

TECHNICAL NOTE

**FIELD STUDY OF SAFETY AND ANTIBODY PRODUCTION FURTHER
TO A COMBINED MYXOMATOSIS AND VIRAL HAEMORRHAGIC
DISEASE (VHD) VACCINATION IN DWARF RABBITS BY
INTRADERMAL ROUTE**

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Abstract: A study of safety of combined vaccination against myxomatosis and VHD was performed using a duly reconstituted vaccine made of a live homologous myxomatosis component SG33 strain and of an inactivated VHD component in adjuvant AG88 strain. The vaccine was administered intradermally to a representative sample of pet rabbits. A local reaction at the vaccine administration area was frequently observed from 2 to 3 days after vaccination in young animals. These local reactions were less frequently observed in adults. The reaction consisted of a local rash which usually disappeared 2 to 3 days after vaccination (maximum 1 week). The immune response following vaccination was monitored by antibody production against VHD and myxomatosis using, for the VHD vaccine component, an IHA titration method, and, for myxomatosis component, an ELISA titration method. Antibody production after vaccination was observed for both components. Maximum VHD IHA titre (192 ± 130) was obtained in vaccinated animals one month after vaccine administration. Antibodies were still detected in these animals one month later (94 ± 39). Mean titre obtained in unvaccinated controls was equal to 0. Maximum myxomatosis ELISA titre (10518 ± 2417) was obtained two months after vaccine administration. Mean titre obtained at the same time in unvaccinated controls was close to 0 (889 ± 744).

Key words: Myxomatosis, VHD, combined vaccine, intradermal route, syringe, safety, antibody production.

INTRODUCTION

The aim of the study was to assess safety and antibody production of vaccination using a combined vaccine in pet rabbits. The reconstituted vaccine enabled vaccination against myxomatosis and VHD at the same time using a single syringe (LEMIÈRE, 2000). Combined vaccine was administered to the animals using a syringe intended to administer products by intradermal route. The vaccine was administered by a small animal veterinarian. Different breeds of rabbits usually found in pet shops were chosen. The animals involved in the first part of the study came from a rabbitry raising rabbits for pets. The combined vaccine was administered intradermally. The same procedure was used during the second part of the study, the antibody production follow-up with rabbits originating from a different rabbitry. The test animals were transferred directly to semi-open traditional facilities where they were kept for the duration of the study.

MATERIALS AND METHODS

Animals

Safety study

Forty vaccinated rabbits were included (Table 1).

Table 1: Safety study.

Groups	Identification	Breed	Age
Group 1	01-10	Dwarf	Adult
Group 2	11 - 20	Dwarf Angora	Adult
Group 3	21 - 30	Dwarf	After weaning (at 7 weeks of age)
Group 4	31 - 40	Dwarf Angora	After weaning (at 7 weeks of age)

Forty rabbits in total were divided into four groups to perform safety study. Rabbits included in the study were young and adult rabbits (Table 1). A clinical follow-up of

each duly identified rabbit was performed during safety study. Observations were reported on an individual. The reference number of the animal, its age, and its characteristics (breed, colour, and size) were first recorded. Visual examination after each vaccine administration was performed on day D0, day of vaccination. The observation of a vaccine vesicle at the ear where the vaccine had been intradermally injected was also reported. Other observations (drowsiness, nervous disorders, aspect of the fur, even the death...) were also recorded on the sheet for each rabbit. General post-vaccine reactions were reported (lesions of the skin and/or mucosa, wounds, oedema, rash...) Local reactions at the injection point were also reported (rash, primary and secondary myxomas...) on D0 + 4 to 6 hours, D1, D2, D3, D7, D14, D21 and D28.

Safety and serological follow-up study

Forty rabbits dwarf various fur were divided into two groups of 20 rabbits to perform serological follow-up study: 'unvaccinated' control animals belonging to group 1 and 'vaccinated' animals belonging to group 2. All rabbits included into the study were adults. The same protocol was implemented during serological follow-up study. Blood samples were taken on the day of vaccination, day 30, and then day 60 for serological follow-up. The animals were anaesthetised at each handling in accordance with a widely used protocol using a unique veterinary injectable product (tiletamin & zolazepam). Blood sampling was performed in anaesthetised rabbits using intra-cardiac route. Each blood sample was duly identified. Serum obtained after centrifugation were stored in a freezer. Each sample was analysed using VHD IHA (inhibition of haemagglutination) method (Laboratoire Vétérinaire Départemental du Maine-et-Loire LDA49 BP 943 F-49001 Angers cedex 01) and myxomatosis ELISA method (UMR960 Microbiologie moléculaire Ecole Nationale Vétérinaire 23 chemin des capelles F-31076 Toulouse cedex 3). VHD and myxomatosis serological results were expressed using ELISA tires calculated from measures the optical density in the wells in which the antigen antibody reactions occurred. VHD IHA method was performed by a reference VHD laboratory in France. The myxomatosis ELISA method preformed was the one described in published data (GELFI *et al.*, 1999).

Vaccine

The vaccine used during both studies is a combined vaccine against myxomatosis,

live attenuated homologous SG33 strain and against VHD, killed virus AG88 strain in aluminum hydroxide adjuvant. Both components were prepared in the same vial. The vaccine used in the safety study was batch L82485, shelf life 21.09.2002, intended for sale in France. Reconstituted vaccine was administered using the intradermal route. Injection of 0.2 ml of vaccine was performed intradermally at the lowest part of the ear pavilion. The vaccine used during serological follow-up study was the same combined vaccine, batch L19088, shelf life 06.04.2003, also intended for sale in France. Injection of 0.2 ml of vaccine was performed intradermally at one the flanks of the animal.

RESULTS

Safety

Visual examination after vaccination showed no vaccine vesicle was observed in 4 / 40 rabbits at the injection point at the lowest part of the ear pavilion (2 cases among adults and 2 other cases among young rabbits). In those 4, the injection was probably done deeper than initially planned, into the derma of the ear. The other rabbits (36 / 40) did display a vaccine vesicle at the injection point. General behaviour and external appearance in the 40 rabbits were not influenced by the vaccination during the entire 4-week-long observation period. No general post-vaccine reactions were observed any of the 40 rabbits. A rash, which was observed locally at the injection site 2 to 3 days after vaccination in 40% of the adults, disappeared one week later. All the 7-week-old vaccinated rabbits displayed this local reaction, which also resolved within a week. No difference was noted between dwarf and Angora dwarf rabbit populations.

Serological follow-up

Visual observation after vaccination showed that all the 40 rabbits from the study displayed a local vesicle of vaccine when administered intradermally *i.e.* in one of the flanks. Health of the animals was unchanged after vaccination. Anaesthetic protocol implementation led to animal losses during the study (2 / 40 after the second anaesthesia and then 2 / 38 after the third anaesthesia). Observed mortality was due either to anaesthetic product overdose before blood sampling or blood sampling technique.

Antibody production after vaccine administration was observed for both vaccine components. Maximum mean VHD IHA titre was obtained one month later and antibodies were still detected one month later. Mean VHD IHA titre following vaccine administration was higher in vaccinated animals than in unvaccinated control animals. In this latter group, medium VHD IHA titre was equal to 0 for the duration of the study (Table 2).

Table 2: Serological VHD IHA results.

	Mean VHD antibody titre on day of vaccination	Mean VHD antibody titre on D30	Mean VHD antibody titre on D60
Group 1 (n=20)	0 ± 0	0 ± 0	0 ± 0
Group 2 (n=20)	0 ± 0	192 ± 130	94 ± 39

Maximum mean myxomatosis ELISA titre was obtained two months further after vaccine administration. Medium titre was higher in vaccinated animals than in unvaccinated control animals the latter of which approached 0 (Table 3).

Table 3: Serological myxomatosis ELISA results.

	Mean myxomatosis antibody titre on day of vaccination	Mean myxomatosis antibody titre on D30	Mean myxomatosis antibody titre on D60
Group 1 (n=20)	40 ± 24	429 ± 352	889 ± 744
Group 2 (n=20)	355 ± 327	3300 ± 671	10518 ± 2417

DISCUSSION

Safety

The choice to include into the study dwarf rabbits was to validate the feasibility of the vaccine administration procedure, as they are often chosen as pet rabbits because of their size. Vaccine volume of 0.2 ml is important with regard to the size of the animals

especially in young animals. Additionally, it has to be administered once at one particular point into a very thin derma. The choice of Angora rabbits was to assess the safety of the live attenuated component of the vaccine in a breed known to be particularly sensitive to the myxomatosis viruses in field conditions. Three weeks after vaccination, 3 / 40 rabbits, all three of which were 7-week-old, displayed inflated bellies due to paresis of the caecum. A classical treatment based on antibiotics was implemented. The authors felt there was no correlation between these observations and vaccine administration.

Serological follow-up

The choice of different breeds of dwarf rabbit was led by the desire to validate the feasibility and the safety of the administration by intradermal route 0.2 ml of vaccine in a representative sample of animals currently chosen as pet rabbits in many countries. SG33 homologous vaccine component was well tolerated in comparison with Shope fibroma vaccines currently used to immunise pet rabbits. The choice of the analytical methods implemented in the laboratory was to establish references in serological follow-up in field conditions. Both laboratories duly validated both serological methods, LDA49 for VHD and Ecole Nationale de Toulouse for myxomatosis. Post-vaccine serological follow-up results were already published (LEMIÈRE, 2000). They already showed using two different analytical methods (another laboratory for VHD and immunofluorescence for myxomatosis) a seroconversion against VHD and myxomatosis. Two unvaccinated rabbits belonging to group 2 displayed seroconversion against myxomatosis. This observation was not confirmed by VHD serological results. As all the rabbits included into the study were adults possibly two of them had encountered a myxomatosis virus before their inclusion into the study. No signs of myxomatosis in any of the rabbits, young or adult, were noticed during the study.

CONCLUSION

Local and general safety of combined myxomatosis SG33 and VHD vaccine in dwarf rabbits using the intradermal route with a syringe was demonstrated in a representative sample of animals (80 rabbits in total). VHD IHA and myxomatosis

ELISA antibody production demonstrated vaccine efficiency of both components.

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