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VIRAL VETERINARY VACCINES

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ABSTRACT

The value of animal models for assessing the quality of veterinary viral vaccines is not to be despised, particularly since one has access to target animal models which are often more relevant than those in the laboratory, especially for challenge/protection studies. Immune protection involves complex immunological phenomena and processes. It is particularly true whenever cellular immunity plays a crucial role because it is still easier to measure antibody responses than cellular ones in vitro. Nevertheless the trend is to replace animal models by in vitro system whenever possible. The problem of the replacement of in vivo by in vitro models is further impeded in Europe by the necessity to comply with Pharmacopoeia monographs where the use of laboratory and/or target animals is often requested.

Recent advances have been made with several inactivated viral vaccines such as equine influenza, where strain variability poses a special problem, or rabies, for which the use of inactivated instead of attenuated vaccines for vaccimation of animals became compulsory in many countries.

Introduction

Animals are frequently used in vaccine research, especially in veterinary medicine, for the development of new vaccines and for the quality control of vaccine batches. Safety testing, efficacy evaluation and potency testing are essential elements of vaccine development and control. The value of animals for vaccine research is not to be despised [1].

Investigating complex immunological phenomena and processes, such as antigen processing, cellular interactions and the subsequent cell-mediated and/or humoral immune responses, is rarely possible without making use of an intact living organism. In the case of veterinary vaccines, there is the possibility of doing this research in the target species and therefore obtaining data which are directly relevant. In vitro methods cannot, at the present time, entirely replace the use of animals. Nevertheless the use of alternative methods can be of great importance, not only from the point of view of animal welfare [2] but also for gaining insight into the mechanisms of action of immunobiologicals [3]. This paper focuses on potential improvements in the area of viral veterinary vaccines with an emphasis on the use of target animal models for safety and efficacy studies with practical examples, keeping in mind that for potency testing of attenuated live viruses, titration of infectivity will generally be sufficient.

Marked vaccines used for epidemiological purposes

In the field of veterinary viral vaccines, most of the previous vaccines were mainly used to prevent clinical signs of the disease without peculiar attention given to their epidemiological impact on virus excretion and circulation after field infection. One of the best example is Infectious Bovine Rhinotracheitis (IBR) caused by Bovine herpesvirus 1 [4]. Previous IBR vaccines were mainly developed to prevent clinical consequences of wild virus infection; therefore in most cases the challenge models in cattle for efficacy studies used highly virulent strains to score efficacy according to the lesions and the clinical signs in vaccinated versus control animals. IBR belongs to list B of the Office International des Epizooties and this infection can therefore impede international trade if it is implemented in some national elimination programmes. In veterinary medicine, health authorities may either choose to vaccinate against a disease as a method of prevention or decide to eliminate the infection using slaughter programmes either on a large scale or on a case-by-case basis. In Western Europe most of the countries have chosen or are forced to implement a programme of IBR elimination.

IBR virus, like other herpesviruses, remains latent after infection. Unfortunately, wild virus can establish latency in animals vaccinated either with an inactivated or an attenuated vaccine. Conversely, an animal remains a latent carrier of a wild virus if it is vaccinated after field infection. So far, all the attenuated strains of IBR virus remain latent after vaccination, even those with deleted gE which can be reactivated later [5]. Therefore, in areas where cattle are vaccinated with

either an inactivated or an attenuated conventional vaccine, one cannot distinguish between animals either vaccinated or infected, whilst in areas where vaccination is not authorized, all the animals seropositive against IBR virus must be considered as infected. If an elimination programme is implemented in a vaccination area, all seropositive animals, either simply vaccinated or infected, must be eliminated from the herds. Unfortunately, due to vaccination programmes against clinical disease, some Western European countries show a high prevalence of seropositive animals (60-70%).

This has led to the development and use of marker vaccines. A marker vaccine can be a strain deleted in a glycoprotein (namely glycoprotein gE) or a subunit vaccine containing the major protective immunogen (gD subunit vaccine) as the one developed by Babiuk's group.

The principle is to have at least one protein deletion to use as a marker. This protein must show several characteristics :

-be non essential (to be able to produce the vaccine) ;

-not be a major immunogen (to keep vaccine efficacy) ;

-give a long living serological response when present (to be a marker) ;

-be present in all the wild strains so far studied;

-induce a serological response in already vaccinated animals.

In this case, whenever an animal is seropositive against the deleted protein, even after vaccination, it is infected and must be eliminated.

In these instances, during vaccine development, one must look at the prevention of infection after vaccination, that is the reduction of primary virus multiplication after challenge instead of clinical sign scoring. Therefore use may be made of less virulent strains for the challenge. If multiplication is reduced, it also shows that the vaccine is efficacious, without too detrimental an effect on the control animals.

Currently the European Pharmacopoeia monograph (freeze-dried IBR live vaccine; Vaccinum rhinotracheitis infectivae bovinae vivum cryodessicatum) requires for the potency test the use of susceptible calves, two to three months old and free from antibodies which neutralize IBR virus and to administer to five calves by the route stated on the label a volume of the reconstituted vaccine containing a quantity of virus equivalent to the minimum virus titre stated on the label or the leaflet. Two calves are kept as controls. After 21 days, a quantity of IBR virus sufficient to produce typical signs of disease such as fever, ocular and nasal discharge and ulceration of the nasal mucosa in a susceptible calf is administered intranasally to the seven calves. The animals are observed for 21 days. The vaccinated calves should show no more than mild signs; the controls should show typical clinical signs. In not fewer than four of the five vaccinated animals, the maximum virus titre found in the nasal mucus should be at least 100 times lower than the average of the maximum titres found in the control calves, the average number of days of excretion being at least three days fewer in vaccinated than in control calves. Following our proposal only this last section is currently useful and should be maintained leading to a «Refinement» of the methodology

in current use. In veterinary medicine, marker vaccines are already available for Aujeszky's disease virus infection (pseudorabies) in pigs, IBR in cattle; marker vaccines against Classical Swine Fever are under development.

Equine Influenza inectivated Vaccines

Horses are peculiar animals in the sense that they represent the only domestic species allowed to behave like man that is, it is free to circulate from one country to another, even from one continent to another, without necessarily being quarantined, provided they are vaccinated against equine influenza at the latest 15 days before travel. Equine influenza belongs to list B of the Office International des Epizooties.

Equine influenza has remained among the main acute contagious respiratory diseases of horses worldwide. Equine influenza occurs as two subtypes: Influenza A/ equine 2 virus (H_3N_8), which is the most important cause of respiratory disease in the horse, and Influenza A/equine 1 virus (H_7N_7), which is still circulating sub clinically but is considered as almost extinct. However, a divergence in the evolution of A/equine 2 (H_3N_8) viruses has occurred since 1987 and two families of viruses are now circulating. These were designated European-like and American-like, although representatives of both families had been isolated in both continents [6], There is increasing evidence from field studies that antigenic drift in the gene coding for the haemagglutinin (HA), which is the major surface protein of influenza A strains, eventually renders vaccine strains obsolete and is likely to compromise vaccine efficacy [7, 8]. The more the vaccine strain is related to field viruses, the more the vaccine can protect against field virus excretion and circulation. A formal reporting mechanism on antigenic/genetic drift or shift of equine influenza viruses and a vaccine strain selection system has been set up, so that vaccine manufacturers and regulatory authorities are informed of the potential need to update vaccine virus strains. An expert surveillance panel, including representatives from three WHO reference laboratories and from three OIE reference laboratories reviews every year the epidemiological and virological information and makes recommendations about suitable vaccine strains. These recommendations are published annually by the OIE in its bulletin (OIE, 1996). As antigenic drift in equine influenza occurs at a slower rate than in human influenza, it is considered that a regular update of the strains could be necessary only every three to five years. What is even more important is the fact that the development of effective vaccines can now be facilitated by the availability of reliable in vitro assays such as:

-Single Radial Diffusion (SRD) to measure vaccine bulk antigen content in terms of HA content ;

-Single Radial Haemolysis (SRH) to measure serological responses.

For in-process controls, SRD provides a reliable method of measuring the HA content of equine influenza bulk antigens, although it cannot be used on final adjuvanted products [9], while SRH is a sensitive and reproducible method for measuring antibody to HA.

The European Pharmacopoeia monograph on Inactivated Equine Influenza Vaccine (Vaccinum influenzae equi inactivatum) still requires the use of five susceptible seronegative horses or 10 seronegative guinea pigs.

This could be replaced by the use of in vitro testing. Moreover, the EMEA has taken the initiative to shorten the procedure of strain replacement in equine influenza vaccines when required according to epidemiological circumstances in case of an antigenic shift. This will allow not only the acceleration of the procedure but also reduction of the number of animals necessary for vaccine development and control.

Rabies Vaccines for Wildlife

Rabies may be the most dreadful challenge infection to be used in animals. Both in the United States and within the European Union, vaccination against rabies has recently concentrated on the use of wildlife vaccination to eliminate rabies [10]. There is a European monograph devoted to rabies vaccine (live, oral) for foxes (Vaccinum rabiei perorale vivum ad vulpem), which requires a large number of animals. The following modifications of the potency test could be recommended:

-reduction of vaccinees in the challenge test to fifteen foxes;

-reduction of controls to five foxes with the requirement of a 100% disease rate;

-establishment of serological methods to minimise use of the challenge test as far as possible (e.g. studies on the duration of immunity without challenge).

Reduction of the number of animals used for safety testing during the licensing procedure:

-by combining safety (single-dose) and potency tests in 15 foxes ;

-by reducing to five the number of animals used to test a 10-fold dose and to test safety in other species.

The number of animals used for rabies vaccine development and control could be reduced since in vitro assays are available [11,12] which allow a good evaluation of protection.

Conclusions

It is difficult to generate a generic approach for reducing the use of animals for vaccine development and control in the field of viral veterinary vaccines. Nevertheless, on a case by case basis, one can propose modifications which allow major improvements as shown by the three examples given in this paper. The field and the scope of veterinary vaccines is evolving rapidly and whenever possible Pharmacopoeia monographs should be modified according to scientific developments and the evolution of practical and field requirements.

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