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# Live coccidiosis vaccine for breeders and layers (Evalon®) immune modulation and enhancement of immunity by the use of an adjuvanted solvent (HipramuneT®)

**M. PAGÈS[1], G. BECH[1]. R MARCH[1], M. SITJÀ[1], H. LILLEHOJ[2], M. DARDI[3*], J. RUBIO PEREZ[3]and E. DEL CACHO[4]**

**[1]Hipra Scientific S.L.U., Avda. La Selva 135, 17170, Amer (Girona), Spain. [2]Animal Biosciences and Biotechnology Laboratory, USDA, Beltsville. [3]Hipra, Avda. La Selva 135, 17170, Amer (Girona), Spain. [4]Department of Parasitology, University of Zaragoza, Spain.**
***email: martina.dardi@hipra.com**

**In order to study the mechanisms by which an adjuvant could modulate the immune mechanisms that trigger the host after vaccination, a study is performed in which birds are vaccinated with a live attenuated coccidiosis vaccine (EVALON®)at day old with or without a selected adjuvant to study important markers of Th1 and Th2 responses. It has been proposed that the Th1 response plays a major role in protecting birds against an *Eimeria* infection (E.del Cacho 2011 and E.del Cacho 2012). It is hypothesized that a live vaccine when administered together with the correct adjuvant, increases the efficacy of the vaccine due to the capacity of the adjuvant to polarize the immune response towards a Th1 response.**

**Con el objetivo de estudiar los mecanismos por los cuales un adyuvante puede modular la respuesta inmune activada por el hospedador después dela vacunación, se ha llevado a cabo un estudio en el cual un grupo de aves se han vacunado con una vacuna viva frente a coccidiosis (EVALON®)aplicada el primer día de vida -con o sin un adyuvante seleccionado- para estudiar marcadores importantes de la respuesta de Th1 y Th2. Se considera que la respuesta de Th1 juega un papel relevante en la protección de aves frente infección de la especie *Eimeria* (E. del Cacho 2011 and E. del Cacho 2012). Se ha planteado la hipótesis de que una vacuna viva cuando se administra junto a un adyuvante apropiado, incrementa la eficacia de la vacuna debido a la capacidad de ese adyuvante de dirigir la respuesta inmune hacia una respuesta de Th1.**

**Keywords:** coccidiosis; immune modulation; adjuvant

**Palabras clave:** coccidiosis; inmuno modulación; adyuvante

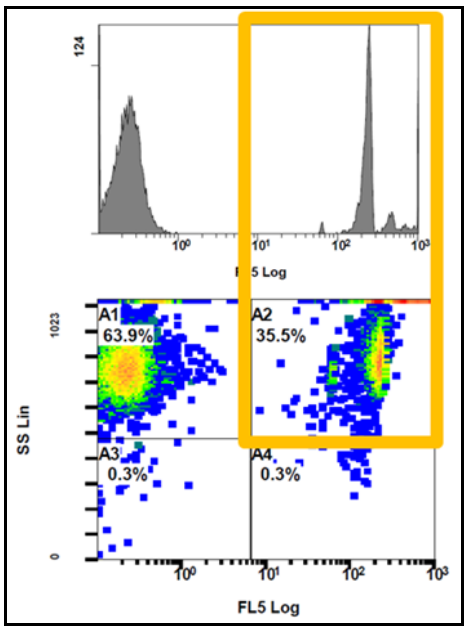
## Introduction

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 2014, 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).

## Materials and methods

In this study, 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 fluorescein and studied with flow cytometry to detect lymphocytes producing IL-2, IFN-Y, IL-4 and IL-10 (see figure 1).

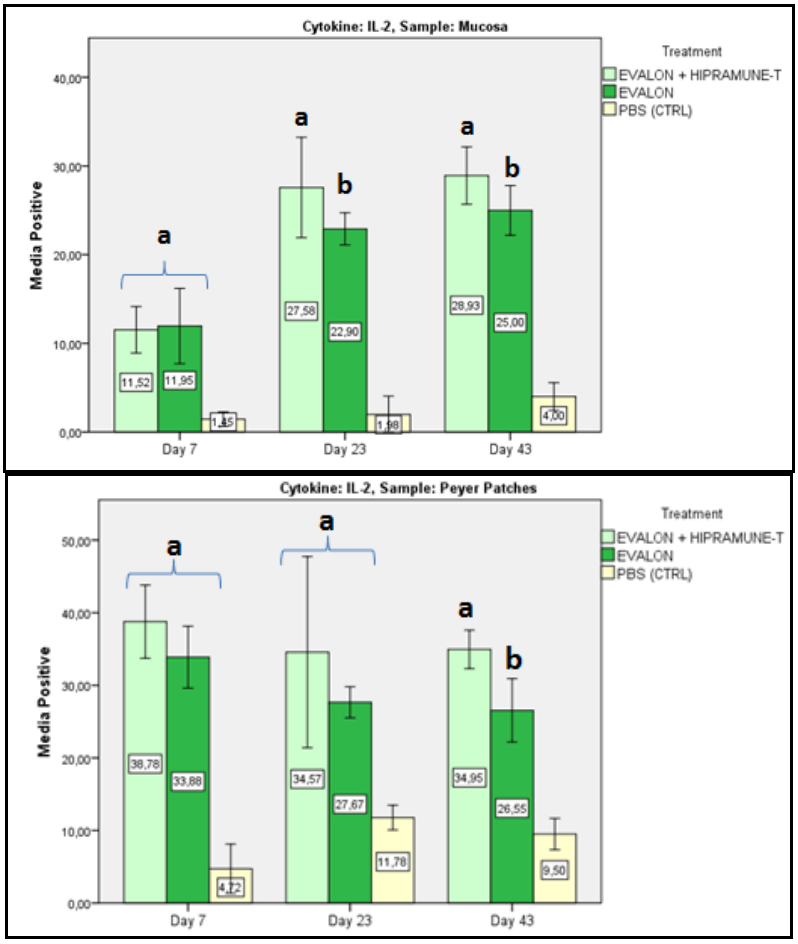
**Figure 1 Flow cytometry images show detection of lymphocytes stained with IFN-Y monoclonal antibody. (Top) Graphic representation of positive lymphocytes and (bottom) the program output where square A2 indicates the percentage of positive lymphocytes to IFN-Y.**



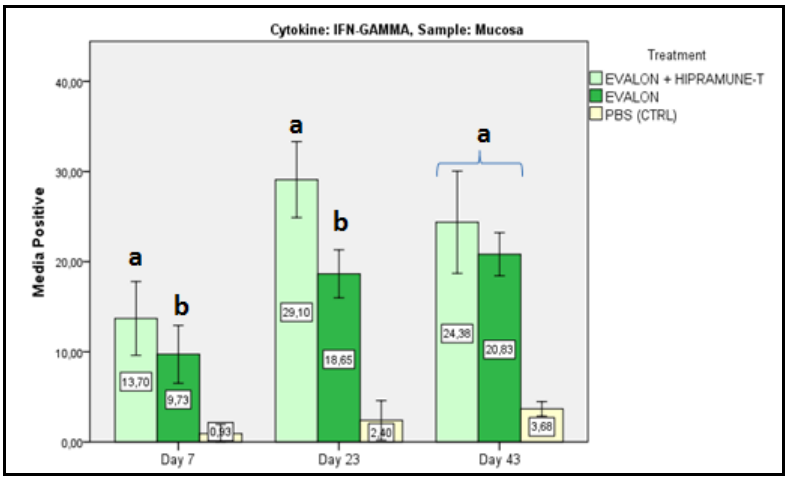
## Results and discussion

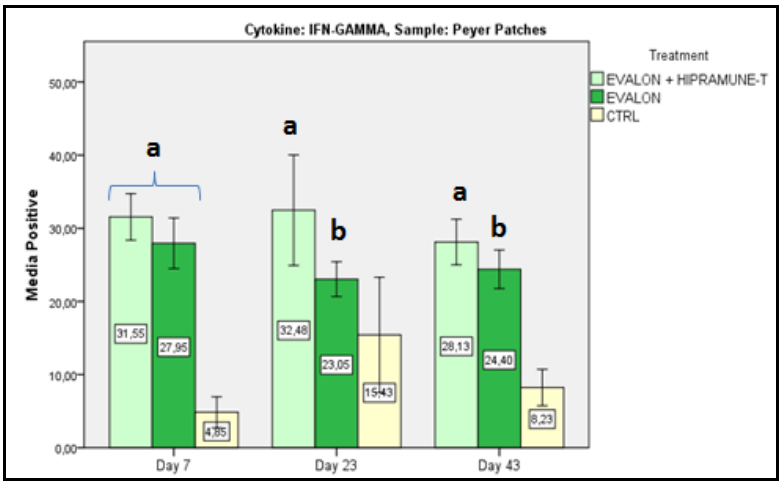
Results obtained in the first experiment indicated that HIPRAMUNE T® is able to increase the level of Th1 cytokines as indicated by the results obtained for IL-2 (Figure 2. Significant differences were detected at days 23 and 43 for mucosa and at day 43 for Peyer's Patches) and IFN-Ɣ (Figures 3. Significant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

**Figures 2 Mean percentage of positives for IL-2. Results obtained with lymphocytes isolated from mucosa and intestinal Peyer's Patches.[a,b] different superscripts indicate statistical differences.**



**Figures 3 Mean percentage of positives forIFN-Y. Results obtained with lymphocytes isolated from mucosa and intestinal Peyer's Patches.[a,b] different superscripts indicate statistical differences.**




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-Y, 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 this study, 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.In the case of EVALON®, its efficacy is boosted by co-administration with HIPRAMUNE T®.

## References

**DALLOUL, R.A., LILLEHOJ, H.S.** (2005)Recent advances in immunomodulation and vaccination strategies against coccidiosis.*Avian Diseases* **49(1)**: 1-8.

**DEL CACHO, E., GALLEGO, M., LEE, S, LILLEHOJ, H.S., QUILEZ, J., LILLEHOJ, E., SÁNCHEZ-ACEDO, C.**(2011)Induction of protective immunity against *Eimeria tenella* infection using antigen-loaded dendritic cells (DC) and DC-derived exosomes.*Vaccine* **29**: 3818–3825.

**DEL CACHO, E., GALLEGO, M., LEE, S., LILLEHOJ, H.S., QUILEZ, J., LILLEHOJ, E., SÁNCHEZ-ACEDO, C.** (2012)Induction of protective immunity against *Eimeria tenella, Eimeria maxima,* and *Eimeria acervulina* infections using dendritic cell-derived exosomes**.** *Infectious Immunology* **80**: 1909–1916.

**JANG, S.I., LILLEHOJ, H.S., LEE, S.H., KIM, D.K., PAGÈS, M., HONG, Y.H., MIN, W., LILLEHOJ, E.P.** (2011)Distinct immunoregulatory properties of macrophage migration inhibitory factors encoded by Eimeria parasites and their chicken host.*Vaccine* **29(48)**: 8998-9004.

**MISKA, K.B., KIM, S., FETTERER, R.H., DALLOUL, R.A., JENKINS, M.C.**(2013)Macrophage migration inhibitory factor (MIF) of the protozoan parasite Eimeria influences the components of the immune system of its host, the chicken.*Parasitology Research* **112(5)**: 1935-1944.

**SCHMID, M., HEITLINGER, E., SPORK, S., MOLLENKOPF, H-J. , LUCIUS, R., GUPTA, N.** (2014)*Eimeria falciformis* infection of the mouse caecum identifies opposing roles of IFNϒ-regulated host pathways for the parasite development**.** *Mucosal Immunology***7(4)**:969-982.