Regulations and procedures in parasite vaccine development

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SUMMARY

Although immunisation protocols for a wide variety of parasitic diseases have been developed, it is often questioned why these do not always reach the market. In this review information about the regulations and procedures that apply to licensing the production and marketing of medicinal preparations, especially parasite vaccines, is presented. These general regulations specify issues on product (quality, safety, efficacy and potency) and production (facilities and consistency). Vaccine developers and manufacturers have to comply with these regulations, which may involve years of research and development. Moreover, where the manufacturer claims specific features of the product, these claims have to be corroborated by (experimental) data. A series of principles has been used to develop vaccines against parasite infections varying from the use of (attenuated) live vaccines to killed vaccines and subunit vaccines. The implications of some specific regulatory issues associated with these approaches are discussed.

Key words: Vaccine, development, manufacture, requirements, guidelines, parasite.

INTRODUCTION

When evaluating the literature about parasite vaccine development one very often encounters arguments that should explain why few vaccines have actually reached the market (Dalton and Mulcahy, 2001; Vercruysse *et al*. 2004). There are a few comments to make here. First, it is not true that vaccines against parasites are an exception. A whole series of parasite vaccines has been developed and used to control parasitic diseases in animals (Cornelissen and Schetters, 1996; Vercruysse *et al*. 2004). These vaccines vary from non-attenuated live vaccines to recombinantly produced subunit vaccines against parasites from very different taxonomic groups; multicellular organisms like helminths and unicellular protozoan parasites. Second, although some of the arguments that explain market failure are correct in their own right, there are many more factors that affect commercial success (Schetters, 1995). In this review regulatory aspects of the commercial development and marketing of these products are discussed.

REGULATIONS

To date, for veterinary medicinal products to be marketed a marketing authorisation (MA) must be obtained from regulatory authorities. Before the 1980s there was generally no MA required in most countries, and if there were, these were usually very basic and easily fulfilled. Clearly, the implementation of legislation on authorisation of veterinary medicinal products has influenced the pace at which new products are introduced to the market.

European Union

On a European level the first step in the process was the adoption of EU Directive 81/851/EEC, in which common requirements for manufacturing and marketing authorisation were laid down, based on the evaluation of the quality, safety and efficacy of the product (see Brunko, 1997 for a detailed review). Later on, the requirement was added that the manufacture should be done according to the principles of Good Manufacturing Practice (GMP), which are described in a detailed guide. Similar principles must be followed in safety studies (Good Laboratory Practice; GLP) and field studies (Good Clinical Practice).

Until 1987 authorisation was obtained on a strictly national evaluation. Due to newly emerged techniques of vaccine development and manufacture, e.g. recombinant DNA technology, a new directive was issued which stated that decisions on marketing authorisations of a high technology product (or which presented significant therapeutic interest) could not be taken unless considered at the community level (Directive 87/22/EEC; effective from 1987). A committee was formed to facilitate the adoption of common scientific opinions on a European level; this Committee for Veterinary Medicinal Products (CVMP) consists of nominees of the Member States. National marketing

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Parasitology (2006), **133**, S189–S195. © 2006 Cambridge University Press
doi:10.1017/S0031182006001879 Printed in the United Kingdom

https://doi.org/10.1017/S0031182006001879 Published online by Cambridge University Press

authorisations were based in this European assessment. In the 1990s the possibility of mutual recognition (MR) of national authorisations was introduced. According to this MR procedure (MRP) the applicant applies first for a marketing authorisation in one Member State (Reference Member State). After a MA is granted on a national level, this MA is recognised by other Member States (Concerned Member States). Until 1993 this legislation excluded specific requirements for immunological veterinary medicinal products not falling under Directive 87/22/EEC such as classical vaccines. Already licensed products had to be reviewed by the national competent authorities and additional data had to be provided by the pharmaceutical companies to comply with the latest EU standard (92/18/EEC). Further harmonisation of these procedures was realised in 1995 by implementing the CVMP into a new central procedure for new/innovative products. For this central procedure applications are sent to the European Medicines Agency, which are evaluated by the CVMP. The EU Commission then issues one single MA that is valid in the entire Community.

In November 2005, the decentralised procedure (DCP) was added to the existing procedures. The DCP is similar to the MRP with the difference that it is not foreseen to wait until the RMS has issued a national MA but all Member States involved issue the MA at the same time.

In summary, one now differentiates in the EU the central, decentral and mutual recognition procedure. Additionally, for products of local interest a national procedure, in principle limited to a single Member State, is still maintained.

United States of America

In the United States the situation is different and there is no mutual recognition of marketing and manufacturing authorisation between the USA and the European Union. This means that for the USA this authorisation must be acquired separately. The basic regulation is the Virus-Serum-Toxin (VST) Act of 1913. This Act aimed at the prevention, importation and interstate shipment of 'worthless, contaminated, dangerous or harmful veterinary biological products' (see Espeseth, 1997 for a detailed review). The Act has been amended on several occasions but the need to ensure purity, safety, potency and efficacy of veterinary biological products has remained the same. In order to produce and market a veterinary biological product in the USA two kinds of licences are required, as described in 9 CFR (Code of Federal Regulations) Part 102: (1). A United States Veterinary Biologics Establishment License for each production facility; and (2). A United States Veterinary Biological Product License for each product produced in a licensed establishment.

Within the United States Department of Agriculture (USDA) it is the Animal and Plant Health Inspection Service (APHIS) that is the authority for administering the VST Act. Within APHIS it is the Center for Veterinary Biologics (CVB) that is responsible for: licensing and policy development, inspections and compliance, test methods and references for use in quality control tests, and investigation of alleged violations and ensuring compliance with the Act. Especially in the USA the use of conditional licenses is found useful for instance when dealing with emergency situations (e.g. disease outbreaks).

Other countries

In most other countries of the world the authority to license vaccine producers and products is with national ministries of agriculture or health. Most of these have their own legislation with emphasis on the quality, safety and efficacy aspects of products. In a number of countries national pharmacopoeias are being used.

Dynamics of legislation

Regulations in the EU and USA are continuously amended and updated, which may affect the development of new vaccines, and sometimes even marketing of licensed product. For example, after the recognition of prions as causative agents of transmissible spongiform encephalomyelitis (TSE) all ingredients used for the production of vaccines had to be reviewed and those products of which it could not be documented that they were free of prions had to be withdrawn from the market.

Currently, the first ever monograph on a parasite vaccine is being written. The monograph intends to describe the particulars of a live coccidiosis vaccine for chickens and is written by the Group of Experts 15V of the European Pharmacopoeia Commission (Pharmeuropa 17.3; June 2005). It is expected that this monograph will become effective within approximately two years.

With increasing globalisation there is also more harmonisation in legislation. For example, VICH is an international cooperation between EU, USA and Japanese authorities with vaccine manufacturers with the aim of harmonisation of technical requirements for registration of veterinary medicinal products. VICH develops guidelines that provide a unified standard for government regulatory bodies to facilitate mutual acceptance by relevant authorities.

REQUIREMENTS

Basic requirements

Although regulations may differ, in general, all authorities aim at licensing only those products that

https://doi.org/10.1017/S0031182006001879 Published online by Cambridge University Press

meet a number of basic requirements. Independent of the Regulatory Agency with oversight, veterinary vaccines must meet the following criteria.

Quality. A veterinary medicinal product must be sterile i.e. free from any contamination with live microbiological agents. In the specific case of live vaccines the product should not contain other live microbiological agents than the vaccine strain(s) (purity). In order to achieve this, it is demanded that the master seed stock from which all subsequent vaccine batches will derive is absolutely free from extraneous agents (bacteria, *Mycoplasma* species and viruses). This may pose specific problems as validated assays must be used i.e. spiking of the seed with a series of extraneous agents should reveal positive test results. In cases where seeds are stored frozen with e.g. cryoprotectants, these may interfere with the extraneous agents testing and solutions must be sought. The quality of the product must be guaranteed. To that order, a Quality Assurance system is implemented at all levels of production as part of GMP. The quality of starting materials used for production, whether bought from a commercial supplier or produced in-house, must be evidenced. In the former situation the commercial supplier has his own quality assurance system. In other cases, additional incoming-goods controls are performed by the vaccine manufacturer (e.g. growth promotion assays for serum batches used to cultivate parasites) to further warrant the quality of the material.

A special situation is the production of vaccines from faeces of live animals (e.g. coccidiosis vaccines). The quality assurance system comprises the microbiological status of these animals, and preferably Specific Pathogen Free (SPF) animals must be used. Additionally a validated final product test on sterility or purity is required.

Regular internal and external audits aim at surveying the quality control procedures. Moreover, a vaccine producer must employ Qualified Persons who is, without prejudice to his relationship with the holder of the manufacturing authorisation, responsible for release of vaccine for marketing.

Safety. Clearly the product must be safe to the target animal, but it must also be documented that the product does not pose a danger to other animals or man that may come into contact with the product, or to the environment. Experimental data obtained with batches with the highest potency or titre (see below) must be generated in GLP experiments (Europe) or GLP-like experiments (USA).

In addition, the safety of an overdose or repeated doses of the vaccine must be shown. A special requirement is that live vaccine strains must be stable, i.e. should not revert to virulence during consecutive passages. In general, a vaccine is produced within a limited number of passages from the master seed stock. Usually this is limited to five passages. The safety of the parasite at the lowest and highest passage number is shown in animal studies using the most sensitive target animal/species.

Efficacy. Data must be provided that supports the efficacy claims. In other words: a product must be able to do what is claimed, for instance, limit parasite proliferation or the development of clinical signs. Preferably, these data are obtained from laboratory studies and field experiments performed under GCP conditions (Europe) or GLP/GCP-like (USA), but if that is not feasible, experimental (challenge-) models may be used. Data must be provided with batches with the lowest potency (see below).

Potency. The manufacturer must provide data that guarantees the efficacy of a product over the entire shelf-life. This is a major hurdle in vaccine development. The discovery of the protective effect of a specific immunogen (parasite strain or partially purified parasite fraction) is usually done or further established by vaccination-challenge experiments. Such experiments are preferably not used as potency tests as these involve animal experimentation and take a long time (in case of some live vaccines this is a serious problem). An alternative test must be developed; a potency test carried out at the time point of batch release, which has predictive value as to the efficacy of that particular batch at the end of shelf-life. Hence, the dynamics of the signal of the potency test must be studied over the period of the shelf-life and correlate with the level of efficacy (real time stability data). If the protective mechanism of immunity against a specific pathogen is not known, it may take years before an accurate potency test is developed.

Consistency of production. As mentioned above, and perhaps more clearly from the legislation in the USA, a licence must be obtained for each production facility where the vaccine (or part thereof) is being produced, in addition to a licence to sell the product at a certain market. Data must be presented to show that at least three consecutively produced batches of product meet the quality requirements (safety, purity and potency) specified for that product.

SPECIFIC CLAIMS

Aside from the general requirements described above, more specific claims are made that add to the market value of the product. This is where the commercial company tries to make the difference. However, any claim made by the manufacturer must be supported by experimental data. Together these specifications form the product profile

https://doi.org/10.1017/S0031182006001879 Published online by Cambridge University Press

(in Europe also known as Summary of Product Characteristics).

Product profile

The product profile is a description of the composition of the product, the target animal (species, minimal age etc.), the specific safety and efficacy claims, the route and method of administration, shelf-life, and physical presentation. A multitude of approaches has been and is being used to discover parasite strains or fractions thereof that induce protection in the host (see below; Cornelissen and Schetters, 1996). One should realise that this is only the first step in vaccine development; the critical component must induce a significant level of protection. The next step is to formulate specific safety and efficacy claims that add to the product profile.

Target animal

Clearly, it must be specified for which animal species the product is intended. In addition, the category must be stated, e.g. minimal age of the target animal, whether the product can be used safely in pregnant animals, or is intended for specific use e.g. a vaccine for broiler chickens as opposed to breeder chickens or layer flocks. An important factor may be the presence of maternal immunity in young animals. Should this possibly affect the induction and onset of vaccine-induced immunity then this must be studied. Results may prompt the manufacturer to recommend not vaccinating animals below a certain age.

Route of administration

Some products can be administered through different routes e.g. oral application or injection. For each of these routes of administration safety and efficacy experiments must be performed to support the claims made. These claims may differ depending on the route of administration, and this must be clearly stated in the dossier and on the leaflet.

Onset and duration of immunity

Apart from the requirements imposed by regulatory authorities, most of these factors are market driven. Any of the claims must be documented, either supported by existing literature or by experimental data. This may take years of research, and is evident for instance if one claims a shelf-life of three years. But also specific efficacy claims, such as a duration of immunity of one year followed by a yearly vaccination to sustain this level of immunity, must be supported by data that show that one year after the primary vaccination animals are significantly protected (in some cases by experimental challenge infection) and also that animals that receive a single booster vaccination one year after initial vaccination are still protected one year later. This involves more than two years of experimentation.

Compatibility

Should one claim that the vaccination may be carried out within two weeks of vaccination with another product (concurrent use) then this must also be documented with supportive data. This can become a complicated task depending on the target animal. As an example, the life-span of the average broiler chicken is 6–7 weeks and these animals very often need to be vaccinated against a series of pathogens (amongst others, Marek Disease virus, Newcastle Disease virus, infectious bronchitis virus, Gumboro Disease virus) very early in life i.e. before the age of 14 days. Compatibility of the new product with all of these must be shown if this use is claimed. This comprises safety studies as well as efficacy studies. Evidently, if it is claimed that a vaccine can be physically mixed with another product and subsequently administered, data of safety and efficacy studies must be presented to support such simultaneous use.

Recommendations

Specific recommendations have to be provided that aid in the most efficient use of the product. Such recommendations vary with the particular product. Obvious recommendations are to vaccinate only healthy animals in case of vaccines for prophylactic use. (Although not registered yet, it is envisioned that therapeutic vaccines may also come to the market e.g. for the treatment of leishmaniosis). In the case of live vaccines against pathogens that can be controlled by chemotherapeutics, it may be important to recommend a withdrawal period after chemotherapeutic treatment before administering the live vaccine. Likewise, it may be advised to minimise the risk of concurrent infections during the vaccination period, as these could interfere with the induction of the proper immune response.

IMPLICATIONS FOR PARASITE VACCINES

A series of principles has been used to develop vaccines against parasite infections varying from the use of (attenuated) live vaccines to killed vaccines and subunit vaccines. Some specific regulatory issues associated with these approaches are discussed below.

Live vaccines

Specific issues regarding live vaccines are the shelf-life, stability of the biological characteristics of the

https://doi.org/10.1017/S0031182006001879 Published online by Cambridge University Press

vaccine strains (reversion to virulence; see safety, above), and the risk associated with spreading to other susceptible animals. In this respect one can distinguish two groups of live vaccines: those that induce self-limiting infections and those that result in chronic infections.

Self-limiting infections. Vaccines based on the use of parasite strains that cause self-limiting infections usually do not present a risk to the environment since the life cycle of the parasite is not perpetuated. Requirements are restricted to providing evidence that the biological characteristics of the vaccine strains do not change upon consecutive passage. This is referred to as 'reversion to virulence' even in cases that a wild-type strain is used for vaccination. One way to deal with this problem is by comparing the safety of the vaccine when containing parasites at the lowest passage level and at the highest passage level. Presently, no rules apply to the parasite vaccines but in general the guideline for viral vaccines is often followed, which means that no vaccine will be produced from parasites that are passaged more than five times from the master seed stock.

Complete life cycles. Examples of the simplest form of such a vaccine are the live vaccines against coccidiosis in chickens (Williams, 2002). As this type of infection is transient (the parasite 'passes' through the chicken) the infection is self-limiting and no chemotherapeutic treatment is necessary to cure the infection. Similarly, strains with reduced virulence can be selected from *Eimeria* isolates. Some commercially available coccidiosis vaccines for broilers contain strains that are selected after repeated passage through chickens. These so-called precocious strains require less time to develop into oocysts, and the number of progeny is reduced as compared to the wild type parent population (Williams, 1994). The infective stage, the sporulated oocyst, is relatively stable at 4–8 °C, which results in a shelf-life of 9–12 months for most of these vaccines. In addition, a live vaccine is available that allows the simultaneous use of a therapeutic dose of certain ionophores to control *Clostridium perfringens* infection in broilers, a bacterial disease that may cause damage to broiler chickens especially if raised without prophylactic medication in the feed (Vermeulen, Schaap and Schetters, 2001).

The virulence of parasite strains derived from a single isolate can be variable. For example, using *Babesia bovis* isolates passage through splenectomised animals can select for strains of reduced virulence. Such parasite strains are being used to vaccinate cattle in Africa and Australia. The infection develops less virulently and the animals develop immunity against subsequent challenge infection (De Waal and Combrink, 2006). These vaccines are presently distributed by governmental institutions, mainly because the shelf-life of the vaccine is short (in Australia the vaccine needs to be administered within a week from production [de Vos; personal communication]).

Attenuation can also be brought about by repeated passage *in vitro*, which is the basic technique used to select for the vaccine strains of *Theileria annulata* that are used in e.g. India and Israel (reviewed by Shkap and Pipano, 2000).

Incomplete life cycles. Many parasite species have complicated life cycles characterized by distinct life cycle stages, sometimes involving more than one host. In cases where the early life cycle stages are sufficiently immunogenic to induce protective immunity, selection for parasite strains with truncated life cycles is another strategy to develop vaccines. A major advantage is that spreading of the vaccine strain in the environment does not occur. Examples are the *Toxoplasma gondii* S48 strain that is used in a vaccine against abortion in sheep due to primary *T. gondii* infection during pregnancy. This strain has lost the capacity to develop from the tachyzoite into the bradyzoite stage, and does not form tissue cysts. The tachyzoites induce a transient infection in the host, while triggering protective immune reactions (Buxton, 1993). Stability of this biological characteristic must be shown (see above 3.1.2) as for all live vaccines.

Irradiation of parasites has also been used as a mechanism to truncate the life cycle. The live vaccine against lungworm infection in cattle contains L3 larvae of *Dictyocaulus viviparus* that do not develop further than the L4 stage. Vaccinated cattle are immune to challenge with L3 larvae (Urquhart, 1985). As irradiation is regarded as a form of inactivation, safety aspects are of major concern. It must be shown that vaccination with larvae inactivated by irradiation is safe to the target animal, and procedures that ensure that the treatment of each batch of vaccine is adequate have to be implemented.

Chronic infections. In case the parasite has a tendency to survive in the host for longer periods of time, chemotherapeutic cure of the infection is required. An example of this approach is the live vaccine against *Theileria parva* infection (Marcotty *et al*. 2001). This vaccine is based on isolates of virulent *T. parva* strains which are used to infect cattle that are simultaneously treated with a long-acting tetracycline preparation to control the infection. This method is still being used in Africa, and the vaccine is produced by International Center for Ticks and Tick-borne Diseases (CTTBD) in Malawi.

The specific safety issues that apply to this method are difficult to deal with. Pertinent questions are the spread of these parasites in the environment and the effects of the use of long-acting tetracyclines.

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It can be argued that, as a result of infection, immunity develops that will ultimately lead to a reduction of the parasite numbers in the environment. In these cases it is important to reach consensus with regulatory authorities before actual development and submission of a registration dossier of the product.

Killed vaccines

Such preparations by themselves usually do not induce protective immunity and an appropriate adjuvant and formulation must be developed. In these cases special attention must be given to the safety of the adjuvant used (Sutkowski and Gruber, 2006). Many experimentally use adjuvants, of which Freunds Complete Adjuvant is the best known, are not acceptable for commercial purposes, mainly because of safety issues. This may become a delicate issue as side-effects are less when the amount of adjuvant is decreased, yet, enough adjuvant must be added to acquire the intended level of protection. Aluminum salts and saponins are commonly used adjuvants, apart from a number of other (water/oil and oil/water) preparations of which the composition is proprietary (for a review see: Schijns and Tangeras, 2005).

Whole organisms. If no live vaccine strains are available, or the use of live vaccines is undesirable, one may want to inactivate the parasites prior to the formulation of a vaccine. Examples of such vaccines are the vaccine against abortion in cattle due to *Neospora caninum* infection (Schetters, 2004) and a vaccine against giardiosis in dogs (Olson, Ceri and Morck, 2000). Evidently the major issue with these vaccines is the efficiency of inactivation, and experimental evidence must be provided that supports the efficacy of inactivation.

Subunit vaccines. A more detailed analysis of the immune response acquired after natural infection, or vaccine-induced immunity, can lead to the discovery of critical antigenic components of an organism that can be used in a vaccine. An adjuvant is required for the induction of protective immunity. The vaccine against babesiosis of dogs due to *Babesia canis* infection is one such example. It contains soluble antigens secreted/excreted from the parasite and saponin is used as adjuvant (Schetters, 2005). A vaccine against leishmaniosis in dogs, based on partially purified fucose-mannose ligand (FML), has been developed commercially and is used as a prophylactic vaccine (Noguiera *et al*. 2005). A special product is a vaccine against coccidiosis in broilers that is based on the protective effect of vaccination of the breeder hens with gametocyte antigens from *Eimeria maxima* (Wallach *et al*. 1995). The protective effect resides in the maternal immunity induced in the chicks derived from vaccinated hens.

In some cases the antigens are produced using recombinant DNA technology. The best example is the vaccine against *Taenia ovis* in sheep, which is based on recombinant parasite antigens that induce antibodies that block the attachment of oncospheres to the gut epithelium (Harrison *et al*. 1999). Saponin adjuvant was shown to be most efficacious. Another example is the vaccine against the cattle tick *Boophilus microplus* (Willadsen, 2004). The vaccine contains recombinantly-produced gut wall antigens of the tick. Upon vaccination of cattle high levels of antibodies to the gut wall of ticks are produced. During feeding of the tick on the vaccinated animal these antibodies are ingested and destroy the gut epithelium of the tick thus killing the parasite.

FUTURE DEVELOPMENTS

It is anticipated that more harmonization of legislation will be realized in future. This should facilitate the production and marketing of commercial products. Specific requirements will be formulated for parasite vaccines in pharmacopoeias, of which the monograph of the (live) coccidiosis vaccines will be the first. With the introduction of molecular biological techniques in vaccine development and production, new regulations will be formulated. Still, a number of conventional techniques will continued to be used in the developed of new products.

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