13 July 2023
EMA/331788/2023
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for YURVAC RHD (EMEA/V/C/005992/0000)

Vaccine common name: Rabbit haemorrhagic disease and RHDV2 vaccine (recombinant)

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

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Table of contents

Introduction

The applicant Laboratorios Hipra, S.A. submitted on 29 April 2022 an application for a marketing authorisation to the European Medicines Agency (The Agency) for YURVAC RHD, through the centralised procedure under Article 42(2)(a) of Regulation (EU) 2019/6 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 9 September 2021 as YURVAC RHD has been developed by means of a biotechnological process, i.e. using recombinant DNA technology (Article 42(2)(a)(i)).

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

For active immunisation of rabbits from 30 days of age onwards to reduce mortality of rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV) and variant strains (RHDV2), including highly virulent strains.

The active substance of YURVAC RHD is the recombinant RHDV2 virus capsid protein, which, administered to the animals, creates an active immunity against the RHD virus. The target species is rabbit, including pet (dwarf) rabbits.

YURVAC RHD emulsion for injection contains the recombinant RHDV2 virus capsid protein antigen - relative Potency (ELISA test) (RP) ≥ 0.7 and it is available in glass vials of 0.5 ml (1 dose) or 5 ml (10 doses) and PET vials with 20 ml (40 doses) or 100 ml (200 doses). The pack sizes available are:

- Cardboard box of 10 glass vials of 1 dose (0.5 ml)
- Cardboard box of 1 glass vial of 10 doses (5 ml)
- Cardboard box of 1 PET vial of 40 doses (20 ml)
- Cardboard box of 1 PET vial of 200 doses (100 ml)

The rapporteur appointed is Ricardo Carapeto García and the co-rapporteur is Leona Nepejchalová.

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

On 13 July 2023, the CVMP adopted an opinion and CVMP assessment report.

On 11 September 2023, the European Commission adopted a Commission Decision granting the marketing authorisation for YURVAC RHD.

Scientific advice

Not applicable.

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 which fulfils the requirements of Article 23 of Commission Implementing Regulation (EU) 2021/1281. Based on the information provided the applicant has in place a pharmacovigilance system master file (PSMF) with reference number PSMF-HIPRA-AH-01, 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 substance

The manufacturing, primary packaging and physical processing of the active substance of this vaccine take place at LABORATORIOS HIPRA, S.A., Amer, Girona, Spain.

A GMP declaration for the active substance manufacturing site is provided from the Qualified Person (QP) at the proposed EU active substance manufacturing site. The declaration is based on an on-site audit which has taken into consideration the GMP certificate available for the active substance site issued by AEMPS (Spanish Medicines and Medical Devices Agency) following inspection carried out on 22/07/2022.

Finished product

The quality control testing, the biological quality control testing, the chemical/physical quality control testing and the microbiological sterility testing of the vaccine take place at LABORATORIOS HIPRA, S.A., Avinguda La Selva, 135, Amer, 17170 (Girona), Spain. Also, the activities of primary and secondary packaging, storage and/or distribution and batch release are carried out at this site.

The site has a manufacturing authorisation issued on 22/09/2021 by AEMPS (Spanish Medicines and Medical Devices Agency) and the last GMP inspection on this site was completed on 22/07/2022 by the Competent Authority - AEMPS.

Overall conclusions on administrative particulars

The GMP status of the active substance and of the finished product manufacturing sites has been satisfactorily proven and is in line with legal requirements. The applicant provides updated GMP certificates based on inspections carried out in July 2022 for both sites.

A complete list of the organisms handled at the different production sites is provided, according to the requirements of Commission delegated Regulation (EU) 2021/805 of 8 March 2021 amending Annex II to Regulation (EU) 2019/6 of the European Parliament and of the Council.

Part 2 - Quality

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

Qualitative and quantitative composition

A table is presented with the qualitative and quantitative composition of the vaccine per dose of 0.5 ml. The active ingredient is the recombinant RHDV2 virus capsid protein, since the plasmid included in *Komagataella phaffii* exclusively encodes for the RHDV2 capsid protein, named VP60. The potency is 0.7 RP (Relative Potency). The product contains light mineral oil as adjuvant which corresponds to USP requirements. In the dossier, the ability of capsid proteins VP60 to self-assemble into VLPs are described, revealing that YURVAC RHD contains VLPs. The applicant has followed the requirements of the Ph. Eur. monograph 0784 (Products of recombinant DNA technology) established to characterise the active substance.

The other constituents are: polysorbate 80, sorbitan mono-oleate, sodium chloride, potassium chloride, disodium phosphate dodecahydrate, potassium dihydrogen phosphate and water for injections.

The vaccine is intended to be available in multidose presentations and does not contain any preservative.

A filling overage volume has been established to guarantee that the number of doses stated on the label can be withdrawn for each vial. An overage in the antigen content has also been established to guarantee that only efficacious batches are released.

Container and closure system

The product is available in Type I colourless glass vials (for 1 and 10 doses – 3 ml and 10 ml vials) and Type I colourless PET vials (for 40 and 200 doses – 20 ml and 100 ml vials). The minimum fill volume for all presentations is the nominal volume plus 5%. The vials are contained in cardboard boxes as described in section 5.4 of the SPC.

Each vial is closed with a Type I rubber stopper and sealed by an aluminium cap.

The pack/container sizes are consistent with the vaccination schedule and intended use.

The certificates of analysis, the suppliers' certificates, the drawings and copies of the relevant Ph. Eur. monographs are provided for both the containers and the stoppers. The containers and closures are in compliance with the pharmacopoeia requirements.

The sterilisation methods of the containers and stoppers have been provided and they are considered in compliance with Ph. Eur. requirements.

Product development

YURVAC RHD is a recombinant vaccine intended for active immunisation of rabbits from 30 days of age onwards to reduce mortality caused by classical Rabbit Haemorrhagic Disease Virus (RHDV) and variant strains (named as Rabbit Haemorrhagic Disease virus-2 (RHDV2)). The classical form of the disease (caused by RHDV) was detected in Europe in the late 1980s and the variant (caused by RHDV2) was detected for the first time in 2010, in France, and it has been since then spreading across many European countries. Both diseases, both viruses, are present nowadays in Europe and no treatment is currently available.

The applicant already owns a marketing authorisation for a vaccine against RHDV (CUNIPRAVAC-RHD, authorised by National Procedure) and one against RHDV2 (ERAVAC, authorised by Centralised Procedure).

Neither the RHDV nor the RHDV2 are able to grow in culture cells. Therefore, the vaccines have to be produced using live rabbits: the animals are infected with the virus, euthanised and their livers extracted.

In order to comply with the 3Rs principles, the applicant has developed YURVAC RHD which can be manufactured without the use of live animals. It is in fact a recombinant vaccine using a *Komagataella phaffii* strain as a host to express and produce a recombinant protein: the recombinant RHDV2 virus capsid protein.

The applicant's target was also to develop a vaccine which confers cross-protection to both viruses, and it is claimed that YURVAC RHD is able to protect rabbits against both RHDV and RHDV2.

The protein selected to be produced is the capsid protein named VP60 which is considered the major structural protein of the RHD virus.

The yeast host-vector *Komagataella phaffii* (formerly *Pichia pastoris*) is one of the most extensively used eukaryotic systems for the production of recombinant proteins. The method of production is based on a seed lot system.

The batch potency test selected to control the potency of the vaccine is a capture antigen ELISA which is also able to identify the active substance.

After testing several formulations, the adjuvant chosen was light mineral oil. It is a known adjuvant, used in other applicant's vaccines intended for rabbits and it is considered well tolerated when administered by subcutaneous route. Based on a combination of efficacy and safety studies results, the final concentration of light mineral oil was selected.

The other excipients were chosen for their ability to be mixed in the final formulation and to improve the stability of the emulsion. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. No novel excipients are used in the finished product formulation. The full list of excipients is included in section 2 of the SPC.

A filling overage volume has been established to guarantee that the number of doses stated on the label can be withdrawn for each vial: the minimum fill volume for all presentations is the nominal volume plus 5%. An overage in the antigen content has also been established: the applicant decided to formulate all batches with the standard dose which has a higher antigen content compared to the protective dose. This guarantees that only efficacious batches are released.

The relevant issues of the pharmaceutical development have been addressed by the applicant.

Description of the manufacturing method

The manufacturing process established for the antigen is based on the "seed lot system", as indicated in the general monograph of the Ph. Eur. no. 0062 (Vaccines for veterinary use). A flowchart illustrating the controls performed at the different steps is included.

The manufacturing process consists of a system of successive batches of the product derived from one Master seed, which is described in the dossier. This production process is considered the most appropriate for the manufacture of the vaccine, since it ensures the manufacture of homogeneous batches of antigen and also complies with the requirements of the European Pharmacopoeia.

The number of passages from the Master seed lot required to obtain the desired volume of the harvest has been properly established.

Once the harvest is obtained, it is concentrated by means of a continuous centrifugation step. Then, a downstream process is carried out in order to obtain the final antigen.

The applicant assesses the manufacturing process and the potential presence of host cell's proteins (HCPs) in the final product to demonstrate that the level of HCPs in the vaccine is acceptable and safe.

The aqueous and oil phases are prepared separately. The antigenic phase is mixed with the aqueous phase, stirred and homogenised. The oil phase (containing the adjuvant and the excipients) is loaded in a stainless-steel tank through a sterilising filter. The emulsion formed is homogenised and stored, and this period is justified with the results of one industrial batch.

Later, the filling process is carried out.

A table with the blending details of 1 litre of vaccine is included in the dossier.

Production and control of starting materials

Starting materials listed in pharmacopoeias

The applicant provided a list including the name, the function and the applicable monograph to each starting material listed in a Pharmacopoeia. All of them are monographs of the European Pharmacopoeia with the exception of monosodium glutamate and light mineral oil, for which USP criteria are applicable in absence of specific European Pharmacopoeia monograph.

For each starting material listed in pharmacopoeias, the applicant included certificate of analysis of LABORATORIOS HIPRA, S.A., certificate of analysis from the supplier(s) and copy of the relevant monograph.

All the certificates of analysis that have been provided conform to the specifications of the Ph. Eur. and USP.

The nature of the starting materials, the controls and the treatments applied guarantee sterility of the vaccine and absence of introduction of any extraneous agent.

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

Before describing the Master Seed, the applicant describes the biotechnological process to obtain it. The appropriateness of the use of *Komagataella phaffii* for the production of the vaccine is justified in terms of 3Rs. The choice is also appropriate considering the avoidance of using antibiotic resistance genes in the host yeast (although the plasmid contains zeocin resistance gene). Both the RHD virus capsid protein gene and the plasmid were synthesised.

Master Seed (BMS)

Original Stock vials were reconstituted and, after an appropriate incubation period, the Master Seed was obtained. The controls performed on the Master Seed are: identity, viability, purity and titre.

Working Seed (BST)

Master Seed vials were reconstituted and, after an appropriate incubation period, the Working Seed was obtained. The Working Seed was controlled for identity, viability, purity and titre.

The applicant indicates the number of vials produced of Original Stock, Master and Working seeds and describes the incubation conditions and the expected shelf life of the seeds. The certificates of analysis of the Master and Working seeds are provided. The controls carried out on the Master and Working seeds are described.

In line with the Ph. Eur. monograph 0784 (Products of recombinant DNA technology), the applicant provided a study on the validation of the cell bank system and production consistency of the recombinant RHDV2 virus capsid protein. The Master Seed was tested confirming the stability of the integrity and viability of the insert. A molecular method was used to analyse the expression vector for copy number, insertion, deletions and the number of integration sites. The coding region was shown to correspond to that expected for protein sequence. Microbiological purity of the cell bank was tested and the validation of the purity controls carried out on the Master and Working seeds have been provided. The effect of the presence of process related impurities, such as HCPs, residual antifoam and residual flocculation reagent, has been addressed and the applicant has provided enough evidence that the manufacturing process is able to reduce process related impurities at levels that can be considered safe for the target species and the food consumer.

The active substance consists of VLPs and the applicant provided the necessary scientific justification for the absence of controls of non-auto-assembled VP60 proteins .

Sabouraud agar dextrose, peptone, yeast extract and yeast nitrogen base are the other biological starting materials used. Details on the source of the materials and information on the sterilisation process applied have been provided together with the supporting appropriate documentation. As required per the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3), the applicant provided a risk assessment. Gelatin, as starting material included in a pharmacopoeia, is also included in this risk assessment. The appropriate documentation was provided.

The applicant provides a report on the management of extraneous agents according to Ph. Eur. 5.2.5. The risk assessment takes into account all the starting materials used during the manufacture of the vaccine and all the production steps. When necessary, appropriate documentation is provided. It is concluded that the presence and the likelihood that a potential extraneous agent (included in Annex I of Ph. Eur. 5.2.5.) can be present in the vaccine is negligible.

Starting materials of non-biological origin

Certificates of analysis have been provided for the antifoam, the PMSF (phenylmethasulfonyl fluoride) and the pDADMAC (Poly-diallyldimethylammonium chloride solution) and all of them are conforming to in-house specifications. Appropriate documentation was provided.

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

During the production of the vaccine, several media are used. Detailed information on the qualitative and quantitative composition, methods of preparation, sterilisation and storage of media and solutions are provided for the in-house prepared media and solutions. The suppliers are listed as applicable and the medium or solution are linked to their respective certificate of analysis.

Control tests during the manufacturing process

The following in-process controls are carried out on the final antigen:

- Identity by Gram stain
- Purity

- Turbidity
- Identity
- pH
- Antigen quantification
- Bacterial and fungal sterility

The integrity of the filters, before and after filtration, is tested for each batch of oil phase.

For the in-process control tests, the method, frequency and timing of testing, function of the test and acceptance criteria are presented as well as the respective description of the test method. When necessary, validation of the methods has been performed.

A test for residual DNA from *K. Phaffii* was performed for which a description and validation of the test is required. The limit has been established taking into account recommendations for human injectable medicines. The applicant proposes not to perform this test as routine in-process control test in the light of the successful results (no residual DNA) obtained in three antigen batches. This proposal is acceptable as these batches were industrial batches. Determination of host cell proteins (HCP) in the recombinant RHDV2 capsid protein antigen is performed by quantitative ELISA. In the validation of this method, the applicant detects foreign proteins by the WB method using antibodies which allow selection of the detected proteins. A comparison of the Western blot results with the Coomassie blue-stained gel is provided, in order to prove that most of the proteins are recognised by both techniques.

The antigen quantification by means of haemegglutination of human red blood cells was included in a SOP which has been divided in two SOPs: one for the HA assay for detection of RHDV in tissue samples and the other one for the HA assay for the quantification of recombinant RHDV2 virus capsid protein in the YURVAC RHD antigen and for the detection in tissue samples of RHDV2. As in this case the HA test is used to titre the antigen of YURVAC RHD and neither RHDV nor RHDV2 virus have been used in the manufacture, it is acceptable. The applicant indicates that the production of vaccines against RHD (CUNIPRAVAC-RHD (against RHDV) and ERAVAC (against RHDV2)) are carried out at other HIPRA facilities.

Control tests on the finished product

The following control tests are described in the dossier:

1) General characteristics of the finished product

The appearance of the product in the bulk and filled is controlled by macroscopic observation.

pH is controlled with a pH-meter.

2) Identification of the active substance(s)

The Batch Potency Test (BPT) is used for identification purposes.

3) Batch titre or potency

The batch potency test is a capture ELISA, able to measure the quantity of antigen in the sample.

The batch of vaccine used as Reference vaccine comes from a dose-response study where different vaccines with different amount of antigen were assessed. The minimum dose and the standard dose were found efficacious for both viruses with challenge efficacy studies. The specificity of the monoclonal antibody used as capture antibody and the polyclonal antibody used as detection antibody are explained and information in relation to their replacement has been provided.

The correlation between the amount of antigen (measured in Richness Units (RU)) and the potency of the finished product (in Relative Potency (RP)) is explained. Information about the batch used to demonstrate that the proposed BPT is able to detect deterioration in the antigen has been provided. The retest period of the reference standard and internal control vaccine has been described. The validation of the method is provided. The batch release specifications were properly established.

4) Identification and assay of adjuvants

The viscosity test is carried out by using a viscometer.

The concentration of light mineral oil is also tested, and it is performed by means of HPLC. The validation of the method has been performed.

5) Identification and assay of excipient components

N/A

The excipients included in YURVAC RHD are commonly used in other vaccines and it is considered unnecessary to have a specific control of them in the finished product.

6) Sterility and purity tests

It is carried out by the method stated in Ph. Eur. General Chapter 2.6.1 (Sterility). The validation of the method is provided. Specific description of the integrity of the filter test is provided.

8) Filling volume

The extractable volume of containers is controlled with the use of graduated cylinders.

Batch-to-batch consistency

The applicant provided the results of the control tests carried out on three consecutive production runs of YURVAC RHD. Batches of different sizes were tested but not at the upper limit. The justification provided is acceptable. However, the consistency with industrial batches close to the upper limit (1200 litres) should be shown (please refer to the list of recommendations). It is recommended to communicate to the CVMP any out of specification detected if it occurs.

The Manufacturer's Batch Protocols provided contains the in-process and finished product control tests described previously.

Stability

Bulk antigen: the proposed storage period for the antigen is 12 months at 2 – 8 ºC and it has been demonstrated with satisfactory results of three batches manufactured according to the method described in the dossier.

Finished product: the proposed shelf-life of the vaccine is 1 year at 2 – 8 ºC in the final container of the vaccine. It has been demonstrated with three consecutive batches and the tests and intervals checked are considered appropriate.

In-use stability: the proposed in-use stability period after first broaching is 10 hours. It has been demonstrated with two different batches and the largest presentation (PET vials of 100 ml). The results of the tests performed are satisfactory and the claim is considered demonstrated.

New active substance (NAS) status

The applicant requested the active substance recombinant RHDV2 virus capsid protein antigen contained in YURVAC RHD to be considered a new active substance in comparison to a similar one previously authorised in the European Union. The applicant claimed that the recombinant RHDV2 virus capsid protein antigen differs significantly in properties with regard to efficacy from the similar substance already authorised in the EU.

Based on the review of the data, the rapporteurs consider that the active substance recombinant RHDV2 virus capsid protein antigen contained in the medicinal product YURVAC RHD can be qualified as a new active substance in comparison to the known virus capsid protein of RHDV2 included in a previously authorised vaccine in the European Union.

Overall conclusions on quality

YURVAC RHD is a vaccine intended for the active immunisation of rabbits from 30 days of age onwards to reduce mortality of rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV) and variant strains (RHDV2), including highly virulent strains. The active substance of YURVAC RHD is the viral capsid protein of RHDV2 (VP60 protein). The ability of these capsid proteins to self-assemble to produce VLPs (Virus Like Particles) is adequately described and the advantages of them are also noticed: VLPs present the shape and size of a virus but lacking the genetic material so they are not capable of infecting the host cell while they are highly immunogenic.

The vaccine contains light mineral oil as adjuvant and is presented as a w/o emulsion for injection.

YURVAC RHD is produced by means of recombinant DNA technology using a yeast (*Komagataella phaffii*) strain as a host strain to express and produce the recombinant VP60 virus capsid protein.

The choice of the sequence coding for VP60 protein has been justified by the efficacy data provided.

The applicant has implemented the necessary requirements of Ph. Eur. monograph 0784 for this product.

The Batch Potency Test is an ELISA assay able to quantify and identify the antigen present in the vaccine. The general characteristics of the finished product and sterility are also tested. The adjuvant (light mineral oil) is tested by means of HPLC.

The stability of the finished product has been demonstrated for 1 year.

Based on the review of the data on quality, the manufacture and control of YURVAC RHD is considered acceptable.

However, a post marketing authorisation measure is recommended.

Recommendation:

- the applicant should provide consistency results on batches representatives of the batch size close to the upper limit of 1200 litres. Any out of specification should be communicated immediately.

Part 3 – Safety documentation (safety and residues tests)

General requirements

The active substance of YURVAC RHD is a recombinant RHDV2 virus capsid protein antigen, which, administered to the animals, creates an active immunity against the RHD virus. The applicant states that, being the active substance the viral capsid protein of the variant (RHDV2), cross- protection has been demonstrated between classical RHDV and variant RHDV2. This subject will be further discussed in the Efficacy part of the dossier.

The target species is rabbit, including pet (dwarf) rabbits.

The objective of this part of the dossier is to demonstrate and confirm that YURVAC RHD is a safe vaccine, which does not cause adverse effects on vaccinated animals, non-vaccinated animals or other species, including humans, and that it is also safe for the environment.

For all safety studies, batches with standard antigen content were used. The applicant has provided justification for the deviation in this part of dossier. In the case of vaccine YURVAC RHD, the antigen concentration is fixed so that all batches will contain the same quantity of antigen. In view of this information, the vaccine batches used in the safety trials can be standard batches produced according to the manufacturing process described in Part 2B of the file and it can be acceptable.

Additionally, also to answer some of the quality concerns detected (e.g. impurities), the applicant submitted an overdose study and a clinical safety study.

The Hemagglutination Inhibition Test (HAI) was used for serological response determination in safety studies. This method is considered fit for purpose.

Safety documentation

Four pre-clinical trials have been performed for the assessment of the safety of YURVAC RHD. One to investigate the safety of the administration of one and repeated dose in dwarf rabbits and two to assess the reproductive performance (e.g. safety in pregnant rabbits and safety of the offspring from vaccinated lactating rabbits). Although not compulsory, as it is an inactivated vaccine, the applicant also submitted an overdose study to address the quality concerns raised during the first assessment in relation to the content of HCP.

Additionally, a clinical trial was provided to further support the safety of the vaccine.

The vaccine was administered by the subcutaneous route, as recommended in the SPC. The pre-clinical studies were reported to be GLP compliant and carried out in rabbits of the minimum age recommended for vaccination, using production batches containing 58.4 x 10^6 RU/l of the antigen component per 0,5ml.

The clinical study was a GCP compliant multicentre trial. The objective of the study was to determine the potential anaphylactic reactions produced by potential impurities present in the vaccine. The applicant followed as far as possible the requirements described in the current Ph. Eur. monograph 5.2.6: "Evaluation of safety of veterinary vaccines and immunosera". The requirements described in the Ph. Eur. monograph 2325: "Rabbit Haemorrhagic Disease vaccine (Inactivated)" have also been taken into account to demonstrate the safety of this vaccine. However, it should be noted that the specific monograph Ph. Eur. 2325 is applicable to RHDV and not to RHDV2.

Study title
Safety of administration of one and repeated dose

Study title
Safety assessment of a single dose administration of the YURVAC RHD vaccine in pregnant rabbits.
Safety of YURVAC RHD when administered to lactating rabbits was conducted to evaluate the safety of YURVAC RHD on the offspring
One dose and overdose study with high levels of HCP
Clinical safety, efficacy and immunogenicity under field conditions of YURVAC RHD vaccine in rabbits

Pre-clinical studies

The following four laboratory studies, investigating the safety of the vaccine, have been performed: administration of one and repeated dose in dwarf rabbits, two studies carried out in New Zealand White (NZW) rabbits to assess the reproductive performance and one dose and overdose study with high levels of HCP.

Safety of the administration of one dose

The safety of the administration of one dose has been assessed. A second dose was administered to the animals on D14 in order to assess safety of a repeated dose.

The experimental study aimed to evaluate the safety of a single dose and repeated dose of YURVAC RHD (0.5 ml) administered subcutaneously to animals of 30 days of age.

In the study, a standard batch (58.4 x 10^6 RU/l) was used. A total of sixteen 30 days-old dwarf rabbits, free from RHDV and RHDV2 antibodies, were included in the study. Dwarf rabbits are expected to be the most sensitive category of the target species.

The animals were randomly distributed in two groups and kept in the same location. One group of 10 rabbits was vaccinated subcutaneously at Day 0 of the study (group A), while the other group of 6 rabbits received sterile PBS, through the same route, with the same administration scheme (group B). The animals, throughout the course of the experiment (during 14 days of observation after the single dose), were monitored daily for morbidity (depression, body condition, dyspnoea, nasal discharge, ocular discharge) and mortality.

The rectal temperature of the rabbits was recorded the day before vaccination, at the time of vaccination, four hours post-vaccination and then daily for 4 consecutive days, as required by the Ph. Eur. monograph 5.2.6. The body weights were measured three days prior to vaccination and on D14 and D35 post-vaccination.

Additionally, serology was monitored. All vaccinated animals seroconverted by Day 35 (after second dose administered on D14), while all the control animals remained negative for RHDV and RHDV2 antibodies.

The observations and examinations for signs of systemic and local reactions gave the following results: the mean rectal temperature per group did not exceed 1.5ºC in none of the groups and the vaccine did not produce significant adverse effects on the body weight gain.

Safety of one administration of an overdose

The applicant submitted an overdose study in order to demonstrate the safety of a vaccine containing the double of the antigen content to discard any anaphylactic reaction produced by the administration of the IVMP in a worst-case scenario. The rabbits enrolled in the study were divided in the following groups:

Group A: overdose 2x (1mL)

Group B: one dose and repeated dose after 14 days (0.5 mL)

Group C: placebo.

After the vaccination, all animals were observed in order to record any abnormal local or systemic reactions. The rectal temperature of the rabbits was recorded one day before vaccination, at the time of vaccination, four hours post-vaccination and then daily for 4 days. In addition, before the vaccination, on day 14 and at the end of the study, animals were weighted in order to evaluate the affectation of body weight performance.

None of the animals showed general clinical signs during the 14 days of observation after the overdose and the single dose administration and after the 21 days of observation following the repeated dose administration.

Slight inflammation was detected after the first dose administration in five animals which disappeared after 3 days. After the repeated dose, inflammation was detected in some animals and lasted a maximum of 10 days after this administration. In the group administered with an overdose, some animals presented a slight inflammation which disappeared 3 days after vaccination.

Regarding the rectal temperature of the animals, all were within normal ranges during the study. The highest mean rectal temperature increase was obtained in the overdose vaccinated group, specifically 0.45ºC. The highest individual rectal temperature increase was obtained in the control group after the single dose administration, specifically 1.14ºC. Therefore, it is confirmed that the average body temperature increase for all animals does not exceed 1.5ºC and no animal showed a temperature rise greater than 2.0ºC, complying with the described safety criteria of the current monograph 2325 of the European Pharmacopoeia "Rabbit Haemorrhagic Disease Vaccine (Inactivated)".

The outcomes obtained are satisfactory and therefore the safety of the administration of an overdose is demonstrated. The applicant has included the results of this overdose study under section 3.10 of the SPC.

Safety of the repeated administration of one dose.

The vaccination schedule of YURVAC RHD includes one single dose of 0.5 ml administered by subcutaneous route to rabbits from the age of 30 days. The applicant designed the study in a way to evaluate both the safety of one dose and that of a repeated dose administration.

A total of sixteen 30 day-old dwarf rabbits free from RHDV and RHDV2 antibodies were enrolled in the study. They were distributed in two groups. Ten animals were vaccinated with a standard batch (58.4 x 10^6 RU/l). The other six animals received PBS. In order to evaluate the safety of the vaccine after a repeated dose, a second dose, through the same route, was administered at Day 14 of the study for both the vaccine and the PBS treatment.

The safety of the repeated dose administration was tested by monitoring the rectal temperature for four days (same measurement as per the single dose) and the health status for twenty-one days after revaccination. Additionally, the body weight was monitored before the repeated dose administration and at the end of the study (on D14 and D35).

The results showed that no statistically significant differences were observed among control and vaccinated groups regarding systemic reactions when a repeated dose of the vaccine is administered. Safety findings in relation to the increase of temperature of one animal (this animal presented a rectal temperature of 40.22ºC (day 4 after vaccination) which returned to normal values 24 hours later) and identified local reactions (inflammation grade 1 (<2cm), which disappeared 24h later) in other three animals were noticed and included in the SPC.

Examination of reproductive performance

YURVAC RHD is intended to be used in breeding animals, therefore, the reproductive performance was investigated in two studies summarised below.

One GLP safety laboratory trial was conducted on pregnant does. Thirty-eight pregnant does, divided based on their gestation phase, were enrolled in the study. One group of 9 pregnant does in the first third of gestation (group A), another group of 8 pregnant does in the second third of gestation (group B) and 8 pregnant does in the last third of gestation (group C) were vaccinated with YURVAC RHD according to the vaccination plan. Other 3 groups (group D, E and F) of 4 or 5 pregnant does each, in the three different thirds of gestation, were administered 0.5 ml of PBS with the same administration and route. A standard batch was used for the study.

After the single dose administration, all animals were observed until parturition to examine any harmful effect during gestation or on the progeny. Any abnormal local or systemic reaction was adequately recorded for at least 14 days post-parturition and the effects on the progeny were recorded up to 3 days post-parturition. The rectal temperature of the does was recorded one day before vaccination, at the time of vaccination, four hours post-vaccination and then daily for 4 days post vaccination. The temperature of the non-vaccinated animals was not recorded due to welfare reasons.

Serology was also evaluated to demonstrate that animals were adequately vaccinated.

Moreover, the following reproductive parameters were evaluated: litter size, number of live offspring per litter, abortion and malformation of newborns. Raw statistical analysis data on these parameters have been provided.

The results showed that none of the animals enrolled in the study presented a compromised health status that could be attributable to the vaccine. Moreover, no local reactions were appreciated during the follow-up period and the rectal temperature monitoring was in accordance with Ph. Eur. monograph 2325.

Abortion was detected in one doe from one of the vaccinated groups (group B: 2nd third of gestation). The cause of the abortion was investigated by the applicant and the cause identified was related to the manipulation in the last phase of gestation.

In addition to the one described above, a study with administration to lactating rabbits was conducted to evaluate the safety of YURVAC RHD on the offspring.

In this study, sixteen NZW rabbits lactating does were enrolled and distributed in two groups. One group of 8 lactating animals was vaccinated with YURVAC RHD according to the vaccination plan (group A). The other group of 8 lactating does was administered 0.5 ml of PBS (group B) with the same plan of administration and route.

After the single dose administration, all animals were observed until weaning to examine any harmful effect during lactation or on the progeny. Any abnormal local or systemic reaction was adequately monitored during the whole study (until the weaning day).

The effects on the body weight of progeny were recorded at the end of lactation. The statistical analysis of the growth performance data was provided together with also data on the age, date of conception, number of kids etc. The rectal temperature of the does was recorded one day before vaccination, at the time of vaccination, four hours post-vaccination and then daily for 4 days after vaccination. The serology was also assessed and results provided. Based on the results obtained, no adverse effect attributed to the vaccine on the reproductive function of the females nor on the health status of the offspring was detected.

No safety study on the reproductive performance has been conducted in male rabbits (bucks). Therefore, the relevant advice has been added to the product information.

Examination of immunological functions

No further studies were conducted to investigate the effects of the product on immunological functions. However, no adverse effects were observed in any of the safety or efficacy studies. It is therefore unlikely, due to the nature of the product, that this vaccine will have a negative impact on the immunological functions.

Special requirements for live vaccines

Not applicable since YURVAC RHD is not a live vaccine.

User safety

The applicant has provided a user safety risk assessment which has been conducted in accordance with the CVMP guideline EMEA/CVMP/IWP/54533/2006 (and EMEA/CVMP/543/03-Rev.1).

The main potential routes of accidental contact with the product have been considered. It was concluded that the most likely are those of accidental self-injection of the person who administers the vaccine and the persons assisting in restraining the rabbits.

The active substance is an inactivated protein and it is not infectious to humans. The excipients are substances commonly used in other vaccines and none of these present a safety concern. Since the formulation includes light mineral oil as adjuvant, the standard warnings for mineral oil-containing vaccines are included in the product information.

As a result of the user safety assessment several advices to users/warnings for the user were considered appropriate under section 3.5. of the SPC.

It is also stated in the SPC that the product is available for use only for animal treatment, under veterinary prescription and should be kept out of the sight and reach of children.

Study of residues

No specific study on residues has been performed. This is considered acceptable.

MRLs

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 listed in section 2 of the SPC, including the adjuvants, 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.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The applicant has not provided data investigating interactions of the vaccine with any other veterinary medicinal product and therefore proposes to include a statement in Section 3.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis.' This is considered acceptable.

Clinical studies

A safety clinical trial has been included in the dossier to study the impact of impurities on safety. Taking into account that may cause allergic or anaphylactic reactions that, because of their low frequency, might be more difficult to detect in a laboratory study with a limited number of animals, the applicant has provided additional information by submitting a clinical trial.

The clinical study was conducted in two commercial farms from an RHD endemic area with standard management and husbandry conditions, representative of the rabbit industry. The total number of animals was 5,789 kits (2,887 vaccinated with YURVAC RHD).

In each farm, the included 30 days old kits were randomly distributed in two groups. One group received the YURVAC RHD vaccine whereas the other group received the placebo (PBS). The route of administration was the recommended one (subcutaneous).

During the study, all animals were observed daily for adverse reactions and were followed-up until the slaughterhouse. The safety of the vaccine was assessed through the evaluation of systemic reactions, local reactions, rectal temperature and body weight.

Regarding the safety results, no adverse reactions were reported. Some slight inflammatory local reactions grade 1 (<2cm) were observed after vaccination with this vaccine, which disappeared without treatment. The administration of the vaccine did not induce any clinically relevant increase in temperature. In addition, the vaccine did not have any negative effect on the body weight of the rabbits.

These results confirm the conclusions obtained in the preclinical studies regarding the safety of the vaccine.

Environmental risk assessment

According to Directive 2004/28/EC of the European Parliament and Council amending Directive 2001/82/EC, the application for marketing authorisation of any immunological veterinary product must be accompanied by a study of ecotoxicity. In addition, as indicated in the Guideline for Environmental Risk Assessment for Immunological veterinary medicinal products (EMEA/CVMP/074/95), it is necessary for any veterinary medicinal product assessing the risk associated with each of the components.

Considerations for the environmental risk assessment

Based on the data provided, the ERA can stop at Phase I. YURVAC RHD is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

The applicant has provided four pivotal laboratory studies.

One of them was to investigate the safety of one dose and the repeated administration of one dose to target animal species, in animals of the minimum recommended age, via the recommended route. Dwarf rabbits are used as they are expected to be the most sensitive category of the target species. The batch used in the study was a standard one. Based on the results, it was concluded that the safety of the targeted animals, when the vaccine is administered according to the recommended schedule and via the recommended route, is acceptable.

Two safety studies were conducted to investigate reproduction safety. Both studies used standard batches.

A fourth study was conducted to address the safety of the administration of an overdose in order to discard any anaphylactic reaction produced after the administration of the IVMP, with satisfactory results.

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

The applicant has not provided any data for the safety in reproductive males. This is reflected in section 3.5 of the SPC.

The data presented are considered adequate overall to characterise the safety profile of the vaccine YURVAC RHD as acceptable.

A user safety assessment, in line with the relevant guidance document, has been presented. The worst-case scenario identified for user safety is that of a self-injection. No hazard has been identified other than that related to the light mineral oil adjuvant included in the vaccine. The standard warnings are included in the product literature, which is acceptable. Based on the assessment presented, the product does not pose an unacceptable risk to the user when used in accordance with the SPC. The appropriate warnings for the user have been included in the product literature.

A clinical trial on safety has been performed. The results confirm the conclusions obtained in the preclinical studies regarding the safety of the vaccine.

An environmental risk assessment was provided. Based on the data provided, the ERA can stop at Phase I. YURVAC RHD is not expected to pose a risk for the environment when used according to the SPC.

According to the applicant, the product is not expected to pose a risk for the environment when used according to the SPC. In view of the data presented, it is accepted.

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

General requirements

The vaccine is intended for active immunisation of rabbits from 30 days of age onwards to reduce mortality of rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV) and variant strains (RHDV2), including highly virulent strains.

Efficacy was assessed in compliance with Regulation (EU) 2019/6, the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7. as well as Ph. Eur. monograph 2325 on Rabbit Haemorrhagic Disease, EMEA/CVMP/682/99-FINAL "Duration of protection achieved by veterinary vaccines" and the Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals" (EMA/CVMP/IWP/439467/2007).

Challenge model

The challenge model is considered adequately validated and therefore appropriate for using in the efficacy trials in order to mimic the natural conditions for infection. The applicant has justified the selection of the challenge strains and the representativeness of the strains in the field.

The doses of the challenge strains chosen in the different efficacy studies are adequately justified by the applicant.

The applicant has adequately characterised the challenge strains.

Efficacy parameters and tests

The efficacy parameters investigated in the efficacy studies are serology, clinical signs and overall, mortality. The tests performed to evaluate them were hemagglutination (HA) method and hemagglutination inhibition (HAI) test. The parameters chosen are considered appropriate for evaluating the efficacy of the product. The tests used are deemed as fit for the purpose to study the efficacy of the vaccine under laboratorial conditions.

Efficacy documentation

Seven pre-clinical studies were conducted to investigate the efficacy of the product against RHDV2. Additionally, four studies were conducted to investigate the efficacy of the product against RHDV. No efficacy field clinical trials are available. The field trial submitted was to address the safety concerns raised in part 2 of the dossier (impurities, HCP). The laboratory studies were well documented and carried out in rabbits of the minimum age recommended for vaccination, using production batches.

Deviation on the age of animals included in the immunogenicity test for the onset of immunity (OOI) study of the RHDV were accepted to comply with the specific Ph. Eur. monograph 2325.

Dwarf rabbits were not enrolled in the efficacy studies.

Study title
Study for the determination of the antigen dose and the efficacy of YURVAC RHD vaccine against the variant of Rabbit Haemorrhagic Disease Virus (RHDV2) in rabbits
Study of the onset of immunity (OOI) of the single dose administration of YURVAC RHD vaccine against the variant of Rabbit Haemorrhagic Disease virus (RHDV2) in rabbits.
Study on the duration of immunity (DOI) of the single dose administration of YURVAC RHD against the new variant of Rabbit Haemorrhagic Disease Virus (RHDV2) in rabbits.
Study on the influence of maternally derived antibodies (MDA) against the variant of Rabbit Haemorrhagic Disease virus (RHDV2) on vaccine efficacy of YURVAC RHD in rabbits.
Study of the efficacy of the single dose administration of YURVAC RHD vaccine against a current highly virulent strain of the variant of Rabbit Haemorrhagic Disease Virus (RHDV2) in rabbits.
Study of the onset of immunity (OOI) of the single dose administration of YURVAC RHD against the classic Rabbit Haemorrhagic Disease Virus (RHDV) in rabbits.
Study on the duration of immunity (DOI) of the single dose administration of YURVAC RHD against the classic of Rabbit Haemorrhagic Disease Virus (RHDV) in rabbits.
Study on the influence of maternally derived antibodies (MDA) against the classical Rabbit Haemorrhagic Disease Virus (RHDV) on vaccine efficacy of YURVAC RHD in rabbits.

Study title
Study on the duration of immunity (DOI) of the single dose administration of YURVAC RHD vaccine against a current highly virulent strain of the variant of Rabbit Haemorrhagic Disease Virus (RHDV2) in rabbits.
Efficacy study against the classical rabbit haemorrhagic disease virus (RHDV) of the YURVAC RHD vaccine in rabbits
Study of the onset of immunity (OOI) of the single dose administration of YURVAC RHD vaccine against a current highly virulent strain of the variant of Rabbit Haemorrhagic Disease Virus (RHDV2) in rabbits.

Pre-clinical studies

Dose determination

A laboratory study was conducted to determine the efficacious dose of active substance contained in the vaccine by performing challenge infections with RHDV2.

In the study, four groups of 20 rabbits (30 days old) each were included. The rabbits were naïve to RHDV and RHDV2. Three different batches containing 3 different formulations with different antigen content were inoculated to three groups (A, B and C) of 20 animals each. Additionally, a group of 20 animals was kept as control group and inoculated with PBS (group D).

The animals received 0.5 ml of vaccine (or PBS) subcutaneously as recommended.

On day 14 post-vaccination, all of them were challenged. The efficacy parameters assessed in this study are as follows: mortality, clinical signs (depression, body condition, dyspnoea, nasal discharge, ocular discharge, other) and serology. Those vaccine formulations that showed significant differences in the main efficacy parameter (mortality) compared to the controls, were considered efficacious.

For RHDV, a laboratory study was carried out to demonstrate that the substandard dose and the standard dose established for YURVAC RHD vaccine are also efficacious against RHDV.

The efficacy of the same formulations as for the RHDV2 study above has been tested against a virulent RHDV challenge (V-4764) by intramuscular route 14 days after vaccination. The study was carried out using the recommended route of administration (subcutaneous) and using the schedule recommended (1 single dose).

The main efficacy parameter evaluated was the mortality caused by RHDV after challenge. In addition, local and general clinical signs were also assessed. Based on the results for mortality related to RHDV, it is demonstrated that that the standard dose (Group A) and the substandard dose (Group B) are efficacious against RHDV.

Onset of immunity

Four studies were carried out in New Zealand White rabbits for the recommended administration route: two for RHDV and two for RHDV2 (against two different strains, one of them highly virulent).

Animals of 30 days of age were enrolled to study the onset of immunity (OOI) for RHDV2. Animals from 10 weeks of age were enrolled to demonstrate the OOI for RHDV in compliance with Ph. Eur. monograph 2325.

In the study of the OOI against the RHDV2, two groups of 22 and 21 animals of 30 days of age were used. A vaccine dose of 58.4 $x10^6$ RU/L per dose from a production scale batch, was administered to the vaccinated group A by the subcutaneous route. Group B was unvaccinated. The vaccinated group

and the unvaccinated group were challenged with a dose of 2^{15} HA/ml of virulent RHDV2 (strain V-1037) administered intramuscularly one week after vaccination. Following the challenge, the animals were investigated for survival rates, seroconversion levels and clinical signs.

The survival rate of the vaccinated group was 100%. The survival rate for the control group was 14%. A necropsy was conducted on every death animal. No clear macroscopic lesions were observed in none of the necropsied animals. Liver samples were collected and tested for the presence of RHDV2. In the control group, 14 out of 18 animals were confirmed as positive to RHDV2 in the liver, whereas no animals in the vaccinated group were positive. Two control animals died after challenge but for causes unrelated to the RHDV2 (digestive problems), whereas two other animals had not clear cause of death. These results show that at least 67% of the control animals died due to RHDV2 (14/21), which was significantly greater than the 0% of the YURVAC RHD vaccinated animals.

No detectable antibodies against RHDV and RHDV2 were found in animal sera taken at day 0. Seven days after vaccination, all animals vaccinated with YURVAC RHD seroconverted whereas all control animals remained seronegative. On day 21 of the study, all the survival animals from both groups were seropositive.

It was concluded that vaccination by the recommended route, with the recommended dose, as described in the SPC, was efficacious and met the efficacy requirements.

In the study of the efficacy against a current highly virulent RHDV2 strain, thirty-six 30 days-old New Zealand White rabbits were enrolled: 17 animals in the vaccinated group (group A) and 19 more in the control group (group B). A standard vaccine dose of YURVAC RHD of 58.4 $x10^6$ RU/L per dose from a production scale batch, was administered to group A by the subcutaneous route. Group B was unvaccinated and received a PBS dose instead. The vaccinated group and the unvaccinated group were challenged with 2^{15} HA/ml of highly virulent RHDV2 (strain V-1171) administered intramuscularly two weeks after vaccination. Following the challenge, the animals were investigated for morbidity, mortality and seroconversion levels.

The mortality rate of the vaccinated group was 0%. The mortality rate for the mock-vaccinated group was 78,95%.

All rabbits were negative for RHDV and RHDV2 antibodies prior to vaccination. Blood samples were collected from all animals at days 14 and 28. The serology results show that after challenge, all survival animals were seropositive to RHDV2. These results validate the fact that the challenge was properly performed. No serology data for RHDV was provided as the challenge was carried out with RHDV2. It was concluded that vaccination by the recommended route, with the recommended dose, as in the SPC, was efficacious and met the efficacy requirements.

In order to set up an OOI claim for highly virulent challenge strains, an additional study was submitted. Forty rabbits of the minimum age recommended for vaccination (30 days of age) and free of antibodies against RHDV and RHDV2 were enrolled in the study. The rabbits were divided in two groups of 20 animal each; one of the groups was vaccinated with YURVAC RHD vaccine (group A) and the other group (group B) received a placebo (PBS).

The study was carried out using the recommended route of administration (subcutaneous) and using the schedule recommended for this vaccine (1 single dose).

All animals were challenged with a recently isolated highly virulent RHDV2 strain by the intramuscular route 7 days after vaccination. Afterwards, all animals were observed for 14 days. On day 14 post-challenge, all survival rabbits were humanely euthanised and liver samples from dead animals were analysed.

The main efficacy parameter evaluated was the mortality caused by RHDV2 virus after challenge. In addition, general clinical signs were also assessed.

Regarding the mortality observed after challenge, the mortality rate of the control group was statistically higher ($p < 0.05$) than that observed for the vaccinated group with YURVAC RHD. It should be noted that all animals of vaccinated group survived the challenge whereas the control group had a mortality rate of 95%. Therefore, an OOI of 7 days was demonstrated for the highly virulent strain.

In the study of the OOI against the classic RHDV, twenty 10 weeks-old New Zealand White rabbits were enrolled: 10 animals in the vaccinated group (group A) and 10 more in the group control (group B). A standard vaccine dose of 58.4 $x10^6$ RU/L per dose from a production scale batch was administered to vaccinated group by the subcutaneous route whereas the other group was unvaccinated and received a PBS dose instead. The vaccinated group and the unvaccinated group were challenged with 2^{12} HA/ml of RHDV (Strain V-4764) administered intramuscularly one week after vaccination. Following the challenge, the animals were investigated for morbidity, mortality and seroconversion levels.

The mortality rate of the vaccinated group was 20%. The mortality rate for the control group was 90%. Liver samples were collected and tested for the presence of RHDV. In the control group, 89% animals were confirmed positive for RHDV, whereas, in the vaccinated group, only one animal was positive. Hence, the total mortality rate due to RHDV in the vaccinated group was 10%.

All rabbits were negative for RHDV and RHD2 antibodies prior to vaccination. Blood samples were collected from all animals at D7 prior to challenge. The serology results show that all animals from the vaccinated group were seropositive to RHDV2 before challenge. Serological results are available post-challenge to validate that the challenge was properly performed.

According to monograph Ph. Eur. 2325:

"The test is not valid if fewer than 80 per cent of control rabbits die with typical signs of RHD within 120 hours of challenge. The vaccine complies with the test if not fewer than 90 per cent of vaccinated rabbits show no signs of RHD".

Therefore, it was concluded that vaccination by the recommended route, with the recommended dose, as in the SPC, did not meet the efficacy requirements set by the specific Ph. Eur. monograph 2325, as 2 animals from the vaccinated group died after challenge (i.e. 80% of vaccinated rabbits show no signs of RHD, in contrast with the 90% required in the monograph). Because of the issues in the above study, the applicant has provided a RHDV dose determination study. The animals received three different antigen concentrations and were challenged 14 days later. The main efficacy parameter evaluated was the mortality caused by RHDV after challenge. Based on the results for mortality related to RHDV, it is demonstrated that the standard dose (Group A) and the substandard dose (Group B) are efficacious against RHDV, obtaining 100% and 91% of survival rates respectively.

According to these results, the OOI for RHDV might be acceptable at 14 days post-vaccination.

Duration of immunity

The applicant provided three studies to support the duration of immunity.

These studies were designed following the requirements of the Note for guidance EMEA/CVMP/682/99-FINAL "Duration of protection achieved by veterinary vaccines". To demonstrate 1-year DOI claim for RHDV2 (high virulent strain included) the applicant has presented two studies.

Study on the duration of immunity (DOI) against RHDV2: one vaccinated group (group A, 22 animals, vaccinated at 30 days-old) and one control group (group B, 17 animals). All animals from vaccinated

group were vaccinated with YURVAC RHD vaccine by the recommended route of administration (subcutaneous) and according to the proposed schedule of administration (1 single dose) whereas the animals from control group were administered with a placebo (PBS) using the same vaccination plan.

In order to demonstrate the efficacy of YURVAC RHD one year after vaccination, all animals were challenged with a RHDV2 virulent strain (V-1037).

General clinical signs of all animals and mortality were recorded throughout all the study until 14 days post-challenge (Day 375). Fourteen days after challenge, rabbits were humanely euthanised and liver samples from dead animals were collected for RHDV2 detection.

Regarding mortality rates observed after challenge, the mortality rate of the control group was statistically higher ($p < 0.05$) than that observed for the vaccinated group. It should be noted that the mortality rate of the vaccinated group was 0% and the mortality rate of the control group, confirmed as positive to RHDV2 in the liver, was 53%. No clinical signs were recorded during the challenge period in none of the animals.

The mortality rates are variable depending on the challenge strain. The study demonstrates that there is a statistically significant difference when comparing the mortality rates in both groups and therefore the claim for DOI one-year post-vaccination is demonstrated for virulent strains of RHDV2.

Additionally, in order to demonstrate the DOI of the administration of YURVAC RHD vaccine against a current highly virulent RHDV2 strain, a study with the same design has been performed against said current highly virulent RHDV2 strain.

Forty-eight animals were divided in two different groups: one vaccinated group (group A, 24 animals, vaccinated at 30 days-old) and one control group (group B, 24 animals). All animals from vaccinated group were vaccinated with YURVAC RHD vaccine by the recommended route of administration (subcutaneous) and according to the proposed schedule of administration (1 single dose) whereas the animals from control group were administered with a placebo (PBS) using the same vaccination plan.

In order to demonstrate the efficacy of YURVAC RHD one year after vaccination, all animals were challenged with a RHDV2 highly virulent strain (V-1171).

General clinical signs of all animals and mortality were recorded throughout all the study until 14 days post-challenge (Day 381). Fourteen days after challenge, rabbits were humanely euthanised and liver samples from dead animals were collected for RHDV2 detection.

The mortality rate of the control group was statistically higher ($p < 0.05$) than that observed for the vaccinated group. No clinical signs were recorded during the challenge period in none of the animals.

Therefore, the duration of immunity against a virulent strain and a recent highly virulent RHDV2 strain was demonstrated and established at 1-year post-vaccination.

The applicant also submitted a study on the duration of immunity (DOI) of the single dose administration of YURVAC RHD vaccine against RHDV in rabbits.

Twenty rabbits of the minimum age recommended for vaccination (30 days) and free from antibodies against RHDV and RHDV2 were enrolled.

The animals were divided in two groups: one vaccinated group (group A, 10 animals) and one control group (group B, 10 animals). All animals from vaccinated group were vaccinated with YURVAC RHD vaccine by the recommended route of administration (subcutaneous) and according to the proposed schedule of administration (1 single dose) whereas the animals from control group were administered with a placebo (PBS) using the same vaccination plan.

In order to demonstrate the efficacy of YURVAC RHD one year after vaccination, all animals were challenged with a RHDV virulent strain (V-4764).

General clinical signs of all animals and mortality were recorded throughout all the study, since Day 0 until 14 days post-challenge (Day 374). Fourteen days after challenge, rabbits were humanely euthanised and liver samples from dead animals were collected for RHDV detection.

Regarding mortality rates observed after challenge, the mortality rate of the control group was statistically higher ($p < 0.05$) than that observed for the vaccinated group.

In addition, the results comply with the efficacy requirements for immunogenicity of the Ph. Eur. monograph 2325. These results for the duration of immunity to be established at 1-year post-vaccination against RHDV are valuable as the RHDV is commonly affecting older animals rather than younger.

The DOI for RHDV is demonstrated 1 year after administration of the vaccine.

Maternally derived antibodies (MDA)

To evaluate the possible interference of residual passive immunity with an efficient active immunisation, two specific studies were performed.

The first study was designed to assess if the presence of MDAs against RHDV2 could have any impact on the efficacy of YURVAC RHD when administered to animals of the minimum age recommended. Efficacy was tested by means of challenge.

Recommendations from the Ph. Eur. monograph 5.2.7 and from the" Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals (EMA/CVMP/IWP/439467/2007)", were followed.

Sixty-nine rabbits of the minimum age recommended for vaccination (30 days old) were divided in three groups. Two groups (group A and B) were vaccinated with YURVAC RHD (58.4×10^6 RU/L). One of these groups, with presence of MDAs (+) against RHDV and RHDV2 and the other group without MDAs (-) against RHDV and RHDV2. The third group of rabbits with presence of MDAs (+) against RHDV and RHDV2 (group C) was administered with PBS in order to keep as control group and used to follow the decay of MDAs. The study was carried out using the recommended route of administration (subcutaneous) and using the schedule recommended (1 single dose).

After the rabbits were challenged intramuscularly with a dose of 2^{15} HA/ml of virulent RHDV2 (strain V-1037), the mortality rate (due to RHDV2 and no other causes) was monitored in all groups. In the control group was 44% whereas the mortality rates observed in the vaccinated groups A and B were 0% and 8% respectively. In contrast, between vaccinated groups A (MDA-) and B (MDA+) no significant differences in mortality were observed. The results demonstrated that the maternally derived antibodies do not affect the efficacy of YURVAC RHD vaccine.

A second study was designed to assess if the presence of MDAs against RHDV could have any impact on the efficacy of YURVAC RHD when administered to animals of the minimum age. Efficacy was tested by means of challenge.

As for the study previously summarised, recommendations of Ph. Eur. monograph no. 5.2.7 and the "Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals (EMA/CVMP/IWP/439467/2007)", were followed.

Thirty-six rabbits of the minimum age recommended for vaccination were divided in three groups. Two groups (group A and B) were vaccinated with YURVAC RHD (58.4×10^6 RU/L). One of these groups

with presence of MDAs (+) against RHDV and RHDV2 and the other group without MDAs (-) against RHDV and RHDV2. The third group of rabbits with presence of MDAs (+) against RHDV and RHDV2 (group C) was administered with PBS in order to keep as control group and used to follow the decay of MDAs. The study was carried out using the recommended route of administration (subcutaneous) and using the schedule recommended (1 single dose).

After the rabbits were challenged with RHDV challenge strain V-4764 (2^{12} HA/ml) intramuscularly, the mortality rate of the control group (due to RHDV) was 92% whereas the mortality rates observed in both vaccinated groups A and B were 0%. It is worth mentioning that, between vaccinated group A (MDA-) and vaccinated group B (MDA+) no significant differences in mortality were observed. Both groups had a survival rate of 100%.

Serology results provided for RHDV for groups A and B after challenge demonstrate that challenge was adequately carried out.

Therefore, the results demonstrated that the maternally derived antibodies do not affect the efficacy of YURVAC RHD vaccine.

Interactions

No specific studies have been carried out to investigate the possible interactions of YURVAC RHD with other veterinary medicinal products. Therefore, an appropriate text is included in section 3.8 of the SPC:

"No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis."

Clinical trials

A clinical trial has been included in the dossier: "Clinical safety, efficacy and immunogenicity under field conditions of YURVAC RHD vaccine in rabbits".

While this study was supportive of the safety profile of the vaccine, no conclusions could be obtained on the efficacy. In the absence of a disease outbreak during the follow up period, none of the efficacy variables could be assessed.

Despite the field study being inconclusive, the efficacy has been demonstrated by laboratory data and the omission of clinical data is acceptable in line with the EMA guideline on clinical trials with veterinary immunological products EMA/CVMP/IWP/260956/2021. A relevant laboratory model of infection was used, and results of the pre-clinical efficacy studies fully support the efficacy claims; the intended method of administration of the vaccine was mimicked under laboratory conditions and the design and execution of pre-clinical studies is such that the results are sufficiently reliable to allow assessment of the benefit-risk balance of the vaccine.

Overall conclusion on efficacy

The results from eleven laboratory trials show that the product is effective for active immunisation of rabbits from the age of 30 days to reduce mortality of Rabbit Haemorrhagic Disease caused by RHDV2, (including highly virulent strains) and RHDV at the proposed dose of 0.5 ml when administered subcutaneously.

Efficacy against RHDV has been demonstrated in accordance with Ph. Eur. monograph 2325 in a dose determination study. Animals received three different antigen concentrations and were challenged 14

days later. The main efficacy parameter evaluated was the mortality caused by RHDV after challenge. Based on the results for mortality related to RHDV, it is demonstrated that the standard dose and the substandard dose are efficacious against RHDV, obtaining 100% and 91% of survival rates respectively.

According to these results, the OOI for RHDV is 14 days post-vaccination. This has been included in the SPC.

The challenge model is adequate.

In the studies supporting efficacy against RHDV2, the challenge against a highly virulent strain was initially performed 14 days after vaccination. In order to obtain the 7 days OOI claim for highly virulent challenge strains, an additional study was submitted with satisfactory results.

Duration of immunity of 1-year post-vaccination is demonstrated for RHDV and RHDV2 (including highly virulent strain).

Influence of MDA on vaccine efficacy can be excluded. The results are satisfactory.

A field clinical study was included in the dossier. Despite the field study being inconclusive, the efficacy has been demonstrated by laboratory data and the omission of clinical data is acceptable in line with the EMA guideline on clinical trial with veterinary immunological products EMA/CVMP/IWP/260956/2021. A relevant laboratory model of infection was used, and results of the pre-clinical efficacy studies fully support the efficacy claims; the intended method of administration of the vaccine was mimicked under laboratory conditions and the design and execution of pre-clinical studies is such that the results are sufficiently reliable to allow the assessment of the benefit-risk balance of the vaccine.

For this specific product, the efficacy claims for RHDV and the RHDV2 variant are supported by the laboratory trials, as the applicant has provided clarifications to the questions formulated to efficacy concerns and, additional efficacy studies, with satisfactory results, to demonstrate protection against RHDV, have been provided.

Part 5 – Benefit-risk assessment

Introduction

YURVAC RHD is an emulsion for subcutaneous injection. It is intended to be used in rabbits (including pet (dwarf) rabbits) for active immunisation to reduce mortality of rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV) and variant strains (RHDV2), including highly virulent strains. One dose of vaccine (0.5 ml) should be administered to rabbits from 30 days of age onwards. Revaccination should be performed annually.

The active substance of YURVAC RHD is a recombinant RHDV2 virus capsid protein antigen. It is obtained by means of recombinant DNA technology (yeast host-vector system).

The product is available in glass vials of 0.5 ml (1 dose) or 5 ml (10 doses) and PET vials with 20 ml (40 doses) or 100 ml (200 doses) with different combination of pack sizes.

The applicant requested the active substance contained in YURVAC RHD to be considered a new active substance since it has a different nature and a completely different manufacturing process, which consist of using a recombinant *Komagataella phaffii* as a vector for expressing and producing a recombinant protein of therapeutic interest, i.e., the recombinant RHDV2 virus capsid protein.

Benefit assessment

Direct benefit

YURVAC RHD is of value in the treatment of rabbit haemorrhagic disease virus caused by RHDV and RHDV2 which causes high mortality rates in young and adult rabbits.

Well conducted controlled laboratorial trials demonstrated that the product is efficacious in decreasing the mortality of rabbits when challenged with virulent strains of RHDV and RHDV2.

The duration of protection is established at one year.

Additional benefits

YURVAC RHD increases the range of available treatment possibilities for a minor species, obtaining the active substances by means of biotechnology and therefore avoiding the use of rabbits in the production of the vaccine.

YURVAC RHD is easy to apply by the veterinarian.

Risk assessment

Quality

The information provided by the applicant in relation to the quality of the vaccine is considered sufficient to show consistency of the production process. The capability to detect sub-potent batches has been demonstrated since the link between the amount of antigen (VP60 forming VLPs) and the efficacy against RHDV has been shown.

Safety

Measures to manage the risks identified below are included in the risk management section.

Risks for the target animal

Administration of YURVAC RHD in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include a limited increase of temperature and local reactions. The potential for mild and transient adverse effects, such as those mentioned above, cannot be excluded.

The safety of YURVAC RHD in dwarf rabbits was confirmed in a pre-clinical study. Safety in the reproductive performance was studied in NZW female rabbits.

An overdose study and a clinical trial demonstrate that impurities present in the IVMP are not capable to produce anaphylactic reactions.

Risk for the user

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice is included in the SPC.

Risk for the environment

YURVAC RHD is an emulsion for injection containing a recombinant capsid protein as active substance and having rabbit as the only one target species. As it contains no live organisms or agents capable of replicating within the host, the probability of causing any negative impact into the environment is negligible and therefore the product is not expected to pose any risk to the environment when used as recommended.

Risk for the consumer:

The vaccine does not contain any ingredients that are likely to pose a risk for consumers of rabbit meat. Residue studies are not required. The withdrawal period is set at zero days.

Special risks

Not detected.

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 active immunisation of rabbits from 30 days of age onwards to reduce mortality of rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV) and variant strains (RHDV2), including highly virulent strains.

The product has been shown to be efficacious for these indications, and the CVMP accepted the indications as proposed by the applicant.

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

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

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for YURVAC RHD 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 medicinal product.