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Dr. Gregori Bech Sàbat obtained his degree in Agricultural Engineering at the University of Lleida (UdL) in 2005. He started his research career working at the Animal Production department of the UdL. Before obtaining his PhD on animal reproduction in 2010, he performed research stays at the veterinarian faculties of the University of Liège (Belgium) and the Complutense University of Madrid (UCM). He also did post-graduate studies on methodology of research and statistics in health sciences at Autonomous University of Barcelona. After finishing his PhD, he returned to the UCM to work on epidemiological and diagnostic aspects

of reproductive diseases in domestic animals, mainly with protozoan parasites, such as neosporosis. He joined HIPRA in 2012 at the R&D department, working in the preclinical and clinical development of poultry vaccines. He is in charge of developing poultry diseases experimental models and designing safety and efficacy trials for vaccine registration.

## STUDY OF THE EFFICACY OF A NEW LIVE COCCIDIOSIS VACCINE (EVALON®) FOR BREEDERS AND LAYERS UNDER FIELD CONDITIONS IN FARMS WITH A HISTORY OF CLINICAL COCCIDIOSIS

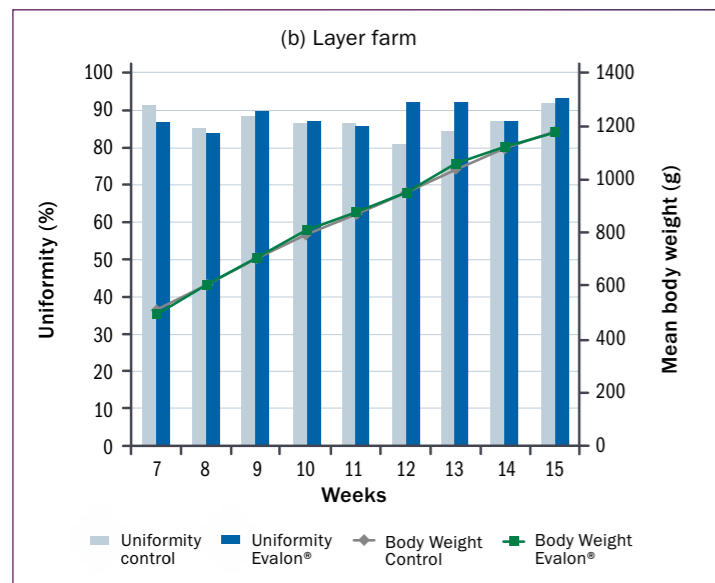
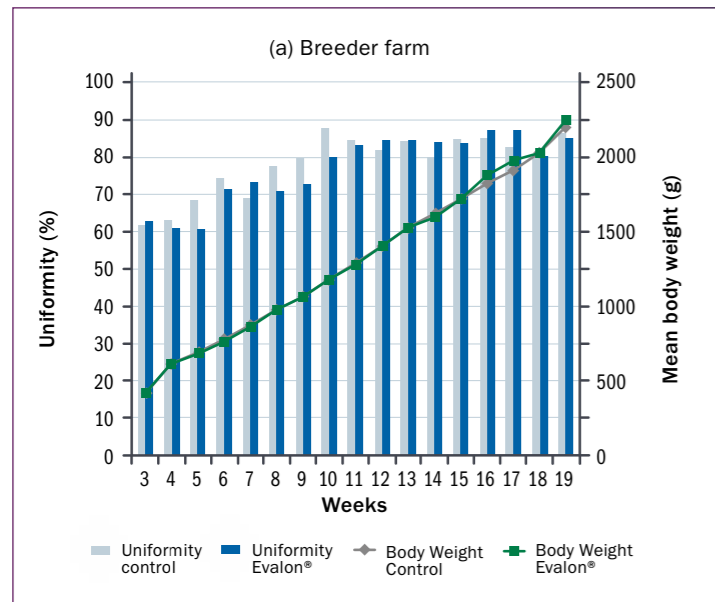
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After the efficacy and safety of a new live vaccine against avian coccidiosis (EVALON®) had been demonstrated by experiments under laboratory conditions, these were then evaluated in a *Good Clinical Practice*-compliant multicentre trial under field conditions. The vaccine was administered via coarse-spray in breeders and layers at 1 day of age, in farms with previous clinical coccidian problems.

Farms were selected on the basis of previous records of problems related to clinical coccidiosis. In addition to the information from the veterinarians responsible for the farms, faeces were sampled at different time points prior to the beginning of the trials. Analyses of these samples indicated high levels of oocysts per gram (OPG) and the presence of all *Eimeria* species. The *Eimeria* species in the farm samples were studied using a combined method of PCR and a morphological study of the oocysts.

The trial was performed on four commercial farms from Spain and Germany. The type of management employed and housing conditions were considered representative of those used in standard laying and breeding production of the European Union. A total of 171,254 day-old chicks (74,154 layers and 97,100 breeders) was included in the trial. Chicks were allocated to two identical units within each farm receiving either the new live vaccine or a commercial live vaccine (control group). The control group received the marketed vaccine administered according to farm's normal practice. The use of the positive comparator was introduced for welfare and ethical



**Figure 1.**

Uniformity and body weight changes during the rearing period on one breeder (a) and one layer (b) farm. Body weight is represented as the mean of 100 animals per group. Uniformity represents the percentage of birds within the range  $\pm 10\%$  of the mean.

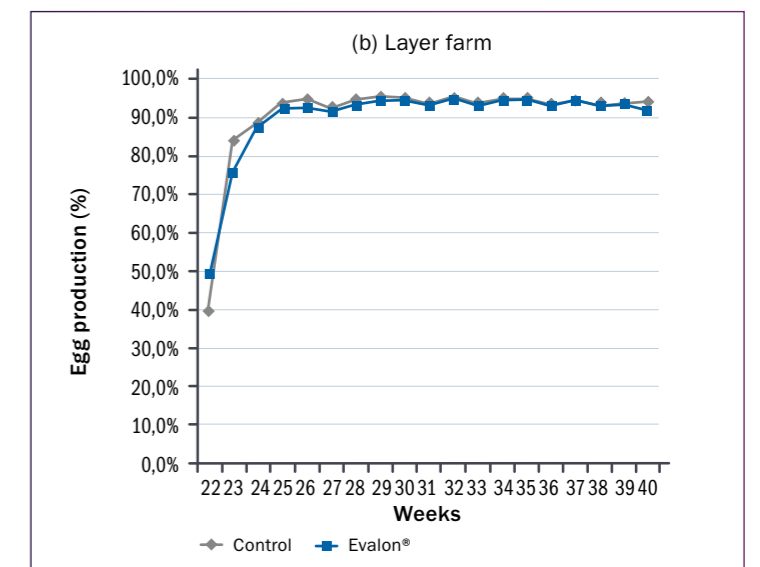
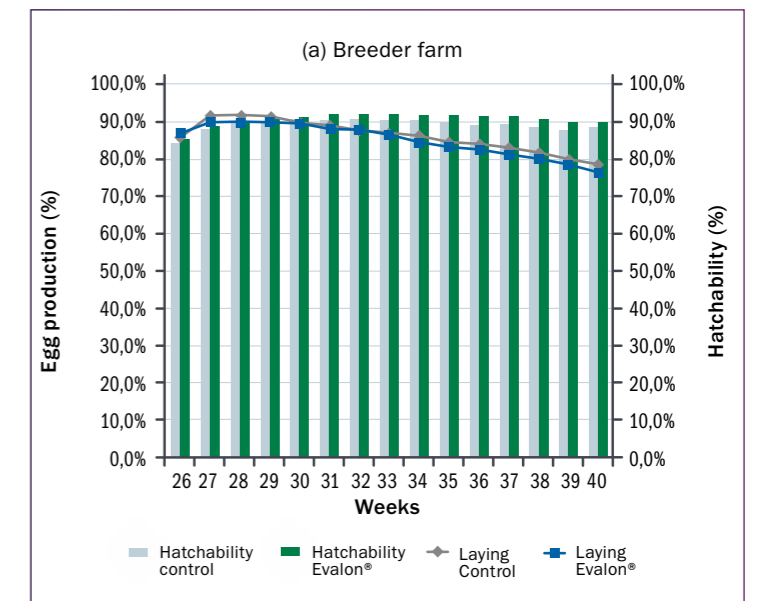
was calculated as the percentage of birds within the range  $\pm 10\%$  of the mean body weight. Local reactions in all sections of the intestine were assessed in a sample of 15 animals from each farm and group, on several days after vaccination (day 6, 7, 24 and 35). Oocyst counts were performed on fresh faeces during the first 9 days after vaccination and on litter faeces weekly throughout the study period. Finally, productive parameters such as egg production and hatchability were also monitored.

reasons, since untreated animals are likely to suffer the disease. Furthermore, the option of using a negative control group had to be ruled out from the beginning due to the fact that the most common practice under field conditions is the routine vaccination of the chicks. Therefore, given the fact that *Eimeria* are ubiquitous and cosmopolitan, efficacy was evaluated on the assumption that a natural outbreak of coccidiosis generally occurs (clinical or sub-clinical) in birds not treated.

After vaccination, several parameters were recorded in order to have an adequate assessment of the vaccine's safety and efficacy under field conditions. Adverse events after vaccination were carefully monitored in all the chicks. Moreover, general health status, including clinical signs, changes in faeces and abnormal mortalities, was recorded and evaluated. A weekly sample of 100 birds per farm and group was weighed in order to study weight changes and uniformity. Uniformity

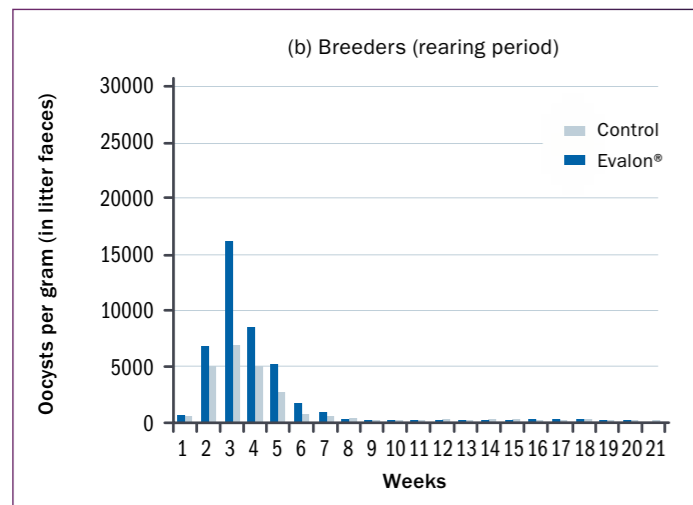
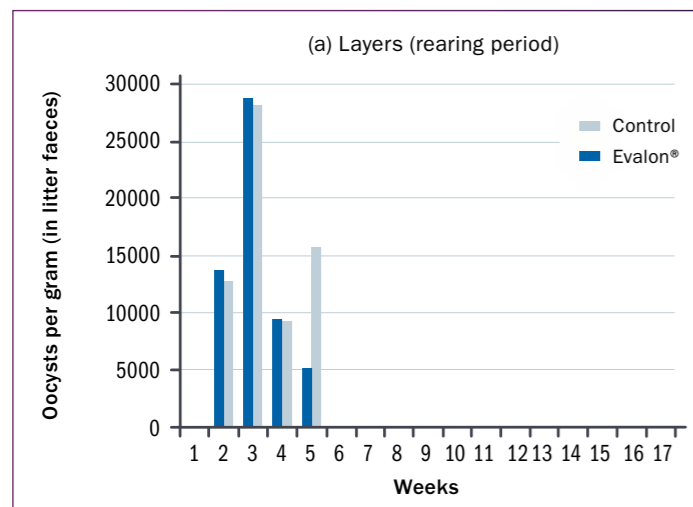
No severe or unexpected adverse effects attributable to the vaccination with EVALON® were observed. No general clinical signs were detected in the vaccinated chicks. Necropsies at days 6, 7 and 24, which are considered critical from a safety point of view, were used to determine local reactions to the vaccine. No lesions were found on any of these days for EVALON® vaccinated birds. Mortality on the study farms was not related to the vaccine, and no differences between groups were observed. As expected, most mortality occurred during the first few days after arrival at the rearing farm, as a result of the chicks having to adapt to the new environment and in consequence of the length of time involved in travelling long distances from the hatchery to the rearing facilities. As regards weight changes, no significant differences between groups were observed. As an example, Figure 1 shows body weight changes and uniformity during the rearing period for one of the breeder farms and one of the layer farms, respectively. Finally, productive parameters remained similar between groups, and no significant differences between groups were observed for laying performance and hatchability (Figure 2).

Oocyst counts in fresh faeces confirmed that *Eimeria* species included in the vaccines were eliminated to the litter from days 3 to 9 post-vaccination. Oocyst counts from litter faeces is a parameter widely used



**Figure 2.**

Laying during the first 40 weeks on one breeder (a) and one layer (b) farm. For the breeder farms, hatchability is also presented.



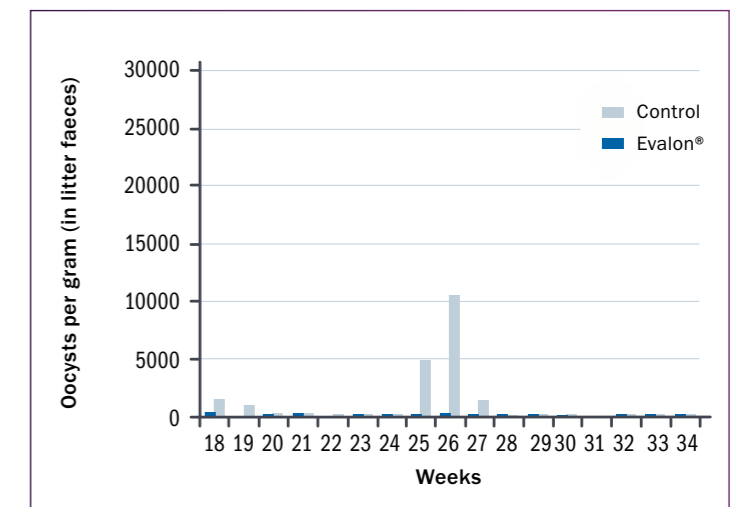
**Figure 3.** Average weekly oocyst counts (oocysts per gram (OPG) of litter faeces) during the rearing period for layer (a) and breeder (b) farms.

to study the pattern of elimination of *Eimeria spp.* under field conditions. Thus, the excretion of oocysts by the group vaccinated with EVALON® was analysed and compared with the excretion in the control group. Figure 3 shows weekly OPG during the rearing period. During the laying period, all weekly OPG were very low for both treatments (ranging from 0 to 333 OPG), except on one farm for the control group (Figure 4). It is known that outside the host oocysts sporulate and, once ingested, they become infective again, due to the fact that they finish their life cycle inside the host. The first peak of elimination in litter is frequently detected at day 7 (excretion of the oocysts from vaccine origin) and a second profile of elimination of vaccine oocysts can be detected between 21-28 days. This second profile of elimination can be higher than the initial one because the number of oocysts ingested during the recycling of the oocysts in the litter is also higher. This fact was observed in both groups, with high OPG during the first weeks (Figure 3), but from week 5 onwards (after the second peak) such recycling of oocysts through the faeces was stopped, because only low levels were detected. This indicated that birds became immunized. The levels of oocysts in both groups were similar during the whole rearing period. The oocyst counts at the end of the rearing period were low in both groups from all the farms, and this clearly indicates that the vaccination was effective against coccidiosis.

Necropsies at day 35 after vaccination were chosen because it is generally considered that there is a peak of replication of field oocysts around this date depending on the load of field strains in the farm when birds arrive and on the presence of immunity. For the EVALON® groups, whenever lesions were detected, they were very mild and not statistically different from the control group, and the majority of necropsied birds did not show any lesions. In the control group a few birds with moderate lesions were recorded. These results are in accordance with low levels of oocysts detected during those days, indicating that vaccines prevented oocysts from replication within the host.

More importantly to confirm the efficacy, no outbreak was registered in the EVALON® groups. On the contrary, on one of the farms for the control group an outbreak of coccidiosis due to *E. necatrix* was detected at 26 weeks (Figure 5). The parameters studied during the outbreak included intestinal lesion scoring, oocyst counts in litter faeces (note the increase in weeks 25-27 in Figure 4), faeces appearance (bloody appearance was observed), clinical signs (birds with abnormal appearance and with a marked lower activity) and an increase in water consumption, which confirmed the presence of a natural outbreak of *E. necatrix* in the control group. In the parallel EVALON® group for the same period there was no report of changes in faeces, clinical signs, intestinal lesions or an increase in water consumption. The parameters studied confirmed the good degree of protection in this group.

The outbreak identified and monitored in the control unit did not include a high degree of mortality. Probably, the fact that the problem was detected



**Figure 4.** Average weekly oocyst counts (oocysts per gram (OPG) of litter faeces) during the laying period on the farm where an outbreak occurred. Note the increase in weeks 25-27 due to an *Eimeria necatrix* outbreak.

on time and the birds were immediately treated with an anticoccidial drug (Amprolium) greatly reduced the outbreak severity. The treatment was effective in reducing the pressure of the outbreak and, as indicated by the oocysts counts (Figure 4) and water consumption, the situation returned to normality.

The outcome of field trials was consistent with the findings under laboratory conditions. Safety and efficacy of EVALON® were confirmed throughout the whole productive cycle for layer and for breeder farms.



**Figure 5.**

Picture taken during the coccidiosis outbreak detected in the control group. Extensive haemorrhage in the lumen of the mid-intestine can be observed. The serosal surface is covered with red petechiae and it is rough and thickened with many pinpoint haemorrhages. This intestinal lesions are compatible with an *E. necatrix* lesion score of 3 (Grading system from Johnson and Reid, 1970). (Photo courtesy of: Meritxell Pérez)

## REFERENCES

Johnson J, Reid WM. *Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens*. Exp Parasitol (1970) 28: 30-6.