EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Cevac Salmune ETI K (EMEA/V/C/006118/0000)

Vaccine common name: Salmonella Enteritidis, Salmonella Typhimurium and Salmonella Infantis vaccine (inactivated) 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 CEVA-Phylaxia Zrt. submitted on 5 December 2022 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Cevac Salmune ETI K, through the centralised procedure under Article 42(4) of Regulation (EU) 2019/6 (**optional scope**).

The eligibility to the centralised procedure was agreed upon by the CVMP on 12 May 2022 as no other marketing authorisation has been granted for the veterinary medicinal product within the Union.

At the time of submission, the applicant applied for the following indication:

"For the active immunisation of chickens (breeders and layers) from 10 weeks of age to reduce faecal excretion with *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Infantis."

The target species are chickens. The active substances of Cevac Salmune ETI K are *Salmonella enterica* subsp. *enterica* serovar Enteritidis, strain 038-90 (inactivated), *Salmonella enterica* subsp. *enterica* serovar Typhimurium, strain 076-94 (inactivated) and *Salmonella enterica* subsp. *enterica* serovar Infantis, strain SM-595 (inactivated). These substances should actively immunise chickens to the above-mentioned serovars of *Salmonella enterica* subsp. *enterica*, which should lead to the reduction of their number in the organism and, consequently, reduce their faecal excretion.

Cevac Salmune ETI K suspension for injection contains per dose (0.5 ml) at least 122 ELISA units of *Salmonella* Enteritidis, at least 212 ELISA units of *Salmonella* Typhimurium and at least 157 ELISA units of *Salmonella* Infantis and is presented in packs containing 1 bottle of 1000 doses and 5 bottles of 1000 doses.

The rapporteur appointed is Esther Werner and the co-rapporteur is Kristina Lehmann.

The dossier has been submitted in line with the requirements for submissions under Article 8 of Regulation (EU) 2019/6 – full application.

On 18 July 2024, the CVMP adopted an opinion and CVMP assessment report.

On 30 August 2024, the European Commission adopted a Commission decision granting the marketing authorisation for Cevac Salmune ETI K.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Summary of the Pharmacovigilance System Master File

The applicant has provided a summary of the pharmacovigilance system master file (PSMF), which in general fulfils the requirements of Article 23 of Commission Implementing Regulation (EU) 2021/1281. Based on the information provided, the applicant should have in place a PSMF, has the services of a qualified person responsible for pharmacovigilance, and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

Manufacturing authorisations and inspection status

Active substances

Manufacture, quality control testing (chemical/physical, microbiological, biological), storage and/or distribution of the active substances *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Infantis take place at Ceva-Phylaxia Zrt. (English: Ceva-Phylaxia Co. Ltd.), Hungary. The site has a manufacturing authorisation issued on 11 March 2022 by the competent authority of Hungary.

Valid GMP certificates confirming compliance with the principles of GMP are provided by the competent authority of Hungary (National Food Chain Safety Office, Directorate of Veterinary Medicinal Products).

A GMP declaration for the active substances manufacturing site was provided from the Qualified Person (QP) at Ceva-Phylaxia Veterinary Biologicals Co. Ltd. The declaration was based on an audit performed on 16 June 2022 by the Manufacturing / Importer Authorisation Holder (MIAH, i.e. Ceva-Phylaxia Veterinary Biologicals Co. Ltd., Hungary). It is confirmed that the manufacturing of the active substance complies with the principles and guidelines of EU GMP.

Finished product

Bulk product manufacturing, primary packaging, secondary packaging, quality control (chemical/physical, microbiological, biological), finished product batch release, storage and/or distribution take place at Ceva-Phylaxia Zrt., Szállás utca 5, 1107 Budapest, Hungary. The site has a manufacturing authorisation issued on 11 March 2022 by the competent authority of Hungary.

In addition to the GMP certificates already mentioned for the active substances, a supplementary certificate issued on 30 May 2018, referencing an inspection of another building and expedition warehouse at Ceva-Phylaxia Veterinary Biologicals Co. Ltd., has been provided.

Further activities of quality control (chemical/physical, i.e. thiomersal content control test) take place at Eurofins Analytical Services Hungary Kft., Hungary. Manufacturing authorisation for this site was issued on 2 February 2023 by the competent authority of Hungary. A valid GMP certificate confirming compliance with the principles of GMP is provided by the competent authority of Hungary. The certificate was issued on 2 February 2023 referencing an inspection of this manufacturer on 13 October 2020.

An alternative site for secondary packaging is Ceva Santé Animale, France. Manufacturing authorisation for this site was issued on 3 January 1985 by the competent authority of France. The decision was updated on 8 April 2022. A valid GMP certificate confirming compliance with the principles of GMP is provided by the competent authority of France. The certificate was issued on 26 August 2022 referencing an inspection of this manufacturer on 9 June 2022.

Overall conclusions on administrative particulars

The summary of the PSMF is considered to be 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

Quality documentation (physico-chemical, biological, and microbiological information)

Qualitative and quantitative composition

The finished product is presented as suspension for injection containing at least 122 ELISA units (EU) of inactivated bacteria of *Salmonella enterica* subsp. *enterica* serovar Enteritidis, at least 212 ELISA units *Salmonella enterica* subsp. *enterica* serovar Typhimurium and at least 157 ELISA units *Salmonella enterica* subsp. *enterica* serovar Infantis as active substances per dose of 0.5 ml. The product contains aluminium hydroxide as adjuvant. Other ingredients are trometamol, maleic acid, sodium chloride, sodium hydroxide and water for injections. The vaccine is intended to be available in multidose presentations and consequently contains thiomersal as a preservative.

For the excipients trometamol and maleic acid, further information has been provided, which allows the conclusion that the risk for human health can be considered negligible.

Container and closure system

The product is filled into plastic containers made of low-density polyethylene (in accordance with Ph. Eur. chapter 3.1.4) with a capacity of 500 ml. The vials are closed with silicone-coated bromobutyl rubber stoppers (type I in accordance with Ph. Eur. chapter 3.2.9) and sealed with aluminium plastic caps having a tear-off strip. The pack sizes are 1 x 1000 doses and 5 x 1000 doses. The pack/container sizes are consistent with the vaccination schedule and intended use.

The containers and closures comply with the pharmacopoeial requirements, and their sterilisation is adequate.

Product development

An explanation and justification for the composition and presentation of the vaccine has been provided. Reasonable justification is given regarding the relevance of the chosen vaccine strains within the EU. The adjuvant aluminium hydroxide is well known and widely used for both human and veterinary immunologicals. As the vaccine is presented in multidose containers, the choice of thiomersal as preservative is justified. Its efficacy has been adequately demonstrated. All excipients are well-known pharmaceutical ingredients, and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in Section 2 of the SPC.

The production of the active ingredient and finished product is considered conventional and uses

processes well known in the manufacture of veterinary vaccines. Adequate information on the manufacturing process of the active substance and the finished product, the in-process and final product control tests and the corresponding validations as well as stability and overages has been provided. The development and validation of the ELISA methods used for antigen quantification of antigen bulks and as in-vitro potency test are explained. The reasons for selecting the competitive ELISA concept, a justification for the monoclonal antibodies used and a description for the replacement procedure for internal standards have been provided. Apart from these aspects, the link between formulation ELISA values and in-vitro potency ELISA values as well as their relations to efficacy studies and the approach for setting of the minimum release titre have been appropriately explained.

The fixed formulation target per ml of bulk vaccine is justified by data from manufacturing process validation, by the minimum guaranteed requirement from the potency release tests (considering the assay variability and recovery aspects), by stability studies and by results of the safety and efficacy studies.

Batches used in the pre-clinical and clinical trials were produced according to the manufacturing process described in Part 2 of the registration dossier, with the following predefined alterations: for safety testing a batch was used that was intentionally over-formulated beyond the target formulation, whereas for the demonstration of the efficacy (immunogenicity studies, onset and duration of immunity studies) batches were used that were under-formulated compared to the target formulation. This approach is considered acceptable as the results of the studies were taken into account for minimum release and end-of-shelf-life specifications in terms of potency testing.

Description of the manufacturing method

The manufacturing process of the vaccine is based on the seed-lot principle and consists of four main steps: up-scaling and main fermentation, inactivation, formulation and finally, filling and packaging. The process is considered to be a standard manufacturing process.

The production steps are identical for each of the three *Salmonella* strains.

For the first step of up-scaling, the prepared sterile media are inoculated with one or more vial of the respective *Salmonella* working seed bacteria (WSB) in flask(s) in order to produce the first pre-culture. For the production of each of the active substances, a stepwise increase in incubation volume is performed. Depending on the target volume of the production culture, the pre-culturing step can be repeated up to (in total) 3 times. The pre-cultures are inoculated into the next pre-culture medium under aseptic conditions. The incubation for the scale-up pre-cultures is carried out in fermenters. The prepared pre-cultures are either immediately used for inoculation of the next pre-culture of the final production culture or can be kept cooled before use. The intermediate storage times are properly justified. Each of the pre-cultures is sampled for purity testing. The last pre-culture is used for the inoculation of the main culture that will be used for the particular strain. The incubation is carried out in a fermenter, during continuous agitation. For all culturing steps, the pH of the medium is regulated, and an antifoaming agent may also be added. Process parameters are checked and recorded by the equipment.

The inactivation is carried out using formaldehyde solution. Then the culture is transferred into the inactivation tank and kept stirred to complete the inactivation process. After the completion of inactivation, the antigen is cooled down before being harvested into storage containers.

To validate the manufacturing process as described above, tabulated manufacturing data are provided for three pilot batches and one commercial size batch of inactivated antigen for each of the

three active substances, i.e. *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Infantis.

To prepare the bulk vaccine, the adjuvants and excipients are blended with the antigens under aseptic conditions. Thiomersal solution is used as preservative.

The product is filled by automated filling equipment into sterile plastic bottles. Sterilised rubber stoppers are placed on the vials under aseptic conditions by the filling machine. The sealing of each vial with an aluminium plastic cap is performed by an automatic capping machine. Until packing, the product is stored under recommended conditions at 5±3 °C.

Major steps of the manufacturing process have been adequately validated.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Starting materials of Ph. Eur. quality are used as media components, pH regulators, adjuvant and excipients. The nature of the starting materials and treatments applied guarantee sterility of the vaccine and absence of introduction of any extraneous agent. Representative internal specifications and/or representative certificates of analysis (CoA) have been provided. The materials in question are in full compliance with the requirements of the respective Ph. Eur. monograph.

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

Starting materials of biological origin, which are not listed in the Ph. Eur., are *Salmonella* Enteritidis, strain 038-90, *Salmonella* Typhimurium, strain 076-94, *Salmonella* Infantis, strain SM-595 and soy peptone.

For the active ingredients, a seed lot system was satisfactorily established. Details of source, passage history, manufacturing, controls and storage conditions for the master seed bacteria (MSB) and WSB have been provided and are considered appropriate. A TSE risk assessment complying with the current regulatory texts, Ph. Eur. monograph 5.2.8 "Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" and the TSE Note for Guidance EMEA/410/01-Rev.3, has been provided. In conclusion, the risk that the seed materials or the final product may transmit TSE to the target animals (chickens), which are not known to be susceptible to TSE, was estimated as negligible.

Soy peptone is of non-animal origin and, hence, there is no risk of viral contamination or TSE transmission. Growth medium is sterilised using the Ph. Eur. reference method.

Starting materials of non-biological origin

Antifoaming agent is a component in growth medium to prevent excessive foaming during fermentation. It is of non-biological origin and, hence, there is no risk of viral contamination or TSE transmission. Material is sterilised at a minimum of 121 °C for at least 15 minutes before use.

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

Information regarding the qualitative and quantitative composition of all media and solutions (pre-culture and production culture media for the active ingredients, thiomersal solution, sodium hydroxide solution and hydrochloric acid solution), their treatment processes and their storage conditions are provided in the dossier. All components are treated to ensure that there are no contaminants.

Control tests during the manufacturing process

For each antigen, the applicant presented in-process data for the manufacture of three small-scale antigen batches and one commercial size antigen batch. During the manufacture, the following tests are carried out: purity, germ counting, control of inactivation, sterility, antigen content by means of ELISA and pH value.

Test descriptions and the limits of acceptance are presented. No specific limits are set for the ELISA tests, as the determined amounts of antigen are informative for further calculation of the fixed formulation targets.

The in-process tests are deemed to be sufficient to control all critical steps in the manufacturing process. Validation studies have been provided for all relevant tests. Further detailed information has been provided for the validation of the inactivation tests and for the validation of the ELISA tests used to quantify the antigen amounts on the inactivated antigen bulks. The latter test is considered crucial for a consistent antigen amount per dose.

Control tests on the finished product

Finished product controls are: appearance, pH value, aluminium hydroxide content, free formaldehyde content, thiomersal content, extractable volume, bacterial and fungal sterility and identification and potency for all three active ingredients.

The descriptions of the methods used for the control of the finished product and the specifications have been provided. The relevant test methods are satisfactorily validated.

Regarding the control of the correct antigen amount, the applicant has developed serovar-specific in-vitro potency tests (competitive ELISA techniques) for each antigen. The methods are the same antigenic content ELISAs as used during in-process control (IPC). The release and end-of-shelf-life specifications set for the potency tests have been adequately justified. Briefly, the minimum antigen content in the vaccine at the proposed end of shelf life is based on batches used in efficacy studies to demonstrate onset and duration of immunity, whereas the release specifications are higher to consider the assay variability and to compensate for losses as observed during real-time stability studies. The replacement procedures for critical reagents like standard antigen preparations and test internal control items have been adequately described.

The proposed tests on the finished product are considered suitable to control the product quality. However, for 10 batches produced post-authorisation, the recovery rates of the antigen should be monitored using potency ELISA (post-authorisation recommendation).

Batch-to-batch consistency

The applicant presented final product data for the manufacture of three small-scale batches and one commercial scale batch indicating both the consistency of the manufacturing of antigen batches for each active substance and a consistent composition of the finished product in a quantitative and

qualitative manner. The three small-scale batches have also been included in the stability studies.

Stability

Stability of active ingredient (bulk antigens)

Data on the stability of the active substance was provided for two batches of each of the three serovars (*S*. Enteritidis, *S.* Typhimurium, *S.* Infantis). The data provided so far support the proposed shelf life of 12 months for the active substance. It was confirmed that the storage bags used for these batches were the same as used during routine production. The antigen batches used are representative of commercial scale batches and they were manufactured in full compliance with the dossier description in the quality part.

Preliminary real-time stability data from one vaccine batch that was blended with antigens older than 12 months are additionally provided. For the time being, it can be concluded that the vaccine manufactured with aged antigen complies with the established specifications for relevant quality parameters throughout a storage period of 15 months. The stability study for this batch is ongoing and will be continued until T27 (April 2025). It is recommended that statistical analysis should be performed on the complete T0-T27 stability data set in order to investigate the impact of aged antigens on vaccine stability (post-authorisation recommendation).

Stability of the finished product

Real-time stability data of three small-scale batches of finished product for at least 27 months at 2-8 ºC were provided. The batches of Cevac Salmune ETI K are representative of those proposed for marketing and were filled in the LDPE bottles proposed for marketing. These batches were closed with silicone-coated bromobutyl rubber stoppers (type I) as described in the quality part. Except for potency, all parameters tested appear reasonably stable over time within the inherent variability of the tests. The statistical evaluation of the potency ELISA titres observed during the stability studies demonstrate a decline in potency over time. To compensate for the observed decrease in potency, the monthly loss over time (90% CI) was considered for calculating the minimum release requirements. The approach is adequately explained by the applicant and is considered acceptable. The data provided are sufficient to justify a 2-year shelf life, when the vaccine is stored at +2 °C to +8 °C.

In addition, stability data for one commercial scale batch have been provided. The results obtained from this batch show that the vaccine remains within the established specifications throughout a storage period of 27 months.

Overall, the data provided are sufficient to justify a 24-month shelf life for this vaccine.

In-use shelf life - efficacy of antimicrobial preservation

Preservative efficacy has been tested in accordance with Ph. Eur. 5.1.3. based on the data provided. The efficacy of antimicrobial preservation has been demonstrated for the proposed 10 hours shelf life after first opening of the immediate packaging. The preservative thiomersal provides adequate protection from adverse effects that may arise from microbial contamination or proliferation during storage and use of the vaccine.

Overall conclusions on quality

Cevac Salmune ETI K is a trivalent vaccine for chickens consisting of inactivated bacteria of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S*. Enteritidis), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S*. Typhimurium) and *Salmonella enterica* subsp. *enterica* serovar Infantis (*S.* Infantis).

The antigens are adjuvanted with aluminium hydroxide. Trometamol, maleic acid, sodium chloride, and sodium hydroxide are included as excipients, as well as water for injections. Thiomersal is added as a preservative. The pharmaceutical form of the final vaccine is a suspension for injection. One dose consists of 0.5 ml. The vaccine is intended to be available in a multidose presentation with 1000 doses (500 ml) filled in plastic containers made of low-density polyethylene (LDPE) with a capacity of 500 ml. The pack sizes are 1 x 1000 doses and 5 x 1000 doses.

The applicant has provided a comprehensive description of the development of the product, including the choice of antigens, adjuvant, presentation including container-closure system, vaccine dose, vaccination regime, route of administration as well as the vaccine production and validation of the production process. Furthermore, the applicant explained comprehensively the rationale for choosing the test methods with special focus on the suitability and development of the ELISA methods used for antigen quantification of antigen bulks and as in-vitro potency test. Reasonable justification is given for selecting the competitive ELISA concept including the selection of monoclonal antibodies used. A replacement procedure for internal standards and control items is explained. Furthermore, the link between formulation ELISA values and in-vitro potency ELISA values and the setting of the minimum potency at the end of shelf life and the minimum release titre for the three *Salmonella* components are explained.

The production process of the antigens is considered as standard manufacture for bacterial vaccines and is identical for each of the three *Salmonella* strains. The active ingredients are produced by culturing bacteria in liquid growth media. The harvest is inactivated by adding formaldehyde solution. The vaccine is blended at a fixed antigen content based on ELISA units obtained from in-process control testing performed on the inactivated antigen bulks. The product is filled by automated filling equipment into sterile plastic bottles. Sterilised rubber stoppers are placed on the vials under aseptic conditions by the filling machine. The sealing of each vial with an aluminium plastic cap is performed by an automatic capping machine. The level of details is sufficient to conclude that the product will be stable and of consistent quality.

In general, the starting materials are properly described. None of the starting materials listed in the Ph. Eur. are of biological origin and, hence, there are no concerns with regard to viral safety and/or transmission of TSE. The sterility of these materials is ensured by either steam sterilisation or by sterile filtration as referenced by Ph. Eur. The only materials of animal origin are the bacterial master and working seeds. The seed lot system was satisfactorily established and sufficient details of the origin, manufacture, storage and testing of the MSBs and WSBs are provided. A risk assessment concerning the prevention of transmission of animal spongiform encephalopathies has been provided. The risk for transmission of TSE to the target animal is estimated as negligible.

The in-process tests are deemed to be appropriate to control all critical steps in the manufacturing process. Validation studies have been provided for all relevant tests. Further detailed information has been provided for the validation of the inactivation test and for the ELISAs used to quantify the respective antigen content of inactivated antigen batches. These ELISAs are considered as key tests for the blending step.

The proposed tests on the finished product are considered adequate to control the product quality. Validation or verification studies have been provided for all key tests, i.e. potency, quantification of the adjuvant, thiomersal, free formaldehyde, extractable volume, and sterility. Regarding the control of the correct antigen amount, the applicant has developed three in-vitro potency tests (competitive ELISA techniques), which, are suitable to detect serovar-specific structures for all three *Salmonella* antigens in inactivated antigen bulks as well as in the finished product. Based on safety and efficacy considerations and in view of the assay variability as well as losses observed during real-time stability studies, the minimum release specifications set for the potency tests have been adequately justified.

Test results of four production runs including in-process data for the manufacture, conforming to the in-process and final product specifications, are provided.

Results of two antigen batches of each of the three *Salmonella* serovars demonstrate that the antigens remain stable for 12 months when stored at 2-8 °C in plastic bags. The antigen batches used for stability testing are representative of commercial scale batches. In addition, preliminary real-time stability data from one vaccine batch that was blended with antigens older than 12 months have been provided. As the stability study for this batch is ongoing, it is recommended that statistical analysis should be performed on the complete T0-T27 stability data set in order to investigate the impact of aged antigens on vaccine stability.

The proposed shelf life of 24 months for the final vaccine when stored refrigerated is substantiated by appropriate data.

The efficacy of the preservative thiomersal has been sufficiently demonstrated. The results support the proposed 10-hour shelf life after first opening the immediate packaging.

In summary, information on the development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner.

Post-authorisation recommendations:

- It is recommended to monitor post-authorisation the recovery data for 10 vaccine batches commercially manufactured, for further confirmation that the recovery rate of the antigen by means of the potency ELISA is constantly between 50-80%. The applicant agrees with the proposal and intends to monitor the recovery rates of batches produced post authorisation.
- It is recommended to perform a statistical analysis on the complete T0-T27 stability data set obtained for one specific batch in order to investigate the impact of aged antigens on vaccine stability once the data will be available. The applicant confirms that the T0-T27 set of stability data will be analysed statistically. In case of any relevant deterioration from the set stability profile that may compromise the quality of the vaccine, the applicant will take the necessary measures. Furthermore, the agency should be informed accordingly.
- The applicant has committed to investigate the possibility of changing the monoclonal antibodies from ascites-derived (for SE and ST ELISAs) to bioreactor-derived ones as already used for the SI ELISA.

Part 3 – Safety documentation (safety and residues tests)

General requirements

Cevac Salmune ETI K is an inactivated liquid vaccine indicated for the immunisation of healthy layer and breeder chickens from 10 weeks of age to reduce faecal excretion of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Infantis.
The vaccine dose is 0.5 ml, injected by intramuscular route, with a second dose of 0.5 ml to be repeated 4 weeks after the first injection.
In order to demonstrate the safety of Cevac Salmune ETI K, tests were carried out both in the laboratory and in the field, using the recommended route of administration (intramuscular) and chickens at the minimum recommended age (10 weeks).
The requirements of Regulation (EU) 2019/6 or of the Ph. Eur. general text 5.2.6 have been fulfilled either through the performance of the tests required or by providing adequate justification. Also, the

requirements of the Ph. Eur. monograph 1947 on *Salmonella* Enteritidis vaccine (inactivated) for chickens, Ph. Eur. monograph 2361 on *Salmonella* Typhimurium vaccine (inactivated) for chickens and the Guideline on User Safety for Immunological Veterinary Medicinal Products (EMEA/CVMP/IWP/54533/2006), Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin and the Guideline on environmental risk assessment for immunological veterinary medicinal products (EMEA/CVMP/074/95) have been considered.

Safety documentation

The following table summarises the designs of the trials:

Laboratory study on SPF pullets		
	Group 1	**Group 2**
Role in the study	Test item (**Cevac Salmune ETI K**)-inoculated group	PBS inoculated control group
No. of SPF chickens on D -7 (at enrolment) and on D0	20	20
Inoculum, inoculation route and volume	**Cevac Salmune ETI K** *i.m.* injection 0.5 ml/ pullet	Reference item (PBS) *i.m.* injection 0.5 ml/ pullet
Administered dose	1 over-formulated dose, per pullet	-
Age and study day at 1st inoculation	10 weeks of age (D0)	
Age and study day at 2nd inoculation	14 weeks of age (D28)	
Post inoculation observation period	D0-D56	

Field safety and efficacy study		
	Group 1	**Group 2**
Role in the study	Test item (**Cevac Salmune ETI K**) inoculated group	**Salenvac T** inoculated control group
No. of layer chickens enrolled on D0	306 day-old layer chickens	306 day-old layer chickens
Inoculum, inoculation route and volume	**Cevac Salmune ETI K** *i.m.* injection 0.5 ml/ pullet (1 commercial dose)	**Salenvac T** *i.m.* injection 0.5 ml/ pullet
Age, study day and No. of chickens at 1st inoculation	10 weeks of age (D63)	
	174	306
Age, study day and No. of chickens at 2nd inoculation	14 weeks of age (D92)	
	153	276
Post inoculation observation period	D63-D273	

Pre-clinical studies

Safety of the administration of one dose

No specific study on the safety of the administration of one dose is included in the application, but the applicant refers to the study demonstrating safety of the administration of the repeated administration of one dose. This is permitted in Regulation (EU) 2019/6 and can be accepted.

Safety of one administration of an overdose and repeated administration of one dose

Cevac Salmune ETI K is an inactivated vaccine, therefore no overdose safety studies have been performed for this vaccine.

For the repeated intramuscular administration of two doses of the vaccine, a batch with an antigen content for each of the three strains containing a 1.5- to 2-fold higher antigen content as a standard batch was used. Forty 10-week-old SPF chickens were randomised into Group 1 (20 chickens), vaccinated with Cevac Salmune ETI K, and control Group 2 (another 20 chickens), vaccinated with PBS. The recommended vaccination schedule was followed with two injections 4 weeks apart. To demonstrate the seronegative status of the birds, their blood was sampled 7 days before study start. The safety was assessed by daily clinical examination including mortality, injection site and body weight (both weekly) until day 56. No general clinical signs were recorded in Group 1 after the first inoculation. One bird was found dead on day 28 with a cardiac failure due to cardiomyopathy. No mortality or clinical signs appeared during the period from the second inoculation until the end of the animal phase (day 56). No significant difference was found between Groups 1 and 2 regarding body weight gain. No birds in Group 2 showed clinical signs after the first and after the second inoculation. No significant difference was found between Groups 1 and 2 regarding mortality. No local reaction was observed by visual inspection and palpation in any bird from Groups 1 and 2 during the observation period after the first and second inoculation. In the macroscopical examination, all birds of Group 1 had yellow discolouration in breast muscle tissue on both sides. All birds of Group 2 had no macroscopical findings on either side. In the majority of samples, the histological examination of the injection sites of the birds from Group 1 showed granuloma Grade 2 with proliferative histiocytes and histological signs of elimination of foreign material, and local cellular immune response without degeneration, necrosis or abscess formation. No histological lesions were observed in the muscle samples of Group 2. In Group 1, normal histological findings were observed in the ovary, oviduct and testes samples. No degenerative or other pathological lesions (inflammation, fibrosis) were detectable in the investigated samples.

Safety of one dose and one repeated dose of Cevac Salmune ETI K in chickens was satisfactory, with no abnormal local or clinical signs. In necropsy, macroscopical local reactions consisting of yellow discolouration in breast muscle tissue were recorded in the vaccinated animals. In the relevant sections of the product information it has been mentioned that yellow discolouration in breast muscle tissues is a possible macroscopical finding.

Examination of reproductive performance

The vaccine is not intended for use in birds in lay or birds within 4 weeks of the onset of laying, as stated in the product information. In a GLP field study, the effect of vaccination on organs of reproductive system and on laying performance was assessed. In all investigated ovary and oviduct samples, a normal histological picture was demonstrated. The egg production of layer chickens

vaccinated with Cevac Salmune ETI K was shown to be significantly better than that of the chickens vaccinated with a comparator product.

Examination of immunological functions

Cevac Salmune ETI K vaccine is an inactivated vaccine. It is not expected to have a deleterious effect on the immune system of target species. Therefore, no study was performed to evaluate the impact on immunological functions.

Special requirements for live vaccines

Not applicable as Cevac Salmune ETI K is an inactivated vaccine.

User safety

A user risk assessment has been provided in accordance with EMEA/CVMP/IWP/54533/2006 – Guideline on User Safety for Immunological Veterinary Medicinal Products. The active substances are inactivated. Maleic acid and trometamol used as buffers are not known to have a harmful effect on the user. The other excipients and the adjuvant (aluminium hydroxide) are commonly used in other vaccines. The risk of accidental self-injection is low, as the users are trained professionals. The overall risk to the user is low. The risks are mitigated through information contained in the product information.

Study of residues

For Cevac Salmune ETI K, being an immunological veterinary medicinal product, no studies on residues were conducted. An assessment is presented considering the substances included in the vaccine. Taking into consideration this information, a "zero day" withdrawal period is proposed. For the excipients trometamol and maleic acid, further information has been provided, which allows the conclusion that the risk for human health can be considered negligible.

The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

The excipients, including adjuvants, listed in section 2 of the SPC are either allowed substances for which Table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

Interactions

Interaction with any other vaccine was not studied. Cevac Salmune ETI K is therefore not recommended to be mixed with or administered with another vaccine or pharmaceutical product at the same time. This is also reflected in the product information.

Clinical studies

The safety of the administration of Cevac Salmune ETI K was demonstrated in one multicentre trial in Hungary in 10-week-old layer chickens after a primary course of two injections at a 4-week interval.

Original study protocols have been provided. 612 birds were originally included in the study. The applicant explained why 130 birds were used for another study and not included in this study. 480 chickens received a primary vaccination (test vaccine Cevac Salmune ETI K: 174 animals, comparator vaccine: 306 animals) and 429 primed chickens received a booster injection after 4 weeks (test vaccine: 153 animals, comparator vaccine: 276 animals). In 20 birds per group, the safety parameters were studied after both vaccinations. Blood sampling was performed several times to quantify the antibody titres against the three *Salmonella* species for efficacy evaluation.

Immediate local and general safety was assessed after both injections by the investigators. Delayed local and general safety including body weight was assessed up to 2 weeks after both injections, mortality up to 4 weeks after each injection. Both the test and comparator vaccines were well tolerated since no adverse events were reported after first and second vaccination. No yellow discolouration of the breast muscle as in the laboratory study was demonstrated. The histological examination of the injection sites in 50% of the birds from Group 1 after first vaccination showed granuloma formation (Grade 2+3) without necrosis and in Group 2, 67% showed granuloma formation (Grade 1+2+3). After second vaccination, 35% of the birds from Group 1 showed granuloma formation (Grade 1+2+3) and in Group 2, granuloma (Grade 1+2) were seen in 15% of birds. In the majority, Grade 2 granuloma occurred. The body weight of the birds in Group 1 four weeks after second vaccination was on average significantly higher than in Group 2. No significant difference was found between groups regarding mortality.

Environmental, cloacal and boot swab samples were collected at several time points for detection of *Salmonella* contaminants. On day 0, the cloacal swab samples of both groups were negative for the presence of *Salmonella* spp. In Group 1, all swab samples remained negative. In Group 2, at several time points cloacal and boot samples with *S. Bredeney* and *S. Livingstone*, which are contaminants, were detected but no *Salmonella enterica* ssp. *enterica* serovars Enteritidis, Typhimurium or Infantis were identified.

Environmental risk assessment

Environmental risk assessment was carried out in accordance with the Note for Guidance: *Environmental risk assessment for immunological veterinary medicinal products* (EMEA/CVMP/074/95).

The active ingredients of Cevac Salmune ETI K are inactivated bacteria and can therefore not replicate in the environment. They do not interact with the ecosystems, as stated in the Note for Guidance EMEA/CVMP/074/95: *"for inactivated vaccines to be administered by injection, the hazards and risks from the active ingredients are likely to be negligible"*.

Consequently, considering the risk of direct and indirect contact with the environment, global likelihood of hazard can be considered as low.

Thiomersal as a preservative, formaldehyde as an inactivating agent and the adjuvant aluminium hydroxide are contained in the vaccine and thus do not pose a threat to the environment. Maleic acid and trometamol are components of the buffer solution for the adjuvant and are not known to have a harmful effect on the environment.

Based on the data provided, the environmental risk assessment can stop at Phase I. Cevac Salmune ETI K is not expected to pose a risk for the environment when used according to the product information.

Overall conclusions on the safety documentation

The safety of Cevac Salmune ETI K was investigated in one laboratory and in one field trial in layer chickens. The studies were carried out with the most sensitive category of target animals, i.e. 10-week-old chickens. In the laboratory study, a batch containing a 1.5–2 times higher antigen content was used. For the field study, a standard batch was used.

Based on the results, it was concluded that the safety of the vaccine in target animals when administered according to the recommended schedule and via the recommended route is acceptable in general. The vaccine is considered safe regarding the administration of one dose and repeated administration of one dose, as no general or local reactions were recorded. Local macroscopical reactions in the form of a yellow discolouration of the muscle are mentioned in the product information.

Reproductive safety has been investigated in both safety studies, but the vaccine is not intended for use in birds in lay or birds within 4 weeks of the onset of laying. This has been reflected in the adequate sections of the product information.

The product is not expected to adversely affect the immune response of the target animals or of their progeny, and therefore no tests on the immunological functions were carried out.

A user safety assessment in line with the relevant guidance document has been presented. Based on that assessment, the potential health risk of the product to the user is considered low and acceptable when used in accordance with the product information.

Residue studies are not required. The withdrawal period can be set at zero days. The quantity of trometamol and maleic acid has been substantiated sufficiently by the applicant.

No claim of compatibility with other immunological veterinary products is included in the product information and therefore no compatibility study was performed.

Cevac Salmune ETI K vaccine is not expected to pose a risk for the environment when used according to the product information.

The vaccine was administered by the intramuscular route as recommended. The laboratory study was reported to be compliant with GLP. Both studies were carried out in the target species of the minimum age recommended for vaccination.

Part 4 – Efficacy documentation (pre-clinical studies and clinical trials)

General requirements

Cevac Salmune ETI K is an inactivated trivalent vaccine recommended for the vaccination of layer and breeder chickens, to reduce faecal excretion of *Salmonella* (*S*.) Enteritidis, *S*. Typhimurium and *S*. Infantis. The vaccine is intended to be given to healthy birds of at least 10 weeks of age. The dose of the vaccine is 0.5 ml, injected by intramuscular route, with a second dose of 0.5 ml to be repeated 4 weeks after the first injection.

In general, vaccination against zoonotic salmonella infection is part of complex control programmes for *Salmonella* infections in poultry. The aim is to reduce or prevent the intestinal colonisation, resulting in

reduced faecal shedding and egg-shell contamination. These measures are essential for reducing *Salmonella* presence in poultry farms and, thus, the risk for food poisoning in humans.

The indication and the vaccination schedule proposed by the applicant is included in the SPC as follows:

3.2 Indications for use for each target species

For the active immunisation of chickens (breeders and layers) from 10 weeks of age to reduce faecal excretion of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Infantis.

Salmonella Enteritidis:
Onset of immunity: 4 weeks after 2nd vaccination
Duration of immunity: until 69 weeks after 2nd vaccination

Salmonella Typhimurium:
Onset of immunity: 4 weeks after 2nd vaccination
Duration of immunity: until 71 weeks after 2nd vaccination

Salmonella Infantis:
Onset of immunity: 4 weeks after 2nd vaccination
Duration of immunity: until 44 weeks after 2nd vaccination

Efficacy was demonstrated in compliance with Annex II, Section I and Section IIIb, to Regulation (EU) 2019/6 as amended by Commission delegated Regulation (EU) 2021/805 and the Ph. Eur. monographs applicable to the product. In particular, the following were taken into account:

- Ph. Eur. monograph 0062: Vaccines for veterinary use.

- Ph. Eur. monograph 5.2.7: Evaluation of efficacy of veterinary vaccines and immunosera.

- Ph. Eur. monograph 1947: *Salmonella* Enteritidis vaccine (inactivated) for chickens.

- Ph. Eur. monograph 2361: *Salmonella* Typhimurium vaccine (inactivated) for chickens.

Field trials were performed in accordance with EMA Note for Guidance "Field trials with veterinary vaccines" (EMEA/CVMP/852/99-FINAL) and EMA Guideline on Good Clinical Practice (CVMP/VICH/595/98-FINAL).

As no Ph. Eur. monograph exists for *S*. Infantis, the requirements of the immunogenicity test of Ph. Eur. monograph 1947 (*Salmonella* Enteritidis vaccine (inactivated) for chickens) were followed, with the exception that, due to the absence of tropism of *S.* Infantis towards internal organs, parameters for internal organ colonisation reduction were not assessed. This is considered acceptable.

The batches used for the pre-clinical and clinical tests were produced according to the manufacturing process described in the quality part of the registration dossier. These batches were intentionally under-formulated (for SE and SI: ca. 4.5-fold; for ST: ca. 8.6-fold) compared to the target formulation to produce batches of the minimum recommended doses used in the pre-clinical trials and one clinical study. For the safety and efficacy field trial, a standard formula batch was used.

Challenge model

The following challenge strains were used in the different studies:

- *Salmonella* Enteritidis (the US origin *Salmonella* Enteritidis Se6-Nar-AP-2SP strain)
- *Salmonella* Typhimurium (the Hungarian origin *Salmonella* Typhimurium SM-167+1SP strain)
- *Salmonella* Infantis (the Hungarian origin *Salmonella* Infantis SM-988-AP28s-3SP strain).

The three chosen serovar challenge strains were able to colonise the enteric system of the chickens adequately. The challenge strains are different from the vaccine strains, and they are relevant for the field situation in Europe.
The challenge strains were always applied by oral route to mimic the natural route of infection. The applied volume of challenge material was 0.3 ml per bird with the titre of the applied challenge material being in the range of 1.29×10^6-1.5×10^7 CFU/0.3 ml (with the exception of one study, in which a lower dose of *S.* Infantis challenge material was used). This bacterial load was found suitable to induce dissemination and shedding in the birds to demonstrate the benefit of vaccination.
The challenge model was considered adequately validated using a reference and, therefore, appropriate for use in the efficacy trials in order to mimic the natural conditions for infection.
Information has been given regarding the source of the challenge strain, the isolation key data, e.g. the species, the year, the cultivation condition and storage until they were used as challenge strains.

Efficacy parameters and tests

The efficacy parameters as provided in Ph. Eur. monographs 1947 and 2361 are as follows:
- the number of *S*. Enteritidis/*S*. Typhimurium in fresh faeces samples of the challenge strains at different days of sampling is significantly lower in vaccinates than in controls and remains lower until the end of the test.
- the number of positive samples of liver and spleen is significantly lower in vaccinates than in controls.

The detection of *S*. Enteritidis, *S*. Typhimurium and *S*. Infantis in faecal and organ samples and the enumeration of these strains were done using the BIDU method. A brief description of the method is included in each study report. The relevant SOPs regarding detection of *Salmonella* spp. in different matrices (presence/absence method) and determination of the quantities (titre) of *Salmonella* spp. in different samples were applied.

The serological examination of the serum samples was carried out with *Salmonella* Group B and D antibody ELISA tests. This assay was used in each study to show seronegativity at inclusion and to confirm seronegativity for non-vaccinated control birds before challenge steps. Additionally, as supportive parameter, the serological answer was monitored after vaccinations with the test items, to assess ratio and extent of seropositivity in vaccinated birds, and also to monitor antibody level changes after challenges.
Serology was also used to monitor the dynamics of changes of maternally derived antibodies in the field studies, and to confirm absence of field infections.

In the laboratory and field studies, clinical signs and mortality were always monitored. Additionally, body weight change was also followed as supportive parameter.

Efficacy documentation

Cevac Salmune ETI K is intended to be given to healthy layer and breeder chickens of at least 10 weeks of age. Immunogenicity studies were carried out in SPF chickens in accordance with the requirements indicated in the respective Ph. Eur. monographs. Additionally, *S.* Infantis efficacy was also tested in commercial pullets. The age of the birds at the first vaccination, in all these studies, was 10 weeks, in line with the youngest age proposed. From these studies (SPF birds for *S.* Enteritidis and *S.* Typhimurium, SPF and commercial layers studies for *S.* Infantis), the onset of immunity was proposed for the three components, as indicated in the "General requirements" section above.
Complementary efficacy trials were carried out in commercial layer chickens to establish the duration of immunity claims for the three components. In line with the youngest age proposed in the product information, the birds were 10 weeks of age at the first vaccination. The chickens were challenged at

different time points, and duration of immunity was proposed for the three components, as indicated in the General requirements section above.
The analyses of presence and quantity of challenge strain in fresh faeces samples after challenge are controlled for type-1 error inflation. The quantitative assessment by the internal method was the primary test for drawing conclusions.

The study protocols for the onset of immunity (OOI) and duration of immunity (DOI) studies have been provided.

Dose determination

The batches used for the pre-clinical and clinical tests were produced according to the manufacturing process described in the quality part of the registration dossier. Intentionally, they were under-formulated compared to the target formulation (see information regarding the batches used under section ”General requirements”) to produce batches of minimum recommended doses. An explanation, based on which the minimum amount of the different antigens per vaccine dose was established, was provided by the applicant. This approach is considered acceptable.

Pre-clinical studies

Onset of immunity

Five studies were carried out to investigate the onset of immunity in 10-week-old chickens by vaccinating each bird with the recommended vaccine dose of 0.5 ml twice, four weeks apart, by the recommended intramuscular administration route followed by challenge four weeks thereafter. The study design meets the requirements of the applicable monographs and therefore, the studies are considered valid. The statistical methods used for each study in this section are considered appropriate.

Evaluation of the immunogenicity of the *S*. Enteritidis component of Cevac Salmune ETI K vaccine in SPF birds 4 weeks after second vaccination

Two groups of 10-week-old seronegative SPF chickens were used to verify the onset of immunity 4 weeks after the completion of the basic vaccination (one dose followed by a second dose) for the *S.* Enteritidis component. One group was vaccinated twice, 4 weeks apart, as described in the product information; the second group was used as unvaccinated control group. In compliance with the requirements of the Ph. Eur. general monograph 5.2.7 “Evaluation of efficacy of veterinary vaccines and immunosera”, an under-formulated batch was used for the vaccination. A certificate of analysis has been provided for this batch. All birds were challenged 4 weeks after the second vaccination and different parameters were examined in compliance with the immunogenicity criteria of Ph. Eur. monograph 1947 “*Salmonella* Enteritidis vaccine (inactivated) for chickens”. After challenge, no mortality or clinical symptoms neither in the vaccinated nor in the control animals could be observed and no significant difference in body weight gain between the vaccinated and the control group could be found.
All birds were seronegative in Groups 1 and 2 on day 0. By the time of challenge, all vaccinated birds became seropositive and all control birds remained seronegative. After challenge, all control birds became seropositive and the ELISA titres in Group 1 (vaccinates) increased further.
The presence and quantity of *S.* Enteritidis in fresh faeces samples were investigated until day 14 after challenge in compliance with the requirements of the monograph. It could be shown that the presence

and quantity of *S.* Enteritidis in fresh faeces samples were significantly lower in vaccinated birds than in controls at the different sampling points. After day 70, half of the birds were euthanised to collect spleen, liver, ovary and oviduct samples. The organs of the remaining chickens were collected at the end of the study on day 84.
In the vaccinated birds, no dissemination of the challenge strain in organs over the time until the end of the study on day 84 was proven. The same applies to the investigated organs of the control group on day 84. When tested on day 70 (14 days post challenge), 4 out of 17 liver and spleen samples in the control group were positive and the difference between the vaccinated birds and control birds was statistically significant. Nevertheless, the dissemination rate was low in general.

In summary, the results of this study show that after the intramuscular vaccination of seronegative SPF chickens, from 10 weeks of age, with two doses of Cevac Salmune ETI K, with an interval of 4 weeks followed by a *S.* Enteritidis challenge 4 weeks later, an onset of immunity of 4 weeks could be proven for the claim reduction of faecal excretion. A reduction of organ colonisation could not be demonstrated taking into consideration the low dissemination rate in general and the results in the control group. Therefore, no claim is made in the SPC for the organ colonisation indication.

Evaluation of the immunogenicity of the *S.* Typhimurium component of Cevac Salmune ETI K vaccine in SPF birds 4 weeks after second vaccination

Two groups of 10-week-old seronegative SPF chickens were used to verify the onset of immunity 4 weeks after the completion of the basic vaccination (one dose followed by a second dose) for the *S*. Typhimurium component. One group was vaccinated twice, 4 weeks apart, as described in the product information; the second group was used as unvaccinated control group. In compliance with the requirements of the Ph. Eur. general monograph 5.2.7 "Evaluation of efficacy of veterinary vaccines and immunosera", an under-formulated batch was used for the vaccination. All birds were challenged 4 weeks after the second vaccination and different parameters were examined in compliance with immunogenicity criteria of Ph. Eur. monograph 2361 "*Salmonella* Typhimurium vaccine (inactivated) for chickens". After challenge, no mortality neither in the vaccinated nor in the control animals could be observed. Only mild clinical symptoms were observed due to the infection in some birds of both groups. No significant difference between the vaccinated and the control group regarding body weight gain could be found.
All birds were seronegative in Groups 1 and 2 on day 0. By the time of challenge, 97% of the vaccinated birds became seropositive and all control birds remained seronegative. After challenge, 76% of the control birds became seropositive while ELISA titres in Group 1 increased further.
The presence and quantity of *S*. Typhimurium in fresh faeces samples were investigated until day 14 after challenge in compliance with the requirements of the monograph. It could be shown that the quantity of *S*. Typhimurium in fresh faeces samples was significantly lower in vaccinates than in controls at the different sampling points. After day 70, half of the birds were euthanised to collect spleen, liver, ovary and oviduct samples. The organs of the remaining chickens were collected at the end of the study on day 84. Because the groups were compiled of male and female birds, the ovary and oviduct samples from only nine birds could be investigated on day 70 and eight birds on day 84 in both groups. As the bacterial colonisation of the challenge strain in organs was very low, a reduction of organ colonisation cannot be considered as proven. Therefore, no claim is made in the SPC for the organ colonisation indication.

In summary, the results of this study show that after the intramuscular vaccination of seronegative SPF chickens, from 10 weeks of age, with two doses of Cevac Salmune ETI K, with an interval of 4 weeks followed by a *S.* Typhimurium challenge 4 weeks later, the claim of reduction of faecal excretion and onset of immunity of 4 weeks could be proven.

Evaluation of the immunogenicity of the *S.* Typhimurium component of Cevac Salmune ETI K vaccine in SPF birds 4 weeks after second vaccination

Two groups of 10-week-old seronegative SPF chickens were used to verify the onset of immunity of 4 weeks after the completion of the basic vaccination (one dose followed by a second dose) for the *S*. Typhimurium component. One group was vaccinated twice, 4 weeks apart, with the proposed minimum antigen content as described in the product information; the second group was used as unvaccinated control group. The manufacturer's batch protocol for the used batch has been provided. All birds were challenged 4 weeks after the second vaccination, and different parameters were examined in compliance with immunogenicity criteria of Ph. Eur. monograph 2361 "*Salmonella* Typhimurium vaccine (inactivated) for chickens". After challenge, one bird from Group 1 died without any clinical signs. In the control group no mortality could be observed. No significant difference between the vaccinated and the control group regarding body weight gain could be found.
All birds in Groups 1 and 2 were seronegative on day 0. By the time of challenge, 83% of the vaccinated birds became seropositive and all control birds remained seronegative. After challenge, 46% of the control birds became seropositive while ELISA titres in Group 1 increased slightly.
The presence and quantity of *S*. Typhimurium in fresh faeces samples were investigated until day 14 after challenge in compliance with the requirements of the monograph. It could be shown that the presence of challenge strain in faeces (direct plating method) was significantly different between the groups (in favour of the vaccinated group at different days). In addition, the quantity of *S*. Typhimurium in fresh faeces samples was significantly lower in vaccinates than in controls at the different sampling points. After D63 and D70, a large proportion of the birds were euthanised to collect spleen and liver samples. The organs of the remaining chickens were collected at the end of the study on D84. The presence and the number of *S.* Typhimurium in liver samples were lower 7 days after challenge (D63) for vaccinates and 14 days after challenge (D70) for the controls. The presence and the number of *S.* Typhimurium in spleen samples from vaccinated chickens were significantly lower than in controls at 7 and 14 days after challenge (D63 and D70). No claim is made in the SPC for the organ colonisation indication.

In summary, the results of this study show that after the intramuscular vaccination of seronegative SPF chickens, from 10 weeks of age, with two doses of Cevac Salmune ETI K, with an interval of 4 weeks, followed by a *S.* Typhimurium challenge 4 weeks later, the claim of reduction of faecal excretion and onset of immunity of 4 weeks could be proven.

Evaluation of the immunogenicity of the *S.* Infantis component of Cevac Salmune ETI K vaccine in SPF birds 4 weeks after second vaccination

No Ph. Eur. monograph exists for *S.* Infantis. Therefore, the immunogenicity was tested in SPF birds following the principle of the *S.* Enteritidis monograph. Reduction in faecal excretion was assessed. Due to the well documented absence of tropism of *S.* Infantis towards internal organs, parameters for internal organ colonisation reduction were not assessed and this is considered acceptable.
Two groups of 10-week-old seronegative SPF chickens were used to verify the onset of immunity 4 weeks after the completion of the basic vaccination (one dose followed by a second dose) for the *S.* Infantis component. One group was vaccinated twice, 4 weeks apart, as described in the product information; the second group was used as unvaccinated control group. In compliance with the requirements of the Ph. Eur. general monograph 5.2.7 "Evaluation of efficacy of veterinary vaccines and immunosera", an under-formulated batch was used for the vaccination. A certificate of analysis has been provided for this batch. Sixty birds were challenged 4 weeks after the second vaccination and different parameters were examined. After challenge, no mortality or clinical symptoms neither in the

vaccinated birds nor in the control animals could be observed, and no significant difference between the group of vaccinated birds and the control group regarding body weight gain could be found.

S. Infantis belongs to serogroup Group C1 and for this reason the Salmonella Group B/D ELISA kit used does not detect changes linked to this component of the vaccine or to the challenge infection with this strain. Therefore, high Salmonella Group B/D titres were detected in the vaccinated group before challenge (due to the *S*. Enteritidis and *S*. Typhimurium component of the vaccine) while after challenge no meaningful changes were seen. In the control group, all the birds except one were seronegative at the time of challenge. After challenge, two birds in the control group were slightly seropositive, most probably attributable to other Enterobacteriaceae infection.
The presence and quantity of *S.* Infantis in fresh faeces samples were investigated until day 14 after challenge in compliance with the requirements of the above-mentioned monographs. It could be shown that the quantity of *S*. Infantis in fresh faeces samples was significantly lower in vaccinated than in the control birds when evaluated by AUC (quantity of the challenge strain) for the total post-challenge period. When assessed separately for sampling days, it was significantly lower on only one sampling day.
The results were not very conclusive as the numbers of the positive birds were high in both groups. However, the rate in the vaccinated group was lower until day 70, compared with the control birds, except for one day, but the difference was not significant. The quantity of the challenge strain was found higher for the control birds, but the difference was only significant for one day.

As an onset of immunity of 4 weeks after the intramuscular vaccination with two doses of Cevac Salmune ETI K 4 weeks apart for the claim "reduction of faecal excretion" for *S.* Infantis could not be supported by this study, the applicant submitted another study to prove the claim for the OOI.

Evaluation of the onset of immunity of the *S.* Infantis component of Cevac Salmune ETI K vaccine in commercial layer birds 4 weeks after second vaccination.

No Ph. Eur. monograph exists for *S.* Infantis. Therefore, the onset of immunity was tested in commercial layer birds following the principle of the *S*. Enteritidis monograph. Reduction in faecal excretion was assessed. Due to the well documented absence of tropism of *S*. Infantis towards internal organs, the parameter for internal organ colonisation reduction was not assessed and this is considered acceptable.
Two groups of 10-week-old seronegative commercial layers were used to verify the onset of immunity 4 weeks after the completion of the basic vaccination (one dose followed by a second dose) for the *S*. Infantis component. One group was vaccinated twice, 4 weeks apart, as described in the product information; the second group was used as unvaccinated control group. In compliance with the requirements of the Ph. Eur. general monograph 5.2.7 "Evaluation of efficacy of veterinary vaccines and immunosera", an under-formulated batch was used for the vaccination. A certificate of analysis has been provided for this batch. Sixty birds were challenged 4 weeks after the second vaccination and different parameters were examined. After challenge, no mortality or clinical symptoms neither in the vaccinated birds nor in the control animals could be observed and no significant difference regarding body weight gain could be found between the group of vaccinated birds and the control group. High *Salmonella* Group B/D titres were detected in the vaccinated group before challenge (due to the *S*. Enteritidis and *S*. Typhimurium component of the vaccine), while after challenge no meaningful changes were seen. In the control group, all the birds were seronegative at the time of challenge. After challenge, one bird in the control group was slightly seropositive, most probably attributable to another Enterobacteriaceae infection.
The presence and quantity of *S*. Infantis in fresh faeces samples were investigated until day 14 after

challenge in compliance with the requirements of the above-mentioned monograph. There was no significant difference in the presence of the challenge strain between Group 1 and Group 2 on any of the sampling days. However, it could be shown that the quantity of *S*. Infantis in fresh faeces samples was significantly lower in vaccinated than in the control birds on sampling days 55, 59, 61 and 63. The AUC for the total post-challenge period was also lower in Group 1 compared to Group 2.

The results are not very convincing, as the numbers of the positive samples were low in both groups, probably due to the lower challenge dose, and there was no difference between the groups. Anyway, the quantity of the challenge strain was found to be higher in the control birds and the difference was significant for four days. As the AUC was significantly lower in Group 1 than in Group 2, an onset of immunity of 4 weeks after the intramuscular vaccination with two doses of Cevac Salmune ETI K 4 weeks apart for the claim "reduction of faecal excretion" for *S.* Infantis could be considered as proven.

Duration of immunity

The efficacy of the vaccine was also assessed under field conditions in two GCP clinical trials in commercial layer chickens. The first study was a combined safety and efficacy trial. In this trial, the efficacy assessment was based primarily on serology. The second study was an efficacy field trial. In this trial, the assessment was based primarily on protective efficacy recorded against experimental *Salmonella* challenge infections applied at different time points. These complementary efficacy trials established the duration of immunity claims for the three components. The birds of both field trials were 10 weeks of age at the first vaccination, in line with the youngest age proposed in the product information.

Layer-type chickens were used in the field studies. According to the applicant's view, as there is no claim set for this vaccine to provide protection to the progeny, the experiments conducted in layers are sufficient to support the claims also in breeders.

Evaluation of the duration of immunity of the *S.* Enteritidis component of Cevac Salmune ETI K vaccine in layer birds at different timepoints after second vaccination

Three studies were carried out to establish the duration of immunity for the *S*. Enteritidis component. For these studies, 34/30/30 10-week-old commercial layer birds, respectively, were vaccinated twice, 4 weeks apart, as described in the SPC. For each study, a second group, 30/30/18 birds, respectively, was used as unvaccinated control group. In compliance with the requirements of the Ph. Eur. general monograph 5.2.7 "Evaluation of efficacy of veterinary vaccines and immunosera", an under-formulated batch was used for the vaccination. A certificate of analysis has been provided for this batch.
The birds were challenged in the first study at 26 weeks, in the second study at 49 weeks and in the third study at 69 weeks after the second vaccination, using the same route and strain but with a slightly deviating challenge dose. Different parameters were examined referring to the immunogenicity criteria of Ph. Eur. monograph 1947 (*Salmonella* Enteritidis vaccine (inactivated) for chickens).
After challenge, no mortality or clinical symptoms neither in the vaccinated nor in the control animals could be observed, except for one vaccinated bird that died on day 7 due to egg peritonitis. No significant difference in body weight gain could be found between the vaccinated and the control group.
In the vaccinated birds in all three studies, moderate levels of seropositivity (between 68% and 83%) were detected 26-69 weeks after the complete vaccination. All of the control birds in Group 2 in all three studies were seronegative on the day of the challenge. After challenge, all vaccinated and control birds in all three studies became seropositive (with the exception of 2 birds in Group 1 in one of the studies), while ELISA titres in Group 1 increased further in all three studies.
The presence and quantity of *S.* Enteritidis in fresh faeces samples were investigated until day 14 after

challenge. It could be shown that the presence of *S*. Enteritidis in fresh faeces samples was significantly lower in vaccinates than in controls on day 5 and day 7 in all three studies after challenge at 26 weeks, at 49 weeks and at 69 weeks after 2nd vaccination, and also on day 9 after challenge at 26 weeks and at 69 weeks after 2nd vaccination.

The quantity of *S*. Enteritidis in fresh faeces samples was numerically lower in Group 1 than in Group 2 in all three studies, except for day 1 in Study 2 and 3. The difference was significant on days 5, 7 and 9 in all three studies, and also on day 14, when challenge was performed at 69 weeks after 2nd vaccination.
In the first study after 14 days and in the second study after 7 days, half of the birds were euthanised to collect spleen, liver, ovary and oviduct samples. The organ samples of the remaining chickens were collected at the end of the study. The organ invasion by *S.* Enteritidis was relatively low. No conclusion could be drawn for liver, spleen, ovary and oviduct samples regarding the presence of challenge strain at the end of the observation period on day 28 as no significant difference was detected in any of the three studies between the vaccinated and control groups. The only exception was the 14 days post-challenge sampling in the first study where the titres of challenge strain in liver and spleen and presence in spleen showed a significantly lower value in the control birds when compared to the vaccinates.

In summary, the results of this study show that after the intramuscular vaccination of 10-week-old commercial layer birds with two doses of Cevac Salmune ETI K, 4 weeks apart, a duration of immunity of 69 weeks for the claim "reduction of faecal excretion" for *S.* Enteritidis could be proven.

Evaluation of the duration of immunity of the *S.* Typhimurium component of Cevac Salmune ETI K vaccine in layer birds at different timepoints after second vaccination

Three studies were carried out to establish the duration of immunity for the *S*. Typhimurium component. For these studies, 45/35/30 10-week-old commercial layer birds, respectively, were vaccinated twice, 4 weeks apart, as described in the product information. For each study, a second group, 45/35/17 birds, respectively, was used as unvaccinated control group. In compliance with the requirements of the Ph. Eur. general monograph 5.2.7 "Evaluation of efficacy of veterinary vaccines and immunosera", an under-formulated batch was used for the vaccination. A certificate of analysis has been provided for this batch.
The birds were challenged in the first study at 39 weeks, in the second study at 51 weeks and in the third study at 71 weeks after the second vaccination, using the same route and strain but with a slightly deviating challenge dose. Different parameters were examined referring to the immunogenicity criteria of Ph. Eur. monograph 2361 (*Salmonella* Typhimurium vaccine (inactivated) for chickens).
No mortality was observed after challenge. Two birds died and one bird was euthanised, but this was not related to the challenge. As regards the clinical symptoms, 8 out of the 35 control chickens had transient diarrhoea for 1-3 days at 6-8 days post challenge in the second study, but this was not observed in the vaccinated group and also not in the other 2 studies. Regarding the results of the body weight gain, a clear conclusion could not be drawn as in two studies it was in favour of the vaccinated group, while in the 3rd study it was in favour of the control group. The applicant argued that no weight gain is expected at 85 weeks of age, which is not quite comprehensible as for the control birds a weight gain was seen. Nevertheless, as there is no real clinical relevance, this will not be pursued further.
In the vaccinated birds in all three studies, moderate levels of seropositivity (between 58% and 79%) were detected 39-71 weeks after complete vaccination. All of the control birds in all three studies were seronegative on the day of the challenge. After challenge, seropositivity increased in both vaccinated (86% and 100%) and control birds (67% and 88%) in all three studies and ELISA titres in Group 1

increased further in all three studies.
The presence and quantity of *S.* Typhimurium in fresh faeces samples were investigated until Day 14 after challenge. It could be shown that the presence of *S.* Typhimurium in fresh faeces samples was numerically lower in vaccinates than in controls except for D1 in study 1 and 3 (equal percentage). The difference was significant on day 9 in all three studies, and also on day 5 and day 7 when challenge was performed at 39 weeks and at 51 weeks post 2nd vaccination, respectively. The difference was also significant on a 4th sampling day, day 14 when challenge was performed at 65 weeks of age (51 weeks post 2nd vaccination).
The difference of the quantity of *S*. Typhimurium in fresh faeces samples was lower in Group 1 than in Group 2 in all three studies, (exception: day 1 in study 1 and day 5 in study 3). The difference was significant on day 7 and day 9 in all three studies, and also on day 5 and day 14, when challenge was performed at 39 weeks and at 51 weeks post 2nd vaccination, respectively. The AUC (the quantity of the challenge strain for the total post-challenge period) was significantly lower in Group 1 than in Group 2 in all three studies.
In the first and in the second study, nearly half of the birds were euthanised after 7 days to collect spleen, liver, ovary and oviduct samples. The organ samples of the remaining chickens were collected at the end of the study on day 28. The organ invasion by the challenge strain was relatively low.
No conclusion could be drawn for liver, spleen, ovary and oviduct samples regarding presence of challenge strain at the end of the observation period on day 28 as no significant difference was detected in any of the three studies between the vaccinated and control groups (exception: one ovary sample in the second study). At the additional earlier testing time point 7 days post-challenge in the first and second study (39 weeks and 51 weeks post 2nd vaccination), the titres and the presence of challenge strain in liver and spleen showed a lower value in the vaccinates when compared to control birds.

In summary, although after the challenge performed 71 weeks after the second injection the difference between vaccinates and controls was smaller than in the challenges performed at earlier time points, it could be demonstrated that the bacterial load was lower in the vaccinates for three out of the four days and, on two sampling days, this difference reached significant level. Therefore, a duration of immunity of 71 weeks after the 2nd vaccination for the claims "reduction of faecal excretion" can be accepted.

Evaluation of the duration of immunity of the S. Infantis component of Cevac Salmune ETI K vaccine in layer birds at different timepoints after second vaccination

Two studies were carried out to establish the duration of immunity for the *S.* Infantis component. For these studies, 30/30 10-week-old commercial layer birds, respectively, were vaccinated twice, 4 weeks apart, as described in the product information. For each study, a second group, 30/17 birds, respectively, was used as unvaccinated control group. In compliance with the requirements of the Ph. Eur. general monograph 5.2.7 "Evaluation of efficacy of veterinary vaccines and immunosera", an under-formulated batch was used for the vaccination. A certificate of analysis has been provided for this batch.
The birds were challenged in the first study at 44 weeks and in the second study at 60 weeks after the second vaccination using the same route and strain but with a slightly deviating challenge dose.
Different parameters were examined referring to the immunogenicity criteria of the Ph. Eur. monograph on "Salmonella Enteritidis vaccine (inactivated) for chickens" as no monograph exists for *S.* Infantis.
No mortality after challenge was observed. One vaccinated bird died unrelated to the challenge. As regards the clinical symptoms, one out of the 17 control chickens had transient diarrhoea for a few days post challenge. Other clinical symptoms were observed neither in the vaccinated nor in the control group in the two studies.

Regarding the results of the body weight gain, a clear conclusion could not be drawn. Before challenge, all of the control birds in both studies were seronegative to group B/D Salmonellae, while in the vaccinated group due to the *S*. Enteritidis and *S*. Typhimurium component of the vaccine, moderate levels of seropositivity were detected. As expected, the ELISA titres only changed slightly after challenge.
The presence and quantity of *S*. Infantis in fresh faeces samples were investigated until day 14 after challenge. It could be shown that the presence of *S*. Infantis in fresh faeces samples was lower in vaccinates than in controls. The difference was significant on sampling days 5 and 7 for the presence of the challenge strain when challenge was performed at 44 weeks after 2nd vaccination. For the quantity of the challenge strain, the difference was significant on 4 sampling days (days 5, 7, 9 and 14). Also, the AUC, reflecting the quantity of the challenge strain for the total post-challenge period, was significantly lower in Group 1 than in Group 2. When challenge was performed at 60 weeks after 2nd vaccination, this reduction effect in faecal excretion could not be demonstrated. Only on one sampling day (day 9), the quantity of *S*. Infantis was significantly lower in the vaccinated group compared to the control birds.

In summary, the results of this study show that after the intramuscular vaccination of 10-week-old commercial layer birds with two doses of Cevac Salmune ETI K a duration of immunity of 44 weeks after 2nd vaccination for the claim "reduction of faecal excretion" for the *S*. Infantis component could be proven.

Maternally derived antibodies (MDA)

Serology was also used to monitor dynamics of changes of maternally derived antibodies in the field studies.

Study to investigate the safety and efficacy of the Cevac Salmune ETI K vaccine

Blood samplings were performed on day 0 from 20 hatch-mate birds of Groups 1 and 2 in this field trial to verify the presence of maternal antibody levels (MDA) against *Salmonella* Group B and D in day-old chickens. After day 0, blood samplings were performed regularly until the end of the study. The *Salmonella* Gp B/D ELISA kit (from BioCheck) was used to quantify the amount of antibodies against *S*. Enteritidis, *S*. Typhimurium and other invasive Group B and D *Salmonella* species in the serum of chickens.
The hatch-mate birds had moderate levels of MDA against *Salmonella* Group B and D species on the day of hatching (mean ELISA titre: 879 units) with 40% positivity ratio. The levels of MDA against *Salmonella* Group B and D decreased nearly to zero (mean ELISA titre: 8 units) at 28 days of age, having no seropositive birds in the groups.

Study to investigate the efficacy of the Cevac Salmune ETI K vaccine

Blood samplings were performed on day 0 from 30 hatch-mate birds of Groups 1, 2 and 3 to verify the presence of maternal antibody levels (MDA) against *Salmonella* Group B and D in day-old chickens. After D0, blood samplings were performed regularly, until the end of the study. The same *Salmonella* Group B/D ELISA kit was used as mentioned above to quantify the amount of antibodies against *S*. Enteritidis, *S*. Typhimurium and other invasive Group B and D *Salmonella* species in the serum of chickens.
The hatch-mate birds had low levels of MDA against Salmonella Group B and D species on the day of hatching (mean ELISA titre: 348 units) with only 13% positivity ratio. The levels of MDA decreased nearly to zero (mean ELISA titre: 3 units) at 34 days of age, with no seropositive birds in any of the groups.

The results of MDA interference investigations have shown that an interference with active immunity development is unlikely as no level of MDA is expected when the birds are vaccinated at an age of 10 weeks as recommended.

Interactions

No studies have been provided regarding coincident use of Cevac Salmune ETI K with other vaccines. Therefore, the product information contains a note that a decision to use this vaccine before or after any other veterinary medicinal product needs to be made on a case-by-case basis.

Clinical trials

The safety and efficacy of the Cevac Salmune ETI K vaccine in 10-week-old layer chickens: Administration of a single dose followed by a repeated single dose

One multicentre trial was conducted in Hungary in 10-week-old layer chickens, which had undergone a primary course of two injections of Cevac Salmune ETI K or the comparator vaccine at a 4-week interval. Six hundred twelve (612) birds were included and vaccinated with either the test vaccine, Cevac Salmune ETI K (Group 1), or a comparator vaccine (Group 2). Four hundred eighty (480) chickens received primary vaccination (test vaccine: 174 animals, comparator vaccine: 306 animals) and 429 primed chickens received a booster injection after 4 weeks (test vaccine: 153 animals, comparator vaccine: 276 animals). In 20 birds per group, the efficacy parameters were studied after both vaccinations. Blood sampling was performed several times to quantify the amount of antibodies against the *Salmonella* species (*S.* Enteritidis, *S*. Typhimurium, *S.* Infantis) for efficacy evaluation. General clinical observation and mortality, cloacal swab sampling and boot swab sampling were assessed after both injections by the investigators. For safety assessment, local examination, *e*gg production, body weights and necropsy were investigated.

No clinical signs and no mortality attributable to the vaccine appeared during the 14-day post-vaccination observation periods after the first or the second vaccination. Exposure to *S.* Enteritidis, *S.* Typhimurium or *S.* Infantis infection of the flock could not be demonstrated on the farm during the trial and no natural outbreak happened. Two birds died in Group 1 before vaccination and 5 birds died after day 120, which was not caused by vaccination.
Environmental, cloacal and boot swab samples were collected at several time points for detection of *Salmonella* contaminants. On day 0, the cloacal swab samples of both groups were negative for the presence of *Salmonella* spp. In Group 1, all swab samples remained negative. In Group 2, at several time points cloacal and boot samples with *S.* Bredeney and *S.* Livingstone, which are contaminants, were detected but no *Salmonella enterica* ssp. *enterica* serovar Enteritidis, Typhimurium or Infantis were identified.
On day 63 (first vaccination), one bird in the comparator vaccinated group showed an ELISA titre of 665 ELISA units due to contamination with *S.* Bredeney. This contamination was confirmed by the bacteriological examination of the cloacal swabs. From day 92 (second vaccination), the *Salmonella* B/D ELISA titres started to increase in both groups and reached their maximum levels on day 119 in Group 1 (4827 ELISA units) and on day 147 in Group 2 (6085 ELISA units). All samples of both groups collected on day 119 and on day 147 were seropositive. The serological results of day 175 demonstrate the start of the decrease in Groups 1 and 2 regarding mean titres and positivity percentage. From this time point, the positivity percentage levels for Group 1 were decreasing from 80% (day 175) to 70% (day 259) and to 40% (day 273). The same parameters in Group 2 were 95% (day 175), 80% (day 259) and 85% (day 273), respectively.

The effect of vaccination was demonstrated by the titre increase from day 92 and the 100% seropositivity detected on day 119 in Group 1. Nevertheless, as there is no proven correlation between the serological titre and the protection rate induced by the vaccination, it is not possible to draw a clear conclusion regarding this parameter in terms of the efficacy of the vaccine.

Study to investigate the efficacy of the Cevac Salmune ETI K vaccine in 10-week-old layer chickens: Administration of a single dose followed by a repeated single dose

A second multicentre field study was conducted in 1049 10-week-old layer chickens in Hungary. Two vaccinations at a 4-week interval were given with either the test vaccine, Cevac Salmune ETI K (Group 1), or a comparator vaccine (Group 2), or no vaccination was given (Group 3). In Group 1, 356 chickens received a primary (D69) and a booster (D96) vaccination. In Group 2, 355 chicken received a primary and 354 a booster vaccination. Additional 30 hatch-mate birds had blood and cloacal samples taken on D0 (at the test facility before settling the study animals) to collect more data about the *Salmonella* infection status of the flock. In 20 birds per group, the efficacy parameters were studied after both vaccinations. Blood sampling was performed several times to quantify the amount of antibodies against the *Salmonella* species (*S.* Enteritidis, *S*. Typhimurium, *S.* Infantis) for efficacy evaluation.
General clinical observation and mortality, including body weights, cloacal swab sampling and boot swab sampling, were assessed after both injections by the investigators. All dead animals were necropsied.

Environmental, cloacal and boot swab samples were collected at several time points for detection of *Salmonella* contaminants. All swab samples remained negative during the completely animal phase in all groups.
No clinical signs and no mortality attributable to the vaccine appeared during the 14-day post-vaccination observation periods after the first or the second vaccination. Exposure to *S.* Enteritidis, *S.* Typhimurium or *S.* Infantis infection of the flock could not be demonstrated on the farm during the trial and no natural outbreak happened. General mortality was very high due to reasons not related to vaccination (cannibalism and hatching weakness; in Group 1, 17.2% (73 animals), in Group 2, 23.1% (98 animals) and in Group 3, 12.4% (31 animals)).
The body weight gain of Group 3 was significantly better than that of Group 1 and Group 2, attributable to the difference in their keeping condition (colonial cages versus deep litter). Anyway, comparison of weight gain revealed significant differences between each pair of groups.
From day 96, the *Salmonella* B/D ELISA titres started to increase in both groups and reached their maximum levels on day 122 in Group 1 (4326 ELISA units, 94% seropositivity) and on day 179 in Group 2 (7356 ELISA units, 90% seropositivity). The *Salmonella* B/D ELISA titres remained above 1481 ELISA units in Group 1 during the whole animal phase of the trial after the second vaccination. In Group 2, the titres remained above 3864 ELISA units during the whole animal phase of the trial after day 96 with seropositivity between 90%-100% among the sampled birds. In Group 3, all samples were negative at each sampling time point.

The effect of vaccination was demonstrated by the titre increase from day 96 and the 94% seropositivity detected on day 122 in Group 1. Nevertheless, as there is no proven correlation between the serological titre and the protection rate induced by the vaccination, it is not possible to draw a clear conclusion regarding this parameter in terms of the efficacy of the vaccine.

The recent study protocol version (before database lock; see VICH GL9, the topic on study documentation) has been provided by the applicant.

Overall conclusion on efficacy

Five efficacy studies were performed for verification of the onset of immunity for Cevac Salmune ETI K, three studies in compliance with the immunogenicity test required in the applicable Ph. Eur. monographs for *S*. Enteritidis and *S*. Typhimurium for these components and two studies referring to this test in the *S*. Enteritidis monograph for the *S*. Infantis component.

In general, two groups of 10-week-old seronegative SPF chickens were used to verify the onset of immunity 4 weeks after the completion of the basic vaccination according to the recommended dose and administration route for the different *Salmonella* components. The vaccine batches used were under-formulated to comply with requirements of the minimum recommended dose. One group was vaccinated twice, 4 weeks apart, as described in the product information; the second group was used as unvaccinated control group. For the *S*. Infantis component, a second study with commercial layer birds was conducted, as the reduction in excretion in faeces of the challenge strain was not pronounced in the initial study. The birds were challenged 4 weeks after the second vaccination. For all three components a significant reduction of excretion of the challenge strain in faeces was proven. The internal organ colonisation reduction was only investigated for the *S*. Enteritidis and *S*. Typhimurium components due to the absence of tropism of *S*. Infantis towards internal organs. For *S*. Enteritidis no invasion was found on day 84. When tested on day 70 (14 days post challenge), 4 out of 17 liver and spleen samples in the control group were positive and the difference between the vaccinated birds and control birds was statistically significant. For *S*. Typhimurium also no invasion was found on day 84. On day 70, organ invasion could be observed but no reduction could be demonstrated. Nevertheless, the results of the studies could support an onset of immunity of 4 weeks for the reduction of faecal excretion for the three vaccine components.
The efficacy of the vaccine was also assessed under field conditions in two GCP clinical trials in commercial layer chickens. The first study was a combined safety and efficacy trial. In this trial, the efficacy assessment was based primarily on serology. The effect of vaccination was demonstrated by the titre increase from day 92 and the 100% seropositivity detected on day 119 in the group vaccinated with Cevac Salmune ETI K. As there is no proven correlation between the serological titre and the protection rate induced by the vaccination, it is not possible to draw a clear conclusion regarding this parameter in terms of the efficacy of the vaccine.
The second study was an efficacy field trial. In this trial, assessment was based primarily on information obtained in pre-clinical studies on protective efficacy against experimental *Salmonella* challenge infections applied at different time points. These eight complementary efficacy trials established the duration of immunity claims for the three components. The birds of both field trials were 10 weeks of age at the first vaccination, in line with the youngest age proposed. The birds were vaccinated twice, four weeks apart, and challenged at different time points. For the *S*. Enteritidis component, a duration of immunity of 69 weeks for the claim "reduction of faecal excretion" was proven. For the *S*. Typhimurium a duration of immunity of 71 weeks for the claims "reduction of faecal excretion" was proven. For the *S.* Infantis component, a duration of immunity of 44 weeks for the claim "reduction of faecal excretion" was proven. Regarding the results of the serology in this study, the effect of vaccination was demonstrated by the titre increase from day 96 and the 94% seropositivity detected on day 122 in the group vaccinated with Cevac Salmune ETI K. Nevertheless, as there is no proven correlation between the serological titre and the protection rate induced by the vaccination, it is not possible to draw a clear conclusion regarding this parameter in terms of the efficacy of the vaccine.

Part 5 – Benefit-risk assessment

Introduction

Cevac Salmune ETI K suspension for injection for chickens contains per dose at least 122 ELISA units of *Salmonella enterica*, subsp. *enterica*, serovar Enteritidis, strain 038-90 (inactivated), at least 212 ELISA units of *Salmonella enterica*, subsp. *enterica*, serovar Typhimurium, strain 076-94 (inactivated) and at least 157 ELISA units of *Salmonella enterica*, subsp. *enterica*, serovar Infantis, strain SM-595 (inactivated).

Cevac Salmune ETI K is given intramuscularly as a single dose of 0.5 ml to chickens (breeders and layers) from 10 weeks of age. The second vaccination should take place 4 weeks later, but no later than 4 weeks before the onset of lay. Cevac Salmune ETI K is intended for active immunisation of chickens to the above-mentioned serovars of *Salmonella enterica*, subsp. *enterica*, which should lead to the reduction of faecal excretion. The proposed withdrawal period is zero days.

Cevac Salmune ETI K suspension for injection is presented in packs containing one bottle of 1000 doses and 5 bottles of 1000 doses.

Benefit assessment

Direct benefit

The proposed benefit of Cevac Salmune ETI K is its efficacy in active immunisation of 10-weeks-old breeder and layer chickens:

- For active immunisation of chickens (breeders and layers) from 10 weeks of age to reduce faecal excretion of *S. enterica* Enteritidis, *S. enterica* Typhimurium and *S. enterica* Infantis.

Onset of immunity and duration of immunity were studied in laboratory studies following relevant Ph. Eur. monographs, when available. A sufficient level of reduced faecal excretion was shown in the studies. Based on the provided results, an OOI of 4 weeks for all three antigens and a DOI of 69 weeks for *S.* Enteritidis, 71 weeks for *S.* Typhimurium and 44 weeks for *S.* Infantis is currently acceptable.

Clinical studies were mainly performed to evaluate the safety of the vaccine under EU field conditions. The influence of maternally derived antibodies on the efficacy of the vaccine did not need to be investigated because from 10 weeks of age onwards no level of residual MDA is present.

Additional benefits

Vaccination with Cevac Salmune ETI K could be a part of complex control programmes for *Salmonella* infections in poultry. These measures are essential for reducing *Salmonella* presence in poultry farms.

Risk assessment

The main potential risks are identified as follows:

Quality

Information on the development, manufacture and control of the active substance and finished product and on stability has been presented in a satisfactory manner.

Safety

Risks for the target animal

Administration of Cevac Salmune ETI K in accordance with recommendations in the product information is generally well tolerated.

The safety of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Infantis vaccine (inactivated) for chickens in layer chickens was confirmed in one laboratory and one field study. Only macroscopical findings in breast muscle tissue (yellow discolouration in all animals) and microscopical granuloma formation (in majority of animals) were observed after administration of Cevac Salmune ETI K at a maximum recommended treatment dose. However, the effects were only observed in the SPF chickens of the laboratory study. In the field safety study only microscopical granuloma formation has been observed. The possible discolouration of muscle tissue has been mentioned in the adequate section of the SPC.

Risk for the user

User safety for this product is acceptable when used according to the product information. Given the nature of the vaccine and its mode of administration, the risk to the user is very low.

Risk for the environment

Cevac Salmune ETI K is not expected to pose a risk for the environment when used according to the recommendations in the product information. The nature of the product makes direct exposure to the environment very unlikely. Standard advice on waste disposal is included in the product information. The standard sentence "Medicines should not be disposed of via wastewater or household waste." has been supplemented.

Risk for the consumer:

A residue study is not required. The withdrawal period is set at zero days.

Special risks

No special risks are indicated.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication: "For the active immunisation of chickens (breeders and layers) from 10 weeks of age to reduce faecal excretion of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Infantis.

Salmonella Enteritidis:
Onset of immunity: 4 weeks after 2nd vaccination
Duration of immunity: 69 weeks after 2nd vaccination

Salmonella Typhimurium:
Onset of immunity: 4 weeks after 2nd vaccination
Duration of immunity: 71 weeks after 2nd vaccination

Salmonella Infantis:
Onset of immunity: 4 weeks after 2nd vaccination
Duration of immunity: 44 weeks after 2nd vaccination"

Based on the data presented, the claim is considered acceptable, and the overall benefit-risk is considered positive.

The product information has been reviewed and is considered satisfactory and in line with the assessment.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) considers that the application for Cevac Salmune ETI K is approvable, since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EU) 2019/6).

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