

The Future of Veterinary Vaccines

Glenn Browning
Professor in Veterinary Microbiology
The University of Melbourne
APVMA Science Fellow in Veterinary Vaccinology

The vaccines that have been the foundation of preventative veterinary medicines until quite recently have not changed fundamentally for over 100 years. These vaccines have been developed by either chemically inactivating the infectious agent or its toxins (to yield bacterins, inactivated viral vaccines or toxoids) or by selecting naturally occurring mutants of wild type agents by passage in an unusual substrate for growth, a concept first developed by Louis Pasteur to create vaccines for pasteurellosis and rabies. The methods used to assess the safety and efficacy of these types of vaccines have generally stood us in good stead, although it could easily be argued that, while we have generally achieved a high level of safety, the assessment of efficacy has been somewhat variable, in part because some jurisdictions have assumed that the market would assess efficacy, and that therefore the focus of their due diligence should be on ensuring absence of harm.

Newer Approaches to Vaccine Development

Over the past 20 years there have been major changes in the approach to veterinary vaccine development in the scientific community. This approach, possibly somewhat arrogantly referred to as “rational vaccine development”, is possibly better described as conscious development, because we know what we have done, but not all the consequences of it. There is a good argument that these newer vaccines have differing risks from the traditional vaccines, and that hence their assessment should differ. Unfortunately, some jurisdictions have instead required these vaccines to meet the all the requirements of the traditional vaccines, as well as additional requirements that recognise their differing types of risk. It seems appropriate that these newer vaccines should be assessed using criteria appropriate to the risks they pose.

The aims in development of the newer inactivated or subunit vaccines are to identify the major protective antigens, develop improved *in vitro* growth conditions for production of these antigens, and in some cases, develop better modes of delivery of the antigen to generate improved immunity. In the newer attenuated vaccines, the aim is to identify optimal parental strains, identify the best targets for attenuation, to delete genes for serological markers, and possibly to generate “hyperimmunogenic” vaccine strains.

The newer vaccines can be categorised into products of first, second and third generation biotechnology. First generation vaccines include temperature sensitive mutants, which have been generated by exposure to a mutagen and then selected on the basis of a phenotypic difference, and bacterins produced from cultures of organisms grown in nutrient-restricted media, which upregulates expression of critical protective antigens. Temperature sensitive mycoplasma vaccines, which grow in the upper, but not the lower, respiratory tract, have been particularly successful in poultry, while inactivated *Pasteurella* vaccines grown in iron-restricted media, inducing production of key protective outer membrane proteins, have been effective in cattle.

Second generation vaccines have been generated by targeted attenuation. The approach has been to create auxotrophic mutants, which can be grown in culture, but are unable to grow *in vivo*. These mutants infect, but are capable of very limited growth in animals. These vaccines can induce heterotypic immunity, but generate very low antibody responses. Currently all the successful examples are based on *aro* gene deletions. These mutants can infect, but can't synthesise aromatic amino acids. Successful examples include *aroA* deleted

Glenn Browning
Professor in Veterinary Microbiology
The University of Melbourne
APVMA Science Fellow in Veterinary Vaccinology

Salmonella Typhimurium, *Escherichia coli* and *Pasteurella multocida*. Third generation subunit and inactivated vaccines, are based on the identification of key novel antigens, optimisation of their production and purification or increased expression of these antigens during growth *in vitro*. In third generation attenuated vaccines the aim is to titrate the degree of attenuation, and if possible simultaneously increase immunogenicity. Optimally these vaccines would also be antigenically marked by deletion of a gene encoding a serological marker.

Poxvirus and herpesvirus vectored vaccines are currently in use in some countries and have significant advantages, particularly when differentiation of infected and vaccinated animals is desirable. However, we still don't understand the reason for variation in performance of different vectors, and the efficacy of vectored vaccines is likely to be dependent on the absence of prior exposure to the vector. It is likely that bacterial vectors will be used for delivery of some antigens in the future.

The development of many novel bacterial vaccines is based on the fact that many key virulence factors are best expressed by bacteria when they are growing in infected animals. This explains why immunity induced by bacterin vaccines is usually serotype specific, and why immunity after natural infection often provides broader coverage. Bacteria detect the unique *in vivo* environment, and then upregulate expression of virulence genes at specific times during infection. However these genes are often only upregulated for a limited period of time and thus the immune response against these virulence factors, which are frequently less antigenically diverse, may be reduced.

Identification of these *in vivo* expressed virulence factors may thus enable more cross reactive subunit vaccines to be produced. In addition, the regulatory systems that control their expression may be manipulated to upregulate the expression of these protective antigens, thus improving the efficacy of inactivated and attenuated vaccines. Finally, blocking upregulation may lead to attenuation.

The other focus of vaccine development has been to use intrinsic adjuvantation, by expressing immunomodulatory genes in vaccine strains (or deleting immunosuppressive genes), thus improving the immune response generated by a vaccine.

The methods being used in development of such vaccines include *in vivo* expression technology (IVET), signature tagged mutagenesis (STM) and reverse vaccinology. IVET is used to identify genes that are only expressed *in vivo*, STM is used to identify genes that are required for, or improve, survival *in vivo*, and reverse vaccinology uses bioinformatics to identify genes that are likely to encode protective antigens (for example cell surface proteins) and then uses high throughput synthesis and expression techniques to generate large numbers for testing.

The most promising approach to veterinary vaccine development at present is to use high throughput sequencing and bioinformatic analysis to identify likely targets for attenuation, then to use targeted mutagenesis to assess the extent of the effect of these candidates on virulence and growth *in vivo*. The genes that offer the desired level of attenuation and extent of growth can then be used, if possible in combination with mutations that upregulate expression of key protective antigens, resulting in attenuated strains that induce higher levels of protection.

Glenn Browning

Professor in Veterinary Microbiology

The University of Melbourne

APVMA Science Fellow in Veterinary Vaccinology

Current Challenges and Opportunities in Regulation of Veterinary Vaccines

Duration of Immunity

There has been some recent consumer pressure to minimise revaccination in companion animals. However it is very difficult to assess maximal safe revaccination intervals experimentally, given the very considerable variation in natural exposure between different animals. In human medicine these intervals have generally been based on epidemiological data, but data of sufficient quality to enable such determinations is not collected in veterinary medicine. This implies that there will be a need for much better collection of epidemiological data after vaccines are released in future.

Efficacy Testing

In the past efficacy has often been assessed using predetermined, defined measures of disease (such as fever, clinical scores, lesion scores or microbiological assessment) that may have little relevance to the consumer. It would seem appropriate that, in assessing efficacy, we should be focussed on what the consumer wants protection from when they purchase a vaccine for their animal.

An additional challenge for the future is properly assessing efficacy for vaccines aiming to control diseases with a complex aetiology. In the absence of very clear evidence that control of a single component will have a significant effect on the disease complex, it may be that examining control of disease experimentally reproduced by infection with just one of the pathogens may not be a suitable test.

Defining Efficacy for Absent Pathogens

The last year or so has seen some consumer demand for availability of routine vaccination against exotic pathogens. This poses particular challenges, not least around meeting requirements for demonstrating efficacy against endemic strains, but there may also be demand for other vaccines in the absence of evidence of a problem for the vaccine to solve. This suggests we need a basis for establishing the need for a specific vaccine. Should evidence of the presence of the infectious organism, be sufficient, or should we require evidence of disease, or evidence of the significance (social or economic) of the disease?

Safety Testing

Reversion to virulence of live vaccines has generally been based on five or so back passages through small numbers of animals. However such a test will not replicate the situation seen in large herds and flocks, where incomplete vaccination may be common, with much greater potential for vaccine cycling. Risks of selection of reversion mutants may be a particular risk when vaccines are mass administered using less reliable methods of delivery or at lower doses than they may have been tested at.

Recombination

Targeted deletion mutant vaccines could be reasonably regarded as having no risk of deletion, and hence there seems little reason to require that they be subjected to reversion to virulence testing. However, like all live vaccines, they may recombine with field strains. It is difficult to quantify the risk of recombination in the field, so perhaps the best way to approach this is to assess whether the parent strain used in generating any mutant live vaccine (not only those generated by genetic manipulation) is significantly more virulent than circulating field strains.

Glenn Browning

Professor in Veterinary Microbiology

The University of Melbourne

APVMA Science Fellow in Veterinary Vaccinology

High Throughput Sequencing

The new DNA sequencing techniques enable generation of very large volumes of data rapidly and cheaply. Large numbers of viral and bacterial genomes can be compared simultaneously, and in addition, samples can be rapidly examined for the presence of unexpected or unknown genomes. This technology offers a new approach to examination of vaccine seedlots. It is now possible to sequence samples of seedlots and look for variation in the vaccine strain or for the presence of sequences of extraneous agents. A challenge for the future use of this technique will be determining the significance of any variation in the vaccine strain, and in assessing whether an extraneous agent detected by sequencing is viable.

Gene Synthesis

It is already possible to chemically synthesise infectious viral genomes, which can then be used to generate a vaccine strain. Synthesis of bacterial genomes may be close. This may facilitate derivation of master seeds free of adventitious agents.

What Else is Coming?

Identification of novel pathogens has always been driven by development of new detection techniques. Each of the technical revolutions in microbiology of the last century has resulted in the rapid identification of significant numbers of previously unrecognised pathogens. These revolutions have taken us from identifying infectious disease solely on the basis of clinical signs, through the use of light microscopy, bacteriological culture, then culture in eggs and cell culture, electron microscopy, several enhancements of serological techniques, and, over the last 20 years, the use of nucleic acid based techniques. A new burst of discovery is already developing from the current nucleic acid sequencing revolution. Many of these pathogens may not be cultivable and will require unconventional approaches to vaccine development and re-evaluation of methods used to assess safety and efficacy.

Glenn Browning

Professor in Veterinary Microbiology

The University of Melbourne

APVMA Science Fellow in Veterinary Vaccinology