13 March 2024
EMA/129981/2024
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Divence Tetra (EMEA/V/C/006222/0000)

Vaccine common name: Bovine viral diarrhoea (subunit), bovine parainfluenza virus 3 (inactivated) and bovine respiratory syncytial virus (live) vaccine

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

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Introduction

The applicant Laboratorios Hipra S.A. submitted on 31 January 2023 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Divence Tetra, 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 6 October 2022 as Divence Tetra 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 indications:

Active immunisation of cattle from 10 weeks of age:

- BRSV and PI-3: to reduce virus shedding, hyperthermia, clinical signs and lung lesions.

- BVD: to reduce viremia, hyperthermia and leukopenia caused by BVDV-1 and BVDV-2 and virus shedding caused by BVDV-2.

Vaccinated animals can be differentiated from field virus infected animals due to the lack of the Non-Structural protein 3 (NS3 or p80) by means of commercial diagnostic kits.

Active immunisation of heifers and cows to protect from births of persistently infected calves and transplacental infection of BVDV (type 1 and 2).

Onset of immunity:	3 weeks after completion of the basic vaccination scheme.
	2 days after completion of the basic vaccination scheme for BRSV.
Duration of immunity:	6 months after completion of the basic vaccination scheme.
	1 year after re-vaccination scheme for BVDV-1 and BVDV-2.

However, during the assessment, these have been modified based on the data provided.

The active substances of Divence Tetra are bovine respiratory syncytial virus (BRSV) strain LYM-56 (live attenuated), bovine parainfluenza 3 virus strain SF4 (inactivated), E2 recombinant protein from bovine viral diarrhoea virus type 1 (BVDV-1) and E2 recombinant protein from bovine viral diarrhoea virus type 2 (BVDV-2). The vaccine contains Montanide IMS, as adjuvant. The target species is cattle.

The rapporteur appointed is Jacqueline Poot and the co-rapporteur is Cristina Muñoz Madero.

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

On 13 March 2024, the CVMP adopted an opinion and CVMP assessment report.

On 9 April 2024, the European Commission adopted a Commission Decision granting the marketing authorisation for Divence Tetra.

Scientific advice

The applicant received scientific advice from the CVMP in June 2019. The scientific advice pertained to the quality part of the dossier (including batch potency test).

The advice given was generally followed.

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), has the services of a qualified person responsible for pharmacovigilance, and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

Manufacturing authorisations and inspection status

Active substances

Manufacture, storage and/or distribution of active substances live attenuated bovine respiratory syncytial virus, strain Lym-56; inactivated bovine parainfluenza 3 virus (PI-3), strain SF4; E2 recombinant protein from bovine diarrhoea virus type 1 (BVDV-1) and E2 recombinant protein from bovine diarrhoea virus type 2 (BVDV-2) takes place at Laboratorios Hipra S.A., Amer, Spain.

A GMP declaration for the active substance(s) manufacturing site was provided from the qualified person (QP) at the EU batch release site. The declaration was based on an on-site audit by the manufacturing site responsible for batch release which has taken into consideration the GMP certificate available for the active substance site issued by the competent authority of Spain (AEMPS) following inspection.

Finished product

Manufacture and primary packaging of the finished product takes place at Laboratorios Hipra S.A., Amer, Spain.

The site has a manufacturing authorisation issued on 28-11-2022 by AEMPS.

GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the activities indicated above, has been provided.

Batch release, manufacture of solvent, quality control testing (biological; chemical/physical; Microbiological), primary packaging, secondary packaging and storage and/or distribution takes place at Laboratorios Hipra S.A., Avda. La Selva 135, Amer, Spain.

The site has a manufacturing authorisation issued on 28-11-2022 by AEMPS.

A valid GMP certificate confirming compliance with the principles of GMP is provided. The certificate was issued on 20 December 2022, referencing an inspection on 7 July 2022, by the competent authority of Spain, AEMPS.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file is considered to be in line with legal requirements.

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

Part 2 - Quality

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

Qualitative and quantitative composition

The finished product is presented as a lyophilisate containing live attenuated bovine respiratory syncytial virus (BRSV), strain Lym-56 ($10^{5.2}$ - $10^{6.5}$ $CCID_{50}$), inactivated bovine parainfluenza 3 virus (PI-3), strain SF4 (≥ 206.2 EU), E2 recombinant protein from bovine diarrhoea virus type 1 (BVDV-1) (≥ 31.6 EU) and E2 recombinant protein from bovine diarrhoea virus type 2 (BVDV-2) (≥ 21.0 EU) as active substances at the potency/titre per dose indicated.

Other ingredients are dipotassium phosphate, gelatin, glycine, potassium dihydrogen phosphate, sorbitol and sucrose.

The solvent contains the adjuvant Montanide IMS. Other ingredients of the solvent are disodium phosphate dodecahydrate, potassium chloride, potassium dihydrogen phosphate, sodium chloride and water for injections.

The product is available as lyophilisate in 10 or 50 ml vials containing 5, 10 or 20 doses combined in carboard boxes with 10, 20 or 50 ml vials containing 10, 20 or 40 ml of solvent as described in section 5.4 of the SPC.

The pack sizes are consistent with the dosage regimen and duration of use.

Container and closure system

The vaccine is presented in type I glass vials of 10 and 50 ml, closed with a type I rubber stopper and aluminium cap. The solvent is presented in colourless PET vials of 10, 20 or 50 ml, closed with type I rubber stoppers and aluminium cap.

The containers and closures are in compliance with the pharmacopoeial requirements and their sterilisation is adequate.

Product development

The vaccine is a fall-out of the larger combination product Divence Penta. Many of the studies included in the dossier for Divence Tetra have been performed with the larger combination vaccine, Divence Penta, which is generally acceptable.

Bovine respiratory disease (BRD) is a multifactorial disease associated with infections with BRSV, PI3, IBR and BVDV as well as bacterial agents. BRD is a major cause of mortality and economic loss in cattle worldwide. Divence Tetra contains three of the four viral agents involved in BRD, BoHV-1 is not included in order for the vaccine to be compatible with BoHV-1 free status and eradication programmes.

An explanation and justification for the composition and presentation of the vaccine has been provided. The lyophilised form was chosen in order to achieve stability of the BRSV component of the vaccine. Although inactivated PI3 and recombinant E2 components are non-live, these antigens are well preserved in lyophilised form. Different freeze-drying components were tested in various compositions to achieve an optimal excipient.

The adjuvant, Montanide IMS, is an immunostimulatory compound. It was chosen from a battery of

adjuvants tested in calves together with the antigens in the Divence Penta vaccine. The selected composition was found to have the best capacity to induce humoral and cellular immune responses.

Appropriate justification is given regarding the relevance of the chosen vaccine strains within the EU. The protective efficacy of the BVDV recombinant antigens against various strains within each genotype is justified.

All excipients are well-known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 2 of the SPC.

The justification provided for the choice of potency tests is acceptable. Tests are considered to quantify relevant (intact) epitopes of the inactivated antigens.

From the calculation of the worst-case scenario for antibiotic remnants in the finished product, it can be concluded that there is no risk to the consumer.

Clinical studies were performed using the larger combination vaccine Divence Penta (including BoHV-1 antigen). Divence Tetra is identical in composition compared to Divence Penta except for the absence of the BoHV-1 antigen. The formulation of the Divence Penta batches used during clinical studies is the same as that intended for marketing.

Description of the manufacturing method

The manufacturing process consists of seven main steps: manufacturing of the four active substances, the freeze-drying excipient, the finished product and the solvent.

The manufacturing process established for the four active substances is based on the "seed lot system", as indicated in the general monograph of the Ph. Eur. no. 0062 (Vaccines for veterinary use). This consists of a system of successive batches of the product derived from one Master seed, which is described in the dossier.

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

Once the harvests are obtained, they are concentrated by means of tangential flow filtration step. Then, a downstream process is carried out in order to obtain the final antigens.

BRSV antigen is produced on suitable cells grown on microcarriers in bioreactors. The different steps are well described.

PI-3 virus antigen is cultured on suitable cells in a bioreactor. The different steps are well described. The inactivation of the PI-3 virus has been appropriately validated.

BVDV-1 E2 recombinant protein is produced by using a suitable cell line. A culture is started from working cell seed (WCS) by mixing with culture medium and scaled up. The culture is further scaled up in a bioreactor. The different steps are well described.

BVDV-2 E2 proteins is produced by using a suitable cell line according to the same process as described for BVDV-1 E2 protein.

For the finished product, the 4 antigens are subsequently transferred to the reactor, then freeze-drying excipient is added, medium and finally water for injections (if necessary). The bulk is mixed and filling and freeze drying is performed. Capped vials are kept a 2 °C – 8 °C for a maximum of 30 months.

The components for the solvent (WFI, disodium phosphate dodecahydrate, potassium dihydrogen

phosphate, sodium chloride, potassium chloride) are introduced into a tank. The adjuvant is added once the rest of the substances has been dissolved. The pH is checked and adjusted if necessary. The solution is sterile filtered into a sterile tank. Samples for bioburden testing are taken and the bulk is filter-sterilised again into the filling machine. If necessary, the bulk can be stored.

The process is considered to be a standard manufacturing process.

Major steps of the manufacturing process have been validated with three consecutive production batches of lyophilised and solvent fractions. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible and consistent manner.

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 simethicone, for which USP criteria are applicable in absence of specific European Pharmacopoeia monograph.

Example certificates of analysis (CoA) have been provided for all substances listed and all substances conform to the relevant Ph. Eur. or USP requirements. Where applicable, certificates of suitability and certificates of irradiation have been provided. The nature of the starting materials, controls and treatments applied guarantee sterility of the vaccine and absence of introduction of extraneous agents (EAs).

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

BRSV strain Lym-56

The original strain was isolated from a clinical case of BRD. The BRSV Lym-56 strain MSV and WSV preparation, control and storage are generally adequately presented. The MSV and WSV are manufactured and handled in a seed lot system in line with Ph. Eur. 0062. The WSV is produced from the MSV by passages in cell cultures again meeting the requirements of Ph. Eur. 0062. The origin of the BRSV antigen has been presented.

In-process controls were carried out on the MSV (titre, identity, sterility, mycoplasma testing, extraneous agents testing). The in-process controls carried out on the WSV include titre and sterility.

Description and validation of all methods is provided. Management of extraneous agents was performed in accordance with Ph. Eur. 5.2.5.

A transmissible spongiform encephalopathy (TSE) and extraneous agents risk assessment for BRSV Lym-56 in the MSV is provided. It can be concluded that the material poses no risk for transmission of TSE or extraneous agents.

PI-3 strain SF4

The SF4 strain was isolated from calves with shipping fever. The MSV and WSV preparation, control and storage are adequately presented. The MSV and WSV are manufactured and handled in a seed lot system in line with Ph. Eur. 0062. The WSV is produced from the MSV by passages in cell cultures

again meeting the requirements of Ph. Eur. 0062.

In-process controls were carried out on the MSV (titre, identity, sterility, mycoplasma testing, extraneous agents testing). The in-process controls carried out on the WSV include titre and sterility.

Description and validation of all methods is provided. Management of extraneous agents was performed in accordance with Ph. Eur. 5.2.5.

A TSE and extraneous agents risk assessment for PI-3 strain SF4 in the MSV is provided. It can be concluded that the material poses no risk for transmission of TSE or extraneous agents.

E2 recombinant protein from BVDV type 1

The E2 recombinant protein from BVDV type 1 was obtained by recombinant DNA technology by using a host-vector system. Before describing the Master Seed, the applicant describes the biotechnological process to obtain it.

The Master Seed was obtained from the original cell stock after several passages. The MCS was tested for general microscopy, viability, karyotype, identity, sterility, mycoplasma, genetic stability and endogenous retrovirus. Absence of extraneous agents was properly assessed, in accordance with Ph. Eur. 5.2.5. Test methods and their validations are provided. A risk assessment for tumorigenicity has been provided and can be considered acceptable.

The working cell seed (WCS) was prepared from the MCS after several passages. The WCS is tested for general microscopy, sterility, mycoplasma, genetic stability and extraneous agents.

The applicant described 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.

A TSE and extraneous agents risk assessment is provided. All reagents used were free of animal or human components.

E2 recombinant protein from BVDV type 2

The E2 recombinant protein from BVDV type 2 was obtained by recombinant DNA technology by using a host-vector system. Before describing the Master Seed, the applicant describes the biotechnological process to obtain it.

The Master Seed was obtained from the original cell stock after several passages. The MCS was tested for general microscopy, viability, karyotype, identity, sterility, mycoplasma, genetic stability and endogenous retrovirus. Absence of extraneous agents was properly assessed, in accordance with Ph. Eur. 5.2.5. Test methods and their validations are provided. The risk of tumorigenicity has been assessed and can be considered acceptable.

The working cell seed (WCS) was prepared from the MCS after several passages. The WCS is tested for general microscopy, sterility, mycoplasma, genetic stability and extraneous agents. The applicant described 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.

A TSE and extraneous agents risk assessment is provided. All reagents used were free of animal or human components.

Vero cells

The Vero cell line is controlled by a cell seed system in line with Ph. Eur. 5.2.4 on cell cultures for the production of veterinary vaccines. The history of the cell line in terms of origin, number of passages, media used, storage conditions and preparation are adequately described.

The MCS was tested for general microscopy, karyotype, identification of species, sterility, mycoplasma and endogenous retrovirus. Extraneous agents were assessed in accordance with Ph. Eur. 5.2.5. Test methods and their validations are provided. A risk assessment for tumorigenicity has been provided and can be considered acceptable.

The WCS is tested for general microscopy, viability, sterility, mycoplasma, and extraneous agents.

A TSE and extraneous agents risk assessment for Vero cells is provided. This includes materials used in the obtainment/storage of the MCS. It can be concluded that the material poses no risk for transmission of TSE or EAs.

MDBK cells

Madin-Darby bovine kidney (MDBK) cell line is controlled by a cell seed system in line with Ph. Eur. 5.2.4 on cell cultures for the production of veterinary vaccines. The history of the cell line in terms of origin, number of passages, media used, storage conditions and preparation are adequately described.

The MCS was tested for general microscopy, viability, karyotype, identification of species, sterility, mycoplasma and endogenous retrovirus. Extraneous agents were assessed in accordance with Ph. Eur. 5.2.5. Test methods and their validations are provided. A risk assessment for tumorigenicity has been provided and can be considered acceptable.

WCS is tested for general microscopy, viability, sterility, mycoplasma and extraneous agents.

A TSE and extraneous agents risk assessment for MDBK Cells is provided. This includes materials used in the obtainment/storage of the MCS. It can be concluded that the material poses no risk for transmission of TSE or EAs.

Tryptose phosphate broth

TPB is a buffered dextrose broth used as an ingredient of the culture medium in different production phases. All components are processed using heat at different temperatures (minimum 80 ºC) and different pH conditions.

An extraneous agents risk assessment for TPB was performed in accordance with Ph. Eur. 5.2.5. In conclusion the risk of contamination with extraneous agents is considered negligible. The material is not considered a TSE risk since it contains porcine materials (non-TSE species) and bovine milk fit for human consumption.

Trypsin

Trypsin is an enzyme derived from porcine pancreas used to detach cells. The trypsin is supplied irradiated.

An extraneous agents risk assessment for trypsin was performed concluding that the risk is considered negligible, since it is terminally irradiated. The material is not considered a TSE risk since it contains only porcine materials.

Cytodex 3 surface microcarriers

Cytodex 3 surface microcarriers are support matrices allowing the growth of Vero and MDBK cells in a

bioreactor. A CoA is provided.

Starting materials of non-biological origin

Certificates of analysis are provided for the starting materials of non-biological origin 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 tests performed during the manufacturing process are detailed below for each production phase, manufacturing of individual antigens, finished product and solvent. The methods listed were all appropriately validated and, for Ph. Eur. methods, suitability was shown.

The in-process tests are deemed to be sufficient to control all the critical steps in the manufacturing of BRSV strain Lym-56: sterility and virus titre.

For the PI-3 strain SF4, sterility, Haemagglutination titre, titre, residual live virus, residual thiosulfate, pH and ELISA control tests are performed.

For E2 BVDV type 1 and E2 BVDV type 2, the in-process controls established during the individual production steps in the production process are all described in detail in the dossier. The in-process controls are considered appropriate, and in line with the expectation for a veterinary vaccine, to assure a well-controlled and consistent production process. The following in-process controls are performed: cellular count, bacterial and fungal sterility, bioburden, appearance, purity, identity, antigen quantification and pH.

Based on the results of the testing for residual host DNA, HCP and Denarase, the applicant considers the level of each of these process-related impurities to be very low and proposes to omit routine testing. This is considered adequately justified and acceptable.

Batch-to-batch consistency shows that the in-process controls are well within the acceptance criteria, and they support that the manufacturing process is able to produce batches of consistent quality.

For the freeze-drying excipient, appearance, sterility by membrane filtration, pH and density are tested on each batch.

For the solvent, bioburden is tested prior to filter sterilisation. During filling, the volume is continuously checked by weight.

<u>Results of control tests carried out on 3 consecutive batches</u>

The results of in-process tests for 3 consecutive batches for all of the antigens is provided. For BRSV and PI-3 antigens the test results are compliant and consistent.

For the E2-1 and E2-2 proteins the three batches complied with the requirements. These batches were also tested for residual DNA, HCP and Denarase.

Control tests on the finished product

The proposed finished product tests are generally considered adequately described and validated and appropriate to control essential properties of the product.

1) General characteristics of the finished product

Appearance is tested on each batch of lyophilised fraction. Solubility is tested on each batch of lyophilised fraction. Any observations are noted. Appearance is tested on each batch of solvent bulk and filled product. The pH is tested on each bulk batch of solvent.

2) Identification of the active substance(s)

Antigens are identified in the individual potency tests.

3) Batch titre or potency

The applicant presents a separate document on the rationale for the batch potency test. Here it is highlighted that Ph. Eur. monographs exist for live BRSV (Bovine respiratory syncytial virus vaccine – live, monograph 1177) as well as for inactivated BVDV (Bovine viral diarrhoea vaccine – inactivated, monograph 1952). For PI3 inactivated vaccine, no specific Ph. Eur. monograph is available.

For the live BRSV vaccine strain, the approach for the potency test is clear -virus titration- and in line with the monograph. For the three inactivated antigens (PI3, and E2 recombinant proteins from BVDV type 1 and 2), *in vitro* potency tests were developed. It is noted that an in vivo test is described in monograph 1952 (BVDV inactivated vaccine), however the *in vitro* approach is in accordance with 3Rs principles.

Appropriate replacement strategies are described for the critical reagents (reference antigens) in all three potency tests for inactivated antigens.

PI3, BVDV-1 E2 protein (E2-1) and BVDV E2 protein (E2-2) potency and identity is determined in each batch of lyophilised product by Sandwich ELISA using monoclonal, polyclonal antibodies and a standard. The method was appropriately validated for each inactivated active substance. A replacement strategy for the reference standards and antibodies is in place.

4) Identification and assay of adjuvants

The identity and concentration of Montanide IMS in the solvent is determined on each bulk batch. The method was appropriately validated.

5) Identification and assay of excipient components

No tests are performed. The absence of testing for excipients is considered justified for the lyophilisate, while for the solvent the buffering capacity is considered adequately controlled by the pH determination.

6) Sterility and purity tests

Each batch of lyophilised product and solvent is tested for bacterial and fungal sterility in accordance with Ph. Eur. 2.6.1. Absence of mycoplasma is tested on each batch of lyophilised product, by culture method, in accordance with Ph. Eur. 2.6.7. No tests for extraneous agents are performed since this is not considered necessary based on the risk-based approach following Ph. Eur. 5.2.5.

7) Residual humidity

Residual moisture is tested on each batch of lyophilised fraction by the Karl-Fisher method in accordance with Ph. Eur. requirements.

8) Filling volume

Filling volume is checked on each batch of filled solvent. The contents of a vial are transferred to a graduated cylinder and volume is measured.

Batch-to-batch consistency

Divence Tetra is a fall-out product of Divence Penta vaccine. The components are identical but for the presence of the BoHV-1 antigen in Divence Penta. The two vaccines are reconstituted with the same solvent and the package, the storage conditions and the storage period are the same. According to the Commission Delegated Regulation (EU) 2021/805 of 8 March 2021 amending the annex II to Regulation (EU) 2019/6 of the European Parliament and of the Council, consistency data obtained from combined products may be used for derivative products containing one or more of the same components. In this context, the consistency data provided for Divence Penta vaccine is also provided in the registration dossier of Divence Tetra vaccine in order to support that the quality of the fall-out product is consistent from batch to batch and complies with specifications.

The results of control tests performed on three consecutive production batches of the 5-dose presentation and three consecutive production batches of the 20-dose presentation of Divence Penta are provided in the dossier. These data are considered to adequately bracket the 10-dose presentation. The results of control tests performed on three consecutive production batches of the solvent are also presented. The batches of lyophilised product conformed to all tests. Results were highly consistent across the batches, noting in particular potency and residual moisture. For the solvent, the batches conformed to all tests and results of adjuvant content testing were consistent. The results are indicative of a well-controlled manufacturing process.

Stability

Stability of pre-inoculum and inoculum

The stability of the inoculum of BRSV antigen and the inoculum of PI-3 was demonstrated by data. Claimed shelf lives are considered acceptable.

Stability of the bulk antigens

The proposed storage period for PI-3 antigen is 24 months at 2 °C - 8 °C and it has been demonstrated with the satisfactory results of three batches manufactured according to the method described in part 2B of the dossier.

The proposed storage period for BVDV-1 E2 antigen and for BVDV-2 E2 antigen is 24 months at 2 °C - 8 °C and it has been demonstrated with the satisfactory results of three batches of each E2 protein manufactured according to the method described in part 2B of the dossier.

For BRSV antigen, the proposed intermediate storage of 48 hours at 2-8°C is justified.

Stability of freeze-drying excipient

The proposed storage period for the freeze-drying excipient is 12 months at 15 °C - 25 °C and it has been demonstrated with the satisfactory results of three batches manufactured according to the method described in part 2B of the dossier.

Stability of the finished product

Three batches each of the 5-dose and 20-dose presentation were put on a long-term stability study. Batches were stored at 2 °C – 8 °C for 33 months.

All finished product tests were performed at regular intervals.

For the 5-dose batches, data are provided for up to 33 months (two batches) and 18 months (one batch) while for the 20-dose batches, data are provided for up to 33 months (one batch), 27 months (one batch) and 18 months (one batch). The available data show no indication of a trend in any of the parameters, the batches appear stable and remain well within the requirements. The data are considered to support a shelf life of 18 months.

Stability of the finished product lyophilisate after freezing for 6 months at ≤-20 °C was investigated. Two production batches were used, one 5-dose and one 20-dose presentation. All finished product tests were planned to be performed. The batch met all requirements and no remarkable change was observed for any of the parameters. Data at T=-6 and T=0 are provided for the 20-dose batch.

Similarly, stability of the finished product lyophilisate after freezing for 12 months at ≤-20 °C was investigated. The study was set-up as described above, using two 5- and one 20-dose batches. The batches met all requirements.

Data provided support a shelf life of 18 months at 2 °C – 8 °C either with or without prior frozen storage for a maximum of 12 months.

Three consecutive production batches of 10 ml and of 40 ml solvent were put on long-term stability in support of the proposed shelf life of 3 years. An accelerated study was also performed. In the long-term stability study, batches were stored at 2 °C – 8 °C. Samples were taken from each batch at regular intervals. All finished control tests of the solvent were carried out. The results give no indication of a particular trend for any of the parameters. All parameters were within limits at all time points.

For the accelerated stability study, batches were stored at 25 °C ± 2 °C for 6 months, samples were taken from each batch at T=0, 3 and 6. The finished product control tests were checked at each timepoint. The results for three 10 ml batches and three 40 ml batches give no indication of a particular trend for any of the parameters. All parameters were within the limits at all timepoints in the accelerated study.

The data support a shelf life of 3 years at 2 °C – 8 °C for the solvent.

New active substance (NAS) status

The CVMP considers that the active substances included in Divence Tetra are not new active substances given that new active substance status was previously confirmed in the CVMP opinion adopted for the parent product Divence Penta.

Overall conclusions on quality

The quality part of the dossier complies with the Annex to Regulation (EU) 2019/6. General and, where relevant, specific Ph. Eur. monographs have been followed and the data are adequate in support of a consistent and well controlled manufacturing process.

The composition of the product is described in sufficient detail. The development of the product has been adequately described and justified. Justification is given regarding the relevance of the chosen vaccine strains within the EU. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation.

The manufacturing process consists of seven main steps: manufacturing of the four active substances,

the freeze-drying excipient, the finished product and the solvent. The manufacturing process has been described in adequate detail.

Starting materials have been listed and shown to comply with pharmacopoeial or in-house requirements.

Control tests performed during the manufacturing process have been adequately described and appropriately validated. The range of control tests is considered to provide adequate control of the consistency of the manufacturing process and critical points.

Finished product control tests have generally been adequately described and appropriately validated. The range of tests is generally considered to provide adequate control of the quality of the final product with respect to its critical attributes.

Data on stability of the active substances as well as the finished product and solvent have been provided. The results of testing give no clear indication of a reduction in potency or change in the properties of the lyophilisate or the solvent. The data support a shelf life of 18 months for the lyophilisate either with or without prior frozen storage at ≤-20°C for a maximum of 12 months. A shelf life of 3 years at +2 °C to +8 °C is supported for the solvent.

Part 3 – Safety documentation (safety and residues tests)

General requirements

The active substances of Divence Tetra are live attenuated bovine respiratory syncytial virus (BRSV), strain Lym-56; inactivated bovine parainfluenza 3 virus (PI-3), strain SF4; E2 recombinant protein from bovine diarrhoea virus type 1 (BVDV-1) and E2 recombinant protein from bovine diarrhoea virus type 2 (BVDV-2). The applicant states that the BRSV antigen is already part of a centrally authorised vaccine, whereas the PI3 antigen is part of nationally authorised vaccines. The lyophilisate fraction includes all the antigens together with a well-known freeze-drying excipient intended to provide cryoprotection as well as a stability to the antigens. The solvent contains PBS and the adjuvant (Montanide IMS).

The vaccine is intended for the active immunisation of cattle from 10 weeks of age. The recommended vaccination programme includes a basic vaccination scheme, which consists of the administration of two intramuscular injections (2 ml each) with an interval of 3 weeks. Re-vaccination is recommended at an interval not longer than 6 months after completion of the basic vaccination scheme by the administration of a single intramuscular dose. Afterwards, subsequent re-vaccinations are recommended at an interval not longer than 12 months.

A full safety file in accordance with Article 8(1)(b) of Regulation (EU) 2019/6 has been provided.

Safety documentation

Six safety studies were conducted to investigate the safety of the product and included 4 pre-clinical studies investigating the safety of the administration of a 10-fold overdose and repeated dose, reproductive safety and 2 clinical trials. Studies applicable to live vaccines were conducted to investigate the dissemination of a single dose of the BRSV vaccine strain, the spread from vaccinated animals to non-vaccinated contacts and reversion to virulence. For these latter studies, the vaccine strain was administered intranasally instead of via the intramuscular route as recommended which is considered acceptable since it is the natural route of infection and more likely to result in spread. Pre-clinical studies were reported to be GLP compliant and carried out in target animals at or below the minimum age recommended for vaccination, using pilot batches of Divence Penta vaccine. Production

batches of Divence Penta were used in the clinical trials, performed under GCP.

The requirements for safety testing of Ph. Eur. chapter 5.2.6 "Evaluation of safety of veterinary vaccines and immunosera" and the specific monograph no. 1952 "Bovine viral diarrhoea vaccine (inactivated) and monograph no. 1177 "Bovine respiratory syncytial virus vaccine" have been taken into account to demonstrate the safety of this vaccine. VICH target animal safety guidelines (GL 41 and GL 44) have also been taken into account.

Pre-clinical studies

Safety of the administration of one dose

No studies on the safety of one dose were performed since this is considered to be covered by the study of the safety of an overdose. Adverse events observed after the application of an overdose of the vaccine are listed in section 3.6 of the SPC.

Safety of one administration of an overdose

A randomised, blinded, controlled study was performed in calves of two months of age that were free of antibodies to BVDV, BoHV-1 and BRSV and had no or low levels of antibodies to PI3. The study was performed to investigate the safety of an overdose and of a repeated dose. Divence Penta, containing the same antigens as Divence Tetra plus BoHV-1 strain CEDDEL, was used in the study. This is acceptable since the larger combination vaccine can be regarded as a worst-case scenario for safety. The test group (n=8) received a 10-fold dose of Divence Penta in a volume of 20 ml divided over 4 injection sites. This is acceptable considering the vaccine contains both live and inactivated antigens and since the injection volume per site exceeds the standard volume of 2 ml. At 14-day intervals, three further single doses of Divence Penta were applied at alternating sites. The control group received injections with PBS at the same time, in the same volume and locations.

Calves were observed for clinical signs and local reactions for the duration of the study. Rectal temperature was measured after vaccination at different timepoints. In case of rectal temperatures over 39.5 °C at day 7 post-vaccination (p.v.), temperature measurement continued daily until the temperature decreased to below 39.5 °C. Injection site reactions were observed daily for 14 days following 1st, 2nd and 3rd administrations and 21 days following the 4th administration.

No generalised clinical signs were observed in any of the animals after vaccination. The body temperature of control animals remained at baseline level throughout the study. In the vaccinated group, average temperatures increased with a peak (avg. 40.7 °C, max. 41.1 °C) at day 1 and a gradual decrease to baseline level on day 9 for all animals. After the second vaccination on Day 14, average temperatures increases in the vaccinates were close to 0, with a maximum of 40.3 °C on day 14 +4hrs. After the third vaccination on Day 28, average temperature increases in the vaccinates were again close to 0, with a maximum of 40.3 °C on Day 28 +4hrs. After the fourth vaccination on Day 42, rectal temperatures in the vaccinated group increased (in all animals) on Day 43 with a maximum of 40.3 °C. At day 44 temperatures had returned to baseline levels.

No local reactions were observed in the control animals. Local reactions were observed in 5 out of 8 vaccinates, scores up to 3 (max. 10 cm diameter) were recorded. Lesions disappeared completely by day 8. After the second vaccination, local reactions were observed in all vaccinates with a maximum size of 13 cm and a maximum duration of 5 days. After the third vaccination local reactions were observed in all vaccinates, with a maximum size of 7 cm and a maximum duration of 6 days. After the fourth vaccination local reactions were observed in 5 out of 8 vaccinates, with a maximum size of 6 cm and a maximum duration of 6 days.

The adverse events are considered to be acceptable for the type of vaccine and did not appear to affect the overall health of the calves.

Safety of the repeated administration of one dose

Repeated administration of one dose has been investigated in the study summarised above for safety of an overdose. A tenfold maximum dose of Divence Penta was tested, as appropriate for the live components of the vaccine. The study schedule included vaccinations at Day 0 (10-fold overdose), 14, 28 and 42 (one dose). Some adverse events were reported after the repeated administrations. Slight increases in rectal temperature occurred (max. 1.3 °C) for 1 day. Injection site reactions with a swelling up to 13 cm and a duration up to 6 days were observed. No indication of an increase in adverse events with repeated vaccinations was found.

Examination of reproductive performance

A study was performed with the aim to investigate the safety of a basic vaccination (two doses with an interval of 3 weeks) followed by one booster vaccination 6 months later, in pregnant cows. The study was appropriately designed, in accordance with Ph. Eur. 5.2.6 and monograph 1952 (BVDV vaccine, inactivated), and performed in compliance with GLP. A total of 28 pregnant FH and FH-crossbreed cows, free from BVDV-1, BDVD-2, IBR, BRSV, PI3 antibodies or with very low PI3 antibodies and not vaccinated against bovine parainfluenza virus, were included in this study. Divence Penta was used in the study and this is acceptable since the larger combination vaccine can be regarded as a worst-case scenario for safety. A batch of Divence Penta containing antigens at maximum or high potency was used. Nine cows in the third trimester of gestation received two vaccine doses with a 21 days interval; in addition, 9 animals in the second trimester of gestation also received 2 vaccine doses with a 21 days interval and 10 animals in the first trimester of gestation received 3 vaccine doses, two doses with a 21 days interval and one dose 6 months after the basic vaccination schedule. Due to the grouping based on gestation, the study was not blinded. Animals were observed for local reactions (14 days p.v.) and clinical signs (daily), rectal temperature (day -1 to day 7) and adverse effects on pregnancy and offspring (up to three days of age).

No systemic adverse reactions were observed in any of the animals during the study. Local adverse reactions were observed in 5 cows after the first vaccination, with a maximum size of 14 cm diameter and a maximum duration of 16 days. After the second vaccination 9 cows presented local reactions, with a maximum swelling size of 11 cm diameter and a maximum duration of 14 days. No animals presented local reactions after the third vaccination.

Rectal temperatures increased on the day after the first vaccination, to a maximum of 40.9 ºC and returning to baseline levels by day 4. After the second vaccination only slight increases were observed in few animals (up to 39.8 °C) on the day after vaccination, returning to normal two days later. After the third vaccination, a slight increase in temperature occurred in two cows at T=0+4hrs.

One animal in the second group (2nd trimester) had dystocia, with the calf presenting in an abnormal position. The calf was born dead due to hypoxia and showed no clinical abnormalities. A calf born to a cow in the same group was found in the morning with a severe injury to the ribcage, likely due to crushing, but otherwise normal. This calf was euthanised. In the third group (boostered), one cow presented with dystocia, the calf presenting in an abnormal position. The calf was born dead due to hypoxia. The remaining 25 cows gave birth to normal healthy calves.

The study results gave no indication of negative effects of vaccination on the outcome of pregnancy. The components of the vaccine are not expected to negatively affect the development of the reproductive system.

Data from the field study are considered adequate to support the safety for lactating cattle.

Examination of immunological functions

Taking into consideration the nature and composition of the vaccine there is no reason for suspecting an impairment of the immune system under the claimed conditions of use. There are no data suggesting a negative influence on the immune response of the vaccinated animal, in particular for the live BRSV component which is already included in a centrally authorised vaccine. No studies were performed and the absence of specific data is considered justified.

Special requirements for live vaccines

Special requirements for live vaccines are applicable to the BRSV component of the vaccine. It is noted this virus strain is a component of a centrally authorised vaccine. The PI3 and BVDV components of Divence Tetra are inactivated and therefore not discussed in this section.

Spread of the vaccine strain

Spread of the vaccine strain from vaccinated to unvaccinated target animals was investigated in a GLP study. A group of 9 calves, 2 to 14 days of age, was vaccinated by intranasal route with a dose of BRSV strain lym56 strain exceeding the maximum titre. A group of 4 calves and a group of 3 lambs were placed in contact with the vaccinated animals. All animals were followed up for 21 days by daily assessment of clinical signs, respiratory rate and body temperature. Nasal swabs were, faecal, urine and saliva samples were taken at regular intervals. Euthanasia and necropsy was performed on 3 vaccinated animals on day 4, 8 and 12. After vaccination, clinical signs of very mild intensity were observed in in-contact calves. However, molecular and serologic data of sentinel calves indicated that the origin of this mild respiratory process was not the vaccine virus. The virus could only be detected in nasal swabs of 3 out of 9 vaccinated calves on day 4 and in the tracheal epithelium (n=1), bronchial epithelium (n=2) and pharyngeal tonsil (n=1) of vaccinated animals necropsied on day 4. No other swabs or tissue samples were positive and sentinels remained sero-negative. No clinical signs were observed in the group of lambs. The application of the BRSV strain intranasally instead of the recommended intramuscular vaccination route is considered acceptable since intranasal application provides the highest risk of spreading while the presence of the other vaccine components is not expected to significantly affect the BRSV behaviour. The use of very young calves is considered a worst-case scenario. It can be concluded that the study provides adequate evidence of absence of spread of the vaccine strain.

Dissemination in the vaccinated animal

The applicant presents a study performed in the frame of the registration of Nasym (Laboratorios Hipra S.A.), an intranasally applied vaccine containing the BRSV strain Lym56. According to Ph. Eur. 5.2.6, dissemination shall be studied using the route of vaccination most likely to result in spread, whereas intranasal application is not a route of vaccination intended for Divence Tetra, it is the natural site of infection of the virus and may be the most likely route to result in high virus titres and dissemination and is as such considered justified. A group of 9 calves, 2 to 14 days of age that should be justified, was vaccinated by intranasal route with BRSV lym56 (refer also to section on spreading). Nasal, oral, urine and faecal swabs were collected for detection of BRSV in order to study the dissemination of the vaccine. The virus was not detected in saliva, faeces or urine. Indeed, the virus was only detected in the nasal secretion at Day 4 post-vaccination, in 3 of the 9 tested animals. However, the quantity of BRSV RNA in the positive samples was below the quantification limit of the method. Tissue samples were tested for BRSV RNA after necropsy was performed on 3 animals at day

4, 8 and 12. The tracheal epithelium (n=1), bronchial epithelium (n=2) and pharyngeal tonsil (n=1) of animals necropsied on day 4 were BRSV positive (non-quantifiable). No other swabs or tissue samples were positive. The results are considered to support the notion that the virus does not disseminate (or replicate) beyond the upper airways. Since the BRSV virus is not a zoonotic agent, the data presented are considered acceptable.

Increase in virulence of attenuated vaccines

The administration of the BRSV Lym56 strain to 2 calves of the youngest age was performed, as required by Ph. Eur. 5.2.6. The virus was not recovered and the study was repeated in 10 calves. One-week old calves were inoculated with a 4-fold maximum dose of the MSV by intranasal route. Nasal swabs were collected on day 3 to 7 post inoculation and analysed by titration on cell culture. No virus was detected in any of the samples. No calf showed any clinical sign or temperature increase (evaluated daily). Considering that the virus replicates in the upper airways, the intranasal application can be considered a worst-case scenario with respect to reversion to virulence and recovery of virus passages. Albeit a lower than 10-fold maximum titre was applied, it can therefore be concluded that reversion to virulence is highly unlikely to occur in the absence of spreading as found in this study and in the study on spreading.

Biological properties of the vaccine strain

No specific studies have been conducted to determine the intrinsic biological properties of the vaccine strains. BRSV is an enveloped, negative sense, single-stranded RNA virus (pneumovirus). The attenuated strain BRSV Lym-56 was shown to be safe (no adverse reactions), does not spread to in-contact calves (or lambs) and thus has no potential for reversion to virulence. On the basis of the data presented the safety profile of the strain can be considered acceptable, in addition it is noted the BRSV Lym-56 vaccine strains are components of centrally authorised vaccines for the same target species.

Recombination or genomic reassortment of the strains

Regarding the genomic reassortment or recombination/redistribution of the vaccine strain with other strains of BRSV virus, no specific trials have been performed.

The BRSV strain Lym-56 is an attenuated strain, any potential recombination is not expected to increase the virulence to more than the virulence of circulating wild-type strains. Together with the intrinsic characteristics of the recombination events (necessity of closely related parental viruses for successful homologous recombination) and epidemiological reasons (prevalence of different respiratory syncytial virus in different geographical zones, host predilection) it can be concluded that the risk arising from the potential recombination between Lym-56 strain and another respiratory syncytial virus is negligible.

User safety

A user risk assessment performed according to the "Guideline on user safety for immunological veterinary medicinal products" (EMEA/CVMP/IWP/54533/2006) has been provided.

BSRV is not considered a zoonotic agent. The PI-3 component is inactivated and the antigens BVDV-1 E2 and BVDV-2 E2 are non-toxic recombinant proteins that do not pose any risk to the user. Regarding the excipients and the solvent, including the adjuvant, no local or systemic harmful effects have ever been reported, except for the mineral oil component that is known to cause severe pain and swelling particularly if injected into a joint or finger.

The vaccine is presented as a lyophilisate and a solvent for emulsion for injection to be administered

by a veterinary surgeon or under veterinary supervision. Accidental self-injection is considered the most likely route of exposure, although the probability is very low. The probability of exposure as a consequence of accidental breakage of the container is considered to be low and any potential such exposure is deemed to be very short. Deliberate ingestion is considered to be very unlikely.

Except for the mineral oil, no apparent hazard emanating from the product's components has been identified and potential exposure to the vaccine is considered to be very limited; the risk for the user, which would only be a professional or trained personnel under the professional's supervision, is considered to be negligible. Therefore, no measures are considered necessary to reduce the risk of user exposure to the vaccine other than the standard warning sentence for mineral oil- containing products, which is included in the SPC.

Study of residues

MRLs

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

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

The antimicrobial substances used in the manufacturing process are present at low residual levels in the finished product, which is not considered to constitute a risk to the consumer.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

No specific studies have been carried out to investigate the possible interactions of Divence Tetra with other veterinary medicinal products. For this reason, the following recommendation is included in the relevant section of the product information: "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

Two multi-centre, randomised, double-blinded and placebo-controlled clinical trials investigating safety and efficacy have been performed. Divence Penta was applied in these studies which is acceptable as it is considered a worst-case scenario with respect to safety. The first study was performed in calves and the second study in heifers and cows. The studies are summarised in the tables below.

Efficacy and safety assessment under field conditions of Divence Penta vaccine in calves	
Objectives	To evaluate clinical safety and efficacy of the vaccine
Study design	Randomised, blinded, placebo controlled, multicentre study designed to assess superiority of the vaccine over control.

Study sites	Seven farms (feedlots) in Spain, with historical records of respiratory disease and entering batches of 50 calves or more.
Compliance with regulatory guidelines	GCP
Animals	A total of 1,017 calves, 10-12 weeks of age. The animals were obtained from several regions (Czech Republic, Belgium, Germany, France) and were of various breeds (Friesian, Blanc-Bleu, Montbeliarde and Montbeliarde cross-breed). On most farms only male calves were kept, but on one farm only Blanc-Bleu females and on one farm male and female Blanc-Bleu calves were kept. Five hundred and six calves were vaccinated, 511 were treated with placebo, on each farm vaccinated and placebo animals were housed together.
Eligibility criteria	The animals were clinically healthy and not previously vaccinated for BRSV, BVD, IBR and/or PI-3.
Test product	One group received Divence Penta vaccine whereas the other group received the placebo (PBS). The route of administration was the recommended one (intramuscular).
Control product/ Placebo	
Vaccination scheme	Vaccination with 1 dose of 2 ml on Day 0 and Day 21
Safety parameters	Overall safety: recording of adverse events Post-vaccinal safety: 30 calves per group, in 3 different batches from 3 farms were followed closely during the first two days after each vaccination for systemic reactions (scoring), rectal temperature and local reactions at the injection site (scoring).
Statistical method	For all statistical tests, a nominal significance level of 5 % ($p<0.05$) was applied. A descriptive analysis was performed for each variable. For quantitative variables, appropriate tests were used, for qualitative variables appropriate tests for comparison between treatments were used. All analyses were performed including farm as a random factor into the appropriate statistical model.
Results	
Safety parameters	At total of 1017 animals received at least the first vaccination and form the safety dataset: 506 vaccinates, 511 controls. Post-vaccinal safety: 60 calves from 3 farms were closely monitored (10 calves/group/farm). No systemic reactions were reported. Rectal temperature in the vaccinates increased slightly on D1 and was returning to normal on D2 (avg vaccinates D1: 39.5°C, controls: 38.6°C). After the second vaccination, again a slight increase in temperature was observed in the vaccinates, only on D1 (avg vaccinates D1: 39.4°C, controls: 38.9°C). After the first vaccination, local reactions (swelling) were observed in 2 control animals on D1 (and one animal on D2). In the vaccinates, swelling up to 3 cm was observed in 2 and 3-5 cm in 3 calves on D1, decreasing rapidly (within 4 days). After the 2nd dose, no reactions were observed in

	controls, in vaccinates one calf had a swelling of 3-5 cm lasting 1 day. No induration was observed in any calf.
Adverse reactions	Two adverse reactions were observed. One calf experienced an anaphylactic type reaction within 15 minutes after the first application of the vaccine. The calf died and field necropsy revealed acute pulmonary emphysema. One calf after receiving the 2nd dose of vaccine and within 15 minutes presented with loss of balance and prostration. Within 10 minutes the calf started to recover (without treatment), stood up and was totally recovered. (anaphylactic shock in 2 out of 1003 administrations = 0.2%).

Discussion/conclusions further to assessment

The study was appropriately designed and executed to an acceptable standard (GCP). The animals were of the youngest age for vaccination (10-12 weeks of age). The use of a standard/commercial dose is acceptable, the basic vaccination schedule (two applications, three weeks apart) was applied. The general follow-up of animals performed mainly by the farmers and the close follow-up of 30 calves in each group around the days of vaccination revealed no significant safety issues (no clinical signs, no clinically relevant increases in body temperature and no large reactions at the injection site (swelling of max. 5 cm and max. 4 days). Two anaphylactic-type reactions were observed in this study, a warning is included in the SPC section 3.6.

Efficacy and safety assessment under field conditions of Divence Penta vaccine in cattle	
Objectives	To evaluate clinical safety and efficacy of the vaccine
Study design	Randomised, blinded, placebo controlled, multicentre study designed to assess superiority of the vaccine over control.
Study sites	Three dairy farms in Spain and 1 in Hungary.
Compliance with regulatory guidelines	GCP
Animals	A total of 1,255 female HF cattle from 10 weeks of age onward were included. Stratified by (age) category: vaccinated 295 heifers and 336 cows, controls: 296 heifers, 328 cows. At inclusion, around 48.5 % was pregnant, in all stages of pregnancy.
Eligibility criteria	The animals were clinically healthy and not previously vaccinated for BRSV, BVD, IBR and/or PI-3.
Interventions: Vaccine	One group received Divence Penta vaccine whereas the other group received the placebo (PBS). The route of administration was the recommended one (intramuscular).
Control product/ Placebo	
Vaccination scheme	Vaccination with 1 dose of 2ml on Day 0 and Day 21. 3rd dose at 6 months after 2nd dose booster at 12 months after 3rd dose (not yet reported for safety) Follow-up for 24 months total.
Safety parameters	Overall safety: recording of adverse events. Post-vaccinal safety: 24 heifers and 24 cows per group, in 3 farms were

	randomly selected at inclusion and followed closely during the first two days after each vaccination for systemic reactions (scoring), rectal temperature and local reactions at the injection site (scoring). Milk yield was compared between groups for 14 days after each dose in two farms with an automatic daily milk production recording system (40 cows/group, with the highest milk production at the time of vaccination).
Statistical method	Descriptive analysis was performed for each variable.
Results	
Safety parameters	At total of 1255 animals received at least the first vaccination and form the overall safety dataset: 631 vaccinates, 624 controls. Post-vaccinal safety: 8 heifers and 8 cows in each group, in each of 3 farms were closely monitored. No systemic reactions were reported. Rectal temperature in the vaccinates increased slightly on D1 and was returning to normal on D2 (avg vaccinates D1: 39.3°C, controls: 39.1°C). The increase was somewhat higher in heifers (maximum increase 2.16°C from baseline in a vaccinated heifer). After the second and third vaccination, the temperature pattern was very similar to the first administration. After the first vaccination, local reactions (swelling, 3-5 cm) were observed in 12 vaccinated animals on D1 (and 3 animals on D2), none were observed in controls. After the 2nd dose, no reactions were observed in controls or in vaccinates. After the 3rd dose, reactions (up to 3 cm) were observed in 5 controls on the day after vaccination (and in 2 controls on the second day). In the vaccinates, 3 animals had reactions (<3cm) on the first day and one on the second day. No induration was observed in any animal. Milk production was monitored in 20 animals/group/farm on 2 farms. No clinically relevant differences were observed between the groups with respect to average daily milk production for 14 days following the three vaccinations. No pregnancy losses were observed in the vaccinates within the two days follow-up after each vaccination. Pregnancy losses were observed in both groups with the following frequencies: after the 1st dose 0.8% in controls and 1.0% in vaccinates, after the 2nd dose 3.7% in controls and 2.6% in vaccinates, after the 3rd dose 8.5% in controls and 8.4% in vaccinates and after the booster dose 0.2% in controls and 0% in vaccinates.
Adverse events	No adverse reactions were observed.

Discussion

The study was appropriately designed and is performed to an acceptable standard. From the results provided on safety of the vaccine, it can be concluded that the vaccine is generally safe in adult cattle since it did not give rise to general clinical signs and no clinically relevant increases in rectal temperature were observed. The local reactions observed were relatively small (<3 cm) and disappeared within a few days. There was no apparent effect on milk yield after vaccination. The evaluation of reproductive safety was performed by analysing pregnancy losses that occurred within two days after (each) vaccination. No losses were observed in the vaccinated group. The overall outcome of pregnancies was highly similar for the vaccinated and control groups, which give a further indication of

the safety of the vaccine for the pregnant animals.

Environmental risk assessment

An environmental risk assessment was performed in accordance the "Guideline for environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95).

Considerations for the environmental risk assessment

The vaccine contains one live attenuated virus component. The live attenuated BRSV strain Lym56 is also the active substance in Nasym, which was authorised via the centralised procedure on 29 July 2019. The strain is highly attenuated and was shown not to spread from vaccinated calves to in-contact calves or lambs. Regarding the other active substances contained in Divence Tetra, the PI-3 virus component is fully inactivated during the manufacturing process. The E2 recombinant proteins from BVDV-1 and BVDV-2 do not pose a hazard to the environment and are highly purified proteins. In summary, the probability of any of the active substances having a negative impact on the environment is considered negligible.

Apart from the active substances, the rest of vaccine components, i.e. the excipients, including the adjuvant, are well-known ingredients used in numerous vaccines currently authorised. None of the ingredients can be considered as hazardous for the environment. Moreover, the vaccine is administered individually by the intramuscular route, thus, the risk of the product being released into the environment is considered to be negligible.

As the environmental risk associated with the use of Divence Tetra is considered to be very low, no specific mitigation measures are considered necessary in addition to general management recommendations and precautions included in the product information regarding the handling and disposal of unused veterinary medicinal product or waste materials derived from the use of thereof.

Considering the approach outlined in Annex I to the "Guideline for environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95), the risk for the environment when using Divence Tetra can be considered to be effectively zero, based on a low likelihood of hazard occurrence and experience in the use of similar vaccines. Consequently, the environmental risk assessment can stop in Phase I and no Phase II environmental risk assessment is considered necessary.

Divence Tetra is expected to pose a negligible risk to the environment when used as recommended.

Overall conclusions on the safety documentation

The vaccine is intended for the active immunisation of cattle from 10 weeks of age. The recommended vaccination programme includes a basic vaccination scheme, which consists of the administration of two intramuscular injections (2 ml each), the first dose administered to calves from 10 weeks of age, and the second dose three weeks later. Re-vaccination is recommended at an interval not longer than 6 months after completion of the basic vaccination scheme by the administration of a single intramuscular dose. Afterwards, subsequent re-vaccinations are recommended at an interval not longer than 12 months.

A full safety file in accordance with Article 8(1)(b) of Regulation (EU) 2019/6 has been provided.

The use of the larger combination vaccine Divence Penta in the safety studies is accepted since it is considered a worst-case scenario.

No studies on the safety of one dose were performed, this is considered to be covered by the study of the safety of an overdose. A randomised, blinded, controlled study was performed in seronegative calves of two months of age. The calves received a 10-fold overdose of the larger combination vaccine Divence Penta followed by three single maximum doses at 2 week intervals. Calves remained clinically healthy for the duration of the study. Rectal temperatures increased after vaccination with a maximum of 41.1 °C and a gradual decrease to baseline over one week. Local reactions were observed with a maximum diameter of 13 cm and a maximum duration of 6 days. The adverse events are considered to be acceptable for the type of vaccine and did not appear to affect the overall health of the calves. No indication of an increase in adverse events with repeated vaccinations was found.

Reproductive safety of a basic vaccination with the larger combination vaccine Divence Penta followed by one booster vaccination 6 months later, was investigated in seronegative pregnant cows. Cows were vaccinated twice with a vaccine at maximum potency, with a 3-week interval while in the second or third trimester of pregnancy. Animals in the first trimester received the basic vaccination followed by a booster 6 months later. No systemic adverse reactions were observed in any of the animals. Local reactions of up to 14 cm and up to 14 days duration were observed, as were rectal temperature increases to a maximum of 40.9 °C. All cows carried to term. Data from the clinical study are considered to support safety during lactation as well as reproductive safety.

No studies on immunological functions were performed, this is considered justified.

Special requirements for live vaccines are applicable to the BRSV component of the vaccine. It is noted this virus strain is a component of a centrally authorised vaccine. The PI3 and BVDV components of Divence Tetra are inactivated.

For BRSV, spread to in-contact calves and lambs after intranasal application was investigated. This is considered acceptable since this route provides the highest risk of spreading. Calves of a very young age (2 to 14 days) were used, considered to be a worst-case scenario. No virus or serological evidence of infection was detected in unvaccinated in-contact animals. The results support absence of spreading of the BRSV vaccine strain.

Considering the dissemination of the BRSV vaccine strain in the target animals, a study performed with the single BRSV Lym56 vaccine (Nasym) was presented. In this study, calves of up to two weeks of age were vaccinated intranasally with a dose exceeding the maximum titre. Whereas intranasal application is not the route of vaccination envisaged for Divence Tetra, it is the natural site of infection of the virus and may be the most likely route to result in high virus titres and dissemination, and is as such considered justified. The results are considered to support the notion that the virus does not disseminate beyond the upper airways. Since the BRSV virus is not a zoonotic agent, the data presented are considered acceptable.

Reversion to virulence was investigated in accordance with Ph. Eur. requirements. The administration of the BRSV Lym56 strain to 2 calves of the youngest age was performed, however, the virus was not recovered. The study was repeated in 10 calves inoculated with a 4-fold maximum dose of the MSV by intranasal route. No virus was detected in any of the nasal swab samples. Considering that the virus replicates in the upper airways, the intranasal application can be considered a worst-case scenario with respect to reversion to virulence and recovery of virus passages. Albeit a lower than 10-fold maximum titre was applied, it can therefore be concluded that reversion to virulence is highly unlikely to occur since no evidence of spreading was found in this study or in the study on spreading.

No specific studies have been conducted to determine the intrinsic biological properties of the vaccine strains; this is considered acceptable based on the data provided.

Regarding the genomic reassortment or recombination/redistribution of the strains with other strains

of BRSV virus, no specific trials have been performed. The chances of recombination occurring are considered very low. Any potential recombination is not expected to increase the virulence to more than the virulence of circulating wild-type strains.

A user risk assessment performed according to the relevant guideline (EMEA/CVMP/IWP/54533/2006) has been performed. The risk for the user is considered to be negligible, as no hazard was identified and the potential exposure to the vaccine is considered to be very limited, the risk is considered negligible, except for a potential risk to the user posed by the mineral oil component of the adjuvant. An appropriate standard warning is therefore included in section 3.5 of the SPC, which is considered to acceptably address the risk.

The active substances, being a principle of biological origin intended to produce active immunity, are not within the scope of Regulation (EC) No 470/2009. The excipients, including adjuvants, listed in section 2 of the SPC are either allowed substances for which Table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product. The antimicrobial substances used in the manufacturing process are present at low residual levels in the finished product, which is not considered to constitute a risk to the consumer.

The withdrawal period is set at zero days.

No specific studies have been carried out to investigate the possible interactions of Divence Tetra with other veterinary medicinal products. An appropriate warning has been included in the SPC.

Two multi-centre, randomised, double blinded and placebo-controlled clinical trials investigating safety and efficacy have been performed. In the first trial 1017 calves were included, in the second trial 1255 heifers and cows. Vaccination was performed with the larger combination product Divence Penta. The first study was appropriately designed and performed to GCP. Follow-up of the calves revealed no significant safety issues but for the occurrence of two anaphylactic type reactions. Local reactions and rectal temperature increases were comparable to what was found in the pre-clinical studies. From the results of the second field study in heifers and cows, it can be concluded that the vaccine is generally safe in adult cattle since it did not give rise to general clinical signs and no clinically relevant increases in rectal temperature were observed. Local reactions were similar to what was observed in pre-clinical studies. There was no apparent effect on milk yield after vaccination. Reproductive safety is supported by the results of the clinical study.

An environmental risk assessment was performed in accordance with the relevant guidance (EMEA/CVMP/074/95). Divence Tetra is expected to pose a negligible risk to the environment when used as recommended.

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

General requirements

The vaccine is intended for the active immunisation of cattle from 10 weeks of age to reduce:

Virus shedding, hyperthermia, clinical signs, and lung lesions caused by BRSV and PI-3, viremia, hyperthermia and leukopenia caused by BVDV-1 and BVDV-2 and virus shedding caused by BVDV-2. It also actively immunises heifers and cows to protect from births of persistently infected calves and transplacental infection of BVDV (type 1 and 2).

The recommended vaccination programme includes a basic vaccination scheme, which consists of the

administration of two intramuscular injections (2 ml each), the first dose may be administered to calves from 10 weeks of age, and the second dose three weeks later. Re-vaccination is recommended at an interval not longer than 6 months after completion of the basic vaccination scheme by the administration of a single intramuscular dose. Afterwards, subsequent re-vaccinations are recommended at an interval not longer than 12 months.

The efficacy of Divence Tetra has been demonstrated by the results of the laboratory tests and clinical trials, which were carried out in accordance with the general principles and requirements of the Commission Delegated Regulation (EU) 2021/805, as well as with the current version of the general chapter 5.2.7 of the European Pharmacopoeia; "Evaluation of efficacy of veterinary vaccines and immunosera". In addition, the requirements described in the following current specific Ph. Eur. monographs have also been followed: no. 1177 "Bovine Respiratory Syncytial virus vaccine (live)", no. 1952 "Bovine Viral Diarrhoea vaccine (inactivated)" and no. 1176 "Bovine Parainfluenza virus vaccine (live)". However, it should be noted that the specific monograph 1176 is described for live vaccines, whereas the PI-3 vaccine strain included in Divence Tetra is inactivated.

Challenge model

A study was performed to assess the pathogenicity of two heterologous challenge strains of BRSV. Calves were challenged with RB94 or DK9402022 BRSV strain by nebulisation using a face mask. Virus was detected in nasal swabs of all 5 calves challenged with DK9402022. These calves had a higher respiratory clinical score between days 3 to 14. Aerosol challenge with strain DK9402022 was considered appropriate as a challenge model for BRSV.

The PI-3 challenge model is based on published data. In order to confirm the validity, a study was performed in 4-month old calves. The challenge caused virus shedding (nasal swabs) and hyperthermia in all calves, as well as mild lung lesions and clinical signs.

In order to validate the BVDV-1 challenge model, a study was performed in 4-month-old calves challenged by the intranasal route with virulent strain. Nasal virus shedding, viraemia, and mild clinical disease were observed in all challenged animals, leukopenia and hyperthermia were also observed.

In order to design the BVDV-2 challenge model, data from literature were used, however lower doses of the two challenge strains were also tested. The lower dose of the Iguazú strain, by intranasal route gave an adequate reproduction of BVDV when applied to 10-week-old calves.

A challenge dose for reproductive studies was tested in pregnant heifers (79-98 days of gestation) by intranasal challenge of different doses of BVDV-1 virulent strain. In all animals challenged with medium or high doses, only persistently infected foetuses were recovered, confirming an appropriate challenge. No specific study was performed to determine the challenge model for BVDV-2 for the reproductive claims, since it was based on published bibliography and previous experience. The Iguazú strain, is administered intranasally at approximately 78-85 days of gestation.

Adequate information on the development of challenge models has been provided.

Efficacy parameters and tests

The efficacy parameters as provided in the relevant Ph. Eur. monographs and as chosen by the applicant, investigated in the efficacy studies, are listed as claims for the different pathogens. The parameters chosen are considered appropriate for evaluating the efficacy of the product. The tests performed to evaluate these parameters were generally appropriately validated to provide reliable results.

Efficacy documentation

Seventeen studies were conducted to investigate the efficacy of the product and included 15 pre-clinical studies and 2 clinical trials. Laboratory studies were well documented and carried out in target animals of the minimum age recommended for vaccination, or in pregnant animals, using production and pilot batches containing a minimum dose. Production batches of Divence Penta were used in the clinical trials.

Study title
Immunological response induced in young calves after administration of Divence Penta vaccine and other fall-out vaccines containing a smaller combination of antigens
Efficacy of Divence Penta vaccine and influence of maternally derived antibodies (MDA) against BRSV challenge in young calves
Efficacy of Divence Penta vaccine against Bovine Parainfluenza type 3 virus (PI3) challenge in young calves
Efficacy of Divence Penta vaccine against BVDV-1 challenge in young calves
Efficacy of Divence Penta vaccine against transplacental infection after an experimental BVDV-1 challenge in pregnant heifers
Efficacy of the revaccination scheme of Divence Penta vaccine against transplacental infection after an experimental BVDV-1 challenge in pregnant heifers
Efficacy of Divence Penta vaccine and influence of maternally derived antibodies (MDA) against BVDV-2 challenge in young calves
Efficacy of Divence Penta vaccine against transplacental infection after an experimental BVDV-2 challenge in pregnant heifers
Efficacy of the revaccination scheme of Divence Penta vaccine against transplacental infection after an experimental BVDV-2 challenge in pregnant heifers
Influence of maternally derived antibodies (MDAs) on Divence Penta vaccine's efficacy against a PI3 challenge in young calves
Influence of maternally derived antibodies (MDA) on the efficacy of Divence Penta vaccine against BVDV-1 in young calves
Study on the duration of immunity (DOI) of Divence Penta vaccine against Bovine Respiratory Syncytial Virus (BRSV) in calves
Study on the duration of immunity (DOI) of Divence Penta vaccine against Bovine Parainfluenza type 3 virus (PI3) in calves
Study on the duration of immunity (DOI) of Divence Penta vaccine against a BVDV-1 challenge in young calves
Study on the duration of immunity (DOI) of Divence Penta vaccine against a BVDV-2 challenge in young calves
Efficacy and safety assessment under field conditions of Divence Penta vaccine in calves
Efficacy and safety assessment under field conditions of Divence Penta vaccine in cattle

Pre-clinical studies

Studies in support of efficacy of Divence Tetra were performed using the larger combination product Divence Penta. The two vaccines are identical but for the presence of a BoHV-1 vaccine strain in Divence Penta.

One study was performed to assess absence of interference between the active substances by evaluating the immunological (serological) response in calves as induced by Divence Penta and fall-out vaccines. Based on the results of the study, no interactions of the active substances in the larger combination on the induction of protection in the vaccinated animals are to be expected.

The study was appropriately designed and executed to an acceptable standard. The potency of the different antigens in the different vaccines was not identical, but can be considered comparable and acceptable for this exploratory type study. It can be concluded that the data generated with Divence Penta can be used in support of the efficacy of the fall-out vaccines.

The applicant also concludes that Divence Tetra can be used in subsequent re-vaccinations in animals previously vaccinated with Divence Penta vaccine. Whereas no specific data to this extent has been provided, it can be accepted that the study results support the absence of significant interaction between the vaccine components and as such it may be expected that re-vaccination with a fall-out vaccine will induce the same immunological response (to the relevant antigens) as re-vaccination with the larger combination.

Dose determination

No specific studies to determine vaccine dose were reported. For BRSV and PI-3 antigens the titres/dose are similar to those used for registered vaccines containing the same antigens.

Onset of immunity

Onset of immunity to BRSV was studied in MDA+ and MDA- calves of 10-16 weeks of age. MDA levels were comparable to what is observed in the field. The study was randomised, controlled and blinded. Vaccination was performed with two doses of vaccine, three weeks apart, containing a minimum titre of BRSV. Control groups received PBS. After challenge at three weeks post vaccination, virus excretion was significantly lower in the MDA+ and MDA- vaccinates, as was the duration of viral shedding. Respiratory clinical signs and rectal temperatures were significantly lower in both vaccinated groups compared to controls. Lung lesion scores were significantly higher in both control groups compared to the vaccinated groups. All requirements of the Ph. Eur. 1177 were met and the study is considered valid. The results support an onset of immunity at 3 weeks. No difference in level of protection was observed between MDA- and MDA+ vaccinated groups.

Onset of immunity to PI-3 virus was investigated in a randomised, blinded, controlled study in 10-12 week old calves vaccinated twice with a three-week interval with a minimum dose of PI-3. Controls received PBS. After challenge at three weeks after vaccination, vaccinates did not shed virus but controls did. Vaccinates had significantly lower average rectal temperatures and overall clinical scores. Lung lesion scores were significantly lower in the vaccinates. The study designed largely in accordance with Ph. Eur. 1176 and was performed to an acceptable standard. The results support the claimed onset of immunity at 3 weeks.

Onset of immunity against BVDV-1 was studied in calves of 10-14 weeks of age. The study was randomised, blinded and controlled. Calves were vaccinated twice with a three-week interval with a minimum dose of BVDV-1 E2 protein (and a low dose of BVDV-2 E2). Controls received PBS. After challenge at three weeks post vaccination, an increase in rectal temperatures was observed, this was more pronounced in the controls and the difference with vaccinates was significant. Clinical signs were very mild and not different between the groups. Control animals showed a significantly greater decrease in white blood cell (WBC) counts compared to vaccinates. Virus shedding was greater in the controls compared to the vaccinates. The study can be considered valid and results support an OOI of 3 weeks.

Efficacy of the vaccine against transplacental infection with BVDV-1 was studied in a randomised, blinded, controlled study in seronegative heifers. Animals were vaccinated twice with a three-week interval with a minimum dose of E2-1 (and E2-2) antigen. Three weeks after vaccination animals were synchronised and bred. Pregnancy was checked at day 75 and, if needed, animals were re-bred and checked again at day 119. Challenge was performed at day 128 by nasal aerosol. Viraemia was detected in 5/7 controls and 2/15 vaccinates, total virus titre was significantly higher in the control group. BVDV-1 was detected in all of the foetuses from control animals, whereas the virus was detected in foetuses of 7 out of 15 vaccinated animals (46.7 %). This difference was statistically significant. The study was appropriately designed, in accordance with Ph. Eur. 1952, and performed to

an acceptable standard. The study was valid in accordance with Ph. Eur. 1952. The results of the study support a level of efficacy of the basic vaccination scheme with reduction of viraemia and reduction of transplacental transmission after challenge with BVDV-1 at 15-20 weeks post vaccination, in the most sensitive period of gestation. However, the efficacy of the primary vaccination schedule against transplacental infection due to BVDV-1 was not considered sufficiently supported.

A study was performed in calves in order to assess the efficacy of the revaccination at 6 months against transplacental infection with BVDV-1. Calves were vaccinated twice with a three-week interval with a minimum dose of BVDV E2-1 (and E2-2), controls received PBS. After 6 months the calves received a booster vaccination. Animals were inseminated 2 months later and challenge by nasal aerosol was performed 20 weeks after revaccination. After challenge vaccinates had significantly higher interferon gamma (IFNγ) levels compared to controls. Viraemia was detected in all controls and in 3/15 vaccinates with a significantly greater virus titre and duration in the controls. BVDV-1 was detected in all foetuses from control animals and in 1 of 15 foetuses from vaccinated animals. The study was appropriately designed, in accordance with Ph. Eur. 1952, and performed to an acceptable standard. The study was valid in accordance with Ph. Eur. 1952. The results of the study are considered to support efficacy of the re-vaccination scheme (6 months after basic vaccination) with respect to reduction of viraemia and reduction of transplacental transmission after challenge with BVDV-1 at 20 weeks post vaccination, in the most sensitive period of gestation.

A randomised, blinded, controlled study in 10-week old MDA- and MDA+ calves was designed to test the onset of immunity against BVDV-2. Calves were vaccinated twice with a three-week interval i.m. with the minimum dose of BVDV E2-2 (and E2-1). Controls received PBS. At three weeks post vaccination all animals were challenged with BVDV-2 by nasal route. After challenge, an increase in average rectal temperatures was observed that was on average significantly higher and had a significantly longer duration in MDA- and MDA+ control calves. There were no differences in clinical signs between the groups. After challenge, the average WBC count was significantly lower in the control groups compared to vaccinated groups. Viraemia was significantly higher and of longer duration in the control groups compared to the vaccinated groups. The study was considered valid and the results are considered to support an OOI of 3 weeks, with reduction of hyperthermia, virus shedding, viraemia and leukopenia caused by challenge with BVDV-2. No significant differences were observed between MDA+ and MDA- vaccinated calves, which would indicate no effect of MDA. The results of the study are considered to support an OOI of 3 weeks, with reduction of hyperthermia, virus shedding, viraemia and leukopenia caused by challenge with BVDV-2. Reduction of clinical signs could not be observed in this study.

Efficacy of the vaccine against transplacental infection with BVDV-2 was studied in a randomised, blinded, controlled study in seronegative heifers. Animals were vaccinated twice with a three week interval with a minimum dose of BVDV E2-2 (and E2-1) antigen. Three weeks after vaccination animals were synchronised and bred: if needed animals were re-bred. Challenge was performed at day 129 by nasal aerosol. Viraemia was detected in 6/7 controls and 7/16 vaccinates, total virus titre was significantly higher in the control group. BVDV-2 was detected in all of the foetuses from control animals, whereas the virus was detected in foetuses of 4 out of 16 vaccinated animals (25 %). This difference was statistically significant. The study was appropriately designed, in accordance with Ph. Eur. 1952, and performed to an acceptable standard. The study was valid in accordance with Ph. Eur. 1952. The results of the study are considered to support a level of efficacy of the basic vaccination scheme with reduction of viraemia and reduction of transplacental transmission after challenge with BVDV-2 at 15-20 weeks post vaccination, in the most sensitive period of gestation.

A study was performed in calves in order to assess the efficacy of the revaccination at 6 months against transplacental infection with BVDV-2. Calves were vaccinated twice with a three-week interval

with a minimum dose of BVDV E2-2 (and E2-1), controls received PBS. After 6 months the calves received a booster vaccination. Animals were inseminated 2 months later and challenge by nasal aerosol was performed 20 weeks after revaccination. Before and after challenge vaccinates had significantly higher IFNγ levels compared to controls. Viraemia was detected in all controls and in 3/17 vaccinates with a significantly greater virus titre and duration in the controls. BVDV-2 was detected in all foetuses from control animals and in 1 of 17 foetuses from vaccinated animals. The study was appropriately designed, in accordance with Ph. Eur. 1952, and performed to an acceptable standard. The results of the study are considered to support efficacy of the re-vaccination scheme (6 months after basic vaccination) with respect to reduction of viraemia and reduction of transplacental transmission after challenge with BVDV-2 at 20 weeks post vaccination, in the most sensitive period of gestation.

Duration of immunity

Duration of immunity against BRSV was studied in MDA- calves of 11-14 weeks of age. In a randomised, blinded, controlled study, calves were vaccinated twice with a 3-week interval with Divence Penta containing a minimum titre of BRSV. Controls received PBS. Challenge was given at 6 months post vaccination and calves were monitored for 2 weeks. The mean titre and duration of virus excretion was significantly higher in the control group compared to the vaccinates. Average clinical scores were lower in the vaccinates in the second week, but overall differences were not significant. Rectal temperatures were similar between the groups. The total percentage of lung affected was notably greater in the controls (9.2%) compared to the vaccinates (6.6%), but the difference was not significant. The study was appropriately designed in accordance with Ph. Eur. 1177 requirements and can be considered valid. The vaccine complied with the test since vaccinated animals showed a significant reduction in virus excretion and a notable reduction of clinical signs. Based on these results, a DOI against BRSV of 6 months after the basic vaccination scheme is considered supported.

Duration of immunity against PI-3 was studied in MDA- calves of 11-12 weeks of age. In a randomised, blinded, controlled study, calves were vaccinated twice with a 3-week interval with Divence Penta containing a minimum dose of PI-3. Controls received PBS. Challenge was given at 6 months post vaccination and calves were monitored for 2 weeks. The mean titre and duration of virus excretion was significantly higher in the control group compared to the vaccinates, that remained negative. Average clinical scores increased clearly in the controls but not in the vaccinates, the difference was significant. Rectal temperatures were notably higher in controls but not significantly different between the groups. Lung lesions were observed at necropsy and were more extensive in controls (7.3%) compared to vaccinates (1.2%). The study was appropriately designed in accordance with Ph. Eur. 1176 requirements and can be considered valid. The vaccine complied with the test. Based on these results, a DOI against PI-3 of 6 months after the primary vaccination schedule is considered supported. The data provided to support a DOI of one year for PI-3 after revaccination is described above with the DOI for BRSV.

A randomised, blinded, controlled DOI study for protection against BVDV-1 was performed. MDA- calves of 10-13 weeks of age were vaccinated twice with a 3-week interval with Divence Penta containing a minimum dose of BVDV E2-1 antigen. Controls received PBS. Challenge was given at 6 months post vaccination and calves were monitored for 3 weeks. The mean titre and duration of virus excretion was significantly higher in the control group compared to the vaccinates. Average rectal temperatures were significantly higher in controls. After challenge, clinical scores were higher in the control group but were minimal and clinically irrelevant overall. Average WBC was significantly higher in vaccinates compared to controls. The average virus titre and the number of days with viraemia in controls was significantly higher compared to vaccinated animals. The study was appropriately designed and can be considered valid. The results of the study are considered to support an DOI of 6

months, with reduction of hyperthermia, viraemia and leukopenia caused by challenge with BVDV-1.

A randomised, blinded, controlled DOI study for protection against BVDV-2 was performed. MDA- calves of 10-17 weeks of age were vaccinated twice with a 3-week interval with Divence Penta containing a minimum dose of BVDV E2-2 antigen. Controls received PBS. Challenge was given at 6 months post vaccination and calves were monitored for 3 weeks. The mean titre and duration of virus excretion was significantly higher in the control group compared to the vaccinates. Viraemia was significantly higher and of longer duration in controls. Average rectal temperatures were significantly higher in controls. No difference in clinical scores were observed. Average WBC was higher in vaccinates compared to controls. The average virus titre and the number of days with viraemia in controls was significantly higher compared to vaccinated animals. The study was appropriately designed and can be considered valid. The results of the study are considered to support an DOI of 6 months, with reduction of hyperthermia, viraemia, virus shedding and leukopenia caused by challenge with BVDV-2. Reduction of clinical signs could not be observed in this study as a result of mild disease symptoms.

A randomised, blinded, controlled laboratory study investigating the DOI for BVDV-1 and BVDV-2 after revaccination is presented. Twenty calves of 2-3 months of age were included in the study, 10 were vaccinated with Divence Penta with a below-minimum potency of E2 proteins. The remaining 10 calves received PBS. Calves were housed together. The study looked at seroneutralising antibody (SN) titres against BVDV-1 over a 594 day follow-up period. Animals were vaccinated at Day 0, 21, 206 (6 months after the second vaccination) and 573 (one year after the third vaccination). The average SN titre obtained just prior to challenge in another efficacy study was compared to the titres obtained in this study from day 338. No significant differences in titre against either BVDV-1 or BVDV-2 were observed. At the last timepoint, just after the yearly revaccination, titres in this study were higher compared to those in the efficacy study (revaccination at 6 months) prior to challenge. Based on the presence of neutralising antibodies, results are considered supportive of a DOI of one year after revaccination for BVDV-1 and BVDV-2.

Maternally derived antibodies (MDA)

The influence of MDA was studied for BRSV in the OOI study, as summarised above, no differences in protection level between MDA+ and MDA- calves were observed.

A randomised, blinded, controlled study in MDA+ and MDA- calves of the youngest age for vaccination was performed to determine the effect of MDA on protection against PI-3. MDA+ and MDA- calves were vaccinated with Divence Penta batch containing a minimum dose of PI-3. The remaining MDA+ control animals were treated with PBS. At three weeks post vaccination, all calves were challenged intranasally and monitored for 2 weeks. Virus shedding (titre and duration) was significantly higher in controls compared to vaccinates, there was no difference between the vaccinated groups. Rectal temperatures increased in the controls but not the vaccinates, the difference was significant. The average clinical score was significantly lower in both vaccinated groups compared to controls. The average lung lesion score was similar in the vaccinated groups (1.51% and 1.58%) which was lower compared to the control group (3.62%). The study was appropriately designed, in accordance with the requirements Ph. Eur. 1176, and can be considered valid. The vaccine met the requirements of the monograph (although these strictly do not apply for inactivated vaccines), both for MDA+ and MDA- animals. It can be concluded there was no effect of MDA on protection against PI-3 in calves.

For BVDV-1 a randomised, blinded, controlled study in MDA+ and MDA-10-15 week old calves was performed. MDA+ and MDA- calves were vaccinated with Divence Penta batch containing the minimum dose of BVDV-1 E2. The remaining MDA+ control animals were treated with PBS. When MDA levels had dropped to undetectable (49 days post vaccination) all calves were challenged intranasally and

monitored for 3 weeks. Rectal temperatures increased in all groups; the increase was significantly higher in controls compared to the MDA- but not the MDA+ group. The duration of hyperthermia was longer in controls compared to both groups of vaccinates. Virus shedding (titre and duration) was higher in controls compared to MDA+ and MDA- vaccinates but differences were not significant. Average viraemia was similar in both vaccinated groups and significantly lower compared to controls. The average WBC count was lower in the control group and higher in the MDA- vaccinated group, differences between these groups were significant. The difference between the MDA- and MDA+ vaccinated groups was not significant. The study was appropriately designed, and was performed to an acceptable standard. Based on the results of virus shedding, WBC, rectal temperatures and even viraemia the MDA- and MDA+ vaccinated group performed similarly. Based on these findings, the absence of interference by MDA is considered demonstrated.

For BVDV-2 the OOI study summarised above also included MDA+ animals. No differences were observed between MDA+ and MDA- vaccinated groups with respect to rectal temperature, WBC, virus excretion or viraemia. The study supports absence of interference by MDA on protection against BVDV-2 challenge.

Interactions

No specific studies have been carried out to investigate the possible interactions of Divence Tetra vaccine with other veterinary medicinal products. For this reason, the following recommendation is included in the relevant section of the summary of product characteristics and package leaflet: "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 warning sentence is acceptable.

Clinical trials

Two field trials were performed in order to assess safety and efficacy under field conditions of use.

The first study was performed in calves on farms in Spain whereas the second study is a study in heifers and cows on farms in Spain and Hungary. The general outline of the studies has been summarised in the Safety part, Clinical trials.

Briefly, the first study included 506 vaccinated and 511 control calves. The following efficacy parameters were applied:

Primary: incidence of new cases of respiratory disease (RD) during an outbreak.

Secondary: overall incidence of new cases of RD, number of concomitant treatments due to RD, severity of respiratory clinical signs, mortality due to RD, serological response, lung lesions at slaughter, productive performance.

Three outbreaks of RD were reported at 23, 26 and 36 days after the start of vaccination. The causative agent could only be diagnosed in one outbreak; this concerned BRSV as a single (detected) pathogen. Fifty-four (54) controls and 54 vaccinates were located. In the control group 29 new cases of RD occurred whereas in the vaccinated group 11 cases were recorded. The animals in the control group were 4.54 times more likely to suffer a respiratory case during the outbreak and this difference was significant (p=0.0007).

The overall incidence of new cases of RD was numerically lower in the vaccinates (28.6%) compared to controls (34.8%), but this was not statistically significant. When the mean number of episodes of RD during the follow up period was compared between groups, this was 0.62 in the controls and 0.48 in the vaccinated group, the difference was statistically significant with this approach.

The mean number of concomitant treatments was comparable between the groups. The mean severity of respiratory clinical signs as recorded during the whole study follow-up was 0.80 in controls and 0.61 in vaccinates, this difference was significant (p=0.004). The mean severity of RD during an outbreak could only be calculated from the outbreak at BAT farm and was 2.11 in controls and 1.70 in vaccinates (p=0.055). Mortality due to RD was similar in control (2.4%) and vaccinated (2.8%) groups.

Lung lesions scores were evaluated at slaughter in animals from the farm mentioned above, no statistically significant differences were observed and scores were generally absent to low.

With respect to production parameters, there were no differences between the groups.

In conclusion, Divence Penta reduced the incidence and severity of respiratory disease during an outbreak of BRSV, even at 23 days after the start of the vaccination program. The number of sporadic episodes of respiratory disease was reduced, as was overall severity of respiratory disease.

The trial was appropriately designed and performed to an acceptable standard (GCP). The results of the efficacy analysis do not contradict the results of the laboratory efficacy studies for BRSV but provide no additional support with regard to protection against PI3 and BVDV. However, this can be accepted since for every active substance in the vaccine an effect was shown in the laboratory studies and no productivity claims are made. The results and conclusions are considered applicable to Divence Tetra vaccine.

It appears that the applicant proposed a claim for OOI against BRSV of 2 days on the results of the clinical trial. However, on the single farm where a BRSV outbreak was confirmed by detection of the pathogen, no statistically significant differences in clinical disease severity, mortality during follow-up, fatality rate during the outbreak or lung lesions at slaughter could be found. No data on virus shedding were provided. Therefore, the results of the clinical study are not considered to support the claims for BRSV at OOI of 2 days (i.e. to reduce virus shedding, hyperthermia, clinical signs and lung lesions). The claim was changed accordingly.The other study was performed in heifers and cows. The study was a multicentrered, randomised, controlled and blinded trial performed in 4 dairy farms, a total of 1255 animals were included from the age of 10 onward. The vaccinated group received four doses of the vaccine (primary vaccination D0 and D21, re-vaccination 6 months and booster vaccination one year later), whereas the controls received a placebo. The follow-up period was 24 months. For the overall efficacy population, pregnancy loss (including early embryonic death, abortion and stillbirth) was highly similar for the control and vaccinated groups (resp. 13.8% and 13.1%). When the data were analysed separately for cows and heifers, the same result (highly similar for control and vaccinated groups) was obtained. No seroconversion to BVDV could be detected in any of the paired samples collected after pregnancy loss was observed. In one of the four farms, circulation of BVDV was observed from the start of the study. The results for this farm are therefore also reported separately; although the number of persistently infected calves born to controls (n=4, 4.5%) was numerically higher compared to the vaccinates (n=1, 1.2%), the difference was not statistically significant.

Overall conclusion on efficacy

The challenge models developed to test the efficacy of the vaccine against the different pathogens were appropriately validated. The parameters chosen can be considered appropriate and tests used were generally validated and fit-for-purpose. Seventeen studies were conducted to investigate the efficacy of the product and included 15 pre-clinical studies and 2 clinical trials. Laboratory studies were well documented and carried out in target animals of the minimum age recommended for vaccination.

All studies were performed using the larger combination vaccine Divence Penta, this is acceptable as it is considered a worst-case scenario with respect to efficacy. Production and pilot batches containing a

minimum dose of the antigen under investigation were used in the pre-clinical studies. Production batches were used in the clinical trials. A study was performed in calves comparing serological responses to Divence Penta and the respective fall-out products. The results support a highly similar response to the antigens irrespective of the number of antigens in the composition. It can be concluded that Divence Penta is a suitable model for efficacy of the fall-out vaccines and that the fall-out vaccines are acceptable for re-vaccination after basic vaccination with a larger combination.

Adequate evidence of onset of immunity at 3 weeks post vaccination was obtained in four separate studies in seronegative calves, investigating protection against the four individual pathogens: BRSV, PI-3, BVDV-1 and BVDV-2. Where relevant, studies were performed in accordance with the specific Ph. Eur. monographs, in most cases the studies were valid and the vaccine was shown to meet the requirements.

Efficacy against transplacental infection with BVDV-1 and BVDV-2 was investigated in two studies, both performed in accordance with the requirements of Ph. Eur. 1952. In both studies, seronegative heifers were vaccinated and subsequently bred. When animals were 7-12 weeks pregnant, challenge was performed. For both studies, virus was detected in all foetuses from control animals. BVDV-1 was detected in 46.7% of the foetuses from vaccinated animals, BVDV-2 was detected in 25% of foetuses from vaccinates. Both studies confirmed that Divence Penta vaccine significantly reduces the presence of PI animals after BVDV-1 and BVDV-2 challenge. However, the efficacy of the primary vaccination scheme against transplacental infection due to BVDV-1 was not considered sufficiently supported.

Efficacy of the revaccination at 6 months against transplacental infection with BVDV-1 and BVDV-2 was investigated in two studies, both performed in accordance with the requirements of Ph. Eur. 1952. In both studies, seronegative calves were vaccinated twice with a three-week interval and subsequently 6 months later. Two months later animals were bred and challenge was performed when animals were 12 weeks pregnant. For both studies, virus was detected in all foetuses from control animals. BVDV-1 was detected in 7% of the foetuses from vaccinated animals, BVDV-2 was detected in 6% of foetuses from vaccinates. The results support efficacy of the revaccination, in accordance with Ph. Eur. requirements, against viraemia and reduction of transplacental infection due to BVDV-1 and BVDV-2.

Duration of immunity against BRSV and PI-3 was studied in separate challenge studies in calves. Studies were performed in accordance with the specific Ph. Eur. monographs, in each case the studies were valid and the vaccine was shown to meet the requirements. Protection was shown to last 6 months after the primary vaccination schedule. In a serological study in vaccinated animals, neutralising antibodies to BRSV and PI-3 quantified at 6 months and 1 year after revaccination and after the yearly booster vaccination were shown to be at least comparable to antibody levels in vaccinated, protected, animals in OOI and DOI studies. The data are considered supportive of a DOI of 1 year after re-vaccination.

Duration of immunity against BVDV-1 and BVDV-2 was studied in calves after primary vaccination. The studies were appropriately designed and can be considered valid. Protection was shown to last for 6 months. A study investigating the DOI for BVDV-1 and BVDV-2 after revaccination was performed. Seroneutralisation titres against BVDV-1 and BVDV-2 were followed for one year after revaccination and after the yearly booster and compared to titres obtained in the challenge studies just prior to challenge. The titres were similar to titres obtained prior to challenge (in protected calves) and support a one year DOI after revaccination as well as the efficacy of the yearly booster vaccination.

The influence of MDA on the onset of protection was studied for BRSV and BVDV-2 in the respective OOI studies. For PI-3 and BVDV-1, separate studies to investigate effects of MDA were performed. No differences in protection level between MDA+ and MDA- calves were observed for BRSV, PI-3, BVDV-1

or BVDV-2. The absence of significant interference is considered supported.

No specific studies have been carried out to investigate the possible interactions of Divence Tetra vaccine with other veterinary medicinal products. A warning sentence to this extent has been included in the SPC.

Two multicentre field trials were performed in order to assess safety and efficacy under field conditions of use. A study in calves included 506 vaccinated and 511 control animals. Vaccination reduced the incidence and severity of respiratory disease during an outbreak of BRSV (primary efficacy criterion), occurring in one farm at 23 days after the start of the vaccination program. The number of sporadic episodes of respiratory disease was reduced, as was the overall severity of respiratory disease. The trial was appropriately designed and performed to an acceptable standard (GCP). The results of the efficacy analysis do not contradict the results of the laboratory efficacy studies for BRSV but provide no additional support with regard to protection against PI3 and BVDV. However, this can be accepted since for every active substance in the vaccine an effect was shown in pre-clinical studies, in accordance with the claims and no productivity claims are made. The results of the clinical study are not considered to support the efficacy claims for BRSV and can thus not be used to support an OOI of 2 days.

With respect to the field trial performed in heifers and cows, pregnancy loss (including early embryonic death, abortion and stillbirth) was highly similar for the control and vaccinated groups. No seroconversion to BVDV was detected after any of the observed pregnancy losses. BVDV was only shown to circulate in one farm: although the number of persistently infected calves born to controls (n=4, 4.5%) was numerically higher compared to the vaccinates (n=1, 1,2%) in this farm, the difference was not statistically significant. The results of the field study do not contradict the results of the pre-clinical studies and generally confirm the results of the laboratory studies with respect to serological responses to BVDV.

Part 5 – Benefit-risk assessment

Introduction

Divence Tetra is a multivalent vaccine containing live attenuated bovine respiratory syncytial virus (BRSV), strain Lym-56; inactivated bovine parainfluenza 3 virus (PI-3), strain SF4; E2 recombinant protein from bovine diarrhoea virus type 1 (BVDV-1) and E2 recombinant protein from bovine diarrhoea virus type 2 (BVDV-2), as active substances; and Montanide IMS as adjuvant.

The active substances BRSV strain Lym-56, inactivated PI-3 strain SF4, BVDV-1 E2 and BVDV-2 E2 recombinant proteins are known active substances included in centralised or nationally authorised vaccines for use in cattle.

The product is intended for use in cattle from 10 weeks of age to reduce virus shedding, hyperthermia, clinical signs and lung lesions due to BRSV and PI-3 and to reduce viremia, hyperthermia and leukopenia caused by BVDV-1 and BVDV-2 and virus shedding caused by BVDV-2. In addition the product is intended for use in heifers and cows to reduce births of persistently infected calves and transplacental infection of BVDV (type 1 and 2).

The effective dose of $10^{5.2\text{-}6.5}$ $CCID_{50}$ BRSV strain Lym-56, ≥ 206.2 EU inactivated PI-3 strain SF4, ≥ 31.6 EU BVDV-1 E2 recombinant protein and ≥ 21.0 EU BVDV-2 E2 recombinant protein administered intramuscularly twice with a three-week interval has been confirmed.

The application has been submitted in accordance with Article 8 of Regulation (EU) 2019/6 (full application).

Benefit assessment

Direct benefit

The benefit of Divence Tetra is its efficacy in reduction of virus shedding, hyperthermia, clinical signs and lung lesions due to BRSV and PI-3 and reduction of viremia, hyperthermia and leukopenia caused by BVDV-1 and BVDV-2 and virus shedding caused by BVDV-2 as well as reduction of births of persistently infected calves and transplacental infection of BVDV (type 1 and 2), which develops after re-vaccination. This was established in a large number of well-designed pre-clinical studies conducted to an acceptable standard.

The onset of immunity is 3 weeks.

The duration of protection is 6 months after completion of the basic vaccination scheme, this was confirmed by challenge. The duration of immunity of 1 year after re-vaccination was determined by serological studies.

Efficacy was shown not to be affected by the presence of MDA.

Additional benefits

The combination of antigens in Divence Tetra reduces the number of vaccinations required to protect animals from viral pathogens involved in bovine respiratory disease.

Risk assessment

Quality

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out 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. The type of containers and the method of administration are commonly used in veterinary vaccines. A shelf life of 18 months is accepted for the lyophilisate. A shelf life of 3 years is accepted for the solvent.

Safety

Risks for the target animal

Administration of Divence Tetra in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include local swelling and increases in rectal temperature.

The safety of Divence Tetra in pregnant cattle was confirmed. Adverse reactions were local swelling and increases in rectal temperature. The vaccine was found to be safe for use in pregnant and lactating animals.

Risk for the user

The product contains mineral oil, which is known to cause severe pain and swelling particularly if injected into a joint or finger. The applicant has included in section 3.5 of the SPC a standard warning sentence concerning mineral oil. No further hazards were identified and the overall risk to the user is considered to be negligible.

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

Divence Tetra is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

Risk for the consumer:

Potential risks to the consumer due to remnants of antibiotics in production are considered acceptable. The potential formation of anti-human antibodies has been adequately evaluated and the risk was found to be low.

Risk management or mitigation measures

Information has been included in the SPC to inform on the potential risks of this product relevant to the target animal. A user risk warning relating to mineral oil is included.

The veterinary medicinal product is subject to veterinary prescription.

Evaluation of the benefit-risk balance

The product has been shown to be efficacious for the active immunisation of cattle from 10 weeks of age to reduce virus shedding, hyperthermia, clinical signs and lung lesions due to BRSV and PI-3; to reduce viremia, hyperthermia and leukopenia caused by BVDV-1 and BVDV-2 and virus shedding caused by BVDV-2; and for the active immunisation of heifers and cows to reduce births of persistently infected calves and transplacental infection of BVDV (type 1 and 2).

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

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

Conclusion

Based on the CVMP review of the data on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for Divence Tetra is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EU) 2019/6).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product. In addition, the CVMP considers that bovine viral diarrhoea virus 1, E2 recombinant protein and bovine viral diarrhoea virus 2, E2 recombinant protein are not new active substances given that new active substance status was previously confirmed for these active substances in the CVMP opinion adopted for the parent product Divence Penta.