EFFICACY OF ERAVAC® AGAINST A HETEROLOGOUS CHALLENGE WITH A VIRULENT *RHDV-2* STRAIN IN THE PRESENCE AND/OR ABSENCE OF MATERNAL DERIVED ANTIBODIES

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ABSTRACT

Vaccination against the variant of rabbit haemorrhagic disease (RHDV-2) is the principal measure available for the protection of young animals against this lethal virus. However, limited scientific information is available about the impact of maternally-derived antibodies (MDA) on vaccination.
The aim of the present study was to evaluate the efficacy of ERAVAC® (HIPRA, Spain) under the influence of MDA against an experimental challenge using a heterologous RHDV-2 strain, in 1-month old rabbits. Two groups of 20 rabbits were vaccinated subcutaneously. One of these groups consisted of animals without MDA and the other one consisted of animals having MDA. The MDA titres found in the latter were representative of the titres found in animals under field conditions. Additionally, another group of 20 rabbits with MDA was kept as a (non-vaccinated) control and was followed weekly for decay of MDA. When MDA had disappeared in this control group, all the animals were challenged with a heterologous virulent strain of RHDV2 by the intramuscular route. After challenge, mortality rates were followed up to support the efficacy of ERAVAC® in the presence of MDA. The results showed that vaccination with ERAVAC provides full protection against mortality after experimental challenge in the presence of MDA as well as in the absence of MDA in RHDV2-infected young rabbits, whereas the control animals suffered significantly greater mortality. This study helped to demonstrate that MDA have no effect on ERAVAC® vaccination, which is the main alternative for RHDV2 control on farms.

Keywords: RHDV-2, vaccination, maternally-derived antibodies.

INTRODUCTION

The emergence of a new RHDV-related virus (RHDV-2) in 2010 clearly affects the rabbit industry, causing mortality and inducing significant economic losses. This new variant of the virus is characterised by spreading worldwide within a short period of time and showing higher prevalence than classical RHDV isolates in kits and adult rabbits. But one of the most relevant characteristics of this pathogen is that it causes disease in rabbit kits (Le Gall et al. 2013).

During the last few years, inactivated vaccines have been developed and marketed in the European Union Member States (Valls et al. 2016). Vaccination has been one of the main preventive measures against RHDV. The vaccination strategy has allowed the control of RHDV-2 disease on farms, showing early protection against mortality and clinical signs after experimental challenges (Montbrau *et al.* 2016, Le Minor *et al.* 2019).

However, when adult rabbits suffer from this disease and survive, these animals could confer protection of their progeny through maternally-derived antibodies (MDA). No data are available about the influence of MDA on the efficacy of inactivated vaccines.

The aim of this study was to assess the efficacy of an inactivated vaccine against a heterologous challenge with a virulent RHDV-2 strain in the presence of MDA or not.

MATERIALS AND METHODS

Animals and experimental design

Sixty New Zealand White female rabbits of 28 days of age were purchased from two different sources, twenty animals from an animal supplier free of major rabbit diseases, including RHDV-2, and the other 40 from a commercial farm that had been suffering RHDV-2 outbreaks, where rabbit kits had high titres of MDA. After one week of acclimatisation, the animals were distributed into three groups of 20 animals. The 20 rabbits that were free of antibodies against RHDV-2 were assigned to group A, which was immunised with an inactivated vaccine against RHDV-2 (ERAVAC®, Laboratorios HIPRA S.A.) by subcutaneous administration following the manufacturer's instructions. The other 40 animals, which had antibodies against RHDV-2, were randomly allocated into 2 groups of 20 subjects each (groups B & C). Group B was immunised in the same way as group A, whereas group C was vaccinated with sterile PBS (phosphate-buffered saline).

From vaccination to challenge day, serum antibodies of group C were analysed weekly to assess the decay of MDA in this group. Fourteen days after vaccination, none any of the rabbits in group C had positive sera. The challenge was then conducted in all the animals in the three groups with a heterologous virulent RHDV-2 strain, with the administration of 1 ml of viral suspension containing 1000 HAU by intramuscular injection.

General clinical signs and local reactions of all the animals were monitored daily throughout the study. During the vaccination phase, the rectal temperature of group A (MDA-Vac) was recorded one day before vaccination, on the day of vaccination, 4 hours post-vaccination and daily for 6 days. After challenge, all the animals were monitored twice per day to record mortality. Serum samples were collected on the day of vaccination and weekly in the control group to assess the decay of MDA. These serum samples were tested for antibodies against RHDV-2 by competitive ELISA (c-ELISA; OIE, 2010). For the purposes of data representation, doubtful results (cELISA titres below the threshold value) were considered to be negative. The livers of all the animals that died during the study were analysed to check the presence of RHDV-2 virus by hemagglutination.

Mortality and serological response data were analysed using Chi-Square test. These analyses were performed using R-studio and statistical significance is defined as *P*-values less than 0.05.

RESULTS AND DISCUSSION

No general clinical signs or adverse reactions were observed in the vaccinated groups (MDA- and MDA+), suggesting that the administration of ERAVAC® is safe in terms of systemic reactions independently of the MDA. However, one animal in group B (MDA+Vac) and one animal in group C (MDA+Control) suffered unrelated diarrhoea and died 10- and 14-days post-administration, respectively. The liver samples of those animals were tested for the presence of RHDV-2, giving negative results. Consequently, group B (MDA+Vac) and C (MDA+Control) had 19 animals on the day of challenge.

In terms of local reactions, these were only noticed in two animals in group A (MDA-Vac), which showed slight inflammation of grade 1 (less than 2 cm in diameter) on day 2 post-vaccination. However, 24 hours later the slight inflammation could no longer be detected. No local reactions were noticed in group B (MDA+Vac).

After vaccination, specifically 2 and 3 days post-vaccination, all the animals suffered a slight increase in rectal temperature which in no case exceeded 40.5 °C. Furthermore, the maximum average increase

observed two days after vaccination was 0.89ºC compared to rectal temperatures one day before vaccination. On day 4 post-vaccination only 2 vaccinated animals had a rectal temperature slightly above 40ºC, and all the animals were within normal parameters on day 5 post-vaccination and onwards (figure 1).Therefore, all the animals met the requirements of the European Pharmacopoeia. That is, no animal showed an increase of more than 2 °C in rectal temperature and the average increase of the group did not exceed 1.5 °C

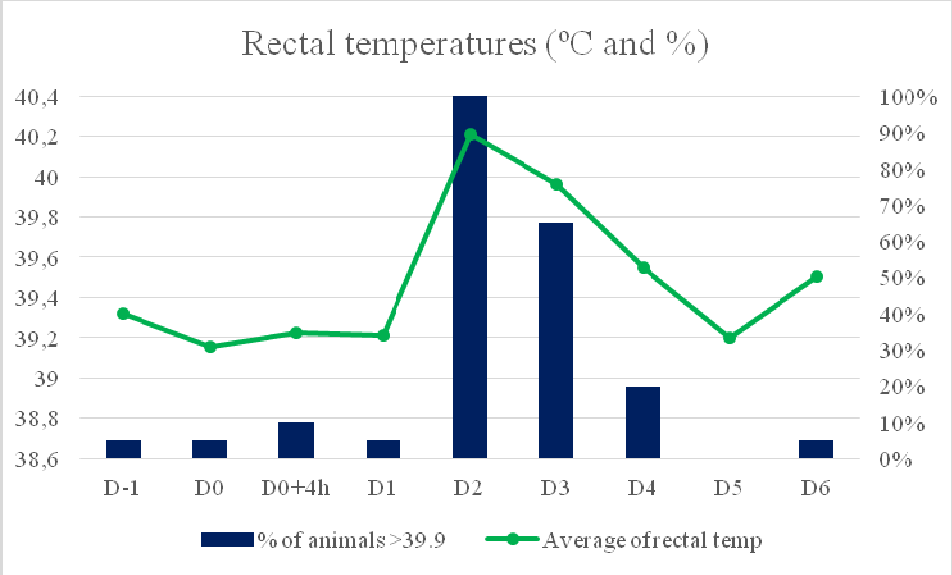

Figure 1: Average rectal temperature and percentage of animals over 39.9ºC in the MDA negative – vaccinated group around vaccination day (D0).

All the animals in group A (MDA-Vac) had no antibodies against RHDV-2 the day of vaccination. On the other hand, all animals in groups B and C had antibodies against RHDV-2 (table 1).

Table 1. Results of cELISA for each group on the day of vaccination (D0)

	Results cELISA		
	Positive	**Doubtful**	**Negative**
MDA-Vac	0^{b}	0^{b}	20^{a}
MDA+Vac	9^{a}	11^{a}	0^{b}
MDA+Control	4^{ab}	16^{a}	0^{b}

^{ab}Values with different superscripts are statistically different between groups (rows) using Chi-Square test ($p<0.05$).

After vaccination, group C (MDA+Control) was sampled weekly to collect serum samples. Those samples were analysed using cELISA. Seven days later (D7), 8 control animals were doubtful and 12 were negative. Fourteen days after vaccination, only 4 animals were doubtful and 15 were negative. Consequently, the challenge was conducted 14 days after vaccination.

The challenge strain V-1035 was chosen. It consists of a different strain (heterologous) from that included in the composition of the vaccine, as required by the European Pharmacopeia (Ph. Eur. monograph on the Efficacy of Veterinary vaccines 5.2.7). It was isolated from the liver of 28-days-old rabbits that died during an outbreak of RHDV2 in Spain. The strain was isolated by the diagnostic centre of Laboratorios Hipra (Diagnos) on 2012. Thus, it consists of a virulent field strain, recently isolated in Spain and representative of the current strains circulating in EU.

Then, following the challenge, all the vaccinated animals (MDA+ and MDA-) survived the experimental infection using a virulent heterologous strain of RHDV-2, whereas 9 out of the 19 control animals (MDA+Control) died during the seven days after challenge (Table 2).

Table 2. Number of rabbits, dead animals and survival rate for each group after challenge.

Group	No. rabbits (Ch. Day)	Dead animals	Survival rate
MDA (-) Vac	20	0[b]	100%[a]
MDA (+) Vac	19	0[b]	100%[a]
MDA (+) Control	19	9[a]	53%[b]

[ab]Values with different superscripts are statistically different between groups (rows) using Chi-Square test ($p<0.05$).

The livers of all the animals that died were analysed to check for the presence of RHDV2 virus by hemagglutination. The results obtained confirm the presence of RHDV2 in the livers of the animals that died after challenge.

In view of these results, the efficacy of ERAVAC® against the new variant RHDV2 in the presence of MDAs is clearly demonstrated. The vaccine has been shown to protect vaccinated animals with and without MDAs by obtaining 100 % survival rates after challenge at 14 days post-vaccination. Differences between the control and vaccinated groups were statistically significant.

CONCLUSIONS

The present study demonstrates that maternally-derived antibodies did not interfere with or affect the immune response generated after the administration of ERAVAC®. This inactivated vaccine is clearly effective in providing protection against RHDV-2 experimental challenge using a heterologous strain. Therefore, this study helps to describe the capacity of some inactivated vaccines to induce immunisation independently of the maternally-derived antibodies.

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