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Insights into veterinary vaccinology: Bygone and Future

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Abstract

Development of modern veterinary vaccines are challenging as against conventional vaccines. In the current era of genomics, intense progress has been made in the field of vaccinology and next generation vaccine approaches. Modern approaches in veterinary vaccinology greatly demands novel strategies for controlling emerging pathogens in livestock. This review corrugates a birds eye view of live attenuated vaccines, inactivated vaccines, DNA vaccines, subunit vaccines and genetically engineered vaccines. Reverse vaccinology approaches paves a new beginning for development of novel vaccines against infectious pathogens affecting the livestock and poultry sector. This conceptual framework for veterinary vaccinology leads to the rational development of next generation vaccines.

Keywords: Attenuated, inactivated, vector, recombinant, DNA, conjugated, DIVA

Introduction

Infectious diseases remains a major challenge in veterinary medicine. In the golden era of veterinary vaccines against infectious disease, the best challenge on some of the pandemic diseases like cattle plague (Charles *et al.* 2018) [6], sheep pox, foot and mouth disease, bovine tuberculosis, (Wood 2011) [33] canine distemper vaccine, canine hepatitis vaccine, (McVey and Shi 2010) [21] Avian Influenza H5N1, Severe Acute Respiratory Disease (SARS), Aflatoxicosis, African swine fever, Anthrax, Australian bat lyssa virus, Avian influenza, Blue tongue, Botulism, Classical swine fever and other emerging zoonotic diseases has been reviewed (Gibbs *et al.* 2012) [12]. Diseases of animals are of major concern due to the economic losses and zoonotic potentials (Van Oirschot, 2000, Graham *et al.* 2008) [26].

Some of the vaccines available for livestock, poultry and pets are canine distemper virus (MLV/recombinant non replicating in canary pox), canine adenovirus (modified live virus), canine parvovirus (modified live virus inactivated), rabies virus for canine and feline (Inactivated recombinant non replicating in canary pox (feline), feline panleukopenia virus (modified live virus and inactivated), feline herpesvirus (modified live virus), feline calicivirus (modified live virus), canine coronavirus (modified live virus and inactivated), canine parainfluenza virus (modified live virus), Bordetella bronchiseptica for canine and feline (Bacterin and inactivated), Leptospirosis bacterins, multiple serotypes (inactivated), Borrelia burgdorferi (Inactivated bacterin and OspA recombinant vaccine) (McVey and Shi 2010; Charleston, and Graham, 2018) [21, 6, 7].

Vaccines ought to have the ability of stimulating proper response without side effects. Modern vaccinology aims at approaches to develop vaccines with increased immunogenicity and without side effects. (Brewer and Schijns, 2009) [5]. In current era majority of licensed veterinary vaccines are in the form of live attenuated, recombinant, killed/inactivated microorganisms, cell membrane compounds, toxoids, vectored, DNA, conventional subunit vaccines. Modern vaccines are developed based on next generation sequencing and reverse vaccinology approaches (McVey & Shi, 2010; Unnikrishnan, Rappuoli, & Serruto, 2012) [21].

Unlike the traditional strategies, modern vaccinology exploits the new approaches on molecular structure and mechanism of immunity of targeted pathogens. Processing and recognition of antigens has been studied in laboratory animal models for the immune response and different ways to control the pathogen infection, mainly by the secretory antibody response pathways. (Townsend and Bodmer 1989) [29]. Antibody response is initiated principally by the recognition of antigens by B and T lymphocytes. Immunoglobulins and T cells receptor are generated by the process of gene rearrangement which directly recognize the antigen and get degraded and presented on the surface of the cells by antigen-presenting cells

such as macrophage, dendritic cells, and major histocompatibility complex. The cell surface molecules such as CD4 and CD8 cells recognize and helps in mediating the B cell response and delayed hypersensitivity by CD4+ and CD8+ T cell for cell mediated cytotoxicity (Davis and Bjorkman 1988). The main implications of modern vaccinology is to induce the antibody response against the targeted infection using short peptide fragments or synthetic peptides.

Live attenuated vaccines

These vaccines are manufactured by passage of bacteria or virus in an unnatural host or animal cell or chicken embryos. Continuous passages are done to attenuate the virulence of the infectious agent. (Meeusen *et al.* 2007) [22]. The process of producing live- attenuated vaccine requires 50 to 60 passages for effective decoding of virulence. These vaccines does not require adjuvants and needs minimal down streaming processing. For example, an infectious agent causing severe entero- pathogenic diarrhea in pigs are Porcine epidemic diarrhea virus (PEDV) strain DR13, a field isolate was attenuated on Vero cells by serially passaging the virus and testing its virulence in piglets and sows (Song *et al.* 2003) [28]. The modified avian influenza virus- low pathogenic avian influenza (LPAI) H9N2 has been used in different countries with moderate efficacy and requires multiple injections to develop protective immunity.

Inactivated vaccines

Compared to live attenuated vaccine, inactivated vaccines are more safe and easy to produce. The infectious agent is completely inactivated and does not replicate in the host. It has no risk of reversion to virulence. But the immunity that inactivated vaccines offer is moderate, particularly the cell-mediated immunity is lower than live attenuated vaccine. It is for this reason that inactivated vaccines are administered with potent adjuvants to boost up immunity by slow release of the agent from the site of vaccination. However booster doses of the vaccine is required for enhanced immunity. (Van Gelder and Makoschey 2012) [30].

Modern vaccinology approaches

Conventional vaccines in veterinary medicine contains live microorganisms like bacteria or virus that has been attenuated or killed. However these conventional methods of producing killed or attenuated microorganisms rely on multiple passages and complete inactivation. On the other hand, modern vaccinology techniques are using the genetic engineering technology to target specific genes or regions of the genome which are responsible for virulence, aiding in the development of non-pathogenic virus to be used as the immunogen. Some of the common approaches followed in the development of novel vaccines are discussed.

Molecularly defined attenuation

Producing vaccines via molecularly defined attenuation results in long lasting immunity. Advances in modern vaccinology results in developing novel vaccines with changes in the molecular structure of virus and its replication pattern, resulting in the production of targeted mutations of gene with altered virulence. For example, the genes encoding the GI glycoprotein and thymidine kinase gene has been disrupted in Aujeszky's disease virus resulting in development of avirulent strain of the pathogen, which

ultimately results in long lasting immunity when compared with the native virus. (Moormann *et al.* 1990).

Genetically engineered vaccines

To develop genetically engineered vaccines we need to understand the gene function to allow specific deletions. For example in Aujeszky's disease in swine, gene deleted vaccines are used against pseudorabies. Here the glycoprotein I and thymidine kinase genes are deleted to improve the safety of vaccines (Maes *et al.*, 2004). Gene deletion enhances the specificity of the vaccine. Gene specific deletions serve as good targets for key metabolic processes resulting in inhibition of spreading infection. For example deleting the HA hemagglutinin protein from H5N1 Avian influenza viruses and combining with NA gene from H2N3 virus by placing it in the H1N1 backbone of the virus, will result in inactivating the H5N3 virus expression in chickens and also against the highly pathogenic H5N1 strain in ducks which can be used as an effective vaccine (Park *et al.* 2006) [25].

The porcine reproductive and respiratory syndrome virus (PRRSV) genome was reverse-genetically engineered into plasmid DNA to form an infectious clone. The genome was altered at the position of N34 and N51 of GP5 (changing from glycosylation to deglycosylation) resulting in epitopes that trigger neutralizing antibody (Ansari *et al.*, 2006) [3]. The resulting vaccine produces high titer of neutralizing antibody against wild type virus as well as in double mutant virus. Recent improvements in the genetically engineered viral vaccines, with effective molecular biological methods by eliminating the virulence factors or by functionally silencing the gene has led such vaccines to maintain the properties of live vaccines, without risk of reversion of virulence of the organisms. For Bovine viral diarrhoea virus (BVDV) deletion and modification of two non-structural proteins resulted in developing an inactivated vaccine with the efficacy and safety of a modified Live virus. Similarly for porcine circovirus, Chimeric circovirus type 1-2 vaccine was developed by inserting immunogenic capsid gene of circovirus type 2 into the backbone of nonpathogenic circovirus type 1, resulting in conferring protection to pigs which were challenged with virulent circovirus type 2. (Park *et al.* 2006) [25]

While using recombinant proteins for vaccination, the best expression system should be used to produce the protein without altering the antibody recognition sites. Examples of such type of vaccines are human hepatitis B vaccine (McAleer *et al.*, 1984), a recombinant protein vaccine for *Boophilus microplus* tick in cattle (Willadsen *et al.* 1989; Rand *et al.* 1989) [31]. Tapeworm *Taenia ovis* for sheep (Johnson *et al.* 1989) recombinant sporozoite surface antigen from *Theileria parva* (Musoke *et al.* 1992), Bluetongue virus capsid protein (Roy 1992) [27]. In baculovirus expression system, the open reading frame 2 (ORF2) protein of porcine circovirus type 2 (PCV2), a major agent responsible for developing post-weaning multi-systemic wasting syndrome in pigs were produced.

Subunit vaccine

Subunit vaccines usually contain a part of the target pathogen which enhances the immune response against the component only. Such subunit vaccine can be achieved by isolating a particular immunogenic protein from the targeted pathogen and presenting it as an antigen on its own to the host system. The immunogenic subunit vaccines may be purified proteins, peptides, or polysaccharide. Subunit vaccine contains short,

specific proteins of a targeted pathogen which are noninfectious as they lack replication in the host system. Subunit vaccines are administered as safe, non-replicating vaccines with weak immunogenicity. To overcome this potent adjuvant are administered with multiple doses to attain the required immunological responses especially the cell mediated immunity. Example for subunit vaccines are PCV2, Newcastle disease virus (NDV), FMDV, Japanese encephalitis (JEV) (Kim *et al.* 2002).

Vector-based vaccines

Vector-based vaccines are typically excellent candidates. Vectors are used to deliver viral protective proteins to the unvaccinated or vaccinated host. These vectors are immunogenic and are able to display multiple antigens. Recombinant vector vaccine or vector based vaccines are classified as naked DNA vaccines and live vector vaccines. As a successful carrier vector, classical live vectors example vaccinia, fowlpox, and canarypox viruses are used to express the immunogenic antigens of other pathogens. Due to the phenomenal ability of pox virus, which accommodate the large amounts of foreign genes can also be used to infect mammalian cells, resulting in large amount of expression of the encoded protein. The canarypox virus vector system has been used as a platform for a range of veterinary vaccines including those against canine distemper virus, equine influenza virus, feline leukemia virus, rabies virus, and West Nile Virus. So the 'DIVA' vaccines which can differentiate vaccinated from naturally infected animals holds the future key for the eradication of infectious diseases.

The earliest vector-based vaccines in veterinary medicine were canary pox-vectored rabies vaccine for cats (Purevax®) and a herpes-based infectious bursal disease virus (vHVT-IBD) vaccine for chickens (Vaxxitek®). Avipox and canary pox developed as vaccine vectors for New castle disease and infectious bursal disease virus (Bournsnell *et al.* 1990, Bayliss *et al.* 1991) [4]. A recombinant BCG expressing a surface antigen from the protozoan parasite leishmania major (Connell *et al.*, 1993, Leroux-Roels, G., 2010). The potential DIVA vaccine for Classical swine fever virus (CSFV) was a gE deleted pseudorabies virus vector that expresses the E2 subunit of Classical Swine Fever.

DNA Vaccines

DNA vaccines have been produced for a variety of diseases with successful immunity elicitation. The DNA vaccines has an efficient immunity to the antigen encoded, offering the potential for the pavement of further advancement in the production of effective vaccines. (Cox *et al.* 1993) [10]. For examples, Infectious bursal disease virus (IBDV) DNA vaccine provides overall protection against IBDV for chickens, IBDV genome containing VP2 protein is responsible for an acute and highly contagious disease in chickens. Vaccination with DNA vaccine showed higher survival rate and low bursal atrophy as compared with the non-immunized groups of chickens after challenge. It was reported that the DNA vaccine showed high level immune responses and conferred overall protection of chickens against IBDV (Kim *et al.* 2004) [16]. Some other examples for DNA vaccine are the Foot and mouth disease virus (FMDV) VP1 gene which carries the neutralizing epitopes responsible for inducing the immune responses for FMD. Production of DNA vaccine via modern vaccinology approaches against FMDV containing the VP1 epitopes elicits both FMDV neutralizing

antibody against FMD and specific T cell proliferation in swine (Wong *et al.* 2002) [32].

Veterinary bacterial vaccines

Food borne pathogens of bacterial origin causes infections among livestock. Some of these pathogens are zoonotic, with increased risk of transmitting the infection to humans through the consumption of meat products. Generally Bacterial vaccines are available as inactivated vaccine, attenuated live vaccine, or antigen component based vaccines. Novel vaccines should be developed to control the zoonotic pathogens which are re-emerging in the animal industry. Live bacterial vaccines elicit good immune response similar to natural infection with humoral and cell mediated immune response. For example vaccine for Porcine proliferative enteropathy (PPE) which is caused by an obligate intracellular bacteria "*Lawsonia intracellularis*," whose immunological activities and virulence are not largely known has been produced in cell culture by serial passages. Killed bacterial vaccines do not revert back to virulence, but the immunity is short lived in comparison to live vaccine, so boosters are required to create a long term immunity. For example to produce vaccine for *Vibrio cholera*, the B subunit was cloned, expressed and purified; the purified protein enhances the antigen specific IgG antibody response with altered antibody specific pattern toward Th1-type response. Subunit vaccine provides a satisfactory immunological response when compared to killed vaccines. Examples for bacterial subunit vaccines are *Pasteurella multocida*, from this organism the outer membrane protein H (OmpH) was expressed in *E. coli* and resultant protein showed strong protection against *P. multocida* infection. The recombinant protein of *Pasteurella multocida*, middle-C-terminal regions of *P. multocida* contains a toxin (PMT) which was expressed in *E. coli* and induced high titers of PMT-specific antibodies with effective protection. (Lee and woo 2010) [18].

Conjugated vaccines

Conjugated vaccines are almost similar to recombinant subunit vaccines, with two different components, produced by chemically linking the polysaccharide with a strong carrier protein derived from bacteria. Conjugated vaccines are generated against pathogens whose polysaccharide capsule protects them from phagocytosis. Polysaccharide, a poorly immunogenic protein enables the immune system to recognize them as antigens. Such types of vaccines are currently in use for *Streptococcus pneumoniae*, *Clostridium tetani*, *Salmonella Typhimurium*. For examples, a conjugated vaccine, composed of Vi capsular polysaccharide of *S. Typhi* conjugated with diphtheria toxoid, cholera toxin B subunit immunized via peritoneal or intranasal route, evoked mucosal and systemic immune responses (An *et al.*, 2012).

Vaccinology in high throughput biology

High throughput techniques such as DNA sequencing, RNA sequencing, microarray, high resolution mass spectrometry proteomics/metabolomics, RNAi screening integrated with ELISA, ELISPOT, FACS and neutralization assays (Nakaya and Pulendran 2015) are used in modern vaccinology for frame working the vaccines. Example the Trivalent influenza vaccine was designed by analyzing and integrating the data from different levels of molecular information and produced live vaccine intranasal spray which resulted in antibody response with induction of antiviral type I interferon genes.

High- Throughput techniques of DNA and RNA (Transcriptomic assays) sequencing helped to identify the specific mechanisms that regulate gene expression associated with differentiation, functionality of different cell lineages including immune cells. Gene set enrichment analyses (GSEA) has been developed to improve immensity and power of transcriptomic data, to analyse genes signalling pathways. Usually the targeted protein analysis assayed by ELISA and Western Blot, analyses only the protein quantification. To overcome this hurdle, different high-throughput methods have been developed is Mass spectrometry based proteomics to define the major histocompatibility complex (MHC) in the context of profiling T cell and also the triggered antigenic determinants of B cell activity.

Reverse vaccinology

Recent technologies such as glycoconjugates in the field of modern vaccinology leads to introduction of novel vaccine adjuvants. However the biggest change came with the sequencing of *Haemophilus influenzae* whole genome, which paved the birth of "Reverse Vaccinology" (Rappuoli. R 2000)^[26]. Sequencing of *Neisseria meningitidis* serogroup B strain and analyzing the whole genome, resulted in the identification of novel candidates for development of a four-component meningococcus B vaccine (4CMenB) (Parikh *et al.*, 2016)^[24]. This approach lead to the identification and characterization of *Ctenocephalides felis* candidate protective antigens for the control of cat flea infestations. Vaccine against cat flea results in reducing the disease risk, with an overall vaccine efficacy of 32–46%. (Contreras *et al.*, 2018)^[8]. For the porcine circo virus, Chimeric circovirus type 1, vaccine has been developed by inserting immunogenic capsid gene of circovirus type 2 into the backbone of nonpathogenic circovirus type 1, resulting in protection of pigs which are challenged with virulent circovirus type 2 by using reverse-genetics tools, a bivalent vaccine expressing the H and N gene of different Avian Influenza viruses was constructed on a single New castle Disease Virus backbone.

Conclusion

Developing novel vaccines by genetic engineering and reverse vaccinology approaches confer protection against an array of infectious pathogens affecting animals. Modern veterinary vaccines have already made enormous impacts regarding animal health, welfare, and production. Continuous intervention strategies for emerging and re-emerging pathogens should be explored to further refine vaccine development for livestock and poultry in the evolving era.

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