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SEROLOGICAL RESPONSE TO MYXOMATOSIS VACCINATION BY DIFFERENT INOCULATION SYSTEMS ON FARM RABBITS

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Abstract: In order to assess the serological response to different vaccination systems against myxomatosis, five groups of 20 rabbits each were inoculated with a vaccine prepared from the Shope fibroma virus (Mixohipra-FSA®). Administration systems differed between groups: subcutaneous injection (group A), intradermic inoculation in one-shot one-impact (group B), one-shot three- impacts (group C), two-shots one-impact (group D) and two shots three-impacts (group E). Clinical signs, general and local, as well as serological response at 28 days post-vaccination were compared among the different groups. Vaccination did not affect health status of rabbits but it produced nodules at the inoculation site, mainly in groups vaccinated by intradermic route where the presence and size of the nodules were closely related to serological response. Although no differences due to the administration system were found in the serological response ($P>0.05$), subcutaneous injection and two-shot intradermic administration provided the most homogeneous responses.

Key words: serological response, myxomatosis vaccination, inoculation systems.

INTRODUCTION

Myxomatosis is a viral disease caused by a *Poxvirus*. It affects domestic rabbits causing persistent problems in industrial rabbitries. The only way to control myxomatosis is through vaccination and bio-security measures. At present there are two types of vaccines to prevent myxomatosis: heterologous and homologous. In the first case, the antigen is the live Shope fibroma virus while in the second case it is the live attenuated myxoma virus, both belonging to the *Poxvirus* family, *Leporipoxvirus*

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genus.

Factors such as production intensity, stress or diseases like epizootic rabbit enteropathy (ERE) are at present undermining health status in rabbitries, mainly affecting young rabbits. We have currently seen an increase in adverse reactions in weaned rabbits following administration of homologous vaccines against myxomatosis (data not shown). These vaccines, even if they are more immunogenic and protect longer than heterologous types, may be immunosuppressive and show residual pathogenicity, specially in young animals (Pagès-Manté, 1995; Vautherot *et al.*, 1997; Brun *et al*., 1981). Although Marlier *et al.* (2000) reported that vaccination with Shope fibroma virus alone failed to prevent clinical signs, shedding of virus or tissue infection after a challenge with a virulent amyxomatous or a virulent nodular myxoma virus strain, our experience is that an adjuvanted heterologous vaccine protects rabbits from weaning to slaughter in industrial rabbitries without causing adverse reactions (Pagès-Manté, 2001). This difference could be due to the adjuvant in the vaccine and/or to the infectious pressure (experimental infection *vs* natural infection in field conditions).

Unfortunately, in Spain, it is not an extended practice to regularly vaccinate farm rabbits, although they are susceptible to infection (Pagès-Manté, 2002). Reasons for not vaccinating are usually cost and time. In order to facilitate vaccination and reduce transmission risks, different mechanical devices are available in the market as an alternative to injection needles. These tools allow intradermic inoculation of the vaccine but sometimes their improper keeping and handling lead to unsuccessful vaccinations and, consequently, contagious myxomatosis. Differences have also been reported regarding the homogeneity, magnitude and duration of immunity after vaccination with these devices (Espuña *et al.*, 1984).

The aim of this study was to compare the serological response of farm rabbits after myxomatosis vaccination by different inoculation systems.

MATERIALS AND METHODS

Animals and conditions

One hundred weaned crossbred New Zealand White/Californian male and female rabbits were used in the study. At the age of 35 days, when the trial started, they were separated into 5 groups (A, B, C, D and E; 20 rabbits/group) and housed in 10 flat-deck wire-mesh cages (100x40.5x38cm, 10 rabbits/cage) in an independent unit of an industrial rabbitry. They were fed a balanced granulated diet *ad libitum* during the whole trial.

Treatments and controls

The first day of the trial clinical signs were observed in all animals and individual blood samples were taken by puncture in the marginal vein of the left ear (1-1.5ml/rabbit). After blood sampling all rabbits were vaccinated with a live heterologous adjuvanted vaccine against myxomatosis (Mixohipra-FSA®, Laboratorios Hipra, S.A.- one dose/rabbit). Vaccine was used immediately after reconstitution. To vaccinate group A, the 25 doses contained in the freeze-dried tablet were diluted in 12.5ml of water for injection. For groups B and C 2.5ml of water for injection were used to dissolve the tablet whereas for groups D and E it was dissolved in 5ml. After vaccinating the 20 rabbits in each group, the remaining vaccine was eliminated as recommended by the manufacturer (incineration). The amount of virus received by each animal in the different groups was similar (live Shope fibroma virus strain OA $^{3}10^{3.5}$ $TCID_{50}$) but the inoculation system differed between groups, as it is detailed in Table 1.

For subcutaneous vaccination, needles of 0.9x40mm ($20G_{1/2}$ Nr.1 Microlance3®) were used and injections were done in the scruff of the neck. Intradermic vaccination was done with an automatic dermojet® (Societé Akra Dermojet) holding a one-orifice injection head (groups B and D) or a 3-orifice multi-jet head (groups C and E). In both cases, the jet head was placed in the mid region of the inner part of the right ear in close contact with the skin. Hairy areas were avoided as much as possible to ensure a correct penetration of the vaccine. The same person did the vaccination

of the five groups, as would have been done in the field routine.

Clinical signs were evaluated seven days after vaccination: general reactions such as anorexia, lethargy, etc., and local reactions at the inoculation site. When a nodule was found, its size was assessed by palpation and recorded using the following scores: small (<0.5cm), medium (0.5-1cm) or large (>1cm). Twenty-eight days after vaccination clinical evaluation was repeated and individual blood samples were taken as described before.

Blood samples were collected in tubes containing pearls. The day after sampling they were centrifuged (2500 rpm, 10 minutes) and, the following day, the extracted serums were analysed for the detection of specific antibodies against myxomatosis virus. An indirect ELISA was used (CIVTEST CUNI MIXOMATOSIS ®, Laboratorios Hipra, S.A.) and results were presented as a relative index (RI) obtained through the following formula (using mean DO_{450} values for controls):

Rabbits were considered to be seropositive when RI was higher than 20.

$$RI = \left[\frac{(DO_{450}\ \text{Sample} - \text{Mean}\ DO_{450}\ \text{Negative Control})}{(\text{Mean}\ DO_{450}\ \text{Positive Control} - \text{Mean}\ DO_{450}\ \text{Negative Control})} \right] \text{x}\ 100$$

Statistics

In each group, the frequency of rabbits showing clinical signs and the frequency of seropositive rabbits were calculated. χ^2 was used to compare theses frequencies. The mean value, standard deviation and coefficient of variation of the serological response (RI) were calculated in each group and compared by ANOVA. Simple correlations between the serological response and the presence and size of local reactions at the inoculation site were calculated in each group. Statistical analysis was performed with the SPSS 9.0 software.

Table 1: Design of the trial.

Group	Nº rabbits	Vaccination Route	Vaccine volume[2]	Vaccine administration	
A	20	Subcutaneous	0.5ml/rabbit	1 injection	-
B	20	Intradermic[1]	0.1ml/rabbit	1 shot	1 impact/shot
C	20	Intradermic[1]	0.1ml/rabbit	1 shot	3 impacts/shot
D	20	Intradermic[1]	0.2ml/rabbit	2 shots (0.1ml/shot)	1 impact/shot
E	20	Intradermic[1]	0.2ml/rabbit	2 shots (0.1ml/shot)	3 impacts/shot

[1]With Dermojet (Societé Akra Dermojet), [2]Independently of the inoculated volume, each dose contains live Shope fibroma virus strain OA≥ $10^{3.5}$ $TCID_{50}$.

RESULTS

Clinical signs

Rabbits showed no general signs before or after vaccination. Only one animal of group A had slight diarrhoea 7 days after vaccination. It was not included in the statistical analysis because it was not considered to be a post-vaccination effect.

Local signs were seen at 7 and 28 days post-vaccination (Table 2). In both cases the proportion of rabbits with nodules at the inoculation site was higher in groups vaccinated by intradermic route (groups B, C, D and E; $P<0.001$). In all groups the number of rabbits showing nodules decreased from 7 to 28 days. In group A no nodule was palpated at 28 days post-vaccination.

There were more rabbits with large nodules (>1cm) in groups vaccinated with one-impact dermojet (groups B and D) and more rabbits with medium nodules (0.5-1cm) when vaccinated with three-impacts dermojet (groups C and E) at 7 days post-vaccination. Three weeks later the same tendency was seen, with groups C and E showing a higher proportion of rabbits with small nodules (<0.5cm) compared to groups B and D.

Serological response

None of the rabbits had antibodies against myxomatosis virus before vaccination (day 0). Four weeks later there was a seroconversion in response to vaccination in all groups (Table 4). The proportion of rabbits that seroconverted was high in groups A, C, D and E 90%) and significantly lower in group vaccinated with one-impact dermojet (group B 70%, $P<0.05$).

The variability of the relative index (RI) was high in all groups (coefficient of variation 0.42, 0.76, 0.56, 0.28 and 0.44 in groups A, B, C, D and E respectively), but it decreased when only seropositive rabbits were included in calculating the mean RI of each group (coefficient of variation 0.36, 0.36, 0.42, 0.28 and 0.28 in groups A, B, C, D and E respectively).

Correlations between local clinical signs and serological response

There was a significant relationship between the presence and size of nodules and the serological response in rabbits vaccinated by intradermic route (Tables 5 and 6). This relationship was more pronounced when nodules were assessed at 7 days post-vaccination. At this time, rabbits vaccinated with one-impact dermojet (group B) showed a higher correlation with their serological response than those

Table 2: Number (and percentage) of rabbits showing local clinical signs after myxomatosis vaccination by different inoculation systems.

Group	Day 0	Day 7 p.v.[1]	Day 28 p.v.[1]
A	0 (0%)	2 (10%)	0 (0%)
B	0 (0%)	14 (70%)	6 (30%)
C	0 (0%)	18 (90%)	14 (70%)
D	0 (0%)	20 (100%)	14 (70%)
E	0 (0%)	19 (95%)	8 (40%)
Significance	-	***	***

[1]p.v.: post vaccination. *** $P<0.001$

vaccinated with the three-impact dermojet, either with one or two shots (groups C and E). At 28 days post-vaccination, rabbits in group E showed no relationship between the presence or size of nodules and their level of antibodies, while groups B, C and D all presented a significant and quite similar relation.

DISCUSSION

Vaccination of weaned seronegative rabbits did not affect their health status as described previously (ESPUÑA *et al.,* 1984) but, on the other hand, it caused local reactions at the inoculation site, specially in rabbits vaccinated by intradermic route (Table 2). The vaccine antigen, Shope fibroma virus, is known by the appearance of a localized fibroma when injected into European rabbits (*Oryctolagus cuniculus*) (FENNER, 1994). These fibromas were easier to detect when localized in the ear than at subcutaneous level. They were transient, decreasing in size or even disappearing (Table 3). Nodule size seemed to be related to the amount of antigen injected at the inoculation site; the more diluted and spread the antigen , the smaller the nodule .

Instead of considering nodules as an adverse reaction, they could serve as an indicator of a positive humoral response to vaccination, as suggested by the high

Table 3: Number (and percentage) of rabbits showing nodules of diverse sizes after myxomatosis vaccination by different inoculation systems.

	Nodules size[2] 7 days p.v. [1]			Nodules size[2] 28 days p.v. [1]		
	Small	Medium	Large	Small	Medium	Large
A	0 (0%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)
B	3 (15%)	2 (10%)	9 (45%)	1 (5%)	5 (25%)	0 (0%)
C	5 (25%)	13 (65%)	0 (0%)	13 (65%)	1 (5%)	0 (0%)
D	0 (0%)	4 (20%)	16 (80%)	7 (35%)	4 (20%)	3 (15%)
E	9 (45%)	10 (50%)	0 (0%)	8 (40%)	0 (0%)	0 (0%)

[1]p.v.: post vaccination, [2]Small <0.5cm, Medium: 0.5-1cm, Large >1cm.

correlations found in this study (Tables 5 and 6). TRIPATHY and REED (1997) described something similar after vaccination of poultry against pox (Family *Poxviridae*, genus *Avipoxvirus*). They reported the presence of "takes" after vaccination. A "take" consisted of swelling of the skin or a scab at the site where the vaccine was applied and it was considered as evidence of successful vaccination. In rabbits, JACOTOT *et al.* (1955) did not report a correlation between the size of the vaccination nodule and the level or extent of the immune response but, in that study, heterologous vaccines were applied by a subcutaneous route, coinciding with our results (group A) and those of NOUGAYREDE and GAYOT (1980).

We saw a higher percentage of seronegative rabbits in group B, vaccinated with one-shot one-impact dermojet (Table 4). The chance of the vaccine entering the skin by this system is smaller if compared to the other devices (3 impacts or two shots). If compared to subcutaneous injection, there are more critical points related

Table 4: Serological response of rabbits after myxomatosis vaccination by different inoculation systems.

	Number (and percentage) of seropositive rabbits		Relative Index[2] Mean (SD)		
Group	Day 0	Day 28 p.v. [1]	Day 0	Day 28 p.v. [1]	Day 28 p.v. [1, 3]
A	0 (0%)	19 (95%)	1.20 (0.89)	74.15 (30.79)	77.53 (27.57)
B	0 (0%)	14 (70%)	1.05 (0.22)	53.55 (40.77)	75.36 (26.75)
C	0 (0%)	18 (90%)	1.20 (0.70)	80.55 (44.81)	89.39 (37.64)
D	0 (0%)	20 (100%)	1.00 (0.00)	82.75 (23.49)	82.75 (23.49)
E	0 (0%)	18 (90%)	1.00 (0.00)	75.85 (33.30)	83.83 (23.74)
Significance	-	*	NS	NS	NS

[1]p.v.: post vaccination, [2]Cut-off ELISA: seropositive when Relative Index>20, [3]Mean value of seropositive rabbits. NS: Non significanc, $P>0.05$, * $P<0.05$.

to the handling of the dermojet which can explain the higher proportion of failures. This reflects what is happening in the field and allows us to understand vaccination failures in industrial rabbitries. Factors such as pressure, dosage, placing of the dermojet, penetration of vaccine at an intradermic level, etc. are critical points that need to be checked when vaccinating with this device. We thought that there were penetration failures while vaccinating group B because there was a coincidence between the absence of serological response and reaction at the inoculation site (Tables 2 and 4).

The magnitude of the serological response (Table 4) was in the same range as in previous works (ESPUÑA *et al.*, 1984; PAGÈS-MANTÉ and Alfonso, 2002). The dispersion of the RI revealed that both subcutaneous and two-shot intradermic were the administration systems which produced the most homogeneous humoral responses. When only seropositive rabbits were considered, there were no observed differences in the serological response due to the vaccination system used. In this case it was also seen that two-shot intradermic inoculation induced a high, uniform serological response.

Table 5: Correlations between serological response (RI) and the presence of local reactions at the inoculation site after myxomatosis vaccination by different inoculation systems.

Relative Index	Local reaction day 7 p.v. [1]	Local reaction day 28 p.v. [1]
Group A	0.27	-
Group B	0.84**	0.52**
Group C	0.61**	0.56**
Group D	-	0.57**
Group E	0.49*	0.05

[1]p.v.: post vaccination. ** $P<0.01$, * $P<0.05$

Other authors comparing administration systems also reported a good initial serological response after one-shot one-impact dermojet vaccination (ESPUÑA *et al.*, 1984) but, as we found, it was more heterogeneous and also less persistent in time. In our study only humoral response in broiler rabbits was assessed (lasting no longer than the ordinary fattening period in Spain) but, if breeders were to vaccinate in the future, it should be amplified to see if the extent of the serological response is affected by the administration system.

Even if we did recommend vaccinating farm rabbits by a subcutaneous or two-shot intradermic route, it should be remembered that the present results have been obtained with a reduced group of healthy rabbits, if compared to certain field circumstances where stress and other diseases are common. These immune-depressing factors could negatively affect the rabbit's serological response to vaccination by reducing or increasing its heterogeneity. Both a correct injection with hygienic needles and a proper handling of the dermojet are critical points that have to be ensured for successful vaccination.

Table 6: Correlations between serological response (RI) and the size of local reactions at the inoculation site after myxomatosis vaccination by different inoculation systems.

Relative Index	Local reaction day 7 p.v.[1]	Local reaction day 28 p.v.[1]
Group A	0.26	-
Group B	0.89**	0.46*
Group C	0.62**	0.45*
Group D	0.17	0.64**
Group E	0.49*	-0.01

[1]p.v.: post vaccination. ** $P<0.01$, * $P<0.05$.

CONCLUSIONS

Vaccination by the different systems did not affect the health status of rabbits but it did produce nodules at the inoculation site, mainly in groups vaccinated by intradermic route, where the presence and size of the nodules were closely related to serological response. Although no differences due to the administration system were found in the serological response, subcutaneous injection and two-shot intradermic administration provided the most homogeneous responses.

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