Vaccination of immuno-suppressed animal
! Immune suppression induced by the vaccine
! Excessive induction of cytokine release
! Multiple vaccines administered concurrently
! Hypersensitivity to vaccine antigens < Type I -- Immediate Type < Type II — Cytotoxic Type < Type III — Immune complex Type < Type IV — Delayed Type
! Triggering or exacerbation of hypersensitivity to non-vaccine antigens < Allergies < Autoimmune Disease
! Induction of neoplastic changes
! MLV BVD vaccine triggering mucosal disease in persistently infected cattle

Vaccine Failure

Vaccines that are licensed by the United States Department of Agriculture for sale in the USA have been tested to determine that they are safe and effective. However, "effective" is a relative term. It does not mean that the vaccine must be able to induce complete immunity under all conditions which may be found in the field. This would not be realistic since the immune system is not capable of such potent protection under adverse conditions.

To be licensed by the USDA, the vaccine must have been tested under controlled experimental conditions. The vaccinated group must have had significantly less disease than the non-vaccinated control group. This testing is typically done on healthy, non-stressed animals under good environmental conditions and with a controlled exposure to a single infectious agent. Vaccines may be much less effective when used in animals that are under stress, incubating other infectious diseases, or exposed to a high dose of infectious agents due to overcrowding or poor sanitation.

It is important to remember that for most diseases the relationship between the infectious agent and the host is sufficiently complicated that vaccination cannot be expected to provide complete protection. The vaccine can increase the animal's resistance to disease, but this resistance can be overwhelmed if good management practices are not followed. Some of the causes for vaccine failure are summarized in Table 2 are discussed in a recent review article(1).

Table 2: Potential reasons for vaccine failure

- Insufficient time after vaccination to develop immunity
- Something happened to the vaccine to make it ineffective
- The physiologic status of the animal impaired the response to the vaccine
- The animal was immunosuppressed at some point after vaccination
- The animal was exposed to an overwhelming challenge dose of infectious agent
- The duration of immunity after vaccination was not adequate
- Important antigenic differences exist between the vaccine and field strains
- Interference when multiple vaccines are administered concurrently

Immune Suppression by Bovine Respiratory Viruses

The best evidence that viruses play an important role in predisposing to bacterial pneumonia comes from epidemiological data indicating that a recent serologic conversion to a respiratory virus is associated with bacterial pneumonia and from challenge experiments in which cattle are infected with a virus and then infected a few days later with an aerosol of *P. haemolytica* or *P. multocida*(2). The cattle that are preinfected with either BHV1, parainfluenza 3 (PI3), or bovine viral diarrhea (BVD) virus will develop a severe bacterial pneumonia, while the non-virus-infected control cattle are able to clear the bacteria from their lungs. These viruses may have a number of effects on the antibacterial defense mechanisms in the lung including impairment of mucociliary clearance, suppression of phagocytic cell function, and interference with lymphocyte function. The relative importance of each of these effects is not known, but it is probably a combination of activities that is responsible for the predisposition to bacterial pneumonia. Studies have shown that simultaneous infection with BVD and BRSV can synergistically increase the pathologic effects of each individual virus.(3)

A number of respiratory viruses of cattle can inhibit mucociliary clearance in the ciliated respiratory epithelium (BHV1, PI3, BVD, bovine respiratory syncytial virus [BRSV])(2). Decreased mucociliary clearance is often cited as a primary reason for greater susceptibility to bacterial pneumonia. Evidence suggests, however, that this is not as important as impairment of bactericidal mechanisms within the lung. This conclusion is based on the observations that the rate of bacterial killing within the healthy lung greatly exceeds the rate of mucociliary transport out of the

lungs and that the period of increased susceptibility to bacterial pneumonia does not coincide with the timing of the inhibition of mucociliary clearance after viral infection(4).

The consensus of opinion seems to be that suppression of the function of phagocytic cells in the lungs (both alveolar macrophages and neutrophils) is a primary factor in predisposing to bacterial infection. The phagocytic cells are essential for killing bacteria that find their way to the lower respiratory tract and removing them from the lung. The alveolar macrophage is the predominant phagocyte in the healthy lung and is very important in surveillance and removal of foreign material (including bacteria) from the alveoli. If the alveolar macrophages are unable to control the infection or if the lung is exposed to a large challenge dose of bacteria, neutrophils will migrate into the alveoli and bronchioles very rapidly and soon (within a few hours) become the predominant cell type. Neutrophils are very active phagocytically and have potent bactericidal mechanisms including the generation of toxic oxygen products (hydrogen peroxide, superoxide anion, hydroxyl radical) and the release of cationic antibacterial peptides and hydrolytic enzymes. In addition to being bactericidal, these products also can damage pulmonary tissue. If the infection is not brought under control relatively rapidly, the neutrophils will induce considerable damage in the lung.

There is evidence that BHV1, PI3, BVD, and BRSV can each impair alveolar macrophage function(2). BHV1 and BVD also can inhibit neutrophil function(2). The effects of PI3 and BRSV on neutrophil function apparently have not been determined. An important aspect of alveolar macrophage and neutrophil function is that they can be activated by cytokines secreted by T lymphocytes. When these phagocytes are activated they become more "aggressive" and are more effective at controlling bacterial infection. By interfering with lymphocyte function, the BRD viruses may inhibit alveolar macrophage and neutrophil activation and leave the animal more susceptible to bacterial pneumonia.

The BVD virus has been shown to inhibit aspects of lymphocyte,(5-7) macrophage, and neutrophil (7,8) function; to impair bacterial clearance from the blood; (9) to lessen the ability of calves to clear BHV1 virus from the lung; (10) and to facilitate pulmonary infection with *P. haemolytica* (11). At least one modified-live vaccine strain of BVD virus also was shown to be capable of suppressing lymphocyte and neutrophil function (12). The suppression of neutrophil function lasts for three to four weeks after infection with either a virulent or modified-live strain of the virus. Cattle that were given ACTH to increase their serum cortisol levels at the same time that they received modified-live-virus BVD vaccine had more marked suppression of neutrophil function than cattle that received either the modified-live BVD virus or the ACTH only (12). This implies that stress and the BVD virus act synergistically to cause an immunosuppression that is worse than either would cause alone. The clinical importance of immunosuppression by currently used MLV BVD vaccines is unknown. It is probably not a problem when used in healthy animals under good management conditions.

The bovine immunodeficiency virus (BIV) is a lentivirus that has antigenic and genetic homology with the human immunodeficiency virus. The true prevalence of BIV infection of cattle in the U.S. is unknown. Various serologic surveys have detected infection rates of from 4 to 18% (13). Experimental infection with BIV has been associated with changes in circulating lymphocyte numbers (14) monocyte function (15-17) and decreases in neutrophil function (18,19). However, these changes were relatively minor, and experimental BIV infection has not been shown to lead to a clinically apparent immunodeficiency syndrome. The potential impact of naturally occurring infection with BIV on susceptibility to diseases in feedlot cattle is still unknown.

Several other viruses have been associated with the BRD complex (including bovine adenovirus, coronavirus, DN599 herpesvirus [Movar], rhinoviruses, reoviruses, and bovine parvovirus) (2,20). Little is known about the immunosuppressive effects of these viruses in cattle; however, it is logical to assume that infection with any of them may render cattle more susceptible to bacterial pneumonia.

CHARACTERISTICS OF PROTECTIVE IMMUNITY TO BOVINE RESPIRATORY DISEASE PATHOGENS

Bovine Herpesvirus 1

Bovine Herpesvirus 1 (BHV1) also referred to as Infectious Bovine Rhinotracheitis (IBR) is in alpha herpes virus. The characteristics of protective immunity against BHV1 is similar to that of other alpha herpes viruses. Antibody

titers as measured by a serum virus neutralization (SN) test can protect the animal against infection. The evidence for this is that passive antibodies that a calf receives from the colostrum can provide solid protection against infectious challenge. The passively acquired antibody can also prevent a modified live virus (MLV) vaccine from inducing an antibody response in a calf. This maternal antibody blockage of an MLV vaccine can even occur if the serum neutralizing titer is very low. If the calf receives a lot of colostrum with a high titer against BHV1, it may be 6 to 8 months old before it is capable of responding to an MLV vaccine by the production of antibody. Even though the MLV vaccine may not induce an antibody response it is possible that it will induce a memory response in the face of maternal antibody so that if the calf is subsequently exposed to the virulent virus it may be capable of responding more rapidly to the viral challenge and have some degree of protection. There is evidence to indicate that vaccination in the presence of maternal antibody against pseudorabies virus in pigs (another alpha herpes virus) stimulates immunologic memory even though an antibody response doesn't occur. This immunologic memory has been shown to provide partial protection against disease challenge with pseudorabies virus in pigs. (21) There is also evidence that the same is likely to be true for MLV BHV1 vaccines used in the presence of maternal antibody in calves. (22,23)

An important characteristic of herpes viruses is that after an animal recovers from disease it will be latently infected with the virus for the rest of its life. The virus resides in ganglia of nerves in a quiescent state. Even modified live BHV1 vaccine has been shown to latently infect cattle. (24) The immune system is not capable of clearing this latent infection. If the animal is stressed, or treated with glucocorticoids later in life, the virus is likely to recrudesce and be shed, even if the animal has a high serum neutralizing antibody titer. (24-26) Therefore serum neutralizing antibody can prevent infection but it cannot prevent recrudescence and shedding of the latent BHV1. The latently infected animal which is shedding BHV1 may not show any clinical signs but will be a source of infection for other animals in the herd.

Once an infection with BHV 1 is established a cell-mediated immune response is probably needed in order to bring the infection under control. Cytotoxic T lymphocytes are thought to be important in controlling the infection.

There do not seem to be important antigenic differences between BHV1 isolates. Immunity to one isolate of BHV1 or one vaccine strain of virus appears to provide good cross protection against all field isolates. Therefore an SN titer measured against any BHV1 virus in the laboratory probably will more or less equally neutralize any BHV1 virus. An antibody titer determined by ELISA may or may not measure protective (serum neutralizing) antibodies, depending on the nature of the antigen used in the ELISA assay.

Bovine Viral Diarrhea Virus

Serum neutralizing (SN) antibody titers of approximately greater than 1:32 have been shown to protect against disease induced by BVD virus (27). A major problem however, in immunity to BVD virus is that there is a great deal of antigenic diversity among BVD virus isolates (28). The BVD virus, like most other RNA viruses, has a high mutation rate, resulting in almost unlimited antigenic diversity among isolates. The E2 gene of BVD codes for the GP53 protein. This is the major surface glycoprotein and is immunodominant for antibody production. It is a major epitope for virus neutralization. The E2 gene represents one of the hypervariable regions of the BVDV genome. This hypervariability may be due to selective pressure from the immune system (29). The heterogeneity of the GP53 protein (and other less important virus neutralizing epitopes) limits the ability of an antibody response to one strain of BVDV to protect against a wide array of other possible strains the animal may be exposed to. The SN antibody titer of a single serum sample may vary from 10 to 100 fold depending on which BVD isolate is used in the SN assay (28,30). The animal is probably protected against the isolates that the serum can neutralize at a titer of approximately 1 to 32 or greater, but the antibody in the serum won't protect against the other isolates of BVD virus. In a field outbreak, it is impossible to predict which antigenic type of BVD virus the animal will be exposed to. There is apparently no single vaccine strain of BVDV (or even a combination of vaccine strains) that is capable of providing cross protective SN antibody titers against all potential virulent BVD virus isolates that may be encountered.

Very little is known about the role of cell-mediated immunity (either cytotoxic T cells or T helper 1 cells) in protection against BVD virus induced disease. It is likely that cell-mediated immunity is important for recovery from infection. It is quite possible that cell-mediated immunity, especially cytotoxic T lymphocytes will provide

better cross protective immunity among different BVD virus isolates than antibody will. If this is true, then an animal that has developed cell-mediated immunity will have better protection against BVD virus challenge. Since MLV vaccines are more likely to induce cytotoxic T lymphocytes than are killed vaccines, they may provide better cross protective immunity to a variety of BVD virus isolates. This hypothesis fits the commonly held perception that MLV vaccines provide better immunity to BVD virus than killed vaccines, but it remains to be proven experimentally. Recently, two separate genotypes of BVD virus have been defined (31), type 1 and type 2. The type 2 BVD viruses have the potential to produce severe acute infection even in adult animals. The homologous genotypes tend to induce better cross neutralizing antibody titers than the heterologous genotypes. A type 1 modified live BVD vaccine has been shown to provide protection from a virulent challenge with a type 2 BVD virus (32), probably due to cross protective cell-mediated immune responses.

A critical factor in controlling BVD virus infection in a herd, and probably in the cattle population as a whole, is to prevent infection of the fetus and development of persistently infected calves. There is very little data on the ability of vaccines administered to the cow to prevent infection of the fetus if the cow should become exposed to virulent BVD virus. There have been some experiments conducted using killed BVD virus vaccines in cows which were subsequently challenged with virulent BVD virus during pregnancy. In most cases these vaccines did not provide adequate immunity to prevent fetal infection. In one experiment using three doses of a killed vaccine, evidence of fetal protection from experimental challenge was obtained (33). Considering all of the evidence, it is likely that a killed vaccine that induces a titer of greater than 1 to 32 in the cow against a particular isolate of BVDV will protect the fetus from becoming infected. However there will likely be strains of BVDV that are antigenically different from the vaccine virus and which the cow and fetus will not be protected from. It is possible that an MLV vaccine administered to the cow prior to pregnancy may provide better cross protective immunity against a variety of isolates as described above.

Bovine Respiratory Syncytial Virus

Circulating antibody does not seem to provide good immunity against Bovine Respiratory Syncytial Virus (BRSV) induced disease. The evidence for this is the observation that calves with passive antibody are not usually protected from BRSV induced infection or disease. However, calves that recover from disease are protected from reinfection, at least for a while (34). The nature of protective immunity is not clearly understood, but there is some evidence to suggest that a strong IgA memory response is associated with protection and that a cytotoxic T lymphocyte response to the F protein of BRSV may protect from disease. In one series of experiments where calves with and without maternal antibody were primed with live BRSV via the respiratory tract, protection was associated with a strong and rapid mucosal antibody memory response after challenge but not with serum or mucosal antibody present at the time of challenge (35).

A problem with BRSV vaccination and immunity is that maternal antibody doesn't provide good protection but it does interfere with active immunization of the calf as assayed by antibody production (34). This presents a real problem since BRSV tends to cause disease in calves that are too young to effectively vaccinate because of maternal antibody. Additional research is needed to further characterize the nature of protective immunity to BRSV and to develop vaccines that can effectively immunize a young calf in the presence of maternal antibody.

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