17 June 2021
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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Fatrovax RHD (EMEA/V/C/005301/0000)

Vaccine common name: Rabbit haemorrhagic disease vaccine (inactivated, recombinant)

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

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Introduction

The applicant Fatro S.p.A submitted on 4 September 2019 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Fatrovax RHD, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 21 February 2019 as Fatrovax RHD has been developed by recombinant DNA technology.

On 17 June 2021, the CVMP adopted an opinion and CVMP assessment report.

On 16 August 2021, the European Commission adopted a Commission Decision granting the marketing authorisation for Fatrovax RHD.

Indications:

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

For the active immunisation of rabbits from 28 days of age to prevent mortality and to reduce infection, clinical symptoms and organ lesions of rabbit haemorrhagic disease (RHD) caused by rabbit haemorrhagic disease virus RHDV and RHDV2.

Onset of immunity: 1 week (7 days) after vaccination

Duration of immunity: 1 year (12 months)

The current proposal for the indication is: For active immunisation of rabbits from the age of 28 days to reduce mortality, infection, clinical signs and organ lesions of rabbit haemorrhagic disease caused by RHDV1 and RHDV2.

Onset of immunity: 1 week (7 days) after vaccination

Duration of immunity: 1 year

The active substances of Fatrovax RHD are two major capsid proteins (VP1) of classical (VP1a) and type 2 (VP1ab) RHD viruses, respectively, which auto-assemble into virus-like particles (VLPs). These VLPs trigger an active immune response against both types of RHD viruses. The target species is rabbit. The product is intended for administration by the subcutaneous route.

Fatrovax RHD is presented as suspension for injection containing ≥1 relative potency (RP) VP1a of RHDV1 and ≥1 relative potency (RP) VP1ab of RHDV2 per dose of 0.5 ml.

Fatrovax RHD is presented in boxes:

- Box of 5 pre-filled syringes of 1 dose (5 x 0.5 ml)
- Box of 1 polypropylene bottle of 50 doses (25 ml)
- Box of 1 polypropylene bottle of 200 doses (100 ml).

The rapporteur appointed is Cristina Muñoz Madero and the co-rapporteur is Petra Falb.

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

Scientific advice

Not applicable.

MUMS/limited market status

The applicant requested eligibility of this application for MUMS/limited market by the CVMP, and the Committee confirmed that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) data requirements would be applied when assessing the application. MUMS/limited market status was granted as rabbits are considered minor species.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated 17 November 2015), which fulfils the requirements of Directive 2001/82/EC.

Manufacturing authorisations and inspection status

The manufacturer of the product (all steps) is Fatro S.p.A (Via Emilia 285, 40064, Ozzano Emilia, Bologna, Italy).

The manufacturing authorisation was issued on 5 October 2018 by the Ministry of Health, General Directorate for animal health and veterinary medicines, Italy (Annex 5.6).

A valid GMP compliance certificate was provided for the production site, which was issued on 5 October 2018 by the by the Ministry of Health, General Directorate for animal health and veterinary medicines, Italy, after inspection (20-23 February 2017; Annex 5.9). Based on the inspection carried out in February 2020, the GMP certificate NBF/20/2020/V is provided, issued on 7 April 2020 and available on EudraGMP.

Overall conclusions on administrative particulars

The GMP status of the active substance(s) and of the finished product manufacturing sites have been satisfactorily established and are in line with legal requirements.

Part 2 – Quality

Fatrovax RHD is a vaccine intended to induce active immunity in rabbits against two types of rabbit haemorrhagic disease virus: RHDV1 (classical strain) and RHDV2 (also called new variant or RHDVb). The vaccine contains two recombinant proteins, RHDV1 VP1a and RHDV2 VP1ab that auto-assemble into virus-like particles (VLPs). Each VLP is produced separately. These proteins are produced by means of recombinant baculoviruses grown in pupae of the *Lepidoptera* order. VLPs are formed by structural viral proteins and can self-assemble. They resemble viruses but are non-infectious because they lack viral genetic material. VLPs mimic the 3D conformation of native viruses and display a high density of repetitive effective antigenic epitopes on their surface, which stimulate the desired immune response. VLPs of Fatrovax RHD are immunogenic for rabbits since they mimic the wild behaviour of the RHDV1 and the RHDV2, respectively.

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The finished product is presented as a suspension for injection containing the two VLPs formed by recombinant proteins from RHDV1 and RHDV2 as active substances. Potency is established as relative potency (RP) to a reference batch of serum from vaccinated mice and measured by ELISA. The potency of the reference vaccine is assigned 1, therefore RP should be ≥1 per dose of 0.5 ml for both the active substances. The product contains aluminium hydroxide as adjuvant.

Other ingredients are sodium dihydrogen phosphate dihydrate, disodium phosphate dodecahydrate, sodium chloride and water for injections.

The vaccine is intended to be available in multi-dose and single dose presentations and consequently contains thiomersal as preservative. According to Ph. Eur. monograph 0062, inclusion of preservatives in single dose presentations can be acceptable when the same vaccine is filled in single-dose and multi-dose containers.

The product is available in polypropylene bottles for multi-dose presentations and pre-filled syringes for single dose presentation, as described in section 6.5 of the SPC.

Regarding the description of the active substances, the applicant proposes:

Rabbit haemorrhagic disease virus 1 (RHDV1) VP1a* ≥1 RP**
Rabbit haemorrhagic disease virus 2 (RHDV2) VP1ab* ≥1 RP**
* recombinant capsid protein;
** relative potency, ELISA by comparison with a reference serum in vaccinated mice

This is acceptable.

Since this vaccine is of a particular quality standard, section 5 of the SPC (Immunological properties) includes the following information: "The active substances of the vaccine are two recombinant proteins: rabbit haemorrhagic disease virus VP1a (capsid protein VP1 and VP2 of strain Ast89) and rabbit haemorrhagic disease virus 2 VP1ab (chimera of strains Ast89 and N11), which auto-assemble into virus-like particles (VLPs)".

Container and closure

The product is filled into polypropylene bottles according to Ph. Eur. of 25 ml (50 doses) and 100 ml (200 doses) with elastomer stoppers and aluminium caps. For the single dose presentations, pre-filled type I borosilicate glass syringes are used.

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

The containers and closures are in compliance with the pharmacopoeial requirements and their sterilisation has been satisfactorily demonstrated. Relevant documentation (drawings from suppliers and Fatro's certificates of analysis) and sterilisation processes of all the containers in contact with the product are provided: polypropylene bottles, elastomer stoppers, syringes, integrated tip caps and needles.

Product development

An explanation and justification for the composition and presentation of the vaccine as well as a description of the history of the disease and the causative agent, including occurrence of the new variant of the RHDV, have been provided. The applicant refers to the absence of cross protection between the two different types of RHDV and the need to include both antigens in the same vaccine. Reasonable justification is given regarding the relevance of the chosen vaccine strains within the EU.

The method of manufacturing is considered appropriate for Fatrovax RHD. It is performed by the use of infected pupae with recombinant baculovirus. The choice of the species of pupae as substrate for the production of the vaccine has been properly justified and it is considered in line with 3Rs. The baculovirus grown in pupae can express the main capsid protein of both strains of RHDV virus. The chosen capsid protein is VP60 (or VP1), which is highly immunogenic for rabbits. It is known that RHDV1 and RHDV2 do not grow in cell cultures. So hitherto infection of rabbits, necropsy and extraction of the liver were needed to obtain a considerable amount of virus for the production of a vaccine.

The production of both recombinant baculoviruses was carried out in Algenex, S.L. and later they were provided to Fatro where the MSVs are located. Both recombinant baculoviruses were generated at Algenex with the plasmids and sequences donated from external sources. The strain of RHDV1, strain Ast89, originated from an ill animal, which was isolated in Spain in 1989 (Genbank accession Z49271) and was selected for optimal antigen supply for an inactivated vaccine. For RHDV2, the strain selected was the strain N11, also isolated in Spain in 2011 (Genbank accession KM878681). In both cases, *E. coli* was transformed by heat shock with 100 ng of RHDVa plasmid in the expression bacmid. The bacmid was analysed by PCR to verify successful incorporation of the sequence. A mixture of the bacmid was transfected to cells to produce the passage 0 of the virus.

The other 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 6.1 of the SPC. Thiomersal is included as preservative in the vaccine and the applicant has addressed the impact on the immunogenicity and antigenicity of the VLPs based on the chemical composition of both proteins (only one Cys residue in their sequence).

The formulation of most of the batches used during clinical studies is the same as that intended for marketing.

Detergents are used in the inactivation process and, according to the applicant, they have demonstrated to inactivate the baculovirus quantity of up to 10^6 plaque-forming units (PFU)/ml within 5 minutes by mixing with the disrupted frozen pupae after a homogenisation step. According to Ph. Eur. monograph 0062, appropriate tests should be carried out to demonstrate that the inactivating agent has been removed or reduced to an acceptable residual level. Also, it is stated that it is essential to take account of the possibility that under the conditions of manufacture, organisms may be physically protected from the inactivant (pupae structures in this case). The applicant has developed and validated two methods based on chromatography in order to detect the presence of detergents in finished product. The presence of pupal proteins is tested by western blotting, using sera from rabbits immunised with a semi-purified extract of non-inoculated pupae.

Test for the absence of live virus using a validated method in a suitable cell line is performed, but not immediately after adding detergents. This is accepted, since no inactivating agent (physical or chemical) is applied to the live viruses during a fixed time period and the whole process (from inoculation of pupae to sterilising filtration) is the inactivation procedure.

Validation of inactivation of recombinant baculoviruses used in the infection of pupae has been

performed by the applicant. Previously, the applicant has carried out three studies to assess the interference due to the pupal material, the composition of the extraction buffer and the composition of the storage buffer in the titration assay (that is, the inactivation control test).

For the validation of inactivation, a study has been performed to demonstrate the inactivation of baculovirus with titres higher than those obtained in routine production. For this study, pupae inoculated with 500 pfu/pupa were processed as they are in the manufacturing process since higher titres are obtained. Validation of inactivation is considered demonstrated.

Description of the manufacturing method

The manufacturing process includes different steps and each VLP is produced separately.

The production process of the vaccine is described adequately.

SOPs and/or codes for the processes have been included in the description; the hold times between different steps are stated; containers used in different steps are explained in detail. The consistency of the production can be considered demonstrated. The selection of the inoculation dose has been justified since the quantity of each recombinant protein is higher compared to when other parameters are used (different amount of PFU and different incubation periods). Validation studies of different steps of the process have been provided, as well as the respective reports and protocols. Possible contaminations, as well as the ability of the manufacturing process to remove any potential product- and process-related impurities have been addressed by the applicant.

Controls on the finished product are also described. Validation of the antibody ELISA test used in the batch potency has been provided, and the replacement of critical reagents of the ELISA test used for potency test of Fatrovax RHD, including the reference serum, has been properly established. The main concern was the absence of any impurity control (identification and quantification) and it was resolved: the applicant has introduced control tests in order to identify and quantify these impurities (detergent residues, proteins from pupae and residual baculoviruses). A purity acceptance limit of ≥80% is specified for the recombinant proteins contained in the vaccine. The applicant has explained that pupal proteins do not interfere with the immunogenicity of the vaccine, according to the safety and efficacy studies. In a new safety study in dwarf rabbits animals were vaccinated at the double dose of active ingredients, therefore, with the double amount of potential impurities contained in the vaccine, and satisfactory results were obtained.

The in-process controls are adequately described.

Production and control of starting materials

The composition of the product is appropriately presented.

Starting materials listed in pharmacopoeias

Certificates of analysis have been provided and are in line with Ph. Eur. requirements. The raw materials and their controls ensure sterility and absence of introduction of any extraneous agents. The function of these starting materials is stated.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

*P*upae and recombinant baculoviruses for both strains, RHDV1 and RHDV2, are described in the

dossier. Insect cells are used for the production of MSV and WSV and their description is included in the dossier according to Ph. Eur. monograph 5.2.4 (Cell culture for the production of veterinary vaccines).

For the recombinant baculoviruses, previously to be acquired by Fatro, the following parameters were controlled: titration, productivity, the expression cassette for the protein of interest and sequencing of DNA by comparing different passages to ensure the absence of genetic modifications.

The MSV, produced in insect cells, is controlled for: identification, viral titre, sterility, absence of mycoplasmas and absence of extraneous agents. Since the vaccine has a MUMS status, only the extraneous agents for those agents that may occur in the source species should be tested (i.e. insect viruses). So, the MSVs were tested by PCR to verify the absence of rhabdovirus and wild baculovirus. The applicant has demonstrated that no rabbit viruses are handled in Algenex since the donor plasmid for RHDV and the foreign gene bearing the sequences for the production of recombinant baculoviruses were produced externally and supplied to Algenex.

The WSV are controlled for viral titre, sterility and absence of mycoplasmas.

Starting materials of non-biological origin

Appropriate information is provided in relation to starting materials of non-biological origin including detergents.

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

Qualitative and quantitative composition, method of preparation, sterilisation procedures and control tests, where applicable, have been described.

Control tests during the manufacturing process

The applicant presented in-process control data from various antigen bulks. Additional consistency data with the results of two further antigen batches produced for each active substance and two finished product pilot batches for the manufacturing, of which said antigens were used, are provided. The applicant provided in tabulated form the data from all the antigen batches used, to show whether the manufacturing process can be considered consistent. Manufacturer's Batch Protocols of these batches are included.

During the manufacture of the antigen, the following tests are carried out: sterility, absence of mycoplasmas, identification of the active substances, quantification of active substances, control of inactivation of baculoviruses, determination of yield, determination of purity and sterility of excipients. For the yield control test, the applicant included a control for the total amount of VP1 comparing different steps of the process.

The applicant identified and quantified the proteins of interest (VP1a and VP1ab). Furthermore, the proportion of remaining single VP1a and VP1ab (without auto-assembling) was demonstrated by means of electron microscopy. Regarding this control strategy, quantification and identification of other substances possibly present in addition to the antigen (product- and process-related impurities) are controlled. The reference batch has been properly characterised and described.

The applicant included two new in-process control tests: determination of residual baculovirus DNA and determination of residual baculovirus proteins. After the filling, uniformity of the filled weight, self-sealing and packaging are controlled.

Control tests on the finished product

The control tests on the finished product are the following:

- General characteristics: appearance and pH
- Identification of active substance by western blot
- Batch titre or potency: this is an *in vivo* test. The applicant stated that an *in vitro* test is under validation. Currently, an ELISA test is carried out with sera from 10 mice vaccinated with one dose of the vaccine. A reference batch has been established to compare the serological response in mice. The potency of the vaccine is expressed as relative potency (RP) and it should be ≥1. Validation data have been provided
- Identification and assay of aluminium hydroxide as adjuvant
- Identification and assay of thiomersal as preservative
- Sterility and purity test. Sterility test is performed by membrane filtration according to Ph. Eur. monograph 2.6.1.

Regarding the purity, the applicant has qualified and identified other components that could be present in the vaccine and a purity control has been included as in-process control test. In relation to product-related substances, if degradation products would arise during manufacturing and/or storage of the active substances, they would be detected by the established control. Moreover, it has been demonstrated with batches stored for up to 3-4 years where degradation products have been found. Therefore, they are not expected to be found in the finished product since the storage period is shorter. In relation to the structural integrity of the VLPs, the applicant has provided two studies for the analysis, by means of electron microscopy, of the ratio of T1/T3 forms and the presence of monomers (VP1 proteins that are not forming VLPs).The ratio of T1/T3 forms was maintained constant for both VLPs, and VP1 were the majority found in the permeate of the filtration while VLPs were mainly found in the retentate.

Inactivation: by culture in cells, it is performed at bulk level. Three subsequent passages of the sample under examination on adherent cells are performed, daily checking for appearance of any cytopathic effect. At the end of the third passage, if no CPE is detected, western blot analysis for baculovirus, is performed, to verify the absence of replicating virus. A new control has been included in final batch testing that is the determination of impurities as residues from the manufacturing method. Detergent residues will be controlled and thresholds have been included in Manufacturer's Batch Protocol.

The description of the methods used for the control of the finished product is considered satisfactory.

Batch-to-batch consistency

The applicant provided batch protocols of five batches of Fatrovax RHD. To summarise the results and to provide a better overview, the applicant has provided the data (including all in-process control data) from all relevant batches in tabulated form. Batches presented up to now are pilot scale batches and the applicant has committed on verifying consistency with an additional batch at industrial scale including all the controls required during the assessment, and also to provide real-time stability data on the two pilot batches and on the first industrial batch. The commitment is provided. This is in line with the MUMS Guideline.

The applicant has included the titre of the WSV used in the production of the batch in the Manufacturer's Batch Protocol. The maximum titre for each recombinant baculovirus was in line with the inactivation validation studies. Control on uniformity of filled weight, self-sealing and final packaging are included in Manufacturer's Batch Protocol.

Stability

A first **stability study of infected frozen pupae** is provided. Two batches of pupae each were infected and incubated as it is described in section 2B and three aliquots were processed at fixed intervals (0, 1, 3, 6, 9 and 12 months). Total crude extracts were evaluated. According to the results provided in two tables, the quantity of recombinant protein remains stable. The applicant has provided information to confirm the stability of the frozen pupae for 12 months: protocol of the study, batch of pupae used and information about the recombinant baculovirus used in the study (WSV of both recombinant baculoviruses) have been provided.

A study for **stability of the antigens** is provided. Three consecutive batches of each active substance were used. At T0, 1, 3, 6 and 12 months after production, the following parameters were controlled: pH and VP1 quantification. Regarding the quantification of VP1, limits are specified by the applicant. Results were statistically analysed comparing the antigen concentration at different time points during storage. Raw data of statistical analysis for each of the six batches are provided. Since quantification control of VP1a and VP1ab is performed at bulk level, it can be considered acceptable to omit setting of limits but to investigate the loss of antigen during storage. It is concluded that the antigen can be stored for a 12-month period at +2 to +8 °C. Furthermore, the applicant was asked to address the following parameters in the stability studies: purity, possibility of degradation, presence of degradation products. The applicant explains that in all the pilot batches of antigen manufactured until now, no degradation products or formation of protein dimers have been observed.

A study for the **stability of the finished product** is provided. The stability study was performed on 3 consecutive batches at pilot scale. Samples were taken on the final bulks at time 0 and on one pack size for each primary packaging material: the 200-dose presentation filled in polypropylene bottles and the single dose in glass syringes. Controls were carried out at T0 and every three months over a period of 15 months. On each batch, the control tests described in part 2E were performed: appearance, pH, identification of the active substances, potency, determination of aluminium hydroxide, determination of thiomersal and sterility. Also, a quantification of the total amount of antigens (RHDV1 VP1a + RHDV2 VP1ab) was performed and the method is described in part 2D (in-process control tests). Samples were stored at the same temperature as recommended in the product literature: +2 to +8ºC. The identification control test and sterility test were performed at T0 and T12. Aluminium hydroxide and thiomersal determinations were performed at T0, T12 and T15. All results were in line with the proposed specifications. In relation to the purity, control tests for determination of residual baculovirus DNA, determination of residual baculovirus proteins and determination of residual detergents are included as finished product control tests. Although the vaccine is formulated with a fixed amount of antigen (5 µg/dose), the sources of variability in the potency test have to be taken into account. Also, the possible effect of the nature of the sample (mainly for the presence of aluminium hydroxide) on the performing of electrophoretic run can affect the quantification of the total amount of VP1. No industrial batches have been manufactured up to now and the applicant has committed to provide real-time stability data on two pilot batches and on the first industrial batch. The shelf life of the finished product is set to 9 months, as during this period the RP is consistently greater than 1.

For the proposed **in-use shelf life** of the broached product of 10 hours, a study is presented in line with EMA/CVMP/IWP/250147/2008 where it is stated that "For inactivated vaccines, if the proposed in-use shelf life is less than one working day (maximum 10 hours) it is acceptable to **omit the potency testing** from the in-use shelf life stability study". Since not only the potency is necessary to guarantee the stability of the product after 10 hours in routine practice, pH has been also controlled to guarantee that denaturation of proteins caused by pH changes does not occur. Also, in line with Ph. Eur. monograph 0062, the microbial safety of the vaccine has been demonstrated over the proposed in-use shelf life. The in-use shelf life is demonstrated and the test for efficacy of antimicrobial

preservation study has been provided. Culture media and test microorganisms were used according to Ph. Eur. monograph 5.1.3 (Efficacy of antimicrobial preservation) and a validation of the method is presented. The study can be considered satisfactory. According to Ph. Eur. monograph 0062, in addition to the evaluation of the efficacy of the antimicrobial preservative, samples are tested at suitable intervals over the proposed in-use shelf life.

Overall conclusions on quality

The product is produced and controlled using validated methods and tests, which ensure the consistency of the product.

Based on the review of the data on quality, the manufacture and control of Fatrovax RHD are considered acceptable.

In addition, the applicant is recommended to provide the following information post-authorisation: real-time stability data on the two pilot batches and on the first industrial batch. The applicant has provided a commitment to submit data post-authorisation and the Committee considers this to be acceptable.

Part 3 – Safety

Introduction and general requirements

Fatrovax RHD is a subunit vaccine consisting of the modified capsidic recombinant proteins of the calicivirus of the rabbit haemorrhagic disease as active substances (VP1a and VP1ab proteins from classical and variant RHDV types, respectively).

Safety documentation

Five safety studies were conducted to investigate the safety of the product, comprising four laboratory studies on the safety and reproductive performance of the administration of single and repeated dose, and one field trial to demonstrate safety of the vaccine in dwarf rabbits. The vaccine was administered by the subcutaneous route, as recommended in the SPC. The laboratory studies were reported to be good laboratory practice (GLP)-compliant and carried out in rabbits of the minimum age recommended for vaccination, using batches containing 5 µg of each of the antigen components VP1a and VP1ab from RHDV1 and RHDV2 per 0.5 ml. One field trial was also performed.

Study title
Safety of administration of a single dose
Safety of the repeated administration of one dose
Safety in pregnant does
Safety in dwarf rabbit (field trial)
Safety of administration of a single dose and a repeated dose in dwarf rabbits

Laboratory tests

Four laboratory studies investigating the safety of the administration of one dose and of repeatedly administered doses (in NZW and Netherland dwarf rabbits) as well as of reproductive performance were conducted.

Safety of the administration of one dose

The safety of the administration of one dose in NZW rabbits has been assessed in one study.

The experimental study aimed to evaluate the safety and efficacy of a single dose of vaccine CUNIVAX-RHD (0.5 ml), former name for Fatrovax RHD, used during the development phase, administered subcutaneously to animals of 30 days of age.

In the study, a pilot scale batch (5 µg RHDV1 and 5 µg RHDV2/0.5 ml) was used in agreement with the reduction in requirements for MUMS-classified products stated in guideline EMA/CVMP/IWP/123243/2006 (no maximum potency requirement). The animals were monitored daily, for morbidity (asthenia, anorexia, gastrointestinal, respiratory and neurological symptoms) and mortality throughout the course of the experiment.

The rectal temperature of the rabbits was recorded daily from the day before vaccination, at the time of vaccination, four hours post vaccination and then daily for 4 days, as required in Ph. Eur. monograph 50206. The body weights were measured for 14 days.

The following observations and examinations for signs of systemic and local reactions were performed in rabbits after administering the recommended dose via the subcutaneous route: the vaccine does not cause an increase in rectal temperature. The vaccine did not produce significant adverse effects on the body weight gain of vaccinated rabbits. The applicant presented a score of the general clinical signs (normal, mild, moderate and serious). During daily clinical observations, no specific examination of the injection site was performed; only the presence/absence of noticeable lesions was investigated. For this reason, information about the absence of local reactions was not included in the study.

The subjects used in the study (age: 30 days) were supplied by Granja San Bernardo, an authorised farm for the supply experimental New Zealand rabbits with minimal disease level (regular tests for the detection of RHDV1 and RHDV2 are performed). The relevant health monitoring certificate provided by Granja San Bernardo has been included.

An additional laboratory study named "Safety of the vaccine Fatrovax RHD administered with a single dose and a repeated dose in dwarf rabbits" was performed in the Experimental Laboratory Animal Facility of Fatro S.p.A.

A total of fifteen 28-day old Netherland dwarf rabbits free of antibodies against RHDV1 and RHDV2 were enrolled in the study. Ten of them were vaccinated with a single and a repeatedly administered dose of Fatrovax RHD, whereas five subjects of the same age were kept as non-vaccinated control and treated with a placebo.

For the single dose administration, a dose of 0.5 ml of a vaccine batch formulated with a double amount of active substances (and double amount of process-related impurities) was used to verify the safety of the vaccine. The control group received 0.5 ml of PBS.

Scoring of general clinical signs has been provided: dyspnoea, diarrhoea, apathy and anorexia were monitored (normal, mild, moderate and severe).

Scoring of injection site reactions has been provided: redness, swelling, pain and nodules were monitored.

Clinical observations, body temperature recording, weight measurements and local reactions evaluation were performed in the 26 days following vaccination. Neither the body weight gains nor the body temperatures (being in full compliance with Ph. Eur. monograph 2325 requirements) presented statistically significant differences between both groups. Following the administration of the first dose at 28 days of age, a small painless nodule of maximum 5.2 mm diameter was observed at the injection site in two of the 10 vaccinated dwarf rabbits and disappeared by day 11–13 after vaccination.

Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines. Nevertheless, according to the results obtained in a safety study with the use of a batch formulated with a double dose of antigens, the following sentence has been included for section 4.10 of the SPC:

"In dwarf rabbits, small transient nodules at the injection site were commonly noted after administration of a 2X dose that completely disappeared in the first two weeks."

Safety of the repeated administration of one dose

The vaccination schedule of Fatrovax RHD consists of one single dose of 0.5 ml administered by the subcutaneous (SC) route to rabbits from the age of 28 days. Hence, it should not be necessary to assess the safety of the administration of a repeated dose for this vaccine (and considering additionally that the product in question has been granted MUMS status).

Nevertheless, the applicant presented a study to evaluate the safety of a repeated dose administration of the vaccine Fatrovax RHD and to test the safety of the vaccine in pregnant does, in addition to the laboratory study in dwarf rabbits.

In this study, a total of sixty New Zealand White pregnant does were enrolled. They were distributed into four groups.

In this study, a pilot scale batch of the vaccine (5 µg RHDV1 and 5 µg RHDV2/0.5 ml) was used in agreement with the reduction in requirements for MUMS-classified products stated in guideline EMA/CVMP/IWP/123243/2006 (no maximum potency requirement).

In relation to the safety of the repeatedly administered dose only animals from groups C and D were used (group C for vaccinated animals and group D as control). Both groups consisted of 15 does each in the second phase of gestation (22 days of pregnancy). After animal selection, the veterinarian weighed all the animals and performed a physical examination. Vaccination was performed subcutaneously in the neck region for both inoculations. No incidents were recorded while vaccinating/inoculating the rabbits.

Evaluation of general clinical signs and local reactions on daily basis of groups C and D was carried out after the first inoculation, but measurement of rectal temperature was not performed for animal welfare reasons, i.e. to reduce stress by handling and manipulation of test subjects as much as possible. Re-vaccination was performed 6 weeks after the administration of the first dose, in order to not interfere with the maternity period.

After the second inoculation (revaccination for group C), animals were monitored again: observation of general clinical signs and local reactions on daily basis for fourteen days, as well as measurement of rectal temperature at time of vaccination and 4, 24, 48, 72 and 96 hours after vaccination.

Results showed that no statistically significant differences were observed between control and vaccinated groups regarding systemic reactions when a dose of the vaccine was administered repeatedly.

In two of the 15 does receiving repeated vaccination, a nodule of less than 1 cm was found in the subcutis at necropsy performed 2 weeks after the second vaccination, although these nodules were not detected during *in vivo* palpation of the injection site.

In the laboratory study carried out in Netherland dwarf rabbits, a repeated dose of the vaccine, 26 days after the first treatment (vaccine batch formulated with a double dose of active substances), this time with 0.5 ml of a standard formulation , was administered to group A and a repeated dose of the placebo solution to group B. Administration was performed subcutaneously on the opposite side of the neck region to which the single dose was administered. None of the animals died or presented clinical signs after the second vaccination. No local reactions at the injection site were recorded in the fourteen days following administration.

The maximum and mean recorded increases in body temperatures in the vaccinated subjects were lower than the limits stated in Ph. Eur. monograph 2325. No statistically significant differences were recorded in body weight gains between the two groups.

Examination of reproductive performance

The safety of the reproductive performance was investigated in a study where one dose of a pilot scale batch of the vaccine (5 µg RHDV1 and 5 µg RHDV2/0.5 ml) was administered by the recommended subcutaneous route to female animals of the target species in the first half of gestation (vaccination at 10 days of pregnancy) and in the second half of gestation (vaccination at 22 days of pregnancy). Safety during pregnancy was tested by vaccinating animals and monitoring health status and reproductive parameters in does until eight days after giving birth. At each phase of gestation tested, a control group was included to compare the results with the vaccinated group.

General clinical signs and local reactions were evaluated on a daily basis for all the groups after inoculation of the vaccine.

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 could be identified after fourteen days of vaccination.

Pregnancy length of all groups was in the normal range.

In relation to the litter characteristics, the number of animals born was similar in all test groups.

Weight of the litters at birth and growth after one week of birth was also recorded as a health indicator of the offspring of each study group.

On the basis of these results, no adverse effects concerning reproductive function neither in females nor on the health status of the offspring attributable to the vaccine were detected. The SPC has therefore been updated as follows:

"Can be used during pregnancy."

The safety of reproductive performance in male rabbits was not evaluated and this is stated in section 4.5 of the SPC.

Examination of immunological functions

No further studies were conducted to investigate the effects of the product on immunological functions.

Section 4.4. of the SPC reflects adequately the lack of studies in relation to the interference of MDA in vaccinated animals.

User safety

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

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

The active substances are inactivated proteins and are not infectious. The vaccine is intended to be administered by healthcare professionals and is not a cause for concern to the user.

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

As a result of the user safety assessment, the following advice to users/warnings for the user are considered appropriate:

"In case of accidental self-injection, seek medical advice immediately and show the package leaflet or the label to the physician."

Study of residues

Since the active ingredients are substances of biological origin intended to produce active immunity, they do not fall within the scope of Regulation (EC) 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin.

The excipients, including adjuvants, listed in section 6.1 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.

The consumption of products derived from rabbits vaccinated with Fatrovax RHD does therefore not present a risk for human health.

Consequently, a withdrawal period of zero days is justified.

Interactions

The applicant has not provided data investigating interactions of the vaccine with other veterinary immunological products and therefore proposed to include the following statement in section 4.8 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".

Field studies

According to the guideline EMA/CVMP/IWP/123243/2006-Rev.3, field studies might not be performed, as long as the laboratory safety studies, as well as safety information collected during the laboratory efficacy studies, supply clear evidence of the absence of a significant target animal safety risk. Nevertheless, the applicant included a safety study for dwarf rabbits that was developed in field conditions. The final report of the study was provided by the applicant to demonstrate the safety of the vaccine when administered to pets.

Dwarf rabbits are pets and the study was carried out at owners' houses.

A production standard batch (5 µg RHDV1 and 5 µg RHDV2/0.5 ml) used in accordance with the reduction in requirements for MUMS-classified products stated in the guideline EMA/CVMP/IWP/123243/2006 (no maximum potency requirement).

Safety in pets was tested by monitoring occurrence of clinical symptoms, local reactions at the injection site and measurement of rectal temperature for four days.

Clinical observations were performed on alternate days until the end of the entire study period, which was established to be 28 days after the administration of the vaccine.

In case of any deviation, clinical observations were performed on a daily basis until normalisation.

The clinical symptoms and local reactions scoring system and the individual raw data were provided by the applicant. The clinical examinations were performed by the veterinarian performing the study. Examinations were performed at the animal owners' residences to lower the stress for the animals due to transportation to veterinary clinics.

One of the animals died on the second day of the study due to a urinary tract occlusion. Another animal showed anorexia and prostration over three days. This unspecific symptom may be a cause of stress induced by the handling during clinical examination.

With regard to local reactions at the injection site, in one of the 15 animals, a mild skin reaction was recorded. In none of the animals a temperature increase of ≥ 2 °C was seen, and the average increase in body temperature for all animals did not exceed 1.5 °C. This is in accordance with the safety testing as described in Ph. Eur. monograph 2325 ("Rabbit Haemorrhagic Disease Vaccine [Inactivated])" for evaluation of safety study results.

This study is complementary to the laboratory study carried out in Netherland dwarf rabbits to demonstrate the safety of the administration of a single dose and the safety of the administration of a repeated dose in pet rabbits.

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 medicinal product must be accompanied by an environmental risk assessment (ERA). 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 immunological veterinary medicinal product that the environmental risk of each of the components is assessed.

Considerations for the environmental risk assessment

Fatrovax RHD is a suspension for injection containing recombinant proteins as active substances, with rabbit as the only target species. The vaccine is intended to be administered by the subcutaneous route to rabbits from the age of 28 days. As Fatrovax RHD contains no live organisms or agents capable of replicating within the host, the probability of causing any negative impact to the environment is negligible. There is no capacity of live organisms to be transmitted to non-target species.

Based on the data provided for the ERA, a higher tier assessment is not necessary. Fatrovax RHD is not expected to pose a risk for the environment when used according to the SPC.

Controls to determine the absence of detergents used during manufacture and controls for the detection of proteins from the baculovirus or *pupae* different to the active substances in the final product have been performed.

Overall conclusions on the safety documentation

The applicant has provided four pivotal laboratory studies to investigate the safety of one dose, repeated administration of one dose to the target animal species in animals of the minimum recommended age via the recommended route and safety of the reproductive performance. Batches used in these studies were pilot scale batches.

On the basis of the results provided, it was concluded that the safety of the target animals, when the vaccine is administered according to the recommended schedule and via the recommended route, is acceptable.

Reproductive safety was investigated. The product was found to be safe when used in pregnant animals at the first and second trimester of gestation. The SPC has been amended accordingly.

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 provided data on administration of an overdose in dwarf rabbits. Section 4.10 of the SPC includes the following information: "In dwarf rabbits, small transient nodules at the injection site were commonly noted after administration of a 2X dose that completely disappeared in the first two weeks." The data presented are considered adequate to characterise the safety profile of Fatrovax RHD as acceptable. 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 is considered low and acceptable when used in accordance with the SPC.

The worst-case scenario for user safety is considered to be accidental self-injection. Appropriate safety advice/warning statements are included in the SPC to mitigate the risks.

An environmental risk assessment was provided. According to the applicant, the product is not expected to pose a risk for the environment when used according to the SPC. Controls to determine the absence of detergents used during manufacture and controls for the detection of proteins from the baculovirus or *pupae* different to the active substances in the final product have been performed.

Part 4 – Efficacy

Introduction and general requirements

The vaccine is intended for active immunisation of rabbits from the age of 28 days to reduce mortality, infection, clinical signs and organ lesions due to rabbit haemorrhagic disease caused by RHDV1 and RHDV2. Immunity is intended to be established 1 week after a single injection and the immunity was observed to last 12 months.

Efficacy was demonstrated in compliance with the European Directive 2001/82/EC (as amended by Directive 2004/28/EC and Directive 2009/9/EC), and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7. as well as Ph. Eur. monograph 2325 Rabbit haemorrhagic disease vaccine (inactivated).

Challenge model

The challenge model was considered adequately validated and therefore appropriate for use in the efficacy trials in order to mimic the natural conditions for infection.

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

Characterisation of the challenge strains is included.

Efficacy parameters and tests

The efficacy parameters, as chosen by the applicant and investigated in the efficacy studies, are: virus neutralising antibody titres, the viral load, clinical signs and mortality. The tests performed to evaluate them were ELISA test, immunohistochemical and immune-chromatographic detection of antigens and specific qRT-PCR analyses. The parameters chosen are considered appropriate for evaluating the efficacy of the product. Validation studies were presented to confirm that the tests chosen are adequately validated to provide reliable results.

Efficacy documentation

Eight laboratory trials were conducted to investigate the efficacy of the product. Laboratory studies were well documented and carried out in rabbits of the minimum age recommended for vaccination, using pilot and production batches.

Study title
Determination of the efficacious dose against RHDV2
Determination of the efficacious dose against RHDV
Efficacy trial against RHDV2
Evaluation of safety and efficacy of administration of a single dose against challenge with RHDV
Validation of two challenge strains of Rabbit Haemorrhagic disease virus and efficacy study of the interference of passive immunity on vaccination in rabbits born from vaccinated does
Efficacy study for the evaluation of the 6-month duration of immunity
Study on duration of immunity based on serological response
Efficacy study for the evaluation of the 12-months duration of immunity

Laboratory trials

Dose determination

Two laboratory studies were conducted to determine the efficacious dose of each of the two active substances contained in the vaccine by performing challenge infections with RHDV1 and RHDV2; in the same studies two routes of administration (subcutaneous and intradermal) were also evaluated.

R&D batches were used in explorative studies to determine the antigen doses needed for efficacious protection against challenge by RHDV1 and RHDV2. As these batches were used in the initial stage of product development, potency values are not available because the potency test was developed at a later stage.

In the validation report of the batch potency test, all vaccine batches produced to be used for pivotal efficacy studies and the pilot batches used for production process validation were potency-tested in mice and individual serological responses of the 10 animals of each group were determined.

Onset of immunity

Two studies were carried out in New Zealand White rabbits of 30 days of age in compliance with Ph. Eur. monograph 2325 requirements to investigate the onset of protection, for the recommended administration route. For each of the antigens a study has been conducted: RHDV1 and RHDV2.

As the eventually chosen administration route is the subcutaneous route, the results for the vaccinated group via intradermal route are not further discussed. The efficacy against challenge infection with RHDV2 was determined in one of the studies where two groups of 12 animals each of 30 days of age were used. A vaccine dose of 5 µg RHDV1 VP1a + 5 µg RHDV2 VP1ab from production-scale batch was administered to the vaccinated group by the subcutaneous route. Another group received a placebo by intradermal route.

The group vaccinated subcutaneously, and the unvaccinated group were challenged with 100 x LD_{50} (Lethal Dose 50%) of virulent RHDV2 (strain Gal08/13) administered subcutaneously one week after vaccination. Following challenge, animals were investigated for survival rates, seroconversion levels and presence of genome and RHDV antigen in vaccinated animals.

Survival rate of the vaccinated group was 100%. Survival rate for the control group was 0%.

No detectable antibodies were found in animal sera taken at day 0 or day 7 post-vaccination.

The quantitative PCR analysis conducted on liver samples from dead and euthanised rabbits, confirmed the presence of very high amounts (10^{11} copies/g) of RHDV2 genome in all control subjects, while samples from vaccinated rabbits were all negative. It was concluded that vaccination by the recommended route with the recommended dose as outlined in the SPC was efficacious and met efficacy requirements one week post-vaccination.

The efficacy against challenge infection with RHDV1 was determined in a second study, where thirty-four New Zealand White rabbits were used. Ten animals in the control group (group 3) and two groups of 12 animals (groups 1 and 2) of 30 days of age were vaccinated. A vaccine dose of 5 µg RHDV1 VP1a + 5 µg RHDV2 VP1ab per dose from pilot-scale batch was administered to groups 1 and 2 by the subcutaneous route. Animals from group 1 were vaccinated at 30 days of age. Animals from group 2 were vaccinated at 51 days of age. Placebo was administered to group 3. The vaccinated groups and the unvaccinated group were challenged with 16,000 hemagglutination units (HAU) of virulent RHDV1 (strain Ast89) administered subcutaneously four weeks after the vaccination in group 1 and one week after the vaccination in group 2. Following challenge, animals were investigated for morbidity and

mortality, seroconversion levels and presence of genome and RHDV antigen in vaccinated animals.

Survival rate of group 1 was 100%. Survival rate for group 2 was 91.6%. Survival rate for the control group was 10%.

All rabbits were negative for anti-RHDV antibodies prior to vaccination. Blood samples were collected from all animals at days 21, 28, and 42 (14 days post-challenge). At 21 days post-vaccination, six rabbits (50%) in group 1 were positive for anti-RHDV antibodies. All vaccinated rabbits in group 2 were still seronegative at the time of challenge infection (7 days post-vaccination), but promptly seroconverted after challenge. No detectable antibodies were found in animals from group 3 (control) prior to challenge.

The PCR analysis performed on the liver samples of dead control rabbits showed very high concentration of RHDV genome (10^{12}-10^{13} copies/g); the surviving control rabbit, euthanised fourteen days after challenge, had a 1,000 times lower load (10^{10} copies/g).

All subjects in group 1 survived the RHDV challenge and no viral genome was detected in their livers 14 days after challenge.

Vaccinated group 2 presented a survival rate of 91.6% (11 survivors out of 12 subjects in the group). Seven among the eleven survivors had RHDV genome content in the liver ranging from 10^8 to 10^9 copies per gram of examined sample; in four subjects RHDV genome was not detected, while the rabbit which died 72 hours post-challenge showed a viral load similar to control subjects (10^{12} copies/g).

It was concluded that vaccination by the recommended route with the recommended dose as outlined in the SPC was efficacious and met efficacy requirements. From these results, the OOI one week post-vaccination can be accepted to reduce mortality and infection for both types of the virus.

Duration of immunity

Three different laboratory efficacy studies have been performed to evaluate the duration of immunity (DOI) conferred by a single vaccination with Fatrovax RHD against challenge with RHDV1 and RHDV2:

In the "Efficacy study for the evaluation of the 6-month duration of immunity", forty-six (46) unvaccinated New Zealand White rabbits of 28-29 days of age, born to unvaccinated does were used, subdivided at random into two groups: one consisting of 30 rabbits (group V, intended for vaccination) and the other one, consisting of 16 rabbits (group C, acting as control group), respectively. A vaccine dose of 5.0 µg RHDV1 VP1a+0.5 µg RHDV2 VP1ab per dose from a production batch was administered to group V by the recommended route. Group C was unvaccinated.

Around six months after vaccination (day 173), 20 subjects from group V were randomly selected from the 30 subjects available and assigned 10 to group V1 and 10 to Group V2, while 10 subjects from group C were randomly selected from the 12 available and assigned 5 to group C1 and 5 to group C2.

Animals in groups C2 and V2 were blood sampled before challenge. All control rabbits were still seronegative for both RHDV1 and RHDV2 antibodies at the time of challenge infection. In group V2, one rabbit was seronegative for both RHDV1 and RHDV2 antibodies. All the remaining rabbits were still seropositive against RHDV2. All the animals were challenged with virulent RHDV2 strain Ve/2019, 6 months after vaccination.

Animals in groups V1 and C1 were blood sampled on day 192 of the trial and infected by intramuscular injection in the thigh with 0.5 ml of homogenised liver suspension of the RHDV strain Te5/88.

All rabbits in control group C2 died within 48 hours from infection. In the vaccinated group, one rabbit showed apathy and anorexia; it died 36 hours post-challenge.

All rabbits in control group C1 died within 48 hours from infection without showing any clinical sign

apart from mild anorexia. In the vaccinated group, all subjects survived without showing any notable clinical sign, thus satisfying the requirements in Ph. Eur. monograph 2325.

Conclusion: In this study 6 months post-vaccination, significant difference in protection was demonstrated between vaccinated groups and controls, supporting sufficiently the proposed duration of immunity.

In the laboratory study "Fatrovax RHD – Study in duration of immunity based on serological response", twenty-one healthy New Zealand White rabbits of 28-30 days of age, born to unvaccinated does and seronegative to both RHDV1 and RHDV2, received one dose (0.5 ml) of vaccine subcutaneously. Three Fatrovax RHD batches were used for immunisation of seven rabbits per batch. The composition was the same for all the batches: 5.0 µg RHDV1 VP1a+0.5 µg RHDV2 VP1ab.

Two additional healthy seronegative rabbits were kept as sentinels for the entire duration of the study.

The kinetics of both RHDV1 and RHDV2 antibodies were shown to follow the same pattern, even with constantly lower titres for RHDV2, with a slow increase after vaccination and a very slow decrease during the 12 months of observation. Although the presence of antibodies post-vaccination is indicative of an immune response, no correlation has been established between the ELISA titres and protection neither for RHDV1 nor for RHDV2.

To demonstrate the 12 months DOI by means of a challenge, a supplementary study was performed. Two distinct experiments, one for each challenge, were conducted, following the same protocol, identified as Experiment 1 (challenge with new variant RHDV2) and Experiment 2 (challenge with classic RHDV1).

The Experiment 1 (challenge with the new variant RHDV2) involved the use of twenty-two (22) unvaccinated NZW rabbits of 28-30 days of age, born to unvaccinated does. They were subdivided at random into two groups of 14 (group A intended for vaccination) and 8 subjects (group KA acting as control group), respectively. Seronegativity of all subjects was ascertained before enrolment in the study. Each rabbit in Group A received one dose (0.5 ml) of Fatrovax RHD S.C.

Group KA was maintained as unvaccinated control group.

In the time interval between vaccination and challenge, rabbits were observed daily to record systemic clinical signs and local reactions at the injection site attributable to vaccination.

During this period, three rabbits died for different causes not related to vaccination (urethral obstruction, gastroenteritis).

On D180 blood samples were taken from both groups: the serological analysis performed six months after vaccination highlighted the persistence of both anti-RHDV1 and anti-RHDV2 antibodies in the vaccinated rabbits.

At D358, 15 subjects were randomly selected (10 vaccinated + 5 controls) and blood sampling was carried out. The serological analyses demonstrated the seronegativity of control subjects at the time of challenge, while confirmed the presence of specific antibodies though at low titres, in the vaccinated rabbits.

On D359 animals were challenged with RHDV2 (intramuscular injection in the thigh with 0.5 ml (strain Ve/2019) The inoculum for each rabbit contained 0.0047 ng of strain genome (corresponding to $4.713x10^7$ copies/0.5 ml).

On the following fourteen days, the animals were examined every 12 h, to record mortality and the appearance of any clinical signs:

- All rabbits in control group KA died within 96 hours from infection. Lethargy, fever, apathy and in

one subject neurological symptoms (paddling and crying) were observed. The data fulfil the requirements in Ph. Eur. monograph 2325 for a valid challenge (at least 80% of control subjects dead within 120 hours).

- In the vaccinated group, two rabbits showed clinical symptoms. One rabbit showed only fever (40.7 °C) on day 2 post-challenge for a day, while another rabbit showed anorexia from the third day post challenge; it died 7 days post-challenge. At necropsy, a pulmonary oedema was found. At the end of the observation period, all survivors were blood sampled and euthanised to analyse organ lesions and collect liver samples for quantitative RT-PCR for the detection of RHDV2 genome. The same analysis was also carried out on the liver samples taken from rabbits that died during the observation period.

On D373 blood samples from survivors and euthanasia: a sharp increase in antibody titres was detected due to anamnestic response (titres >160 ELISA Units in all subjects) for both RHDV2 and RHDV1. Quantitative PCR on liver samples showed genome load in control subjects ranging from 10^7 to 10^8 copies/g of liver. No viral genome was found in the liver samples of any vaccinated subjects, including the one that died. For this rabbit, PCR was performed also on lung and spleen, in both cases with no genome detected.

The study demonstrated that protective immunity against RHDV2 is still present 12 months after a single dose of Fatrovax RHD at a level that fulfils Ph. Eur. requirements (survival rate in vaccinated subjects $\geqslant$90%).

The Experiment 2 (challenge with classic RHDV1) involved the use of twenty-two (22) unvaccinated NZW rabbits of 28-30 days of age, born to unvaccinated does, subdivided at random into two groups of 14 (group B intended for vaccination) and 8 rabbits (group BK acting as control group), respectively. Seronegativity of all subjects was ascertained before enrolment in the study. Each rabbit in group B received one dose (0.5 ml) of Fatrovax RHD S.C.

Group KB was maintained as unvaccinated control group.

In the time interval between vaccination and challenge, rabbits were observed daily to record systemic clinical symptoms and local reactions at the injection site attributable to vaccination. During this period, two rabbits died of different causes not related with vaccination (enteritis).

On D180 blood samples were taken from both groups: The serological analysis performed six months after vaccination highlighted the persistence of both anti-RHDV1 and anti-RHDV2 antibodies in the vaccinated rabbits.

On D367, 15 subjects were randomly selected (10 vaccinated + 5 controls) and blood sampling was carried out. The serological analyses demonstrated the seronegativity of the control subjects at the time of challenge, while confirming the presence of antibodies in all the vaccinated rabbits.

On D368 animals were challenged with RHDV1 (intramuscular injection in the thigh with 0.5 ml of homogenised liver suspension of the RHDV strain Te5/88, received from the Istituto Zooprofilattico Sperimentale of Perugia in 2004, containing 0.0022 ng of strain genome (corresponding to $1.912x10^7$ copies/0.5 ml).

In the following fourteen days, the animals were examined every 12 h, to record mortality and the appearance of any clinical signs. All rabbits in control group KB died within 48 hours from infection showing in some subjects, fever, lethargy and anorexia.

In the vaccinated group one subject showed fever (40.1 °C) on day 2 post-challenge for one day. All subjects survived without showing any notable clinical sign.

Challenge infection therefore satisfied the criteria for challenge validity in Ph. Eur. monograph 2325 (at

least 80% of control subjects dead within 120 hours).

On D382, at the end of the observation period, all vaccinated rabbits were blood sampled and euthanised to collect liver samples to perform a quantitative RT-PCR for the detection of RHDV1 genome. The same analysis was also carried out on the liver samples taken from rabbits that died during the observation period.

-In the fourteen days post-challenge, a sharp increase in anti-RHDV1 titres was observed, with only slight increase in anti-RHDV2 titres.

-The quantitative PCR performed on liver samples showed genome load in control subjects ranging from 10^7 to 10^8 copies/g, while viral genome was not detectable in the liver of vaccinated subjects.

The present study demonstrated that protective immunity against RHDV1 is still present 12 months after a single dose of Fatrovax RHD at a level that fulfils Ph. Eur. requirements (survival rate in vaccinated subjects $\geqslant$90%).

DOI 12 months post-vaccination has been demonstrated by means of a challenge, to reduce mortality and infection.

Maternally derived antibodies (MDA)

To evaluate the possible interference of residual passive immunity with an efficient active immunisation, a specific study was performed, entitled: "Validation test of two challenge strains of Rabbit Haemorrhagic Disease Virus and efficacy study for the evaluation of the interference of passive immunity on vaccination in rabbits born from vaccinated does".

The study involved 150 animals, from which 30 subjects were maintained as control groups and 120 were intended for single or double vaccination.

The 30 rabbits of (8 weeks of age) composing the control groups were born to non-vaccinated does; they were later sub-divided into two groups (1 and 2), intended to act as control groups in the challenge infections with RHDV1 and RHDV2, respectively.

The remaining 120 rabbits (4 weeks of age) were divided into four groups of 30 subjects each based on their characteristics, identified as following:

- A and C, born to vaccinated does and therefore expected to have a certain degree of MDA.

- B and D, born to un-vaccinated control does.

At the study start, all subjects were blood sampled and sera were analysed for the determination of the presence/absence of residual maternally derived antibodies in subjects born to vaccinated does or for the confirmation of seronegativity in subjects born to unvaccinated does.

Presence of MDAs was detected in 30% of the subjects born to vaccinated does (groups A and C) at 25-27 days of age, when the samples have been re-analysed using the more sensitive ELISA test from OIE Laboratory of IZSLER.

In the study presented, the vaccination was carried out in 32- to 34-day old animals. To demonstrate the lack of interference of the MDAs, animals at the minimum age recommended for vaccination should be used, i.e. 28 days old.

At the present moment and according to the sera results obtained from animals 25-27 days old in the study, the possible interference of MDAs cannot be excluded at the recommended age for vaccination, and it is therefore reflected in section 4.4 of the SPC.

Interactions

The applicant has not provided data investigating interactions of the vaccine with other veterinary immunological products and therefore proposes to include the following statement in Section 4.8 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.*'

Field trials

No specific field efficacy study and no field efficacy data have been presented by the applicant. As stated in the introduction to part 4, field studies were not conducted, as allowed by EMA/CVMP/IWP/123243/2006-Rev.3: "Guideline on data requirements for immunological veterinary products intended for minor use or minor species (MUMS)/limited market", because data obtained from the laboratory studies adequately show the vaccine's efficacy and are representative of efficacy under field conditions.

Overall conclusion on efficacy

The results from eight laboratory trials show that the product is effective for active immunisation of rabbits from the age of 28 days, at the proposed dose of 0.5 ml. To demonstrate the indication for reducing clinical signs, clinical signs score and raw data were provided. The indication regarding reduction of mortality and reduction of infection has been verified based on the results achieved in the studies for both types of the virus. Monitoring of organ lesions is considered suitable for the proposed indication.

Onset of immunity is established to be 7 days post-vaccination and duration of immunity is demonstrated one year post-vaccination by means of a challenge.

MDA study is not acceptable as MDA were detected in animals born from vaccinated does at the age of 25-27 days. Considering that the minimum age recommended for vaccination is as short as 28 days, the interference of the maternally derived antibodies in the vaccination cannot be excluded, so section 4.4 of the SPC has been adequately amended.

In conclusion, the product has been shown to be efficacious for active immunisation of rabbits from 28 days of age to reduce mortality, infection, clinical signs and organ lesions due to rabbit haemorrhagic disease caused by RHDV1 and RHDV2.

Part 5 – Benefit-risk assessment

Introduction

Fatrovax RHD is an inactivated vaccine to be used for the active immunisation of rabbits to reduce mortality, infection, clinical signs and organ lesions due to rabbit haemorrhagic disease caused by RHDV1 and RHDV2. The active substances are the rabbit haemorrhagic disease virus 1 recombinant capsid protein VP1a and rabbit haemorrhagic disease virus 2 recombinant capsid protein VP1ab. These proteins auto-assemble into virus-like particles (VLPs). Aluminium hydroxide gel has been used as adjuvant and thiomersal has been used as preservative.

The vaccine is produced as a suspension for injection.

Fatrovax RHD contains injection ≥1 relative potency (RP) VP1a of RHDV and ≥1 relative potency (RP) VP1ab of RHDV2 per dose of 0.5 ml.

Benefit assessment

Direct therapeutic benefit

Fatrovax RHD is of value in the treatment of rabbit haemorrhagic disease caused by RHDV1 and RHDV2, which causes high mortality rates in young and adult rabbits.

Well conducted controlled laboratory trials demonstrated that the product is efficacious in decreasing mortality of rabbits when challenged with virulent strains of RHDV1 and RHDV2.

Additional benefits

Fatrovax RHD increases the range of available treatments for a minor species. The active substances are obtained by means of biotechnology using pupae, avoiding the use of rabbits in the production of the vaccine in line with the principles of 3Rs.

Fatrovax RHD is easy to apply by a veterinarian.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried put indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

Fatrovax RHD has been demonstrated to be safe when one dose is administered to 28-day old rabbits by subcutaneous route.

Risk for the user:

The user safety for this product is acceptable when used as recommended and taking into account the safety advice in the SPC.

Risk for the environment:

Fatrovax RHD is a suspension for injection containing recombinant proteins as active substances and having rabbit as the only target species. As it contains no live organisms or agents capable of replicating within the host, the probability of causing any negative impact to the environment is negligible and therefore the active substances contained in the product are not expected to pose any risk to the environment when used as recommended. The potential presence of residual detergents used during the manufacture of Fatrovax RHD is controlled in all batches of finished product.

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 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 rabbits from 28 days of age to prevent mortality and to reduce infection, clinical signs and organ lesions of rabbit haemorrhagic disease (RHD) caused by rabbit haemorrhagic disease virus RHDV and RHDV2."

The CVMP agreed to the following indication: "For active immunisation of rabbits from the age of 28 days to reduce mortality, infection, clinical signs and organ lesions of rabbit haemorrhagic disease caused by RHDV1 and RHDV2".

Information on development, manufacture and control of the active substances and finished product has been presented and it is considered appropriate. 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 Fatrovax RHD is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

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