SEROLOGICAL EVALUATION OF THE IMMUNITY INDUCED IN COMMERCIAL RABBITS BY VACCINATION FOR MYXOMATOSIS AND RHD

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ABSTRACT

For both Rabbit Haemorrhagic Disease and Myxomatosis viruses, vaccination is made in order to increase the specific humoral and/or cellular immune response of the animals and protect against disease. The aim of our work is to detect the humoral immune response, using competition ELISAs (cELISA) as diagnostic tests, in breeders and fattening rabbits vaccinated with different types of vaccines and by variable route of administrations against myxomatosis and RHDV. Difference in serological response were analysed by mixed model on a logaritmic transformation of titres. In all the vaccinated groups a specific seroconversion was observed. As expected the highest average values were detected among multiparous breeders, which had been previously vaccinated several times. For breeders, the linear mixed models for serological responses show a very important interaction effects between “Day of Sampling” and “Kind of Vaccination” group. The most relevant difference in fattening rabbits vaccinated for the first time against myxomatosis was, as expected, the absolute lower levels of antibodies detected. The linear mixed model for Myxomatosis serological response in fattening rabbits show a significant interaction between “Day of Sampling” and “Kind of Vaccination”. Moreover, by using RHDV serological methods (cELISA and anti-isotype IgG-IgA-IgM ELISAs) all the rabbits tested at 28 days of age showed passive antibodies of maternal origin against RHD. Therefore we cannot exclude that the presence of antibodies at different titres at the moment of first vaccination could have conditioned the effect of immunization. The results obtained in this study demonstrate that serological methods could be a valid aid in monitoring the efficacy of vaccination for both Myxomatosis and RHD. Using methods having a high level of specificity, it was possible to detect seroconversion in groups of rabbits vaccinated with different type of vaccines. Indeed, serology and particularly the combination of RHDV anti-isotypes ELISAs could help to improve the quality of vaccination by determining the more convenient moment to administrate the first vaccination in relation to the disappearance of maternal antibodies.

Key words: serology , vaccination, RHD, myxomatosis, ELISA.

INTRODUCTION

Rabbit haemorrhagic disease (RHD) is a highly contagious and acute fatal disease characterised by a high morbidity and mortality (40-90% of affected rabbits). In Italy, RHD was first reported in 1986 (CAPUCCI et al 1991). Infection can occur in rabbits of all ages but clinical disease is observed only in animals >40-50 days of age. Where RHD is endemic, an indirect control of the disease in industrial rabbitries is successfully achieved by vaccination. Vaccines are usually prepared by using clarified liver suspension of experimentally infected rabbits, subsequently inactivated and adjuvated. Vaccinated animals quickly produce strong humoral immunity.
Myxomatosis is a contagious, diffusive and fatal disease of domestic and wild rabbits, caused by a Leporipoxvirus (Fam. Poxviridae). It has been firstly described one century ago in *Sylvilagus* spp. in South America and then introduced in Europe on '50, where rapidly became endemic (FENNER 1994). Two clinical forms are described: the classical form that prevalently affects rural breeds, and the respiratory form that is typical of industrial units. The disease can be controlled by the adoption of strict measures of direct prophylaxis *plus* attenuated vaccines. However myxomatosis could prove to be very difficult to control because: a) the virus elicits mainly cellular immunity, thus determining a variable individual response and a high variability of the level and period of protection; b) the clinical signs and epidemiological aspects are extremely variable and 3) the available vaccines could show residual pathogenicity difficult to differentiate from wild strains and need repeated inoculations to be efficacious. For both RHD and Myxomatosis viruses the vaccination is made in order to increase the specific humoral and/or cellular immune response of the animal and protect against disease. Since the presence of maternal antibodies induces a neutralisation of vaccine effects, it is therefore very important to ascertain the presence and duration of passive antibodies in order to choose the optimal period for vaccination and induce an active immunisation.
The aim of our work is to show the variability of the humoral immune response due to vaccination with different vaccines and route of administrations in breeders and fattening rabbits, through taking of blood samples at different time points post vaccination. In our study we used two types of vaccine against myxomatosis for each group of breeders and fattenings, and two different types of vaccine against RHDV in the group of breeders. We also searched for passive antibodies against RHDV in non-vaccinated fattening rabbits in order to predict the optimal period for the vaccinal prophylaxis.

MATERIAL AND METHODS

Animals

We considered two categories of animals:
Breeders: 4 groups of 20 multiparous does each, all originating from the same farm. In order to have two replicas of the same experiment, we selected two pairs of groups (A-C and B-D) recovered in separate sheds. These rabbits had been already vaccinated against RHD and Myxomatosis using two separate vaccines. The serological status at the moment of vaccination (D0) was established by randomly testing 20 rabbits from each shed. After that, the rabbits belonging to each of the 4 groups were vaccinated following the scheme reported in table 1 and serologically controlled at 15 (D15) and 30

days (D30) post vaccination. A similar trial was repeated in a different farm. After having determined the serological status at the moment of vaccination (D0) by randomly testing 12 rabbits, two groups of 10 multiparous already vaccinated does (X and Y) were vaccinated applying the same vaccination schedule as before. Then, they were kept under observation for a longer period and thus serologically controlled not only at D15, D30 but also at 45 dd post vaccination (D45).
Fattening rabbits: 4 groups of 20 rabbits each, all coming from the same farm. Again, in order to have two replicas of the same experiment, we selected two pairs of groups (E-F and G-H), recovered in separate sheds and originating from two consecutive production cycles with a ten days interval. The serological status at the moment of vaccination (D0) was established by randomly testing 15rabbits from each shed. The rabbits were vaccinated at 28 days of age only for myxomatosis, using two different types of vaccines and following the scheme reported in table 1. They were kept in two separated sheds, weaned at 42 days of age and serologically controlled at D15 and D30.

Table 1: Vaccination scheme and tests performed

Groups	Vaccine	Number of rabbits	Day p.v.	ELISA Myxo	ELISA RHD	ELISA RHD isotype
Breeders						
AB – CD - XY	pre vaccination	20 + 20 + 10	0	Y	Y	N
A - C	Dercunimix®	20	15 - 30	Y	Y	N
B - D	Rabbit Mixo® + Rabbit MEV®	20	15 - 30	Y	Y	N
X	Dercunimix®	10	15 – 30 - 45	Y	Y	N
Y	Rabbit Mixo® + Rabbit MEV®	10	15 – 30 - 45	Y	Y	N
Fattenings						
EF - GH	pre vaccination	15 + 16	0	Y	Y	Y
E - G	Dervaximyxo®	20	15 - 30	Y	Y	Y*
F - H	Rabbit Mixo®	20	15 - 30	Y	Y	Y*

Y = yes ; N = no ; *only at D15

Vaccines

Dercunimix® is a vaccine against both Myxomatosis and RHD. It is obtained from the union of a live attenuated vaccine prepared with the SG 33 strain of myxoma virus and an inactivated vaccine prepared with the AG 88 strain of RHDV and aluminium hydroxide as adjuvant. It is administered by intradermic via in the internal face of the ear. The characteristics and efficacy of this vaccine were described by LEMIERE (2000). *Dervaximyxo®* is used for a vaccinal prophylaxis against myxomatosis and it has the same characteristics of the previous concerning this valence. *Rabbit Mixo®* is a live attenuated vaccine prepared with the BTK/RB/84 strain of myxoma virus and it is administered by intradermic via inside the ear. *Rabbit Mev®* is an inactivated vaccine derived from a field Italian strain of RHDV and aluminium hydroxide as adjuvant. It is administered by subcutaneous via in the neck region.

Serological methods

The serological tests used in routine are based on a competitive ELISA (cELISA). The anti RHDV antibodies test was standardized using sera from commercial rabbits. The technical procedure and the steps for performing such method are already described in details elsewhere (CAPUCCI and LAVAZZA 2000). The cELISA for myxomatosis has been similarly developed and validate by ourselves (CAPUCCI, personal data). It is based on the use of two different MAbs (one as catcher and the other for competition) and a purified virus, strain California grown on RK13 cell culture, as antigen. According to the test procedures applied, for both cELISAs the serum titre corresponds to the dilution giving an absorbance value equal to 50% (±10) of the value of the negative serum at dilution 1/160 (reference value). In order to improve the serological interpretation and to correctly classify the RHDV immunological status of the rabbits belonging to some groups (Table 1), a combination of ELISA techniques that distinguish IgA, IgM and IgG antibody responses was employed and used to detect anti-isotype specific anti-RHD antibodies (COOKE et al, 2000). The technical procedure and the steps for performing such method are already described in details elsewhere (CAPUCCI and LAVAZZA 2004). Sera are considered to be positive if the OD value at the 1/40 dilution is more than 0.2 OD units (two standard deviations) above the value of the negative serum used as a control. The titre of each serum is taken as the last dilution giving a positive value.

Statistical Analysis

Difference in serological response were analysed by mixed model (VENABLE and RIPLEY 2002) on a logaritmic transformation of titres, according to formula log (titre + 1), in order to take account of zero value of negative results. In the model the “Day of Sampling” i.e. the repeated measures at D15, D30, D45 of rabbits belonging to each group and the “Kind of Vaccination” i.e. the different types of vaccination, were considered as fixed effects, while the “Subjects” (units of analysis) were considered as random effects nested in Herds; the “Herd” was considered a random effect as well. For Breeders animals we fit two different models: for serological response to Myxomatosis and to RHD respectively. For Fattening animals we fit one model on serological response to Myxomatosis. All statistical analysis and plot were made with statistical software R (R DEVELOPMENT CORE TEAM 2003).

RESULTS AND DISCUSSION

The results of ELISA tests and of their statistical evaluation are schematically reported in the Table 2 and 3, and Figure 1 and 2. In all the vaccinated groups a specific seroconversion was observed.

Breeders

In table 1 are shown mean and standard deviation of log Titre for Breeders animals. As expected the highest titres were detected among multiparous breeders, which had been previously vaccinated several times. Indeed, the values of D0 titres, calculated in order to determine the serological status of the herd at the moment of vaccination, clearly indicated the presence of a relevant immunity against both diseases. A booster effect

was particularly evident in almost all breeders' groups at D15 but it should be noted that the titre values detected at D30 and D45 were equal or even lower than those detected prior vaccination for Myxomatosis while they were equal or higher for RHD. These results agree with those previously reported in literature concerning the lasting of antibodies after vaccination, which indicate a quite short period of persistence of anti-myxomatosis antibodies, i.e. 12 weeks, using the attenuated SG33 strain (PICAVET et al 1989) and a much longer life of anti-RHD antibodies, i.e. more than one year, using an inactivated and adjuvated vaccine (ARGUELLO VILLARES J.L. 1991).

Table 1: Serological Results in Breeders

	D0	D 15	D 30	D45
Myxomatosis	5.65(1.03)	6.31(1.27)	5.66(1.58)	4.33(0.94)
Dercunimix®		6.45(1.15)	5.46(1.05)	4.79(0.90)
Rabbit Mixo® + Rabbit Mev®		6.17(1.25)	5.87(1.23)	4.60(0.97)
RHD	5.16(2.09)	6.20(1.27)	6.06(1.32)	4.33(0.94)
Dercunimix®		6.53(1.26)	6.08(1.44)	3.71(0.64)
Rabbit Mixo® + Rabbit Mev®		5.92(1.22)	6.05(1.21)	5.08(0.65)

Both linear mixed models for serological responses show a very important interaction effects between "Day of Sampling" and "Kind of Vaccination" group. Comparison between Vaccination groups in the different Day show the existence of a significative difference in mean of log titres for RHD serological response at D15 ($p=0.016$) and at D45 ($p<0.0001$). For myxomatosis, the difference between Vaccination groups at D30 correspond to a p value = 0.06, i.e. around the threshold of statistical significance ($p=0.05$). In Figure 1, are reported the serological responses in the different Vaccination groups for the two viral diseases.

Fattening

In table 2 are shown mean and standard deviation of log Titre for myxomatosis. In these rabbits, vaccinated for the first time against myxomatosis at 28 days of age, the most relevant difference towards breeders was the absolute lower levels of antibodies detected both at D15 and at D30. Nevertheless, at vaccination (D0) the rabbits did not tested negative as expected but they showed low titres, most likely of maternal origin, of both anti-RHD and anti-myxomatosis antibodies In order to get the proof of the passive origin of these antibodies we tested the D0 and D15 sera using the anti-isotype ELISAs for RHD. We detected high IgG titres but not IgM titres, thus excluding a recent immunization, neither IgA, which are normally produced only after natural infection (COOKE et al 2000). The values of the IgG titres were higher at D0 than D15, indicating a progressive decline of maternal antibodies. Even if we did not get a similar result for anti-myxomatosis antibodies due to the absence of similar methods for detecting Ig subclasses, we can suppose that the titres found at D0 could similarly be of maternal origin. Therefore we cannot exclude that the presence of antibodies at different titres at the moment of the first vaccination could have conditioned the effect of immunization.

Table 2: Serological Results in Fattening animals

	D0	D 15	D 30
Myxomatosis	2.88(0.82)		
Dervaximyxo®		3.57(1.27)	3.74(1.50)
Rabbit Mixo®		2.85(1.21)	1.76(2.00)
RHD cELISA	3.78(0.71)	2.56(1.22)	n.d.
RHD isotype (IgG)	7.35(0.90)	5.84(1.05)	n.d.

The linear mixed model for Myxomatosis serological response in fattening rabbits shows a significant interaction between “Day of Sampling” and “Kind of Vaccination”. In fact, the comparison between Vaccination groups in the different Day, show a significative difference in mean of log titres, at D15 ($p<0.05$) and at D30 ($p<0.0001$). In Figure 2, are reported the serological response in different vaccination groups for different disease.

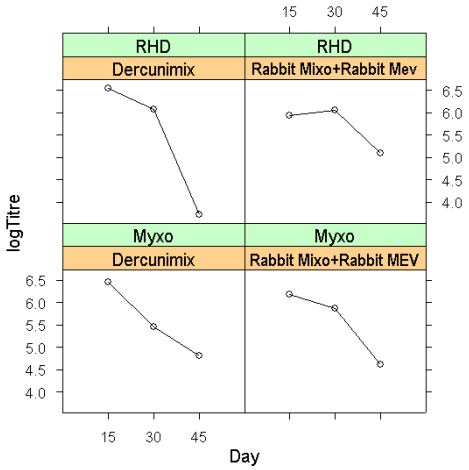

Figure 1: Serological response by Disease and Vaccination Group in breeders animals

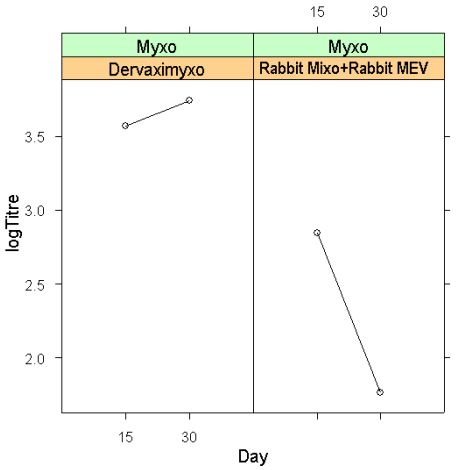

Figure 2: Serological response by Vaccination Group in fattening animals

Indeed, a discrepancy was observed between the two replicates of the same trial (data not shown). In particular, the seroconversion resulted more evident in the rabbits of the first replicate that show the lower initial level of antibodies: D0=2.75(0.60), D15=2.91(1.20), D30=3.45(2.10), than in those of the second replicate: D0=3.02(0.99), D15=3.5(1.31), D30= 2.05(1.69). In addition, in animals of the second replicates we detected at D30 a sharp decrease of the level of antibodies. This situation could be due to a unknown cause that could have hampered the immune response of some rabbits but it can not be also excluded a lower capacity of live attenuated vaccines to stimulate the immune system in presence of residual specific antibodies of maternal origin, and in this case Rabbit Mixo® seems to be even more impaired than Dervaximyxo®.

CONCLUSIONS

The results obtained in this study demonstrate that serological methods could be a valid aid in monitoring the efficacy of vaccination for both Myxomatosis and Rabbit Haemorrhagic Disease. Using methods presenting high level of specificity (cELISAs) it was possible to detect seroconversion in groups of rabbits (breeders and fattening rabbits) vaccinated with different type of vaccines. In particular the combined RHD + Myxomatosis vaccine (Dercunimix®) was as effective as separate vaccines to induce immunity or create a booster effect in breeders. Indeed, serology and particularly the combination of anti-isotypes RHDV ELISAs could help to improve the quality of vaccination by determining the more convenient moment to administrate the first vaccination in relation to the disappearance of RHDV maternal antibodies.

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