28 June 2021
EMA/364209/2021
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Strangvac (EMEA/V/C/005309/0000)

Vaccine common name: *Streptococcus equi* vaccine (recombinant proteins)

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000

Introduction

The applicant Intervacc AB submitted on 27 February 2020 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Strangvac, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 16 April 2019 as Strangvac has been developed by recombinant DNA technology.

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

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

The active substances of Strangvac, recombinant proteins from *S. equi*, are new active substances not authorised for a veterinary medicinal product in the EU before.

Indications

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

For the active immunisation to reduce clinical signs of disease and bacterial shedding caused by infection with *Streptococcus equi (S. equi)*.

Onset of immunity: Two weeks after the second primary vaccination dose.

Duration of immunity: At least two months as demonstrated by protection against challenge.

The full duration of immunity is not yet known but based on serological data, priming antibodies are induced for at least 12 months after the primary vaccination or after a single revaccination.

The active substances of Strangvac are recombinant proteins from *S. equi* CCE and Eq85, and endopeptidase protein IdeE from *S. equi*. This is considered a new active substance. The target species are horses. The product is intended for administration by the intramuscular use.

Strangvac, suspension for injection contains recombinant proteins from *S. equi* CCE (≥ 111.8 µg/dose) and Eq85 (≥ 44.6 µg/dose), and a single endopeptidase protein IdeE (≥ 34.6 µg/dose) from *S. equi* and is presented in packs containing 8 x 2 ml vials.

The applicant is registered as an SME pursuant to the definition set out in Commission Recommendation 2003/361/EC.

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

Reduced data requirements in accordance with CVMP MUMS/limited market guidelines may apply.

Scientific advice

The applicant requested scientific advice from the CVMP in several occasions and it was provided on 16 June 2010 (EMA/SAWP-V/056/2010), 16 May 2013 (EMA/CVMP/SAWP/95065/2013), 11 December 2014 (EMA/CVMP/SAWP/609665/2014) and finally on 10 October 2019 (EMA/CVMP/543577/2019).

The last scientific advice given in 2019 pertained the quality part of the dossier.

For the quality part of the dossier, overall the pieces of scientific advice given have been followed.

MUMS/limited market status

The applicant requested eligibility of this application for MUMS/limited market by the CVMP, and the Committee confirmed on 17 April 2019 that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) data requirements would be applied when assessing the application. MUMS/limited market status was granted as horses is considered a minor species. The indication accepted by the CVMP was for the protection from morbidity and mortality caused by the bacterium *S. equi* subspecies *equi* in horses.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The MAH must ensure that the system of pharmacovigilance is in place and functioning before the product is placed on the market and for as long as the marketed product remains in use (Art 12(3)(k) and o) of the amended Directive 2001/82/EC.

The applicant has provided documents that set out a detailed description of the system of pharmacovigilance.

The applicant states that the QPPV is employed by Intervacc AB and serves as a QPPV for the entire Intervacc group including Intervacc AB, Nordvacc Läkemedel AB and SIA Nordvacc Latvia. The applicant Intervacc AB will use the same system and the same QPPV as Nordvacc AB.

On 23-24 January 2020 the Swedish Medical Products Agency (MPA) performed a pharmacovigilance inspection of Nordvacc AB (part of the Intervacc group) and noted 17 findings, all majors and all describing lack of processes for each pharmacovigilance (PV)-process.

The applicant has submitted the CAPA plan as requested. In the CAPA plan the following was decided:

The QPPV function will be outsourced to a PV-vendor and the current QPPV will take the deputy QPPV function. Yearly training plans have been established to ensure ongoing training of the QPPV and deputy QPPV.

The applicant has informed that the Medical Products Agency (MPA) has closed the pharmacovigilance inspection on the 2 October 2020.

A revised Detailed Description of Pharmacovigilance System version 4.0 has been submitted where the proposed actions have been incorporated.

In the revised Detailed Description of Pharmacovigilance System a statement signed by the applicant and the qualified person for pharmacovigilance, indicating that the applicant has the services of a qualified person responsible for pharmacovigilance (QPPV) and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country has been provided. In general, the description of Intervacc pharmacovigilance system – version 4.0 fulfils the current legal requirements. Nevertheless, the applicant will submit a revised DDPS when distribution agreements have been finalised; this is subject to a post authorisation recommendation.

Manufacturing authorisations and inspection status

Manufacture of the active substance takes place within the EU at 3P Biopharmaceuticals SL, Spain. The site has a manufacturing authorisation issued on 07 March 2018 by the Spanish medicines and medical

devices agency (AEMPS) for the manufacture of veterinary medicinal products and manufacture and control testing of sterile or biological active substances. The site has a certificate of GMP compliance issued on 22 October 2020 by the AEMPS; it is noted that the last inspection was conducted on 13 December 2017, however, the validity of the certificate is extended to until 13 December 2021 due to restrictions caused by Covid-19; Once the restrictions are over an on-site inspection will be performed.

Manufacture of the finished product takes place within the EU at LIOF-Pharma S.L., Spain. The site has a manufacturing authorisation issued on 07 November 2019 by AEMPS for the manufacture and batch certification of immunological veterinary medicinal products and quality control testing for biologicals. The site has a certificate of GMP compliance issued on 14 July 2020 by the AEMPS; it is noted that the last inspection was conducted on 21 September 2017, however, the validity of the certificate is extended to until 21 September 2021 due to restrictions caused by Covid-19; Once the restrictions are over an on-site inspection will be performed.

Secondary packaging and batch release within the EU take place at LIOF-Pharma S.L., Spain which holds a manufacturing authorisation issued by the Spanish medicines and medical devices agency (AEMPS).

A good manufacturing practice (GMP) declaration for the active substances 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 Spanish medicines and medical devices agency (AEMPS) following inspection.

Overall conclusions on administrative particulars

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

The detailed description of the pharmacovigilance system was considered in line with legal requirements for the time being. Nevertheless, the applicant will submit a revised DDPS when distribution agreements have been finalised; it is subject to a post authorisation recommendation.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The Strangvac finished product is presented as a suspension for injection. The vaccine contains three recombinant protein antigens; the chimeric recombinant proteins CCE and Eq85, which contain amino acid sequences from respectively five (CCE) and two (Eq85) different cell-surface proteins from *S. equi*, and the *S. equi* IgG endopeptidase IdeE. The finished product contains a standard amount (in µg) of the three antigens: CCE ≥111.8 µg; Eq85 ≥44.6 µg and IdeE ≥34.6 µg per dose of 2 ml. Strangvac contains Matrix V as adjuvant, consisting of purified Quillaia saponin QS-21 (Fraction C) (≥260 µg), cholesterol and phosphatidyl choline. Other ingredients are trometamol (buffer component), sodium chloride and polysorbate 80 (surfactant). The list of excipients is included in section 6.1 of the SPC.

The active substances of Strangvac, recombinant proteins from *S. equi*, are new active substances not authorised for a veterinary medicinal product in the EU before.

The vaccine is presented as single doses of 2 ml in glass vials as described in section 6.5 of the SPC. The finished product does not contain preservatives.

The composition of the vaccine is adequately described.

Container and closure

The product is filled into 3-ml type I borosilicate clear glass injection vials (Ph. Eur 3.2.1) closed with silicone coated bromobutyl rubber stoppers (Ph. Eur. 3.2.9) and sealed with aluminium caps. The vials have a nominal fill volume of 2.3 mL to ensure that a 2 mL volume can be removed for administration. Certificates of analysis have been supplied for containers and closure demonstrating compliance with the proposed specifications.

Vials and rubber stoppers are steam sterilised. Vials are depyrogenized prior to filling.

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

Product development

The strategy for the pharmaceutical development of Strangvac was aimed at blocking infection via the generation of an immune response against virulence factors on the bacterial cell surface. Therefore, the antigens were selected from a panel of mainly cell-surface proteins from *S. equi* based on their biological properties, which have been described as far as they are known and based on a number of literature studies. The applicant states that isolates of *S. equi* are very closely related genetically and the selected antigens are expected to give a broad protection against strains from outbreaks spread over time and between geographical locations. The selection of antigens and the role of each antigen in

the vaccine is considered sufficiently described and justified.

Strangvac contains the ISCOM-matrix adjuvant, Matrix V. According to the background information provided in Scientific advice (EMA/CVMP/SAWP/95065/2013), the applicant has gained extensive experience about this adjuvant, since it has been used in equine influenza vaccination. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. The selected container materials are considered standard for this type of product.

The antigens are produced, using recombinant DNA technology. The manufacture is based on a seed lot system with master cell bank and working cell bank for each construct. The manufacturing process is almost identical for the CCE, Eq85 and IdeE antigens. The manufacturing process is a standard biomass scale-up in bioreactors, followed by initiation of protein expression, isolation of the recombinant proteins from the biomass, purification of the proteins, formulation of the antigen bulks, and formulation and filling of the final product. The proposed lower specifications limits for potency for each antigen are based on pivotal efficacy studies and the upper release specifications are based on dose-finding studies.

Some changes of manufacture have been made during development. These include minor changes in downstream process to facilitate scale-up, and introduction of new manufacturing sites. The upstream manufacturing process, including the applied cell banks, was not changed during development. A comprehensive comparability study of the antigen and finished product batches produced at the earlier and the proposed manufacturing sites has been performed. Comparability is overall considered demonstrated and in line with the provided scientific advices. From the comparability study, as well as from the validation and SOP for the SDS-PAGE analysis, it was noted that several additional bands of both lower and higher molecular weight are visible for the antigen samples, and that the total amount of these additional bands seems to make up a relatively large part of the protein in some cases. The nature of the additional bands, as well as their potential immunogenicity in terms of creating a protective immune response to equine strangles in the target species, was questioned. Subsequently, the impurity bands have been characterised, and it has been demonstrated that they consist primarily of minor variants of the antigen products. For Eq85, however, two persistent truncated variants are found. Limits for impurities, and the truncated variants have been added to the antigen specifications.

Overall, the product development is considered adequately described and the composition and presentation of the vaccine has been presented and explained appropriately.

Description of the manufacturing method

Antigen production

The recombinant protein antigens CCE, Eq85, and IdeE are manufactured in three separate manufacturing processes using the working cell banks *E. coli* BL21 (pBmK CCE), *E. coli* BL21 (pBmK Eq85), and *E. coli* BL21 (pBmK IdeE), respectively. The antigen manufacturing processes are considered to be standard.

The expression system is designed so that the protein antigens are overproduced and accumulate in the *E. coli* cytoplasm as soluble protein. The antigens are produced by fermentation in bioreactors. The upstream process is essentially the same for the CCE, Eq85, and IdeE processes and consists of three up-scale steps, followed by a protein expression step. Antifoam may be added to control foaming. Relevant fermentation parameters and in-process controls are defined. The bacterial biomass is harvested by continuous centrifugation and the harvested biomass is stored. Overall, the descriptions of the upstream manufacturing processes are considered sufficient, and the processes are considered adequately controlled.

The recombinant proteins are isolated from the resuspended biomass. For the following purification steps some differences exist between CCE/Eq85 and IdeE. For CCE and Eq85, the proteins are further clarified. This is followed by chromatography to separate CCE/Eq85 from host cell proteins, filtration, dilution and a second chromatography to remove host cell DNA and residual contaminant proteins. Prior to the second chromatography, the protein solution may be stored. Chromatography is followed by filtration, concentration by ultrafiltration to a target concentration, buffer exchange by diafiltration, formulation, final filtration and finally filling into storage bags. The CCE and Eq85 bulk antigens are stored. For IdeE, the protein is purified by a number of clarification membrane filtrations, followed by buffer adjustment, another filtration and chromatography to remove host cell DNA and contaminant proteins. Chromatography is followed by filtration, concentration to defined target concentration, buffer exchange by diafiltration, formulation, final filtration and finally filling into storage bags. The IdeE bulk antigen is stored. For all downstream processes, relevant process parameters and in-process controls are defined where appropriate. Overall, the description of the downstream manufacturing processes is considered sufficient and the processes are considered adequately controlled.

The applicant has provided a risk assessment with regard to a possible transfer of the kanamycin resistance gene into other microorganisms or to the environment when the vaccine is used in the field. The risk is considered negligible.

Vaccine Production

Bulk vaccine formulation is done by mixing the appropriate amounts of CCE, Eq85, and IdeE antigens, Matrix V adjuvant, and buffer salts. More than one antigen batch may be used to formulate a vaccine batch. Details of blending are provided. pH of the final bulk is controlled and adjusted if necessary. The final bulk is sterile filtered immediately before filling; the final sterilising filter is integrity tested and bioburden is tested for information prior to sterile filtration. Finally, the vaccine bulk is filled into vials; the filled vials are closed with the stopper and crimped. The finished product is stored at +2 to +8 °C for not more than 24 months. Overall, description and control of the blending and filling process is considered adequate.

Validation

Validation of the manufacturing process as a whole is demonstrated with the provision of results data from two commercial scale GMP vaccine batches and one non-GMP vaccine batch manufactured and filled at pilot scale. Process consistency for final product formulation is considered demonstrated and in compliance with the guideline (GL) on data requirements for immunological veterinary medicinal products (IVMPs) intended for minor use, minor species (MUMS)/limited market *EMA/CVMP/IWP/123243/2006-Rev.3* and in line with the provided scientific advices.

For validation of the upstream manufacturing process, only one fermentation was performed at commercial scale for each of Eq85 and IdeE antigen, whereas for CCE antigen two commercial scale fermentations were performed. The two GMP vaccine batches were formulated with antigens from a single fermentation for each antigen, but from two separate downstream processes for each antigen (based on bacteria stored as frozen cell paste). Thus, consistency cannot be assessed with regard to the antigen upstream manufacture part of the process; instead, consistency demonstration has been performed for three downstream process batches for each antigen. The approach was discussed with the CVMP and was deemed acceptable (EMA/CVMP/SAWP/195581/2015). The rapporteur agrees with this position and further notes that the three antigen upstream manufacturing processes are based on the same host cell and expression system and are almost identical, and that the in-process controls during upstream for the three antigens demonstrates consistency. The results overall demonstrate consistency of the antigen manufacturing process, including consistent removal of host-cell proteins (HCP) and removal of DNA to a very low level.

Production and control of starting materials

Starting materials listed in pharmacopoeias

All starting materials are listed, and the function of each material is described. Example certificates of analyses are provided; the quality of the materials complies with Ph. Eur. with the exception of ammonium sulphate and Antifoam Dow Corning Medical C Emulsion, which complies with the United States pharmacopeia (USP). This is acceptable since no Ph. Eur. monograph exists for these materials; the relevant USP monographs have been included.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

Starting materials of biological origin includes the master and working cell banks for manufacture of CCE, Eq85, and IdeE antigen, respectively. Furthermore, it includes components used in fermentation media, and Matrix V adjuvant. In addition, LB media used in manufacture of cell banks contains tryptone of animal origin. Finally, glycerol is used as cryoprotectant in the cell banks.

Cell banks

The three antigens CCE, Eq85, and IdeE are derived from *S. equi* subsp. *equi*. CCE and Eq85 are chimeric recombinant proteins, which contain amino acid sequences from a total of seven different cell-surface proteins from *S. equi* as antigens; IdeE is an IgG endopeptidase, also from *S. equi*. All the antigens are produced using recombinant DNA technology in *E. coli* host strains containing plasmids expressing the antigen gene coding region, using one specific host strain for each antigen.

The plasmid expression vector and the *E. coli* host strain are the same for the three antigens CCE, Eq85, and IdeE. A commercially available plasmid was used.

The manufacturing process of the master cell banks (MCB) and working cell banks (WCB) is the same for all antigens. In brief, the bacteria were sub-cultured from single discrete colonies to a target optical density, glycerol was added as cryoprotectant, the cultures were dispensed into cryovials and frozenand stored.

Testing of the MCBs includes: species confirmation, confirmation of β-galactosidase activity, confirmation of plasmid encoded kanamycin resistance, viable count, plasmid genetic stability, colony morphology, Gram stain, purity test, plasmid identity and absence of bacteriophage. Testing of WCBs includes: species confirmation, viable count, plasmid genetic stability, colony morphology, gram stain, purity test, plasmid identity and absence of bacteriophage. Certificates of analysis (CoA) for each MCB and WCB are provided.

The manufacture, testing, and storage of the cell banks is overall adequately described and documented, and compliance with the requirements of Ph. Eur. 0062 *Vaccines for veterinary use* and Ph. Eur. 0784 '*Recombinant DNA technology, products of*' is considered demonstrated.

Risk of transmissible spongiform encephalopathies (TSE) transmission

Risk materials of animal origin used in the production of the active substances are tryptone derived from casein of bovine origin and enzymes of porcine origin. It is confirmed that tryptone complies with the TSE Note for guidance (*EMA/410/01 rev.3*). Signed general statements have been provided for each cell bank, certifying compliance with the TSE Note for guidance (*EMA/410/01 rev.3*. The risk of

transmissible spongiform encephalopathies (TSE) transmission is considered negligible.

Starting materials of non-biological origin

Specifications and an example CoA are provided for the starting materials of non-biological origin.

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

Information on the qualitative and quantitative composition, methods of preparation, sterilisation, and storage conditions of all media and solutions is provided in the dossier. All fermentation media and solutions are sterilised by autoclaving where possible; Kanamycin solution and IPTG solution are sterilised by filtration due to heat sensitivity. Buffers and solutions for downstream, including the formulation buffer, are filter sterilised.

The nature of the raw materials, and the applied controls and treatments of media and solutions are overall considered adequate to assure sterility of the vaccine and absence of introduction of extraneous agents.

Control tests during the manufacturing process

The in-process control tests performed during the production processes of CCE, Eq85, and IdeE antigens are listed in the dossier. Routine in-process controls during upstream production of antigens include analyses of optical density (OD) at the individual culture steps and culture wet weight at end of fermentation; specific target OD levels are defined for the initiation of induction of protein expression by addition of IPTG. Control of culture purity is performed at end of fermentation. Routine in-process controls during midstream process include pH and total protein at 660 nm (UV_{660}) on clarified filtrate. Routine in-process controls in downstream process include total protein load on columns and on column eluates, total protein on concentrated antigens, and integrity tests of 0.2 µm filters. A test for bioburden of the antigens prior to formulation is also established. Acceptance limits are stated for all in-process controls. In-process controls during final product formulation is test of pH, only. Overall, the in-process controls are considered sufficient to control the critical steps of manufacturing; however, the proposed bioburden limit for the antigen bulks remains to be justified; this will be handled as post authorisation recommendation.

The routine release tests for bulk antigens include appearance and description (clarity and degree of opalescence, degree of coloration), antigen identity and potency/content by ELISA, product impurities (SDS-PAGE), process impurities (endotoxin, bioburden) and pH. Acceptance requirements are stated for all relevant release tests. Antigen specifications are provided for all three antigens. However, the current reference standards should be reanalysed according to the revised antigen specifications and methods; this will be handled as post authorisation recommendation.

Extended sampling was implemented during the qualification/scale-up and validation. For in-process controls during antigen manufacturing, extended testing includes plasmid retention at different culture steps, and content of host-cell protein (HCP) and host-cell DNA (DNA) at a number of purification steps. For bulk antigens release tests, the extended testing includes content of HCP and DNA. The applicant has decided to continue the extended testing as routine testing until more data are available and if, subsequently, the testing program will be changed, a variation application will be submitted.

Brief descriptions of test method are provided for all methods, and detailed SOPs are appended for non-pharmacopoeia methods. Endotoxin testing is performed according to Ph. Eur. 2.6.14 and

bioburden testing is performed according to Ph. Eur. 2.6.12; for both analyses test of method suitability was performed.

The quantity of CCE, Eq85 and IdeE antigens are determined by similar individual ELISA assays. The same ELISA methods are used for quantification of the individual antigens in the final Strangvac vaccine. In-house reference material of the specific antigens is used as standard. Positive control and spiked sample control are included, too. The final antigen concentration is given in µg/ml. The proposed acceptance limits reflect the levels found in the validation batches and are considered acceptable. Each ELISA test method was validated for linearity, specificity (lack of interference from diluent buffer, the other two antigens, and Strangvac vaccine), precision and robustness. Additional information has been provided to answer several questions regarding the ability of the ELISAs to distinguish between intact and potentially non-immunogenic truncated or aggregated forms of antigens. Furthermore, the suitability of the ELISAs to control the actual potency (*i.e.* the relevant immunogenicity) of the bulk antigens at release and during shelf life was adequately justified.

The purpose of the SDS-PAGE analysis is to analyse identity and purity of the antigens, as well as identity of the antigens in the final product. The SDS-PAGE was validated for specificity and limit of detection. A number of questions were raised which have all been solved.

Residual content of HCP is analysed using a commercial ELISA kit, and residual DNA is analysed using a commercial quantitative PCR test kit. Both methods are validated.

Data from in-process control testing and antigen testing are presented for three commercial-scale batches of finished antigen for each component CCE, Eq85 and IdeE; overall, compliance with current specifications is demonstrated.

Control tests on the finished product

Control tests of Strangvac finished product include analyses of general characteristics, identification, batch titre/potency, product impurities, identification and assay of adjuvant, sterility and purity and dose volume. A final product specification including acceptance limits is provided. The proposed specifications are overall acceptable and are considered in line with Ph Eur 0062 *Vaccines for veterinary use*. Acceptance limits are considered adequately justified. However, an issue on the Mw of the individual antigen bands remains; this will be handled as post authorisation recommendation.

The finished product specification does not include tests for host cell DNA or host cell protein (HCP). This is acceptable since controls for DNA and HCP are performed at the active substance level and since the applicant has justified that a worst-case content of residual HCP in each of the three antigens would not lead to a total level of HCP posing safety concerns in the final product. For DNA, the validation demonstrated that DNA is effectively removed during downstream processing and the proposed acceptance criterion for residual DNA of the individual antigens is very low; it is therefore considered acceptable that no specification for total residual DNA is included in the finished product specification.

Brief descriptions of test method are provided for all methods and detailed SOPs are appended for non-pharmacopoeia methods. The tests for clarity and degree of opalescence, degree of coloration, visible particles, identity, pH, sterility, and endotoxin are carried out according to Ph. Eur. and test for method suitability was performed for all test methods. All methods are considered acceptable. Non-pharmacopoeia methods are validated. However, the current and candidate reference materials should be reanalysed according to the revised specifications and methods; this will be handled as a post authorisation **condition** (see Conclusion).

Potency

Antigen potency in the finished product is measured by capture ELISA: The vaccine is formulated to contain a fixed quantity of CCE, Eq85, and IdeE antigen per batch, and upper and lower release specifications are set based on studies of efficacy and safety, as well as stability studies. The potency is controlled by measuring the content of the individual antigens (CCE, Eq85, and IdeE), in µg/ml, against a reference standard of each antigen. The methods of analyses are the same as those used for the individual bulk antigens CCE, Eq85, and IdeE, respectively; the ELISA assays are validated as described above (refer to 'Control tests during the manufacturing process' above). The ability of the ELISAs to distinguish between intact and potentially non-immunogenic truncated or aggregated forms of antigen was questioned; accordingly, the suitability of the ELISAs to control the actual potency (*i.e.* the relevant immunogenicity) of the finished product at release and during shelf life was questioned, entailing a risk of release of sub-potent batches and/or undetected decrease in potency during shelf-life of the vaccine. This has been adequately addressed by the applicant.

Antigen identity

An additional identification test (in addition to the potency tests using antigen-specific antibodies) and a test assessment of purity (degradation) in the finished product are performed. Questions were raised on the degree of purity of the antigens in the finished product and potential degradation during storage, and addition of the attribute 'Product impurities', including acceptance limits, was requested in the finished product and shelf-life specification. Upon request, specification limits for impurities have been added to the finished product release and stability specification. Furthermore, the applicant agrees that an orthogonal method to verify purity/integrity of the antigens should be implemented for release testing; thus, it is stated, that analytical development of another method is ongoing, and the method will be validated for release analyses; implementation of the additional analysis for batch release and stability testing will be handled as a post authorisation recommendation.

Batch-to-batch consistency

Batch data for three finished product batches have been provided. All batches were manufactured at 3P Biopharmaceuticals (antigens) / LIOF-Pharma S.L (finished product) and covered the proposed batch size. One batch is designated 'non-cGMP'; however, it is stated that apart from the scale, the manufacturing process was identical to the 70L-scale process but as the batch was not QP-released it is not considered a cGMP batch. Batch data for in-process results for upstream processes of antigens, and from three constitutive batches of each antigen (the ones which are part of the above finished product batches) can be found in provided in Part 2B of the dossier. In general, the batch results submitted fulfil the proposed in-process control specifications, antigens specifications, and specifications for finished product. Batch-to-batch consistency is considered demonstrated and in compliance with the *GL on data requirements for IVMPs intended for MUMS/limited market* EMA/CVMP/IWP/123243/2006-Rev.3. Certificates of analyses have been provided for each finished product batch. Full batch protocols including results of all tests performed during production, as well as on the finished product, have been provided.

Stability

Antigens

The proposed shelf life for all antigens is 36 months when stored at +2 to +8 °C. For each of the three antigens CCE, Eq85 and IdeE, 12/18 months stability data has been provided for two GMP process validation batches and 18 months data for one non-GMP process qualification batch. The provided

stability data would support 18 months stability for CCE and IdeE antigen. However, for the Eq85 antigen stability has been demonstrated for 9 months only as the percentage of truncated variants exceeds the specification at several occasions after 12- and 18 months of storage.

As for the impact of aged antigens on the vaccine stability, the applicant has confirmed that stability studies will be performed for finished product formulated with antigens at, or close to, the end of antigen shelf life, and that data from these studies will be provided; this will be handled as post authorisation recommendation.

Finished product

The proposed shelf life for the final vaccine is 2 years when stored at +2 to +8 °C. Currently, 12 months stability data have been provided for the two GMP process validation batches and 18 months data for the non-GMP process qualification batch for long-term (+2 to +8 °C) storage conditions. 6 months data were provided for accelerated conditions. In addition, 24 months supporting stability data have been provided for one clinical batch (comparability demonstrated), and two early development batches. Overall, based on *Guideline on data requirements for immunological veterinary medicinal products intended for MUMS/limits marked* (EMA/CVMP/IWP/123243/2006-Rev.3), the provided stability data could support 24 months stability for finished product.

However, confirmation has been requested that the real time stability studies will be finalised, and that data will be provided immediately to the competent authorities if outside specifications or potentially outside specifications at the end of the approved shelf life (with proposed action); this will be handled as a post authorisation recommendation.

Data on in-use stability have not been provided and the relevant section of the SPC includes the statement: 'Shelf life after first opening the immediate packaging: use immediately'.

Overall conclusions on quality

Strangvac is a suspension for injection, containing three recombinant proteins, intended for active immunization of horses against equine strangles. The finished product is a suspension for injection presented in single-dose glass vials.

The antigens are produced, using recombinant DNA technology, in three different *E. coli* host strains. The manufacturing is based on a seed lot system for each *E. coli* construct. The manufacture, testing, and storage of the cell banks is overall adequately described and documented, and compliance with the requirements of Ph. Eur. 0062 *Vaccines for veterinary use* and Ph. Eur. 0784 '*Recombinant DNA technology*, products of' is demonstrated. The quality of the other starting materials is considered adequate and the risk of TSE transmission is considered negligible.

The antigen manufacturing processes for the three antigens are almost identical and are considered to be standard. Information on the development, manufacture and control of the active substances and the finished product has been presented in a satisfactory manner. The manufacturing process including in-process controls and quality controls on the antigen bulks and finished product are described in sufficient detail and are overall considered adequate. Based on the data from three consecutive finished product batches, batch-to-batch consistency is considered acceptable. Compliance with Ph. Eur. monographs 0062 *Vaccines for veterinary* use is generally considered demonstrated.

One **condition** for marketing authorisation, concerning re-analyses of the current finished product reference material, has been recommended.

The provided data from stability studies would support 18 months stability for CCE and IdeE antigens, whereas for Eq85 only 9 months stability has been demonstrated. The data provided for the finished

product are sufficient to justify a 24 months shelf life when the vaccine is stored at 2 °C – 8 °C. Regarding the impact of aged antigens on the vaccine stability, the applicant has confirmed that stability studies will be performed for finished product formulated with antigens at, or close to, the end of antigen shelf life. This will be handled as post authorisation recommendation.

As the vaccine does not contain a preservative and a study of the efficacy of the antimicrobial activity has not been conducted, the vaccine is intended to be used immediately after first opening.

The quality of the product is described in sufficient detail and is overall considered adequate. One condition for marketing authorisation, concerning re-analyses of the current finished product reference material, is recommended. There are also some issues that will be addressed as post-authorisation recommendations.

Part 3 – Safety

Introduction and general requirements

Strangvac is an adjuvanted vaccine for the active immunisation of horses for:

- Reduction of body temperature increase, coughing, difficulty swallowing, and signs of depression (inappetence, changes in demeanour) in the acute stage of infection with *Streptococcus equi*.
- Reduction in number of abscesses within submandibular and retropharyngeal lymph nodes.

The vaccine contains three recombinant proteins as active ingredients and the adjuvant Matrix V, as described in Table 1 and in the Summary of Product Characteristics (SPC).

Two of the antigens, CCE and Eq85, are chimeric recombinant proteins, which contain amino acid sequences from a total of seven different cell-surface proteins from *S. equi*. The third, IdeE, is an IgG endopeptidase also stemming from *S. equi*. Matrix V comprises purified Quillaia saponin QS-21 (fraction C) formulated in immune-stimulating complexes (ISCOMs) with cholesterol and phosphatidyl choline. Matrix C was renamed Matrix V following vaccine development. The adjuvant is referred to as Matrix V throughout the following text.

The vaccine is presented as a suspension for injection and the proposed route of administration is intramuscular (i.m.).

The vaccine is presented in the proposed SPC for vaccination of horses from 8 months of age. The primary course is two doses administered four weeks apart which should induce protection for at least two months.

The dose used for the safety studies was the quantity of the product that is recommended for use (2 ml).

The safety of Strangvac was evaluated on the basis of following general requirements:

- requirements for immunological veterinary medicinal products as described in the Directive 2001/82/EC (amended by Directive 2004/28/EC and Directive 2009/9/EC),
- guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010-Rev.1),
- monographs 04/2013:0062 ('Vaccines for veterinary use') and 04/2013:50206 ('Evaluation of safety of veterinary vaccines and immunosera') of the European Pharmacopoeia (EP), and the
- guideline on veterinary medicinal products for minor use, minor species (EMA/CVMP/IWP/123243/2006-Rev.3).

The studies were carried out in accordance with the principles of good laboratory practice (GLP), although the facilities where the studies were carried out were not GLP-compliant.

The presented safety study was carried out in Swedish standardbred horses. The vaccine was administered by the recommended route (i.m.) to horses approximately 7 (6.3–9.1) months old at first vaccination. Three doses of the vaccine were administered within a 4-week interval, and the last (fourth) vaccination was given following a 2-week interval. The dosing paradigm according to recommendations stated in Ph. Eur. monograph 'Evaluation of safety of veterinary vaccines and immunosera', stating that the number of administrations must be not less than the maximum number recommended; for vaccines, this shall take account of the number of administrations for primary vaccination and the first revaccination. Since primary vaccination should be repeated, as no single dose

revaccinations has been documented, the safety of administering four separate doses is considered to comply with the Ph. Eur. monograph 'Evaluation of safety of veterinary vaccines and immunosera'. The animals were observed and examined for 14 days after each vaccination for signs of systemic or local reactions.

A single batch of vaccine, GMP-compliant was used. This batch was close to minimum potency and adheres to the final formulation of the vaccine. This is considered acceptable according to EMA/CVMP/IWP/123243/2006-Rev.3 as: *"Laboratory safety studies for inactivated IVMPs may be combined with laboratory efficacy studies and, therefore, standard batches may be used with no requirement to demonstrate the safety with batches formulated with maximum antigen content."*

In addition, five combined safety and efficacy studies have been presented. These laboratory studies were carried out in Welsh Mountain ponies at the Animal Health Trust in the UK.

The two vaccine batches for use in the combined safety and efficacy trials were prepared at IDT-Biologika, Germany (Table 11). Only one batch of each antigen (CCE, Eq85 and IdeE) was prepared, and these were combined into a bulk vaccine that was used to prepare both vaccine batches. It is stated in the scientific advice provided on October 2019 that, in principle, this approach was considered to be acceptable for a MUMS product.

During the manufacturing transfer from IDT Biologika to 3P Biopharmaceuticals/LIOF-Pharma S.L., some changes were made to the downstream purification processes of the three antigens to improve robustness and yield. In addition, a detergent was added to each antigen in the final formulation step, so the final formulation of the vaccine has changed. The approach to demonstrate comparability of vaccine batches at both sites was addressed by requesting scientific advice, which was given on 10 October 2019.

A comparability study has been performed between Strangvac batches manufactured at IDT Biologika and at 3P Biopharmaceuticals/LIOF-Pharma S.L. The comparability study is presented in part II of the dossier. Based on quality issues[1], the results could indicate that the clinical trials which were performed with the IDT batch are still suitable (IVV-OCR-091).

Additionally, the safety of the vaccine was evaluated in laboratory studies for efficacy.

These studies were carried out in consideration of the requirements stipulated in Ph. Eur. monograph 04/2013:50206 ('Evaluation of safety of veterinary vaccines and immunosera'), GLP principles and according to good clinical practice (VICH guideline GL9, CVMP/VICH/595/98). The facilities were not GLP-compliant (stated in part 3.B). Animal welfare provisions were observed in all tests.

A study for the investigation on residues is not relevant for the vaccine Strangvac (see section MRL below).

No studies were carried out to test the interactions of Strangvac with other veterinary medicinal products.

Comments to the importance of the vaccine in the context of ecotoxicity testing are given in part 3.D.

Composition of the vaccine

Table 1 shows the general composition of the vaccine Strangvac used for the safety testing. The composition corresponds to that given in part II A of the dossier.

[1] The comparability study was based on results of in-process testing, final release testing, stability testing, immunological cross reactivity testing, endotoxin testing and data generated with several analytical methods, e.g. mass spectrometry, SDS-PAGE and western blotting, SDS-PAGE and gel scanning analysis, SEC-HPLC and a host cell protein (HCP) ELISA.

The batches of the vaccine used for testing in the safety studies are a batch with close to minimum potency and two batches at minimum potency.

Indication and administration of the vaccine:

Strangvac is intended for the active immunisation of horses from the age of 8 months onwards to reduce clinical signs in the acute stage of infection with *S. equi* and to reduce the number of abscesses within submandibular and retropharyngeal lymph nodes.

The vaccine is intended for i.m. injection to be applied as follows:

Primary vaccination:

1st immunisation: 2.0 ml i.m. from the age of 8 months on,

2nd immunisation: 2.0 ml i.m. 4 weeks later

Onset of immunity:

Two weeks after the second vaccination dose.

Duration of immunity:

Two months after the second vaccination dose.

The vaccine is intended for use in horses for which a high risk of *Streptococcus equi* infection has been clearly identified from areas where this pathogen is known to be present.

Safety documentation

Strangvac is an adjuvanted vaccine intended for i.m. administration to horses from the age of 8 months. Two doses of the vaccine should be administered 28 days apart to induce active immunity against *S. equi*.

The active ingredients are recombinant proteins, which are not infectious.

Laboratory tests

Study title
A randomized, double-blinded and placebo-controlled study on efficacy of vaccination with Strangvac against *S. equi* challenge; 2 weeks onset of immunity study (8-11 months, mean age 10.9 months at first vaccination)
A study on the efficacy of vaccination with Strangvac against *S. equi* challenge; 2 months duration of immunity (4-6 months, mean age 5 months at 1st vaccination)
A randomized, double-blinded and placebo-controlled study on efficacy of vaccination with Strangvac against challenge; 2 weeks onset of immunity following three vaccinations 'revaccination study' (4-6 months, mean age 5 month at 1st vaccination)
A pilot study on the immunological response of basic vaccination with Strangvac, plus revaccination (mean age 11 months at 1st vaccination)
A pilot study on the immunological response of basic vaccination with Strangvac, plus revaccination (mean age at 1st vaccination 26 months)
Safety study in young Swedish standardbred horses following vaccination with the recombinant vaccine STRANGVAC (mean age 7.5 months at 1st vaccination)

Developmental studies:

Study title
Efficacy of Vaccination against *S. equi* Challenge
Streptococcus equi vaccination study
Streptococcus equi vaccination study
Efficacy of vaccination against *S. equi* challenge
Efficacy of vaccination against *S. equi* challenge
Efficacy of vaccination against *S. equi* challenge
Efficacy of vaccination against *S. equi* challenge

Safety of the administration of one dose

Safety study in young Swedish standardbred horses following vaccination with the recombinant vaccine STRANGVAC against strangles.

Strangvac was investigated in the safety study AB19-03 using the final formulation of the vaccine (batch GMP-compliant, close to minimum potency). The Strangvac vaccine was administered at four timepoints to 12 young Swedish standardbred horses (six females and six males). Two 'sentinel' horses were left untreated. The horses were reportedly approximately 6–9 months of age at the start of the study. No animals demonstrated increases in body temperature to 39 °C and above for two days or

more following vaccination, although a number of horses presented with transient increases in body temperature of 39 °C and above for one day. The range of normal body temperature in horses is between 37.5 °C to 38.5 °C.

Vaccine administration was very commonly associated with mild to moderate swelling of the injection site (up to 7 cm in diameter), which in some cases was associated with pain. The injection site reactions were transient and lasted for up to five days.

Throughout the study, animals presented intermittent submandibular lymph node swelling, serous and sometimes purulent nasal discharge and cough. These changes in health observations were made before vaccinations, and also included the two untreated sentinels.

No animals demonstrated significant increases in plasma fibrinogen concentration following vaccination. Several horses demonstrated increased neutrophil counts following vaccination. However, as increases were also evident in the male sentinel at the end of the study, it is not possible to ascertain whether increases were vaccine-related.

Transient swellings of the injection site after repeated intramuscular administrations of Strangvac were observed.

Issues concerning minimum potency of the vaccine batch used and health of the enrolled horses before vaccination and during the study have been satisfactorily addressed. The study is considered to be acceptable considering the MUMS status of the application.

Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines.

Safety of the repeated administration of one dose

Please refer to section B.1 above, as the study to demonstrate the safety of a repeated dose administration is identical to the study to show safety of a single administration.

The studies with the vaccine Strangvac have also been submitted in this part of the dossier as well as in part 4, efficacy.

In one study, 16 male and 16 female ponies were randomised into two groups. Sixteen ponies were vaccinated with Strangvac batch and a control group of 16 ponies was vaccinated with a placebo vaccine batch on day 0 of the study (V1). A second dose of each vaccine was administered 28 days after the first vaccination (V2). All personnel were blinded throughout the study, except for the quality assurance manager who allocated doses. Vaccine batches (GMP) prepared at IDT Biologika were used. These batches were comparable to final formulation of the vaccine.

Safety parameters related to the administration of the vaccine were investigated. Rectal temperatures, clinical observations and injection sites were investigated.

Serum samples were taken at intervals and analysed for IgG titres by using an ELISA method (CCE, Eq85 and IdE). Some ponies had measurable titres of IdE antibodies at the start of the study, which were suggested to represent cross-reactivity with of the test system with *Streptococcus zooepidemicus*. On day 13 post 2nd vaccination, serum samples were tested in an iELISA for exposure to *Streptococcus equi*, and no antibody titres were detected in vaccinated ponies. Nasal swabs were taken at intervals and analysed by qPCR to detect *S. equi* DNA. Swellings were detected in vaccinated ponies after 1st and 2nd vaccination, some associated with heat and pain. Details from maximal temperature increase in individual animals, size of swellings and characteristics of heat are covered in the SPC. One pony scored

poorly for demeanor and feed intake on day 1 post 2nd vaccination, this is covered in the SPC. Ocular scores in the vaccination phase are also covered in the SPC.

Another study tested the two-dose primary vaccination scheme. An unbalanced and limited number of ponies was included in this study (12 ponies in the vaccine group and only 4 placebo vaccinated animals).

A number of ponies had raised temperatures during a period between day 1 and day 6 post 1st vaccination and for the first two days after 2nd vaccination. Swellings at the injection site were detected in vaccinated ponies after the 1st and 2nd vaccination, some associated with increased injection site temperature and pain. Rectal temperatures were only considered elevated, by the applicant, when equal or higher than 39 °C. Details from maximal temperature increase in individual animals, size of swellings and temperature increase at the injection site are covered in the SPC. Ocular and nasal scores occurred in the vaccination phase. One pony scored poorly for demeanor and feed intake on day 1 post 2nd vaccination.

During the vaccination phase of the trial, the ponies experienced an episode of respiratory disease after the second vaccination. Elevated ocular and cough scores were observed in all ponies due to disease spreading among the ponies. *S. zooepidemicus* was detected in a few tested animals, while the etiology of the disease was not investigated further. Briefly, nasal discharge symptoms were identified in 5 ponies during the pre-second vaccination phase, and the vaccination schedule was continued as planned. The nasal discharge became more purulent and 15 ponies became pyretic. *S. zooepidemicus* DNA was recovered from 4 ponies. All ponies were treated with a 5-day course of penicillin by i.m. injection. This resolved the signs in the majority of ponies, but clinical signs persisted in 7 animals. These ponies were given an additional 5-day course of trimethoprim-sulfonamide orally (p.o.).

In a third study, 32 ponies, equally divided into vaccinated and placebo groups, were administered one dose of Strangvac vaccine and placebo, respectively, at day 0 (V1). A second dose (V2) was administered 28 days post V1 and a third dose (V3) 91 days (13 weeks) post V2. One of the placebo-vaccinated control ponies was withdrawn on day 98, unrelated to study objectives. Following vaccination, disease outbreaks among the ponies confounded the study. Animals were treated with antibiotics at least twice.

Safety parameters related to administration of the vaccine were investigated. Rectal temperatures, clinical observations and injection sites were investigated. Serum samples and nasal swabs were taken at intervals and analysed for IgG titres by using an ELISA method (CCE, Eq85 and IdE).

Signs of disease were observed in 14 of 16 Strangvac-vaccinated and in 11 of 16 placebo-vaccinated ponies in the period prior to and after the second vaccination, which manifested as fever as well as elevated ocular, cough and nasal scores. Otherwise, these manifestations are some type of adverse reactions related to the vaccine components. The only explanation provided by the applicant was that these clinical signs reflected mild *S. zooepidemicus* upper airway infections. No diagnostic samples were presented to support this hypothesis.

Three Strangvac-vaccinated ponies, but no control ponies, became pyretic with a rectal temperature ≥ 39°C on two out of three consecutive days during the three days immediately after the second vaccination. The clinical signs of respiratory disease had resolved by the time of administration of the third vaccination. Two Strangvac and no control ponies showed transient elevated temperatures for one day following the third vaccination.

Some ponies experienced transient pain, heat and swelling after the first, second and third vaccination doses, and poor demeanor and feed intake scores one day after the 1st vaccination. Nasal and cough scores in the vaccination phase were not scored as adverse events. However, ocular scores were at

significantly higher levels in the vaccinated ponies compared to the placebo controls; ocular manifestations are considered adverse events and stated in the SPC.

A pilot study on the immunological response of basis vaccination with Strangvac, plus re-vaccination has been presented. Injection site reactions and transient rises in body temperature were very commonly observed.

A pilot study on the immunological response of basic vaccination with Strangvac, plus revaccination has been presented. This study investigated whether a reduced amount of adjuvant used generates a significant different immunological response. An increase in injection site reactions appeared after second and third vaccination compared with first vaccination.

Developmental studies

Seven developmental studies related to Strangvac have also been submitted in part 3 of the dossier as well as in part 4. Overall, for the developmental study, differences in vaccine composition, administration route and the vaccination schedule preclude direct comparisons or any conclusions on safety of the final vaccine used in the pivotal trials. Please refer to part 4 B for assessment of these studies.

Examination of reproductive performance

The safety of the vaccine on reproductive performance was not determined.

This is considered acceptable.

No reproductive studies were provided, and the product is not indicated to be used during pregnancy. Section 4.7 of the SPC has been updated accordingly to provide a statement concerning the use during pregnancy and lactation as follows: 'The safety of the veterinary medicinal product has not been established during pregnancy or lactation. In the absence of data, the use of this vaccine is not recommended'. In addition, the following statement occurs in the SPC: 'The safety and efficacy of the vaccine has not been established in breeding animals. The vaccine should be used only according to the benefit-risk assessment by the responsible veterinarian.'

Examination of immunological functions

No specific investigations were carried out on the effects of Strangvac on immunological functions. The investigations are not considered necessary for this recombinant proteins vaccine.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with the CVMP 'Guideline on user safety for immunological veterinary medicinal products' (EMEA/CVMP/IWP/54533/2006).

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are those of accidental self-injection and dermal and/or oral exposure. The active substance is an inactivated protein and is not infectious.

The excipients, including adjuvants, may provoke an allergic reaction, which should be treated symptomatically. Strangvac contains saponins, which have little toxicity for humans when ingested but have haemolytic effects when injected intravenously.

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

In case of accidental self-injection, seek medical advice immediately and show the package leaflet or the label to the physician. An allergic reaction may occur. Treat symptomatically.

Additionally, the following text is included in the product information:

- For animal treatment only — to be supplied only on veterinary prescription.
- Keep out of the sight and reach of children.

Based on the above risk assessment, the conclusion is that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

Strangvac is an inactivated adjuvanted vaccine without any component in a concentration that may pose a risk to human health.

The active substances which are of biological origin are not within the scope of the Regulation (EC) No 470/2009. The vaccine does not contain a preservative. It contains purified Quillaia saponin QS-21, cholesterol and phosphatidyl choline as adjuvants.

Quillaia saponin QS-21

A study for the investigation on residues is not relevant because the adjuvant Quillaia saponin QS-21 is considered as not falling within the scope of Council Regulation (EC) 470/2009. Quillaia saponin QS-21 is not a source of harmful residues.

Cholesterol and phosphatidyl choline

For cholesterol and phosphatidyl choline MRLs are not necessary since (1) cholesterol is a normal constituent of animal tissue including that of horses, and (2) phosphatidyl choline is an EFSA-approved food additive which was re-evaluated as safe for the consumer in 2017 (E322 lecithins; EFSA J. 2017;15[4]:4742).

In conclusion, Strangvac will not give rise to residues which will be harmful for the consumer. No withdrawal periods are considered to be necessary before vaccinated horses are slaughtered for human consumption.

Excipients

The excipients included in the product do not raise any safety concern.

MRLs

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

The excipients, including adjuvants, listed in section 6.1 of the SPC are either allowed substances for which Table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

In conclusion, Strangvac will not give rise to residues which will be harmful to the consumer. No withdrawal periods are considered to be necessary before vaccinated horses are slaughtered for human consumption.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

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

This is considered acceptable.

Field studies

None submitted. This is acceptable considering the MUMs status of the product.

Environmental risk assessment

The environmental risk assessment was carried out according to the CVMP guideline 'Environmental risk assessment (ERA) for immunological veterinary medicinal products' (EMEA-CVMP/074/95).

Phase 1:

Strangvac is an adjuvanted vaccine for the active immunisation of horses for:

- Reduction of body temperature increase, coughing, difficulty swallowing, and signs of depression (inappetence, changes in demeanour) in the acute stage of infection with *Streptococcus equi*.
- Reduction in number of abscesses within submandibular and retropharyngeal lymph nodes.

The vaccine contains three recombinant proteins as active ingredients. Strangvac does not contain any live organisms as active substance and is tested for the absence of any infectious organisms.

The phase I assessment made by the applicant allows the following conclusions:

Strangvac does not contain any live organisms, thus shedding of live organisms will not occur.

Strangvac is administered by intramuscular injection. If the vaccine is used according to the SPC, the potential exposure to the environment is considered negligible.

The use of the vaccine does not lead to any residues that could cause harm to the environment.

The vaccine does not contain any components of toxic or pathogenic concerns.

Since no hazards concerning the environment are indicated, no consequences need to be considered.

Since no precautions need to be taken, a phase II assessment is not deemed necessary.

Considerations for the environmental risk assessment

Based on the data provided, the ERA can stop at phase I. Strangvac is expected to pose a negligible risk to the environment when used according to the SPC.

Overall conclusions on the safety documentation

Taken together, the 6 trials presented on the safety of Strangvac performed in 12 Standardbred horses and 87 Welsh Mountain ponies, clinical signs were observed in the animals within the vaccination phase.

Appropriate wording for the relevant sections of the proposed SPC has been provided, including frequency, severity, duration and characteristics of the manifestations:

- A transient increase in body temperature of up to 2.6°C for one to five days is very common following vaccination.

- Transient local tissue reactions at the injection site, characterised by heat, pain and swelling (approximately 5 cm diameter) are very commonly seen and last for up to five days. Frequency of injection site reactions are more pronounced after the second primary dose and further doses and increased swelling of up to 8 cm diameter can occur.

- Loss of appetite and demeanour changes for one day are common.

- Ocular discharge which may be mucopurulent and present from both eyes is very commonly seen for one to five days after vaccination.

Overall, the accumulated results concerning adverse reactions, which have been reported from investigations of safety parameters after vaccine administrations, are considered covered fully in the wording of section 4.6 of the SPC.

Justification for the use of batches overall at minimum potency is considered acceptable, considering the 'Guideline on veterinary medicinal products for minor use, minor species' (EMA/CVMP/IWP/123243/2006-Rev.3).

Strangvac will not give rise to residues which will be harmful to the consumer. No withdrawal periods are considered to be necessary before vaccinated horses are slaughtered for human consumption. The vaccine is considered to be safe for the environment.

Part 4 – Efficacy

Introduction and general requirements

Strangvac is an adjuvanted vaccine for the active immunisation of horses to reduce clinical signs in the acute stage of infection with *S. equi* and to reduce the number of abscesses within submandibular and retropharyngeal lymph nodes.

The vaccine contains three recombinant proteins as active ingredients and the adjuvant system.

Two of the antigens, CCE and Eq85, are chimeric recombinant fusion proteins, which contain amino acid sequences from a total of seven different cell-surface proteins from *S. equi*. The third antigen, IdeE, is an IgG endopeptidase, also from *S. equi*. The adjuvant system comprises purified Quillaia Saponin QS-21 (Fraction C) formulated in immune stimulating complexes (ISCOMs) with cholesterol and phosphatidyl choline.

The efficacy of Strangvac was evaluated on the basis of the following general requirements:

Directives:

- Requirements for immunological veterinary medicinal products as described in the Directive 2001/82/EC (amended by Directive 2004/28/EC and Directive 2009/9/EC).

Guidelines:

- EudraLex - Volume 6 - Notice to applicants and regulatory guidelines for medicinal products for veterinary use.
- OECD Principles on Good Laboratory Practice.
- VICH GL9 (Good Clinical Practice)
- CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals)
- CVMP Note for Guidance on the use of adjuvanted veterinary vaccines.
- Guideline on veterinary medicinal products for Minor Use, Minor Species (EMA/CVMP/IWP/123243/2006-Rev.3).
- The relevant monographs of the European Pharmacopoeia (EP):
- "Vaccines for veterinary use" (04 /2013: 0062) and
- "5.2.7.: Evaluation of efficacy of veterinary vaccines and immunosera" (50207).

Indication and administration of the vaccine:

Strangvac is presented as a suspension for injection by the intramuscular route.

The indication is:

For active immunisation of horses from 8 months of age for:

- Reduction of body temperature increase, coughing, difficulty swallowing, and signs of depression (inappetence, changes in demeanour) in the acute stage of infection with *Streptococcus equi*.
- Reduction in number of abscesses within submandibular and retropharyngeal lymph nodes.

Onset of immunity: Two weeks after the second vaccination dose.

Duration of immunity: Two months after the second vaccination dose.

The vaccine is intended for intramuscular injection to be applied as follows:

Primary vaccination:

1st immunisation: 2.0 ml i.m. from the age of 8 months.

2nd immunisation: 2.0 ml i.m. 4 weeks later.

The vaccine is intended for use in horses for which a high risk of *Streptococcus equi* infection has been clearly identified from areas where this pathogen is known to be present.

Re-vaccination

Data for prolonged clinical immunity from the administration of single dose revaccinations is not available.

Therefore, in horses at high risk of *S. equi* infections it is recommended to repeat the primary vaccination regimen after two months.

Scientific advice was given concerning most aspects of the application (table 1 below);

3. SCIENTIFIC ADVICE REPORTS

EMA Scientific advice concerning the development of Strangvac has been sought on four occasions as summarized Table 1 and Strangvac Scientific Advice. Clarifications to responses from the scientific advice were sought on three occasions.

Table 1. EMA Scientific Advice Reports

Procedure	CVMP Meeting date	Response date	Procedure number
EMA Scientific Advice 1	5-17 June 2010	16 June 2010	EMA/CVMP/237830/2010 EMA/SAWP-V/056/2010
Clarification to Scientific Advice 1	14-16 September	16 September 2010	EMA/581224/2010
EMA Scientific Advice 2	14-16 May 2013	16 May 2013	EMA/CVMP/SAWP/95065/2013
Clarification 1 to Scientific Advice 2	8-10 October 2013	10 October 2013	EMA/CVMP/SAWP/495158/2013
Clarification 2 to Scientific Advice 2	5-7 May 2015	7 May 2015	EMA/CVMP/SAWP/195581/2015
EMA Scientific Advice 3	9-11 December 2014	11 December 2014	EMA/CVMP/SAWP/609665/2014
EMA Scientific Advice 4	8-10 October 2019	10 October 2019	EMA/CVMP/SAWP/405290/2019

The study protocols for the studies have been provided.

Efficacy documentation

Laboratory trials

All efficacy studies were performed in Welsh mountain ponies. A vaccine batch which were close minimum potency and comparable to final formulation (Strangvac batch; placebo batch) was used in the studies. All clinical trials were conducted using batches manufactured by IDT. The applicant provided a comparative quality study to show that the trials performed with the IDT batches are still suitable.

The experimental intranasal challenge model for *S. equi* in Welsh mountain ponies is based on a virulent strain, *S. equi* strain 4047 (Se4047), isolated from a horse with strangles in the New Forest, UK, in 1990 (Kelly et al., 2006, Guss et al., 2009, Hamilton et al., 2006). The complete genome sequence of this strain has been determined (Holden et al., 2009) as a reference genome for *S. equi*. The vaccine has not been tested against challenge with any other recent field strain of *S. equi*.

A total of 35 placebo ponies were included in the presented studies concerning onset and duration of immunity and revaccination. Pyrexia (defined as 39ºC and above for two out of three consecutive days) with lethargy was typically the first sign of infection occurring 3–14 days after exposure in the challenge model (Boyle et al., 2018).

Study title
A randomized, double-blinded and placebo-controlled study on efficacy of vaccination with Strangvac against *S. equi* challenge; 2 weeks onset of immunity study (8-11 months, mean age 10.9 months at first vaccination)
A study on the efficacy of vaccination with Strangvac against *S. equi* challenge; 2 months duration of immunity (4-6 months, mean age 5 months at 1st vaccination)
A randomized, double-blinded and placebo-controlled study on efficacy of vaccination with Strangvac against challenge; 2 weeks onset of immunity following revaccination study (4-6 months, mean age 5 month at 1st vaccination)
A pilot study on the immunological response of basic vaccination with Strangvac, plus revaccination
A pilot study on the immunological response of basic vaccination with Strangvac, plus revaccination

The comparability of vaccine doses manufactured at IDT-Biologika and final formulation (3P Biopharmaceuticals) has been studied with respect to quality parameters.

Developmental studies:

Study title
Efficacy of Vaccination against *S. equi* Challenge
Streptococcus equi vaccination study
Streptococcus equi vaccination study
Efficacy of vaccination against *S. equi* challenge
Efficacy of vaccination against *S. equi* challenge
Efficacy of vaccination against *S. equi* challenge
Efficacy of vaccination against *S. equi* challenge

Overall, for the developmental studies differences in vaccine composition, administration route, and the vaccination schedule preclude direct comparisons or any conclusions on safety and efficacy of the final vaccine used in the pivotal trials.

Challenge model

No studies have been presented on the establishment of the challenge model.

It is stated in file 4b-lab-trials that an experimental challenge model for *S. equi* in Welsh mountain ponies has been developed at the Animal Health Trust (AHT, Newmarket, UK). This model is based on a virulent strain isolated from a horse with strangles in the New Forest, UK, in 1990 (Kelly et al., 2006, Guss et al., 2009, Hamilton et al., 2006).

Dose determination

For the developmental study "Exploratory dose-finding study" differences in vaccine composition, and the vaccination schedule preclude direct comparisons or any conclusions on efficacy of the final vaccine used in the pivotal trials.

Importantly, a stepwise dilution of vaccine antigen and investigation for a potential clinically relevant and significant dose-effect association (and distinction between potent and subpotent batches) has not been identified after IM administration.

Onset of immunity

A randomised, double-blinded and placebo-controlled study on efficacy of vaccination with Strangvac against S. *equi* challenge; 2 weeks onset of immunity study

Study on **onset of immunity** tested the efficacy of the recommended two-dose primary vaccination scheme with challenge exposure 2 weeks after last administration of Strangvac vaccine.

In this study, 16 male and 16 female ponies were randomised into two groups. 16 ponies were vaccinated with Strangvac batch and a control group of 16 ponies were vaccinated with a placebo vaccine batch on day 0 of the study (V1). A second dose of each vaccine was administered 28 days after the first vaccination (V2). All personnel were blinded throughout the study, except for the quality

assurance manager who allocated doses. Vaccine batches (GMP) prepared at IDT Biologika, were used. These batches were comparable to final formulation of the vaccine.

Following challenge, ponies were monitored for the onset of clinical signs of disease over a period of 21 days based on the primary and secondary endpoints described below.

Challenge dose was between 2.0 x 10^8 CFU and 3.3 x 10^8 CFU of *S. equi* strain Se4047 per animal. Clinical signs were assessed pre- and post-vaccination according to a scoring system defined at the discretion of the applicant (e.g. lymph node scoring: normal/moderate /severe; demeanour scoring: normal/depressed/markedly depressed; injections site swelling: slight/moderate/severe) given in Table 2 below.

Table 2. Scoring system for clinical signs observed during AHT16.

Area		Observation	Score
Temperature		°C	Not applicable
Ocular		0=normal, 1=serous, 2=mucopurulent, bl=bilateral	0, 1, 2 or double
Nasal		0=normal, 1=serous, 2=mucopurulent, bl=bilateral	0, 1, 2 or double
Lymph		0=normal, 1=slight, 2=moderate, 3=severe, 4=abscessate, 5=sinus and drain, bl=bilateral	0, 1, 2, 3, 4, 5 or double
Cough		0=not present, 1=present, 2=marked	0, 1 or 2
Swallow		0=normal, 1=painful	0 or 1
Feeding		0=normal, 1=reluctant, 2=off feed	0, 1 or 2
Demeanour		0=normal, 1=depressed, 2=markedly depressed	0, 1 or 2
Injection site reaction	Heat	0=normal, 1=slight, 2=moderate, 3=severe	0, 1, 2 or 3
	Pain	0=normal, 1=slight, 2=moderate, 3=severe	0, 1, 2 or 3
	Swelling	0=normal, 1=slight, 2=moderate, 3=severe	0, 1, 2 or 3

The primary endpoint of the study was the proportion of ponies remaining free from pyrexia at day 21 following challenge. Ponies were classified as pyretic if the rectal temperature $\geq$39°C for two out of three consecutive days.

Secondary endpoints included number of days of pyrexia, as well as clinical observations (ocular, nasal, cough, lymph node, swallow, feeding, demeanour, and injection site scores), and blood measurements (fibrinogen, neutrophil levels).

Nasal swabs were taken for quantification of *S. equi* DNA shedding, using a triplex qPCR assay for eqbE and SEQ_2190 genes.

Ponies were euthanised at the end of the study (on day 21 after challenge) or on welfare grounds before end of the observation period if the demeanour and/or feeding score reached 2 or lymph node score was classed as 3 or above during the study. All 16 placebo-vaccinated ponies were euthanised on welfare grounds before the end of the study on days 6-14 post challenge, whilst 10 Strangvac-vaccinated ponies were euthanised on days 8-21 post challenge.

Post mortem findings were scored based on number/and or severity of the lesions according to a scheme, based on a scoring system defined at the discretion of the applicant (Table 3).

Table 3. Pathology scoring system.

Pathology	Score
Retropharyngeal, submandibular, cervical or tracheal/bronchial lymph node abscess	15
Retropharyngeal, submandibular, cervical or tracheal/bronchial lymph node microabscess	10
Empyema of guttural pouch	5
Scarring/Thickening of guttural pouch	5
Enlarged lymph node	1
Follicular hyperplasia of guttural pouch	1

All vaccinated ponies seroconverted for all three antigens and titres were increased after both vaccination doses for all three antigens.

None of the placebo ponies remained free from pyrexia during the 21 days following challenge (i.e. "pyrexia" definition for the studies: having body temperatures of ≥ 39°C on two days in any three-day period) compared with five of the 16 ponies that had been vaccinated with Strangvac. This finding was statistically significant using a two-sided Fisher's exact test (P=0.043). For this Onset of Immunity study 10 out of 16 vaccinated ponies were euthanised based on humane endpoint before end of trial period. Fourteen out of 16 vaccinated ponies developed one or more abscesses in the investigated submandibular and retropharyngeal lymph nodes at post mortem, which means that 12.5% of the vaccinated ponies were fully protected from lymph node abscesses and 31% were protected from the applicant's chosen pyrexia level (rectal temperatures of $\geq$ 39.0°C for two out of three days).

For the study, there was a wide age-span of ponies used. For example, the age of the ponies at first administration of the vaccine was reported from 7-12 months. The susceptibility to development of severe clinical disease after *S. equi* infection may, everything else equal, decline with age. A minimum age of 8 months has been adequately supported by the presented data.

This wide age-span is not considered in compliance with the principal of employing a worst-case scenario with use of MDA negative animals of minimum age.

Concerning the high incidence of lymph node abscesses in vaccinated ponies (P = 0.5, two-tailed Fisher's exact test) at post mortem), the applicant provided a post-hoc comparison of the median number of lymph node abscesses found in the two groups. Results pointed toward a significant difference in ponies vaccinated with Strangvac (median 2) compared with placebo-vaccinated control ponies (median 3), P = 0.011 (two-sided Mann-Whitney U test). However, due to the limited data points the statistical assumptions behind this test is not considered to have been convincingly met. Also, several submandibular and retropharyngeal lymph nodes examined at post-mortem contained 'micro-abscesses'. It remains unclear as to the fate of these micro-abscesses, to either be resolved by the lymph node or remain chronically infected as a potential future source of shedding/transmission.

The post-mortem scores for placebo ponies (55) were higher than that for vaccinated ponies (44). There was also a statistically significant difference in post-mortem scores when analysed using the Mann-Whitney text (P=0.0056).

There was a strong correlation between number of days to pyrexia and post-mortem score when Spearman's r was calculated (r=-0.8803, 95% confidence interval -0,9674 to -0,6071, P<0,0002).

Early euthanasia of ponies on welfare grounds took place, and a "last observation carried forward" (LOCF) approach was used. However, since the majority of the placebo ponies were euthanised early in the 21-day period after challenge, the statistics based on such scores do not appear to be a valid overall approach to evaluate efficacy endpoints for this challenge model in ponies.

The criteria for euthanasia are considered only moderately objective and robust, and the resulting large proportion of animals euthanised prematurely reduces significantly the quality of the obtained efficacy data.

In this study, the clinical scores were not found to be significantly different between vaccinated and placebos for all the clinical observations.

A trend is identified that the clinical signs associated with infection appeared to be delayed and at a lower average level in the Strangvac-vaccinated compared to placebo group.

As a supplement to the initial analysis of pyrexia, the primary endpoints, the applicant has introduced Kaplan Meier survival analysis. Based on data up to the time of euthanasia, Strangvac delayed the onset of clinical signs, primarily the defined pyrexia. This is considered a better analysis, based on the study design compared to the previous LOCF method, which employed repeating data on the day of euthanasia of mostly placebo ponies, to fill in the gaps to the end of the challenge at 21 days.

The clinical trials, specified by humane endpoint parameters, ended before any animals could demonstrate either the severity of clinical symptoms or likelihood/speed of recovery. An indication narrowed to the "acute stage" of clinical onset of disease more accurately expresses the data from this clinical trial.

Shedding of *S. equi* was reduced in Strangvac-vaccinated ponies compared to placebo when nasal swabs taken from the ponies were used to quantify the number of copies of *S. equi* DNA that were present using a triplex qPCR assay for the *eqbE* and SEQ_2190 genes. Only in a small number of ponies, *S. equi* DNA was detectable in the samples investigated, and differences were not statistically significant.

Notably, elevated ocular and cough scores were observed in all ponies due to disease spreading among the ponies during the vaccination phase. *S. zooepidemicus* was detected, while the aetiology of the disease was not investigated further by laboratory diagnostics.

The effect of vaccination on the later stages of the clinical infection, including the fate of the developed lymph node abscesses and further the recovery phase of the disease, have not been investigated. The SPC has been updated to narrow the indication to the acute stage of the infection.

Duration of immunity

A study on the efficacy of vaccination with Strangvac against S. *equi* challenge; 2 months duration of immunity

Study on duration of immunity tested the efficacy of the recommended two-dose primary vaccination scheme with challenge exposure 63 days after last administration of Strangvac vaccine in the challenge model also used for the onset of immunity trial. An unbalanced and limited number of ponies were included in this study (12 ponies in the vaccine group and only 4 placebo vaccinated animals).

Nasal discharge, pyrexia, ocular and cough scores were elevated again among ponies during the vaccination phase coinciding with administration of the second vaccination dose.

Safety parameters related to administration of the vaccine were investigated.

Serum samples and nasal swabs were taken at intervals and analysed for IgG titres by ELISA method (CCE, Eq85 and IdE). Swabs tested by qPCR for shedding. Two weeks post 2nd vaccination serum samples were tested in iELISAs for exposure to *S. equi*. None of the ponies tested positive for anti-SEQ_2190 or SeM antibodies. Some background levels of antibodies to IdE were detectable in both placebo and Strangvac ponies prior to vaccination. These results were suggested to be related to cross-

reacting antibodies against *S. zooepidemicus* or through the functional activity of IdeE that binds IgG non-immunologically.

Vaccinated ponies developed antibodies against CCE, Eq85 and IdeE after first vaccine dose; antibody titers increased after 2nd vaccination. Antibody kinetics in nasal swabs were similar to serology results, but with lower titres.

Sixty-three days after second vaccination challenge was applied with a dose between 1.7 x 10^8 CFU and 2.3 x 10^8 CFU per animal of *S. equi* strain Se4047 (somewhat less amount when compared to the study on onset of immunity). Monitoring for clinical signs of disease was carried out using the scoring and principles as for the Onset of Immunity study.

Ponies vaccinated with Strangvac responded with high levels of serum antibodies for all three antigens from seven days after the first vaccination (V1) and the serum antibody response peaked between one and two weeks after the second vaccination (V2). Nasal mucosa antibody levels were also elevated in the Strangvac-vaccinated ponies from 7 days after V1 and the levels peaked at seven days post-second vaccination.

Seven of twelve ponies vaccinated with Strangvac did not become pyretic (i.e. "pyrexia" as defined for the studies: having body temperatures of ≥ 39°C on two days in any three-day period) post-challenge, whereas all four placebo vaccinated ponies exhibited pyrexia. This was not a statistically significant difference when analysed with Fisher's exact test as specified in the study protocol (P=0.088).

Again, a trend could indicate that the average number of days-to-pyrexia (i.e. as defined for the study) was postponed for the Strangvac vaccinated ponies (14.7 days) compared to that of placebo vaccinated ponies (4.5 days). A Kaplan-Meier survival analysis for the time-to-onset of pyrexia was statistically significant. Considering the two tests together, it gives information both on the proportion of horses with pyrexia and the rate of onset of pyrexia. Survival analysis shows an effect of vaccination based on the actually observed clinical data sets, but is neither a tool to demonstrate differences in severity of symptoms nor duration of the symptoms. It has been explicitly taken into account that the observations were restricted to the acute stage of infection with *S. equi* (i.e. before maturation and rupture of either submandibular or retropharyngeal lymph nodes). The SPC has been updated to specify the indication to the acute stage of the infection.

There was a trend for reduced clinical signs of infection in Strangvac-vaccinated ponies for the secondary endpoints investigated.

The post mortem scores for placebo ponies (49) were higher than that for vaccinated ponies (17.2).

Overall, non-significant results for the primary endpoint, and ongoing spread of disease among the ponies in the vaccination phase were reported.

An unbalanced and limited number of ponies was employed in the trial set-up; non-significant results were obtained for the primary endpoint, four of 12 vaccinated ponies were euthanised for animal welfare reasons and half of the Strangvac ponies had lymph node abscessation at post mortem examination, with high numbers of copies of *S. equi* DNA copies found. Vaccination does not protect ponies from developing lymph node abscessation with *S. equi* colonisation when challenged 8-week post vaccination. The effect of vaccination on the later stages of the clinical disease, including the fate of the developed lymph node abscesses, and the recovery phase from the disease have not been investigated. The proposed SPC has been updated and the indication is specified to the acute stage of the infection. Wording has been added to the SPC on potential complications from vaccination and *S. equi* infection following challenge, which were not investigated. The following text has been added to the Section 4.4 of the SPC: Effect of vaccination on further stages of the infection, rupture of developed lymph node

abscesses, prevalence of subsequent carrier status, bastard strangles (metastatic abscessation), purpura haemorrhagica and myositis and recovery, is not known.
Therefore, continued after marketing authorisation monitoring of the application of the vaccine under field condition is strongly recommended especially the monitoring of possible long-term consequences for vaccinated horses at high risk, infected with *S. equi*, with special focus to potential associated complications like bastard strangles, purpura haemorrhagica, myositis, myocarditis and/or persistent carriers.
Efficacy has been demonstrated for the individual horse to reduce clinical signs of disease in the acute stage of the infection. Vaccinated horses can be infected and shed *S. equi*.

A randomised, double-blinded and placebo-controlled study on efficacy of vaccination with Strangvac against challenge against *S. equi* challenge; 2 weeks onset of immunity following revaccination

This study addressed protection conferred by vaccination with the recommended two-dose primary vaccination schedule together with a dose of Strangvac three months after the second dose of the primary vaccination schedule. In this study 32 ponies, equally divided into vaccine and placebo group, were administered one dose of Strangvac vaccine and placebo, respectively at D0 (V1). A second dose (V2) was administered 28 days post V1 and a third dose (V3) 91 days (13 weeks) post-V2. One of the placebo-vaccinated control ponies was withdrawn on day 98, unrelated to study objectives. Two weeks post-third vaccination the remaining 31 ponies were challenged by the administration of challenge doses of *S. equi* strain Se4047 in a range similar to those of the onset of immunity study (doses were between 2.1 x 10^8 CFU and 3.3 x 10^8 CFU of Se4047 per animal).

Fifteen of the 16 vaccinated ponies and two of the placebo ponies reached the study endpoint at 21 days post-challenge, while 12 of the 15 placebo ponies had been euthanised by day 12.

Again, disease outbreaks among the ponies confounded the study. Animals were treated with antibiotics at least twice. Study design was amended to allow animals to recover. Originally, the study was planned to investigate onset of immunity with challenge exposure 2 weeks after the second dose of the primary vaccination schedule. Instead, the ponies received a 3rd vaccination 3 months after primary vaccination and challenge was carried out 2 weeks after third vaccination.

None of the ponies vaccinated with the placebo control remained free from pyrexia during the 21 days following challenge compared with 15 of the 16 ponies that had been vaccinated with Strangvac. This finding was statistically significant using a two-sided Fisher's exact test ($P < 0.0001$) by per protocol statistical analysis.

Post-mortem scores for placebo ponies were statistically significantly higher than for vaccinated ponies when analysed with the Mann-Whitney's test ($P<0.0001$).

Strangvac-vaccinated ponies, but not placebo vaccinated ponies, developed a robust antibody response to the vaccine components from day seven post-first vaccination, which increased further following the administration of the second vaccine dose. Serum samples and nasal swabs were taken at intervals and analysed for IgG titres by using an ELISA method (CCE, Eq85 and IdE). None of the ponies tested positive for anti-SEQ_2190 or SeM antibodies in serum samples taken 2 weeks after the 2nd vaccination. Vaccinated ponies developed antibodies against CCE, Eq85 and IdeE after the 1st vaccine dose, and antibody titers increased after 2nd and 3rd vaccinations. Antibody kinetics in nasal swabs were similar to serology results, but with lower titres.

The response was boosted following the administration of a third vaccination three months post-second vaccination. Antibody levels in nasal mucus were also elevated in Strangvac-vaccinated ponies from day seven post-first vaccination and peaked at seven days post-second vaccination. Again, significant

disease outbreaks in the vaccination phase confounded the study and the originally planned challenge was postponed and trial set-up was modified.

There were potential confounding effects of significant disease outbreaks in the ponies within the vaccination phase. The effect of vaccination on the later stages of the clinical disease, including the recovery phase from the disease, has not been investigated. The proposed SPC has been updated to narrow the indication to the acute stage of the infection.

The 3rd vaccination took place 3 months after basis vaccination, while data to support a duration of immunity of 3 months have not been provided. This leaves outstanding issues concerning clinical protection, beyond a 2 months duration of immunity and behind the chosen vaccination schedule.

The vaccination schedule stated in the SPC is a two dose vaccination schedule.

Maternally derived antibodies (MDA)

No studies have been provided.

No information is available on the use of the vaccine in seropositive animals, including those with maternally derived antibodies.

Interactions

No studies have been provided. The SPC includes the following text in section 4.8.: 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.'

Field trials

No studies have been provided.

Pilot studies

A pilot study on the immunological response of basis vaccination with Strangvac, plus re-vaccination has been presented.

Analysis of immune responses were investigated with vaccine batch (not final, but comparable formulation) in study to evaluate the immunological response in Welsh Mountain ponies.

Antibody responses were investigated after primary vaccination with two doses of Strangvac followed by re-vaccination after three and six months (13 and 26 weeks) respectively after the second vaccination dose. Clinical signs were assessed pre- and post-vaccination according to the scoring system described in Table 2. The study was not blinded, and there was no placebo-control group.

The kinetics of the antibody responses indicated that levels did not decline to pre-immunisation levels during the study periods.

In conclusion, the results of this pilot study of immunological responses indicate that vaccinated ponies developed an antibody response to the vaccine antigens within 8 days post-first vaccination, which increased further following the administration of the second vaccine dose. This response was boosted

following the administration of the vaccine 3- or 6-months post-basis vaccination, and antibodies were detectable in both serum- and nasal swab samples.

Three ponies were seropositive against IdeE prior to the start of the study. The applicant proposes that these responses are most likely to cross-reacting antibodies primarily directed at *S. zooepidemicus*, which produces a homologue of IdeE or through the functional activity of IdeE, which binds to IgG non-immunologically. However, clear differences were apparent in the magnitude of antibody responses post-vaccination.

The study provides only supportive data on antibody responses after three vaccinations.

A pilot study on the immunological response of basic vaccination with Strangvac, plus revaccination has been presented.

This study investigates whether a reduced amount of adjuvant used generate a significant different immunological response. Furthermore, the study was used to demonstrate a booster effect following re-vaccination at week 56 after basic vaccination (D0, D28).

There was no clear difference in the slope of increase in antibodies against the components of the vaccine or the maximum levels of the antibodies after second and third vaccination.

It seemed like the higher amount of adjuvant gave a higher antibody titre after first vaccination for Eq85 and after first and second vaccination for IdeE.

Considering that this pilot study involved a very limited number of animals in each treatment group and the vaccine batch used differed from the composition of the final formulation, the results are not considered pivotal or supportive.

Overall conclusion on efficacy

The vaccine is intended for use in horses for which a high risk of *Streptococcus equi* infection has been clearly identified from areas where this pathogen is known to be present.

Efficacy of vaccination was demonstrated in studies using an experimental challenge model of the acute stage of the infection with the heterologous strain, *Streptococcus equi* 4047 (isolated in New Forest, UK in 1990).

In two pivotal studies, efficacy of the recommended two-dose primary vaccination schedule was investigated in ponies (from 8 months of age) following challenge with *S. equi* two weeks and two months after the second vaccine dose. Based on the findings of these studies, it is accepted that administration of the primary vaccination schedule (one dose by intramuscular injection, followed by a second dose four weeks later) resulted in reduced acute clinical signs compared to unvaccinated controls. Of the vaccinated animals,

- 43% (12 out of 28 ponies) remained pyrexia free (pyrexia defined as 39°C or above for two out of three days). The number of days with pyrexia was significantly lower in the vaccinated compared to non-vaccinated animals.

- 36% (10 out of 28) did not show signs of coughing.

- 43% (12 out of 28 ponies) did not show signs of difficulty in swallowing.

- 43 % (12 out of 28) did not show signs of marked depression (inappetence, marked change in demeanour) after challenge.

A third study has been provided on efficacy 15 days after 3 vaccinations, where the third vaccination was administered 3 months after primary vaccination (first 2 injections given with a 4-week interval). However, there were potential confounding effects of significant disease outbreaks in the ponies within the vaccination phase and animals were treated with antibiotics at least twice. Study design was amended to allow animals to recover. In addition, no data on duration of immunity are provided after three vaccinations. Therefore, these data were not considered an adequate basis to make a recommendation for a three-dose vaccination scheme.

There are insufficient data to support any revaccination schedule based on single dose administrations. Therefore, in the event that revaccination is considered appropriate (for animals at risk), it is recommended that the primary vaccination schedule is repeated.

Continued after marketing authorisation monitoring of the application of the vaccine under field condition is strongly recommended especially the monitoring of possible long-term consequences for vaccinated horses at high risk, infected with *S. equi*, with special focus to potential associated complications like bastard strangles, purpura haemorrhagica, myositis, myocarditis and/or persistent carriers.

Part 5 – Benefit-risk assessment

Introduction

Strangvac is a vaccine presented as suspension for injection containing recombinant proteins from *S. equi*. The vaccine is intended for use in ponies and horses.

Strangvac contains three novel recombinant proteins derived from the amino acid sequences of *S. equi* subspecies *equi* and the adjuvant Matrix V. The active substances are innovative.

The mode of action is stimulation of the immune system to generate adaptive specific immune response with the purpose of protection against clinical manifestation in the acute stage of infection with *S. equi* in horses.

The product has been shown to be efficacious for active immunisation of horses from 8 months of age for:

- Reduction of body temperature increase, coughing, difficulty swallowing, and signs of depression (inappetence, changes in demeanour) in the acute stage of infection with *Streptococcus equi*.
- Reduction in number of abscesses within submandibular and retropharyngeal lymph nodes.

Onset of immunity:

2 weeks after the second vaccination dose.

Duration of immunity:

2 months after the second vaccination dose

The vaccine is intended for use in horses for which a high risk of *Streptococcus equi* infection has been clearly identified from areas where this pathogen is known to be present.

The CVMP agreed on the above mentioned indications.

The vaccine is intended for intramuscular injection to be applied as follows:

Primary vaccination:

1st immunisation: 2.0 ml i.m. from the age of 8 months on

2nd immunisation: 2.0 ml i.m. 4 weeks later

The vaccine is intended for use in horses for which a high risk of *Streptococcus equi* infection has been clearly identified from areas where this pathogen is known to be present.

Revaccination:

Data for prolonged clinical immunity from the administration of single dose revaccinations are not available.

Therefore, in horses at high risk of *S. equi* infections it is recommended to repeat the primary vaccination regimen after two months.

Benefit assessment

Direct therapeutic benefit

S. equi is a pathogen transmitted between horses in Europe and worldwide.

Direct benefits could be expected in form of reduction of acute clinical signs in the acute stage of the infection with *Streptococcus equi* in horses and in reduction in number of abscesses within submandibular and retropharyngeal lymph nodes.

Additional benefits

Strangvac could provide a new prophylactic possibility against the acute stage of the infection with *Streptococcus equi* in horses (MUMS).

Risk assessment

Quality:

The quality of the product is described in sufficient detail and is overall considered adequate. One condition for marketing authorisation, concerning re-analyses of the current finished product reference material has been added as post-authorisation measure. There are also some issues that will be addressed post-authorisation as recommendations.

Safety:

Risks for the target animal:

All findings on adverse events are reflected in the SPC. Potential complications from vaccination and *S. equi* infection following challenge were not investigated. The SPC has been updated sufficiently on these issues (section 4.4 of the SPC).

Risk for the user:

The CVMP concluded that the low risk for the user is considered acceptable and sufficient safety advice has been included in the SPC.

Risk for the environment:

Strangvac is expected to pose a negligible risk to the environment when used according to the SPC.

Advice on waste disposal is included in the SPC.

Risk for the consumer:

No risks have been identified.

Special risks:

No special risks have been identified.

Risk management or mitigation measures

Evaluation of the benefit-risk balance

Overall, Strangvac presents an acceptable risk for users, the environment and consumers when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

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, potential complications from vaccination and *S. equi* infection following challenge were not investigated. Some adverse events were observed either very commonly or commonly in vaccinated animals. Strangvac presents an acceptable risk for users and the environment when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Conclusion

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

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

In addition, the CVMP has recommended one condition for the marketing authorisation:

The current Strangvac finished product reference material as well as the candidate reference material should be (re)characterised according to the current revised finished specification and using the updated methods for assessment of purity.