6 December 2018
EMA/861413/2018
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for EVANT (EMEA/V/C/004902/0000)

Common name: Coccidiosis vaccine live for chickens

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted

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Introduction

The applicant LABORATORIOS HIPRA, S.A. submitted on 1 December 2017 an application for a marketing authorisation to the European Medicines Agency (the Agency) for EVANT, through the centralised procedure under Article 3(2)b of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 7 September 2017 as the applicant showed that the product would provide a significant scientific innovation.

The proposed indication is for active immunisation of chicks from 1 day of age to reduce intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria (E.) acervulina*, *E. maxima*, *E. mitis*, *E. praecox and E. tenella* and to reduce clinical signs (diarrhoea) associated with *E. acervulina, E. maxima* and *E. tenella*.

EVANT suspension and solvent for coarse spray vaccination for chickens contains the following active substances:

E. acervulina, strain 003	332 – 450*
E. maxima, strain 013	196 – 265*
E. mitis, strain 006	293 – 397*
E. praecox, strain 007	293 – 397*
E. tenella, strain 004	276 – 374*

* Amount per dose of sporulated oocysts derived from precocious attenuated lines of coccidia, according to *in vitro* procedures of the manufacturer at the time of blending.

EVANT is presented in packs containing 10 ml, 50 ml or 100 ml type I colourless glass vials containing 7 ml, 35 ml or 70 ml of suspension (1,000, 5,000 and 10,000 doses).

The vaccine suspension is to be diluted with the solvent HIPRAMUNE T and water before spray administration to chickens from 1-day-old.

The HIPRAMUNE T solvent is presented in volumes of 50 ml, 250 ml and 500 ml (for the 1,000, 5,000 and 10,000 vaccine doses, respectively) in colourless polypropylene plastic (PP) bottles.

Type I polymeric elastomer closures and aluminium caps are used for the vaccine and solvent presentations.

The rapporteur appointed is Jeremiah Gabriel Beechinor and the co-rapporteur is Petra Falb.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

On 6 December 2018, the CVMP adopted an opinion and CVMP assessment report.

On 5 February 2019, the European Commission adopted a Commission Decision granting the marketing authorisation for EVANT.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (dated 02/03/2012) which fulfils the requirements of Directive 2001/82/EC was provided. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

EVANT is manufactured in the European Union (EU) by LABORATORIOS HIPRA, S.A. at three sites in Amer, Girona, Spain.

Secondary packaging and batch release takes place at LABORATORIOS HIPRA, S.A. Avda. La Selva, 135 Amer, Spain.

Manufacturing authorisation issued on 21 October 2017 by the Spanish competent authority covers the manufacturing activities at these sites. Good Manufacturing Practice (GMP) certification, which confirms the date of the last inspection of the sites (6 November 2015) and shows that the sites are authorised for the manufacture and batch release of such veterinary dosage forms, has been provided.

Active substance manufacturing sites are listed as LABORATORIOS HIPRA, S.A., Lloret Salvatge production plant, Amer, Spain and LABORATORIOS HIPRA, S.A., Ciamer production plant, Amer, Spain. A GMP declaration for the active substances manufacturing sites was provided from the Qualified Person (QP) at the EU batch release site. The declaration has taken into consideration the GMP certificates available for the active substance sites issued by the Spanish competent authority following inspection of the sites on 6 November 2015.

A copy of the GMP certificate for each active substance manufacturing site issued by the Spanish authorities following inspection on 6 November 2015 is also provided.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of the active substances and of the finished product manufacturing sites has been satisfactorily established and is in line with legal requirements.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

EVANT is a live vaccine which contains as active substances sporulated oocysts derived from five precocious attenuated lines of the following *Eimeria* species: *E. acervulina, E. maxima, E. mitis, E. praecox* and *E. tenella*.

The vaccine suspension contains the sporulated oocysts suspended in a sterile phosphate-buffered saline solution containing disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, potassium chloride and purified water. Polysorbate 80 is included as an excipient in the vaccine suspension. Each vaccine dose is formulated to contain a standard amount of sporulated oocysts of each *Eimeria* strain; the ranges referred to in SPC section 2 reflect the variability of the oocyst counting procedure.

The solvent HIPRAMUNE T is recommended for use with EVANT. This solvent contains montanide IMS as adjuvant, vanillin as flavouring agent and two colouring agents (Red AC (E129) and Brilliant Blue (E133)). HIPRAMUNE T is currently approved for use as a solvent for the applicant's centrally authorised EVALON vaccine which contains sporulated oocysts of three of the *Eimeria* species included in EVANT, i.e. *E. acervulina, E. maxima* and *E. tenella* at the same quantitative composition as EVANT.

The vaccine is intended to be available in multidose presentations. There is no preservative – the claimed 10 hours in-use shelf life is satisfactorily justified in the stability section of the dossier.

Container and closure

The vaccine suspension is filled into 10 ml, 50 ml and 100 ml colourless type I glass vials for the 1,000 dose, 5,000 dose and 10,000 dose presentations respectively. The vials comply with European Pharmacopoeia (Ph. Eur.) chapter 3.2.1 requirements.

The HIPRAMUNE T solvent is filled into 50 ml, 250 ml and 500 ml colourless polypropylene plastic (PP) bottles. The PP material meets the requirements of Ph. Eur. 3.1.3 and Ph. Eur. 3.1.6 chapters. As the same HIPRAMUNE T presentations are approved as a solvent for the centrally authorised EVALON vaccine, the PP bottles are acceptable.

Type I rubber stoppers composed of polymeric elastomer are used as closures for both the vaccine and solvent containers which comply with Ph. Eur. chapter 3.2.9.

Details of the sterilisation method for the containers and rubber stoppers have been provided. All sterilising conditions are in accordance with the reference conditions specified in Ph. Eur. chapter 5.1.1 and are acceptable.

Caps of anodised aluminium are used to ensure the correct closure of the stoppers for both the vaccine and solvent containers. The caps are not in direct contact with the product.

Product development

EVANT was developed based on another of Hipra's coccidiosis vaccine, HIPRACOX BROILERS, which has been authorised for vaccination of broiler chicks for > 10 years in 25 EU countries via the Mutual Recognition procedure. Both vaccines contain sporulated oocysts of the same five *Eimeria* species.

There are two novel aspects in this vaccine: the inclusion of the adjuvant montanide IMS in the solvent to enhance the immune response and specific steps in the production of the antigens.

It is explained that avian coccidiosis is distributed worldwide with clinical signs ranging from decreased growth rate to a high percentage of sick birds with severe diarrhoea, an increased mortality percentage and decreased egg production.

Seven species of *Eimeria* are considered to be the causative agents of avian coccidiosis in domestic fowl: *E. acervulina, E. brunetti, E. maxima, E. mitis*, *E. necatrix, E. praecox* and *E. tenella.* Each species can be differentiated by characteristics such as oocyst size, reproductive capacity, pathogenicity, immunogenic properties, pre-patency period, minimum sporulation time and several molecular characteristics.

The five species included in EVANT, i.e. *E. acervulina, E. maxima, E. mitis*, *E. praecox* and *E. tenella* are considered important for a vaccine intended for short-lived birds such as commercial broilers. *E. praecox* and *E. mitis* infections generally appear in the first weeks of life, resulting in a negative effect on weight gain – as such, *E. praecox* and *E. mitis* infections have a higher impact in short-lived birds (e.g. commercial broilers) than in long-lived birds (e.g. layers or breeders), as the longer production cycles for the latter mean that infections at the start have minimal impact.

For the other three *Eimeria* species, detection rates of >70% for farms surveyed in France in 1994 are quoted in a literature publication provided. While more recent reporting rates for the EU are not given, as these three strains are included in the applicant's currently authorised HIPRACOX BROILERS (authorised for >10 years) and EVALON (authorised April 2016) vaccines, it is accepted that *E. acervulina, E. maxima* and *E. tenella* strains are representative of current EU *Eimeria* strains relevant for broiler chicks and as such their inclusion in EVANT is justified.

The *Eimeria* vaccine strains have been selected so that they have reduced or no pathogenicity. Attenuation of pathogenicity is achieved by repeated passage in chickens with selection for early appearance of oocysts. By selection for precocious development, populations can be selected with an important reduction of pre-patent periods (i.e. the time from ingestion of sporulated oocysts to emergence of oocysts in the faeces) and a significant reduction of pathogenicity.

The attenuation of the *Eimeria* species in EVANT is satisfactorily described and involved inoculation of the purified parental strains to coccidia-free specified pathogen free (SPF) chicks to obtain the initial passage (P0). The first oocysts eliminated were re-inoculated to new coccidia-free SPF chicks to perform the next passage. A number of similar successive passages were performed until populations of oocysts with greatly reduced pre-patent times and pathogenicity compared to the parental strains were obtained.

The sporulated oocysts need to be in a liquid suspension to remain viable and immunogenic: therefore, a suspension was chosen as the pharmaceutical form.

As outlined earlier, the HIPRAMUNE T solvent is already approved for use with the applicant's centrally authorised EVALON vaccine. As well as the montanide IMS adjuvant, HIPRAMUNE T also contains two colouring agents (Red AC (E-129) and Brilliant Blue FCF (E-133)) and one flavouring agent (vanillin). Literature publications support the claim that the colour and smell of vaccine droplets

is important to facilitate correct ingestion and, as such, the inclusion of these agents is justified, as the efficacy of the vaccine depends on the correct ingestion of the vaccinal oocysts at vaccination.

The quality of the excipients in the vaccine suspension is compliant with Ph. Eur. standards and all are listed in the SPC section 6.1. There are no novel excipients in the HIPRAMUNE T solvent – the flavouring agent (vanillin) is compliant with Ph. Eur. requirements and E129 and E133 are listed as suitable colouring agents in Annex 1 to Directive 94/36/EC.

Vaccine batches are blended to contain a fixed amount of sporulated oocysts of each *Eimeria* species, hence no overages are used in vaccine blending. Considering the fixed amount of sporulated oocysts of each species, it was possible to use the same vaccine batch for the safety and efficacy studies described in the dossier. The production of this batch was the same as that described for batches intended for marketing.

An acceptable rationale for the vaccine and solvent packaging materials used was provided.

The proposal not to test routine vaccine batches for the extraneous agents listed in Ph. Eur. chapter 2.6.25 'Avian viral vaccines: tests for extraneous agents in batches of finished product' is acceptable, as Ph. Eur. 2326 'Coccidiosis vaccine live for chickens' monograph allows for the omission of this testing where the oocysts are disinfected by a validated procedure. The sodium hypochlorite disinfection procedure used in the production of each *Eimeria* species in EVANT was validated to reduce the virus titre of representative contaminating viruses by at least 6 $\log_{10}$ in accordance with Ph. Eur. chapter 5.2.5 'Substances of animal origin for the production of immunological veterinary medicinal products'. Furthermore the seed materials have been satisfactorily tested for freedom from relevant extraneous agents (Ph. Eur. chapter 2.6.24 'Avian viral vaccines: tests for extraneous agents in seed lots') and four vaccine batches tested to date were negative for the extraneous agents listed in Ph. Eur. chapter 2.6.25.

Description of the manufacturing method

The manufacturing method and the in-process controls for the production of sporulated oocysts of each of the *Eimeria* components of the vaccine are similar, with only minor differences to take account of their different characteristics. Several passages in coccidia-free SPF chicks are used to produce the vaccine active substances as described below.

The manufacturing process established for the five Eimeria antigens is based on the "seed lot system", as indicated in the general monograph of the Ph. Eur. 0062 (Vaccines for veterinary use). It consists of a system of successive passages derived from one master seed lot. For each Eimeria species, the number of passages from their master seed parasite (MSP) is identical and fixed. The MSP and all subsequent passages are only propagated in coccidia-free SPF chicks.

The production process for each Eimeria species included in the vaccine is very similar. The chicks are inoculated with a suspension of sporulated oocysts. Their faeces are collected after a concrete period of time and the oocysts are recovered; then they are sporulated and the resulting oocysts are re-inoculated to a new group of coccidia-free SPF chicks. This process is repeated a further three times and finally antigens are disinfected with sodium hypochlorite solution and processed. Production of the finished product involves mixing equal volumes of each strain with the excipients and then aseptically filling into vials.

Production data are provided from batches of each *Eimeria* species which support the consistency of manufacture.

Data supporting the storage time for intermediates are provided.

Batch protocols are provided for batches. These demonstrate that the manufacturing process is capable of producing batches of acceptable quality in a reproducible and consistent manner.

Data from batches are provided to support the consistency of the manufacturing process. Acceptable validation data for the sterilising filtration step are provided.

Production and control of starting materials

Starting materials listed in pharmacopoeias

The following starting materials listed in a pharmacopoeia are used and the correspondent chapters and monographs are indicated in brackets: betamethasone sodium phosphate (Ph. Eur. 810), dimethyl sulphoxide (Ph. Eur. 763), disodium phosphate dodecahydrate (Ph. Eur. 118), foetal bovine serum (Ph. Eur. 2262), glycerol (Ph. Eur. 496), polysorbate 80 (Ph. Eur. 428), potassium chloride (Ph. Eur. 185), potassium dihydrogen phosphate (Ph. Eur. 920), purified water (Ph. Eur. 008), sodium bicarbonate (Ph. Eur. 195), sodium chloride (Ph. Eur. 193), SPF eggs (Ph. Eur. 5.2.2), vanillin (Ph. Eur. 747) and simethicone emulsion (USP 30/NF 25).

Representative certificates of analysis are provided supporting compliance with the pharmacopoeial standards. The foetal bovine serum is gamma irradiated with a minimum dose of 30 kGy.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

These include the five *Eimeria* strains (*E. acervulina* 003, *E. maxima* 013, *E. mitis* 006, *E. praecox* 007, *E. tenella* 004), tryptose phosphate broth and coccidia-free SPF chicks.

All strains were isolated from Spanish poultry farms. For all five strains the isolation, characterisation, purification and attenuation for precocity by serial passage in coccidia-free SPC chickens are adequately described.

Master Seed Parasites (MSP) were prepared for each of the strains as follows: The oocysts from the last passage of the attenuation process were identified. Sporocysts were prepared from these oocysts inoculated into coccidia-free SPF chicks, the faeces collected and sporulated. The resulting sporulated oocysts were used to prepare sporocysts that were stored frozen in liquid nitrogen as the MSP. Protocols are provided outlining the steps involved in the preparation of each MSP – these are sufficiently detailed.

The tests done on each of the MSPs included sporocyst concentration, identity by polymerase chain reaction (PCR), viability, bacterial and fungal sterility, freedom from mycoplasmas, freedom from adventitious viruses (using embryonated hen's eggs, chicken kidney cells and chicks), freedom from avian leukosis virus (ALV), avian reticuloendotheliosis virus (REV) and chicken anaemia virus (CAV). A certificate of analysis is provided for each MSP outlining the satisfactory results. The tests for adventitious viruses using embryonated hen's eggs, chicken kidney cells and chicks were done according to Ph. Eur. 2.6.24 and it is explained that a crystal violet stain is suitable to detect cytopathic effect (CPE) as an alternative to the stains specified in the monograph. The PCR tests used

to detect ALV, REV and CAV have been satisfactorily validated and acceptable details of the positive controls used in the PCR identity tests are provided.

Working Seed Parasites (WSP) were prepared by inoculation of the MSP into coccidia-free SPF chicks followed by recovery of oocysts which were then sporulated. Sporocysts were stored in liquid nitrogen as the WSP. It has been confirmed that all future WSPs will involve several passages from the MSP.

Each WSP is tested for sporocyst concentration, identity (PCR), viability and bacterial and fungal sterility. Certificates of analysis are provided for each WSP which are satisfactory.

Tryptose phosphate broth (TPB) is a component of the MEM Glasgow medium included in the freezing medium of the MSP for each *Eimeria* species. The animal-origin raw materials are derived from porcine tissues and bovine milk. A certificate of analysis, an animal origin position statement and a satisfactory risk assessment in accordance with Ph. Eur. chapter 5.2.5 on Substances of animal origin for the production of immunological veterinary medicinal products are provided.

Coccidia-free SPF chicks used for MSP, WSP and successive passages are hatched from SPF eggs. Representative certificates of analysis from each of the suppliers confirm compliance with Ph. Eur. chapter 5.2.2 on Chicken flocks free from specified pathogens for the production and quality control of vaccines. An acceptable justification is given for the tests done on the chicks between hatching and their use for Eimeria production to confirm the absence of specific pathogens.

The risk of contamination of the vaccine with extraneous agents due to the above referenced biological origin starting materials is considered to be negligible on the basis that all MSPs have been tested for freedom from the extraneous agents listed in Ph. Eur. 2.6.24, the satisfactory Ph. Eur. 5.2.5 risk assessment for TPB and as the SPF chicks are from eggs meeting Ph. Eur. 5.2.2 requirements. On this basis, the applicant's proposal that routine vaccine batches will not be tested for extraneous agents can be accepted, particularly as vaccine batches tested to date were negative for extraneous agents.

A transmissible spongiform encephalopathies (TSE) risk assessment for each of the biological starting materials in accordance with the Note for Guidance (NfG) on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products *(*NfG EMA/410/01 rev.3) and Commission Directive 1999/104/EEC is provided, which supports the negligible risk of TSE transmission as follows:

Eimeria species MSPs/WSPs:

Each Eimeria species was isolated from infected chickens or their faeces – chickens are not considered to be susceptible to TSE infection. The foetal bovine serum used in the freezing medium of the MSPs and WSPs is covered by European Directorate for the Quality of Medicines (EDQM).

Valid TSE certificates of suitability have been provided. Tryptose phosphate broth, which is included in the freezing medium for the MSPs, is derived from bovine milk and porcine tissues, which are considered to represent a negligible risk. Chickens which are not considered to be susceptible to TSE infection are used for vaccine production. No material of ruminant origin is included in the composition of the excipient or the solvent or used in the manufacturing process.

Tryptose phosphate broth (TPB):

The animal origin materials in TPB are porcine enzymes and bovine milk. Pigs are considered non-TSE relevant species (NfG EMA/410/01) and the bovine milk is obtained from healthy animals under the same conditions as milk for human consumption and is, thus, unlikely to present any TSE risk.

SPF chicks:

Birds are not considered to be a TSE-relevant species.

Starting materials of non-biological origin

These are Red AC (E-129), Brilliant Blue FCF (E-133), Hank's balanced salt solution (HBSS), MEM Glasgow medium, montanide IMS, propionic acid, potassium dichromate and sodium hypochlorite. Acceptable quality control documents describing tests, specifications and results have been provided for all of the materials.

In-house preparation of media and solutions consisting of several components

Preparation of the following media is described: freezing medium, propionic acid solution, saturated salt solution, potassium dichromate solution, PST VC dilution medium and sodium hypochlorite solution. Details of the qualitative and quantitative composition and the treatment and storage of the media are satisfactorily described.

Control tests during the manufacturing process

The applicant presented in-process data for the manufacture of three consecutive antigen bulks. During the manufacture of the antigen the following tests are carried out for each of the 5 *Eimeria* species contained in each of the bulks: total oocysts before flotations, total sporulated oocysts after sporulation, percentage of sporulation, appearance, concentration of sporulated oocysts and percentage of sporulation, sterility, identity/purity (PCR) and pH. The testing carried out during manufacturing of the vaccine was integrity filter testing before and after each sterilising filtration of PBS and polysorbate solution and volume control. The control tests performed on three batches of solvent HIPRAMUNE T are appearance, pH, filter integrity before and after pre-filtration, bioburden, integrity of filters before and after sterilising filtration and volume control. Test descriptions and the limits of acceptance were presented. Total oocysts and sporulated oocysts, and percentage of sporulation are counted and validation of the counting method is given in Part 2E. Sterility testing is in line with Ph. Eur. chapter 2.6.1 on sterility.

Overall, based on the data obtained, the in-process tests are deemed to be sufficient to control all the critical steps in the manufacturing. The test methods for in-process controls are satisfactorily validated.

Acceptable validation for the test methods for extraneous agents testing are presented; therefore, in line with Ph. Eur. 2326 monograph on Coccidiosis vaccine live for chickens, all of the tests for extraneous agents testing can be omitted as routine tests on each batch.

Control tests on the finished product

The applicant presented finished product testing data for three consecutive antigen bulks, each bulk is split into three batches of 1000 doses, 10,000 doses and 5000 doses, control testing on the filled batches is performed. Ph. Eur. monograph 2326 requires that batch testing includes identification, sterility testing, mycoplasma, extraneous agents testing, sporulated oocysts count and potency testing; these requirements are met, however there are some questions to be resolved.

Finished product testing on the bulk for EVANT includes; appearance, pH, concentration of sporulated oocysts, concentration of sodium hypochlorite, mycoplasma and batch potency. Control testing on the filled product includes appearance, pH, volume, sterility and concentration of sporulated oocysts. Identification is by microscopic examination to confirm the presence of oocysts in the batch of vaccine using the validated counting method, the potency test is also used to confirm the presence of oocysts; the test provides evidence on the identification of the *Eimeria* species and is able to confirm viability.

Sterility is determined according to the Ph. Eur. chapter 2.6.1, which is acceptable. Mycoplasma testing was performed in line with the Ph. Eur. chapter 2.6.7 on the bulk before filling, which is acceptable. The sodium hypochlorite oocyst disinfection procedure has been validated to reduce representative contaminating viruses by at least 6 $\log_{10}$ and all batches tested to date meet Ph. Eur. chapter 2.6.25 requirements.

The extraneous agents testing data are appropriately presented as part of the finished product/consistency data in Parts 2E and 2F. Ph. Eur. monograph 2326 states that it is acceptable to omit extraneous agent tests from routine testing, if validated. Satisfactory consistency data are used to validate the process for the omission of the extraneous agents testing from routine testing, in line with Ph. Eur. monograph 2326.

The batch potency test is described, validated, and includes an appropriate acceptance criteria in line with the requirements for immunogenicity in Ph. Eur. monograph 2326.

The data provided show that all of the required specifications were fulfilled for all *Eimeria* species and repetitions performed during the validation of the potency test. The test has been shown to acceptably discriminate between potent and subpotent batches. Results from routine batches are shown to be statistically significant when treatment groups are compared (vaccinated versus non-vaccinated challenged), as opposed to results from subpotent batches, where treatment groups (vaccinated versus non-vaccinated challenged) give results that are not statistically significant.

Batch-to-batch consistency

The applicant presented final product data for the manufacture of three consecutive final product batches. During the manufacture of the antigen the following tests are carried out (total oocysts, total sporulated oocysts, percentage of sporulation, concentration of sporulated oocysts, percentage before blending, sterility, identity and pH) for harvest for each *Eimeria* species. Test descriptions and the limits of acceptance were presented. In-process control testing (appearance, pH, identification of E-129, E-133, vanillin, colour study, montanide IMS, sterility and volume control) of the diluent bulks is acceptable; all results are within the required limits. Final batch testing of the 3 bulks met all of the required specifications for appearance, pH, concentration of sporulated oocysts, concentration of sodium hypochlorite, mycoplasma and batch potency. Sterility is tested in line with Ph. Eur. 2.6.1 and mycoplasma testing is in line with Ph. Eur. chapter 2.6.7. The concentration of sporulated oocysts for each of the 3 bulks was within the required limits.

The batch potency was determined for the 3 final bulks. Each of the 5 *Eimeria* species met all of the required specifications; therefore, requirements were met for each final bulk. The final batches (x 3 batches of 1,000, 5,000 and 10,000 doses) from each of the 3 bulks also met the required specifications for appearance, pH, volume, sterility, concentration of sporulated oocysts and containers (secondary packaging).

Overall the batch-to-batch consistency data provided was acceptable.

Stability

For antigen:

Satisfactory stability data to 13 months for batches with aged antigens for the Eimeria species, excluding *E. praecox*, stored at 5±3 ºC for 2 months, was provided. The stability study with aged antigen *E. praecox,* to 13 months, is ongoing.

For finished product:

The applicant has provided stability data for three consecutive batches to 13 months, all results met with required specifications. Therefore, a finished product shelf life of 10 months is considered acceptable in line with Ph. Eur. 0062.

A 24-month shelf life has been assigned to the solvent HIPRAMUNE T. This is consistent with the shelf life authorised for the solvent for a similar vaccine, as the same solvent is used for both vaccines and real-time data have already been assessed with the other vaccine.

The applicant is proposing an in-use shelf life of 10 hours for EVANT, based on data already approved for their other similar vaccines. The proposed 10-hour in-use shelf life is considered acceptable in this instance.

Overall conclusions on quality

The qualitative and quantitative particulars of the vaccine suspension and solvent and the containers are described adequately. The necessary certificates are provided.

The applicant gives a detailed description of the development of the vaccine strain and its manufacturing process. Summaries of studies which were performed in the course of the development of the vaccine, and studies on attenuation of the active substances are provided.

The method of production of the sporulated oocysts used as active substances is provided. Overall an adequate description of each process is given and the procedure for disinfection of the oocysts is validated for removal of relevant extraneous agents. Data supporting the storage of the sporulated oocysts prior to vaccine blending is provided.

Vaccine manufacture involves mixing of standard volumes of the sporulated oocysts of each strain and the excipients under aseptic conditions followed by aseptic filling into vials. Data from three batches support the manufacture of consistent batches of acceptable quality.

The production of the HIPRAMUNE T solvent is described in sufficient detail. A bioburden test and acceptable limit in accordance with GMP requirements is performed prior to sterilising filtration and acceptable validation data for the sterilising filter are provided. Satisfactory data supporting the consistency of the manufacturing process are given.

The representative certificates of analysis provided for all pharmacopoeial grade materials support the quality of each material. Foetal bovine serum is irradiated at a minimum of 30 kGy before use.

A detailed description of the preparation and testing of each master and working seed parasite is given. The testing of the seed materials is satisfactory.

For the other biological origin starting materials (tryptose phosphate broth (TPB) and coccidia-free SPF chicks) a satisfactory risk assessment according to Ph. Eur. chapter 5.2.5 has been carried out. The SPF chicks are hatched from SPF eggs meeting Ph. Eur. chapter 5.2.2 requirements. Appropriate tests

are performed on the chicks for the absence of specific pathogens during the quarantine period prior to their use in production, to support a negligible risk of extraneous agent contamination.

A TSE risk assessment in accordance with NfG EMA/410/01 rev.3 supports a negligible risk of TSE transmission.

The non-biological materials are satisfactorily described.

The composition, treatment and storage of the media are satisfactory.

In-process data for the manufacture of three consecutive antigen bulks and three batches of HIPRAMUNE T solvent is presented. Test descriptions and the limits of acceptance are presented. Total oocysts and sporulated oocysts, and percentage of sporulation are counted using a validated counting method. Sterility testing is in line with Ph. Eur. chapter 2.6.1. Based on the data provided, the in-process tests are deemed acceptable to control all of the critical steps in the manufacturing process. The test methods for in-process controls are satisfactorily validated.

The applicant presented finished product testing data for three consecutive antigen bulks. The testing meets the batch testing required in the Ph. Eur. monograph 2326. Identification to confirm the presence of oocysts in the batch of vaccine is determined using a validated counting method; the potency test is used to confirm the presence of oocysts for the Eimeria species. Sterility and mycoplasma are appropriately determined. The results of the extraneous agents testing are appropriately included.

The batch potency test is described satisfactorily. Validation of the potency test showed that all of the required specifications were met for all *Eimeria* species. The test has been shown to acceptably discriminate between potent and subpotent batches.

In-process testing for the manufacture of three consecutive final product batches and the limits of acceptance were provided. In-process control testing of the three diluent bulks was provided and met all of the required limits. Sterility and mycoplasma were tested in line with Ph. Eur. 2.6.1 with Ph. Eur. 2.6.7, respectively. In-process testing and final batch testing of the 3 bulks for each of the 5 *Eimeria* species met all of the required specifications. The concentration of sporulated oocysts for each of the 3 bulks was within the required limits. The batch-to-batch consistency data provided was acceptable.

All *Eimeria* aged antigens were tested in vaccine batches, as part of the ongoing stability programme. From the data provided, a 2-month shelf life is acceptable for bulk antigens. Appropriate real-time stability data for 3 consecutive batches, filled into the smallest and largest presentations, has been presented to 13 months, proposing an acceptable product shelf life of 10 months.

A shelf life of 24 months can be supported for HIPRAMUNE T solvent. A 10-hour in-use shelf life is considered acceptable.

Part 3 – Safety

Introduction and general requirements

EVANT is a live vaccine containing 5 different *Eimeria* species, intended to stimulate active immunity against avian coccidiosis caused by *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox* and *E. tenella.* The strains included have been attenuated for precocity, by repeated passage in chickens with selection for early appearance of oocysts. The time from ingestion of sporulated oocysts to emergence of oocysts in the faeces is termed the pre-patent period and differs between each *Eimeria* species. By selection for

precocious development, populations can be selected with a reduction of pre-patent periods and a significant reduction of pathogenicity.

A single dose of the vaccine is intended for oral use in chickens from one day of age to be administered by coarse spray. The vaccine suspension, which also contains polysorbate 80 and PBS as excipients, is diluted in water and the solvent provided, HIPRAMUNE T, which contains montanide IMS as adjuvant, in addition to vanillin, Red AC (E129) and Brilliant Blue (E133) which are included to facilitate vaccine uptake by birds. Instructions for the appropriate volumes of water and HIPRAMUNE T to use for vaccine dose presentations of 1,000, 5,000 and 10,000 doses are included in the SPC. The final volume of diluted vaccine to be administered to each bird via spray corresponds to 0.28 ml.

The active substances of EVANT are:

Strain	**Range of sporulated oocysts for standard dose***
E. acervulina, strain 003	332 – 450*
E. maxima, strain 013	196 – 265*
E. mitis, strain 006	293 – 397*
E. praecox, strain 007	293 – 397*
E. tenella, strain 004	276 – 374*

* The range established correlates to ± 15% of the standard concentration per dose (fixed concentration).

A full safety file in accordance with Article 12(3) has been provided. The safety of the immunological veterinary medicinal product has been investigated in accordance with the requirements of Directive 2001/82/EC, as amended. In addition, Ph. Eur. chapter 5.2.6 'Evaluation of safety of veterinary vaccines and immunosera', and the specific requirements outlined in Ph. Eur. monograph 2326 'Coccidiosis vaccine (live) for chickens' have been taken into account in order to demonstrate the safety of the vaccine.

Safety documentation

Eleven laboratory trials and one multicentric field trial were carried out to assess the safety of EVANT. The laboratory and the field studies were conducted according to Good Laboratory Practice (GLP) standards and Good Clinical Practice (GCP) guidelines, respectively.

The minimum age of chickens proposed for vaccination is one day; however, specific requirements for the investigation of the safety of the vaccine outlined in Ph. Eur. 2326 specify the use of chickens of the category that is expected to be the most sensitive, i.e. 14-day-old chickens. Thus, the applicant has conducted all laboratory safety studies in 14-day-old SPF chickens as required by Ph. Eur. 2326. The animals enrolled in the field studies were chicks of one day of age, which is the youngest age recommended for vaccination. This approach is considered acceptable.

The investigation of the safety of the vaccine was performed at the maximum content of sporulated oocysts that will be present in a dose. In order to assure that each animal receives the dose established in each protocol, the inoculum (master seed parasite or the vaccine) was administered by oral gavage (directly into the oesophagus of each bird) in all safety laboratory trials. This is in line with

Ph. Eur. 2326. However, in the field trial, the recommended method of vaccination, coarse spray, was used. The use of oral gavage is considered acceptable in the laboratory studies as this route of administration ensures that the correct dose is administered to birds.

One batch of vaccine has been used in the laboratory safety and efficacy studies, and the field study. This was a batch stated to have been manufactured in accordance with the manufacturing process described in the dossier and at the same manufacturing facilities that will be used for future production batches. The applicant has confirmed that batch of vaccine contained coccidian harvest from each *Eimeria* species at the least attenuated passage level that will be present in a batch of vaccine.

During the safety studies, clinical signs, mortality, weight, faeces appearance, oocyst production and intestinal lesions were monitored.

The scoring of intestinal lesions followed the system described by Johnson and Reid (1970), incorporated in Ph. Eur. 2326, for the species-specific lesions visible in the intestine for *E. acervulina*, *E. maxima* and *E. tenella*. For *E. praecox* and *E. mitis*, which are known not to induce macroscopic lesions, the intestinal tract is required to be examined for microscopic evidence of infection such as demonstration of oocysts or developing oocysts; for these two strains the presence of parasites, macroscopic lesions and histological changes were evaluated. The scoring of clinical signs of coccidiosis was performed according to a scale developed by the applicant as follows:

- 0: Animals with normal appearance. Respond to stimuli, eat and drink normally and no remains of blood or diarrhoea are found in the faeces.
- 1: Animals with slightly puffed feathers, movement is slightly less than usual and the faeces may present slight diarrhoea. Respond rapidly to stimuli.
- 2: Animals with puffed feathers, tend to stay in corners due to hypothermia, although they eat and drink normally. Slightly arched body. May present a little blood in faeces or diarrhoea. Respond rapidly to stimuli although after a while they recover their initial state. Feet and crests are a little pale.
- 3: Animals stay in corners, have puffed up feathers, feet and crests are pale due to anaemia caused by haemorrhage. An important diminution is observed in regard to the ingestion of food and drink. Their eyes are shut (sleep-like posture) and an arched body. Do not respond rapidly to stimuli and, even if they do, they rapidly recover their initial appearance. They tend to kneel down. Haemorrhagic faeces as well as diarrhoeas may be quite abundant.
- 4: Death.

Laboratory tests

Eleven laboratory studies have been conducted for the assessment of safety of EVANT. The special requirements for live vaccines have also been addressed by the applicant. Studies were in compliance with the Ph. Eur. 2326 monograph requirements for the examination of residual pathogenicity of live coccidiosis vaccines.

Safety of the administration of one dose

The safety of the administration of one dose was investigated together with the safety of the administration of an overdose; please refer to the following section.

Safety of one administration of an overdose

One laboratory study was conducted to investigate the safety of administration of a single dose and of an overdose. On Day 0 of the study, 14-day-old SPF, coccidia-free chickens were administered a 1X dose (group A), a 10X overdose (group C) or PBS (negative control, group B) by oral gavage. Birds were housed under appropriate circumstances to favour reinfection with oocysts. Feed free from anticoccidials and tap water were freely available.

Animals were observed for 14 days with monitoring of clinical signs, mortality, body weight and feed consumption. Oocyst counts were performed during the 14-day follow-up period. On specific study days (which corresponded to the optimal time for scoring of intestinal lesions for each *Eimeria* species), birds from each group were euthanised and intestinal lesions were scored.

The results demonstrated that no clinical signs, mortality or abnormal faeces were observed in the 1X dose group.

In the 10X overdose group, with the exception of very mild clinical signs observed in 4% of animals, no abnormal clinical signs, mortality or abnormal faeces were observed.

No significant differences in growth rate or mean weight were detected between groups.

Intestinal lesions were found only in the duodenum or upper mid-intestine. No lesions with a score greater than 1 were found in any group during the study. The mean lesion score per group (1X dose group, 10X overdose group) for each study day did not exceed 0.25 with no significant differences between groups, therefore the study complies with the Ph. Eur. 2326 requirements that no individual score is greater than 3 points, and the average lesion score on each of the sampling day is not greater than 1.5 points.

The elimination profile of oocysts and number of oocysts eliminated followed the expected curve for the vaccine and confirmed the administration of a single dose and an overdose.

It is accepted that this study demonstrates the safety of the administration of a single dose, and of a 10X overdose in SPF birds of 14 days of age. No adverse reactions were observed following the administration of a single dose, therefore the proposed wording under section 4.6 of the SPC ('None') is considered acceptable. Although mild coccidial lesions were observed following the administration of a single dose, these lesions did not correlate with clinical signs or any other adverse effects in the single dose group, and such lesions would only (barely) be noticeable if birds were slaughtered shortly after vaccination, which would not occur under field conditions. It can also be accepted that lesions were not more severe in the 10X overdose group and therefore are not required to be mentioned in the SPC. However, after the administration of a 10X overdose, mild clinical signs are commonly transiently observed. Appropriate text has been included in the SPC section 4.10 to reflect these data.

Safety of the repeated administration of one dose

As EVANT is intended for single use only, the applicant has not conducted any studies to investigate the safety of the repeated administration of one dose. This is considered to be acceptable.

Examination of reproductive performance

Since EVANT is intended for use in short-lived birds such as commercial broilers, the applicant has not conducted any studies to investigate any potential effects on reproductive performance. This approach is supported. However, while the vaccine is intended for use in short-lived birds, it is noted that the

SPC does not restrict use to any particular production type of chicken. The CVMP considers that this is acceptable, given that the vaccine is indicated for single use only from one day of age, and no adverse impact on reproductive performance would be expected taking into account the species-specific affinity to different areas of the intestine. An appropriate statement is included in the SPC to indicate that the vaccine should not be used in lay or within 4 weeks before the start of the laying period, or in breeding birds. It is not considered that it is necessary to restrict use in the SPC to broilers only, as future layers/breeders could potentially be vaccinated with this vaccine and then at a later stage with a vaccine containing *Eimeria* strains relevant for longer-lived birds (i.e. *E. necatrix* and *E. brunetti*).

Therefore, by not restricting the vaccine to broilers only (when no restriction is called for on safety grounds), it maintains flexibility for vaccination regimes for poultry farmers. However, it is clearly indicated in the SPC that the vaccine is intended for use in short-lived chickens only and that no data is available on protection of longer-lived birds such as future layers/breeders.

Examination of immunological functions

The applicant has not carried out a specific study to examine any adverse effects on immunological function, since, taking into consideration the nature and composition of EVANT, no negative influence on the immune response is expected. This is considered acceptable, taking into account that there are no known immunomodulatory effects associated with live coccidiosis vaccines.

Special requirements for live vaccines

Spread of the vaccine strain

It is a well-known feature of live coccidia vaccines that the vaccinal oocysts spread. The *Eimeria* species included in the EVANT are live attenuated species; the parasites complete their life cycle in the vaccinated animals (triggering the immune response) and, afterwards, they are eliminated through the faeces to the litter. Then, the oocysts sporulate outside the host and are able to re-infect other in-contact target animals, either previously vaccinated or not. The elimination of oocysts in vaccinated birds was confirmed throughout the safety studies. Spread to non-target species was not investigated because chickens are the only animals that are susceptible to the *Eimeria* species used in EVANT due to its strong host specificity.

Dissemination in the vaccinated animal

Dissemination in the target animal was not investigated in a specific study; the trait of each *Eimeria* species relative to their affinity to a specific portion of the intestine is well-recognised and no further studies are considered necessary.

Reversion to virulence of attenuated vaccines

Reversion to virulence and testing for residual pathogenicity were evaluated in ten laboratory studies. Two studies were conducted for each *Eimeria* strain, the first study to conduct five passages in chickens of the master seed parasite (MSP) to obtain passaged material for reversion to virulence testing, the second to test for residual pathogenicity of both the MSP and the passaged MSP. The test for residual pathogenicity for each *Eimeria* strain involved inoculation of at least ten times the number of oocysts that will be included in a standard dose of EVANT of the MSP or the passaged material

(MSP+5) to 14-day-old SPF birds. Mortality, clinical signs, faeces alteration, body weight, oocyst output and species-specific intestinal lesions on the appropriate day post-inoculation were evaluated. Intestinal lesions were also scored on Day 14 and at study end on Day 21 post-inoculation.

The results of the studies demonstrated that for both the MSP and the MSP+5, for each of the three *Eimeria* strains included that are known to induce specific lesions in the intestine (*E. acervulina, E. maxima* and *E. tenella*), the intestinal lesions in the target area were very mild, indicating attenuation of the strain. For both *E. praecox* and *E. mitis*, again there were no differences between the MSP and the MSP+5 groups; parasites were detected in the intestine for a limited period and correlated only with very mild (or no) histological changes, indicating that only limited signs of infection were observed. In terms of oocyst output, the pre-patency and patency period, there were no differences between the MSP and corresponding MSP+5 for each of the five *Eimeria* strains. No notable clinical signs, mortalities related to treatment or notable changes in faeces appearance were observed for the MSP or MSP+5 in the data presented for each of the *Eimeria* species included in the vaccine.

Overall, it is accepted that the data presented comply with the Ph. Eur. 2326 requirements for the examination of residual pathogenicity of live coccidiosis vaccines, and demonstrate that the vaccine strains are stably attenuated and that no reversion to virulence was observed following five passages *in vivo*.

Biological properties of the vaccine strain

EVANT contains strains of *Eimeria* that have been attenuated for precocity; the precocious lines were obtained from the virulent parent strains by serial passages in SPF chickens, with the collection of oocysts from faeces within the first few hours after excretion. The biological properties are those of the *Eimeria* species without the pathogenic properties of the wild strains; the vaccine strains have retained the property to stimulate an antigen response but are not associated with adverse clinical signs in the target species, and can spread from vaccinated to non-vaccinated animals without reversion to virulence.

Recombination or genomic reassortment of the strains

No specific trials regarding the genomic reassortment or recombination/redistribution of the *Eimeria* strains with other different vaccine strains have been performed. The absence of such studies is considered acceptable, because in the event that genomic recombination between one of the vaccine *Eimeria* strains and the respective wild-type *Eimeria* parasite were to occur in the field, the resultant strains would be expected to be of equal or less pathogenic characteristics than the parent strains.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guideline EMEA/CVMP/IWP/54533/2006.

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are those of accidental dermal or ocular exposure. The vaccine strains are not pathogenic for humans and therefore do not pose a risk for the user.

The excipients including adjuvants are commonly used in other vaccines and do not pose a risk for the user.

As a result of the user safety assessment the following advice to users, included in the product

literature submitted with the application, is considered appropriate:

- Wash and disinfect hands and equipment after use.

Based on the above risk assessment, it can be concluded that EVANT does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

No studies on residues have been performed.

MRLs

The active substance being in principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009. All other components of the final product (vaccine and solvent) are either allowed substances according to Table 1 of Regulation (EC) No. 37/2010, or are substances considered as not falling within the scope of Regulation (EC) No 470/2009.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The applicant has not provided data investigating interactions of the vaccine with other veterinary immunological products and therefore proposes to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis'. This is considered acceptable.

In addition, the *Eimeria* species included in EVANT are sensitive to the most frequently used anticoccidials and to the other agents having anti-coccidial activity. Thus, an incompatibility statement on this point has also been included in section 4.8 of the SPC to warn against use of anticoccidial drugs via feed or water for at least 3 weeks following vaccination of the chickens. However, given that the duration of immunity is dependent on continuous recycling of oocysts in the environment in which vaccinated birds will be housed, and that the claimed benefit of EVANT is a prolonged duration of immunity, that is, compared to a predecessor vaccine marketed by the same applicant, the three-week timeframe for avoidance of anticoccidial substances may be considered limited. Thus, an appropriate warning is also included in Section 4.8 to highlight that the use of anticoccidial substances in the period after 3 weeks post-vaccination should be made taking into account the potential impact on the duration of immunity.

Field studies

One GCP compliant, multicentre, randomised, double blind, positive-controlled clinical field trial was conducted to evaluate the safety and efficacy of EVANT under field conditions. The study was conducted in four commercial broiler farms in one EU member state (Belgium), which was considered to be representative of management and conditions for standard broiler production in the EU, with historical records of clinical or subclinical coccidiosis. A total of 243,210 one-day-old broiler chicks were randomly assigned to two treatment groups and received either the test product (EVANT, n=120,620)

or the control product (positive control, commercially available vaccine against coccidiosis, n=122,590). The vaccination schedule consisted of a single dose administered on Day 0 by the oral route (coarse spray).

During the study, on each farm the test and control groups were housed in separate housing units, both with identical handling conditions, similar density of animals and similar number of feeders and drinkers. Animal housing and management was conducted according to the common rearing practices. Standard feed free of coccidiostats and water were freely available. No drugs to which *Eimeria* could be sensitive were administered during the study. Litter used was wood shavings in three farms and straw in one farm.

Animals underwent a follow-up period of 40 days after vaccination, the approximate period of fattening in broiler chicks in the EU. During this time, daily monitoring of adverse reactions, clinical signs, faeces appearance and mortality was conducted. Euthanasia and intestinal lesion scoring was performed on 15 animals in each group on days 7, 22, 35 and 40 on each farm. Body weight, feed consumption and oocyst output in fresh faeces and in litter samples were also evaluated.

The study was well conducted and confirmed that the product is safe for use in one-day-old broiler chickens, i.e. birds at the proposed minimum age for vaccination, under field conditions. No adverse effects, clinical signs related to vaccination or changes in faeces appearance were observed in either the test or control group. No significant differences in body weight were observed between groups. The mortality rate in both groups was within the common ranges under rearing conditions at the selected farms; the mortality rate in the EVANT group was marginally (and statistically significantly) lower than the mortality rate in the positive control group. Intestinal lesions at Day 7 post-vaccination were not observed in any of the birds sampled. Other parameters evaluated in the study as efficacy variables (refer to Part 4 of the EPAR) can also be considered supportive of safety; feed conversion rate, oocyst counts, intestinal lesions on Days 22, 35 and 40, and body weight at the end of the study, for which it was demonstrated that there were no differences between treatment groups, or if differences existed, they were in favour of the test group.

Environmental risk assessment

An environmental risk assessment, conducted in accordance with the CVMP note for Guidance EMEA/CVMP/074/95, was provided.

Considerations for the environmental risk assessment

It is widely described that the parasites of the genus *Eimeria* are characterised by their rigid and strong host specificity. Not only are the *Eimeria* species naturally limited to a narrow range of host species, but also to a specific site of infection in the intestine. The potential hazard of the vaccine strains included in EVANT to be transmitted to non-target species is effectively nil.

Vaccinated chicks are expected to shed oocysts of each *Eimeria* species included in the vaccine, however this does not involve any risk to non-vaccinated target animals since the vaccine strains are attenuated and data are provided that demonstrate the absence of risk of reversion to virulence. Shedding of the *Eimeria* strains by vaccinated animals is an expected event and is important for re-infection and maintenance of immunity.

Genetic recombination of the strains included in EVANT with field strains is possible, but the resultant strains would be of equal or less pathogenic characteristics than the parent strains.

Apart from the antigen, the rest of the vaccine components are well-known excipients widely used in pharmaceutical formulations. They are generally regarded as non-toxic at these low concentrations used.

It is not considered that there is any likelihood of the live attenuated vaccinal parasites to cause hazards to the environment, taking into account that use as recommended ensures that all the resuspended vaccine is taken up by the vaccinated birds and no parasites are released into the environment. If transmission of vaccine parasites to non-vaccinated chicks occurs, clinical signs of disease will not occur due to the attenuation of the vaccine strains, and shedding to non-vaccinated chicks may even prove beneficial. Transmission to non-target species, including humans, is not expected.

Conclusions on the environmental risk assessment

Based on the data provided, the environmental risk assessment can stop at Phase I. EVANT is not expected to pose a risk for the environment when used according to the SPC. No specific control measures are needed in addition to the general management recommendations of poultry farms and the standard precautions included in the package leaflet concerning the handling and disposal of unused veterinary medicinal product or waste materials derived from the use of such product.

Overall conclusions on safety

Eleven GLP laboratory safety studies, conducted in SPF birds of two weeks of age, and one GCP combined field safety and efficacy study in commercial broiler chickens of one day of age, were conducted to investigate the safety of EVANT. The laboratory studies are considered acceptable and in line with the requirements of the specific Ph. Eur. monograph for avian live coccidiosis vaccines (Ph. Eur. 2326) for the investigation of safety.

During the safety studies, clinical signs, mortality, weight, faeces appearance, oocyst production and intestinal lesions were monitored. One laboratory study was conducted to investigate the safety of administration of a single dose and of an overdose. Given that EVANT is intended for single use only, the safety of the administration of a repeat dose was not necessary to investigate. With the exception of very mild clinical signs, no abnormal clinical signs, treatment-related mortality or abnormal faeces were observed in either of the test groups. No significant differences in growth rate or mean weight were detected between groups.

Since EVANT is intended for use in short-lived chicks, it is considered acceptable that the applicant has not conducted any studies to investigate any potential effects on reproductive performance. In addition, no specific investigations have been performed to examine any adverse effects on immunological function, since EVANT is not expected to have a negative influence on the immune response.

The special requirements for live vaccines were satisfactorily addressed for EVANT, with acceptable justification provided for studies which were omitted.

Concerning spread of the vaccine strain, it is a well-known feature of live coccidia vaccines that the vaccinal oocysts spread. The elimination of oocysts in vaccinated birds was confirmed throughout the safety studies. Spread to non-target species was not investigated because chickens are the only animals that are susceptible to the *Eimeria* species used in the vaccine.

Dissemination in the target animal was not investigated in a specific study; the trait of each *Eimeria* species relative to their affinity to a specific portion of the intestine is well-recognised and no further studies are considered necessary.

Reversion to virulence and testing for residual pathogenicity were evaluated in compliance with the Ph. Eur. 2326 requirements for the examination of residual pathogenicity, with the data demonstrating that no reversion to virulence was observed for the vaccine strains after five passages *in vivo*.

It is accepted that the use of EVANT does not pose a risk to the user, when used in accordance with recommendations. The proposed withdrawal period of zero days is considered acceptable.

One multicentre, randomised, double blind, positive-controlled clinical field trial, conducted in accordance with GCP guidelines, was performed to investigate the safety and efficacy of EVANT under field conditions. The field study confirmed the safety of administration of EVANT when used in accordance with recommendations; administration by coarse spray to commercial broiler chickens of one day of age.

EVANT is not expected to pose a risk for the environment when used according to the SPC.

Part 4 – Efficacy

Introduction and general requirements

The vaccine is intended for the active immunisation of chicks from 1 day of age to reduce intestinal lesions and oocysts output associated with coccidiosis caused by *E. acervulina, E. maxima, E. mitis, E. praecox* and *E. tenella* and to reduce clinical signs (diarrhoea) associated *with E. acervulina, E. maxima* and *E. tenella*. A single dose of the vaccine is intended for use in chickens from one day of age to be administered by coarse spray, following dilution in water and the solvent provided for use with the vaccine (HIPRAMUNE T).

The onset of immunity (OOI) is claimed as 14 days post-vaccination and the duration of immunity (DOI) is proposed as 63 days in an environment that permits oocysts recycling.

Efficacy was demonstrated in compliance with the European Directive 2001/82/EC (as amended by Directive 2004/28/EC and Directive 2009/9/EC), and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7 as well as Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens.

Generally, seven species of *Eimeria* that affect chickens are internationally accepted to be the causative agents of avian coccidiosis, a disease which is distributed worldwide and causes substantial economic losses throughout the world. These species are: *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, *E. tenella*, *E. praecox* and *E. mitis*. The pathogenicity of the first five strains is widely recognised, since they can lead to clinical coccidiosis in which the affected birds generally show typical symptoms of the disease, such as bloody droppings and increased mortality. *E. necatrix* and *E. brunetti* mainly affect birds of more than 8 – 9 weeks of age and thus they are not needed in vaccines intended for use in chickens that are slaughtered at an early age.

The other two *Eimeria* species which can affect chickens, *E. praecox* and *E. mitis*, are known to cause subclinical coccidiosis, as the affected birds show no clear symptoms of the disease; however this subclinical form may lead to uncontrollable body weight and uniformity (out of standard).

The repercussions on the productive performances are due to its early impact in the cycle; it is especially important in broiler meat productions where the first weeks are crucial for the overall

performance of the bird. Thus, five species should be considered as essential when dealing with a vaccine intended for broilers: *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox* and *E. tenella*.

The CVMP considers that the inclusion of the five selected *Eimeria* strains for EVANT is adequately justified, considering the intended target species, broiler chickens. While not strictly relevant, given that this marketing authorisation application is evaluated as a stand-alone application, it is noted that the applicant holds a marketing authorisation for another coccidiosis vaccine which is authorised in many EU member states, contains the same *Eimeria* species and is indicated for use in broilers; the main differences between the applicant's predecessor vaccine and EVANT is the adjuvant used in the solvent HIPRAMUNE T, and a claimed longer DOI following vaccination with EVANT.

Efficacy parameters and challenge model

The efficacy parameters as provided in Ph. Eur. 2326, oocyst production, intestinal lesions, signs of disease and growth rate, were investigated in the efficacy studies. The parameters chosen are considered appropriate for evaluating the efficacy of the product.

No specific studies for the development of the challenge model for each *Eimeria* strain were presented. The *Eimeria* species used to perform the challenge were heterologous from the strains included in the vaccine. The vaccinal strains are Spanish field isolates whereas the challenge strains were isolated in the UK at the coccidia reference laboratory of Houghton Poultry Research Station in Compton (UK). For each *Eimeria* species, the challenge consisted of administration of virulent coccidia by gavage to birds in the vaccinated and control groups, and evaluation of clinical signs, oocyst production in faeces and intestinal lesions.

Considering that the challenge studies were conducted in accordance with the immunogenicity requirements of Ph. Eur. 2326, it is accepted that the challenge models used for each *Eimeria* species were appropriate.

Efficacy documentation

Four studies were conducted to investigate the efficacy of the product and included three laboratory studies and one field trial. Laboratory studies were well documented and carried out in target animals of the minimum age recommended for vaccination. The correct ingestion of vaccinal oocysts at vaccination and during its recycling is an important factor for the efficacy of live coccidiosis vaccines. In line with the Ph. Eur. 2326, the animals included in the laboratory trials were housed in suitable floor pens to favour reinfection with vaccine oocysts. Under field conditions, commercial farms were enrolled and thus standard housing conditions were used.

One batch of EVANT was used in the efficacy trials and was produced at the same manufacturing facilities that will be used for future production batches, in accordance with the proposed manufacturing process. Vaccine batches that are used to test efficacy are required to contain the most attenuated passage to be used for production. According to the manufacturing method for EVANT, the passage level of the antigen for production is a fixed value from the MSP; there is no range.

The investigation of the efficacy of the vaccine was performed at the minimum content of sporulated oocysts that will be present in a dose.

Laboratory trials

Three GLP laboratory studies were performed to investigate the efficacy of EVANT.

The first study was conducted in order to obtain a homogenous population of chickens (vaccinated and controls) to be used in the OOI and the DOI studies. In the first challenge study only the chicks required for the test were used and moved to the challenge unit at 2 weeks post-vaccination. The remaining animals were maintained under the same conditions in the vaccination units until they were required for the DOI study at 63 days post-vaccination.

In the first study, 1-day-old SPF chicks were randomly distributed in two equal-sized groups; one group of chicks was vaccinated by coarse spray with EVANT while the other group was a control group, mock-vaccinated via coarse spray with dye (which had the same composition as HIPRAMUNE T, without the adjuvant montanide; i.e. Red 40, Brilliant Blue and vanillin) in accordance with the requirements of Ph. Eur. 2326. Birds in each group were maintained under the same housing conditions on solid floors with shavings or similar covering to favour reinfection with oocysts. Feed free from anticoccidials and tap water were freely available. Daily monitoring of clinical signs, faeces appearance and mortality was conducted. Body weight on the day of vaccination was recorded in a subset of birds per group and feed consumption was measured when birds were withdrawn for the OOI and for the DOI studies. Oocyst counts were performed on fresh and litter faeces.

There were no clinical signs or mortalities related to vaccination/mock-vaccination, or alterations of faeces observed. The average weight in both groups on Day 0 was the same. In the vaccinated group, the elimination profile of oocysts confirmed that vaccination had been performed correctly. No oocysts were detected in faeces in the control group.

It is accepted that the animals included in this study were suitable for use as vaccinates/controls in the subsequent OOI and DOI challenge studies.

Onset of immunity

One study was carried out in the target species of minimum age, in compliance with Ph. Eur. requirements to investigate the onset of protection, using birds vaccinated from the previous study, as discussed. In the study, five separate challenge sub-studies for each *Eimeria* species were conducted, in accordance with the requirements of Ph. Eur. 2326. The *Eimeria* species used to perform the challenges were heterologous to the strains included in the vaccine and were inoculated by oral gavage to the vaccinated and control birds at 14 days post-vaccination.

Clinical signs, faeces appearance, mortality, body weight and oocyst counts were evaluated during the 14-day observation period. Intestinal lesions were evaluated in 18 birds/group at the time at which lesions were expected to be most severe for the three *Eimeria* strains known to be associated with characteristic intestinal lesions: *E. acervulina, E. maxima* and *E. tenella*. Intestinal lesions were also evaluated for *E. mitis* and *E. praecox* on an appropriate day post-challenge, in addition to histological analysis and evaluation of number of parasites present in the intestine. Lesions were scored in the remaining birds in each group on day 14. Intestinal lesions were evaluated using the scoring system outlined in Ph. Eur. 2326 for *E. acervulina*, *E. maxima* and *E. tenella*. For *E. mitis* and *E. praecox*, these species are known not to induce macroscopic lesions (microscopic evidence of infection is evaluated to compare differences between vaccinated and control groups, based on the count of parasites in a section of tissue and histological changes due to infection), however the applicant did evaluate macroscopic intestinal lesions (based on an in-house developed scoring system on a three-point scale).

The assessment of appearance of faeces of a group of poultry was performed using the scale: 0; normal, 1; less than 25% of faeces are affected, 2; between 25-50% of faeces are affected, 3; between 50 – 75% of faeces are affected, 4; more than 75% of faeces are affected. The clinical signs

of poultry were assessed by evaluating the general state of health of the group and also the appearance of faeces, with the same scoring system for clinical signs of coccidiosis as used in the safety studies (refer to introduction to Part 3).

In each challenge study, the study complied with the requirements of Ph. Eur. 2326 for establishment of infection in the control group (intestinal lesions with a score not less than 2 in ≥80% control birds) and protection in the vaccinated group (≥80% vaccinated birds have no or minimal lesions in the intestine, e.g. mean lesion scores not greater than 1, no bird has a score of 4).

Intestinal lesions were statistically significantly reduced in the vaccinated group compared to the control group for each *Eimeria* challenge study, as indicated below (mean lesion scores indicated in parenthesis):

- *E. acervulina*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.11) and control group (2.44).
- *E. maxima*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.72) and control group (1.83).
- *E. mitis*: no statistically significant difference ($p=0.059$) was found in intestinal lesion scores between the vaccinated (0.50) and control group (0.94) for macroscopic lesions, which is acceptable for this strain. Statistically significant differences in parasite score ($p=0.003$) and in histological changes ($p<0.001$) were observed following microscopic evaluation of intestinal lesions.
- *E. praecox*: a statistically significant difference ($p<0.001$) was observed between the vaccinated (0.29) and control group (1.83) for macroscopic changes (unexpected given that macroscopic lesions are not a feature). A statistically significant difference in parasite score ($p<0.001$) and histological changes ($p<0.001$) were observed following microscopic evaluation of intestinal lesions.
- *E. tenella*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.67) and control (2.41) group. Lesions were slightly more severe in the vaccinated group relative to lesions observed with the other *Eimeria* strains (2 birds had a score of 3), however the mean score was not greater than 1 and no bird had a score of 4, therefore the data are in compliance with Ph. Eur. 2326.

Oocyst production was decreased in the vaccinated group compared to the control group, with a percentage reduction of 88.7%, 50.3%, 62.5%, 72.2% and 90.7% for *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox* and *E. tenella*, respectively. Although it is noted that oocyst output was not statistically compared between groups, it can be accepted that the data support a reduction in oocysts output considering that the percentage reduction was >60% in the vaccinated group compared to the control group for four of the *Eimeria* challenges. For *E. maxima*, while it is noted that the percentage reduction was somewhat lower in the vaccinated group compared to that observed for the other challenge studies (50.3%), numerically there is a clear beneficial effect of vaccination on the reduction of oocyst production, with an average count of 6.2 million oocysts in the vaccinated group compared to 12.4 million in the control group.

Given that the proposed indication includes a claim for reduced oocyst output, the CVMP considers that while it would have been preferable if the reduction of oocyst production had been supported by an appropriate statistical analysis, based upon the data provided, the proposed claim has been adequately demonstrated.

Clinical signs evaluation demonstrated that for each of the challenge studies, mild to moderate changes in faeces were observed in the control group (scores 1 and 2, exceptionally score 3), and no changes (*E. acervulina, E. maxima*) or mild changes (*E. mitis, E. praecox, E. tenella*) in faeces (score 1 only) were observed in the vaccinated group after challenge. The duration of changes in faeces in the vaccinated group (when mild changes were detected) also appeared to have been reduced compared to the control group. With respect to other clinical signs of disease, the frequency and/or severity of signs in the control group was insufficient to allow a meaningful comparison of clinical signs of disease between groups, and thus the challenges were considered inadequate for the purpose of supporting a reduction of clinical signs of disease. For the three *Eimeria* species that are known to cause clinical coccidiosis (*E. acervulina*, *E. maxima* and *E. tenella*), a reduction in the % average daily prevalence of clinical signs in the respective vaccine group compared to the control group was demonstrated. However, the statistically significant differences obtained in the % average daily prevalence of clinical signs between groups were primarily driven by the scores relating to the proportions of birds with changes in faeces. While it is accepted that the main clinical sign of coccidiosis is the effect on faecal consistency, the CVMP considered that a broader claim for a reduction of typical clinical signs of disease was insufficiently substantiated. However, it was considered that adequate support for a reduction in clinical signs (diarrhoea) associated with *E. acervulina*, *E. maxima* and *E. tenella* had been provided.

Finally, regarding growth rate, while a claim for a positive effect of vaccination to reduce infection-related growth impairment is not sought for this vaccine, the Ph. Eur. monograph 2326 requires that the growth rate is significantly greater in the vaccinates that in the controls. A significant difference in growth rate was observed in each of the challenge studies, during the immediate post-challenge phase for *E. acervulina*, *E. maxima*, *E. praecox* and *E. tenella,* and during the period day 4.5 to 14 for *E. mitis*, supporting that growth rate was significantly greater in the vaccinated group during the acute challenge period (or just after, for *E. mitis*). However, the growth rate in the control group quickly recovered after the challenge, and a difference in overall growth rate between Days – 1 to 14 post-challenge was observed only in the *E. maxima* challenge study. It is accepted that compliance with the Ph. Eur. monograph 2326 requirement that the growth rate in the vaccinated group is significantly greater than in the control group was demonstrated, when the growth rate was most affected by challenge.

In summary, it is accepted that a reduction in intestinal lesions and a reduction in oocyst output for each of the *Eimeria* strains included in EVANT has been demonstrated at 14 days post-vaccination following vaccination of 1-day-old SPF birds by coarse spray with a minimum dose of EVANT. In addition, a reduction of clinical signs (diarrhoea) associated with *E. acervulina*, *E. maxima* and *E. tenella* has been adequately supported.

Duration of immunity

One study was carried out in the target species of minimum age, in compliance with Ph. Eur. requirements to investigate the duration of protection, using birds vaccinated in the previous study, as discussed. In the DOI study, five separate challenge sub-studies for each *Eimeria* species were conducted at 63 days post-vaccination.

The study designs and parameters measured were the same as for the OOI study, with evaluation of clinical signs, faeces appearance, mortality, body weight and oocyst counts during the 14-day observation period.

Intestinal lesions were statistically significantly reduced in the vaccinated group compared to the control group for each of the five *Eimeria* strains included in the vaccine. There were no indications of any decrease in the level of protection in vaccinated birds at 63 days post-vaccination in an environment which permitted recycling of oocysts.

- *E. acervulina*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (1.00) and control (2.61) groups. While it is noted that the mean score increased slightly in the vaccinated group compared to that of the vaccinated group in the OOI study (mean score 0.11), birds were still fully protected in accordance with Ph. Eur. requirements and no vaccinated bird had a lesion score of 4.
- *E. maxima*: a statistically significant difference ($p=0.004$) was found in intestinal lesion scores between the vaccinated (0.41) and control (2.72) group. The mean score in the vaccinated group was lower than that observed in vaccinated group in the OOI study (0.72), and was higher in the control group than the control group in the OOI study (1.83).
- *E. mitis*: a statistically significant difference ($p=0.016$) was found in intestinal lesion scores between the vaccinated and control group, based on an internally developed scoring system from 0 – 2 (mean lesion score was 0.72 in the vaccinated group and 1.44 in the control group). Statistically significantly lower parasite scores ($p<0.001$) and histological changes scores ($p=0.007$) were observed in the vaccinated group compared to the control group following microscopic evaluation of intestinal lesions.
- *E. praecox*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated and control group, based on a scoring system from 0 – 2 (mean lesion score was 0.17 in the vaccinated group and 1.67 in the control group). A statistically significant difference in parasite scores ($p<0.001$) and histological changes scores ($p<0.001$) were observed following microscopic evaluation of intestinal lesions; in fact, while 14/18 control birds had score 2 (over 100 parasites present in area examined), there were no parasites detected in the vaccinated group. Similarly, mild to moderate histological changes were observed in all control birds, but were absent in the vaccinated group.
- *E. tenella*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.11) and control (3.50) groups. The mean score for lesions for vaccinates was less than that observed for the vaccinated group in the OOI study (0.67), while lesions were more severe in the control group in this study compared to the control group in the OOI study (2.41), with all 18 birds reported with lesion scores of 3 or 4 in the control group.

Oocyst production was decreased in the vaccinated group compared to the control group, with a percentage reduction of oocyst excretion in the vaccinated group compared to the control group during Day 3 to 14 post-challenge of 71.3%, 65.2%, 52.7%, 99.9% and 96.1% for *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox* and *E. tenella*, respectively.

Clinical signs consisting of mild to moderate changes in faeces appearance were observed in the control groups for four of the challenge studies, and no changes (*E. acervulina, E. maxima*) or milder changes *(E. mitis, E. tenella*) in faeces appearance were observed in the vaccinated groups. No changes were observed in the vaccinated or control groups following *E. praecox* challenge.

Clinical signs, apart from changes in faeces appearance, were not observed in study groups, with the exception of isolated reports in the control group in the *E. acervulina* challenge study and in the *E. tenella* challenge study. Therefore, whilst these data demonstrate that mild to moderate changes in faeces appearance were observed in the control groups for each of the challenge studies, and that

none (*E. acervulina, E. maxima*) or milder changes *(E. mitis, E. tenella*) in faeces appearance were observed in the vaccinated groups, as highlighted for the onset of immunity data, no comparison (including statistical analyses) of incidence and/or severity of such signs was initially presented by the applicant. Similar to that discussed for the OOI study, the applicant presented supplementary analyses of the raw data for *E. acervulina*, *E. maxima* and *E. tenella* in the response to the concern raised regarding insufficient support for the proposed claim for a reduction in typical signs of disease. The results demonstrated a statistically significant difference between groups in the % average daily prevalence of clinical signs in the 14-day post-challenge period; 0 compared to 3.83 in the vaccinated and control group, respectively, after *E. acervulina* challenge ($p<0.001$), 0.22 compared to 8.61 in the vaccinated and control group, respectively, after *E. maxima* challenge ($p<0.001$), and 0.28 and 40.6 in the vaccinated and control group, respectively, after *E. tenella* challenge ($p<0.001$). However, since the difference in scores between groups is considered to be mainly attributed to the prevalence of changes in faeces (as discussed for the OOI study), it is considered that the data provided support a reduction of clinical signs (diarrhoea), rather than a wider claim for a reduction of typical clinical signs of disease.

A significant decrease in growth rate was observed in the control group in post-challenge period for the five *Eimeria* strains, in some cases to such a degree that there was an overall difference between the vaccinated and control group from Day – 1 to Day 14 post-challenge (*E. maxima, E. mitis, E. tenella*). However, in the interim period after the day on which lesions were most severe in each challenge study until the end of the study, compensatory growth could be observed in the control group with either the same or a greater growth rate than the respective vaccinated group.

In summary, it is accepted that a reduction in intestinal lesions and a reduction in oocyst output for each of the *Eimeria* strains included in EVANT has been demonstrated at 63 days post-vaccination following vaccination of 1-day-old SPF birds by coarse spray with a minimum dose of EVANT. In addition, a reduction of clinical signs (diarrhoea) associated with *E. acervulina*, *E. maxima* and *E. tenella* infection has been adequately supported.

Maternally derived antibodies (MDA)

Whilst EVANT is proposed for use in one-day-old chickens, no product-specific studies were conducted to determine if the presence of MDA would interfere with the response to vaccination; however, this approach has been justified by the applicant on the basis of a) the nature of the immunological response triggered after an *Eimeria* infection (both humoral and cell-mediated, however the cell-mediated response is likely to play a major role in protection against avian coccidiosis), b) the fact that vaccines with either virulent or attenuated strains have been used since 1950 worldwide, and in the majority of cases chicks vaccinated with live vaccines come from breeders that have been previously vaccinated also with live vaccines, without specific indication of lack of efficacy in such cases, and c) studies conducted with the vaccine HIPRACOX BROILERS, which were considered demonstrative of the absence of interference of MDAs with the response to vaccination during the respective registration procedure.

The CVMP notes that while EVANT and HIPRACOX BROILERS have the same antigenic composition, they differ with respect to the inclusion of the adjuvant montanide in EVANT; however, it is not considered that the inclusion of the adjuvant would alter the accepted lack of interference of MDAs, and therefore it is accepted that the conclusions from these studies that MDAs would not interfere with the response to vaccination are valid for EVANT. In addition, during the application procedure for the centralised product EVALON, it was accepted by CVMP that the presence of MDA does not impact the development of immunity against *Eimeria* species. The CVMP considers that this conclusion can

reasonably be extrapolated to EVANT, considering the similarities between both vaccines (i.e. three of the *Eimeria* strains are the same in both vaccines, the adjuvant is the same, and both vaccines are indicated for use from one day of age).

In summary, on the basis of supportive information generated with another of the applicant's live coccidiosis vaccines, it is accepted that the presence of MDA would not be expected to have a negative impact on vaccination with EVANT.

Field trials

One GCP compliant, multicentre, randomised, double blind, positive-controlled clinical field trial was conducted to evaluate the safety and efficacy of EVANT under field conditions; refer to Part 3 for a summary of the trial design.

The primary efficacy variable was the feed conversion rate (FCR), calculated on each farm by dividing the amount of feed consumed by each group up to slaughter and the body weight of each group at slaughter. Secondary efficacy variables consisted of evaluation of oocyst counts (litter and fresh faeces), macroscopic intestinal lesions on Day 22 and 35, weight evolution, and mortality (and clinical signs and faeces appearance if an outbreak occurred).

The effects to be achieved to claim efficacy were three-fold: in all circumstances under which clinical signs of coccidiosis are not observed in the test group, in all circumstances under which an outbreak of clinical or sub-clinical coccidiosis appears in the positive control group, in all circumstances under which there are no significant differences in the production values (mainly FCR, weight and mortality) of the test group compared to the positive control group. The experimental unit was each unit treated. For qualitative variables, differences between groups were tested using a Chi-2 Test. For quantitative variables, differences were tested using a Student's t-Test if application conditions were satisfied or, alternatively, Mann-Whitney's U-Test was used.

The results demonstrated that, following vaccination of 1-day-old commercial broiler chicks with EVANT (n=120,620) or the positive control vaccine (commercially available vaccine against coccidiosis, n=122,590), no difference in the FCR was found between the test (FCR 1.60) and control group (FCR 1.61), p=0.726.

The secondary efficacy variables evaluated (weight, mortality, oocyst production [demonstrating replication of vaccinal strains], intestinal lesions) also supported comparability between the test and control groups, or if there were differences, they were in favour of the EVANT group. However, although it is noted that the farms included in the trial were selected on the basis of previous history of clinical or sub-clinical coccidiosis, there were no outbreaks of coccidiosis during the trial. In addition, whilst it is noted that the omission of a negative control group has been justified for ethical reasons, the absence of a negative control group limits the conclusions that may be drawn from this study given that it cannot be confirmed that effectiveness of the vaccine has been investigated under field challenge (notwithstanding the claimed history of coccidiosis on the study sites).

Furthermore, deficiencies in the statistical analyses were noted; the statistical comparison between test and positive control groups is considered inadequate for the purpose of definitively concluding upon the effectiveness of the test vaccine compared to the positive control. Notwithstanding the deficiencies highlighted above, the primary efficacy variable, FCR, which is an economically important measure of broiler performance, was not found to be statistically significantly different between the EVANT group and the positive control group. Secondary efficacy variables also appear to generally support comparability between the EVANT group and the positive control group. However, given the

deficiencies noted and the absence of confirmation of adequate infection pressure, the study data can be considered as supportive only regarding the efficacy of EVANT.

Overall conclusion on efficacy

Three laboratory studies were conducted to evaluate the efficacy of EVANT and one field trial to support the laboratory studies.

The challenge models were considered acceptable for the evaluation of oocyst production and intestinal lesions.

The results from the laboratory studies conducted to investigate the OOI and the DOI at the minimum dose according to the proposed range on the label for each *Eimeria* species demonstrate that the product has been shown to be efficacious for the active immunisation of chicks from one day of age against coccidiosis caused by *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox* and *E. tenella* to reduce intestinal lesions and oocyst output. In addition, adequate data was provided to support a reduction of clinical signs (diarrhoea) for *E. acervulina*, *E. maxima* and *E. tenella.*

The OOI and the DOI are accepted as 14 days and 63 days post-vaccination, respectively, when animals are vaccinated in an environment which permits oocyst recycling.

The presence of MDA in the target species are not expected to interfere with the response to vaccination.

One field study was undertaken in four commercial broiler chicken farms, performed in one EU member state, in which more than 120,000 1-day-old chickens were vaccinated with EVANT in accordance with recommendations. Whilst the data provided demonstrated that there were no differences between the group vaccinated with EVANT compared to the positive control group for various parameters including feed conversion rate, there were no outbreaks of coccidiosis and in the absence of a negative control group the level of infection pressure was unknown. Therefore, the field study did not provide additional support for the efficacy of the vaccine.

Part 5 – Benefit-risk assessment

Introduction

EVANT is a live coccidiosis vaccine containing 5 different attenuated species of *Eimeria*. The vaccine is provided with a solvent, HIPRAMUME T, which contains colouring agents, flavour and the adjuvant montanide IMS, which are included to facilitate uptake of vaccine droplets by birds and to enhance the response to vaccination.

The proposed vaccination scheme is one dose by coarse spray vaccination.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

Benefit assessment

Direct therapeutic benefit

The *Eimeria* strains included in EVANT are the five most important *Eimeria* strains which affect broiler chickens. While the applicant also holds a marketing authorisation for a vaccine which is commercially available within many EU member states which has the same strains as included in EVANT, and which is indicated for use in broiler chickens, the duration of protection for EVANT is longer, presumably attributed to the inclusion of the adjuvant. This is of therapeutic benefit considering that according to different production systems for broiler chickens in more recent times, the life span of such birds may be longer.

The benefit of EVANT is its efficacy for the active immunisation of chicks from 1 day of age to reduce intestinal lesions and oocysts output associated with coccidiosis caused by *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox* and *E. tenella*, and to reduce clinical signs (diarrhoea) associated with *E. acervulina*, *E. maxima* and *E. tenella* infection, which was investigated in well-designed laboratory studies and a field study conducted to acceptable standards. The OOI was demonstrated at 14 days post-vaccination, and the DOI was demonstrated at 63 days post-vaccination.

Additional benefits

EVANT increases the range of available treatment possibilities for coccidiosis in chickens. The increase of vaccines available for the reduction of coccidiosis would have an indirect benefit on reducing the use of anticoccidial agents.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out support the consistency and uniformity of important product quality characteristics leading to the conclusion that the product should have a satisfactory and uniform performance in clinical use. The stability data provided can be deemed satisfactory.

Safety:

Risk for the target animal:

The safety data provided for EVANT demonstrate that for the target animal, there were no adverse reactions following vaccination under recommended conditions of use. The vaccine strains have been attenuated for precocity, by selection of oocysts with shorter pre-patent periods, which enables the vaccine strains to maintain immunogenic properties without causing disease. Reversion to a virulent form after *in vivo* passages in chickens could not be demonstrated. No adverse events are expected when administered at the recommended dose and administration route.

Concerning spread of the vaccine strain, it is a well-known feature of live coccidiosis vaccines that the vaccine oocysts spread. The elimination of oocysts in vaccinated birds was confirmed throughout the safety studies.

Risk for the user:

The use of EVANT does not pose a risk to the user, when used in accordance with recommendations. Appropriate risk mitigation measures are described in the SPC section 4.5.

There are no risks identified for consumers of animals vaccinated with EVANT. The active substances included in EVANT are live attenuated *Eimeria* strains which do not infect humans. In addition, the vaccine does not contain any components for which an MRL is required, therefore a withdrawal period is not required.

Risk for the environment:

The *Eimeria* oocysts in the vaccine EVANT will be shed by chickens after vaccination, and will be present in the environment where chickens are maintained. However, the recycling of oocysts is a desired attribute of the vaccine, necessary for the development of immunity, and the risk to the environment of the presence of the attenuated vaccine oocyst strains is considered to be acceptable. The attenuated vaccine strains are highly host specific and do not cause disease in the target species. In addition, the presence of coccidia is considered to be ubiquitous in commercial poultry farms and the risk to the environment would not be expected to be any greater than the risk presented by the naturally present coccidia species. Spread to non-target species was not investigated because chickens are the only animals that are susceptible to the *Eimeria* species used in EVANT due to strong host-specificity. The product is not expected to pose a risk to the environment when used according to SPC.

Thus, the risks for the environment following use of the vaccine as recommended are considered to be negligible.

Risk for the consumer:

The withdrawal period is set at zero days.

Risk management or mitigation measures

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user, consumer, and the environment and to provide advice on how to prevent or reduce these risks.

Evaluation of the benefit-risk balance

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for EVANT is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned medicinal product.