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Vaccines and Vaccination Practices: Key to Sustainable Animal Production

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Glossary

Antigen A substance which the body recognizes as alien and induces an immune response.

Cluster of differentiation (CD) A nomenclature system to identify and characterize cell surface molecules.

Cytokines A group of small proteins that is important in cell signaling. Cytokines include interleukins which are important in the regulation of immune responses.

Proinflammatory cytokines are important during the inflammatory process directly after infection.

Dendritic cells Cells of the immune system which process antigen and present it on the surface to other cells of the immune system.

Differentiating infected from vaccinated animals (DIVA) An approach in which animals are vaccinated with a vaccine that can be differentiated from infections with field virus by serological or other methods.

Major histocompatibility complex (MHC) A set of molecules displayed on cell surfaces that are responsible for lymphocyte recognition and 'antigen presentation'. The MHC molecules control the immune response through

recognition of 'self' and 'nonself,' and consequently, serve as targets in transplantation rejection.

Pathogen-associated molecular patterns (PAMS) PAMS are molecules associated with groups of pathogens. PAMS include lipopolysaccharides, proteins, single stranded RNA fragments, and nonmethylated CpG DNA sequences. PAMS bind to pattern recognition receptors which is important for the initiation of immune responses by the host.

Pattern recognition receptors (PRR) Proteins expressed by cells of the innate immune response. PRR can be expressed on the cell surface and in the cytoplasm. Toll-like receptors are a specific group of PRR.

Quantitative trait loci (QTLs) Stretches of DNA containing or linked to the genes that underlie a quantitative trait.

Toll-like receptors (TLR) A specific group of PRR (see Pattern Recognition Receptors), TLR are evolutionary preserved and were first recognized in the fruit fly (see Pathogen-Associated Molecular Patterns).

Introduction

The projected increase in human population from 6.8 billion in 2010 to more than 9 billion in 2050, combined with the increase in disposable income in countries such as China and India, will have a major impact on the increased need for animal protein for human consumption. The current production of animal protein for human consumption is projected to continue increasing until 2050 (Table 1). To achieve these predicted increases it will be of crucial importance to improve feed conversion, production parameters, and genetic resistance to disease as well as control exposure to pathogens by improved biosecurity and vaccination.

The focus of this article is mostly on vaccines and vaccination technologies used in aquaculture, poultry, swine, and cattle. Unless specifically mentioned as a category, these four groups are referred to as production animals. Although small ruminant production is expected to increase until 2050 (Table 1), the amount of small ruminant products is dwarfed by the other four commodities, and vaccination of small

ruminants is not covered here. Because vaccine-induced protection depends on the degree of genetic resistance to a given pathogen and on the degree of biosecurity, these topics are briefly addressed. In addition, the major groups of pathogens and immune responses relevant to vaccine-induced immunity are briefly reviewed.

There are several extensive publications on the production and quality control of vaccines, the use of vaccines, and related topics. For more detailed information see Gay *et al.* (2007), Jones *et al.* (2007), Lombard *et al.* (2007), Lubroth *et al.* (2007), McLeod and Rushton (2007), Scudamore (2007), O'Brien and Zanker (2007), Schat and Baranowski (2007), and Schudel (2007).

Pathogens

For the purpose of this article pathogens are defined as organisms infecting production animals, leading to disease or causing immunosuppression that leads to increased

Table 1 World production of meat, milk, eggs, and aquaculture from 1961 to estimates for 2050

Commodity	Production in million tons						Increase from 2011 to 2050 (%)
	1961/1963 ^a	2005/2007 ^a	2011 ^b	2012 ^b	2013 ^b	2050 ^a	
Bovine meat	30	64	62.7	66.5	70.2	106	69
Ovine meat	6	13	13.5	13.6	13.8	25	85
Pig meat	26	100	109	112.5	114.2	143	39
Poultry meat	9	82	102.1	104.6	106.4	181	77
Aquaculture	2.6 ^c	49.9 ^d	62.7	66.5	70.2	209.5 ^f	234
Milk products	344	664	745.5	767.4	784.4	1077	44
Eggs	14	62	No data	65 ^e	No data	102	65

^aData for 1962/1963, 2005/2007 and 2050 are derived from Alexandratos, N., Bruinsma, J., 2012. World agriculture towards 2030/2050: The 2012 revision. ESA Working Paper No. 12-03, pp. 1–147. Rome, Italy: FAO, unless superscript *c* and *d* are given for a specific value.

^bData for 2011, 2012 (estimated) and 2013 (forecast) are derived from FAO, 2013. Food Outlook: Biannual Report on Global Food Markets. Rome, Italy: FAO, pp. 137–139, unless superscript *e* is given for a specific value.

^cData for 1970 from FAO Fisheries and Aquaculture Department, 2013. Global Aquaculture Production Statistics for the year 2011. Rome, Italy: FAO.

^dData for 2007 from FAO, 2012. The State of the World Fisheries and Aquaculture. Rome, Italy: FAO, pp. 3–40.

^eData from Evans, T., 2013. Global poultry trends: World egg production sets a record despite slower growth. Available at: www.thepoultrysite.com (accessed 16.04.14).

^fFrom Wijkström, U.N., 2003. Short and long-term prospects for the consumption of fish. Veterinary Research Communications 27 (S1), 461–468 and Brugère, C., Ridler, N., 2004. Global Aquaculture Outlook in the Next Decades: An Analysis of National Aquaculture Production Forecasts to 2030. Rome, Italy: FAO, pp. 1–47, tonnes of aquaculture products required on the basis of stagnating capture fisheries.

susceptibility to infection and disease by other pathogens. Three broad categories of pathogens are relevant to this article: viruses, bacteria, and parasites. The latter category includes protozoa as well as helminths. Fungi constitute a fourth group of pathogens but are not discussed as there are no antifungal vaccines available for production animals. Research to develop vaccines for humans against *Candida albicans* and other fungal infections (Cassone, 2013; Spellberg, 2011) may lead to antifungal vaccines for use in production animals in the future.

Detailed descriptions of human and animal pathogens can be found in several textbooks, such as Medical Microbiology (Murray *et al.*, 2012), Veterinary Microbiology and Microbial Disease (Quinn *et al.*, 2011), and Georgis' Parasitology for Veterinarians (Bowman, 2014). A short description of viruses, bacteria, and parasites relevant for this article is provided for readers not familiar with the key characteristics of these pathogens.

Viruses

Viruses infecting production animals are small (15–300 nm) and cannot be seen by light microscopy with the exception of poxviruses. Viruses are inert outside of living cells and are strictly intracellular pathogens. Entrance into living cells is essential for their replication using cellular organelles for the production of viral proteins. Viruses have double-stranded (ds) or single-stranded (ss) deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) genomes but never both. Some of the larger viruses have genomes coding for proteins that are needed for genome replication (e.g., poxviruses and herpesviruses), whereas other viruses depend on cellular enzymes to replicate their genomes. The genome is surrounded by a capsid that consists of proteins or sometimes by a lipid membrane derived from the host cell, which is referred to as the envelope. Viral proteins are inserted in the envelope during the development of virus particles. Enveloped viruses are in general susceptible to inactivation by disinfectants and external

factors, such as sunlight, whereas viruses with only a protein capsid are more resistant to chemical and physical treatments, which have important consequences for biosecurity measures.

Bacteria

Bacteria are classified as prokaryotic cells lacking a nucleus and organelles in the cytoplasm. Individual bacteria range in size from 1 to 20 µm, but they can form clusters, chains, or biofilms. Bacterial genomes consist of a single ds DNA chromosome, which is often circular but can be linear. Bacteria reproduce by cell division. Small circular, autonomously replicating DNA sequences called plasmids may be present. These plasmids can contain virulence factors and sequences conferring antibiotic resistance. Plasmids can be exchanged between bacteria of the same or different species. Most bacteria have a rigid cell wall and may have flagella or fimbria. The composition of the cell wall is important for a broad classification into two groups: Gram-positive and Gram-negative bacteria. The only important pathogenic bacteria lacking a rigid cell wall are the Mycoplasmas, which have a plasma membrane. Some bacteria (e.g., *Bacillus anthracis* causing anthrax) produce endospores that are extremely resistant to chemical or physical treatments and can remain dormant for very long periods. The classification of bacteria was traditionally based on morphology, culturing on different media, motility, and metabolic activity, which all required the ability to grow the bacteria in inert media. With the advance of next generation sequencing, classification of bacteria can be achieved to the level of strain identification using the 53 genes coding for ribosomal protein subunits (ribosomal multilocus sequence typing (rMLST)) (Jolley *et al.*, 2012).

Parasites

This widely divergent group of organisms includes unicellular organisms, such as coccidia, Plasmodium, and *Theileria parva*,

as well as multicellular organisms that can vary tremendously in size, including 10-m long tapeworms. All organisms in this group are eukaryocytes with a defined nucleus including a nuclear membrane and with different organelles in the cytoplasm. The life cycle of parasites can be very complex, including sexual and asexual reproduction. Several economically important parasites affecting production animals have intermediate hosts (e.g., ticks for *T. parva*), whereas others do not use intermediate hosts (e.g., *Eimeria* species in chickens).

Relevant Immune Responses

To discuss vaccines and vaccination it is important to provide a brief overview of the major immune responses. A good introduction to the basics of veterinary immunology can be found in 'Veterinary Immunology: Principles and Practice' (Day and Schultz, 2011); more in-depth information is provided in 'The Immune System, third ed.' (Parham, 2009). Both books are focused on the immunology of mammalian species. Readers interested in the immunology of birds or teleost fish may consult 'Avian Immunology, second ed.' (Schat *et al.*, 2014) or 'Fish Defenses, vol. 1: Immunology' (Zaccone *et al.*, 2008), respectively. Most of the information on mammalian immunology is based on studies in mice or humans, whereas avian immunology is largely based on studies in chickens. Within these two classes of animals, major differences may exist among species of the same class. Similarly, major differences are expected to exist among the approximately 4000 teleost fish species. Key differences between the three classes of animals and, if appropriate, within classes are indicated.

Immune responses are often divided into innate and acquired (also called adaptive) immune responses, but this division is no longer seen as an absolute. Innate immune responses, although capable of directly killing microbes, are also absolutely required for the generation of adaptive immune responses. This is achieved by specific 'pattern recognition receptors (PRR)' that are located on the cellular membranes and in the cytoplasm of the cells. Toll-like receptors (TLR) are an example of PRR and were originally described in the fruit fly (*Drosophila melanogaster*). PRRs recognize specific 'pathogen-associated molecular patterns (PAMPs)'. PAMPs are conserved among classes of microbes but should not be confused with virulence factors. Binding of PAMPs through TLRs or other PRR on the so-called professional antigen-presenting cells (APC), such as dendritic cells and macrophages or infected cells, results in the production of specific cytokines or interleukins (ILs) by APC. The ILs activate lymphocytes starting the activation of the adaptive immune system.

Innate Responses

Innate responses are activated rapidly within minutes to hours after an infection and have limited specificity and lack of memory. Pathogens first encounter physical and chemical barriers at the skin and epithelial surfaces in the respiratory and intestinal tracts. Mucus on the skin of fish as well as in the upper respiratory tracts acts as a chemical barrier, can trap pathogens, and prevent infection. If a pathogen successfully overcomes the physical barriers, it meets cells of the innate

immune system that are equipped with secreted and cell-associated defense mechanisms. Soluble defenses induce inflammation and include interferons (IFN), defensins, and complements. Complement can promote phagocytosis by cells such as neutrophils (called heterophils in birds) and macrophages, which initiate cell-associated defenses, such as nitric oxide (NO) production. Natural killer (NK) cells exhibit both innate and adaptive-type responses. NK cells can kill tumor and virus-infected cells and are an important source of IFN- γ , which also enhances macrophage and neutrophil phagocytosis and microbial killing. Most of these innate responses are very similar in teleost fish, birds, and mammals, although minor differences among these three groups may exist.

Although innate immune responses are important for the initiation of vaccine-induced immunity, they are generally not considered important vaccine immune responses due to the lack of memory and specificity. However, in intensive production systems, such as the chicken broiler industry, vaccination especially with live attenuated vaccines may briefly boost innate responses, which can have a beneficial protective effect before the development of an adaptive, protective immune response. For example, in chickens, NK cells are activated after vaccination against Marek's disease (MD) herpesvirus (MDV) (Heller and Schat, 1987) and based on studies by Garcia-Camacho *et al.* (2003) may provide protection during the first few weeks when the birds are in the poultry houses. MD is discussed in more detail in the another article of this Encyclopedia.

Antibody Responses

The adaptive immune responses can be divided into antibody and cell-mediated responses. On exposure to an antigen (in this article typically a protein-based part of a pathogen) antibodies are generated by B lymphocytes, which may develop into plasma cells producing large quantities of antibodies. Immunoglobulins (Ig) consist of heavy chains and light chains. IgG (see below) consists of two heavy and two light chains, which represents the basic structure of an immunoglobulin. The heavy chains have a number of constant domains and one variable domain, whereas the light chain has one constant and one variable domain. The variable domains of the heavy and light chains form the antigen-binding site, whereas the constant domains of the heavy chain are important for additional functions, such as activation of complement or binding to cellular receptors. For further details on the general aspects of antibodies, the readers are advised to refer to the different textbooks mentioned earlier.

There are a number of different antibody classes and subclasses that vary among the teleost fish, birds, and mammals (Table 2). For the purpose of this article, the author has focused on the IgG, IgM, and IgA antibodies, or their equivalents. IgG (often referred to as IgY in birds) consists of two heavy and two light chains. It is the dominant antibody in serum and depending on the immunization schedule and vaccine used it is the key antibody produced in vaccine-induced, antibody-based protective immunity. After a primary infection or first vaccination, IgG antibodies are detected approximately 7 days postinfection (pi), which is named the primary response. One of the hallmark characteristics of an

Table 2 Antibody classes and subclasses in cattle, swine, chickens, and teleost fish

Species	Antibody classes and subclasses						Key references
	IgM ^a	IgG	IgA	IgD	IgE	IgT	
Fish	+	–	–	+	–	+	Fillatreau <i>et al.</i> (2013)
Chickens	+	Aka IgY	+	–	–	–	Härtle <i>et al.</i> (2014)
Cattle	+	3 subclasses	+	+	+	–	Zhao <i>et al.</i> (2006)
Swine	+	5 subclasses	+	+	+	–	Butler <i>et al.</i> (2006)

^aTetramer in fish and pentamer in birds and mammals.

Abbreviation: Aka, also known as.

acquired immune response is memory. Memory B cells are generated during the primary response, and on second exposure to the same antigen, they are responsible for a rapid increase of IgG antibody production. This is called the secondary response and is generally long lasting. These characteristics form the basis for vaccination.

Other classes of antibodies include IgM and IgA. IgM consists of five basic structures (pentamer) except in fish, which have tetrameric IgM-like antibodies. IgM antibodies are the first class that can be detected after exposure to a pathogen and are typically not detected in a secondary response. However, IgM is the major antibody response to pathogens in fish. IgA is important for mucosal immunity. IgA can exist as a monomer or a dimer joined by the so-called J chain. In addition, dimeric IgA is associated with a protein, the secretory component, which protects the antibody against degradation by proteolytic enzymes at mucosal surfaces. Fish lack IgA but produce IgT, which performs similar functions to IgA and is present in relatively large quantities in the skin mucosa.

Cell-mediated Immunity

Cell-mediated immune (CMI) responses are especially important for the control of intracellular pathogens and are primarily mediated by cytotoxic T lymphocytes (CTLs). CTLs have been described in all production animals from fish to cattle and swine and are characterized by the surface markers CD8 α and CD8 β . These cells recognize small antigen fragments of 8–12 amino acid peptides if these fragments are presented in the context of major histocompatibility complex (MHC) class I proteins, which are expressed on the surface of virtually all cells in the body. When CTLs recognize a small peptide expressed in the context of MHC class I, they kill the cells presenting the antigen through a rather complex system, eliminating pathogen-infected cells. Memory responses have been described for CTL; thus, once an animal has been immunized and a CTL response has been generated, antigen-specific CTL can rapidly expand after subsequent exposures, similar to memory antibody responses.

Antigen Processing

To understand the differences in immune responses generated by live versus inactivated (also referred to as killed) vaccines it is important to briefly discuss antigen processing. When a killed vaccine or an antigenic protein is injected into an animal it is typically processed by professional APC. The proteins are

broken down in the phagolysosome of the APC to fragments of mostly 10–30 amino acids, which are then presented to B-lymphocytes in the context of MHC class II antigens on the cell surface of the APC. In mammalian species this process occurs in the lymph nodes. Chickens lack lymph nodes and antigen processing occurs mostly in the spleen.

Antigen processing for presentation to CTL differs fundamentally from the processing for presentation to B lymphocytes. For CTL presentation, *de novo* synthesis of proteins is needed, i.e., replication of virus, intracellular bacteria, or protozoa in an infected cell is needed for optimal presentation of antigen to CTL. The newly synthesized pathogen-derived proteins are broken down to small peptide fragments in the cytosol and are transported into the endoplasmic reticulum, where they bind to the MHC class I molecules. These MHC class I–antigen complexes are then transported via the Golgi apparatus to the cell surface for CTL recognition. The practical consequences for vaccinology are that inactivated vaccines induce antibody responses with little or no CMI responses, whereas live vaccines generate both antibody and CMI responses.

Maternal Immunity

The immune system of newborn animals is generally poorly developed and requires time to fully mature after birth. To protect the newborn against infections, antibodies are transferred in mammals from the dam to their offspring through the placenta or the colostrum. There is little or no transfer of IgG through the placenta in cows and sows; thus, it is of crucial importance that newborn calves and piglets receive colostrum during the first 24–48 h after birth (Butler, 2006). The neonatal intestinal tract of many mammals, including cattle and swine, allows efficient absorption of Ig only during the first 24–48 h after birth. In cows, the unique IgG subclass 1 is the major antibody in the colostrum. The intestinal tract allows the absorption of Ig in calves and piglets only during the first 48 h after birth. Sow colostrum consists of 60% IgG, 30% IgA, and 10% IgM. After 48 h the composition of immunoglobulins in sow milk changes to predominantly IgA. (Butler *et al.*, 2006). Maternal immunity in chickens is provided through transfer of IgY from the yolk to the embryo and newborn chicken. Although maternal immunity has been described for fish (Zhang *et al.*, 2013), the level of transfer of maternal antibodies to Atlantic salmon (*Salmo salar* L.) fry is apparently insufficient to provide protection against infection with *Yersinia ruckeri* (Lillehaug *et al.*, 1996). The importance of

maternal immunity for vaccination schedules is discussed in the Section 'Selected Examples of Vaccinations in the Four Groups of Production Animals.'

Immunosuppression and Immuno-evasion

Many factors can influence the immune response to a given vaccine, especially in the more intensive production systems of swine, poultry, and fish units where large numbers of multiple age groups are kept in high density in confined spaces (e.g., the chicken layer industry). Stress levels can be high under these conditions and immunosuppressive viruses (e.g., chicken anemia virus and infectious bursal disease in chickens and porcine circovirus in piglets) often cause subclinical immunosuppression resulting in suboptimal protection after vaccination. In addition, a number of pathogens have developed strategies that interfere with immune responses. Poxviruses, herpesviruses, coronaviruses, and orthomyxoviruses have been especially successful in developing immune-evasive approaches, which have been well documented for chicken pathogens (reviewed in [Schat and Skinner, 2014](#)).

Importance of Biosecurity

Biosecurity is defined here as the complex of precautions taken to protect against the introduction and spread of harmful organisms and diseases into or within animal production systems. Some producers of production animals believe that vaccine manufacturers can always produce better vaccines and therefore biosecurity is not very important or is even irrelevant. However, the use of vaccines can never be an excuse for poor biosecurity. Most vaccines do not provide a sterilizing immunity, meaning that vaccination may prevent disease but not prevent replication of pathogens. As a consequence escape mutants may arise for which the vaccine no longer provides protection. Premises that are poorly cleaned after a previous flock or herd has been removed may allow naïve animals to be exposed by residual pathogens before the immune response

matures or before solid vaccinal immunity has been established. An example of good and poor farm management for poultry is provided in [Figures 1\(a\)](#) and [\(b\)](#), respectively. In [Figure 1\(b\)](#) there is no clean area directly adjacent to the poultry house. The absence of a clean area facilitates the entrance of rodents and other fomites. It will be much more difficult to control diseases under these circumstances even when vaccination procedures are correctly executed.

Ideally, closed houses with proper ventilation and climate control, which are cleaned and disinfected after each cycle, are used for chickens and swine, but this is often impractical in warmer climates, especially in economically poor countries. Multiage farms, a reality in most poultry production systems for layers, are another problem for strict biosecurity, preventing thorough cleanup of the premises.

Biosecurity measures that can always be included are as follows: restriction of access to the farm to only essential personnel, a change of boots and coverall before entering a chicken or swine house, and the use of proper footbath with disinfectant that is changed daily. It is important to ensure that vaccination crews and other persons visiting several farms on one day do not enter the facility without change of clothes and ideally a shower before entering the premises. A specific problem is encountered in several countries where most of the chickens are sold through live markets. Traders come to the farms to get their quota of birds, often using trucks that have not been cleaned for a long time. In this situation it is recommended that the farmer brings the birds to the entrance of the farm so that the trader does not have to enter with his truck. As a consequence of the bird flu situation, biosecurity measures have been more strictly enforced in the poultry industry over the past 5–10 years, but in many instances a critical evaluation by a qualified poultry veterinarian will result in recommendations for further improvements. For more detailed information on biosecurity measures for poultry production, the readers may refer to 'Diseases of Poultry, thirteenth ed.' ([Collett, 2013](#)). Many of the recommendations made for biosecurity on poultry farms are also applicable to intensive production systems for other food animals.



Figure 1 Layer parent farm (a) and commercial layer farm (b) in India. Both are multiage farms with natural ventilation and curtains. Note the clean environment and cement borders around the building in (a) in contrast with the commercial farm with plants growing up to the building and junk lying around in (b).

Genetic Resistance to Disease

Several factors favor the inclusion of genetic resistance in disease control programs for the four major production animal groups (reviewed in Gay *et al.*, 2007). The first factor is the concentration of genetic stock within a limited number of companies. In the poultry industry, for instance, there are only a few major breeders left in the world. These breeders are maintaining pure genetic lines under constant selection pressure for multiple traits. To produce a commercial product (layers or broilers), eight lines are used basically following the outline in Figure 2. Introduction of and subsequent selection for a new trait in one of the eight lines, indicated by the asterisk in Figure 2, will result in the presence of the new trait 4 years later in the production animals. Similar trends in concentration of genetic stocks are evolving in the salmonid industry and have started in the swine industry.

A second factor is the use of artificial insemination. In dairy cattle this had far-reaching implications as is illustrated by the impact of an elite sire and son duo on the distribution of candidate genes related to important production traits, including disease resistance. Each of these two bulls accounts for approximately 7% of the current genomes (Larkin *et al.*, 2012). However, the use of a highly desirable sire can also introduce unexpected new problems, as is illustrated by the case of leukocyte adhesion deficiency (LAD) disease. LAD has been linked to a point mutation in the gene coding for the leukocyte adhesion molecule CD18, and this defect was traced back to a specific sire Osborndale Ivanhoe, who was used extensively as a semen donor in the United States (Shuster *et al.*, 1992). Once the defect was identified at the molecular level, the disease was eradicated. Several other genetic diseases linked to the widespread use of semen from a single bull are mentioned by Shuster *et al.* (1992). The increased use of embryo transfer will have similar positive and potentially negative effects.

A third condition favoring the inclusion of genetic selection for disease resistance is the rapid progress in new DNA sequencing techniques, which has resulted in the (near) release

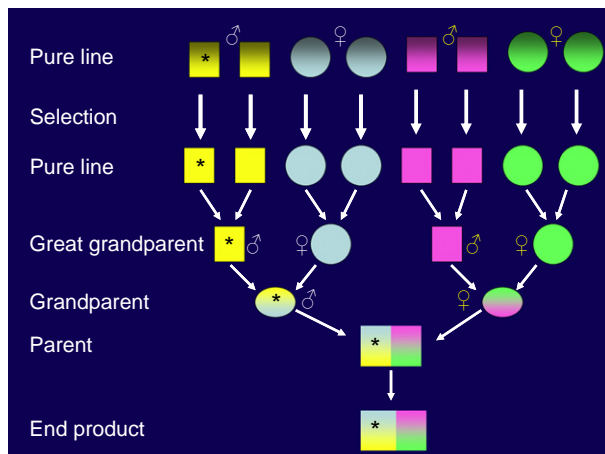


Figure 2 Breeding scheme used by primary poultry breeders to generate a commercial end product. The asterisk represents the introduction of a new trait and subsequent selection for the trait through the generations.

of complete genome maps for chickens, swine, cattle, and salmonids. Genetic resistance to pathogen-induced disease can be at the level of receptors preventing infection. For example, chickens can be resistant or susceptible to infection with avian leukosis virus subgroup A (ALV-A) based on allelic differences in the receptor gene (Nair and Fadly, 2013). The poultry breeders have used this information in their genetic selection program to generate birds resistant to ALV (McKay, 2013, personal communication). In swine, a single-nucleotide mutation at position 307 (G/A) of the alpha-(1,2)-fucosyltransferase (FUT1) gene, the putative receptor for F18 fimbriae of *Escherichia coli*, is strongly correlated with increased resistance to enterotoxigenic and verotoxigenic *E. coli* (Wang *et al.*, 2012). Atlantic salmon are highly susceptible to infectious pancreatic necrosis (IPN) virus, a birnavirus, which can cause 30–80% mortality during the juvenile posthatch freshwater stage. A quantitative trait locus (QTL) has been identified that confers resistance to the infection (Houston *et al.*, 2012; Moen *et al.*, 2009). Including the resistance conferring QTL in the breeding program by AquaGen in Norway has led to a dramatic reduction in the incidence of IPN (Anonymous, 2013b).

These examples suggest that transgenic approaches for disease control may be possible if regulatory and consumer resistance can be addressed. One example is the development of transgenic chickens that were unable to transmit avian influenza virus (AIV) to pen-mates, although the transgenic birds were not resistant to direct challenge. The resistance is based on the expression of a small RNA fragment that acts as a decoy to the viral polymerase complex (Lyal *et al.*, 2011). Transgenesis to increase resistance to specific diseases has been reported for several fish species (Dunham, 2009). Dunham suggests that the environmental risks of transgenic fish are minimal, but transgenic fish resistant to disease have not yet been used commercially. The Food and Drug Administration of the United States Department of Health and Human Services (FDA) has jurisdiction over the acceptance of transgenic fish for human consumption in the United States. In a preliminary document, the FDA concluded that transgenic Atlantic salmon containing the *opAFP-GHc2* recombinant DNA construct, produced by AquaBounty Technologies, are safe as a food source for human consumption.

Genetic resistance to certain diseases can also be based on the presence of specific MHC alleles or on the identification of QTL linked to resistance. For example, in chickens resistance and susceptibility to Marek's disease have been linked to the MHC complex (reviewed by Schat and Nair, 2013) and several QTL (Cheng and Lamont, 2013). In MD-resistant birds, viremia levels are lower than in susceptible birds during the first 14 days pi and vaccines provide better protection against virus replication in resistant than in susceptible chicken lines (Yunis *et al.*, 2004; Schat *et al.*, 1982). In a multigenerational challenge study using elite egg production pure lines, Fulton *et al.* (2013) reported an association between Marek's disease mortality and the MHC of the sire. This information combined with selection for production traits is actively used in the selection program to improve resistance to MD (Fulton *et al.*, 2013; McKay, personal communication). Because this type of selection experiment is not easily reproduced for other pathogens, selection for genetic control of immune responses is used rather than selection for resistance to specific pathogens

(Lamont *et al.*, 2014). In chickens, in addition to the QTL linked to MD resistance, QTL have been identified that are linked to resistance to Salmonellosis, *E. coli*, coccidiosis, and infectious bursal disease (Cheng and Lamont, 2013), but these QTL are currently not included in the breeding programs of at least one major breeder company (McKay, personal communication).

In conclusion, improvement in genetic resistance needs to be an important part of an integrated disease control program in conjunction with strong biosecurity measures and an optimal vaccine program.

Vaccines

To Vaccinate or Not to Vaccinate

It is generally accepted that the use of vaccines is an essential component of disease control, which also impacts the economic production and welfare of production animals. Although disease control is the main reason to use vaccines, there are several additional considerations when determining whether vaccines should be used (McLeod and Rushton, 2007). Of major importance are the restrictions on import and export of animals or animal products imposed by many countries. These exist as a consequence of the presence of specific diseases, such as avian influenza or foot-and-mouth disease (FMD). Vaccination results in the development of antibodies, which may not differ from antibodies present after infection, thus interfering with disease monitoring. As a consequence, countries may not want to import products that are antibody positive. Avian influenza not only causes major losses for the poultry industry but is also a zoonotic disease; however, FMD is an economically important disease that does not affect humans. In the case of avian influenza, some countries allow routine vaccination of poultry, whereas others rely exclusively on 'stamping-out' and inhibition of animal movement. In some countries, ring vaccination is allowed to prevent spread of AIV, whereas poultry flocks within the infected area are euthanized. This is especially attractive if a differentiating infected from vaccinated animals (DIVA) strategy can be used as is the case in avian influenza (Suarez, 2012; Capua *et al.*, 2003) (see Section Vaccines for Poultry). There is currently no DIVA strategy available for FMD (Clavijo *et al.*, 2004) and many countries rely, therefore, exclusively on stamping out; this has led to significant social problems as was the case during the 2001 outbreak in England (Scudamore, 2007). During this outbreak, the Netherlands used a ring vaccination to prevent the spread of FMD, euthanized all animals within the affected area, and subsequently destroyed the vaccinated (antibody positive) animals in order to be declared FMD free ('vaccinate to kill' policy) (Parida, 2009) (FMD vaccination is discussed in another article of this Encyclopedia).

McLeod and Rushton (2007) mentioned the possibility of eradication of a specific disease by international vaccination campaigns as a second reason to vaccinate. This approach has been successful in the case of rinderpest, a viral disease of cattle, which was officially declared eradicated in 2011 (Njeumi *et al.*, 2012). Other national or international campaigns are often initiated to reduce risks of transmission of zoonotic diseases to humans, for example, rabies and *Brucella abortus*, but these campaigns have not led to the global eradication of the diseases.

The third reason to vaccinate is to control economically important diseases. Decisions to vaccinate or not are often based on a cost/benefit analysis. For example, in the broiler industry, economic benefits were realized after the decision was made in 1983 to add the SB-1 vaccine strain to the herpesvirus of turkeys (HVT) vaccine against Marek's disease when HVT alone no longer fully protected birds against the disease. Within 3 months the industry had calculated that the addition of SB-1 was financially advantageous by reducing the condemnation rate of diseased birds (listed as leukosis in the USDA database on condemnations in poultry) enough to offset the increased vaccine costs. Another example is related to the overall costs of vaccines and medicines in relation to the overall production costs. The cost of all vaccines and medicines for an average broiler farm in the United States is US\$ 0.05 per pound (=0.45 kg) live weight. In comparison, the chick cost is US\$ 5.47 per pound live weight (Anonymous, 2013a), with profits ranging from approximately US\$ 0.10 to a loss of US\$ 0.01 per pound live weight. MD vaccines are the most expensive vaccines for broilers with the average prices in the United States ranging from US\$ 2.65 per 1000 doses for HVT alone to US\$12.00 per 1000 doses for HVT combined with the CVI988 vaccine strain (prices quoted for 2014). To reduce these costs, MD vaccines are often diluted, especially when the condemnation rates for 'leukosis' are very low (Schat and Baranowski, 2007; Schat and Nair, 2013). However, dilution of the vaccine reduces protection to virus replication and tumor development when birds are challenged with very virulent plus (vv+)MDV strains (Gimeno *et al.*, 2011) increasing the possibility of selection for more virulent strains (Atkins *et al.*, 2013).

Although vaccination of production animals against zoonotic and economically important diseases seems to be non-controversial, there are some impediments, which are often regional. In Europe, consumers have expressed reservations about consuming meat from vaccinated animals, especially since the outbreaks of the highly pathogenic H5N1 avian influenza in poultry and the 2001 outbreak of FMD in the United Kingdom (Scudamore, 2007; O'Brien and Zanker, 2007). A second problem is related to the production and evaluation of vaccines that often involves animal testing for safety and efficacy, which has become problematic (O'Brien and Zanker, 2007). This is even more of a problem when new vaccines need to be developed for emerging or reemerging diseases. A third impediment is related to the control of transboundary diseases. Control of these diseases is frequently based on political decisions to vaccinate or to use 'stamping-out.' (Inter)national or regional vaccination campaigns may be needed if vaccination is allowed, which requires the willingness to commit economic resources and the cooperation of farmers. These conditions can be problematic, especially in politically unstable parts of the world (Lubroth *et al.*, 2007), but it can be done as has been shown with the eradication of rinderpest.

Characterization of Vaccines

Traditionally, vaccines have been developed by attenuating pathogens or using closely related agents that are not pathogenic in the target hosts. Since the advance in biotechnology,

vaccines have been developed using molecular approaches. In this section the author has first discussed the traditional vaccines and then the different approaches for biotechnology-based vaccines. Both categories can consist of live organisms or inactivated products. The latter group includes whole organisms, protein products, and DNA-based vaccines. A good overview of the different phases for the production of veterinary viral vaccines was recently published (van Gelder and Makoschey, 2012).

Determining vaccine efficacy

Independent of the type of vaccine, there is a need to establish methods for monitoring vaccine efficacy. When a new vaccine is submitted to the veterinary authorities, data are normally included showing protection against challenge. These tests are expensive and subject to animal use regulations, which vary among countries. To monitor vaccine 'takes' in field conditions, serology tests are most often used to determine whether adequate antibody titers have been achieved and are maintained over time. Most of these tests use different enzyme-linked immunosorbent assay (ELISA) approaches and commercial ELISA kits are available for most pathogens in cattle, swine, and poultry. Determining specific CMI responses is complicated and not used to measure vaccine responses. In chickens, a quantitative polymerase chain reaction (qPCR) assay can be used to determine whether the birds are adequately vaccinated by measuring CVI988, HVT, or SB-1 genome copies in the feather pulp between 7 and 14 days postvaccination (Baigent *et al.*, 2006; Renz *et al.*, 2013).

Traditional live vaccines

Most of the current vaccines for production animals are directed against viral and bacterial pathogens. Typically, a virus was isolated and passed a number of times in experimental animals, embryonated chicken eggs, or cell cultures, resulting in attenuation while remaining immunogenic. Probably the first example of attenuation of a virus was the use of rabbits by Pasteur to attenuate rabies virus (reviewed in Lombard *et al.*, 2007). Cell culture attenuation can be achieved by passaging the virus at its optimal temperature or at lower temperatures as has been done for the cold-adapted, live human influenza vaccine (Maassab and DeBorde, 1985). These approaches have been used successfully over the years, but the development of attenuated vaccines was empirical with often unpredictable results. Key requirements for the successful use of live virus vaccines are ease of (1) production, i.e., cell cultures using roller bottles or suspension cultures in bioreactors; (2) transport, i.e., liquid nitrogen (essential for Marek's disease vaccines), need of cold chain (either -20°C or 4°C , important for most vaccines), or ambient temperature (heat tolerant); (3) administration, and (4) good replication in the vaccinated animals without causing clinical or subclinical problems. The ease of administration is discussed in more detail in the Section 'Vaccination Procedures' and potential issues with subclinical problems discussed in the section on poultry vaccines. Production of live vaccines follows detailed protocols adhering to strict standards, which are determined by governments of individual countries (e.g., the Veterinary Services of APHIS-USDA), a group of countries (e.g., the

European Union), or by the World Organization for Animal Health (OIE) (Jones *et al.*, 2007; Schudel, 2007).

Similar to viruses, bacteria have been attenuated by passage in animals or suboptimal culture conditions on artificial media. Pasteur and his coworkers have been credited with the production of an attenuated vaccine against swine erysipelas by passage of *Erysipelothrix rhusiopathiae* through rabbits and fowl cholera by using aged *Pasteurella multocida* cultures (reviewed in Lombard *et al.*, 2007). Bacterial vaccines are made in large bioreactors.

Different approaches have been used for some vaccines available for the control of unicellular and multicellular parasites (Lightowers, 2014; Williams, 2002). Poultry coccidia vaccines have been developed by selecting precocious lines of coccidia, which replicate faster than wild-type strains and induce immunity without disease when given to young chicks. Control of lungworm, *Dictyocaulus viviparus*, in cattle is achieved by using irradiated attenuated L3 lungworm larva.

Since the advance in rapid sequence techniques, many of the attenuated vaccines have been properly characterized at the genomic level and shown to contain insertions or deletions. The finding of deletions has been helpful for the development of vaccines using recombinant technologies (see below).

Traditional inactivated vaccines

In general, the production of inactivated or killed vaccines follows similar guidelines, although somewhat more relaxed than for live vaccines. The more relaxed guidelines are best demonstrated for chicken embryo-produced killed virus vaccines. Although the production of live vaccines in embryos or chicken embryo-derived cell cultures requires the use of specific pathogen-free eggs, inactivated vaccines can be made in the so-called 'clean eggs,' which are not necessarily free of all pathogens. Inactivation can be achieved by several methods, such as treatment with alkylating agents (β -propiolactone and aminoethyl ethylene imines), different concentrations of formalin or glutaraldehyde, temperature, pH, and UV or gamma irradiation (Delrue *et al.*, 2012). Key concerns with the preparation of inactivated vaccines are immunogenicity and safety. Depending on the virus, some of the inactivation procedures damage the immunogenic epitopes, resulting in insufficient induction of protective immune responses. Viral safety can be compromised by incomplete inactivation of the vaccine virus or by the presence of extraneous pathogens in the cell culture media or embryos. Sera, trypsin, and embryonated chicken eggs can contain extraneous pathogens, some of which have only recently been described. The use of fetal bovine serum, for example, frequently results in contamination of vaccines with bovine viral diarrhea virus (BVDV). To avoid this problem, chemically defined cell culture media without animal components have been developed but may not be widely used for vaccines in, for example, the poultry industry. Another potential problem is incomplete inactivation of viruses belonging to the Circoviridae and the proposed new group of Anelloviridae. For example, chicken infectious anemia virus (CAV or CIAV) and porcine circovirus (PCV), belonging to the Gyrovirinae and Circovirinae subfamilies, respectively, of the Circoviridae are highly resistant to chemical inactivation (Schat and van Santen, 2013) and could remain present in inactivated vaccines. Application of PCR techniques has

become a major tool to determine the absence of known extraneous agents (Mackay and Kriz, 2010).

The choice of live versus inactivated vaccines

The choice between live and inactivated vaccines is not always simple and depends on many factors, such as the type of immune response that is required for protection. For example, a live vaccine will be preferred if cell-mediated immune (CMI) responses are essential for solid protection (see Section Antigen Processing), although inclusion of some adjuvants can stimulate CMI responses (see below). A live vaccine may also be preferred if mucosal antibody responses (IgA) are needed for protection. Production of IgA antibodies is stronger after natural exposure (see Section Vaccination Procedures) than after parenteral injection, which is typically used with inactivated vaccines. However, if IgG responses are critical for protection, inactivated vaccines will be highly effective, although adjuvants (see below) will be needed in most if not all inactivated vaccines.

In addition to the need to inject inactivated vaccines, which is a distinct disadvantage in the poultry and aquaculture industries, there is a second disadvantage: the need to produce high-titered products because there is no amplification of the vaccine in the vaccinated host. This is not always a problem; high titers can be obtained, especially when vaccines are made in embryonated chicken eggs. However, it makes the inactivated product more expensive than a live vaccine. Inactivated vaccines also have certain advantages over live vaccines. Because attenuation is not a linear process, live attenuated vaccines most likely contain populations that differ in the degree of attenuation, which may cause problems, especially if the animals are immunosuppressed by stress or subclinical immunosuppressive infections. Moreover, there is always the potential that the vaccine reverts back to become pathogenic or recombines with the wild-type viruses generating a recombinant. Live vaccines induce immune responses by replicating in the host, which can cause some degree of tissue damage. These vaccine reactions can be complicated by secondary infections leading to economic losses. Certain live vaccines are also contraindicated during pregnancies. None of these problems occur with properly inactivated vaccines except for some local tissue damage caused by adjuvants.

Inactivated vaccines are sometimes the only choice because virus cannot be attenuated as is the case for FMD. In other cases a new disease appears causing devastating losses, and a vaccine needs to be developed in a very short time span preventing the development of a live attenuated or recombinant vaccine. This was the case when Schmallenberg virus, a novel insect-transmitted Orthobunyavirus, appeared in 2011 in Europe, causing severe fetal malformation and still births in ruminants. The rapid development of inactivated vaccines has significantly reduced the impact of this pathogen (Wernike *et al.*, 2013). Autogenous vaccines are sometimes developed to solve local or regional problems by isolating the pathogen and producing a killed product. This type of vaccine is subject to authorization by the State Veterinarian in the United States.

If long-lasting immunity with high antibody titers is important, for example, for the transfer of IgY from the hen to her offspring through the yolk, a primer with a live vaccine

followed by a killed vaccine is the best option. Boosting the immune response by second vaccination with a live vaccine is in general not recommended because the primary immune response will prevent replication of the booster vaccine.

Biotechnology-based vaccines

Since the advance in recombinant DNA technology several approaches have been used or proposed to produce new vaccines, but in actuality few have been authorized for use in the United States or elsewhere in the world. The following technologies have been used to develop commercial products: deletion mutants, vectored vaccines expressing antigens to different pathogens, subunit vaccines including virus-like particles (VLP), and DNA vaccines. The basic science on which these vaccines are based is briefly discussed in this section. More detailed information can be found in several review papers and the actual use in production animals is discussed in the four sections on vaccination in production animals. Many of the biotechnology-based vaccines can be used in DIVA strategies, and specific tests to differentiate between vaccine responses and pathogen infection are an integral part of the development of these vaccines. Tests include ELISAs as well as PCR-based assays. The former confirms the absence of antibodies against the deleted gene products after vaccination, although these antibodies are present after infection with the field strains of the pathogen. In the case of AIV, recombinant vaccines produce antibodies to the neuraminidase protein that is different from the field virus. PCR-based assays can also be used as in the case with the gE deletion mutant of bovine herpesvirus 1 (BoHV-1). In this test, a gE DNA fragment is amplified and analyzed by restriction enzymes (Schyns *et al.*, 1999). With the advance in new sequencing techniques, single-nucleotide polymorphism analysis could also be used in this example.

The possibility of deleting specific genes without significantly altering their immunogenicity was the basis for the first recombinant vaccine licensed in the United States. Deletion of two genes, Tk and glycoprotein III, in swine herpesvirus 1 (aka pseudorabies virus) led to the development of a marker vaccine that was successfully used to eradicate Aujeszky's disease in the commercial swine population in the United States in 2005 (USDA, 2008; Kit, 1990; CAST, 2008). Since then, several deletion and insertion techniques have been developed – mostly for herpesviruses and poxviruses but also for RNA viruses. The use of bacterial artificial chromosomes has increased the possibilities drastically by allowing the cloning of large DNA sequences, such as herpesvirus and poxvirus genomes and infectious cDNA (complementary DNA) clones of RNA viruses (Tischer and Kaufer, 2012). The use of *en passant* mutagenesis facilitates the deletion or mutagenesis of specific genes as well as the generation of vectored vaccines expressing foreign genes (Tischer *et al.*, 2010). Currently, several vaccines for chickens using fowlpox or HVT as a vector and expressing genes for AIV, infectious laryngotracheitis virus (ILT), Newcastle disease virus (NDV), or infectious bursal disease virus (IBDV) have been licensed in the United States as well as in other parts of the world. There are currently no vectored vaccines for cattle and swine licensed in the United States (Anonymous, 2013c), but the gE deletion mutant of BoHV-1 has been licensed in the European Union for the control and

possible eradication of BoHV-1, which is the cause of infectious bovine rhinotracheitis.

In addition to poxvirus and HVT, NDV vaccine strains Lasota or B1 are also used as a vector for the expression of foreign genes, such as the HA gene of AIV (Park *et al.*, 2006). Recombinant NDV-HA vaccines are currently produced in Mexico. Adenoviruses have also been proposed as a vector for use in poultry and swine vaccines (Toro *et al.*, 2010) but are not yet available commercially.

Bacteria can also be used to generate apathogenic deletion mutants by removing pathogenicity genes. Curtiss and Hassan (Curtiss and Hassan, 1996) deleted genes for adenylate cyclase (*cya*) and cAMP receptor protein (*crp*) in *Salmonella enterica* serovar typhimurium (*Sa. typhimurium*) strain χ 3985. Vaccination of 1-day-old chicks generated significant antibody responses and booster vaccination at 16 or 18 weeks of age prevented egg transmission after challenge with highly invasive strains of *Sa. typhimurium* and *Sa. enterica* serovar enteritidis. The vaccine strain is currently licensed in the United States, Canada, New Zealand, and the Dominican Republic as AviPro Megan Vac 1 and AviPro Megan Egg (Lohmann Animal Health). This strain can also be used as a vector to immunize against other pathogens, especially when strong mucosal immunity is important; for example, to protect against *Eimeria acervulina* (Konjufca *et al.*, 2006). Since then several papers have been published showing protection against different pathogens after immunization with recombinant *Sa. typhimurium*, but it is not currently used commercially as a vector.

Subunit vaccines comprise the second group of biotechnology-derived vaccines and include proteins and VLP. Recombinant proteins and VLP can be produced by transfecting or infecting different cells, including *E. coli*, yeast, insect and mammalian cells. The choice of cells depends on a number of factors, such as posttranslational modifications, yield of protein, and purification methods. The platform of choice is currently the baculovirus system using insect cell lines or *Trichoplusia ni* (cabbage looper) larvae (Mena and Kamen, 2011; Crisci *et al.*, 2012). Recombinant protein vaccines that consist of the envelope glycoprotein E2 of classic swine fever (CSF) virus, a pestivirus, and produced in the baculovirus system have been licensed in Europe for the control of CSF. Vaccines that consist of recombinant proteins need to be injected and require adjuvants to induce strong immune responses. A problem with this type of vaccine is that the protein folding may not produce conformational (tridimensional) epitopes, which are often important for the production of relevant antibodies. This is not a problem if the antibodies are generated against linear epitopes.

VLP are self-forming virus-like structures lacking a virus genome that are generated when virus structural genes are expressed simultaneously (CAST, 2008). This can be achieved by infection of insect cell lines with recombinant baculoviruses expressing the structural genes. Crisci *et al.* (2012) listed several advantages for the use of VLPs: (1) the well-defined geometric structure with a highly repetitive expression of proteins on the surface presents PAMP motifs for triggering the immune response, (2) good cross-presentation to both MHC I and II by uptake by the APC resulting in CMI and antibody responses, (3) presents conformational epitopes, (4) is not infectious, and (5) can present foreign epitopes and can be

used in DIVA strategies. One of the disadvantages of VLP vaccines prepared with the baculovirus system is that baculovirus particles that need to be removed can also be produced (Crisci *et al.*, 2012). Thus far only one VLP vaccine has been licensed for animals: Porcilis[®] PCV (Merck) for protection against PCV.

A slightly different approach has been used by Harrisvaccines (Ames, IA, USA) to generate RNA replicon vaccines for a H3N2 vaccine for swine. This vaccine has been licensed by the USDA (Anonymous, 2013c). The platform technology is based on alphavirus-derived nonstructural genes to which genes of interest can be fused. The construct is transfected into VERO cells, which then produce particles containing the recombinant RNA molecules. Although their website indicates that the method for particle production is proprietary, it is likely that these particles have the characteristics of VLP.

The finding that injection of mice with plasmid DNA encoding proteins resulted in expression of these proteins led to the concept that DNA could be used to immunize animals (reviewed by Dunham, 2002; Fowler and Barnett, 2012; CAST, 2008, and references therein). DNA vaccination is of interest because the DNA fragment codes only for part of the pathogen; thus, there is no risk that the pathogen escapes into the environment. The unmethylated CpG sequences in the DNA interacts with Toll-like receptors (TLR), thus enhancing innate responses. Interestingly, DNA vaccination sometimes induces strong CMI immune responses without the antibody responses, which complicates monitoring if appropriate levels of protection have been achieved. The proposed mechanism for the induction of both types of immune responses is that APC are directly stimulated by transfection of the plasmid into the APC or by proteins expressed in transfected somatic cells. Although DNA vaccines have attracted strong interest for their potential use in humans, the development of vaccines for production animals has been lacking with one exception. A DNA vaccine to protect salmonids against infectious hematopoietic necrosis virus has been licensed in Canada (Alonso and Leong, 2013) (see Section Vaccines for Fish). One of the problems is that injection of plasmids into the muscle or dermis does not always result in adequate responses without the addition of an immunogenic protein and or cytokines or unless multiple injections are given. The use of a gene gun administering gold particles coated with DNA has improved the results probably by delivering the vaccine into the epidermis, which is rich in professional APC (keratinocytes and Langerhans cells) (Fuller *et al.*, 2006).

Adjuvants

Killed vaccines, including recombinant protein vaccines, are frequently used with adjuvants to increase the immunogenicity. Most of the adjuvants used in vaccines for production animals and humans consist of aluminum- or oil-based emulsions (reviewed in Fox and Haensler, 2013; Schijns and Lavelle, 2011; Schijns *et al.*, 2014). Mineral oil or natural oils together with surfactants are used to prepare the oil-based emulsions. Squalene, a naturally occurring substance in plants and animals, including humans, has replaced mineral oil-based formulations in human vaccines (Fox and Haensler, 2013). Final compositions of adjuvants are frequently proprietary information and in animal vaccines are based in part

on price considerations, in addition to immunostimulatory properties.

Although the use of adjuvants has a long history, the actual mechanisms involved are still poorly understood (reviewed in Schijns and Lavelle, 2011; Schijns *et al.*, 2014; Mount *et al.*, 2013). The aluminum- and oil-based adjuvants remain present for some time after vaccination, providing a depot function for antigen release, which has been named signal 1 facilitators. However, the depot also causes tissue damage, thus facilitating an inflammatory response (signal 2 facilitators). The former results in gradual release of the antigen to macrophages and APC, whereas the latter activates the innate immune response pathway by providing ligands to PRR, such as TLR (see Section Relevant Immune Responses). The use of adjuvants in production animals can cause economic problems if the resulting tissue damage decreases the value of the product (see Section Selected Examples of Vaccines in the Four Groups of Production Animals).

Based on the current understanding of the initiation of innate immune responses, research to develop new and improved adjuvants stimulating CMI and antibody responses is being conducted (e.g., Mount *et al.*, 2013). Most of this research is directed toward the development of adjuvants for human vaccines. Incorporation of new adjuvants into vaccines for production animals will only be done if it provides economic value, for example, by less damage to meat products.

Vaccination Procedures

To obtain optimal protection, vaccines must be administered using the correct procedures as outlined by the manufacturers.

Unfortunately, these recommendations are not always followed, especially if vaccines are provided by other methods than injection. In the case of cattle and swine, vaccines are mostly administered by subcutaneous (sc), intradermal, or intramuscular (im) injection. The location of injection may depend on the preference of the veterinarian and the owner of the animals. Some vaccines can be given intranasally, such as the recombinant vaccine against BoHV-1 (Schynts *et al.*, 1999) in cattle or deletion mutant vaccines for Aujeszky's disease in swine, but this may not always be practical. Oral vaccination can be used for some of the bacterial vaccines in swine by adding the vaccine to the drinking water. Aerosol vaccination to protect piglets against *Mycoplasma hyopneumoniae* is possible (Murphy *et al.*, 1993; Feng *et al.*, 2013), but it is not clear whether this is currently used.

Poultry vaccines can be given by *in ovo* injection or in chickens by spray, intraocular or eye drop, in the drinking water, wing-web stab, or by sc or im injection. In this article the author has briefly reviewed these different techniques: detailed information on the advantages and disadvantages has been discussed by Collett (2013). The concept of *in ovo* vaccination was developed by Sharma and Burmester (1982) for the HVT Marek's disease vaccine. The development of machines for *in ovo* vaccination was pioneered by Embrex (now Zoetis) and is widely used to vaccinate broiler embryos. Current machines can vaccinate up to 70 000 eggs h⁻¹ and at the same time remove infertile eggs and eggs with embryos that died within the first 10 days of incubation (Figure 3). The mechanisms involved in early protection are not completely understood but probably involve early activation of innate immune responses and, if the machine is properly calibrated,



Figure 3 An automated *in ovo* vaccination device shown with an egg candling module and a capacity to inoculate up to 70 000 eggs h⁻¹. At approximately 18 days of incubation the flats with eggs are loaded on an egg remover module (right front) where infertile eggs and eggs with dead embryos are removed, thereby reducing chances of contamination. The flats are then moved to the next module for vaccine injection/delivery through a multiple-head, injector assembly that consists of a punch and needle system. After injection, the flats are moved to the transfer table module where eggs are automatically transferred to hatching baskets to be moved into the hatching incubator. Photo of the Embrex® systems (Invoject® vaccination device with Vaccine Saver® feature and egg remover module) courtesy of Zoetis Inc.

approximately 100% of the embryos will receive the correct vaccine dose. This is in contrast with vaccination of newly hatched chicks at the hatchery by sc injection, where chicks are often not at all or improperly vaccinated. One-day-old chicks are vaccinated before leaving the hatchery by sc injection, spray, and ocular or nasal drop. Spray vaccination at the hatchery is done in a cabinet where the boxes with chicks receive the spray. At the farm, spray vaccination is often employed for respiratory vaccines using adaptations of insecticide spray equipment. Droplet size is crucial in both situations with 100–150 μm being optimal. Smaller droplets may cause undesirable vaccine reactions. The labor-intensive eye or nasal drop vaccination is probably not used in many countries due to labor costs, although it is used in countries like India at the farm level instead of spray vaccination. Vaccination by drinking water is effective but requires proper preparation of the equipment and birds. Flushing the system to remove all disinfectants is important and if the only water supply is chlorinated water it will not work at all. Before vaccinating, water must be withheld from the birds so that they drink as soon as water becomes available. Proper preparation of the vaccine solution is done by adding buffers to the drinking water (e.g., skim milk), which needs to be well mixed with the vaccine. Improper application of any of the vaccination techniques can cause disastrous results.

Vaccination of fish has become very important in aquaculture. Originally, it was thought that this could be done by immersion of fish in a tank with vaccine. However, this required large concentrations of vaccine, which made this method prohibitively expensive. Moreover, protective immunity was not uniformly achieved by this method. Currently, vaccination is done by intraperitoneal injection in almost all instances (Rødseth and Moen, personal communication). Fish are placed briefly in an anesthetic solution before being injected manually (Figures 4(a) and (b)). The Norwegian company Skala Maskon has developed a machine that can vaccinate 20 000 salmon h^{-1} and separate the fish into different weight classes while rejecting malformed fish (Figure 5). In some countries oral vaccination is used by encapsulating the vaccine in different components (reviewed by Gomez-Casado *et al.*, 2011).

Selected Examples of Vaccines in the Four Groups of Production Animals

In this section, the author has highlighted some of the specific aspects for each group of animals focusing on diseases of global interest. It is not possible to provide detailed vaccination schemes for production animals for several reasons. First of all, it is almost impossible to obtain reliable information on regional disease incidence and vaccine use. Two examples illustrate the geographic differences in vaccine use. The United States has been free of FMD virus (FMDV) since 1929 (McReynolds and Sanderson, 2014), whereas FMDV is still endemic in large parts of Asia. Similar examples can be given for the poultry industry in regard to H5N1 highly pathogenic (HP)AIV. Several countries in Asia and Egypt routinely vaccinate against the H5N1 AIV, whereas countries in Europe may use stamping out; however, the United States is free of HPAIV. Decisions on how to deal with these two diseases are determined by the national governments or the European Union.

The second reason is that veterinarians working for poultry producers often have to follow vaccination schemes that are implemented by the veterinary staff at the headquarters of large producers. These vaccination schedules can be based on regional differences in disease incidence, density of poultry farms in a given region, etc. This situation is very similar to that of the aquaculture industry. The list of bovine vaccines (Table 3, Figure 6) that are currently used in the United States is provided only to demonstrate the type of vaccines that are available, although these vaccines are not always used. Additional information on vaccines and their regional use can be obtained by accessing websites of the large vaccine manufacturers and government licensing agencies. Different articles in this Encyclopedia deal with descriptions of the common diseases in the four groups of food animals and the interested reader may refer to those articles for additional information.

Vaccines for Cattle

Cattle receive many vaccinations starting after 3 months of age when maternal immunity no longer interferes with vaccination by neutralizing the vaccine virus. Table 3 provides an example



(a)



(b)

Figure 4 (a) Manual vaccination of Atlantic salmon by intraperitoneal injection. (b) Manual mass vaccination of Atlantic salmon performed by a professional team of vaccinators. Photos: MSD Animal Health.



Figure 5 A vaccination system for intraperitoneal injection of salmon. Fish enter in the Maskon anesthetic and buffer tank (not shown) and are transported to the vaccination unit, which has injection sites. Location of the injection site and needle depth is adjusted for every fish based on the length of the fish. The unit is operated by one person and depending on the configuration, up to 20 000 fish can be vaccinated per hour.

Table 3 Vaccines for cattle available in the United States

Viruses	Bacteria	Protozoa
Infectious Bovine Rhinotracheitis (IBR)	Leptospira	Neospora
Bovine Viral Diarrhea (BVD)	Clostridia	Trichomoniasis
Bovine Respiratory Syncytial Virus (BRSV)	Pasteurella (Mannheimia)	
Parainfluenza (PI3)	Brucellosis	
Rotavirus	Vibriosis	
Coronavirus	Enteric <i>E. coli</i>	
Rabies	Salmonella	
Papilloma	Gram-negative core bacterins	
	Footrot	
	Hemophilus	
	Anthrax	
	<i>Staphylococcus aureus</i>	
	Johnes	
	Pink eye	
	Anaplasmosis	
	Foot warts	
	Mycoplasma	

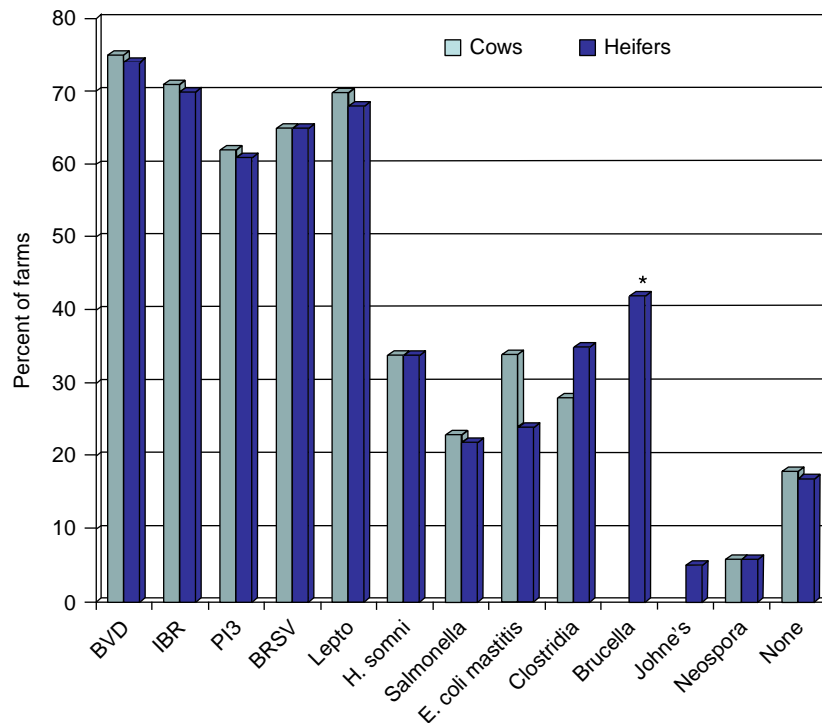
Source: Warnick, L., Department of Population Medicine and Diagnostic Sciences, Cornell University.

of the types of vaccines available in the United States, whereas **Figure 6** indicates the frequency of the use of the different vaccines (Data for 2007, provided by Professor L. Warnick,

Department of Population Medicine and Diagnostic Sciences, Cornell University). It is noteworthy that the use of *Brucella* vaccines is different in the western part compared with the eastern part of the United States. This is most likely the consequence of the presence of *Brucella abortus* in free-living bison (*Bison bison*) and elk (*Cervus canadensis*) (Olsen, 2013) combined with the extensive ranching of cattle in the western half of the United States.

The following diseases are of global interest for which vaccines are available and are briefly discussed: mastitis, a major worldwide economic disease in dairy cattle; brucellosis is a major zoonotic disease, caused by *Brucella abortus* and a potential bioterrorism agent; and FMD.

Mastitis is an infection of the udder frequently caused by intramammary infection with *Staphylococcus aureus* (*St. aureus*) or different coliforms (e.g., *E. coli*, *Klebsiella* spp, and *Enterobacter* spp). The host response and pathogenesis of bacterial intramammary infections have recently been reviewed (Schukken *et al.*, 2011) and the interested reader may refer to this article for additional information. Production losses are caused by clinical mastitis, an obvious disease state, or subclinical mastitis when the somatic cell count (mostly leukocytes and some epithelial cells) is increased above 100 000 ml⁻¹ of milk. Prevention of mastitis is primarily based on sound management practices and vaccination is considered an adjunct to the overall herd health management programs (Erskine, 2012). Vaccines against coliforms consist of Gram-negative ‘core antigen’ bacterins, referred to as GNCABs, using the Rc mutant strain O111:B4 of *E. coli*.



*38% of the heifers in East, 87% West

Figure 6 Graphical representation of the use of different vaccines for cattle in the United States. Note the difference in use of *Brucella abortus* vaccine between the eastern and the western half of the United States. Courtesy of Warnick, L.D., Department of Population Medicine and Diagnostic Sciences, Cornell University.

Vaccination with this vaccine, referred to as J5, provides moderate protection against coliforms. More problematic is the control of *St. aureus* mastitis by vaccination. Pereira et al. (2011) and Erskine (2012) reviewed the literature on *St. aureus* vaccines and found mixed results for the current commercial vaccines. New vaccine formulations using recombinant Target of RNAPIII-Activating Protein (TRAP) (Leitner et al., 2011) and ISCOMATRIX™ (Camussone et al., 2013) show promising results for improved protection.

Brucella abortus, a Gram-negative intracellular bacterium, causes abortion and other reproductive diseases in cattle. Infection with *B. abortus* induces rather poor immunity because it can subvert innate and acquired responses. Vaccination using live attenuated strains (strain19 is most commonly used) together with elimination of positive animals is the preferred strategy. However, the currently available vaccine strains have some serious drawbacks. Vaccination can induce abortions in pregnant animals and does not prevent superinfection and seroconversion. Vaccinations need to be conducted with great care, because the vaccine strains can also cause infection in humans (Olsen, 2013).

Control of FMD by vaccination is complicated for several reasons (Parida, 2009). First of all, vaccine-induced protection lasts for the relatively short period of approximately 6 months; thus, yearly vaccinations are essential in endemic parts of the world. Second, there are at least seven distinct serotypes with considerable antigenic diversity within serotypes. As a consequence, vaccine formulations need to be carefully evaluated for efficacy, which is difficult to achieve. Challenge

experiments for such evaluations require strict isolation facilities to prevent outbreaks. Third, current inactivated FMD vaccines require a cold chain, which is also a complicating factor, especially in tropical regions, impeding efficient immunizations.

In many parts of the world, tick-borne diseases are important in cattle with control often done by pushing the cattle into dip tanks that contain acaricides. The use of a recombinant vaccine based on gut glycoprotein BM86 of the *Rhipicephalus microplus* tick has been successfully used in Australia and Latin American countries (de la Fuente et al., 2007). Unfortunately, the BM86 vaccine has limited activity against non-*Rhipicephalus* ticks, but it is likely that new candidate vaccine antigens will be discovered in the near future (de la Fuente and Merino, 2013).

Vaccines for Swine

The intensive swine industry uses several production systems, but all consist of farrowing, nursery, growing, and finishing units, with the latter two frequently combined into one unit. On some farms, all units are used in a continuous production system, preventing the use of an all-in/all-out system and thorough cleaning of the facilities. Other systems use fully separated facilities and the grower/finishing units may be completely different farms than the farrowing/nursery farms. Piglets are moved to nursery units after weaning at approximately 3 weeks of age, which is also the prime time for vaccinations by injection. In some instances a booster vaccination is given at 6 weeks of age. Sows can be vaccinated between

2 and 5 weeks before farrowing to increase antibodies in the colostrum. Although most vaccines are given by injection, attenuated live strains of bacteria (*Lawsonia intracellularis* causing ileitis and *Salmonella* and *E. coli* causing diarrhea) can be given in the drinking water. Over the past 20 years, two important new diseases have appeared worldwide in swine: PCV serotype 2 (PCV2) causing immunosuppression and wasting pigs, and porcine reproductive and respiratory syndrome (PRRS) caused by Arterivirus. Recombinant vaccines have been developed against PCV2 using VLP (Merck), a recombinant protein produced in a baculovirus system (Boehringer Ingelheim) or a recombinant hybrid inserting the VP2 gene of PCV2 into the genome of the nonpathogenic PCV serotype 1 virus (Zoetis). The latter is the only live vaccine against PCV2. Development of vaccines against PRRS is problematic for several reasons (Cruz *et al.*, 2010). Natural infection or attenuated vaccines induce weak innate immune responses and low levels of VN antibodies and virus-specific CTL. Moreover, PRRSV strains are highly diverse, especially for the immunodominant protein GP5. It is also suggested that CTL responses are more important for protective immunity than VN antibodies. Development of recombinant vaccines inducing strong CTL responses may solve the shortcomings of the current vaccines. Cruz *et al.* (2010) used a vectored vaccine with transmissible gastroenteritis virus as the backbone expressing two PRRSV antigens eliciting strong VN antibody and CTL responses as well as good mucosal immunity. Currently, this type of recombinant vaccine has not been licensed.

Vaccines for Poultry

Regional and national differences in prevalence of specific poultry diseases are well recognized. As a consequence, decisions on the selection of vaccines are frequently made by the veterinary staff of large integrated companies based on the regional needs and national policies. It is, therefore, not possible to provide detailed information on all poultry vaccines used in the world and hence only some general concepts are discussed.

Vaccination schedules depend on the type of bird; the needs for vaccination of grandparent and parent flocks are different than those for commercial layers and broilers. Because maternal antibodies are important for the protection of chicks, (grand) parent flocks are frequently vaccinated with a live vaccine to prime the immune response followed by a killed adjuvanted vaccine before lay to boost the antibody titers. Boosting with a live vaccine is not recommended because the neutralizing antibodies produced by the first vaccination interfere with vaccine virus replication from the booster vaccination.

The use of recombinant vectored vaccines has become a common practice in many parts of the world, mostly using HVT as the vector. Interestingly, HVT-vectored vaccines cannot be used together with conventional HVT, nor can different recombinant vaccines be used at the same time (e.g., HVT-IBD and HVT-NDV) unless the two inserts are present in the same vector. Possible explanations are that the insert negatively influences the replication rate of the vectored vaccine in the bird with the consequence that conventional HVT initiates immune responses before the recombinant HVT, thus curtailing the infection of the latter. The second explanation could be that the vectored vaccine has a higher passage level *in vitro* and

therefore replicates slower in the bird with the same consequences. Similar circumstances may explain the recommendation against using two different recombinant HVT vaccines. The consequence of using recombinant HVT instead of the standard HVT is that protection against MD may be suboptimal and therefore protection to MD depends more on the inclusion of SB-1 and or CVI988 (aka as Rispens). The slower replication of, for example, recombinant HVT expressing VP2 of IBDV may still protect against IBD because maternal antibodies will provide sufficient protection against IBD during the first 14 days after hatching.

Vaccination against AIV provides a challenge, especially since the occurrence of highly pathogenic H5N1 AIV. Some countries do not allow vaccination, whereas others use killed adjuvanted vaccines. AIV, a member of the orthomyxoviridae, has a genome that consists of eight different RNA molecules, one of which codes for the hemagglutinin (H) protein and a second one for the neuraminidase (N) protein. Chickens make antibodies against both proteins, but the antibodies against H are the key for neutralizing the virus. By changing the N gene to a different neuraminidase gene (e.g., N2 in the case of a vaccine against H5N1), it is possible to monitor flocks for field exposure to H5N1. Recombinant vaccines expressing a different N protein than the field virus have successfully been used in Italy during outbreaks of avian influenza (Capua *et al.*, 2003).

Vaccines for Fish

Globally, approximately 600 fish species are farmed (Brudeseth *et al.*, 2013), but only a few species are produced at high densities, such as salmonids. Vaccine development has become an integral part of aquaculture management for these species and several review papers have summarized the availability of vaccines against viral and bacterial diseases (Gomez-Casado *et al.*, 2011; Salgado-Miranda *et al.*, 2013; Brudeseth *et al.*, 2013). One of the problems identified in these papers relates to the fact that almost all vaccines consist of water-in-oil emulsions that need to be injected. The adjuvants in the vaccine can cause adhesions in the peritoneal cavity, which negatively influences the quality of the fish meat. To reduce the impact of adjuvants on the meat quality, multivalent vaccines that contain antigens against different pathogens are frequently used. The type of combinations depends on the geographic locations (Brudeseth *et al.*, 2013). The other major problem is that a number of viral diseases can cause high mortality in swim-up fry. Unfortunately, the very young fish have an immature immune system, are too small to be injected, and maternal immunity is not very effective in fish (see Section Maternal Immunity). Live, attenuated vaccine strains cannot be used mainly because attenuation in one fish species does not translate to attenuation in other fish species. Vaccination by immersion is in general not cost effective, although it is used with some bacterins in channel catfish and salmonids (e.g., *Yersinia ruckeri* bacterin).

As mentioned in the Section 'Vaccination Procedures,' DNA vaccines for IHNV are licensed in Canada. IM inoculation of plasmid DNA that contain the IHNV glycoprotein G gene provides long-term protection against challenge without causing long-term lesions at the site of inoculation (Kurath *et al.*, 2006). Protective immunity is likely achieved by

a combination of VN antibodies, CTL, and NK cells based on studies in rainbow trout (*Oncorhynchus mykiss*) (Utke *et al.*, 2008). Interestingly, vaccination with plasmid DNA for IHNV glycoprotein G induced not only rapid protection against challenge with IHNV but also cross-protection against another rhabdovirus (viral hemorrhagic septicemia virus) infection for the first 2 months postvaccination (Lorenzen *et al.*, 2002). An experimental DNA vaccine expressing VP2 of IPNV encapsulated in alginate microspheres showed good protection against challenge 15 and 30 days after oral vaccination (de las Heras *et al.*, 2010). It is expected that DNA vaccines will become an important part of fish health management if oral vaccination works in general and if safety concerns can be overcome.

Conclusions

Vaccines remain an important part of health management programs in food animal production systems. It is expected that progress will be made over the next 5 years toward safe vaccines using different recombinant technologies. The use of vectored live vaccines in poultry and DNA vaccines in fish has shown that these applications are safe and provide strong protection. Research into the mechanisms of adjuvants will also lead to the development of science-based approaches stimulating innate and acquired immune responses.

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See also: Animal Health: Foot-and-Mouth Disease. Asian Aquaculture. Beef Cattle. Dairy Animals. Detection and Causes of Bovine Mastitis with Emphasis on *Staphylococcus aureus*. Genomics of Food Animals. Marek's Disease and Differential Diagnosis with Other Tumor Viral Diseases of Poultry. Poultry and Avian Diseases. Swine Diseases and Disorders

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