CHARACTERIZATION OF THE MATERNAL DERIVED ANTIBODY IMMUNITY AGAINST *RHDV-2* AFTER ADMINISTRATION IN BREEDING DOES OF AN INACTIVATED VACCINE

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ABSTRACT

Inactivated strain-specific vaccines have been successfully used to control rabbit haemorrhagic disease (RHD) caused by RHDV-2 in the rabbit industry. It is unknown whether and how vaccination of breeding does contributed to protect the population of young susceptible rabbit kits. The present study investigates whether the immunity against RHDV-2 produced by vaccination of breeding does is transmitted to their progeny and its dynamic once inherited by kits. For this purpose, New Zealand female rabbits of 8-9 weeks of age were allocated into 2 groups of 40 subjects each and bred during 6 reproductive cycles. The first experimental group was vaccinated with a commercially available inactivated vaccine against RHDV-2 whereas the second group was inoculated with PBS. Six months later, 10 vaccinated rabbits were re-allocated into a new group and re-vaccinated with the same vaccine. Moreover, the present study was also meant to identify the mechanisms of transmission of that maternal immunity. For this reason, rabbit Kits of vaccinated and non-vaccinated breeding does were cross fostered before milk uptake. The RHDV-2 antibody response was monitored in the blood serum of breeding does and of their Kits by ELISA techniques. Results showed that RHDV-2 antibodies were inherited by Kits up to one year from vaccination of breeder does and that the revaccination increased but not significantly the antibody response. Once inherited, the maternal derived antibody response against RHDV-2 lasted at least until 28 days of life. Finally, the study also elucidated that the major contribution to the maternal derived immunity against RHDV-2 in Kits was provided during gestation and probably transmitted through transplacental mechanisms. The present study contributed to elucidate the characteristics of the maternal antibody immunity produced by vaccination and its mechanisms of transmission; however, the extents of protection of this response are still not fully clarified.

Keywords: RHDV-2, vaccination, maternal immunity.

INTRODUCTION

The emergence of a new RHDV related virus (RHDV-2) in 2010 partially changed the epidemiology of this disease. The lack of a prior protective immunity permitted this to spread through Europe causing high mortality in wild and domestic rabbit populations. Inactivated homologous vaccines were developed and successfully used to control the disease in the rabbit industry. Almost ten years later, some questions about the mechanisms of action of those inactivated vaccines are still open. RHDV-2 is able to cause disease in rabbit Kits (Le Gall et al. 2011). The mechanisms by which vaccines, administered to rabbit does, can contribute to protect the population of rabbit Kits are still unclear. Humoral immune response, either active or passive, has been suggested essential in protection against RHD (Abrantes et al. 2012). In mammals, maternal derived antibodies (MDA) are a type of passive immunity transmitted from mothers to offspring during the gestation and/or lactation and which

generally aid to protect during their early life. Rabbits have a haemochorial placentation and thus it is known that maternal antibodies are transmitted from the mother to the offspring through placenta; in contrast, no much is known about transmission through lactation (DeSesso et al. 2012). However, Peri et al. 1986 provided evidences of the existence of both mechanisms of antibodies transfer in rabbits. The aim of this study was to evaluate the capacity of an inactivated RHDV-2 vaccine to produce passive antibody immunity in rabbit Kits by means of active immunization of breeding does. Beside this, it was also meant to elucidate the mechanisms of the RHDV-2 antibody immunity transmission and to evaluate its dynamic once inherited by Kits. Finally, this study was also meant to evaluate the duration of transmission of RHDV-2 MDA to new-born rabbit Kits during the life of vaccinated breeding does.

MATERIALS AND METHODS

Animals and experimental design

Eighty New Zealand White female rabbits of 8-9 weeks of life (wol) were purchased from a minimal disease grade animal supplier free of major rabbit diseases, including RHD. After one week of acclimation, animals were randomly allocated into 2 groups of 40 subjects each. Group A was immunized with an inactivated vaccine against RHDV-2 (ERAVAC®, Laboratorios HIPRA S.A.) by subcutaneous administration following manufacturer's instructions, whereas group B was vaccinated with sterile PBS (phosphate-buffered saline). After 6 months, 10 female rabbits of group A were re-allocated in a new group (C) and re-vaccinated with ERAVAC®. The immunization status of all rabbits was checked 25 days post vaccination (dpv). Breeding program started at 18-19 wol and continued up to 6 reproduction cycles (inter-partum interval: 49-56 days).

The duration of the immune response in breeding does was monitored up to 351 dpv. Blood samples were collected 2 days after giving birth, from the central auricular artery of 10 breeding does per group (A, B and C). The presence of MDA in Kits was simultaneously evaluated. For this purpose, Kits of 2 days of life (dol) from the 10 above selected mothers were bled intracardiacally and then humanly euthanized. The duration of the MDA was monitored in rabbit Kits up to 58 dol. Forty Kits per group (groups A and B) were randomly selected, weaned at 30-35 dol and grown up to 60 dol. Blood samples were collected periodically by intracardiac puncture or from the central auricular artery from 15 Kits per group.

The mechanisms of maternal immune transmission were determined by cross fostering Kits between group A and B breeder does. At the 3rd farrow, birth was induced in breeding does with oxytocin. Kits born from 6 mothers of group A were cross fostered just after their birth with Kits born from mothers of group B (group AB and BA); the procedure was performed before the start of the maternal milk intake. The cross fostering was also performed between Kits born from breeding does of the same group to evaluate the influence of possible confounding bias associated to the implemented procedures; for this purpose, the same process was performed among 4 mothers of group A (group AA) and also among 4 mothers of group B (group BB). MDA were monitored in rabbit Kits at 2, 9, 19 and 29 dol; for this purpose, between 4-12 randomly selected Kits per group were bled either by intracardiac puncture or from the central auricular artery.

Laboratory analysis

Blood samples were processed and then tested to detect and quantify the antibody response against RHDV-2 by using a competition ELISA (cELISA) (OIE, 2016). The cut-off of the assay was 3.32 Log_2 cELISA titres.

Statistical analysis

For the purposes of data representation, doubtful results (cELISA titres below the threshold value) were considered negative and negative results were transformed as 1 Log_2 titre. Statistic comparison was performed by Kruskal-Wallis or Mann-Whitney U tests implemented in SPSS® and with a significance level of 5%.

RESULTS AND DISCUSSION

Transmission of the vaccine produced maternal antibody immunity against RHDV-2 to Kits during the breeding does reproductive life

Vaccination produced in breeding does (Group A) an active antibody immune response against RHDV-2 (7.66 to 8.99 Log_2 cELISA titres) which persisted up to 351 dpv. Revaccination slightly improved, but not significantly, the antibody response against RHDV-2.

Vaccination of breeding does produced a specific passive antibody response in their Kits. In fact, the presence of antibodies against RHDV-2 was detected in Kits of 2 dol born from groups A and C (Figure 1A). Moreover, this was detected up to the sixth reproductive cycle (349 days post vaccination). Revaccination of breeding does (group C) did not significantly improve the MDA response against RHDV-2 in rabbit Kits' blood.

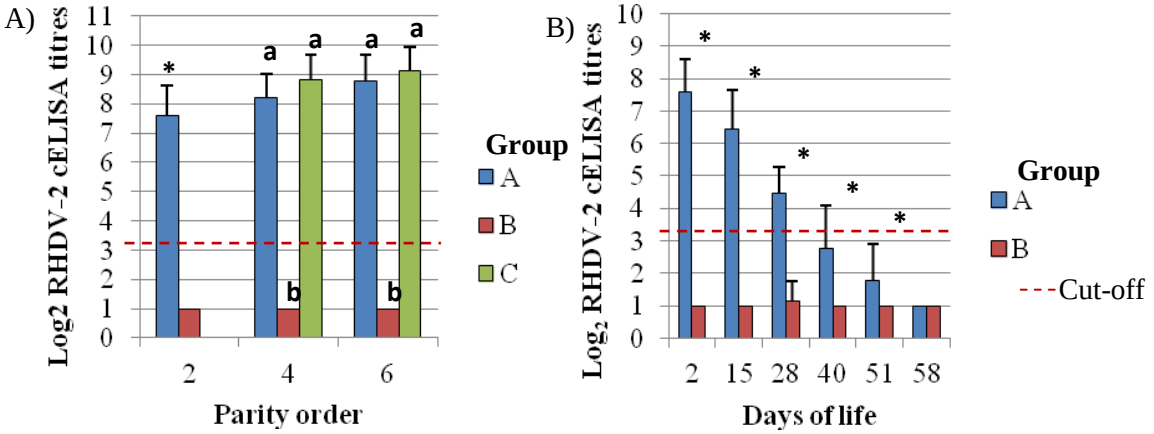

Figure 1: RHDV-2 antibodies in rabbit Kits at A) 2 dol but different parities and B) during an entire grow-up cycle (2nd parturition). Asterisks and letters indicate statistically significant differences (* Mann-Whitney U test, $p<0.05$; a-b Kruskal-Wallis test $p<0.05$).

Dynamic of the maternal derived antibody immunity against RHDV-2 in rabbit Kits

The passive immunity against RHDV-2 produced in Kits by vaccination lasted at least 28 dol but did not reach 58 dol (Figure 1B). In particular, the average antibody titre was maintained above the cut-off of the assay up to 28 dol; however, few positive Kits were still observed up to 51 dol. Negative rabbits started to be firstly observed at 40 dol (33.33%) and then their frequency increased over time up to 58 days of life when all Kits were negative.

Mechanisms of transmission of the maternal derived antibody immunity against RHDV-2 to rabbit Kits

The major contribution to the RHDV-2 MDA immunity in Kits was transmitted during gestation and probably by transplacental transmission (Figure 3). Kits taken from vaccinated and moved to non-vaccinated breeding does (group AB) showed RHDV-2 antibodies in blood similar to Kits' cross fostered between vaccinated breeding does (group AA). These Kits received maternal antibodies during gestation, likely through transplacental transmission, but not through lactation because the cross fostering was performed before Kits uptake any mammal gland secretion. Kits moved from non-vaccinated to vaccinated breeding does (group BA) showed much lower antibody titres compared to AA and AB but similar to group BB; moreover, the majority of them were negative. Therefore, results suggest that lactation has little or no detectable contribution to RHDV-2 maternal derived antibody immunity. These findings are in line with the results obtained by previous authors; in particular, a previous study showed the same mechanisms of transmission of total IgG and antigen specific IgG (Peri et al. 1986).

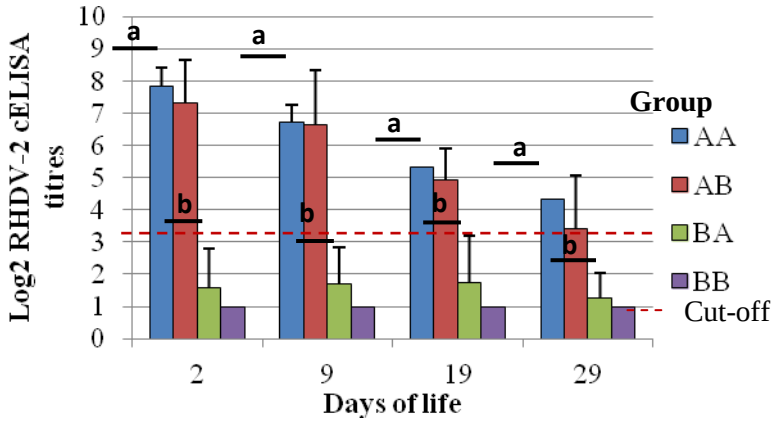

Figure 3: Mechanism of RHDV-2 antibody transmission from breeding does to their Kits. Kits were cross fostered between vaccinated (AA) or non-vaccinated breeding does (BB) or between them (AB, BA). Different letters indicate statistically significant difference (Kruskal Wallis test $p<0.05$).

CONCLUSIONS

The tested inactivated RHDV-2 vaccine produced passive antibody immunity against RHDV-2 in rabbit Kits by mean of active immunization of breeding does. Indeed, breeding does were able to provide maternal derived antibody immunity to new born rabbit Kits up to almost one year after the first vaccination. The present study also identified the transplacental as the main transmission mechanism contributing to the described passive antibody immunity in Kit rabbits. Moreover, the dynamic of the transmitted immunity was also described and the duration corresponded to 28 days of life. Therefore, this study contributed to describe the capacity of inactivated vaccines to produce passive antibody immunization against RHDV-2; however, the protective extents of this last need to be better elucidated in the future.

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