Viral haemorrhagic disease of rabbits: vaccination and immune response

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Summary: Results are presented for the large-scale use of a tissue vaccine, inactivated with beta-propiolactone and containing aluminium hydroxide adjuvant, against viral haemorrhagic disease of rabbits.

The kinetics of haemagglutination-inhibiting antibodies over eighteen months, the response of vaccinated animals to challenge infection with field virus between six and fifteen months after vaccination, the serological response to revaccination, and the immunological mechanisms involved in primary vaccination and revaccination were investigated.

KEYWORDS: Immune response - Immunisation - Rabbit diseases - Vaccines - Viral diseases - Viral haemorrhagic disease of rabbits.

INTRODUCTION

The appearance of viral haemorrhagic disease (VHD) among Angora rabbits imported from Germany into the People's Republic of China in 1984 (14), and the subsequent spread of the disease to Europe (possibly from Eastern countries) and then to North Africa and the Americas, where Mexican rabbits were affected, immediately led to numerous proposals to control the disease.

Although vaccination was the first measure recommended by some authors, the implementation of sanitary measures, under which the unaffected areas tried to remain free from the disease while affected areas were conducting control and eradication measures, was the most commonly adopted procedure as soon as outbreaks occurred in a country.

These opinions are reflected in the notice issued by the OIE on 20 January 1989 (21), when reports of the new disease arrived from various European countries, including Italy (16), Spain (2), France (19) and Germany (15). Similar occurrences were notified in countries of Eastern Europe.

Among disease control measures recommended in this notice were emergency slaughter of entire populations of rabbits on affected breeding farms, with repopulation after an interval of four weeks. General control procedures were recommended for disease-free regions or premises.

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When Mexico was confronted with a severe outbreak and lacked access to vaccination, the eradication programme included quarantine of infected premises, prohibition of movement and sale of rabbits, voluntary slaughter of sick animals with cleaning and disinfection of affected premises, and an interval of at least two months before repopulation (1, 17). These measures resolved the problem and were followed by a significant decline in the rabbit population.

Considering the Mexican experience, the United States Foreign Animal Disease Report (10) recommended the concept of “sentinel rabbits”, introduced in small numbers before complete repopulation of affected premises and after an unpopulated period of two months.

Abundant information exists on precautions to be adopted, particularly with regard to the early stages of investigation of the new disease; however, developments in research have somewhat modified the previous approach. For instance, Rosell (25) reported the appearance of the disease in well-fed rabbits which were free from intercurrent disease; general disease precautions had been strictly implemented in the colonies where these rabbits lived. Cancellotti (7) reviewed the situation and concluded that severe outbreaks of viral haemorrhagic disease, with 90% mortality, could occur even in intensive establishments with good technical assistance and where disease control precautions were in force. Despite isolation, disinfection and safe disposal of carcasses, cases of the disease continued in relentless progression.

In the opinion of Rosell (26), hygiene control is indispensable before and after outbreaks of VHD, although the adoption of such measures cannot guarantee protection.

All these findings agree with the experience gained in the People’s Republic of China (29) which showed that the disease could be easily controlled by the slaughter of affected animals, strict quarantine and, most importantly, vaccination.

Vaccination was contemplated in the beginning, in the hope of controlling the disease (2). Not until research had been conducted (3, 4, 5, 6, 22, 23) and the results of more than two years of field trials of general vaccination had been completed, however, was it possible to conclude that vaccination was valuable in controlling the new disease. The reduction in the number of new outbreaks and in the spread of the disease since vaccination has been adopted confirms the efficacy of vaccination as a control method.

**VACCINE**

A commercially available vaccine has been used in Spain against VHD virus (VHDV) from the beginning. It was first used experimentally (in collaboration with the Animal Health Sub-Directorate of the Spanish Ministry of Agriculture, Fisheries and Food) and, subsequently, in a field trial according to protocols dictated by the disease emergency. This vaccine has also been widely used in Germany, Belgium, France, Greece, Italy, Portugal and Tunisia.

Like other vaccines which were either officially licensed or under trial, this vaccine was prepared from tissues which had been obtained by inoculating susceptible animals, then collected from the target organs shortly before death.
The animals used for inoculation were always selected from colonies which had previously shown high susceptibility to the disease. The animals had been reared under satisfactory health conditions and kept in strict quarantine upon arrival. During the quarantine, bacterial infections were eliminated by broad-spectrum chemotherapy.

The quarantine period and the known origin of the animals guaranteed that no animal which had experienced hyperthermia before inoculation was inoculated. Also excluded were animals yielding a positive blood culture to standard bacteriological testing during inoculation, as this implied an intercurrent septicæmic process.

Inoculation was performed by intramuscular injection (although other routes can also be used) of 0.5 ml of an inoculum which had previously been titrated to contain at least 100 LD₅₀; this led to death within an average of 24-36 h. In all cases, the inoculum was prepared from strains capable of haemagglutination (HA) at room temperature with slow elution. As reported by Badiola (6), cases occurring in the field are associated with HA only at 4°C, or with rapid elution. In the experience of the author, however, such cases are rare.

Following inoculation, the moment for collecting samples is determined by the clinical course in the inoculated animals. Those animals which had previously experienced pronounced hyperthermia and are in pre-agonal depression, with a temperature below the normal physiological range, are selected for slaughter.

The target organs showing the characteristic lesions of VHD (2, 16) and which are free from signs of intercurrent diseases, including presumptive signs, are removed after death. The organs are triturated; phosphate-buffered saline (PBS) at pH 7.2 is added in the proportion 1:2 (w/v) and the mixture is placed in a blender, operating at 18,000 rpm in a refrigerated environment for 5 min. It is then triturated, also at 4°C, for 5 min at 24,000 rpm.

The fluid resulting from clarification of the suspension is assayed for virus content by its haemagglutinin titre against human group O erythrocytes. This is performed by means of a microplate technique with 0.025 ml of each reagent at 4°C and a pH of 6.4.

Once the number of haemagglutinating units (HAU) from the initial titration is known, more PBS at pH 7.2 is added in sufficient volume to provide, after inactivation of the suspension and adsorption on adjuvant, a concentration of 128-256 HAU in the commercial product.

In view of these explanations, the vaccine produced by the author does not, in principle, differ from those described by other authors in other countries (2, 3, 4, 5, 8, 11, 12, 13, 18, 22, 23, 27, 28, 29).

However, the vaccine used by the author differs from most of the products used in the field, including those tried experimentally, in two respects.

Firstly, the inactivating agent is beta-propiolactone, originally chosen for two important reasons:

- The product has given good results as an inactivating agent for canine parvovirus. There are various opinions on the classification of the VHDV, but the prevailing opinion when the author made his decision was that it belonged to the Parvoviridae family. In any case, beta-propiolactone has since proved to be a satisfactory inactivating agent.
- The product enables easy verification that it has not undergone degradation due to partial hydrolysis before use and that it therefore remains fully active. Once inactivation is complete, the substance is readily hydrolysed at 37°C into beta-propionic acid and hydroacrylic acid, both of which are innocuous.

Secondly, the final component to be incorporated in the vaccine, the adjuvant, can be selected from the two options most frequently employed: Freund’s incomplete adjuvant and aluminium hydroxide.

The selection of aluminium hydroxide, which is stable in the final product over a long period, is supported by reports in the literature (4, 8, 9, 11, 12, 13, 18, 27, 28, 29) and by the results of experiments conducted by the author. Given the special features of the antigen, there is no doubt that stability is better in the presence of aluminium hydroxide than in an oil emulsion and that the use of aluminium hydroxide avoids the risk of anaphylactic phenomena upon revaccination.

**VACCINATION PROCEDURE**

Previous studies (4) have established the dose at 1 ml for adult animals and 0.5 ml for animals vaccinated at weaning. The latter amount is based on the protection conferred by a reduced dose, taking into consideration the short period of protection required during the production cycle, which corresponds to the fattening period.

Revaccination was initially recommended after six months. Although data existed to indicate that protection would last for a long period, experimental evidence to support this was lacking. Now, however, evidence has been obtained from revaccination in the field and from vaccination experiments which indicates that revaccination is unnecessary. For this reason, it is not used much.

**SEROLOGY**

All the serological data for the present study, whether from experimental animals or from intensive rabbit colonies, were obtained by using the haemagglutination inhibition (HI) test described above (2). This technique underwent a number of modifications, such as the choice of pH 6.4 and a reaction temperature of 4°C, in different laboratories.

Such modifications are justified because the first observations by the author were made so soon after the disease had first been notified. This meant that the author had to incorporate all the data obtained up to that time in order to establish the kinetics of the antibodies and that he was unable to use those techniques, such as ELISA, which appeared later (20). In any case, given the similarity between the results of the HI test and the ELISA technique (20), the author believes that his data reflect the true serological situation.

**RESULTS OF VACCINATION**

Earlier publications (4, 5, 6, 11, 12, 13, 22, 23, 24, 26, 27, 28) reveal two distinct aspects of the response to vaccination, depending on whether it was performed experimentally or in the field. The results indicated below reflect current knowledge.
Innocuity

Innocuity in a broad sense is demonstrated and confirmed by the adequate inactivation of a virus originally lethal for rabbits, by the safety of the inactivating agent, and by the absence of reaction to and the suitability of the adjuvant.

At the control stage, before each batch of vaccine is offered for sale, at least ten rabbits older than ten weeks are inoculated; when possible, rabbits of country origin are chosen because of their high susceptibility to the virus. Each is inoculated with 5 ml of vaccine. The absence of clinical manifestations, including hyperthermia, and a weight increment similar to that of the unvaccinated controls, demonstrates both the adequate inactivation of the virus and the absence of undesirable side effects. When the animals are slaughtered seven days later, the absence of definite or suspect lesions of VHD confirms the inactivation.

Finally, the absence of local reactions at the inoculation site confirms that the excipient suitably stimulates bodily defences.

Vaccination experiments were conducted on animals of different ages and on females at various stages of gestation, between mating and parturition. In all cases, the number and viability of offspring were completely normal.

These laboratory findings were fully confirmed in the field and included less carefully controlled, though unproblematic applications, such as the vaccination of all categories of animals encountered in commercial rabbit breeding: animals bred for meat, breeding males and females, and does at various stages of pregnancy and lactation.

Serology of vaccination

The serological response of vaccinated animals has been examined in previous studies (3, 4, 5). What follows are the most commonly encountered features of serological behaviour:

- Before vaccination, and during the first three days thereafter, the animals show no serological response as measured by inhibition of HA by 4 HAU of VHDV.

- Between four and seven days there is obvious seroconversion in all animals, attaining titres of 1:320 by the end of this period.

- A titre of 1:320 is maintained for three months after vaccination.

- When four, five or six months have elapsed since vaccination, the response falls to a new plateau of 1:160.

- At seven and eight months after vaccination there is another fall in serum titre by one logarithm to the base of two, with HI titres of 1:80 still capable of providing protection, as shown by the results of challenge infection.

- The subsequent serological picture in the ninth and tenth months after vaccination in animals kept under experimental conditions, and in the sixth to sixteenth months in field trials, shows that the same titres become stabilised, with all animals possessing an HI titre of 1:80 to 4 HAU.
Results of challenge infection

Animals kept in the laboratory or in field trials (culled for poor productivity) were inoculated with 100 LD_{50} of virus at various times after vaccination.

Those animals vaccinated 2-3 days previously and possessing titres between 1:20 and 1:40 died after field virus inoculation. A less acute form of illness, however, lasted six to seven days after challenge in some animals, particularly those with the higher titre.

All animals with initial titres of 1:80 at 4-5 days after vaccination survived, a fact which indicates that this titre conferred protection. Seven days after challenge the antibody titre usually reached 1:640, subsequently stabilising (at 14 days) at 1:320. In some animals the titre reached 1:1280, while in others it increased by only two logarithms, to 1:320.

Recent vaccination followed by challenge with a field strain of virus in animals having initial titres of 1:160 or 1:320 resulted in an increase in some animals to 1:640, although the most common HI titre was 1:320. This demonstrates that, with high initial titres, the challenge infection failed to stimulate a new serological response.

Among animals vaccinated some months previously, challenge infection under the conditions described above met with the following responses:

- animals with titres of 1:160 or 1:80 at six, seven or eight months after vaccination showed no significant seroconversion after challenge, the titres increasing only to 1:320, but without any clinical illness;
- between nine and fifteen months after vaccination, challenge infection produced increasing seroconversion, almost always without symptoms (there were some exceptions in the periods furthest removed from initial vaccination), with titres reaching 1:5120 one week after challenge. In every case the titres then subsided slowly, falling to the previous titres by 3-4 weeks after challenge.

REVACCINATION

The following is a synopsis of events after revaccination of laboratory and farmed rabbits:

- The vaccine was found to be entirely safe upon second inoculation, as there was no local or general reaction and no difference in response from the first inoculation.
- With regard to the serological response, revaccination after seven to eight months invariably resulted in an increase in titres in all animals, reaching 1:320 within 2 to 3 days. There was no further increase in titre in this group and no exceptional incidents were reported.
- The starting titres of 1:80 increased to 1:320 in 60% and to 1:640 in 40% of the animals revaccinated after nine to ten months.
- The serological response always increased to 1:640 in animals which had received a primary vaccination 11-13 months previously. Because the first test was not conducted until seven days after revaccination, the onset of titre increase was
not determined in this group. This titre persisted for three weeks, however, after which it declined (by the fourth week) to 1:320 in the majority of animals tested.

- Animals revaccinated 14-16 months after primary vaccination responded early and intensely, giving titres between 1:1280 and 1:2560 at 2-3 days after revaccination. The persistence of these elevated titres lasted for approximately four weeks; in most cases, they then declined to 1:320.

**DISCUSSION**

The results obtained by the author and those reported by Pagés (23) proved beta-propiolactone to be a suitable inactivating agent. While preserving the immunogenicity of the virus, it also provided a valid test of the integrity of those antigenic determinants involved in the immune response.

The results obtained by the author, and supported by those of Galassi (9), showed that the adjuvant which was used potentiated the immune response and contributed to a completely effective vaccine.

In addition to enhanced efficacy due to the right sort of adjuvant, the careful selection of a strain of virus for vaccine production, based on its haemagglutinating property and slow elution, was an important factor in avoiding ineffective batches. This has been a problem experienced by numerous research workers in different countries.

The accumulation of results of hundreds of serological tests and the narrow ranges of HI titres obtained provided solid criteria for the validity of the serological procedure used. Nevertheless, studies on the relationship of this test to others, such as ELISA, have yet to be conducted.

The innocuity of the product conforms with the experience of all those who have used vaccine to control VHD. In none of the field trials did the author encounter any impairment of fertility when animals were mated 5-15 days after vaccination, as stated by Kelemen (13) when using other products.

Unvaccinated animals either possessed no antibody or had titres < 1:20 in serological response to primary vaccination. As these animals were procured in the early stage of the disease in Spain, this result was not unexpected. The results obtained in an endemic situation would be different, as witnessed by Morisse in France (personal communication) and other authors, where some animals, selected randomly from colonies free from the disease, had clearly positive titres (20). This, however, differs from the experience of Xu, who found a decrease in both susceptibility and mortality in areas where the disease had occurred, the HI test giving doubtful or mildly positive results (29).

The precocity of the serological response, confirmed by the disappearance of clinical cases on infected premises only 4-5 days after vaccination, was a constant finding in the trials conducted by the author and by others. In any case, the use of inactivated vaccine provides no easy explanations.
Xu mentioned the possible participation of interferon (29). The author believes that the antigenic material to which the animals respond is comprised more of the modified surface of virus-containing cells than of the virus itself. The response to these novel antigenic determinants, readily accessible to the immune system through their superficial location, no doubt occurs sooner than the response to intracellular virus.

If this happens, the vaccinal protection would involve, among other mechanisms, cytotoxic mechanisms which destroy infected cells and stop viral replication.

Challenge infection of vaccinated animals at various intervals (prolonged intervals, in many cases), has confirmed that, given the high proportion of replacements required to increase yield, revaccinating rabbits is unnecessary in commercial colonies. If not clearly expressed, this idea has been assumed in numerous earlier studies.

The absolute innocuity of revaccination is to be expected from a vaccine high in protein derived from the organs of rabbits and free from excipient, other than aluminium hydroxide: the protein available to the immune system of the vaccinated animals is homologous and would not elicit anaphylactic shock.

The serological response of animals revaccinated after seven to eight months was not typical of a secondary response. The antibody titres achieved, and the fact that they appeared promptly and persisted, is clear proof of the activation of memory cells and of the type of gamma globulin on which the secondary immune response is based.

Enhancement of the serological response at a greater interval between primary vaccination and revaccination may owe to the adjuvant function of an immunity which forms its own antibodies. These antibodies consist of aggregates which are not formed earlier by an excess of circulating antibody. Better presentation of antigenic material to macrophages and the consequent improved management of the antigen as a whole, which results in an enhanced immune response, may explain how this process occurs.

Finally, the response elicited by challenge infection at a variable interval demonstrates an improved serological response after challenge. This response is directly related to the time which has elapsed since the primary vaccination and does not permanently influence the initial serological titre of the animal. The absence of clinical signs proves that the aetiologcal agent has not multiplied. It also signifies that challenge infection is the equivalent of revaccination in that the immune mechanisms mentioned above no doubt play their part.

**CONCLUSION**

Without giving a detailed account of the presumably complex immunological mechanisms linked to vaccination against VHD, but considering the actual production cycles in rabbit farming, which usually involve the maintenance of breeding stock for no more than a year, the author concludes that vaccination of future breeding stock at ten weeks of age will confer solid protection throughout the productive life of the rabbits.

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REFERENCES


