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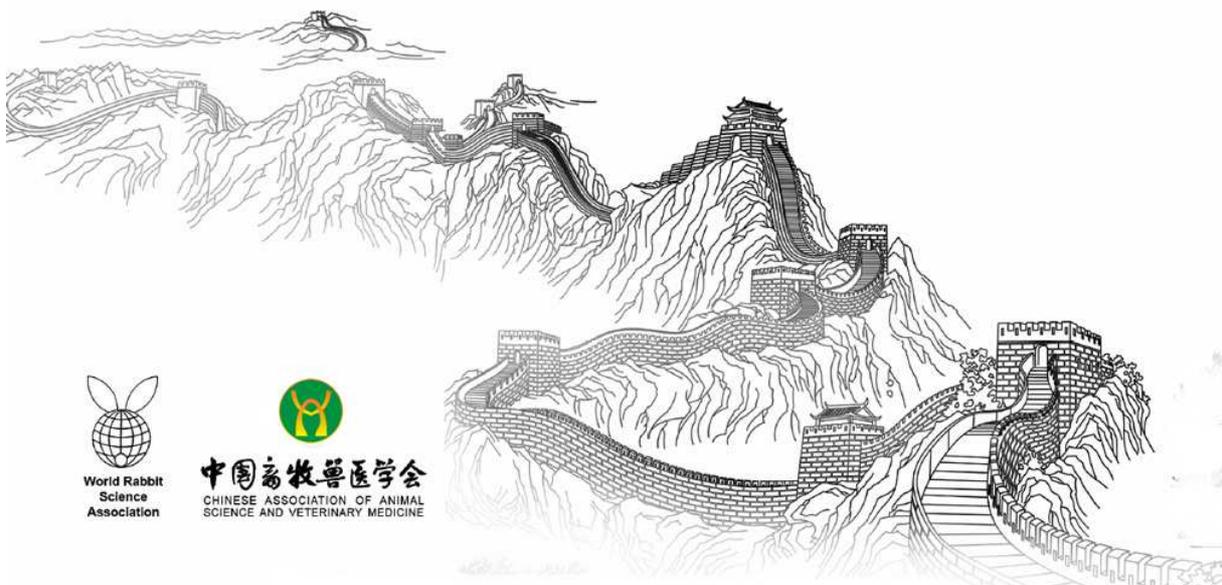
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EFFICACY AND SAFETY OF A NEW INACTIVATED VACCINE AGAINST THE RABBIT HAEMORRHAGIC DISEASE VIRUS 2-LIKE VARIANT (RHDV-2)

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ABSTRACT

In 2010 a new variant of rabbit haemorrhagic disease (RHDV-2) emerged in France and quickly spread through several European countries, causing atypical outbreaks in commercial rabbitries. Classical RHDV vaccines showed low cross-protection against RHDV-2, revealing the need for a specific vaccine against the disease. In this study the efficacy and safety of ERAVAC, an inactivated vaccine developed by HIPRA for preventing RHDV-2, was evaluated and compared with its simultaneous administration with a classical RHDV vaccine (Cunipravac RHD). Sixty 28-day-old New Zealand white rabbits were randomly distributed into three groups of equal size; the first group was vaccinated with ERAVAC (group A), the second group was vaccinated with ERAVAC + Cunipravac RHD (group B), and the third group received PBS (group C – control). Clinical signs and mortality were monitored after vaccination and after challenge (performed seven days post-vaccination). Blood samples were collected at day 0 (before vaccination) and at day 7 (before challenge) in order to determine the antibody response against RHDV-2 by competition ELISA. No clinical signs or adverse reactions were observed in the two vaccinated groups (A and B), suggesting that ERAVAC, alone and simultaneously administered with Cunipravac RHD is safe in terms of local reactions. All animals from both vaccinated groups (A and B) survived the experimental challenge, whereas the mortality rate in the control group was higher (0% groups A and B vs 63 % group C; $p < 0.05$). All animals from the vaccinated groups showed clear seroconversion (ELISA titer equal to or higher than 10) seven days post-vaccination. No statistically significant differences were observed between ELISA titers among groups A and B ($p < 0.05$). These results suggest that the administration of ERAVAC alone or simultaneously with Cunipravac RHD was equivalent in terms of safety and efficacy performing a challenge seven days post-vaccination. Furthermore, serological analysis demonstrated that the simultaneous administration of Cunipravac RHD does not interfere in the serological response against RHDV-2.

Key words: RHD, RHDV-2, vaccine, efficacy, seroconversion

INTRODUCTION

Rabbit haemorrhagic disease (RHD) is a fatal and highly infectious disease of the European rabbit (*Oryctolagus cuniculus*) that was first documented in China in 1984 (Liu *et al.*, 1984). It is currently endemic in Europe, where it causes large economic losses to commercial rabbit farms (Xu, 1991; Villafuerte *et al.*, 1995; Campagnolo *et al.*, 2003). Over the last 20 years an effective control of the disease was achieved through the following factors: aetiological agent (RHDV) isolation, effective inactivated vaccines development, and low antigenic variability of the field virus strains (Lavazza *et al.*, 2012).

However, in 2010 atypical outbreaks of the disease were reported in France (Le Gall-Reculé *et al.*, 2011) and rapidly spread through several European countries over the following years, such as Spain, Italy, Portugal, Germany and the United Kingdom (Dalton *et al.*, 2015). These outbreaks differed from the classical ones in two main aspects: susceptibility of young rabbits (< 40 days of age) and lower mortality rates. In addition, classical RHD vaccines proved not to be effective enough to face these atypical outbreaks.

The aetiological agent was isolated and characterized. It was proved to be antigenically different from the classical strains (Dalton *et al.*, 2012; Le Gall-Reculé *et al.*, 2013) and it was named RHDV-2. Recent studies confirmed that there is a lack of cross-protection immunity between classical vaccines and RHDV-2 (Bárcena *et al.*, 2015), highlighting the need of specific RHDV-2 vaccines to protect rabbitries from disease outbreaks.

The main objective of this study was to assess the efficacy and safety of ERAVAC, an inactivated vaccine for preventing the rabbit haemorrhagic disease virus 2-like variant (RHDV-2) developed by HIPRA, and to compare it with the simultaneous administration of ERAVAC and a classical RHDV vaccine (Cunipravac RHD).

MATERIALS AND METHODS

Animals and experimental design

Due to the lack of specific guidelines for RHDV-2 vaccines, this study was designed following the recommendations of the immunogenicity test described in specific monograph 2325 *Rabbit haemorrhagic disease vaccine (inactivated)* of the European Pharmacopoeia and monograph 50207: *Evaluation of efficacy of veterinary vaccines and immunosera*. According to these recommendations, a vaccine is considered efficacious if a percentage equivalent to or higher than 90% shows no signs of disease. In terms of serological response, significant differences must be observed between the vaccinated and control groups.

Sixty 28-day-old New Zealand white rabbits (*Oryctolagus cuniculus*) from a RHDV-2 free farm were included in the study. The animals were randomly distributed into three groups of equal size (A, B and C). All animals were fed with antibiotic-free standard granulated rabbit foodstuff and water *ad libitum* during the whole study period. The experimental design and trial were performed in accordance with the European Union Guidelines for Animal Welfare (Directive 210/63/EU), and it was approved by the Commission for Ethics in animal experimentation of HIPRA.

After an acclimation period of 72h (day 0), all animals from group A were vaccinated subcutaneously with a single dose (0.5 ml) of ERAVAC; animals from group B were vaccinated with a single dose (0.5 ml) of ERAVAC and a single dose (0.5 ml) of Cunipravac RHD simultaneously in a single shot/injection (1 ml); rabbits from group C (the control group) received 1 ml of PBS (phosphate buffer solution).

At day 7, all animals of the three groups were challenged with a heterologous virulent RHDV-2 strain (administration of 1 ml of viral suspension containing 1000 HAU by intramuscular injection). The challenge strain was previously isolated from a farm in Catalonia at the diagnostic center at HIPRA. (Diagnos) on March 2012. A stock of this strain is kept frozen at -80°C on site at the R&D Department of HIPRA.

Since day 0 (vaccination) to day 14 (seven days post-challenge), all animals were monitored twice a day to register clinical signs and mortality. Livers were collected from dead animals after challenge to determine the presence of RHDV-2.

Blood samples were collected twice, at day 0 before vaccination and at day 7 before challenge, from the auricular vein of 10 random animals of groups A and C and 5 random animals from group B to perform serological analysis. These sera samples were sent to the OIE Reference laboratory for rabbit haemorrhagic disease (IZSLER) to be tested for antibodies against RHDV-2 by competition ELISA (c-ELISA) (OIE, 2010).

Statistical Analysis

Mortality and serological response data were analyzed using Chi-Square test and U-Mann Whitney test, respectively. These analyses were performed using SPSS and statistical significance is defined as p-values less than 0.05

RESULTS AND DISCUSSION

No clinical signs or adverse reactions were observed in the vaccinated groups (A and B), suggesting that the administration of ERAVAC alone and its simultaneous administration with Cunipravax RHD, are safe in terms of local reactions.

One animal from the control group (group C) was found dead 24 hours after the administration of the placebo (PBS). A necropsy was conducted on this animal and no clear macroscopic lesions were observed. The whole liver of this animal was collected to determine the presence of RHDV-2 and the result was negative.

Following the challenge, the mortality rate observed in the control group (63 %) was higher ($p < 0.05$) than in the vaccinated groups, in which no deaths were reported at any time (Table 1). All deaths were reported around 24 hours after challenge and liver samples confirmed the presence of RHDV-2 in all dead animals.

Table 1: Mortality rates after challenge

Group	N° of survivors	N° of deaths	Mortality (%)
A = ERAVAC	20	0	0 ^a
B = ERAVAC + Cunipravax RHD	20	0	0 ^a
C = Control (PBS)	7	12	63 ^b

^{a,b} Values with different superscript are statistically different between groups (Chi-Square test, $p < 0.05$).

These results reach the efficacy parameters established for this study, as more than 90% of vaccinated animals showed no symptoms of RHD. Consequently it has been demonstrated that the vaccine ERAVAC is effective in protecting rabbits against challenge with virulent RHDV-2 strain seven days after vaccination. These results support the onset of immunity to be established from seven days post-vaccination.

The mortality rate obtained in the control group is similar to previous reported results by other Authors when experimental infections were performed with the RHD virus 2-like variant. For example, Le Gall-Reculé (2013) obtained a mortality rate of 46% with a virulent strain of RHDV-2; Parra and Dalton (2013) registered mortality rates of 50-55% 72 hours after challenge. Therefore, these results confirm that the mortality rate of RHDV-2 is statistically lower than the rate when a challenge is carried out with classical strains, which show values from 80 to 100% (OIE, 2010).

Regarding serological analysis, all animals were seronegative before vaccination (day 0). Group C rabbits (unvaccinated) remained seronegative until the day of challenge. On the other hand, both vaccinated groups (A and B) showed clear seroconversion (ELISA titers equal to or higher than 10) seven days after vaccination. No statistically significant differences ($p < 0.05$) were observed between the median ELISA titers obtained in groups A and B (see Table 2), demonstrating the equivalency of the administration of ERAVAC alone or simultaneously with Cunipravax RHD, in terms of seroconversion against RHDV-2.

Table 2: Serological response (ELISA) against RHDV-2 seven days post-vaccination

GROUP	RHDV-2 antibodies (ELISA titers)	GROUP	RHDV-2 antibodies (ELISA titers)	GROUP	RHDV-2 antibodies (ELISA titers)
A	320	B	80	C	N
A	160	B	80	C	N
A	40	B	80	C	N
A	160	B	80	C	N
A	40	B	20	C	N
A	40	Median B	80^a	C	N
A	160			C	N
A	160			C	N
A	40			C	N
A	80			C	N
Median A	120^a			Median C	n/a^b

^{a,b} Values with different superscript are statistically different between groups (U-Mann-Whitney test, $p < 0.05$).

ELISA titers equal to or higher than 10 are considered as positive. N=negative, n/a = not applicable.

CONCLUSIONS

These results suggest that the administration of ERAVAC alone or simultaneously with Cunipravac RHD can be considered safe in terms of local and general reactions. Furthermore, in both cases the seroconversion induced in vaccinated rabbits confers complete protection against challenge with virulent RHDV-2 seven days after vaccination, demonstrating that the simultaneous administration of both vaccines does not interfere in the serological response against RHDV-2.

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