

The use of genetically modified microorganisms in animal health

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Genetically Modified Organisms (GMOs) never fail to grasp the public's attention. They appear in the wider press on a regular basis, often relating to crops and crop protection, and perhaps too often with negative connotations (especially in Europe) – a refusal to import food produced using GM plants, or a protest at GM crop trials in the environment

However, when it comes to human pharmaceuticals there appears to be a greater willingness to accept the benefits of GMOs. Their importance in pharmaceutical research is ever increasing, and there are numerous examples of success stories. Injectable insulin (for diabetics) and filgrastim (granulocyte stimulating factor) are good examples, both produced by GM *Escherichia coli* bacteria. Even the infamous tobacco plant is now being harnessed using GM techniques to produce antiviral drugs to promote human health – a little irony exemplifying the potential to achieve good from bad... or at least from the unexpected.

Alongside their human counterpart, the animal health industry has seen similar significant developments thanks to GM techniques and none more so than with GM microorganisms (GMMs). This article seeks to describe some of the techniques involved in genetic modification of microorganisms and their application within animal health. In a future article, we will look at DNA vaccines, the role of GMOs in other fields such as feed additives and the regulatory process involved in developing a new GM veterinary medicine.

Genetically Modified Microorganisms (GMMs) play a significant role in animal health with a number of products containing, or

manufactured using, GMMs already approved. The veterinary vaccine market in particular has seen dramatic growth using Recombinant Vaccine technology.

Recombinant vaccines

Before looking in greater detail at the novel molecular methods that enable the development of vaccines from GMMs it is worth looking at vaccine development prior to what is considered to be “modern” genetic modification.

Traditionally, attenuation of disease-causing microorganisms was achieved by multiple passaging of the microorganism on a growth medium, in animals, eggs or cell cultures or by chemical or physical mutagenesis. This resulted in “random” mutations, some of which resulted in a sufficiently attenuated strain of the original microorganism to be considered for selection for vaccination.

In contrast modern genetic modification allows genes that are particularly suited to induce attenuation or to reduce undesirable effects to be specified and specifically targeted.

These “made to measure” recombinant vaccines are often made using either “deletion mutation” or “insertion mutation”.

Deletion mutation refers to a gene targeting strategy

where the host microorganism is modified in a way that a piece of its genome is cut out. The deleted DNA may render the organism less virulent, incapable of reproduction outside laboratory controlled conditions, or perhaps inhibit toxin production – anything that might attenuate the original strain sufficiently to allow it to be used as a vaccine.

Examples of deletion mutation strategies used to create veterinary vaccines are numerous.

Deletion of the *aroA* gene in bacteria results in auxotrophy, rendering them unable to biosynthesise folate. No longer able to grow in host tissues they may then only be cultured artificially using supplemented media. Vaccine strains of *Aeromonas*, *Salmonella*, and *Escherichia* have all been produced using this technique for use in a variety of species including fish, cattle, pigs and poultry.

Gene deletion can be equally effective in viruses, resulting in attenuated strains that make suitable candidates for vaccines. Rabies remains an important disease in both humans and animals and continues to kill tens of thousands of people every year. New vaccine strains have been developed where the phosphoprotein (P) gene or the matrix (M) gene have been deleted. Since these genes are required for effective virus replication in the host,

the genetically modified virus is thus rendered replication deficient and, critically, is unable to spread into the Central Nervous System (CNS) of the host animal. Thus far, trials in laboratory animals and non-human primates suggest these genetically modified replication deficient vaccines are highly efficacious.

In poultry, the Infectious laryngotracheitis Virus (ILT) causes important losses in commercial production. ILT is an alphaherpes virus, and work with other herpes viruses has shown that they may be successfully attenuated by inactivation of the encoding gene for thymidine kinase (TK), which is essential for DNA synthesis. Several studies in chickens have now confirmed that deletion of the TK gene in ILT causes virus attenuation without impairing vaccine immunogenicity.

Recombinant vaccines such as these, based on deletion mutants, do not contain foreign genome. They are in a way similar to traditionally attenuated vaccines, but in fact they are much more specific in the attenuation achieved thanks to the use of modern gene targeting techniques.

Insertion mutation, by contrast, is where foreign genome piece(s) are integrated into the genome of the host microorganism. For example, in chimerics a non-pathogenic microorganism is used as

backbone (“vaccine vector”) to insert an immunogenic piece of genome of the pathogenic microorganism.

Once again, examples for insertion mutation strategies used in veterinary vaccines are numerous and once again it is the humble chicken that has seen some of the greatest benefits from these advanced techniques.

Chickens suffer significant losses due to Marek’s disease, a herpes virus of chickens. There is a related disease that occurs in turkeys, Herpes Virus of Turkeys (HVT). In a process that neatly mirrors the work of Edward Jenner, (the founding father of vaccination who recognised that you could protect humans from small pox by giving them the closely related cow pox – the first vaccine), poultry scientists realised that giving the herpes virus of turkeys to chickens also conferred protection against the much more serious disease of Marek’s. As a direct result a whole range of commercial HVT vaccines have been used to successfully protect chickens against Marek’s disease. Given this long experience, accepted safety and proven efficacy of using HVT in chickens, it was only natural that HVT would be considered as a potential vector vaccine to carry immunogenic pieces of the genome of other pathogenic organisms in order to also confer immunity to those diseases.

Infectious Bursal Disease (IBD) of chickens, also known as Gumboro disease, was an obvious early candidate for insertion into the HVT backbone. Although there were commercial Gumboro vaccines already available they were less than ideal. The presence of maternal antibodies in young birds that could destroy the vaccine strains meant that it was necessary to administer them only after the maternal antibodies had declined. However, since the

levels of maternal antibodies in commercial flocks are very variable, it makes it almost impossible to protect all of the birds, even when 2 or more vaccinations are made. An additional complication arose from the fact that the Gumboro vaccine strains that were best able to spread and protect most birds were often those that were least well tolerated by the birds, leading to an economic compromise.

Enter then the first recombinant vaccine against both Marek’s and Gumboro diseases. Scientists inserted a viral protein from the Gumboro virus into the genome of the HVT virus. Known simply as Viral Protein 2 (VP2) this protein was selected as it encodes the major antigenic determinants of the Gumboro virus, including those that are important in virus neutralisation. When chickens are exposed to this genetically modified HVT virus they mount an immune response to the HVT component in the usual way, conferring resistance to Marek’s disease. However, thanks to the modified HVT virus also expressing the VP2 protein from Gumboro, the birds also mount an immune response to this protein. When subsequently challenged by wild type Gumboro virus, the birds recognise the VP2 component and are able to mount an immune response that also confers protection against Gumboro disease.

Building on this work there are now numerous commercially available recombinant vaccines using the same principles. Those using HVT as the vector and inserting the appropriate immunogenic component of the disease organism into the HVT genome include vaccines combining protection against Marek’s and Newcastle disease (ND) and Marek’s and Infectious Laryngotracheitis (ILT). However the most significant of these may be

the commercialisation in the USA in 2012 of an HVT vectored vaccine to protect against Avian Influenza, with obvious implications not just for poultry but also for human welfare.

Sticking with poultry there are also a range of commercial vaccines which use another viral vector, Fowl Pox virus. Commercial products are available offering protection against Fowl Pox and ILT, Fowl Pox and ND and Fowl Pox and Avian Encephalomyelitis (AE). Fowl Pox has also been used as the vector for a vaccine against the mycoplasma *Mycoplasma gallisepticum* in chickens.

Fowl Pox also offers possibilities outside the avian world. It has the ability to enter mammalian cells but is then unable to complete reproduction. Canary Pox virus has similar attributes, rendering them both potentially suitable vectors (backbones) for mammalian vaccine development. Within the veterinary world Canary Pox vectored vaccines have been commercialised for Canine Distemper, Equine West Nile Virus and Feline Leukemia, to name but a few. There is considerable potential for human medicine too with on-going clinical trials of canary pox vectored vaccines against not just other harmful viruses but diseases as diverse as cancer, malaria, tuberculosis and AIDS.

Another example of a viral vector being used to create a vaccine against a non-viral disease can be found with adenoviruses. There are numerous adenoviruses that infect cattle, some causing disease, others apparently quite benign. Selecting the more benign types to act as the recombinant vector, scientists have then introduced genetic material from *Neospora caninum* which causes Neosporosis. This is an intracellular protozoan disease which can parasitize a variety of species including

dogs, cattle and sheep. The inserted genetic material from the protozoa encourages the adenovirus vector to express surface proteins normally associated with protozoan adhesion and invasion of host cells. Since these proteins also have a significant role to play in the host immune response the resulting vaccine then also confers significant immunity to the protozoan disease.

Adenovirus vectors have also been used to produce vaccines against Peste des Petits Ruminants virus (PPRV) in goats and other small ruminants. Similarly in pigs recombinant adenovirus vectors have allowed development of vaccines against Classical Swine Fever (CSF) and Swine Influenza Virus (SIV).

It seems the possibilities to combine non-pathogenic microorganisms with immunogenic parts of pathogenic microorganisms appear to be almost unlimited. It would be quite possible to continue listing examples of GM vaccines either commercialised or in development to fill the space for this article many times over. Genetically modified animals, and microorganisms in particular, are clearly playing an increasingly significant role in animal health.

There are clear benefits to animal welfare and production, and many associated benefits to human health. Concerns remain within the wider public, primarily related to safety and environmental impact. It is important that the industry continues to address these concerns through good science and sensible legislation. Nevertheless, it is to be hoped that increasing familiarity with GMOs in the animal health industry, and an awareness of the many benefits, might bring with it an increasing acceptance of this technology. ●