EFFICACY OF INTRADERMAL RHD VACCINATION USING VARIOUS ADJUVANTS ON FATTENING RABBITS

PAGÈS-MANTÉ A., ALFONSO M.

Laboratorios Hipra, S.A. Avda. La Selva, 135. 17170 Amer, Girona. Spain
apm@hipra.com

ABSTRACT

There is a clear necessity to protect fattening animals to prevent the losses that an outbreak of Rabbit Hemorrhagic Disease (RHD) can cause in industrial rabbit farms. Inactivated vaccines against RHD with different adjuvants are available in the market as well as several vaccination devices. The purpose of this study was to assess the efficacy of various RHD vaccines with different adjuvants when administered by intradermal route by measuring the serological immune response and the resistance to an intramuscular challenge with RHD virus in fattening rabbits. Five batches of animals were vaccinated: Batch 1 or control rabbits were inoculated phosphate buffer solution (PBS) by intradermal route. Batches 2 and 3 were vaccinated with half-dose of oil-based Cunipravac-RHD® (Laboratorios Hipra) by subcutaneous and intradermal route respectively. Batch 4 received an experimental aluminum hydroxide-based vaccine with the same antigenic composition as Cunipravac-RHD®. Aluminum hydroxide-based Dercunimix® (Merial) was administered to Batch 5 according to manufacturer's indications. Vaccinations did not affect health status of rabbits but produced transient local reactions at the inoculation site. Serological response at 29 days post-vaccination was not complete and varied widely between groups. In contrast, total protection after challenge was reached in Batches 2, 3 and 5. When using the intradermal route half-dose of the oil-based vaccine (Batch 3) conferred a protection comparable to a complete dose of the aluminum hydroxide-based vaccine (Batch 5) after challenge with RHD virus. Our results suggested that oil-based vaccines were more effective controlling a RHD viral infection regardless of the inoculation route used compared to the aluminum hydroxide-based vaccines based on the identification of RHD virus from vaccinated-challenged animals.

Key words: intradermal vaccination, rabbit hemorrhagic disease, adjuvants, serological response, challenge resistance.

INTRODUCTION

The rabbit haemorrhagic disease (RHD), caused by a Calicivirus, occurs in an acute form in the European rabbit (*Oryctolagus cuniculus*) causing high mortality in non-protected farms (90%).

Young animals are resistant to the disease, although the explanation for this fact is not well known and the age when they become susceptible is not clearly defined. KRONEMAN and HORZINEK (1994) reviewed reports were animals less than 2-month-old did not become ill and other works showed that after 4 weeks of age the natural resistance to the disease quickly diminished. We have observed high mortality in commercial rabbits at the end of the fattening period (6-8 weeks) on repeated occasions frequently associated with large temperature fluctuations in short periods of time. There is a clear necessity to protect fattening animals to prevent the losses that an outbreak of RHD can cause in industrial rabbit farms.

There are inactivated vaccines against RHD with either oil or aluminum hydroxide-based adjuvants that are administered by subcutaneous and intradermal route respectively (LEMIERE, 2000). The type of adjuvant determines the duration of the protection that they confer being longer for oil-based vaccines after subcutaneous injection (PAGÈS MANTÉ, 1989). Intradermal vaccination has been proven to be advantageous in terms of ease of handling and reduction of disease transmission. However, vaccination failures against myxomatosis have been shown (ALFONSO and PAGÈS MANTÉ, 2003). Nowadays there are no oil-based vaccines available in the market to be delivered by intradermal inoculation. The purpose of this study was to assess the efficacy of various RHD vaccines with different adjuvants when administered by intradermal route by measuring the serological immune response and the resistance to an experimental infection with RHD virus in fattening rabbits.

MATERIAL AND METHODS

Animals

Forty one-month-old industrial hybrid rabbits were used in the study. They were housed in cages (100 x 40.5 x 38 cm - 5 animals per cage) in an independent unit of an industrial rabbitry. They were administered feed and water *ad libitum* throughout the test.

Treatments

Five batches of animals were vaccinated as detailed in Table 1.

Batch 1 or control rabbits were inoculated phosphate buffer solution (PBS) by intradermal route. Batches 2 and 3 were vaccinated with half-dose of Cunipravac-RHD® (Laboratorios Hipra) by subcutaneous and intradermal route respectively. Batch 4 received an experimental aluminum hydroxide-based vaccine with the same antigenic composition as Cunipravac-RHD®. Dercunimix® (Merial) was administered to Batch 5 according to manufacturer's indications.

Table 1: Study design.

Batch	No. rabbits	Vaccine[A]	Antigen[B] Titre/0.5ml	Adjuvant	Administration route[C]	Dose/ animal
1	5	Control (PBS)	-	-	Intradermal	0.2ml
2	5	Cunipravac-RHD®	≥640 HAU	Mineral oil	Subcutaneous	0.2ml
3	10	Cunipravac-RHD®	≥640 HAU	Mineral oil	Intradermal	0.2ml
4	10	Cunipravac-RHD experimental	≥640 HAU	Aluminum hydroxide	Intradermal	0.2ml
5	10	Dercunimix®	≥5 DP_{90}	Aluminum hydroxide	Intradermal	0.2ml

[A]PBS phosphate buffer solution; Cunipravac-RHD® Laboratorios Hipra; Dercunimix® Merial. [B]Inactivated RHD virus: Strain 3116-AP in Cunipravac-RHD®; strain AG88 in Dercunimix®. Hemagglutinating Units (HAU); Protective dose (PD_{90})
[C]Intradermal using Dermojet® with 3-orifice multi-jet head (*Societé Akra Dermojet*)

Evaluations

Clinical signs were assessed on the first day of the test. Blood samples were taken from 5 animals of each group by puncture in the marginal vein of the right ear (1-2 ml/rabbit). Animals were vaccinated after bleeding (Table 1). Subcutaneous injection was made in the scruff of the neck with 0.9x40 mm needles (20G½ Nr.1 Microlance3®). Intradermal administration was performed with a Dermojet® with 3-orifice multi-jet head (Societé Akra Dermojet). Two shots (0.1ml/shot) were given in the mid region of the inner part of the left ear. Areas covered by hair were avoided to ensure correct penetration of the vaccine. General clinical signs of vaccine reaction such as: lethargy, anorexia, etc., and reactions at the inoculation site were evaluated five days after vaccination. The assessment of clinical signs was repeated 29 days after vaccination and, in addition, blood samples were extracted from all the animals as described previously. Blood samples were collected in tubes with pellets and centrifuged (2500 rpm 10 minutes) in order to get the serum. An indirect ELISA (INGEZIM Rabbit 1.7.RHD.K.1®, Ingenasa) was used to detect IgG antibodies specific to RHD virus. The results were presented as a relative index (RI) and animals were considered to be seropositive when it was greater than 1.5 (RI>1.5).

Challenge

At 30 days post-vaccination, five animals of each group were transported to experimental isolators where they were challenged by intramuscular inoculation of 0.5ml of a suspension of RHD virus (stock 4764, titer 6000HAU/dose). Clinical signs were controlled daily after the infection. Mortality was submitted to necropsy examination and liver samples were taken for RHD viral identification by hemagglutination test (HA) and Dot-ELISA. Animals surviving the challenge were humanely euthanasied and processed in the same way.

Statistical analysis

Frequency of seropositive animals where compared by Chi-square. Mean, standard deviation, and coefficient of variation of the serological response of each group were also calculated and compared by analysis of variance (ANOVA). Correlation between survival rate and RHD virus detection using different techniques was performed. Statistical analyses were made with SPSS 11.5 software.

RESULTS

Clinical signs

Animals showed no clinical signs at the start of the trail. Health status was good at five days post-vaccination. Small nodules were observed at the inoculation site in intradermal vaccinated animals. In the control batch, nodules were small (0.2 cm). In Batch 3 vaccinated with oil-based Cunipravac-RHD® they were medium sized (0.3-0.4cm) and in the Batches 4 and 5 inoculated with aluminum hydroxide-based vaccines nodules were a little larger (0.5cm). At 29 days post-vaccination, nodules no longer were detected and animals showed a good health status. No lesions or local vaccine granulomas were observed in the carcasses of the animals vaccinated by subcutaneous route. Only one animal died along the trial by causes non-related with vaccination.

Response to vaccination and challenge

All animals were seronegative at the beginning of the test. At 29 days post-vaccination, one rabbit in the control group had seroconverted and the percentage of vaccinated animals that were seropositive ranged between 30 and 80% depending on the batch (Table 2). Higher seroconversion percentages were observed in rabbits vaccinated with Dercunimix® (Batch 5) and with oil-based Cunipravac-RHD® by intradermal route (Batch 3) resulting in higher mean level of antibodies, followed by rabbits vaccinated by subcutaneous route (Batch 2).

Regarding the homogeneity of the response to vaccination (expressed as coefficient of variation), it was better in the animals vaccinated by subcutaneous route (Batch 2, 52.4%), followed by those vaccinated with Dercunimix® (Batch 5; 67.7%) and the group vaccinated with oil-based Cunipravac-RHD® (Batch 3, 82.9%). The most heterogeneous serological response was observed in the batch vaccinated with aluminum hydroxide-based Cunipravac-RHD (Batch 4, 115.1%).

Resistance to challenge was lower in the control animals. Eighty percent of them died 48-96 hours after the infection. One rabbit in Batch 4, which was seronegative at 29 days post-vaccination, also died (Table 2). Dead animals showed similar gross lesions compatible with RHD: friable liver with discolored zones and a visible reticular pattern, congestion in multiple organs (spleen, lungs, liver), and hemorrhagic tracheitis with

frothy exudate. Diagnosis by HA and Dot-ELISA confirmed the presence of RHD virus in the livers in all dead animals.

The survivors showed no clinical signs in the week following challenge. Table 3 shows the number of infected animals in which RHD virus was detected in the liver at 7 days post-challenge. The results differed according to the technique used but in both cases the number of positive samples was greater in the non-vaccinated animals (Batch 1) and in those inoculated with aluminum hydroxide-based vaccines (Batches 4 and 5). Considering all challenged animals there was a negative correlation between the survival rate and the identification of RHD virus in the liver. Pearson's coefficient of correlation was -0.73 for HA and -0.44 for Dot-ELISA.

Table 2: Serological response after vaccination and mortality after intramuscular challenge with RHD virus.

Batch	Level of antibodies (RI) Mean and (Std)		Seroconversion (%)	Mortality after challenge (%)
	Day 0	Day 29 post-vaccination[B]	(Seropositive[C]/Total)	(Dead/Challenged)
1	0.074 (0.009)	0.452a (1.559)	20.0% (1/5)	80.0% (4/5)
2	0.073 (0.007)	1.344ab (0.704)	40.0% (2/5)	0.0% (0/5)
3	0.095 (0.036)	2.516b (2.086)	66.7% (6/9)	0.0% (0/5)
4	0.099 (0.063)	0.715a (0.823)	30.0% (3/10)	20.0% (1/5)
5	0.078 (0.018)	2.661b (1.800)	80.0% (8/10)	0.0% (0/5)
Sig.[A]	n.s.	*	n.s.	***

[A] Sig. p>0.05 ns; p<0.05 *; p<0.01 **; p<0.001 ***
[B] Different letters (a, b) indicate significant differences between batches
[C] Seropositive when RI >1.5

Table 3: Identification of RHD virus by Dot-Elisa and Hemagglutination (HA) in frozen livers from RHD virus-challenged rabbits at 7 days post-challenge.

Batch	Dot-ELISA (Positive/Total)	Hemagglutination (HA) (Positive/Total)
1	5/5	4/5
2	1/5 *	0/5 *
3	1/5 *	0/5 *
4	5/5 [N]	1/5 [N]
5	2/5 [N]	3/5 [N]

* significant p<0.05 respect controls
[N] not significant p>0.05 respect controls

DISCUSSION

None of the vaccinations tested affected the general health status of the animals. At a local level, the reactions were of no clinical significance. They were restricted to the mild traumatism caused by the intradermal inoculation. In spite of what was expected, the different adjuvants used did not cause severe local reactions probably because the vaccine was dispersed in 3 points.

Different vaccinations caused variable serological responses. The number of animals per batch was limited and this could have contributed to the high heterogeneity. No complete seroconversion was reached but total protection was demonstrated after challenge showing the efficacy of vaccination. It would be interesting to try other techniques to assess the serological response (Hemagglutination inhibition, Competitive ELISA) (CAPUCCI *et al*., 1996) and to compare the results.

Resistance to challenge was 100% in rabbits vaccinated with oil-based Cunipravac-RHD® by both subcutaneous and intradermal route, and with Dercunimix® (Batches 2, 3 and 5). The highest serological responses were also seen in those batches. Although a complete dose of Dercunimix® was administered and just half-dose of Cunipravac-RHD® were inoculated both by intradermal route there were no differences in efficacy (100% protection, Batches 3 and 5). The experimental vaccine used in Batch 4 (aluminum hydroxide-based Cunipravac-RHD) failed to protect 20% of the rabbits which could indicate that half-dose was not fully protective. A complete dose or higher antigenic titer should be considered to increase its effectiveness.

A peculiar fact was observed in the control batch: one of the animals seroconverted and died after the challenge. The animal that survived, nevertheless, was seronegative. CAPUCCI et al. (1997) have previously described a similar seroconversion in an industrial unit of rabbits infected with a non-pathogenic rabbit hemorrhagic disease-like virus, but they were protected to challenge.

Although there were slight quantitative differences, the rate of identification of RHD virus by Dot-Elisa and HA in RHD-challenged rabbits, was lower in the batches vaccinated with oil-based vaccines compared to the aluminum hydroxide-based vaccinated and control batches. This would indicate a superior efficacy of the oil-based vaccines to control an RHD viral infection regardless of the inoculation route used. When using the intradermal route half-dose of the oil-based vaccine (Batch 3) conferred a protection comparable to a complete dose of the aluminum hydroxide-based vaccine (Batch 5) after challenge with RHD virus.

According to the present results vaccination of young fattening rabbits is strongly recommended to prevent the economic losses that a RHD outbreak can cause in an industrial rabbitry.

REFERENCES

ALFONSO M., PAGÈS MANTÉ A. 2003. Serological response to myxomatosis vaccination by different inoculation systems on farm rabbits. *World Rabbit Science* 11: 145-156.

CAPUCCI L., FUSI, P., LAVAZZA A., PACCIARINI M. L., ROSSI C. 1996. Detection and preliminary characterization of a new rabbit calicivirus related to rabbit hemorrhagic disease virus but nonpathogenic. *J.Virol.* 70, 8614-8623.

CAPUCCI L., NARDIN, A., LAVAZZA A. 1997. Seroconversion in an industrial unit of rabbits infected with a non-pathogenic rabbit haemorrhagic disease-like virus. *Vet Rec.* 140, 647-650.

KRONEMAN A., HORZINEK M.C. 1994. Rabbit haemorrhagic disease. En *Virus infections of rodents and lagomorphs*, Editor Osterhaus, A.D.M.E. Ed. Elsevier.

PAGES MANTE A. 1989. Consideraciones técnicas de la sueroterapia y de la profilaxis vacunal en la enfermedad hemorrágica vírica del conejo (RHDV). *Med.Vet.* 6, 285-291.

LEMIERE S. 2000. Combined vaccination against Myxomatosis and VHD. An Innovative Approach. 7th World Rabbit Congress Valencia Spain.