# DR. MARC PAGÈS

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Dr. Marc Pagès Bosch obtained a Microbiology and Genetics degree at the University of Barcelona in 1998. He obtained his PhD working on the synaptonemal complexes of *Eimeria tenella* chromosomes during meiosis. He started working in poultry coccidiosis at HIPRA in 2000 when he joined Dr. Emilio del Cacho group at the Department of Parasitology (University of Zaragoza, Zaragoza, Spain). He has been collaborating since then with Dr. del Cacho's group on the development of live attenuated coccidiosis vaccines. He was the R&D Project Manager for the EU registration of Hipracox® in 2005 and now EVALON®. He participated in the working group that published the European Monograph on coccidiosis vaccines. Together with his work in live vaccines he spend two years at the laboratory of Dr. Hyun Lillehoj (Animal Parasitic Diseases, USDA, Beltsville) developing strategies to isolate *Eimeria* protective antigens for a subunit vaccine against poultry coccidiosis. He is currently working in developing classic vaccines as well as novel strategies against coccidia at HIPRA.

# LIVE COCCIDIOSIS VACCINE FOR BREEDERS AND LAYERS (EVALON®) IMMUNE MODULATION AND ENHANCEMENT OF IMMUNITY BY THE USE OF AN ADJUVANTED SOLVENT (HIPRAMUNE® T)

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EVALON® is a live coccidiosis vaccine against avian coccidiosis composed of five attenuated strains. *Eimeria acervulina* 003, *E.brunetti* 034, *E.maxima* 013, *E.necatrix* 033 and *E.tenella* 004 have been selected to maximize immunogenicity, minimizing the side effects of *Eimeria* parasites. Avian *Eimeria* have a complex life cycle with a combination of exogenous and endogenous stages that trigger the immune system of the host. However, *Eimeria* parasites have also been described as being highly elusive to the immune system as well as producing chemokines that can slow or inhibit the immune response (Jang 2011, Schmid 2013, Miska 2013). Although it is well-known that live vaccines can induce an adequate immunity without the combination with an adjuvant, we strongly believe that immune modulation is crucial in providing a strong, fast and long-lasting immunity (Dalloul 2005).

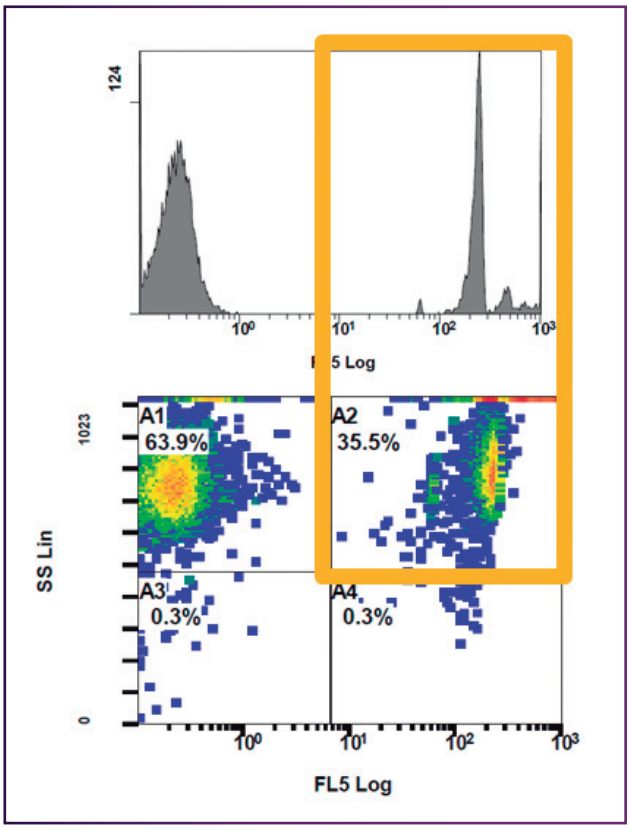
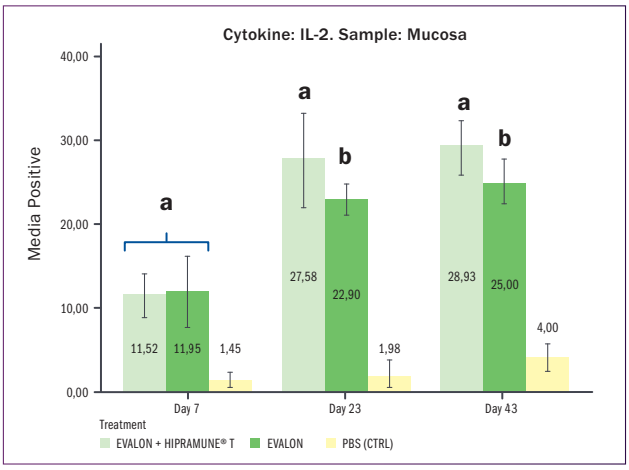
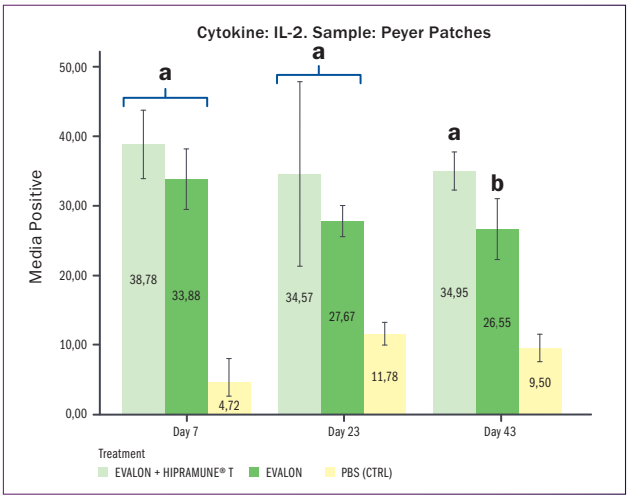



**Figure 1.**

Flow cytometry images that show detection of lymphocytes stained with IFN-γ monoclonal antibody. (Top) Graphic representation of positive lymphocytes and (Bottom) the program output where square A2 indicates the percentage of positive lymphocytes to IFN-γ.

In **Study 1** conducted at the University of Zaragoza with Prof. Emilio del Cacho, four subgroups per treatment received EVALON®, EVALON® in combination with HIPRAMUNE® T or Phosphate Buffered Solution (control group). Five birds from each subgroup were used to obtain intestinal lymphocytes from mucosa and Peyer's patches at different time points post-vaccination (7, 23 and 43 days p.v.). The lymphocytes were then incubated with an appropriate medium and stimulated overnight with *Eimeria* whole antigen. Later, lymphocytes were fixed and stained using monoclonal antibodies marked with fluoresceine and studied with flow cytometry to detect lymphocytes producing IL-2, IFN-γ, IL-4 and IL-10 (see figure 1).




**Figures 2 (top) and 3 (bottom).**

Figures 2 and 3 represent the mean percentage of positives for IL-2. Figure 2 results obtained with lymphocytes isolated from mucosa and Figure 3 isolated from intestinal Peyer's Patches.

[a,b] *different superscripts indicate statistical differences.*

Results obtained in the first experiment indicated that HIPRAMUNE® T is able to increase the level of Th1 cytokines as indicated by the results obtained for IL-2 (figures 2 Nd 3). Significant differences were detected at days 23 and 43 for mucosa and at day 43 for Peyer's Patches.
Regarding IFN-γ (figures 4 and 5), significant differences were detected at days 7 and 23 for mucosa and at days 23 and 43 for Peyer's Patches.

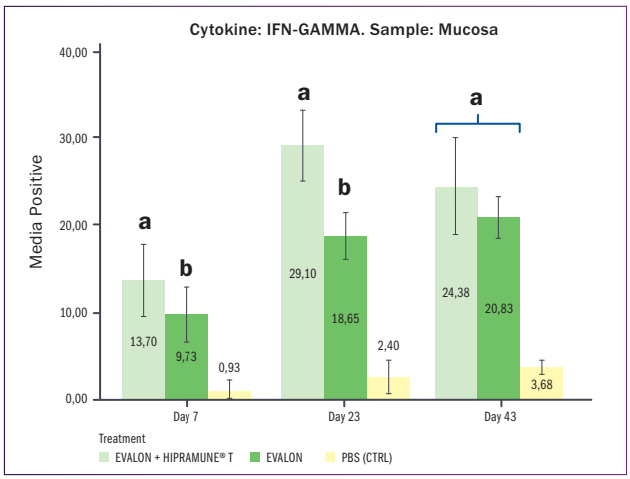
In contrast, the level of IL-4 and IL-10 at days 23 and 43 was equal or lower when EVALON® and EVALON® + HIPRAMUNE® T were compared. These results, combined with the results recorded for IL-2 and IFN-γ, confirm the ability of HIPRAMUNE® T to stimulate a cellular immune response.

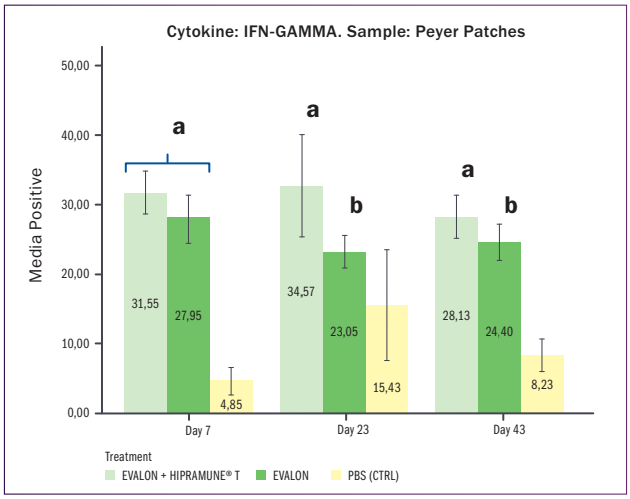
It is therefore hypothesized that EVALON®, when administered together with HIPRAMUNE® T, is able to polarize the immune response towards a Th1 response. This happens, as indicated in study 1, with more intensity than the live vaccine without the adjuvant. The Th1 response is crucial for protection against Eimeria (del Cacho 2011 and 2012).

In vaccines designed for layers and breeders which are long-lived categories, it is of paramount importance to have extended protection throughout the life cycle. Generally, live attenuated vaccines have proved to provide protection until 37 weeks. However, in the case of EVALON®, its efficacy is boosted by co-administration with HIPRAMUNE® T and, we therefore wanted to test the duration of immunity until the end of a production cycle (60 weeks in breeders).

In **Study 2** the duration of immunity was assessed for EVALON® + HIPRAMUNE® T. The laboratory facilities used to perform the trials prevented the introduction of external Eimeria oocysts which could provide trickle infections throughout the rearing and laying period. Together with this, birds were not moved from rearing to laying and on the contrary they were kept in the same room. It is well-known that at farm level and after vaccination, trickle infections occur, and it is important to maintain and enhance long-term immunity against *Eimeria* parasites (Williams 2002). In the present study we wanted to prove that protection was extended in the absence of trickle infections.

At day 0 a group of one-day-old birds was vaccinated via coarse-spray with one dose of vaccine EVALON® + HIPRAMUNE® T while another group of birds received only PBS. The elimination of oocysts was monitored weekly in litter faeces, and as can be seen in Figure 6. After the vaccination peak and once the birds became fully protected, generally no oocysts were detected.




**Figures 4 (top) and 5 (bottom).**

Figures 4 and 5 represent the mean percentage of positives for IFN-γ. Figure 4 results obtained with lymphocytes isolated from mucosa and Figure 5 isolated from intestinal Peyer's Patches.

[a,b] *different superscripts indicate statistical differences.*

To study the efficacy of the vaccine, birds were randomly selected at different time points (14, 28, 40 and 60 weeks) and individual challenge tests for each *Eimeria* species included in the vaccine were performed using highly pathogenic heterologous challenge strains. The vaccinated and non-vaccinated birds were compared, the main parameter under consideration being the macroscopic intestinal lesions after the challenge. Other secondary parameters evaluated also included individual body weight, elimination of oocysts post-challenge, clinical signs and mortality.

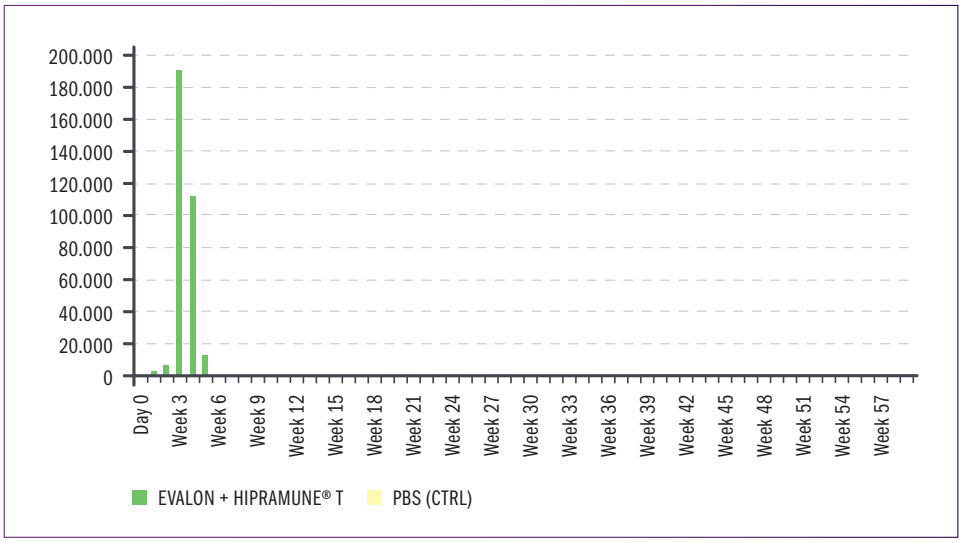



**Figure 6.**

Weekly oocyst counts from litter faeces.

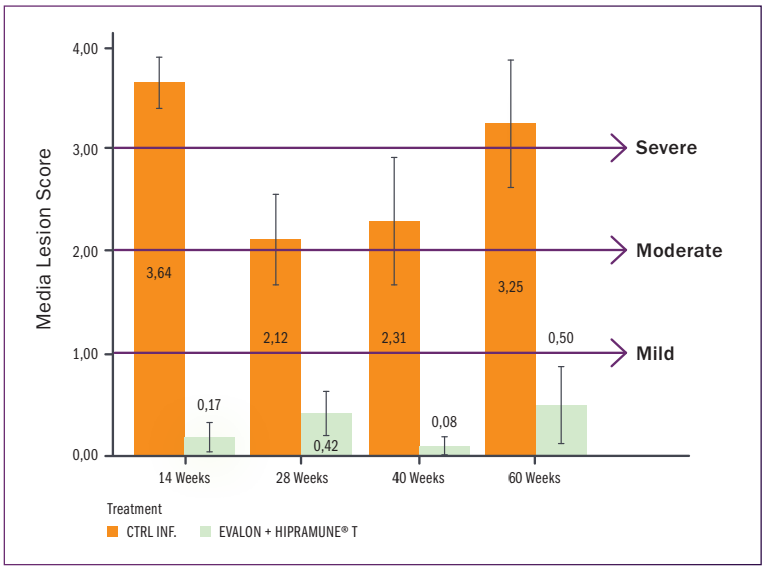



**Figure 7.**

Lesion scores of mid-intestine at 6 days post-challenge with *E.necatrix*. Lesion scoring 0-4 according to Johnson and Reid, 1970.

As an example, data obtained for *E.necatrix* lesion scoring after challenge are included in Figure 7. Similar results in terms of significant reduction of lesions in vaccinated groups were obtained for all the other *Eimeria* species included in the vaccine.

The results obtained indicated an extended duration of immunity with EVALON® when administered together with the adjuvanted solvent HIPRAMUNE® T in conditions that do not favour the presence of oocysts in the litter. The duration of immunity could be confirmed at 60 weeks post-vaccination.

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