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LEMIÈRE S.

COMBINED VACCINATION AGAINST MYXOMATOSIS AND VHD: AN INNOVATIVE APPROACH

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COMBINED VACCINATION AGAINST MYXOMATOSIS AND VHD: AN INNOVATIVE APPROACH

S. LEMIÈRE

Laboratoire MERIAL, BP 7 Saint-Herblon 44153 Ancenis, France

ABSTRACT

Combined vaccination against myxomatosis and VHD is an innovative approach in rabbit production. Safety of the vaccine was tested according to the European legislation in force. SG33 vaccine strain showed no spread amongst SPF rabbits in laboratory conditions, no reversion to virulence and no influence upon humoral immunity. The administration of a double dose of the combined vaccine in females had no influence upon pregnancy and progeny. Challenge tests using myxomatosis and VHD virulent virus strains in laboratory conditions demonstrated the efficacy of the vaccine.

RÉSUMÉ

La vaccination combinée de la myxomatose et de la VHD représente une innovation en production cunicole. L'innocuité du vaccin a été testée selon la législation européenne en vigueur. La souche vaccinale SG33 ne montre pas de diffusibilité chez le lapin EOPS dans les conditions du laboratoire, de réversion vers la virulence et aucune influence sur l'immunité à médiation humorale. L'administration d'une double dose du vaccin combiné chez la femelle n'a eu aucune répercussion sur la gestation et sur la descendance. Des essais d'épreuve virulente utilisant une souche virale de la myxomatose et une souche virale VHD dans les conditions du laboratoire ont démontré l'efficacité du vaccin.

RESUMEN

La vacunación combinada contra la mixomatosis y la enfermedad vírica hemorrágica (VHD) representa una innovación en la producción cunícola. La seguridad de la vacuna ha sido comprobada de acuerdo a la legislación europea en vigor. La cepa vacunal SG33 no mostró ninguna difusión entre conejos SPF en condiciones de laboratorio, tampoco demostró reversión a la virulencia, ni influencia alguna sobre la inmunidad humoral. La administración de una dosis doble de la vacuna combinada a hembras, demostró no tener ninguna repercusión sobre la gestación ni sobre la descendencia. Las pruebas de desafío en las que se utilizaron cepas virulentas de los virus de mixomatosis y de VHD, en condiciones de laboratorio, demostraron la eficacia de la vacuna.

INTRODUCTION

Rabbit pathology is known to include two major viral diseases, myxomatosis and viral haemorrhagic disease (VHD). Myxomatosis, viral disease due to a poxvirus first isolated in Southern America, was introduced intentionally in France in 1952 and spread all over Europe in a few years. First cases of VHD were described in China in 1984. The disease spread all over Europe a few years later. Since, both diseases need to be fought, as they still exist in all the areas in which rabbits are bred. The first vaccines against myxomatosis were produced using the Shope fibroma viral strain. They were the first heterologous vaccines. Homologous strains were later attenuated in order to obtain more immunogenic vaccines. One the homologous strains was the SG33 strain (Saurat *et al*., 1978). Vaccines against VHD have since the beginning of the control of the disease killed vaccines prepared from livers of infected rabbits, as the calicivirus does not grow in cell lines. Up to now, vaccinations were performed separately with two different vaccines so, combined vaccine against myxomatosis and VHD has been an innovation in rabbit production since its launch in France last April 1999. The development of such a new vaccination approach has taken several years and involved the avian and rabbit biological research and development team of Merial laboratory based in Lyon, France. A freeze-dried, live, attenuated vaccine against myxomatosis containing the SG33 homologous viral strain (Fournier, 1993)

and a liquid, killed vaccine prepared with the AG88 field strain isolated in France in 1988, against VHD in aluminium hydroxide adjuvant (Fournier, 1992), both already marketed and widely used on the field, constitute the new combined vaccine: Dercunimix®. A specific vaccination device, delivering three impacts at each shot and intended for the intradermal administration of the vaccine, was tested in order to guarantee that combined vaccination was as efficient as separate vaccinations. A greater number of impacts on one ear (six instead of two) increases the vaccination success rate because of the longer contact period between antigens and immune system cells, hence inducing an immune response against VHD. The dose administered is 2 x 0.1 ml, each impact delivering 0.1 ml in three shots. No difference between the administration of the SG33 strain alone and the administration of the combined vaccine has been found. Both the safety and efficacy of the combined vaccine have been demonstrated in laboratory conditions. The recommended vaccination schedule in rabbits is one SG33 vaccine at 4 weeks of age (Picavet *et al*., 1989), then Dercunimix® at 10 weeks of age. Booster administrations of SG33 vaccine have to be performed every 4 months in adult rabbits. Booster administrations of VHD killed vaccine have to be performed every year with Dercunimix®.

1. SAFETY OF COMBINED VACCINATION AGAINST MYXOMATOSIS AND VHD.

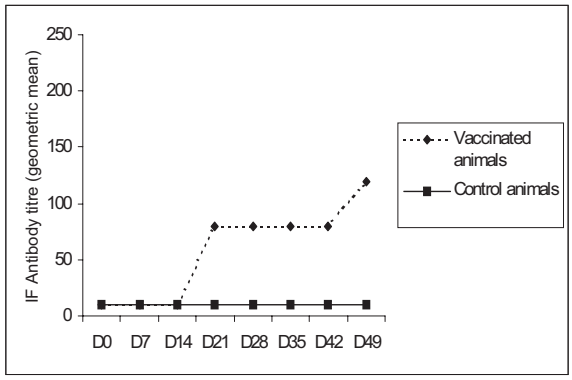
The following trials were carried out to evaluate the safety of the attenuated SG33 strain vaccine: virus spread, reversion to virulence, and influence upon humoral immunity. The safety of the administration of a double dose of Dercunimix® vaccine to pregnant female rabbits was evaluated, too. The experimental conditions were particularly severe in order to ensure the safety of the combined vaccine in field conditions.

1.1 Spread of SG33 vaccine strain.

1.1.1 Material et methods.

The study of virus spread aimed at checking that the SG33 viral strain, administered intradermally, did not spread amongst a group of rabbits. The animals included in the study were 8-week-old specified pathogen-free (SPF) rabbits. The titration of Dercunimix® product under study gave the following result: 5.0 $\log_{10}$ $CCID_{50}$, equivalent to 200 times the minimum guaranteed SG33 dose. The objective was to mix vaccinated rabbits with unvaccinated control rabbits. Myxomatosis antibody production was monitored by immunofluorescence (IF) to check whether the SG33 virus disseminated in the group of rabbits. Ten myxomatosis antibody-free rabbits were distributed into two groups of five animals. One of the two groups was vaccinated. Four days following vaccination, the control group was mixed with the vaccinated group during 16 days. The rabbits were separated at the end of this contact period and observed for 29 days.

Figure 1: Geometric means of IF myxomatosis antibody titres of the rabbits (SG33 virus spread).



1.1.2 Results.

Vaccinated rabbits seroconverted while control rabbits did not (see Figure 1). When observed for 45 days, unvaccinated control rabbits did not show any myxomatosis-related lesion following vaccination. The SG33 viral strain used in Dercunimix® formulation proved not to disseminate in a group of rabbits in laboratory conditions.

1.1.3 Discussion.

Theses results obtained under European marketing authorisation application conditions proved that SG33 viral vaccine strain is likely not to spread in field conditions in rabbit population duly vaccinated. This represents the major interest of the study. Both parameters chosen, serology and follow-up of the development of myxomas, are proven to be relevant because none of the control animals show any positive serology, nor any myxoma further to their long-term contact with the vaccinated animals.

1.2 Reversion to virulence

1.2.1 Material and methods

The study of reversion to virulence aimed at checking whether the SG33 viral strain, following five serial passages in rabbits in laboratory conditions, reverted to its initial virulence before attenuation. The *in vivo* stability of attenuation was checked. The lack of some of the genes involved in the SG33 virus pathogenicity (Guérin *et al.*, 1998) could prevent reversion to the initial pathogenicity of the vaccine virus. Five-week-old SPF rabbits were included in the study. The product tested came from a SG33 working seed lot titrating 4.4 $\log_{10}$ $CCID_{50}$ per dose, i.e. 50 times the minimum release dose of Dercunimix®. The objective was the individual inoculation of an extract of the myxomas that developed in the first properly vaccinated rabbits to other rabbits. These myxomas contained the SG33 virus. Inoculation was repeated four times consecutively. A total of five passages called 'reverse passages' were carried out. At each passage, the presence of SG33 virus in myxomas was checked by examining for the cytopathic effect induced in rabbit dermal cell cultures (IDL_7). Seroconversion to myxomatosis was also evaluated at each passage. The consequences of the fifth *in vivo* passage were compared with those obtained with the initial SG33 virus. Local reactions, weight gains and rectal temperatures were monitored for 49, 21 and 4 days, respectively. The virus was titrated and detected at each passage. Evidence of seroconversion after the fifth passage was given.

Table 1: Comparison of mean live weights (g) between both groups of rabbits (SG33 reversion to virulence).

	D28	D35	D42	D49
SG33	1150	1419	1619	1882
Fifth passage	1232	1456	1667	1944
Difference	NS	NS	NS	NS

NS: not significant ($p>0.05$)

1.2.2 Results

The antibody titre of the rabbits administered with the fifth passage was similar to that of control rabbits after the first passage. These results validate the reality of SG33 virus transmission at each passage from sample to target animal. The number of rabbits showing primary myxomas and the interval between inoculation and the first symptoms (the same for secondary myxomas) were similar both in the rabbits inoculated with the fifth passage and in unvaccinated control rabbits. The monitoring of weight gains, rectal temperatures and local, clinical reactions showed no difference between either populations (see Tables 1 and 2).

Table 2: Comparison of rectal temperatures (°C) between both groups of rabbits (SG33 reversion to virulence).

	D28	D29	D30	D31	D32
SG33	39.1	38.9	39.5	39.3	39.5
Fifth passage	39.1	39.2	39.0	39.3	39.2
Difference	0	P = 0.03	NS*	NS	NS

NS: not significant ($p>0.05$). * $p = 0.054$.

1.2.3 Discussion

Virological and serological evidence confirms the relevance of the study. Results are consistent with the theoretical assumption of the genetic stability of the SG33 viral strain according to Guérin *et al*., 1998.

1.3 Influence of vaccination upon humoral immunity.

1.3.1 Material and methods

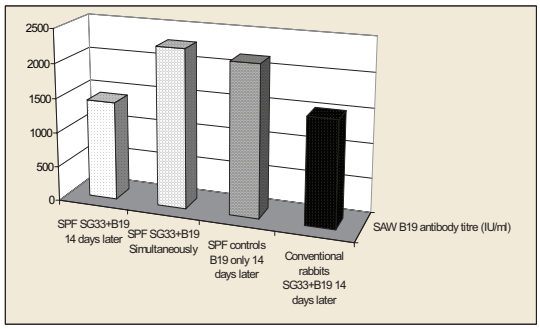
The study of the influence of vaccination upon humoral immunity aimed at checking that the SG33 viral strain was not immunosuppressive. The serological response to known bacterial antigens was assessed. SPF or conventional rabbits aged 4 weeks were included in the study. The product tested titrated 3.7 $\log_{10}$ $CCID_{50}$ i.e. almost 10 times the minimum release dose of SG33 virus in Dercunimix® vaccine. All rabbits were administered with a single dose of live vaccine against *Brucella*, B19 strain. The immunosuppressive capacity of the SG33 strain was evaluated by testing its ability to induce an antibody response to *Brucella* by comparing SG33-vaccinated rabbits with unvaccinated, control rabbits. Anti-*Brucella* antibodies were detected using two different methods: quick agglutination and Wright's seroagglutination, both the latter methods allowing to obtain quantitative results. Four groups of rabbits were constituted: 10 SPF rabbits vaccinated with SG33 strain and administered with the B19

vaccine 14 days later, 10 other SPF rabbits vaccinated with SG33 strain and administered with the B19 vaccine simultaneously, 10 other SPF control rabbits administered only with the B19 vaccine on D14, and 10 conventional rabbits vaccinated with SG33 strain and administered with the B19 vaccine 14 days later. The SG33 seroneutralising antibody response was monitored.

1.3.2 Results

All the rabbits included in the study showed positive reactions following administration of *Brucella abortus* B19 strain antigen using quick agglutination and Wright's seroagglutination, which demonstrates that the SG33 viral strain is not immunosuppressive in rabbits. Serological results showed that rabbits vaccinated with the SG33 strain seroconverted and that the antibody response results obtained in control rabbits were close to the threshold value. The SG33 strain is not immunosuppressive (see Figures 2 and 3).

Figure 2: Geometric mean of SAW anti-*Brucella abortus*, B19 strain, antibody titres (IU/ml) on D28 (influence of vaccination upon humoral immunity)



1.3.3 Discussion

The results obtained validated the study. The experimental design using *Brucella abortus* B19 strain serology was suggested by the Central Veterinary Laboratory of Weybridge, United Kingdom (ADAS, 1998). Four-week-old SPF rabbits are the most sensitive to an immunosuppressive agent. Conventional animals, much closer to field conditions, were included into the study to assess and compare their sensitivity to immunosuppressive agents

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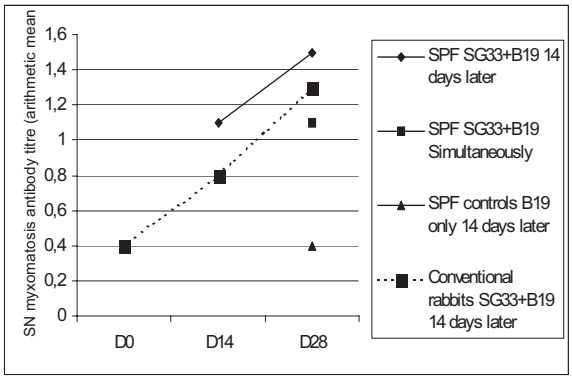
Figure 3: Arithmetic means of SN myxomatosis antibody titres (influence of vaccination upon humoral immunity).



1.4 Safety of a double dose of Dercunimix® vaccine.

1.4.1 Material and methods

The study of the safety of a double dose of Dercunimix® vaccine in pregnant female rabbits aimed at checking that the vaccine had no influence upon pregnancy, by comparing vaccinated females and control females administered with a placebo. Thirty-five (35) artificially inseminated, pregnant, female rabbits were included in the study. The vaccine batch under test came from a Dercunimix® batch whose SG33 virus titre was 80 times the minimum guaranteed dose, administered twice during the same vaccination operation (0.4 ml delivered in 4 shots) at 13 weeks of age. Females were artificially inseminated 10 days before vaccination. Thirty-seven (37) control females, receiving the same volume of placebo by the same route, were also included in the study. Four parameters were monitored: pregnancy rate, abortion rate, birth rate and mortality rate. Three other parameters were monitored in young rabbits: percentage of rabbits born alive, percentage of rabbits further to adoption and selection of viable rabbits, percentage of surviving rabbits at weaning. The percentages recorded for each parameter were compared between vaccinated females and their offspring, and females having received a placebo and their offspring.

1.4.2 Results

Neither abortion nor mortality was observed during the study. No significant difference ($\alpha = 5\%$) in pregnancy rate and birth rate between both groups was found either. 265 and 297 young rabbits were born to 35 properly vaccinated, artificially inseminated females and 37 inseminated females included in the placebo group, respectively. Percentages of rabbits born alive, of rabbits further to adoption and

selection of viable rabbits, and of surviving rabbits at weaning did not reveal any significant difference between the vaccinated and placebo groups ($\alpha = 5\%$) (see Table 3).

1.4.3 Discussion

The administration of an overdose of Dercunimix® further enabled to assess the consequences of vaccination on the reproductive function, in the worst conditions. The absence of adverse reactions following vaccination or administration of a placebo in female rabbits justifies the recommended vaccination date i.e. approximately 11 days after artificial insemination. Dercunimix® vaccine can be used in pregnant female rabbits without inducing any adverse reaction during pregnancy and after the birth of young rabbits during the period from milking to weaning.

Table 3: Safety of a double dose of Dercunimix® vaccine in pregnant female rabbits.

		Vaccinated females	Placebo	Difference
Females	Number of artificial inseminations	35	37	-
	Pregnancy rate	82.9%	83.8%	NS ($\alpha = 5\%$)
	Abortion rate	0%	0%	None
	Birth rate	82.9%	83.9%	NS ($\alpha = 5\%$)
	Mortality rate	0%	0%	None
Young rabbits	Number of young rabbits born	265	297	-
	Percentage of rabbits born alive	96.6%	97.3%	NS ($\alpha = 5\%$)
	Percentage after adoption and selection of viable rabbits	81.2 %	80.8%	NS ($\alpha = 5\%$)
	Percentage of weaned rabbits	62.0 %	68.7%	NS ($\alpha = 5\%$)

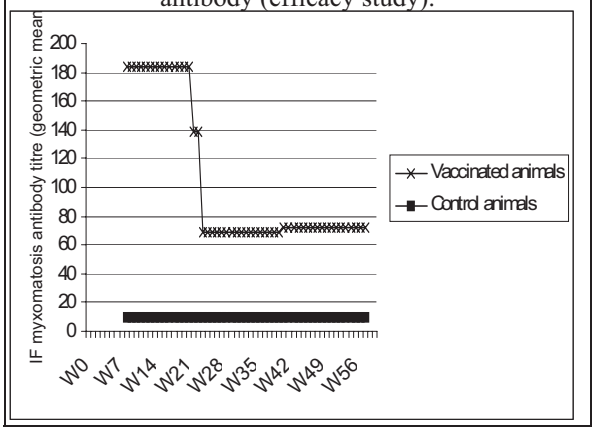
NS: not significant.

2. EFFICACY OF COMBINED VACCINATION AGAINST MYXOMATOSIS AND VHD.

2.1 Material and methods

The efficacy of the vaccination schedule including Dercunimix® vaccine was evaluated in laboratory conditions at the recommended vaccination schedule: 0.1-ml dose of SG33 vaccine at 4 weeks of age, 0.2-ml dose of Dercunimix® delivered in two shots at 10 weeks of age, booster vaccinations with SG33 vaccine 4 and 8 months later and with Dercunimix® 12 months later. Five-week-old, SPF rabbits were included in the study. Serological monitoring by detection of anti-myxomatosis antibody by immunofluorescence was carried out throughout the study in order to validate the efficacy of vaccination against myxomatosis. Unvaccinated, control rabbits were serologically monitored, too. Four months after Dercunimix® vaccination, all vaccinated and unvaccinated, control rabbits were challenged as described by Saurat *et al.*, 1978. Anti-VHD antibody production was monitored by ELISA throughout the study in order to validate the efficacy of vaccination against VHD. Unvaccinated, control rabbits included in the study were serologically monitored in the same manner. The protection conferred by VHD vaccination at 10 weeks of age with Dercunimix® and with the monovalent VHD inactivated vaccine was evaluated and compared using a viral challenge test in-house model.

Figure 4: Geometric means of IF myxomatosis antibody (efficacy study).

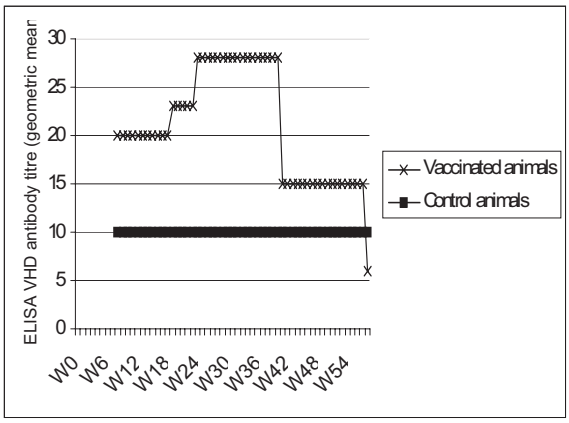


2.2 Results

The protection induced by the monovalent vaccine proved to last at least 12 months as demonstrated by Fournier, 1992. Protection conferred by Dercunimix® was evaluated as part of the same experimental design. Myxomatosis and VHD serological follow-up revealed high titres in vaccinated rabbits as opposed to unvaccinated, control rabbits, throughout the study period (see

Figures 4 and 5). The results obtained following challenge with a myxomatosis virus showed, according to Saurat *et al.*, 1978, that the sickness rate was 0/10 four months following primary vaccination using SG33 alone at 4 weeks of age and Dercunimix® at 10 weeks of age, while the sickness rate in unvaccinated, control rabbits was 10/10. Protection against a virulent myxomatosis virus is total (no case reported) 4 months following primary vaccination using SG33 and Dercunimix®. The experimental design was validated by the 100% sickness rate observed in the control group. The results obtained following challenge with a VHD virus showed that the death rate was 0/10 and 0/8 three months after vaccination with the inactivated VHD vaccine alone and Dercunimix® at 10 weeks of age, respectively, while the death rate in unvaccinated, control rabbits was 7/10. Protection against a virulent VHD virus was total (no case reported) 3 months following a single vaccination with an inactivated VHD vaccine alone and Dercunimix®. The 70% death rate observed in the control group validated the experimental design. Protection conferred both by the monovalent, inactivated VHD vaccine and by Dercunimix® is similar, as shown by challenge results. The duration of immunity is 12 months, which is validated by challenged 12 months following Dercunimix® vaccination. One out 12 rabbits died in the vaccinated group as opposed to 8 out of 10 in the unvaccinated, control group.

Figure 5: Geometric means of ELISA VHD antibody titres (efficacy study).



2.3 Discussion

The proposed efficacy study is relevant as it demonstrates using two challenge test models that the protection induced by the vaccination lasts 4 months after the induction of the immunity in young animals against myxomatosis and that protection against VHD lasts 12 months further to Dercunimix® administration at 10 weeks of age. The proposed vaccination schedule is in these conditions duly validated under laboratory conditions in SPF rabbits. One of the rabbits which died during the VHD challenge test one year after the vaccination showed abnormal low individual ELISA antibody titres ([2.5 on week 24, 5 on week 40 and then 5 on week 58). One of the other rabbits of the vaccinated group showed such a level of antibody, but did not dye from VHD further to the challenge test. Antibody titre is not the only parameter correlated to protection against VHD as demonstrated by both observations.

CONCLUSION

The vaccination schedule recommended for future breeding does, i.e. SG33 virus vaccine at 4 weeks of age and Dercunimix® vaccine at 10 weeks of age, confers protection against both the infective agents involved: myxomatosis poxvirus and VHD calicivirus. The same protection is induced in rabbits following vaccination according to the recommended vaccination schedule. The combined vaccine against myxomatosis and VHD can be used safely in industrial rabbit units.

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