COCCIDIOSIS VACCINES: PAST, PRESENT AND FUTURE.

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Introduction

Coccidiosis, an intestinal disease of intensively reared livestock, is caused by parasites of the genus *Eimeria*. Control of coccidiosis in poultry is absolutely essential because rearing large numbers of birds in contact with their faeces in moist, warm conditions favours the transmission, replication and rapid build-up of parasites in the litter. Without adequate control, outbreaks of severe coccidiosis are inevitable and devastating. Economically, the most important *Eimeria* species are the seven that infect chickens: *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria praecox*, *Eimeria necatrix* and *Eimeria tenella*. Three species, *E. acervulina*, *E. maxima* and *E. tenella* are most frequently diagnosed in coccidiosis of intensively reared poultry so their control is usually top priority, especially in broilers.

For the past ~60 years coccidiosis has been controlled, for the most part, by in-feed prophylactic medication with a range of chemical and antibiotic (ionophorous) anti-coccidial drugs. Live vaccines, based on the established principle that chickens infected with small numbers of *Eimeria* parasites quickly develop protective immune responses against subsequent challenge with the same species of *Eimeria*, were introduced in the 1950's. However, vaccination remained a very minor method of control until the late 1980's when safer, live-attenuated vaccines were introduced and rapidly taken up throughout Europe and other parts of the world by the egg-laying sector of the industry. Coccidiosis control in the intensive broiler sector remained almost entirely dependent on drugs, due in part to the relatively high cost and limited availability of vaccines and in part to the cautiousness of producers in switching intensive operations over to non-drug based coccidiosis control. However, over the past 15 years anti-coccidial drugs have faced enormous pressures, especially within Europe where legislations on drug re-registration and on the administration of in-feed substances to poultry have removed several drugs from use. In addition, the inexorable rise of parasite drug resistance has rendered several compounds useless and compromised the efficiency of even the most potent ionophores. With no new drugs on the horizon and the withdrawal of many large pharmaceutical companies from anti-coccidial research and development, the role of vaccines has recently increased dramatically and a number of new vaccine products are coming to the marketplace, many of which are designed specifically for broilers.

In the beginning – live virulent vaccines

The development of live coccidiosis vaccines was based on the understanding that to protect against disease there is no need to totally prevent infection, but only to limit the numbers of parasites to which naïve chicks are exposed. Vaccines based on the administration of small numbers of sporulated oocysts of fully virulent parasites were developed ~50 years ago and products such as Coccivax, introduced in 1952 (Schering-Plough Animal Health) and Immucox, introduced in 1984 (Vetech Laboratories, Canada) are still widely available in formulations designed for both layers and broilers. All of these contain drug sensitive parasites. Crucial to the success of these vaccines is effective, uniform delivery, because uneven uptake of vaccine within a flock leads to outbreaks of disease when vaccinal oocysts are replicated and subsequently ingested by birds that were not immunised in the initial vaccination. Without careful administration, virulent vaccines can cause disease and therapy with anticoccidial drugs may be required for a period after vaccination. For this reason, despite their relatively low cost, live virulent vaccines were not widely taken up by the intensive poultry industry for many years. However, by the late 1980's when problems with drug resistance were becoming rife, interest in vaccination as a viable alternative to anti-coccidial drugs was renewed. This was partly fuelled by the introduction of the safer, live-attenuated vaccines but also helped by the development of new delivery methods that achieved better vaccine uptake across flocks, thus making virulent vaccines a lower risk for causing disease.

The second generation – live attenuated vaccines

The risk of disease associated with live vaccination can be eliminated by the use of attenuated, rather than fully virulent, organisms. The first live-attenated vaccine, Paracox 8 (Schering Plough Animal Health), which contains attenuated parasites of all seven avian *Eimeria* species was introduced in 1989 and was rapidly adopted by the egg-laying and broiler-breeder sectors within Europe. Livacox Q (Biopharm), which contains four species, was introduced in 1992 and has also been very successful in many countries throughout the world, although it is not registered for use in Europe. More recently different formulations of these two products, containing fewer species of parasites, have been developed for use in the intensive broiler market and although figures of their usage are not generally available, they appear to be making a positive impact.

The success of these first two live-attenuated vaccines has stimulated several small companies to develop and manufacture similar vaccines, most of which are aimed at geographically defined local markets. Examples include Eimeriavax (Australia BioProperties Ltd, Australia) and Gel-Cox (Inmuner Laboratories, Argentina).

Most live-attenuated vaccines contain populations of *Eimeria* that were obtained by repeated passage through birds with selection for the first oocysts to emerge during infection (precocious parasites), a phenomenon first described by Tom Jeffers in 1975. As rounds of selection proceed, the pre-patent time is reduced and the parasites that evolve have shorter endogenous life-cycles than their wild-type parent, usually lacking one or two of the late asexual stages. This drastically reduces the numbers of parasites produced during infection, which in turn causes a marked attenuation of virulence without any significant loss of immunogenicity. To develop precocious parasites as useful vaccines, a balance must be achieved between the degree of attenuation and the reproductive potential of the parasite. It is crucial that the attenuation phenotype is genetically stabilised, usually by propagating the precursor of the vaccine seed stocks from a single sporocyst or oocyst that has the desired precocious phenotype. The approach has been very successful and precocious lines are the major source of laboratory-attenuated organisms incorporated into live-attenuated coccidiosis vaccines, although Livacox vaccines include an attenuated egg-adapted line of *E*. *tenella*.

Like the virulent vaccines Coccivac and Immucox, the attenuated vaccines Paracox and Livacox are composed of drug sensitive parasites. This offers some advantages since the introduction of drug sensitive vaccines into poultry houses has been shown to restore sensitivity to drug treatment in subsequent non-vaccinated grow-outs, thus allowing the possibility for combination of drug and vaccine programmes. The mechanism by which restoration of drug sensitivity is achieved has not been defined but could be due to simple competition between wild-type and vaccinal parasites or may be indicative of genetic recombination between populations, resulting in the substitution of mutated drug resistance genes with drug sensitive alleles.

Whilst attenuated parasites can be selected by passage in the laboratory, naturally occurring strains display a range of virulence and some less virulent strains may be included within vaccines. A recently registered vaccine, Nobilis®COX ATM (Intervet) contains drug-resistant, naturally attenuated field strains and the vaccine is formulated to allow concurrent use of ionophores to achieve control of *Clostridium perfringens*, a gram+ve anaerobe that can cause necrotic enteritis.

Formulation and delivery of live vaccines

The composition of individual live vaccines varies, with products intended for broilers generally containing fewer species of *Eimeria* than those used in layers or breeders that live for much longer and so may need protection against more species. All vaccines contain *E. acervulina*, *E. maxima* and *E. tenella* as it is essential to protect all birds against these three species. Some vaccines for layers contain all seven species of *Eimeria* but others contain the most important three species plus some other problematic species, such as *E. mitis* or *E. necatrix*. For broilers, some vaccines contain only the three main species whereas others incorporate a fourth species, usually *E. mitis*. Formulations take into account factors such as the cost of parasite production (especially for the more expensive live-attenuated vaccines) and the epidemiology of coccidiosis within the target country or husbandry system. For example, antigenic diversity within *E. maxima* is well documented throughout the world and many live vaccines now incorporate two strains of this species in order to guarantee full protection against field challenge.

There are many different delivery systems available for live coccidiosis vaccines, whether virulent or attenuated. Early recommendations were for vaccinal oocysts to be administered within the poultry houses either by spraying onto the food or suspended in the drinking water. More recently, and especially for broilers, there has been a major shift towards vaccinating birds at one day of age within the hatchery and alongside vaccination for other common infections such as Marek's disease, Infectious Bronchitis and Gumboro.. This not only ensures that birds develop immunity to coccidiosis during the first week of life, but also allows for high throughput spray delivery systems that directly apply vaccine onto chicks from where it is rapidly ingested by preening or pecking. When carried out by trained workers, this type of application has a high level of reliability and efficacy. Most recently a new product, Inovocox (Embrex, USA), is going through registration, which is a live vaccine consisting of *E. acervulina*, *E. maxima* and *E. tenella* that is administered by injection into the amniotic cavity of embryonated eggs at day 18 of development using the Inovoject technology developed by Embrex. This approach ensures 100% uptake of the vaccine and early results of trials indicate that good immunity is induced in the hatchlings using *in ovo* vaccination and moreover that the vaccination is compatible with co-administration of other vaccines such as Gumboro.

A novel approach – maternal immunisation to protect the offspring against disease

Another very recent commercial development has been the introduction of the first killed vaccine for coccidiosis, CoxAbic (Abic, Israel). This vaccine consists of a preparation of crude parasite antigens that are extracted from chickens previously infected with *E. maxima*. The main components of the antigen preparation are derived from the macrogametes and when the antigen is inoculated into laying hens it induces a powerful antibody response to several parasite proteins. The antibodies produced by the hen are transferred into eggs, via the yolk, during lay and provided that the antibody titre remains high it is claimed that offspring chicks are passively protected against challenge infection in the field. Interestingly with this approach, which is dependent in the first instance on the passively received antibodies rather than on acquired protection, there appears to be some cross-protection between species such that the chicks are protected not only against exposure to *E. maxima* but also against the other avian species. This vaccine is only now beginning to be used in the field, so it will be interesting to see how it fares over the next few years.

Prospects for recombinant vaccines

Only small numbers of live parasites need to be given to chickens to induce effective protective responses suggesting that *Eimeria* parasites express antigens that are highly immunoprotective. However, not only is it very difficult to pinpoint the antigens that are responsible for inducing protective immunity, it is also clear that the optimal methods for delivering protective antigens so that they stimulate appropriate, and protective immune responses remain to be determined. There are currently a number of very powerful approaches being brought to bear on the parasite that should help to unravel some of these difficulties. These include the derivation of the complete genome sequence of *E. tenella* and the identification and characterisation of all the parasite's genes; the mapping of proteins to immunologically important targets such as the parasite surface and the secretory organelles; the development of transfection techniques that will allow direct genetic manipulation of parasites including the expression of antigens from several parasites within a single species and finally the use of classical parasite genetics to map regions of the genome that are linked to loci encoding important immunoprotective antigens. So, although recombinant vaccines are unlikely to be just around the corner, the longer term prospects of developing such products remain good.

Summary and perspective

It is clear that vaccination now plays an important role in the control of coccidiosis. Debates on the relative merits of vaccines and/or drugs will no doubt continue especially considering the perceived need within Europe for 'greener' chickens and the need also to attend to practical matters such as how to deal with the gram +ve anaerobes that are kept under control by the ionophorous antibiotics but left untouched by the anti-coccidial vaccines. The ease with which live vaccines, both virulent and attenuated, can be developed suggests that this market is likely to continue to expand, especially with the growth of smaller companies that are producing products on a small, local scale. However, even with much expansion it seems unlikely that sufficient stocks of high quality vaccines could be produced to supply the total potential market. For the longer term, a clear objective will be to develop simpler vaccines that require fewer or no chickens for their manufacture and to achieve such a goal it is clear that there needs to be continued investment into the underpinning research that is likely to lead to the development of such products for the future.