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Overview of new vaccines and technologies

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Abstract

Molecular technology has given us a greater insight into the aetiology of disease, the functioning of the immune system and the mode of action of veterinary pathogens. The knowledge gained has been used to develop new vaccines with specific, reactive antigens which elicit protective immune mediated responses (humoral and/or cell mediated) in the host. These vaccines should not burden the immune system by initiating responses against non-essential antigens. However, the efficacy of these vaccines is only as good as the delivery technology or route used to present them to the immune system. Some vaccines, traditionally given by the parenteral route, are now given by the natural route; either orally or intranasally. Two major advantages, often interrelated, are the rapid onset of immunity and stimulation of the local, mucosal immunity. These new technologies are now making an impact on current vaccine development. The balance has to be found between what is technologically feasible and what will provide at least as good a protective immunity as current, conventional vaccines. As new and emerging diseases appear globally, new opportunities arise for molecular and conventional technologies to be applied to both the development and delivery of novel vaccines, as well as the improvement of vaccines in current use.

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1. Introduction

Vaccinology has advanced considerably since Edward Jenner first immunized susceptible ‘volunteers’ with vaccinia (cowpox) virus to prevent infection with the smallpox virus (Le Fanu, 1951). Although vaccine preparations bear no resemblance to the crude scab homogenates of 200 years ago, his concept of vaccination is still the basis of vaccine development today, i.e. that immunization with attenuated or killed pathogens will elicit protective

responses against homologous or closely related (heterologous) pathogens. A better understanding of the immune system has been gained over the last 50 years and this deeper knowledge of innate and acquired immunity is being used for the optimal targeting of vaccines.

Viral and other intracellular infections are more likely to elicit a Th 1 cellular immune response and the subsequent actions of released cytokines such as IL2, INF γ and TNF are well documented. However, both viral and bacterial antigens can also stimulate humoral immunity which involves the Th 2 cell response. Antibodies produced by plasma cells derived from B cells recognize free antigens (viral, bacterial, para-

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sitic) in body fluids, and subsequently bind and inactivate them. Viruses with many identical binding sites for antibodies due to their structural symmetry, can form large aggregates when they are bound by immunoglobulins, which are taken up by phagocytes (opsonisation) and destroyed (Levine, 1992). The function of the vaccine is to aid this process by priming the animal before the pathogen invades, creating memory in the B cells and stimulating cell mediated immunity.

During the last 40 years safe and efficacious vaccines against the major pathogens of dogs (canine distemper, canine adenovirus, canine parvovirus and leptospirosis) and cats (feline herpesvirus, feline calcivirus and feline panleucopenia) have been developed and comprise the core antigens for small animal vaccinations worldwide. In some countries where rabies is endemic or a significant threat, vaccination against this disease is also mandatory. However, breakthroughs have occurred in the field on occasion and vaccinated animals succumbed to disease. This paper will briefly discuss the causes for the seeming lack of efficacy of some vaccines and give an overview how vaccine manufacturers are developing new and improved preparations using the current molecular technology available.

2. Current vaccines and evolving diseases

A plethora of vaccines is widely available to the veterinary surgeon, and an informed choice has to be made as to which vaccines and vaccination regimes should be used. Factors such as prevalence of certain types of disease in a particular region or environment may influence the use of vaccines, such as FeLV, where most cats in endemic areas are vaccinated although not, for example, in a single cat household where the cat is housebound. The core vaccines are, in general, the same throughout Europe and the US, so choice has to be made on product claims such as the efficacy of early vaccination, in the presence of maternally derived antibody (MDA), onset of immunity (OOI), duration of immunity (DOI) and sterilising immunity. In some countries guidelines have been issued by informed bodies recommending which vaccines and regimes to use, and what information should be stated on product literature (Gaskell et al., 2002).

Diseases such as canine coronavirus or feline bordetellosis are not considered by some serious enough to justify vaccination, although such vaccines are readily available. Proceedings from one congress stated that a new feline bordetella vaccine was “A cure in search of a disease” (Wolf, 2001). However, researchers investigating catteries, breeding establishments and multi-cat households may disagree with this statement. (Binns et al., 1999) again indicating that it is the environment which influences vaccine choice.

During the last 20 years, feline leukaemia virus (FeLV) has been rigorously studied down to the molecular level in order to discover how it causes disease. Knowledge of the virus has led to the preparation of various types of vaccine, including those containing whole killed adjuvanted virus, subunits of the envelope glycoprotein or live recombinant viruses expressing envelope proteins (Sparks, 1997). The latter vaccines have shown greater efficacy, on the whole, than whole killed virus preparations and indicate the advantage of selecting the correct immunogen so that the immune system is not overwhelmed by directing responses against non-essential antigens.

Feline immunodeficiency virus has also gained a high profile due to its similarities with HIV. In 2002, the first FIV vaccine, prepared from adjuvanted, inactivated viral Clades A and D, was registered and claims to give protection against Clade B also (Kusuhara et al., 2005). This heterologous, broad protection is an interesting finding as different clades occur from continent to continent, and even within the US, from state to state, so that FIV vaccines need not contain all Clade types but rely on synergistic cross protection of a few. This would reduce the antigenic load on the cat's immune system and dispel fears of some that vaccines contain too many unnecessary constituents.

Effective vaccines against canine parvovirus have been available since the 1980s, when only the CPV2 type virus was present (Churchill, 1987). Since then, the virus has evolved in Europe and the US, producing CPV2a and 2b types but CPV2 vaccines can still protect against these new strains due to the antibody response against the VP2 protein (Greenwood et al., 1995.). This is an example of a current vaccine protecting against evolving pathogens, demonstrating that while viruses change at the genetic level, current

vaccines can still retain their efficacy in the field. This is also true for canine distemper, canine adenovirus, feline herpesvirus and feline panleukopenia in that the strains used in the majority of vaccines, since they were first developed, still protect against field strains currently encountered.

Feline calicivirus is known to have many biotypes and some vaccine manufacturers are now including two strains to give broader protection against the current field isolates. Another vaccine which has been upgraded after many years of stability is canine leptospirosis. Vaccination with *L. canicola* and *L. icterohaemorrhagiae* were part of the core canine vaccination programme, but other strains, such as *L. bratislava*, *L. pomona* and *L. grippotyphosa* are more frequently causing disease in dogs and so vaccines have been updated to include some of these more relevant serovars also.

This short overview of current vaccines show that many are still as effective as they were when they were first introduced and significant improvements would have to be triggered by a lack of efficacy against new field isolates. However, as owners wish to socialise their animals from a young age, vaccines need to confer immunity quickly, and in the presence of maternal antibody. This is a major justification for improvement of the current core vaccines and the results of this type of research are seen in the added claims of today's major vaccine manufacturers.

3. New vaccines and new emerging diseases

Due to recent legislative changes, such as the Pet Passport scheme, controlling the movement of animals between countries, diseases such as leishmaniasis and babesiosis are now appearing in countries such as the United Kingdom where they were never previously encountered. The climatic changes occurring worldwide have also contributed to the spread of vector borne diseases such as West Nile fever. The arthropod vectors for this virus are found as far north as the UK and recent serological studies of British wild birds have shown evidence of the virus in more than half the birds tested. Migrating birds and mosquitoes are thought to be responsible for infection in more than 20 species, including crows, magpies, swallows, chickens, turkeys and ducks. (Buckle et al., 2003).

Although this virus does not appear to cause disease in cats and dogs they nevertheless can be infected. A vaccine has already been developed and has been shown experimentally to protect dogs and cats against viraemia (Karaca et al., 2005). This is a case when the zoonotic aspect of a disease must be considered even though the disease is not clinically apparent in the host animal. Horses, on the other hand, are particularly prone to the disease which reached Southern France in 2000 and infected 131 of the famous horses of the Camargue (Murgue et al., 2001).

Prevention of parasitic diseases by vaccination has proven most challenging due to the parasites ever-changing surface antigens and the carbohydrate moieties which make up the major part of their antigens. The immune system does not respond as well to carbohydrates as it does to proteins so the response must be enhanced by adjuvants which target one or both parts of the immune system. Vaccines against leishmaniasis usually consist of whole freeze-dried organisms of *L. infantum* and have varied in their efficacy. A new adjuvanted vaccine is now being trialled, prepared using only antigen proteins excreted by *L. infantum*. Initial results are promising, demonstrating immunity over a 2-year period in 100% of the vaccinated dogs. It is believed that this is due to Th 1 activation which induced infected cells to produce nitric oxide, killing the parasite and clearing the infection from the cells (Lemesre et al., 2005).

A new vaccine against another parasitic disease, babesiosis, caused by the intracellular parasite *Babesia canis*, claiming to reduce clinical manifestations of the disease, has been developed containing solubilized parasite antigen (SPA) and adjuvant (Schettlers, 2005).

Diseases such as the ones described above are increasingly being found in areas of the world previously disease-free, causing concern not only for the health of endemic animals but also for the zoonotic risk to the human population. The requirement for the availability of efficacious vaccines is therefore paramount. Not only are pathogens spreading across geographical borders, but more alarmingly, from species to species.

Avian influenza is headline news due to reports of humans becoming infected and dying following infection with the H5NI strain, especially on the Asian continent. Wildfowl are carriers of this virus,

spreading it over considerable distances. Kuiken et al. (2004) experimentally infected cats with this virus and found that the cats excreted virus, developed severe diffuse alveolar damage, and transmitted virus to sentinel cats. Signs in animals vary but virulent strains may cause death within a few days after an incubation period of 3–5 days. (Kuiken et al., 2004). This situation will have to be closely monitored as society is sensitive to the spread of disease from small animals ever since the source of the SARS outbreak was associated with civets (Tu et al., 2004).

Equine influenza virus was also considered to be species specific but for the first time it is believed to have crossed the species barrier and be responsible for the deaths of eight greyhounds in Florida. Although this was restricted to dogs in kennels in Jacksonville, Florida, this is still a very important and significant epidemiological event. Genetic sequence analysis has shown the strain of virus isolated from the dogs to be very similar to the strain infecting horses in Wisconsin the previous year. Specific antibodies to the virus were found in dogs implying that the virus replicated sufficiently to stimulate an immune response (Carey, 2004).

This brief account of some of the emerging diseases of veterinary importance highlights the need to be aware of the ever changing nature of pathogens, in particular, the viruses which do not only evolve in their host species to overcome vaccination but by the very nature of antigenic drift, may opportunistically find new hosts in new species and present new challenges for both veterinary and medical research.

4. New technology

The use of molecular technology has advanced veterinary vaccine design significantly in the past 20 years leading to the development of vector, sub-unit and marker vaccines. The application of molecular technology for the prevention of disease was highlighted in the extensive wild life vaccination programme of 1989–1995 against sylvatic fox rabies in which the use of a vaccinia virus recombinant expressing rabies glycoprotein (VRG vaccine) resulted in many European countries now being rabies free (Pastoret and Brochier, 1999). Since then much research has been invested into producing safe and

efficacious vaccines using this technology in a more economical and efficient way. Safety of vaccines is enhanced as reversion to virulence is no longer a risk and excretion of vaccinal virus post vaccination is eliminated. Vaccines containing sub-unit fractions of pathogens, rather than the whole organism, should also be less immune-stressful to the host as the immune system is only directed against specific antigens and not a spectrum of non-essential cell structures.

Genes responsible for immunogenic epitopes of pathogens have been identified and incorporated into bacteria (e.g. *E. coli*), viruses (e.g. baculovirus) and tissue culture cells (e.g. Chinese hamster ovary) where they are then expressed as proteins in growth medium and harvested, sometimes with the production cells if the proteins are surface bound antigens. They may be concentrated and purified before being adjuvanted to enhance the immune response. A typical example of the few sub-unit vaccines which are available today are the feline leukaemia vaccines containing the P45 FeLV envelope protein (Jarrett and Ganiere, 1996).

Recombinant vaccines are produced by inserting genes coding for essential immunogens of pathogens into a vector, usually viral, which then are incorporated into the viral genetic code. When the vector is inoculated into the animal, it infects target cells and upon replication, expresses the foreign gene proteins as well as its own. The infected cell then releases these proteins which are detected by the immune system and a humoral and/or cell mediated immune response is initiated. In this way, protective neutralising antibodies are raised against the epitope(s) of the pathogen without the presence of the whole organism. Choice of an appropriate vector is often made by using ones that are not found naturally in the target species. For example, although canine adenoviruses and feline herpesviruses have been shown to be efficient vectors, inoculation into the respective host species may not be viable due to circulating antibodies present through vaccination or disease. Some of the most successful viral vectors for small animals have been found in the poxviruses—Modified Vaccinia Ankara (Drexler et al., 2004), canarypox (Paoletti, 1996) and myxoma (McCabe et al., 2002) as cats and dogs are not naturally infected by these viruses.

DNA vaccines could be described as the purest of vaccines as the animal is not injected with the antigen

but with the DNA encoding the antigen. These vaccines can be injected into the muscle or intradermally by needle injection for optimal uptake. The DNA, encoding epitopes or complete antigen, is integrated into a plasmid, along with a promoter which is selected for optimal transcription, yielding high expression levels of the encoded protein. Although these vaccines elicit both cell mediated and humoral immune responses in the target species, the immune responses induced by some DNA vaccines may be lower than those elicited by conventional vaccines (Oyaski and Hildegunds, 2000). Experimental DNA vaccines have been shown to protect against a variety of diseases but two concerns are the production of sufficient quantities of DNA to make vaccination economical, and transfection efficiency. Immune responses can be enhanced significantly by the use of gene guns and electroporation which increase transfection but these technologies have not yet advanced to the stage of routine use, especially in companion animals which require user-friendly procedures. Gene guns have been available for the past few years but a practical disadvantage is the noise they produce when the gun is triggered and the pressurised gas in the chamber expels the DNA-coated gold particles into the epidermis. This sudden shock could well make the animal nervous on subsequent visits to the veterinary surgery. A second disadvantage is in deciding where to inoculate an animal whose body is covered with fur. A significant amount of work is now being undertaken to improve the efficacy of DNA delivery.

However, the first two DNA vaccines for veterinary use have recently been granted US approval (Animal Pharm, 2005), West Nile virus vaccine, for horses and infectious haematopoietic necrosis vaccine for farm reared Atlantic salmon. This is a landmark for DNA veterinary vaccine technology which within the next few years may also benefit small animal vaccination.

The ability to manipulate DNA has resulted in the development of 'marker' vaccines which allow the differentiation between infected and vaccinated animals in the field, hence the name DIVA vaccines. The DNA of a vaccine virus can be manipulated to delete a specific gene so the animal does not produce antibodies to the coded protein which is present in the pathogen. Differential diagnostic tests (e.g. ELISA) are then developed to detect that particular protein absent in the vaccine. Serum from vaccinated animals

would test negative while that from infected animals would test positive. The first vaccine to be used in this way was an Aujeszky disease vaccine for pigs in which both the glycoprotein (gE) and thymidine kinase (TK) genes were deleted in the vaccinal virus (Pasick, 2004). Presently, vaccine manufacturers are not required by EU directives to differentiate their vaccines from field pathogens before submitting them for registration, but as this may become a reality, sequence analysis, deleted genes and markers are fast becoming routine technology within vaccine R&D laboratories. The use of reverse genetics also enables the manipulation of RNA viruses.

DNA and recombinant vaccines are not only used as prophylactics against biological disease, but research is ongoing as to their use as therapeutic vaccines, especially against malignancies such as canine melanoma (Bergman et al., 2003). Contraceptive vaccines (Naz, 2005) and anti-allergy vaccines (Thomas et al., 2005) are also being investigated, especially in the medical field and is an area of interest to the veterinary profession also.

5. Vaccine delivery

Regardless of the technological advances in vaccine development, the vaccine itself is only as good as its route of delivery and its ability to overcome natural barriers such as maternally derived or active circulating antibodies in the host animal. Convention for many years meant that vaccines were delivered by the subcutaneous (s/c), or in some cases, intramuscular (i/m) route. For diseases such as parvovirus and distemper, systemic antibody response correlates with protection. Vaccines given by s/c or i/m routes have been shown to produce and maintain significant protective antibody levels for many years which has resulted in major vaccine companies recommending at least bi-annual and tri-annual boosts with certain vaccine components, such as parvovirus, distemper and adenovirus, instead of the annual booster prescribed since vaccination regimes were instigated. However, circulating maternal antibodies in young animals may inhibit the take of certain vaccines when given at a young age. In some instances, as with canine distemper and parvovirus, this can be overcome to a certain degree by increasing the titre of the vaccinal virus (Chalmers and Baxendale,

1994), otherwise a second or even third vaccination has to be given when the MDA levels have decreased sufficiently to ensure vaccine take.

DNA and recombinant vector vaccination is one solution to overcome MDA in young animals. Another simpler solution is to give the vaccine by the natural route by which infection takes place and becomes established. This is particularly relevant for the respiratory pathogens whose main tropisms are the cells of the upper respiratory tract and oral mucous membranes. Kennel Cough (KC) vaccines are a prime example. The organisms associated with the KC syndrome, namely *Bordetella bronchiseptica*, canine parainfluenza and canine adenovirus, included in mono-, bi-, or tri-valent vaccines, are usually given prior to kennelling when a rapid onset of immunity (OOI) is required, usually within 48–72 h post inoculation. Those which offer a duration of immunity (DOI) of 12 months can then be used to vaccinate dogs in the intermediate years between immunization with the core vaccines (CDV, CPV, CAV2) with a 3-year DOI. There are, however, mixed reactions in the veterinary profession as to the practicalities of intranasal/oral vaccination, especially in some of the larger breeds of dogs indicating that a compromise has to be reached between what is practical and what is more efficient from a scientific approach.

Controlled release of vaccines will be an important development of the future giving the potential for single shot vaccination and sustained DOI. As polymer technology improves, it should be possible to coat the vaccinal microorganisms with stable polymeric microspheres capable of controlled release. This has been successful for tetanus toxoid allowing slow release for periods ranging from days to over months (Jaganathan et al., 2005).

The modern veterinary surgeon has many choices today as to which vaccines he should use, including different constructs, combinations, routes of delivery and delivery systems. The days of the needle and syringe may be numbered as new technology supercedes the tried and tested ways. Informed choices have to be made which necessitate the practitioner being kept up to date with advances in veterinary vaccinology. However, the prime objective of any immunisation programme, regardless of the technology, is to protect the animal as quickly as possible and to maintain that immunity for as long as possible,

against prevalent disease. Some vaccine companies have addressed these issues and have taken the ethical view on 'over vaccination' by reducing or eliminating non essential antigenic components and achieving DOIs of at least 2 years for the major components. Veterinary vaccines include a wide range of viral and bacterial antigens but it is now time to for parasitic vaccine development to catch up. These vaccines are the most problematic due to their composition, but because of the increasing incidence of exotic parasitic diseases appearing in new regions, it is imperative that these are part of the future vaccine armoury giving a broad protection against new and emerging diseases.

The ideal vaccine is the one shot, slow release, rapid OOI, long DOI, given in the presence of MDA, cheap to produce and creating sterilising immunity. For some vaccines, this may still be achievable as we obtain a deeper understanding of the immune system and the technology to manipulate it.

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