

VACCINOLOGY



BASICS

OVERVIEW

- The control of existing and emerging diseases with vaccines is an important preventative measure for cattle producers and bovine veterinarians.
- Vaccinology involves the preparation and testing of vaccines, the animal's immune response to the vaccine, and the outcome against a challenge.
- This area of veterinary medicine is often misunderstood and subsequently misused, with vaccination expected to cure the impossible. Unfortunately, there is no “magic bullet” and the myths of vaccinology outweigh the reality. This section aims to enforce basic vaccine concepts.

UNDERSTANDING VACCINES

Modified Live

- Modified live vaccines (MLV) have been used because of the good antibody response, longer duration of immunity, fewer doses needed per animal, and lower cost.
- These vaccines are administered intramuscularly, intranasally, or subcutaneously. These vaccines have drawbacks because they can contain adventitious agents, and the MLV bovine viral diarrhea virus (BVDV) and infectious bovine rhinotracheitis virus (IBR) are immunosuppressive.
- Although the return to virulence in MLV viruses has been minimal, mutations will occur and there is some risk of new strains arising. The selective pressure from an animal's immune response may lead to new phenotypes.

Gene-deleted MLV

- Gene-deleted MLV were first developed against pseudorabies in swine. They have also been developed in Europe for IBR.
- Using recombinant technology, specific genes are removed from vaccine strains of the virus, reducing virulence. Most importantly, the organism is now “marked,” so vaccinated animals can be differentiated from naturally infected animals.
- Vectored MLV are now commercially available for Newcastle disease in poultry, canine distemper in dogs, and West Nile virus in horses. These vaccines seem to have all of the characteristics of an ideal vaccine.
- They are free of adverse side effects, stable, adaptable to mass vaccination, nonadjuvanted, and like the gene-deleted vaccines allow for differentiation between a vaccinee and an infected animal.

- These vaccines are created by recombinant technology in which the vector genes are deleted and one or more genes from the pathogen are inserted into the vector. The vector with the pathogen insert is then administered as the vaccine.
- The vector can be severely attenuated so that it will not be shed from the animal, or be host-restricted so that it will not replicate itself within the animal's tissues. At this time there are no vectored vaccines available for cattle.

Inactivated

- Inactivated vaccines contain chemically or physically treated bacteria, toxins, and/or viruses. This process eliminates the danger of replication of the pathogen or adventitious agent in the vaccinated animal, as is possible with MLV.
- Improved adjuvants have increased the scope and duration of inactivated virus immunity.
- They have several disadvantages including cost, a larger number of doses required per animal, and a shorter duration of immunity. Inactivated vaccines generate cell-mediated responses.
- Hypersensitivity reactions (allergic) occur more often with inactivated vaccines.

Subunit Vaccines

- Subunit vaccines use just the immunogenic portion of an organism. These immunogenic proteins can be purified directly from the pathogen and incorporated into vaccines.
- Another approach is to produce purified antigen subunits by recombinant DNA techniques. This DNA can then be placed in bacteria, yeast, or other cells that will produce the protein. These protein antigens can be produced in large, pure quantities.

Synthetic Peptides

- Synthetic peptides use even smaller pieces of the protein than subunit vaccines. They contain just 10 to 20 amino acids of the most important protective sites on the antigen, that are called epitopes.
- These synthetic epitopes can be made into a vaccine if they are large enough to be immunogenic.
- Experimental peptide vaccines have been shown to be effective against external parasites (i.e. ticks).

DNA Vaccines

- DNA vaccines use recombinant DNA techniques to make DNA plasmids that contain the pathogen genes that produce a protective immune response.
- The plasmid is directly injected in the animal and the DNA plasmid enters the host's cell, where the host actually makes the protein. These DNA vaccines have not been marketed for cattle but a West Nile virus DNA vaccine for horses has been approved. DNA vaccines usually require an initial vaccination with an MLV or inactivated vaccine and are usually used as boosters.

ADJUVANTS

- Adjuvants influence the magnitude and quality of the immune response and function by targeting the antigen, influencing antigen processing, activating immune cells, promoting cytokine release, and regulating cell receptor expression.
- Adjuvants can influence the antibody class (e.g. IgA versus IgM), the T-helper response, the cytokine profile, and cytotoxic T-lymphocyte response. Although they have been used primarily with subunit and inactivated vaccines, they are now also being used with modified live vaccines to enhance immune targeting

Logistical Functions of Adjuvants:

- **Antigen transport:**
The adjuvant must facilitate antigen uptake by antigen-presenting cells (APC) and these APC must deliver the antigen to the draining lymph nodes. This may be achieved by facilitating antigen uptake by APCs or by increased influx of APCs into the injection site or lymph node. Whichever is the case, the result is the same: an increase in the antigen-loaded APCs for stimulation of naive T-cells.
- **Antigen Storage (“depot effect”):**
The depot theory was based on the antigens being stored or sequestered at the site surrounded by APC. This represented what was seen with oil-based adjuvants that developed the so-called sterile abscess. Subsequent research with newer adjuvants has looked at a cellular depot effect. This theory states that these complexes are taken up by APC and can be presented over time so the APC will provide stimulation over time. This process sustains T cell activation and extends an otherwise short-lived response.
- The immune stimulation function of adjuvants is necessary for additional signals (both through signaling receptors and secreted cytokines) to provide the necessary “second” signal from the APC to the T cell to result in T cell memory and help for B and T cells.

Adjuvant Components

- **Vehicles**
 - The goal of vehicles is to enhance the immune response by sustaining the release of the antigen in the animal. Metabolizable oils are mixed with water and form micelles (water-oil-antigen complexes), which form a bilayer that contains the antigens and provides sustained release.
 - Liposomes are a mixture of isolated phospholipids and cholesterol that form a bilayer structure with an internal aqueous space containing the antigen. They are synthesized and can be administered with immunostimulants.
 - Microcapsules are synthetic polymeric materials that release antigen slowly over time. Degradable polymers such as poly-lactide co-glycolide (PLG) can release antigen for >30 days.
- **Immunostimulants**
 - Immunostimulants fall into four classes: bacterial products, saponins, cytokines, and aluminum salts.
 - *Bacterial products* include bacterial cell wall components (TDM and MDP-Tubercle [Mycobacterium] bacilli, MPL-Salmonella) and toxins (cholera toxin-CT).

- *Saponins* are potent immunostimulants that induce the humoral and cellular immune response. Quil A and QS 21 are 2 members of this group that are derived from the tree bark of a South American tree. Making liposomes in the presence of saponin and antigen makes a vaccine mixture called ISCOMS.
- *Cytokines* must be given at the appropriate time and site. Cytokines have multiple effects and the type of response desired must be targeted.
- *Aluminum salts* (alum) have been used for many years in veterinary vaccines. With these adjuvants, the antigen is adsorbed onto the surface of an insoluble salt. Not all antigens are adsorbed well to these adjuvants. The aluminum complex attracts APC, activates complement, and results primarily in an antibody response.

Adjuvant Selection

- The adjuvant selected depends on the immune response that is desired (mucosal immunity, toxin neutralization, humoral versus cellular), the nature of antigen (protein, lipid, carbohydrate), vaccine formulation (single component, complex mixture [viral and bacterial antigens], stability, scalability, consistency), safety (autoimmunity, clinical side effects, tissue reactivity) and regulatory issues (likelihood of government approval, residue levels). However, with all these new adjuvants becoming available, the effectiveness of the vaccine still depends on a good antigen.

LICENSING REQUIREMENTS

- All manufacturers of vaccines in the U.S. are required to test each and every batch of vaccine for potency, purity, safety, and efficacy before it is released to the market place.
- The Center for Veterinary Biologics, Ames, IA, monitors all serials of vaccines produced and marketed. They have a surveillance of each manufacturer, testing from 10 to 18% of all serials on a random basis each year. They can elect to test more often. This cross check helps to ensure that the products that are being sold in the animal markets meet the minimum requirements, and that all manufacturers are judged the same way.
- The potency of a vaccine is correlated to actual protection in the host animal species. Usually, a laboratory animal (because of cost of running a test) is used to judge the relative potency of each and every serial of vaccine that is manufactured.
- Purity in vaccines is very important in protecting the animal populations from contaminated products.
- Good manufacturing processes require that a high degree of testing at virtually every phase of production is essential. Every serial of vaccine that is produced undergoes rigid purity testing before being released to the market. A checks-and-balances system is in place as the USDA randomly tests serials of vaccines for purity.
- Efficacy is tested in cattle and requires at least 20 vaccinates and 10 control animals. The vaccine must protect the animal against challenge.
- Onset of protection, duration of protection, minimum antigen concentration, and interfering antigens are all determined for each vaccine. This is normally one of the most expensive parts of developing vaccines. It requires the procurement of susceptible animals; the disease that one is trying to protect against must be able to be demonstrated, and the vaccine that is being developed must be able to protect against the disease when challenged.

- Most countries have regulations that demand this type of scrutiny of vaccines. One exception to this rule is the manufacture and use of autogenous vaccines, where only safety but not efficacy must be shown.
- All regulatory agencies require that vaccines must be safe in the target species. In addition, studies in laboratory animals provide an important monitoring tool during the manufacturing process to determine how safe a process will be in the formulation of vaccines.
- It is important to consider the impact of vaccines, their uses, and exposure to animals outside of the host species for which the vaccine is intended. This is especially important when developing and using modified live vaccines.

THE ANIMAL'S RESPONSE TO THE VACCINE

The Importance of the Innate Immune System for Antigen Presentation and Lymphocyte Activation—the Keystone for Duration of Immunity

- Vaccination is intended to provide long-term protection after its administration. Activated T- and B-cells last only a few days, so the primary aim of any vaccine is to generate immunological memory.
- There are three steps (3-signals) necessary for a vaccine to activate the immune system. The first step (signal 0) is the vaccine activating the innate immune system. This involves the production of the pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β) and interleukin-6. TNF- α activates a number of liver acute phase proteins and also affects the central nervous system appetite and attitude centers. IL-1 β is associated with high febrile responses and IL-6 is a major chemotactic cytokine for recruiting immune cells. These proinflammatory cytokines are essential for activating the APC. The next two steps involve the APC. The specific immunogen needs to be presented by the APC on the MHC-peptide complex to the T cell receptor on the T cell (signal 1-antigen presentation) along with interaction of several other APC receptors and cytokine signals with T cell surface molecules (signal 2-costimulation).
- The “danger signal” (signal 0) must occur prior to signals 1 and 2 for an effective acquired immune response. The danger signal is required for activation of the inflammatory response to ensure a good acquired immune response, memory, and duration of immunity. This danger signal is induced (cell debris from necrotic cells, heat shock proteins [HSPs], nucleotides, reactive oxygen intermediates, cytokines [e.g. interferon]), by molecules produced or induced by a pathogenic organism infection and/or are from damaged cells.
- The molecules associated with pathogens are called pathogen-associated microbial patterns (PAMPS) and are only present on pathogens. These PAMPS are present on a wide range of pathogens, unlike the adaptive immune response that is epitope specific. For example, all Gram-negative bacteria contain lipopolysaccharide (LPS), one of the PAMPs, whereas another PAMP, CpG nucleotides, are present in bacterial DNA. Flagellum present on protozoa and bacteria and double-stranded RNA produced by viruses are two other PAMPs recognized by the innate immune cells (APC, dendritic cells, macrophages). These PAMPs are recognized by receptors called pathogen recognition receptors (PRRs or toll-like receptors) on innate immune cells. At least 10 groups of these PRRs have been identified. Non-microbial adjuvants such as oil

emulsions or saponins may also be recognized by PRRs or other receptors on innate immune cells.

- Successful vaccines must activate antigen-presenting cells (APC) to initiate antigen processing and produce cytokines. These cells must then migrate to the regional lymph node to activate both T and B cells to give a high yield of memory cells that will provide long-term protection for the animal.
- The ability of a foreign molecule to induce an immune response is antigenicity (or immunogenicity), and the stimulating molecule is an antigen (or an immunogen). These antigens react with the B cell receptor (membrane antibody) on B-cells or T-cell receptor (TCR) on T cells. This immune response must generate populations of T cells (both T-helper and T-cytotoxic cells) to several epitopes of the antigen and be broad enough to overcome the variation in the immune response of the outbred cattle population.
- A good vaccine must also enable the persistence of antigen in APC in lymphoid tissue to elicit continued production of antibody from B cells.
- Activation of the TCR upon interaction with MHC-peptide complexes on APCs is signal 1. Signal 1 alone is insufficient to activate T cells; it leads to tolerance and deletion of the T cells.
- Naive T lymphocytes require a second stimulus, signal 2, to become fully activated. During maturation, APC increase their expression of membrane-bound, co-stimulatory molecules, such as B7, that are recognized by naive T cells.
- APC also up-regulate the synthesis and surface expression of cytokines, including interleukin (IL)-1, IL-12, tumor necrosis factor (TNF), and interferon γ (IFN- γ). These cytokines are important signaling molecules between the cells in the immune system. There are more than 30 IL.
- These cytokines (endogenous adjuvants) together with the membrane-bound B7 molecules are collectively termed Signal 2 (“second signal”). This signal amplifies the up-regulation of T lymphocyte activation in the T cell areas of the lymph node and results in T cell proliferation (by clonal expansion), memory cell formation (in case of re-infection), and finally T-cell differentiation. The non-antigen-specific, innate, immune system regulates signal 2 and dictates the adaptive immune system response.

Basis of Vaccine Protection

- Lymphocytes are the key effector cell. Although they are dependent on APC to initiate the acquired immune response, lymphocytes are the cells responsible for vaccine memory and for the subsequent immune response.
- Lymphocytes respond to presented antigens by the production of cytokines (by T and B cells) or antibodies (by B cells). These have many actions, including control of the adaptive immune response by secondary action on the participating cells, and, in the case of cytotoxic T cells, in killing virally infected host cells.
- B and T cells use 3 lines of attack to fight infection and disease. These approaches can occur alone but often the immune system uses some combination of all 3.
 1. *Elimination of extracellular pathogens*: In response to infection, B cells mature into plasma cells that secrete antibodies. B cells recognize pathogens by using membrane-bound antibody as the antigen receptor. At the time of first vaccination, there is no antibody in blood and the level does not begin to increase until 10 to 14 days after infection. The level of antibody rises slowly, peaks, and

then gradually declines. This is a primary immune response. On subsequent exposure to the same pathogen, the level of antibody begins to increase within 24 hours and reaches a high level in a few days that can last for weeks or months. This is the secondary or anamnestic response. The immune system produces antibodies that recognize many different types of structures on the pathogen. Serum from an immune animal contains many different types of antibodies, each recognizing different epitopes on the surface of the pathogen. Antibody is secreted on mucosal surfaces and diffuses through tissues to target extracellular pathogens. Binding of antibody to pathogens activates two mechanisms to eliminate pathogens: (1) complement activation with opsonization and/or lysis of the pathogen and (2) phagocytosis by neutrophils and macrophages with intracellular killing.

2. *Elimination of intracellular pathogens in macrophages:* Even when antibody and complement opsonize pathogens and phagocytosis occurs, some pathogens are not killed but survive and multiply in macrophages, e.g. *Mycobacterium bovis*, *M. paratuberculosis*, and *Listeria monocytogenes*. Killing of the pathogen occurs through the assistance of T-helper cells (T_h cells). T_h cells recognize macrophages containing intracellular pathogens using their T-cell receptors. They help macrophages to kill pathogens by synthesizing cytokines that stimulate bacterial killing mechanisms in macrophages.
3. *Elimination of pathogens by cell-to-cell killing:* Viruses are obligate intracellular pathogens that can infect many types of cells. During viral replication, virus proteins occur on the surface of the infected cell. A second subset of T cells, cytotoxic T cells (T_c), recognize these viral antigens on the infected cells. These cells then secrete molecules that kill the virally infected cells.

Interval between Doses of Vaccine

In all animals following vaccination there is expansion in the populations of responding T- and B-cells). However, to have a complete and mature immune response, this T- and B-cell expansion must not only stop but an active process of cell death (apoptosis) must also occur. This “waning process” allows “culling” T- or B-cells that may be poor responders or even cause autoimmunity to be removed by apoptosis. This whole process from vaccination to achieving mature immune response homeostasis takes at least 3 weeks. This fully developed mature primary response can then be boosted to get a true anamnestic secondary response. In many cases, cattle vaccine primary and booster doses are administered at two-week intervals (Figure 1). In young calves, this is done to provide an opportunity to make sure that the calves develop a primary response in the face of maternal immunity. The adjuvants that are used with most commercial vaccines provide superior immune development over older generation adjuvants like alum. Therefore, in most instances if primary vaccination occurs after 3 weeks of age, booster vaccination beyond three weeks and even longer will be efficacious. The dogma that revaccination must occur within two weeks of the primary vaccination is not true and the anamnestic response will be better if we wait longer.

Timing and Immune Response- Young Animals Primary vs Booster Response

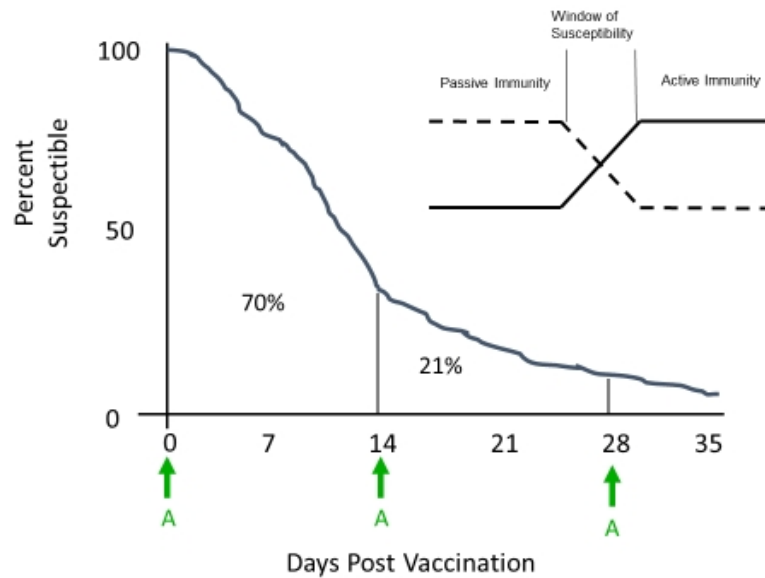
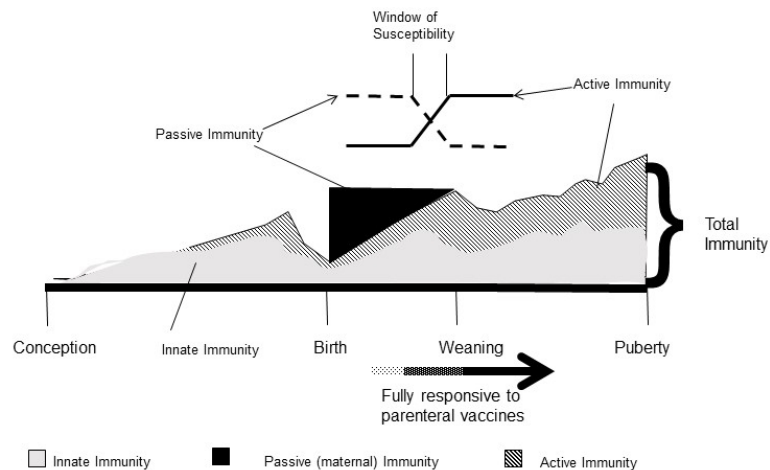


Figure 1

The Immune Response in the Neonate

- The immune response in the ruminant neonate is dictated by 2 factors: the development of active immunity and the decay of maternal passive immunity.
- Neonates (10 to 14 days of age) can develop a protective immune response following vaccination with modified live vaccine (MLV). However, the presence of maternal antibody in young animals can interfere with the immune response to parenterally administered vaccines (Figure 2).

The Components of Immunity in the Calf



Modified from Figure 1, Morein et al Vet Immunol Immunopath 87:202-213, 2006 with permission of Dr. G Blomqvist and Fig 1 Butler et al Vet Res 37:417-441, 2006 with permission of Dr. JE Butler.

Figure 2

- Oral vaccines may not be affected by maternal antibody. Neonates may develop a cell-mediated response but no neutralizing antibodies are produced. The animal develops a specific acquired immune response and memory and develops an anamnestic antibody response after exposure to the pathogen.

Measuring the In-Vitro Immune Responses

- **Cell-mediated responses**
 - *Memory cells:* In proliferation assays, the cells that are measured are the circulating lymphocytes, which may not be an accurate indicator of localized immunity. One of the properties of cytokines is to stimulate the production of more specific memory cells. For example, when memory cells from an animal vaccinated for IBR are mixed with IBR virus, the cells will produce cytokines that will make IBR-specific cells divide and produce more cells. This is called proliferation and is measured by the incorporation of fluorescent or radioactive-labeled nucleotides in the nucleus of the newly produced cells. Although the dividing cells are predominately T cells, B cells may also be measured.
 - *Cytotoxicity:* These assays measure the ability of circulating mononuclear cells (lymphocytes, macrophages) to kill virally infected target cells. This population in the bloodstream may not be an accurate indicator of localized immunity. The target cells

are labeled with a radioactive or color marker and cell killing is measured by the release of this marker.

- **Humoral (antibody response):** The study of antibody responses in animals is serology. There are several types of antibody assays used in the laboratory:
 - *Precipitation/agglutination:* The specific antigen and the corresponding antibody combine to form an antigen-antibody complex. For precipitation, if antibody and antigen are present in roughly similar amounts, precipitation lines will occur in the agar; otherwise, if there are excess antibody or antigen, no precipitate will form. Immunodiffusion tests are used for many pathogens (e.g. bluetongue, brucellosis). Agglutination tests test for antibodies that can react with specific antigens to form visible clumps (e.g., leptospirosis, brucellosis).
 - *Complement fixation:* Complement fixation is an immune response in which an antigen-antibody combination inactivates a complement (so it is unavailable to participate with a second antigen-antibody combination). Complement reacts with antigen-antibody complexes. The lack of specificity of complement allows it to react with almost any antigen-antibody complex. In cattle, the CF test is used for anaplasmosis and brucellosis. With this test procedure, unless complement is fixed by the particular antigen and antibody system in question, it will remain free to react as an indicator, resulting in a “negative” test. If complement is fixed by the antigen and antibody system under study, it is not free to react in the indicator system, resulting in a “positive” test. The indicator system used in CF is sheep red blood cells (RBCs). In a positive test, the complement is bound to an antigen-antibody complex, and is not free to interact with sensitized RBCs so they remain unlysed and settle to the bottom of the well to form a button. On the other hand, in a negative test, the complement remains free since there is no antigen-antibody complex for it to bind to, and interacts with the sensitized RBCs, causing them to lyse.
 - *Virus neutralization:* The virus neutralization (VN) test measures the neutralization of the pathogenic properties of the pathogen (e.g., a virus that kills cells), where the antibody will tightly bind to the pathogen and neutralize the pathogen, keeping it from binding to its normal receptors and preventing infection of the cells. This test is also known as SN (serum neutralization) and is the most commonly used serology test for bovine respiratory viral pathogens (i.e., IBR, BRSV, BVDV, PI3). This is a 2-step test: (a) A virus-antibody reaction step, in which the virus is mixed and inoculated with the appropriate antibody reagents, and (b) an inoculation step, in which the mixture is inoculated into the appropriate host system (e.g. cell cultures) to detect residual virus infectivity. The absence of infectivity constitutes a positive neutralization reaction and indicates the presence of virus-specific antibodies in animal sera.
 - *Enzyme linked immunosorbent assays (ELISA):* ELISA, also known as enzyme immunoassays (EIA), are tests designed to detect antibodies by producing an enzyme-triggered color change. These tests are rapid and used for a variety of pathogens (i.e. Johnes, Bovine leukemia virus, IBR, BVDV).

EVALUATING VACCINE TRIALS

- Many vaccine trials are run exclusively for marketing and not for a label claim. If a company intends to make a label claim, the vaccine study protocol must be approved by

the Center for Veterinary Biologics (CVB) and Animal and Plant Health Inspection Service (APHIS), and the study data and report reviewed by CVB.

- Most studies that originate from marketing or technical service sections in animal health companies are not intended for label claim, making their design less stringent and their data more open to interpretation.
- When presented with a “vaccine study” for any biological product, carefully evaluate the following criteria:
 - How many animals were in the study? Many “studies” are done with fewer than 10 animals that make interpretation variable.
 - How was the statistical analysis done? Many transformations or manipulations are done to make the data fit the authors’ statistical analysis. Beware of studies that assign arbitrary values. Statistical significance in studies may be nothing more than good number massaging.
 - Was a challenge administered to establish that the “findings” have any biological significance to the animal? For example, immunology studies will claim that memory and/or cell-mediated immunity was measured, but the significance to actual protection of the animal is never tested so any positive preventive effect for animal health can be shown. Studies that measure antibody level “titers” are notorious for failing to do any challenge work.
 - Was the vaccine trial done by outside researchers—independent university or contract research organizations—or did the animal health company do the studies? Although animal health companies go to great lengths to properly design and conduct vaccine studies, they are still in the marketplace to make money. This limits the likelihood that studies with mixed or poor results will ever be published or even discussed outside the company. One criteria to determine the objectivity of an outside researcher is to see whether a single company or many companies support research.
 - Were the studies ever published in a peer-reviewed publication? Publishing in peer-reviewed journals requires that one or more of the authors’ peers who are experts in the field review the study. Not all peer-reviewed journals are the same. There are first-tier, second-tier, and third-tier journals, with the first-tier journals being the best. For example, in the field of vaccines, the *Journal of Clinical Immunology* and *Vaccine* would be considered first-tier or best journals and papers accepted in these journals are accepted at 40 to 50% rate. Second-tier journals such as the *American Journal of Veterinary Research* and *Veterinary Immunology and Immunopathology* accept 60 to 80% of the papers submitted. Peer-reviewed journals that contain applied veterinary medicine research along with columns and unsolicited animal health articles are the third tier and are accepted at a very high rate. Many of the first- and second-tier journals will not review applied clinical studies. On the other hand, articles in publications such as *Bovine Veterinarian* are not peer-reviewed. An article that is peer-reviewed has more credibility than an unreviewed article.
- Understanding vaccine trials involves knowledge of the viruses, the immune response, and vaccinology. In no areas of veterinary medicine has information continued to explode exponentially every year as in these areas. However, always use a critical eye in evaluating these trials and their application to your practice and client’s herds.



MISCELLANEOUS

ABBREVIATIONS

- APC = antigen-presenting cells
- APHIS = Animal and Plant Health Inspection Service
- BRSV = bovine respiratory syncytial virus
- BVDV = bovine viral diarrhea virus
- CF = complement fixation
- CVB = Center for Veterinary Biologics
- EIA = enzyme immunoassays
- ELISA = enzyme-linked immunosorbent assay
- HSP = heat shock proteins
- IBR = infectious bovine rhinotracheitis virus
- IFN = interferon
- IL-1 = interleukin-1
- LPS = lipopolysaccharide
- MHC = major histocompatibility complex
- MLV = modified live vaccines
- PAMPS = pathogen-associated microbial patterns
- PI3 = parainfluenza-3
- PLG = poly-lactide co-glycolide
- PRRs = pathogen recognition receptors
- RBCs = red blood cells
- SN = serum neutralization
- TCR = T-cell receptor
- TNF = tumor necrosis factor
- VN = virus neutralization

SEE ALSO

- Specific disease entities

Suggested Reading

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Author Christopher C.L. Chase

Consulting Editor Kaitlyn Lutz