The mammalian body is an excellent growth medium for microorganisms. It is warm, moist and full of nutrients. One of the best media used in the laboratory for culturing bacteria is blood agar. For animals to remain alive, organisms living on or in the body must be kept in check. Three factors help ensure that bacteria and other infectious agents remain in check or in the tissue locations where they belong:

1. **PHYSICAL AND OTHER BARRIERS**

There include intact skin and mucous membranes, coughing-, gag-, vomit- and sneeze-reflexes, urinary flow, diarrhea, upward mucus flow in the respiratory tract, and competition with pathogens by resident innocuous microbial flora. The importance of these physical barriers will be evident to your relatives, but not you, immediately after your death. Organisms, particularly anaerobes that lived contentedly in your gut for years, can now proliferate and cross your gut lining within hours, unless your remains are promptly refrigerated, frozen, deep fried or perfused with formalin. A cow that dies in summer in Wyoming will have clostridia in all organ systems, including brain, within 24 hours of death, due to the breakdown in mucosal barriers and spread via blood vessels. This phenomenon is a major accelerant of post-mortem decomposition. Wounds in skin and mucosal surfaces (e.g., ulcers in GIT, bladder or uterus) are serious because they compromise physical barriers. They allow bacteria and other agents to enter the body. The lumen of your gut, like that of your mouth and nose, is effectively outside your body.

2. **INNATE (non-specific) IMMUNITY**

These comprise chemical and cellular defense mechanisms. The most important is the inflammatory response. This involves two major white blood cell classes (neutrophils and monocytes-macrophages), and a complex set of regulatory chemicals in blood and tissue. Inflammation is the body’s defensive reaction to injury. Animals lacking a robust inflammatory response will die – there are several congenital diseases, such as BLAD (bovine leukocyte adherence defect seen in Holstein-Friesians) where a component of the inflammatory response is defective. Other components of non-specific immunity are innate antibacterial factors in blood and tissue, such as lysozyme, lectins, and iron-binding proteins. Other components are in the complement system, which generates proteins that aid in the clearance and breakdown (= lysis) of infectious agents.

3. **ACQUIRED (specific) IMMUNITY**

Acquired immunity is analogous to a closed, repressive society: it sustains itself by expelling foreigners, it tolerates well-behaved citizens provided they follow specific rules, and eliminates citizens who deviate. As in repressive societies, this approach can go badly awry when mistakes are made. A component of several major diseases in animals and people involve immune reactions misdirected against the body’s own antigens (autoimmune diseases).

Acquired immunity has two major components: antibody- and cell-mediated. These are controlled primarily by lymphocytes and the mononuclear/macrophage system. The immune system is programmed to recognize and destroy antigens, which are defined as substances can induce an immune response. Most antigens are proteins. The best immune responses are to proteins that are glycosylated (sugar residues attached to proteins). Antigens don’t have to be proteins, since polysaccharides and oligosaccharides that occur in the external capsules of some bacteria can serve as antigens. These represent a challenge for vaccine manufacturers since they stimulate only humoral responses (see below). In the case of infectious agents, the best target antigens are those on the surface (rather than internal, and therefore occult) antigens. The immune system evolved so that it can recognize and react appropriately to all antigens other than self-antigens. The ability to distinguish self-antigens from foreign antigens is central to the body’s ability to protect itself from microbial invasion. It also evolved to recognize self-antigens that are expressed inappropriately or in changed form. These include the altered self-antigens of senescent and cancer cells. In the process of becoming cancerous, neoplastic cells throw off antigens (tumor-associated antigens) that are sufficiently unusual to recognized as foreign. Unfortunately, the immune response that is generated is often too weak to attack and eliminate neoplastic cells.

Major components of acquired immunity are twofold: antibody- and cell-mediated.
**ANTIBODY-MEDIATED REACTIONS**

This is humoral immunity. The active component consists of proteins (antibodies) produced by a specific subset of B lymphocytes known as plasma cells. This form of immunity is especially effective against extracellular infectious agents. There are various classes of antibodies, such as IgG, IgM, IgA and IgE.

**CELL-MEDIATED REACTIONS**

This is mediated directly by a subset of specialized cytotoxic lymphocytes, T cells. Cell mediated responses are particularly important for dealing with agents that live inside cells (obligate intracellular parasites). This includes all viruses, many protozoa, and some bacteria including all mycoplasmal and chlamydial agents. To this end, it is important to stimulate effective CD8+ cytolytic T lymphocytes responses so that intracellular pathogens can be attacked and destroyed. This generally requires that the host cell which harboring infection is killed.

Cell-mediated and humoral mediated responses work together to clear infections: for viral infections, there is often a strong cytotoxic T cell response, followed by a robust humor response that to ensure that re-infection does not occur. The complexion of immune responses is determined by whether the response is driven largely by **Type 1 (Th-1)** helper T cells (= cell mediated; good for intracellular pathogens) or by **Type 2 (Th-2)** helper T cell (= predominance of humoral immunity; good for extracellular pathogens). The principal anatomical home of lymphocytes is lymph nodes and lymphoid aggregates (in mucosa of digestive, respiratory, urinary and reproductive tracts), which are dispersed in strategic locations around the body. Other locations are in the circulation, spleen, bone marrow and lymph fluid.

**WHEN IMMUNITY FAILS**

The immune system is critical to protect animals from microbial agents. Sometimes the response fails or is inadequate. It is important to understand the limitations that vaccines operate under. We still lack a vaccine for some agents (e.g., human immunodeficiency virus, the cause of AIDS), in spite of years of work and billions of dollars in research. It happens for a variety of reasons:

1. The infectious agent changes its surface antigens during the course of infection. The immune response is always reacting to the last iteration of viral or parasitic antigens. Example: equine infectious anemia (EIA, the agent tested for with a Coggins test) and antigen shifts.
2. Antigens expressed by infectious agents are masked, inaccessible or weak so that immune responses are muted and therefore ineffective. Example: many metazoan parasites, which are in the lumen of the gut or other hollow organs, and are inaccessible. There is an exception to this. A successful vaccine exists to the bovine lungworm, *Dictyocaulis viviparous*, which lives in the lung and causes a parasitic pneumonia.
3. The agent hides in intracellular locations. As a consequence, although a good immune response exists, it cannot deliver the killer punch to the agent. Example: intracellular organisms (*Salmonella, Listeria, Brucella*) and some viral agents (African swine fever).
4. Some infectious agents bind to antibodies on their surfaces, which do not inactivate them. They can then be taken up by macrophages, which they infect.
5. The immune system confuses infectious agents for a self-antigen. Example: response to *Streptococcus equi* resulting in autoimmune disease (vasculitis and skin disease).
6. The immune system over-reacts to an antigen. Example: cow asthma.
7. The immune system no longer recognizes self-antigens as self, and responds to them as foreign. Example: pemphigus in young horses, and other autoimmune diseases.
8. The immune system can’t get to the anatomical location where an agent is present. There are specific barriers, particularly in brain, joint, testis and eye, where such barriers exist. Example: many forms of bacterial and viral encephalitis.
9. The agent establishes chronic, persistent infection. To do this it must do two things: evade the initial immune response, and suppress or subvert long-term immune surveillance. It does this in many ways: destroys helper T cells (CD4+ T); down-regulate expression of class I MHC molecules; mutation, which changes viral peptide sequences, rendering existing effector T cells ineffective; and inhibiting activity of cytotoxic T cells.

**TYPES OF IMMUNIZATION**

There are two way methods by which an animal becomes immune: by giving it antibodies from a resistant animal, or by exposure to antigens you want it to be able to recognize. These are passive and active immunization, respectively.
PASSIVE IMMUNIZATION

There are two examples of this in herd medicine: antibodies from a mother to her offspring (generally colostral antibodies; in some species, antibodies cross the placenta to the fetus), and injection of hyperimmune serum, when it is important to protect an animal quickly against infectious agents or toxins. Examples of these are commercially available hyperimmune serum against clostridial diseases (tetanus and botulism; the serum is called “antitoxin”), some infectious diseases (*Rhodococcus equi*, West Nile virus, anthrax, rabies, *E coli*), and snake venoms (so-called “antiserum”). Concentrated serum from normal or hyperimmunized horses or cattle can also be given neonatal foals or calves where the latter failed to nurse and therefore did not get colostral antibodies (= failure of passive transfer (FPT) and at high risk of dying of neonatal bacterial infections). Although the antibodies are effective immediately, the products are expensive and antibodies are short lived. If used repeatedly in an animal, they will be recognized as foreign and cause hypersensitivity reactions, including death. Hyperimmune serum is raised commercially, usually in horses, by giving animals first a vaccine and then the pathogenic infectious agents so they are hyperimmune. The blood is drawn off, serum is harvested, and antibodies purified. As with all blood products, the use of hyperimmune serum carries risks. A disease in horses (“serum hepatitis”) is probably due to a virus that may be present in some lots of commercial hyperimmune serum. Horses become sick 30 – 70 days post-vaccination and their liver is severely damaged. More than 50% die as a result. Commercial preparations to treat FPT can occasionally react with the foal’s red blood cells and destroy them, causing anemia and death (a form of neonatal isoerythrolysis).

Example of commercially available antisera:

**Tetanus Antitoxin;** manufacturer: Colorado Serum Company. The product is used in horses after they suffer a deep penetrating wound, if the animals were not previously vaccinated for tetanus. Antibodies take effect immediately, and are cleared within 7 – 14 days. Protection is short-term. If such products are used in the face of an outbreak, the animals should be vaccinated once the antitoxin has worn off (about 21 days after passive immunization). Many owners prefer to vaccinate immediately, rather than do the two-stage business of antitoxin followed by vaccination.

**HiGamm-Equi;** manufacturer: Lake Immunogenetics Inc. This product is used to treat FPT in foals. It contains concentrated antibodies. It is stored frozen, gently warmed, and given intravenously. It has a good shelf life if kept frozen (up to three years). It is expensive and provides short-term protection (10 – 14 days), but is useful for valuable animals. It is also possible to buy freeze-dried antibody preparations (= good shelf life as a powder, but must be reconstituted) from hyperimmunized animals for foals and calves. Some can be given either orally or intravenously (e.g., Equine Lymphmune®; manufacturer: BioQual Inc). They are reconstituted in sterile 5% dextrose for intravenous injection. Such products may not provide protection to all of the diseases that are endemic on the property where they are used.

**Rhodococcus equi hyperimmune serum:** Manufacturer: Lake Immunogenetics Inc. This organism causes a high mortality pneumonia on some farms in the US (see equine respiratory disease lecture). There is no vaccine, due to the nature of the organism, so the ONLY proven protective measure is to use hyperimmune (HI) plasma obtained from donors. Antibodies are maintained for ~30 days in most foals. Administration of 1 L of HI plasma within the first week of life followed by a second administration at approximately 25-30 later, although expensive, may be the best approach on farms with high morbidity rates. In farm with lower morbidity rates, one administration at 10 - 21 days of age may be advisable.

**PolySerum:** manufacturer; Novartis Animal Health inc. This hyperimmune serum is raised in cattle vaccinated against three bacterial causes of pneumonia and two causes of diarrhea. It is confusingly called a vaccine, which it is not. Given by injection, usually to baby calves, immunity is short lived.

ACTIVE IMMUNIZATION (vaccination)

Vaccination is the most efficient and cost-effective method for controlling infectious disease, although not the only one. Other components are good nutrition and hygiene, effective biosecurity, and good management (esp. reducing stress). Vaccination is not necessarily innocuous. It is expensive, it creates a false sense of security, and it may itself cause disease (e.g., post-injection swellings; endotoxemic deaths; immunosuppression; occasionally, reversion to virulence of attenuated agent, accidental contamination at the point of manufacture with wild-type viruses). Some are dangerous if injected into people – a Wyoming veterinarian self-injected himself with the RB-51 *Brucella* vaccine recently, and lost much of the function of one hand as a result. Vaccines can complicate disease diagnosis. For regulatory
reasons it is important to distinguish whether an animal that has antibodies to an infectious agents has them due to vaccination or natural infection. This is sufficiently important that some perfectly adequate vaccines are unavailable or controlled by state or federal agencies (RB51 for brucellosis; FMD vaccines). Vaccination remains one of the humanity's great discoveries. The rarity with which we see rabies, anthrax and rinderpest in livestock and companion animals is due in large part to effective use of vaccines.

As a general rule, vaccines prevent disease, not infection. Vaccinated animals are generally not immune to infection, but the immune response is sufficiently robust that no clinical disease occurs. This is why vaccines often contain the statement "as an aid in the prevention of disease caused by..."

Active immunization involves the administration of antigens derived from an infectious agent so that an animal mounts a fast specific acquired immune response when it next encounters that set of antigens (antigenic memory or anamnestic response). This results in resistance in most of the vaccinated animals. The site where immunity is induced may be important. Systemic immunity is important for agents that spread through blood. Local (mucosal) immunity is important for agents that exert their effects at mucosal surfaces (e.g., equine influenza virus and the nasal passage; some feline respiratory vaccines given into the nose and ocular conjunctiva). For some agents, a good local immune response is essential to control infection.

Vaccines are used to control disease in populations rather than in individuals. Successful vaccination in populations, such as in a flock or a herd, creates herd immunity. If herd immunity is adequate, the probability that an infectious agent will contact a susceptible animal is small, which slows or halts the spread of disease. In general, more than 80% of a herd needs to be solidly immune to ensure that an outbreak of an infectious disease will not occur in the herd. For some highly infectious diseases (e.g., FMD) and in crowded situations (e.g., dairy herds; intensive swine and poultry units), the proportion of animals that must be solidly immune is higher (99%). There will always be some animals in a herd that do not respond to effective vaccines. The occurrence of one or two cases of a disease against which the herd was vaccinated does not mean the vaccine is ineffective.

An IDEAL VACCINE should:

- Confer strong prolonged immunity (years, not months)
- Have few or no adverse side effects.
- Be cheap, stable, and adaptable to mass vaccination.
- Stimulate an immune response distinguishable from that of natural infection so that vaccination and eradication programs proceed simultaneously.
- Stimulate humoral and cell-mediated immunity and deliver an immune response where it is needed (in respiratory tract for respiratory diseases; in digestive tract for GIT diseases)

The government oversees and licenses the production of veterinary biologicals by private corporations. In the USA, this is the USDA’s Center for Veterinary Biologics (CVB). The CVB licenses establishments that produce vaccines and inspects premises to ensure facilities and production methods are satisfactory. All vaccines are checked for safety and potency. Safety tests include confirmation of the identity of the organism used, freedom from extraneous organisms, and tests for potential toxicity. Because living organisms in vaccines will die over time, it is necessary to ensure that they are effective over a defined shelf life. Although properly stored vaccines may be potent after the expiration of the designated shelf life, do not use vaccines that exceed the expiry date (written on each vial). Veterinary vaccines go through less rigorous safety trials than human vaccines in the US, which is why it is generally easier and quicker to bring products to market. A WNV vaccine has been on the market for three years for horses, yet the human vaccine is still not available. The corollary is that veterinary vaccines have more adverse side effects and are less efficacious than licensed human biological products. Unlike the European Community, where access to vaccines is restricted to veterinarians, in the US and Canada producers have access to most vaccines on the market for animals. This imposes on producers the need to understand the products.

Producers can also have companies make custom vaccines. This is an expensive process and of questionable value. It is attractive to some producers, since the vaccine is based on the isolation of a pathogen from their herd, and manufacture of the product takes 6 – 8 weeks. They are generally killed products, and can only be used on the ranch of origin or, with USDA permission, on adjacent premises. The microorganisms used to produce custom vaccines may not be older than 15 months from the date of isolation, so the production cycle is short. Novartis Animal Health produces custom vaccines. The main disadvantages are cost, and the use of a killed, rather than a more effective live attenuated, product.
There are two MAJOR CLASSES OF VACCINES:

1. INACTIVATED VACCINE

These consist of wild-type disease causing infectious agents that have been grown in the laboratory and then inactivated ("killed"). A vaccine containing inactivated bacteria is called a bacterin. These are generally safe with respect to residual virulence, free of contamination with other infectious agents (due to the killing process), can be given to pregnant animals, and relatively easy to store. The down side is that, since organisms are inactivated, they don’t replicate in the host animal and there is no amplification of the amount of antigen other than what you get in the vial. Immunity is shorter-lived that with a modified live vaccine and often requires a booster shot within 4 – 6 weeks.

Manufacturers try to compensate for this by adding chemical adjuvants, which elicit a stronger immune response to the vaccine. Adverse effects of inactivated vaccines are high endotoxin concentration (basis for many so-called anaphylactoid reactions, where animals collapse and die minutes or hours after vaccination) and swellings at sites of injection (due to unduly irritating adjuvant). Very rarely, inactivation may not work and the agent may remain viable - this unfortunately has happened with rabies vaccines for people. Inactivated organisms should be as similar to living organisms as possible. Crude methods of inactivation (e.g., heating) are unsatisfactory since they reduce antigenicity. When chemicals are used, it is essential that they produce little change in the antigens (either damage DNA of the agent or mildly denature proteins). Compounds used in this way include formaldehyde, acetone, alcohol, ethylene oxide, and β-propiolactone. Physical inactivation such as UV irradiation is also used. Animals may react to inactivating chemicals if they are persist in high concentrations at the end of manufacture in the vaccine.

Note: we should refer to these as “inactivated” vaccines rather than “killed” vaccines, as it is debatable whether agents such as viruses considered “alive” – live viruses are more correctly referred to as viable. The terms modified live and killed virus are however entrenched and widely used.

Example of inactivated vaccine:

- **ScourGuard 3 K/C** – manufacturer: Pfizer. This is a widely used anti-diarrheal vaccine. It contains inactivated K99 *E coli* (= bacterin), inactivated rotavirus, inactivated coronavirus, and the neutralized beta toxin (= toxoid) of *Clostridium perfringens type C*. This is therefore a combined bacterin-toxoid-inactivated viral vaccine. Other components are an adjuvant (Quil A-saponin), an antibiotic to inhibit bacterial contamination (gentamicin), a mercury-containing preservative (merthiolate), a second preservative (formalin), and water (>90% of the total volume).

- **Virashield** product line – manufacturer Novartis. This line of 12 products contains inactivated BVDV, and varying combinations of BHV-1, BRSV, PI-3, leptospirosis, *Haemophilus somnus*, and *Campylobacter*. The adjuvant in these products is often quite irritating.

ii. Toxoids and related vaccines

Toxoids do not contain whole organisms. Instead, they utilize particular toxins secreted by pathogens responsible for provoking clinical symptoms of disease. Toxins are altered in such a way that, while no longer toxic to the target animal, the resulting molecules are antigenically intact. Toxoid vaccines do not prevent infection, instead they protect animals from the effects of the toxin secreted by the bacterium. The purified tetanus exotoxin is inactivated by treatment with formalin (= tetanus toxoid) and used for active immunization against tetanus. The attachment pili of enteropathogenic *Escherichia coli* can be isolated and the purified pilus proteins (hair-like projections on the bacterial surface) incorporated into vaccines. Antipilus antibodies protect animals by preventing bacterial attachment to the intestinal wall. On the horizon is the use of peptides, rather than whole proteins, which have the advantage of being chemically defined, stable and safe.

Example of a toxoid:

- **7-way and 8-way vaccines** for clostridial diseases. These are designed to prevent 3 causes of acute death in cattle, caused by a group of related clostridial bacteria. These are acute gangrenous myositis ("blackleg" – most commonly due to *Clostridium chauvoei*, and rarely by *C. septicum, C. novyi, and C. sordelli*), clostridial wound infections ("malignant edema" – most commonly due to *C. septicum*), and bacterial hemoglobinuria ("redwater" – due to *C. novyi type D*). Bacterin-toxoids are cheap and effective. The difference between a 7- and an 8-way is that the former does not contain the cause of bacterial hemoglobinuria, since this disease occurs only in certain areas of the country. It is uncommon in Wyoming. Dr. Montgomery will cover some of these diseases in his lectures.

2. MODIFIED LIVE VACCINES (MLV)

The advantage of MLV is that the agent is alive and replicates in vaccinated animals, conferring stronger immune response than with inactivated vaccines. As a general rule, in food animals, MLV
These are derived from recombinant organisms into which a gene from a pathogenic organism is

1. Making use of the good bits: **Subunit vaccines**

These are derived from recombinant organisms into which a gene from a pathogenic organism is

GENETICALLY ENGINEERED VACCINES

Sometimes a lot of hype attends engineered vaccines, but they are coming. In the animal field we still largely rely on inactivated and MLVs. Engineered vaccines are beginning to make their appearance in the food animal and equine marketplace

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These are derived from recombinant organisms into which a gene from a pathogenic organism is
inserted. The recombinant organism is propagated and the protein encoded by the inserted gene is secreted, harvested, purified, and administered as vaccine. This is usually done in bacteria, such as *E. coli*. It can also be done in plants. Generally the best proteins to target are those expressed on the surfaces of the organism, since these are the first to be recognized by the immune system. **Subunit vaccines** are therefore **inactivated vaccines**. Adjuvants may still be used, along with chemical tricks to improve immune reactions (aggregating the antigen complex into virus-like particles, on antigen coated beads, or as lipid encapsulated microdroplets). The first generation of vaccines have not performed as well as hoped because many engineered proteins lacked the glycosylation step that is essential for antigens to be fully recognized and processed by the immune system. It is also possible to produce antibodies in transgenic plants – pigs fed on potatoes engineered to produce antibodies to TGEV showed reduced illness and death when challenged with wildtype virus.

Example: Foot and mouth subunit vaccine (a protective antigen of FMD in *E. coli*).

2. **Getting rid of the bad bit: Gene-deleted agent vaccines**

Using recombinant technology, specific gene sequences are removed from the pathogenic virus or bacterium to make the agent less pathogenic (attenuation). This is a more efficient way of generating MLV – the various tricks used in the past to produce an attenuated virus (growing it in unusual conditions, cells, or animals for an extended period) depended on chance. These are being be replaced by direct targeting of pathogenic genes to make agents incapable of causing disease (= avirulent). An additional advantage is that the engineered organism is uniquely “marked” so that, based on the presence or absence of a targeted gene, one can differentiate vaccinated and naturally infected animals. Many vaccines currently being developed are against the herpesvirus group. They were first used against pseudorabies in swine (PRV)(thymidine kinase gene removed from virus, which it needed to replicate; the deletion mutant PRV infect neurons, but cannot replicate and cause disease). This vaccine not only confers effective protection but blocks cell invasion by virulent PRV and prevents development of a persistent carrier state. It is possible to alter surface antigens so a virus induces an antibody response distinguishable from that caused by wild strains.

Examples:

1. Pseudorabies virus lacking the TK gene
2. Bayer’s *aro* gene-deleted salmonella vaccine (Argus SC) for use in pigs. The product used to prevent diarrhea, septicemia, pneumonia and mortality caused by *S. choleraesuis*.
3. **Putting the protective antigens into a safe, live organism (a “vectored vaccine”): live recombinant agent vaccines**

This involves inserting a gene of interest into attenuated or host-restricted living carrier organisms (vectors) such as the poxviruses, adenoviruses and herpesvirus. Poxviruses such as vaccinia (derived from cowpox) are favored because they have a big genome, ~10 percent of the large poxvirus genome can be replaced by foreign DNA, and they have the potential to become multivalent vaccines (contain antigens from multiple agents). A good vector infects, but does not replicate in, the host cells of vaccinated animals. Current examples are a Newcastle disease vaccine (in gene-deleted vaccine strain of fowlpox), WNV (in canarypox), equine influenza virus (also in canarypox) and rabies (in vaccinia virus). They are relatively free of adverse side effects, stable, adaptable to mass vaccination, non-adjuvanted and, like gene-deleted vaccines, allow differentiation of vaccinated from naturally infected animals. One advantage is that any protein that is made will be naturally glycosylated, making it more likely to be an effective antigen. Vaccines are created by recombinant technology where one or more of the vector genes are deleted replaced by one or more protective genes from the pathogen. The vector is administered in the vaccine. The inserted gene products are manufactured after the vector infects cells. The vector may be attenuated so that it will shed from the vaccinated animal, or host-restricted so that it will not replicate in the tissues of the vaccinated animal. Genes are sometimes inserted so that the vaccine virus is marked and can be identified later if it is isolated in a disease outbreak. These are known as **marker vaccines**. Some BHV-1 strains in vaccines are labeled in this way.

Examples:

1. **Oral recombinant vaccinia virus with rabies glycoprotein** for wildlife rabies. This vaccine is given in baits (e.g., chicken heads or in manufactured baits that abrade the oral cavity of animals that consume them). It is one of the great success stories in vaccination. The manufacturers deleted the thymidine kinase gene of the vaccinia virus, guaranteeing low pathogenicity of the vector. Oral vaccination of dozens of animal species, including wildlife, has not revealed residual pathogenicity. When administered orally (by direct instillation into the mouth, or in a bait) to young and adult foxes, or raccoons, the vaccine elicits high antibody titers and confers protection against severe racies virus challenge. Studies have shown that the vaccine is not pathogenic in 10 avian and 35 mammalian species, including the majority of rabies reservoir hosts. Regardless of the vaccine dose or route of
administration, vaccinated animals remained clinically normal, with no overt disease. Following oral
administration, the vaccine is cleared quickly (e.g. within 48 hours). No abortifacient, teratogenic, or
oncogenic side effects occur. Large-scale field trials in foxes, raccoons and coyotes have been reported
to date in Europe and North America. The movement of rabies was stopped or slowed in many parts of
Europe and the USA due to use of this vaccine. A bait that works on skunks has unfortunately not been
developed for use in Wyoming.

2. West Nile virus and equine influenza virus RecombiTek®; manufacturer: Merial. This consists
of a modified canarypox virus that contains genetic sequences of West Nile virus or equine influenza
virus. Although Merial makes claims quicker onset of protection and higher antibodies levels, as far as I
am aware the two West Nile virus products on the market (Merial's and Fort Dodge's) are equally
efficacious. Just because the technology is sexy and advertisements are slick does not automatically
make a product better. There is much to be said for a product that is inactivated and has a proven track
record (many units of vaccine sold with few reports of adverse effects). In situations like this, it may be
better to make a decision based on factors such as price and company reputation.

4. Nucleic acid vaccines
Selected sequences of the infectious agents purified DNA can also be used. This requires that the DNA
be modified so that it can be taken up by the vaccinated animals host cells and expressed in the
nucleus. The DNA is inserted into bacterial plamids (circular pieces of DNA) with a suitable mammalian
promoter sequence. Such vaccines cannot be given by injection, and require either a gene gun or
aerosolization. I am not aware of a commercial nucleic acid vaccines for animals, but they too are
coming down the pike. There is ongoing research with developing a nucleic acid vaccine for equine
influenza.

SINGLE vs. MULTIVALENT VACCINES - which ones to use
Vaccines are available as single agent or multiple agent (= "multivalent") vaccines. In most cases it is
more cost-effective to use multivalent vaccines. There is a large, confusing number of vaccines on the
market. The Intervet company, for example, has 6 differently formulated vaccines for BVDV, with the
addition of live or killed versions of respiratory viruses, bacteria and leptospira. At the time of writing,
Novartis had twelve BVDV products. As a general rule, good quality independent comparative studies of
the efficacy of different manufacturers vaccines are not available in the scientific literature. The large
number of products makes it difficult to do a crosscutting comparison of products for protection against
a specific agent. Most studies are commissioned by companies who have a vested interest in showing
their competitors products are worse and will designed accordingly. The comparison is usually to one or
two targeted competing products. Results unfavorable to the commissioning company are unpublished.
When studies are done in universities, the study design is that of the company, including the severity of
the challenge (which may be unrealistic). Veterinarians in private practice tend to learn over time what
appears to work in their area, and are loyal to particular companies. Vaccine preferences are often
based on non-scientific factors (how well the vaccine representative treats a veterinary practice with
price breaks, free trips to veterinary conferences, responsiveness when there is a problem, and steak
dinners; whim; ease of use). Veterinary diagnosticians have some sense of bad vaccines when they see
a wreck, but are not in a position to give a reliable opinion on the best vaccines, which is what producers
often request. Government agencies overseeing vaccine quality will not advise on best, good or bad
products. The USDA is under no obligation to force companies to withdraw ineffective products. The
advertising and producer articles by vaccine company representative are simplistic and often misleading
– please read these claims skeptically. Some companies have a preference for killed products (e.g.,
Novartis Animal Health) whereas others prefer MLVs.

ROUTES OF INOCULATION
The simplest and most common method is subcutaneous (SC) or intramuscular (IM) injection. Vaccines
for food animals should be given in the neck or other cheap cuts of meat, not the thigh or
rump. We recently had a spectacular example of the misuse of an irritating vaccine, resulting in multiple
cases of lameness in cattle due to injection into thigh muscles of late term cattle. In the not so distant
past we had an episode where a vaccine was given close to the vertebral column, resulting in multiple
cases of paralysis - the vaccine entered the spinal canal, caused inflammation, and compressed the
spinal cord. For some diseases, mucosal immunity is more important than systemic immunity. In these
cases, it is more appropriate to administer the vaccine at sites of initial microbial invasion. For example,
intranasal vaccines are effective in protecting cattle against infectious bovine rhinotracheitis, cats
against feline rhinotracheitis and calicivirus infections, and poultry against infectious bronchitis and
Newcastle disease. These techniques require handling each animal. Aerosolization of vaccines enables
them to be inhaled by all animals in a herd, group, or flock—an obvious advantage when the unit is large. This method is used to vaccinate mink against canine distemper and mink enteritis, and poultry against Newcastle disease. Some vaccines may be administered in feed or drinking water, e.g., vaccinating poultry for Newcastle disease and avian encephalomyelitis. With cattle, sheep and horses we are still giving vaccines by injection or intranasally one-by-one.

**VACCINATION SCHEDULES AND PRACTICES**

Certain principles are common to all methods of active immunization. When animals are born, most of their ability to resist infection is due to antibodies from the dam in colostrum. One approach for protection is to vaccinate the dam in late gestation. Generally such products should contain killed infectious agents. If properly timed, peak antibody levels are reached just before the calf, lamb or foal is born, and is delivered in colostrum. Because the exact time of loss of maternal immunity cannot be predicted, young animals are often vaccinated at least twice to ensure successful immunization. The interval between doses varies. Some vaccines require administration every 6 months, whereas vaccines that produce long-lasting immunity may need to be given only once every 2-3 years. The interval between doses may be determined by the disease. Some diseases are seasonal, and vaccines may be given before expected disease outbreaks. Examples include lungworm vaccine given in early summer before the lungworm season, anthrax vaccine in the spring, and WNV vaccine given to horses before mosquitoes become active (in our area, in the spring at least one month before mosquitoes appear).

**VACCINE DO's**

- **DO** read and understand the information on the vaccine insert until you become familiar with the product. Don’t just read the label on the vaccine.
- **DO** vaccinate against diseases known to be a problem in your area.
- **DO** go with subcutaneous rather than intramuscular vaccines, if you have a choice between two equally efficacious inactivated adjuvanted vaccines, one S/C and one I/M. S/C vaccines are slower to administer but cause less tissue damage.
- **DO** be skeptical of advertising claims by vaccine companies, particularly in the producer press. Vaccines are one of the best tools we have to control disease, second only to good management. Many advertising claims are misleading and/or exaggerated.
- **DO** keep the vaccines sites consistent when processing a herd for vaccination.
- **DO** keep a record of which vaccine went where and of the serial/lot number of the vaccine (in case of adverse reactions that you need to report to the company and the USDA's Center for Veterinary Biologics).
- **DO** vaccinate in the neck.
- **DO** clean multiple dose syringes appropriately after use (e.g., recently boiled (= sterile) de-ionized water drawn repeatedly into the syringe) and store the cleaned syringe appropriately (ziplock bag in freezer).
- **DO** heat sterilize plastic syringes (can be done in a microwave).
- **DO** boil disassembled metal syringes.
- **DO** allow plastic and metal syringes to cool before use. Hot syringes kill MLV agents in vaccines.
- **DO** keep bacterins/toxoids/inactivated vaccines cool (fridge temperature).
- **If** you think your herd had a vaccine-related problem, **DO** contact the company **AND** the US CVB **AND** the person who sold you the vaccine. Keep a handwritten record of what happened the herd and any conversations you had with interested parties.
- **DO** involve a reputable veterinary diagnostic laboratory if you suspect the vaccine was responsible. If the company agrees to pay for testing, make sure you will access to the data down the road. Pick a veterinary laboratory that you’ve used before and trust, and not financially tied to the company.

**Vaccine DON'Ts**

- **DON'T** give two vaccines in the same site at the same time.
- **DON'T** vaccinate into prime cuts (i.e., don’t vaccinate in rump or hip), or too close to the spine.
- **DON'T** vaccinate pregnant animals unless company specifically states it is safe to do so and defines the circumstances. Some BVDV products can be given to pregnant animals, but cattle must be vaccinated once before as non-pregnant animals before this is safe.
- **DON'T** vaccinate pregnant animals right before parturition, even if they are stated to be safe. The irritation in muscle may cause heavily pregnant cattle to go down, or become badly lame. We have seen and reported some spectacular episodes of this in large herds.
• **DON’T clean the inside of the syringe with disinfectants or soap** unless the manufacturer indicates this is safe to do. Soap and disinfectant residue inactivate vaccines.

• **DON’T freeze vaccines.** Treat them with respect - they are biological products, and should be treated with the same respect as similar products (milk! blood!). A good practice of keeping them in coolers chute side.

• **DON’T over-vaccinate** – clostridial vaccines (7- and 8-way) tend to be given every time animals change hands. These result in excessive trim due to vaccine-induced muscle injury.

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**WHY VACCINES FAIL**

- Disease induced by strains of organisms or antigens that differ from the strain in the vaccine you used
- Faulty production of specific lot of vaccine (not enough antigen; accidental inactivation during manufacture)
- Unsatisfactory storage, exposure to heat (keeping it in the cab of a truck during the height of summer), freezing (esp. modified live vaccines)
- Administering antibiotics in conjunction with live bacterial vaccines - it kills the bacteria!
- Chemical sterilization of syringes
- Administration by unconventional routes (e.g., given intranasal vaccine intramuscularly, or vice versa)
- Animals incubating disease at time of vaccination
- Disease due to an agent other than the one in the vaccine
- Blocking effects of maternal antibodies
- Immune response is suppressed (heavily parasitized or malnourished animals; stress of pregnancy, extremes of cold and heat, and fatigue)

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**PROBLEMS THAT OCCUR WITH VACCINES**

- Endotoxic shock and/or abortion (Gram-negative bacterial vaccines)
- Idiosyncratic reactions (usually restricted to individual animals)
- Immunosuppression (e.g., with live attenuated BVDV vaccines).
- Hypersensitivity reactions (purpura; big head – esp. with killed products given on multiple occasions to animals)
- Viral contamination of products (usually in MLV products)
- Injection site swelling (common with oil-based adjuvants).
- Clostridial contamination of needle, with clostridial myositis at site of injection, or activation of pre-existing clostridia at sites of injection or trauma. This is a well recognized but sporadic problem in horses, and in other species.

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*Post-traumatic myositis in a 5-year old AQH used in barrel racing. The horse sustained a large bruise (hematoma), and the lesion became larger over time. The horse then died unexpectedly. The veterinarian found gas and dead muscle tissue at necropsy. Clostridium perfringens was cultured. The histology was consistent with a clostridial myositis. WSVL accession #08E5645. Such lesions occur as a rare complication of injections with vaccines or other medications. Images courtesy of Dr. Marshall Kohr, Animal Medical Center, Gillette, WY*

- Induction of disease (MLV of BVDV given to PI cattle; abortion due to BHV-1 in pregnant heifers)
- Cancer (feline vaccines induce soft tissue sarcomas; not a recognized problem in food animals)
Currently, there is no automatic forwarding of suspect adverse reaction (SAR) reports from the veterinary biologics industry to the USDA. This is an extraordinary situation. Automatic forwarding of such reports to the regulatory agency is required in many other industrial countries. The USDA is often in the dark about defective products after they are licensed, except when a catastrophic adverse reaction develops. There is nothing to stop a company from settling with a producer when a SAR occurs, and ensuring that as part of the settlement no report is made to the USDA. The situation is particularly unfortunate since the rules for licensing virus products under the Virus-Serum-Toxins act (Code of Federal Regulations 9 - parts 1 - 199 - Animals and Animal Products) set a low bar for companies - particularly for inactivated products (bacteria and viruses). This is especially in terms of the rigor of efficacy studies. One of the defects of the American vaccine licensing situation is that it is highly proscriptive: the Code of Federal Regulations (above) lays out exactly how studies need to be done to get a license. These requirements are not often updated to reflect current knowledge. There is nothing to stop researchers or companies doing more creative studies to ensure their products work well - the better companies do so. There are surprisingly few published studies of the efficacy of vaccines. For example, it was only in November 2007 that an efficacy study was published on how well a widely used vaccine for bovine herpesvirus-1 product protects animals from abortion. An additional issue is that most US veterinary journals in which vaccine trials are reported do not have a formal conflict-of-interest disclosure policy. As a result it is not possible to know whether a vaccine trial at a university or research institute was done by scientists without financial ties to the company, and who were therefore truly independent. Conflict-of-interest disclosure policies have become the standard in human medicine.

The profusion of vaccines on the veterinary market is largely a result of the ease with which products can get a license. There are about 165 licensed vaccines containing bovine herpesvirus-1 (BHV-1), the cause of rednose. It makes it impossible to do comparison studies of efficacy, and difficult to know which products to recommend. Nevertheless, some good studies are published occasionally of "old fashioned" vaccines in the peer-reviewed literature. One recent example covered in class is the use of a Novartis product to protect against abortion due to BHV-1. Most adverse reports are lodged with the manufacturing company, which usually then deals with the issue by sending out a company veterinarian to investigate the problem. For some years, the USDA has declared its intention to require automatic forwarding to it of adverse reaction reports received by companies. This is still the intent, although when it will happen is unclear. The USDA has begun the welcome practice of publishing in summary form adverse reaction reports for various species.