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SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for UBAC (EMEA/V/C/004595/0000)

Common name: Streptococcus uberis vaccine (inactivated)

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

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Introduction

The applicant Laboratorios Hipra, S.A. submitted on 20 February 2017 an application for a marketing authorisation to the European Medicines Agency (the Agency) for UBAC, through the centralised procedure under Article 3(2)a of Regulation (EC) No 726/2004 (new active substance).

The eligibility to the centralised procedure was agreed upon by the CVMP on 8 September 2016 as UBAC contains a new active substance Biofilm Adhesion Component (BAC) including Lipoteichoic acid (LTA) of *Streptococcus uberis (S. uberis),* strain 5616, which is not yet authorised as a veterinary medicinal product in the Community.

The applicant applied for the following indication:

For active immunisation of healthy cows and heifers to reduce the incidence and severity of mastitis caused by *S. uberis* and to reduce milk production losses after infection.

During the procedure the indication has been revised as follows:

For active immunisation of healthy cows and heifers to reduce the incidence of clinical intramammary infections caused by *S. uberis,* to reduce the SCC in *S. uberis* positive quarter milk samples and to reduce milk production losses after *S. uberis* intramammary infections.

UBAC is an inactivated bacterial subunit vaccine containing LTA within the BAC of *S. uberis* as active substance. The antigen is adjuvanted with Montanide ISA and Monophosphoryl Lipid A (MPLA). The target species is cattle (cows and heifers). The product is intended for intramuscular administration and is presented as emulsion for injection.

UBAC is to be available in single dose presentation of 2 ml filled in 3 ml colourless glass type I vials as well as in multidose presentations (5 doses, 25 doses and 50 doses) filled in colourless plastic containers made of polyethylene terephthalate (PET) with a capacity of 10 ml, 50 ml or 100 ml. UBAC is presented in packs containing either 20 single dose vials or 1 vial for each of the multidose presentations.

The rapporteur appointed is Esther Werner and the co-rapporteur is Ewa Augustynowicz.

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

On 25 May 2018, the CVMP adopted an opinion and CVMP assessment report.

On 26 July 2018, the European Commission adopted a Commission Decision granting the marketing authorisation for UBAC.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (version dated 02/03/2012) which fulfils the requirements of Directive 2001/82/EC was provided. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

UBAC is manufactured in the European Union by Laboratorios HIPRA, S.A. at two sites both located in Amer, Gerona, Spain. Manufacturing of the master and working seed stocks and quality control of the active substance take place at the Laboratorios Hipra, S.A. site Avda. La Selva, 135. The manufacturing of the active substance takes place at Hipra, S.A. site Carretera C-63. Manufacture of the final product is carried out at the Laboratorios Hipra, S.A. site Avda. La Selva.

Both sites have a manufacturing authorisation issued on 4 October 2016 by the National Competent Authority of Spain. GMP certificates which confirm the date of the last inspection (6 November 2015), are valid for three years and show that both sites, Laboratorios Hipra, S.A. site Avda. La Selva and Hipra, S.A. site Carretera C-63, are authorised for the manufacture and batch release of such veterinary dosage forms, have been provided.

A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit of the active manufacturer.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

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

Part 2 – Quality

Qualitative and quantitative particulars

The finished product is presented as emulsion for injection containing LTA from BAC of *S. uberis* as active substance with the quantity of Relative Potency (RP) ≥ 1 (ELISA) per dose. The product contains Montanide ISA and MPLA as adjuvant. Other ingredients as disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride and potassium chloride are included as excipients, as well as water for injections. The vaccine does not contain a preservative.

The product is available in vials as described in section 6.5 of the SPC.

Container and closure

The product is filled in PET containing 10 ml (5 doses), 50 ml (25 doses) and 100 ml (50 doses). The 1 dose presentation is filled in 3-ml glass type I vials. The containers are closed with rubber stoppers (nitrile for the presentation of 3 ml and chlorobutyl nitrile for the 10 ml, 50 ml and 100 ml presentations) and sealed with anodised aluminium capsules in order to ensure the correct closure of the stoppers.

The glass containers and rubber stoppers are in compliance with European Pharmacopoeia (Ph. Eur. chapters 3.2.1 and 3.2.9). For the PET containers information has been provided for demonstration of compliance with Ph. Eur. chapters 3.1.15 and 3.2.2. The sterilisation processes applied are suitable to ensure a sufficient innocuousness with respect to the risk of contamination due to container materials. Details regarding the dimension of the PET vials have been provided.

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

Product development

Adequate information has been provided on the choice of antigen, excipients, container-closure system, challenge model, overages as well as about vaccine production. To justify the vaccine composition with regard to the adjuvant and the addition of MPLA to the adjuvant system sufficient data have been provided. The inclusion of MPLA in the vaccine composition is considered acceptable because the safety profile is not abnormal for this kind of vaccine.

Reasonable justification is given regarding the suitability of the chosen vaccine strain. *S. uberis*, strain 5616, was selected as active component for the vaccine UBAC based on its ability to form biofilm *in vitro*. Published literature suggests that the colonisation of *S. uberis* into the mammary gland causing bovine mastitis may be related to the *S. uberis* ability of biofilm formation. Adhesion and biofilm production of Gram-positive bacterial pathogens is related to teichoic acids (TAs). Gram-positive bacteria contain two types of TAs; wall teichoic acid (WTA), which is covalently linked to the peptidoglycan layer and lipoteichoic acid (LTA) which is embedded in the membrane via lipid anchor. LTA is also involved in pathogenesis as it leads to increased biofilm formation, enhanced resistance to antimicrobial peptides and increased attachment to eukaryotic cells. Therefore, LTA is chosen as target antigen within the BAC. Information has been provided which allows the conclusion that the immunogenicity of the vaccine is mainly induced by the LTA component. The vaccine strain was isolated from a milk sample of a clinical mastitis case in a farm in Spain.

The vaccine is an emulsion for injection containing Montanide ISA and MPLA as adjuvant components. Montanide ISA is a mineral oil based adjuvant which is free of animal origin ingredients. MPLA is a novel adjuvant component. It is a derivative of lipid A, which retains the immunologically active lipid A portion of the parent molecule and preserves the LPS function of being a Toll Like Receptor 4 (TLR4) agonist. It is a potent stimulator of T cell and antibody responses. MPLA is included in the list of substances considered as not falling within the scope of Council Regulation (EEC) No. 70/2009, which means that no MRL evaluation is required if MPLA is used as an adjuvant at concentrations of up to 6 µg per dose.

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

of excipients is included in section 6.1 of the SPC.

The antigen and adjuvant amounts are selected to elicit a suitable immunological response with minimal or no local reactivity at the site of injection. The final formulation of UBAC has been set in view of the results on efficacy, safety and serological results obtained during the assessment of different formulations.

The formulation of batches used during clinical studies is the same as that intended for marketing except for the antigen content. All standard (industrial) batches will be blended with a higher amount of antigen than those included in clinical trials. Thus, it is expected that standard batches will exceed the potency of the minimum protective dose batch which is used as reference vaccine batch. A new laboratory trial was carried out in order to demonstrate the safety of a vaccine batch blended with a higher amount of antigen. The safety profile remains similar to that of a previously conducted safety trial, thus acceptable.

Description of the manufacturing method

The production process of the antigen is considered to be a non-standard manufacture for bacterial vaccines as the vaccine strain is incubated so as to obtain an adequate biofilm formation. The manufacturing process consists of the following main steps: Working seed bacteria are propagated in culture medium and then cultured to obtain biofilm. The biofilm is harvested and thermally treated. The antigen suspension is stored at 2–8 °C.

The final product is produced by mixing the aqueous phase containing phosphate-buffered saline (PBS), MPLA suspension and antigen with the oily phase containing Montanide ISA. All industrial batches are manufactured with a fixed antigen concentration (standard dose). Subsequently, the blend is emulsified by homogenisation and filled into glass vials (single presentation) or PET vials (multidose presentations).

The essential parts of the production process are adequately described with a sufficient level of detail. Further assurance has been provided that the production process generates consistent vaccine batches. The absence of inactivation kinetics has been properly justified by the special manufacturing process which gives sufficient assurance that no residual live bacteria will be present in the final product.

Major steps of the manufacturing process for the antigen have been validated by performing three manufacturing runs at small commercial scale. Additional data for a large scale batch have been provided. All results of the critical process parameters and those of the in-process controls comply with the acceptance ranges and indicate consistent manufacture of the antigen.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Starting materials listed in the Ph. Eur. are disodium edetate (EDTA), disodium phosphate dodecahydrate, gelatine, highly purified water, potassium chloride, potassium dihydrogen phosphate, povidone, sodium chloride, sodium hydroxide, sucrose, triethanolamine, hydrochloric acid and water for injections. Sufficient information is provided with regard to the starting materials listed in a Pharmacopoeia. Internal specifications and/or representative Certificates of Analysis (CoA) have been provided and all conform to the required specifications. Monosodium glutamate

complies with the respective USP requirements.

Specific materials not listed in a pharmacopoeia e.g. active ingredient, adjuvants, cell seeds and some excipients

Starting materials of biological origin

Starting materials of biological origin, which are not listed in the Ph. Eur., are *S. uberis,* strain 5616, tryptic soy agar (TSA), or tryptic soy broth (TSB), yeast extract, trypsin and MPLA.

For the active ingredient a seed lot system was satisfactorily established. Details of source, passage history, preparation, controls, storage conditions and certificates of analysis for the master seed bacteria (MSB) and working seed bacteria (WSB) have been provided and are considered appropriate. The seeds are sufficiently tested for their identity and purity. Based on the risk assessment provided, the seed materials do not pose a risk for TSE transmission.

Gelatine complies with the current regulatory texts related to Ph. Eur. monograph 5.2.8 "Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" and the TSE Note for Guidance (EMEA/410/01-Rev.3). Valid TSE certificates of suitability have been provided for the stated manufacturer.

Tryptic soy agar and tryptic soy broth do not pose a risk for TSE transmission due to its composition. The only relevant substance of bovine origin is milk sourced from healthy animals in the same condition as those used to collect milk for human consumption which is classified as low risk material as per Ph. Eur. 5.2.8. No other ruminant materials are used to manufacture the milk derivatives.

The compliance with Ph. Eur. 5.2.5 on Substances of animal origin for the production of veterinary vaccines has been evaluated for all starting materials of animal origin (gelatine, TSA, TSB, trypsin). The risk of virus contamination and its transmission is considered negligible due to the treatment of the substances which have to be considered in connection with the manufacturing process of the vaccine. MPLA and yeast extract are of non-animal origin.

Starting materials of non-biological origin

A certificate of analysis has been provided for Montanide ISA.

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

Information regarding the qualitative and quantitative composition of all culture media, freeze-drying excipient, sodium hydroxide solution and trypsin solution, their treatment processes and their storage conditions is provided in the dossier. All components are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk.

Control tests during the manufacturing process

The applicant presented in-process data for the manufacture of three small scale antigen bulks and one large scale antigen batch. During the manufacture of the antigen the following tests are carried out: Gram stain, viability-purity, identity, turbidity, bacterial and fungal sterility and ELISA for

antigen quantification. Test descriptions and the limits of acceptance were presented. The established in-process tests are deemed to be sufficient to control all critical steps in the manufacturing of the antigen. The relevant test methods for in-process controls are satisfactorily validated. Additional information regarding the batches used for the validation of the antigen ELISA, regarding the test procedure and the replacement of the reference batch has been provided and considered sufficient. A maximum specification was established to ensure that the antigen quantification ELISA runs with an acceptable accuracy and precision.

Control tests on the finished product

Finished product controls performed on the bulk vaccine are: appearance, pH, Montanide ISA identification and quantification, MPLA presence and maximum limit, viscosity, conductivity, stability of the emulsion and batch potency test by means of antigen quantification based on an ELISA technique. The filled product will be controlled for appearance, bacterial and fungal sterility and correct volume.

The descriptions of the methods used for the control of the finished product and the specifications have been provided. Validation reports have been provided for potency testing, adjuvants identification, quantification for Montanide ISA and sterility testing. Additional information has been presented concerning the quantification of the adjuvant components. The established test for MPLA determination has been optimized and validation criteria have been introduced to ensure that the MPLA content in the final vaccine is not higher than the maximum amount of 3 µg/ml allowed in Regulation 470/2009.

The specifications proposed at release are appropriate to control the quality of the finished product bearing in mind that the initially established specifications for pH and viscosity testing have been tightened based on currently available data.

Regarding the control of the correct antigen amount, the applicant has developed a potency test (ELISA) which is suitable to detect the immunorelevant antigen. As requested the minimum release specification for potency was ≥1 RP (Relative potency). By using this specification it is ensured that the tested batch is at least as potent as a reference batch that has been shown to be efficacious in the target species. The omission of a maximum potency specification is deemed to be acceptable taking into consideration the GMP compliance, the established full set of manufacturing controls performed in process and on the final product as well as the safety results obtained with a batch of vaccine containing a considerably higher amount of antigen. Further clarification for the replacement of the reference vaccine batch has been provided. Whilst it is acknowledged that the principle of monitoring of the reference vaccines is in theory satisfactory, the justification for the selection of the acceptance criterion for new reference vaccines according to "internal know-how" is not acceptable. The equivalence of the proposed and existing reference vaccine needs to be demonstrated by suitable statistical methods, which should be supported by worked example scenarios to ensure that it is clear how equivalence will be ensured. A post authorisation recommendation to the marketing authorisation should be established to solve this issue.

This ELISA has been properly validated and is deemed suitable to quantify the amount of LTA antigen. In addition, the applicant has provided other analytical means to further characterize the active substance (BAC). A post-authorisation recommendation is established to optimize this analytical method.

Batch-to-batch consistency

The applicant presented final product data for the manufacture of 4 consecutive final product batches indicating a consistent composition of the finished product in a quantitative and qualitative manner. Some of these final batches have also been included in the stability studies. For all batches manufacturing protocols have been provided. The process of blending has been verified on a commercial scale batches. The maximum blending volume as currently stated in Part 2B might be acceptable if substantiating data will be provided as soon as a maximum scale batch is available. These data are recommended to be provided post-authorisation.

Stability

Stability of active ingredient (bulk antigen)

According to Part 2B the antigen suspension filled in plastic bags can be stored at 2–8 °C for a maximum of 18 months until being further processed. Stability data demonstrating that the antigen can be stored at 2-8 °C for up to 18 months have been provided. However, the impact of this aged antigen on the vaccine stability is still under evaluation. At present preliminary data are provided to show that the product meets the proposed specifications when formulated with aged antigen. Further data are recommended to be provided post-authorisation.

Stability of the finished product

The proposed shelf-life is indicated as 24 months at 2–8 °C. Applying the concept of bracketing (testing the smallest and largest containers of all proposed materials) the applicant presents preliminary data on three batches. For all currently available points in time the tested batches complied with the set specifications and there is no obvious decline in potency as demonstrated by statistical evaluation. At this stage stability data are available for up to 27 months for two of the three final product batches. Therefore, the data provided so far are sufficient to justify a 24 months shelf life when the vaccine is stored at 2 °C – 8 °C. The applicant committed to complete the stability testing program for the third batch up to 27 months to further support the proposed shelf life of 24 months and to include 3 additional commercial scale batches blended with the higher antigen amount in the stability program.

In use stability of the vaccine

The proposed shelf life after first opening of the immediate packaging was initially indicated as 10 hours when stored at +15 to +25 °C. However, as a study of the efficacy of the antimicrobial activity has not been conducted as requested, SPC section 6.3 has consequently been adapted by inclusion of the recommendation that the vaccine should be used immediately after first opening. This is considered acceptable bearing in mind that the largest presentation consists of 50 doses only.

Overall conclusions on quality

A comprehensive description of the development of the product and the development and validation of the production process has been provided.

Appropriate information has been presented on the active ingredient (BAC) including the LTA and its relevance for the immunogenicity of the vaccine. The composition of the vaccine has been sufficiently described. The inclusion of MPLA in the vaccine composition is deemed to be acceptable

because the safety profile is not abnormal for this kind of vaccine. The production process of the antigen is considered as non-standard manufacture for bacterial vaccines. The production process of the antigen is described with a sufficient level of detail. The manufacturing process of the antigen has been validated.

In general, the starting materials are properly described. For starting materials of animal origin further assurance has been provided that they are either tested for or treated to ensure that there are no contaminants or that the treatment process ensures the removal of any potential risk caused by extraneous agents. Furthermore, compliance of starting materials of animal origin with TSE regulation has been shown. The risk that the final product may transmit TSE to the target animal was estimated to be negligible.

The in-process tests are deemed to be sufficient to control all critical steps in the manufacturing process. Validation studies have been provided for all key tests.

The control tests on the finished product are satisfactorily validated. Clarification has been provided confirming that the production and control processes generate consistent vaccine batches. Additional data for the characterisation of the antigen in terms of molecular size has been provided. The release limit for potency testing has been increased to ensure that the tested batch is at least as potent as a reference batch that has been shown to be efficacious in the target species.

At this stage stability data are available for up to 27 months for two of three final product batches. Therefore, the data provided so far are sufficient to justify a 24 months shelf life when the vaccine is stored at 2 °C – 8 °C.

Stability data demonstrating that the antigen can be stored at 2-8 °C for up to 18 months have been provided. However, the impact of this aged antigen on the vaccine stability is still under evaluation.

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.

In addition, the applicant is recommended to provide the following information [post-authorisation]:

1. Regarding the replacement of the reference vaccine used for potency testing of the final product. This should be supported by worked example scenarios to ensure that it is clear how equivalence will be ensured.
2. Further stability data from the third batch already included in the ongoing stability program to support the proposed shelf life of 24 months.
3. Further data to evaluate the impact of aged antigen (stored 18 months at 2-8°C) on the vaccine stability.
4. Further data demonstrating that large scale final vaccine batches formulated with a higher antigen amount are unchanged with respect to their quality and stability. These commercial batches need to comply with all acceptance limits including the minimum potency specification (≥ 1 RP).
5. To establish an optimized analytical method for further characterisation of the active substance (BAC).

Part 3 – Safety

Introduction and general requirements

UBAC is a subunit vaccine for use in cattle (cows and heifers) consisting of a fixed content of the antigen BAC of *S. uberis* (strain 5616), PBS and the adjuvant Hipramune U (made up of Montanide ISA and Monophosphoryl Lipid A). No preservative is included. The BAC is a new active substance not authorised for a veterinary medicinal product in the EU before. The vaccine is administered by deep intramuscular injection in the neck muscles. The full immunisation program consists of three vaccinations (approximately 60 days before the expected parturition date, at least 21 days before the expected parturition date and about 15 days after calving) and needs to be repeated with each gestation.

A full safety file in accordance with Article 12(3)(j) of the Directive 2001/82/EC has been provided. Studies to determine the safety of the vaccine were performed in accordance with the Ph. Eur. monographs 0062 on vaccines for veterinary use, Ph. Eur. chapter 5.2.6 on evaluation of safety of veterinary vaccines and immunosera, Commission Directive 2009/9/EC amending Directive 2001/82/EC and VICH GL 44.

Safety documentation

Four studies were conducted to investigate the safety of the product. These included three laboratory studies (investigating the safety of the administration of the primary vaccination regimen and the safety of a second vaccination cycle using an antigen overdose and an examination reproductive performance) and one multicentre field trial.

The vaccine was administered by the intramuscular route, as recommended. Two laboratory studies were reported to be Good Laboratory Practice (GLP) compliant and carried out in target animals of the most sensitive category recommended for vaccination (heifers <24 months), using standard batches. The studies were also carried out to assess the efficacy of the product, as the antigen content is fixed and no separate studies with minimum or maximum potency were necessary. Corresponding batch protocols have been provided. In one laboratory trial a batch containing an antigen overdose was used. This GLP compliant trial was designed to assess the safety of the vaccine when administered for a second time in the following gestation.

The field trial was carried out according to Good Clinical Practice (GCP) principles.

The following studies were carried out with UBAC:

Study title	RP used
Assessment of efficacy of an inactivated vaccine in pregnant cows against challenge of *S. uberis*.	RP = 1.07 RP = 1.15
Study for the determination of the efficacy of the vaccine in pregnant cows.	RP = 1
Safety and efficacy assessment of vaccine in controlling *S. uberis* infections in dairy cows under field conditions.	RP = 1 RP = 1.06
Assessment of safety of a second vaccination cycle of the UBAC vaccine in dairy cows. Assessment of the safety of the administration of an antigen overdose in dairy cows.	RP = 2.08

Laboratory tests

Laboratory safety trials were carried out in conformity with the Organisation for Economic Co-operation and Development (OECD) principles of GLP. Both studies were randomised, double-blinded studies.

Safety of the administration of one dose

The safety of administration of the primary vaccination regimen (consisting of three administrations of one dose) was tested in a laboratory study according to the requirements of Ph. Eur. 0062, 5.2.6 and VICH GL 44. The applicant uses a sufficient number of animals of the most sensitive category (14 pregnant heifers), the recommended route of administration (intramuscular) and the recommended application scheme to investigate the safety of the product. An appropriate control group (14 pregnant heifers), receiving a placebo (PBS), was also included (group B).

All study animals were free from antibodies against *S. uberis*.

The animals were observed and examined at least daily for signs of abnormal local or systemic reactions (using a scoring system) and rectal temperature was measured. The safety data regarding reproductive performance (peripartum incidences and calf viability) and milk production were also recorded.

The statistical analyses were conducted with a level of significance (p) < 0.05 to reveal significant differences between the groups. The individual increase in rectal temperature was calculated considering the average measured before each vaccination as basal rectal temperature (1st vaccination: D-1, D0; 2nd vaccination: D37–D39; 3rd vaccination: D73–D75).

Results showed that no general clinical signs attributable to the vaccine were observed after any of the three administrations and only slight swelling was found in a total of 5 vaccinated animals (up to 1.5 cm in diameter) disappearing after a maximum of three days.

After the first administration no important significant differences in rectal temperature could be detected between the treatment groups (most animals had rectal temperatures around the physiological range, apart from one vaccinated animal which showed an individual increase of 1.4 °C to 40.2 °C on the day post vaccination).

After the second administration a significant increase in rectal temperature was observed 3 days after vaccination in group A (vaccinates) compared to group B (controls). Indeed, not all vaccinated animals remained within the physiological range but showed higher temperatures. .

After the third administration a significant difference was observed on day 75+4 h in group A (vaccinates) compared to group B (controls). The maximum average increase in group A was 0.86 °C compared to 0.51 °C in control group B.

Besides the average group values, the individual increases should also be taken into consideration. In two vaccinated animals a maximum individual increase of 2.2 °C was detected after the third administration. However, four of the controls also showed high temperatures up to 41.3 °C from day 75 onwards, so it is not clear if such individual temperature increases are attributable to the vaccine.

Regarding reproductive performance (peripartum incidences and calf viability) and milk production during the vaccination phase no significant differences between groups were detected. For more details see "Examination of reproductive performance".

In conclusion, the vaccination does not cause general clinical signs or adverse effects on reproductive performance or milk production and induces only mild transient local swellings and acceptable rectal temperature increases. The findings of the laboratory studies were also largely supported by the finding of the field trial; however, in a number of animals in the field study, some larger local reactions over 5 cm in diameter were found lasting for up to 28 days in one animal.

Therefore, the vaccine is considered safe for administration of one dose and the primary vaccination regimen.

Given that there is a statistically significant difference with regard to the rectal temperature increase between the vaccinated and the control animals in this laboratory trial and in the field trial, this potential adverse reaction has been reflected in the product literature.

Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines.

Safety of the repeated administration of one dose

The primary vaccination scheme for UBAC consists of three doses (assessed under section "Safety of the administration of one dose") is repeated in each gestation and no booster is proposed. Therefore, in accordance with VICH GL 44, Commission Directive 2009/9/EC amending Directive 2001/82/EC and Notice to Applicants Vol. 6B no repeated dose study was performed, which is acceptable.

Safety of a second vaccination cycle

To evaluate the possible occurrence of adverse events of increasing intensity with regard to the adjuvant when administering the UBAC vaccine for a second time in the following gestation a laboratory study was conducted according to the requirements of Ph. Eur. 0062, 5.2.6 and VICH GL 44.

Nine animals from the field trial, which had already received a first vaccination cycle of 3 doses of UBAC, were enrolled in the study. The cows were vaccinated by the intramuscular route with a second vaccination cycle of three doses of an experimental vaccine batch of UBAC containing an overdose of antigen (RP around 2) according to the recommended program (60 days and 21 days before parturition, and 15 days after parturition).

The animals were observed and examined one day before and every day for a period of 14 days after each vaccine administration for signs of abnormal local or systemic reactions (using a scoring system). After the third vaccination the observation period was prolonged up to 21 days.

Rectal temperatures were measured. Rectal temperatures were only registered if the value was > 39.3 ºC. The safety data regarding reproductive performance (peripartum incidences and calf viability), milk production and SCC were recorded.

Statistical analysis was carried out using a significance level of $p < 0.05$ for all variables evaluated in this trial. The individual increase of rectal temperature was calculated considering the basal values of rectal temperature per animal, which consist of the mean between the evaluations of two consecutive days previous to each administration (D-1 and D0 for the 1st dose; D41 and D42 for the 2nd dose; D14pp and D15pp for the 3rd dose).

The results showed that no general clinical signs attributable to the vaccine were observed after any of the three administrations neither in the vaccinated animals nor in their progeny.

After the first dose local reactions at the inoculation site less than 2 cm were observed only in three animals. However, after the second dose three animals showed slight inflammation up to 5 cm from 4 hours up to 11 days after vaccination. The inflammation decreased progressively until it disappeared at days 16 - 17 after vaccination. After administration of the third dose one cow showed slight inflammation of 1 cm from 4 hours to 2 days after vaccination with no further local reaction. A second animal showed slight inflammation of 1 cm from day 6 to day 9 after vaccination.

With regard to the increase in rectal temperatures, the results indicated that there was no clinically relevant increase after vaccination (after 1st, 2nd and 3rd a mean increase average of 0.54°C, 0.30 °C and 0.22°C, respectively). The highest individual value recorded was 1.42 ºC.

Regarding reproductive performance (peripartum incidences and calf viability), no adverse effects attributable to vaccination were detected. One animal suffered from dystocia due to a twin-calving parturition.

All animals showed similar values of milk production before and after vaccination; so no impact on milk production was observed after vaccination. As regards SCC only 8 samples had a cell count higher than 400,000 cells/ml (5.60 log10 SCC/ml), which represents 3.3% (8 out of 242). 96.7% of the samples remained below 400,000 cells/ml.

In conclusion, in this study, the vaccination does not cause general clinical signs or adverse effects on reproductive performance or milk production and induces only mild transient local swellings.

Therefore, the vaccine is considered safe when used in a second vaccination cycle of three doses, especially with regard to the administration of an antigen overdose. The safety profile remains similar to that of the previous safety trial except for the local reactions at the injection site, which are more pronounced. This has been adequately reflected in the product literature.

Examination of reproductive performance

The safety of reproductive performance was investigated in two laboratory studies using seronegative pregnant heifers. In each study one vaccinated group and one control group receiving PBS as placebo were included (Safety and efficacy trial: 14 vaccinates and 14 controls, Efficacy trial: 16 vaccinates and 16 controls).

At each administration one dose of the product was administered by the intramuscular route which is the recommended one. In the safety and efficacy trial, the recommended vaccination scheme was used, but in the efficacy trial (designed to establish the onset of immunity) the third administration was replaced by the challenge.

Besides other safety parameters (not described in this section) the reproductive performance was evaluated using the parameters "peripartum incidences" and "calf viability until three days after parturition" and compared between the groups. For the evaluation of reproductive performance the omission of the third administration in the efficacy trial is not relevant, as it is intended to be performed around two weeks after parturition. Consequently, the results of both studies are fully applicable.

In total three calves from vaccinated animals died because of problems during parturition and resulting hypoxia, and one control cow aborted 57 days before expected parturition (3 days after administration of PBS, no pathogen was detected during necropsy). The pathological examination

reports for the dead calves indicate cyanosis in all mucosas caused by hypoxia during parturition and is not attributable to the vaccine.

All other animals included in the studies had a normal parturition and the calves were healthy and showed no clinical signs, perinatal mortality or developmental delay (growth delay) during the following three days after parturition.

Data from the new laboratory study carried out with an overdose of antigen confirms that the vaccination does not have adverse effects on reproductive performance.

Data from the field trial revealed comparable incidence and types of reproductive adverse events between the vaccinated and the control group indicating that vaccination with UBAC has no negative influence on reproductive performance.

Examination of immunological functions

According to the legislation in force, where the product might adversely affect the immune response of the animal to which the product is administered or of its progeny, suitable tests on the immunological functions have to be carried out.

No further studies were conducted to investigate the effects of UBAC on immunological functions but no adverse effects were observed in any of the safety or efficacy studies. It is therefore unlikely that this vaccine will have any adverse effect on immunological functions due to the nature of the product (inactivated subunit vaccine).

User safety

A user safety risk assessment has been presented in accordance with 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 are accidental self-injection and dermal and/or oral exposure, with the former representing the worst case scenario. The active substance is an inactivated subunit of *S. uberis* and therefore not infectious. The risk of accidental injection is considered to be adequately mitigated by the standard warning required due to the presence of mineral oil in the product (see below).

Regarding the adjuvant MPLA (which has not been used as an adjuvant in veterinary immunological products so far but only in human products) it is noteworthy that the CVMP accepted to include the substance in the list of substances considered as not falling within the scope of Regulation 470/2009 (with the restriction "for use as an adjuvant at concentrations of up to 6 µg per dose"). With regard to an accidental self-injection, no risk is expected to the end user and the fact that the adjuvant is already used in human vaccines where no harmful effect has been reported.

All other excipients are commonly used and do not pose a risk for the user, except for Montanide. As a result of the user safety assessment, and since the product contains mineral oil (Montanide), the standard warning for mineral oil-containing vaccines is included, appropriately, in the product literature.

Based on the above risk assessment it is concluded that the risk posed to the user by this product is very low when used in accordance with the SPC.

Study of residues

No study of residues has been performed. All substances included in the composition of the vaccine are listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 or are considered as not falling within the scope of Council Regulation (EC) No 470/2009.

As numerical MRLs have not been set for any of the ingredients there was no need to perform residue studies for UBAC and a withdrawal period of zero days can be established.

Interactions

No data has been provided investigating interactions of the vaccine with other veterinary immunological products and therefore inclusion of the standard statement in Section 4.8 of the SPC is proposed.

Field studies

One multicentre randomised, double blinded, placebo controlled study was conducted to evaluate safety and efficacy of UBAC in controlling *S. uberis* infections in clinically healthy dairy cows (heifers or multiparous) under field conditions. The study was conducted on 6 commercial dairy farms located in Spain with historical records of *S. uberis* clinical mastitis and recently confirmed presence of *S. uberis* infection and did adhere to GCP. Randomisation was performed stratifying by farm and heifers/multiparous conditions. A randomisation list was provided by the statistician for each farm.

Cows previously vaccinated against *S. uberis* and multiparous animals positive for *S. uberis* and/or *Staphylococcus aureus* and/or coliforms in milk or with a high SCC were excluded from the study.

The applicant used a sufficient number of animals, the recommended dose (2 ml), the recommended route of administration (intramuscular) and the recommended application scheme to investigate the safety of the product. Standard vaccine batches (RP =1) (RP = 1) and (RP = 1.06) were administered.

The study was well designed and conducted. During the whole study period reproductive adverse events and other adverse events were assessed in a total of 750 cows (382 vaccinates, 368 controls). In a sub-group of cows (29 vaccinates and 27 controls from three farms) general reactions, rectal temperature progression and local reactions were recorded on the day before each administration, at administration (baseline value: average of days -1 and 0), 4 hours post administration and on the following three days. The chosen parameters were appropriate to investigate the safety of the product.

Adverse events, including reproductive adverse events, were recorded at comparable incidence in both groups and also corresponded to levels previously reported for the included farms.

None of the animals showed any general reaction during the first three days after administration.

Regarding the post-vaccination rectal temperature progression a statistically significant difference [with a fixed significance level of 5% or 1% ($p<0.05$, $p<0.01$) could be detected on day 1 (after each administration) and additionally on days 2 and 3 (after the third dose only). However, the mean increases per group and day were below 0.5 °C and, therefore, the temperature increase can be classified as not clinically relevant. The highest individual increase in one vaccinated animal was 1.8 °C.

The vaccinated animals showed a larger number of local reactions than the placebo animals (in total 4 local reactions have been observed in the placebo group and 25 in the vaccine group). Also, even if most reactions were mild and smaller than 5 cm in diameter, 5 vaccinated animals (out of 29) showed bigger local reactions lasting for up to 28 days after the third dose. An appropriate warning has been included in the product literature.

Environmental risk assessment

An environmental risk assessment according to the "Guideline for Environmental Risk Assessment for Immunological veterinary medicinal products" (EMEA/CVMP/074/95) was provided and the likelihood of hazard and resulting consequences can be considered negligible. Based on the phase I assessment a study of phase II has not been considered necessary.

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

Overall conclusions on the safety documentation

The safety of UBAC was investigated in three placebo-controlled, randomised, double-blinded studies which are adequately described; two laboratory studies and one multicentre field trial. Standard batches were used as the antigen concentration is fixed and all batches will contain the same quantity of antigen. In the laboratory studies, heifers <24 months of age were used which represent the most sensitive category of target animals. In the field trial, both primiparous and multiparous cows were included. A third laboratory study using a batch containing an overdose of antigen was conducted to assess safety aspects in a second vaccination cycle.

UBAC is considered safe for administration of one dose and the primary vaccination regimen as recommended in SPC but may induce transient temperature increase and local reactions at the injection site. Based on the available safety data, local swelling more than 5 cm in diameter at the injection site is a very common reaction after administration of the vaccine. This swelling will have disappeared or be clearly reduced in size by 17 days post vaccination. However, in some cases, swelling may persist for up to 4 weeks. In addition, a transient increase in rectal temperature (mean increase of 1 °C but may be up to 2 °C in individual animals) may very commonly occur in the first 24 hours after injection. The potential adverse effects of vaccination are reflected in the SPC.

No overdose administration was carried out as this product is not a live vaccine.

The full immunisation program of three doses is repeated in each gestation and no booster is proposed. Therefore, in accordance with VICH GL 44, Commission Directive 2009/9/EC amending Directive 2001/82/EC and Notice to Applicants Vol. 6B no repeated dose study was performed, which is acceptable.

The possible occurrence of adverse events of increasing intensity when administering the UBAC vaccine for a second cycle in the following gestation was evaluated in a laboratory study. A second vaccination cycle was administered according to the recommended program to animals, which had already received a first vaccination cycle of 3 doses of UBAC. A batch containing an antigen overdose was used. No general clinical signs attributable to the vaccine were observed after any of the three administrations neither in the vaccinated animals nor in their progeny. The observed local reactions at the inoculation site were more pronounced than those of a previous safety trial. After none of the three vaccinations relevant increases in rectal temperatures were registered. No impact on reproductive performance or on milk production was observed after vaccination. Therefore, the

vaccine is considered safe when used in a second vaccination cycle of three doses, as recommended in SPC.

The reproductive performance was evaluated in three laboratory studies and one field trial. The safety data regarding reproductive performance (peripartum incidences and calf viability) were recorded and demonstrated comparable incidence and types of reproductive adverse events between the vaccinated and the control groups indicating that vaccination with UBAC has no negative influence on reproductive performance.

The inactivated subunit vaccine UBAC is not expected to adversely affect the immune response of the target animals or of its progeny, and therefore no specific tests on immunological function were carried out.

A user safety risk assessment in line with the relevant guidance has been presented. No hazard has been identified, except in relation to the mineral oil included in the vaccine. For this ingredient the standard warning was included in the product literature. Overall, the risk posed to the user by this product is considered to be very low when the product is used in accordance with the SPC.

No study of residues has been performed. All substances included in the composition of the vaccine are listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 or are considered as not falling within the scope of Council Regulation (EEC) No 470/2009. As numerical MRLs have not been set for any of the ingredients a withdrawal period of zero days can be established.

No specific studies on interactions with other immunologicals or veterinary medicinal products were performed and the standard warnings were included in the product literature, which is acceptable.

An appropriate environmental risk assessment was provided. The product is not expected to pose a risk to the environment.

Part 4 – Efficacy

Introduction and general requirements

UBAC is an inactivated subunit vaccine for intramuscular vaccination of cattle (cows and heifers) consisting of BAC of *S. uberis* (strain 5616), PBS and the adjuvant Hipramune U. Hipramune U is made up of Montanide ISA (adjuvant) and MPLA (immunostimulant). No preservative is included. The antigen concentration is fixed and the quantity of BAC per dose (2 ml) is RP ≥ 1 (ELISA).

The vaccine is administered by deep intramuscular injection in the neck muscles. The full immunisation program consists of three vaccinations (approximately 60 days before the expected parturition date, at least 21 days before the expected parturition date and about 15 days after calving) and needs to be repeated with each gestation.

Immunity is intended to be established approximately 1 month after the second dose and is boosted by a third vaccination resulting in an induced immunity lasting for 5 months of lactation.

Studies to determine the efficacy of the vaccine were performed in accordance with the Ph. Eur. monographs 0062 on vaccines for veterinary use, Ph. Eur. chapter 5.2.7 on evaluation of efficacy of veterinary vaccines and immunosera, Commission Directive 2009/9/EC amending Directive 2001/82/EC as well as the applicable guidelines. There is no specific Ph. Eur. monograph for vaccines for mastitis caused by *S. uberis* infection in cattle.

Justification was given why BAC was chosen as the active ingredient and why it was considered that

BAC would provide the optimal protection against a clinical mastitis caused by *S. uberis*. The clinical relevance of biofilms in intramammary infections, the mechanism of adhesion, the role of biofilm structural components and the relevance of different virulence-associated factors have been discussed. Furthermore, the strain diversity was justified.

Challenge model

The challenge strain used in the efficacy laboratory trials is a *S. uberis* field isolate from milk of a clinical mastitis case in the UK, isolated by the Quality Milk Management Services of the UK. It is a different strain from the one used in the production of the vaccine. The strain is considered as representative of the EU field circulating bacteria. The applicant has evaluated the pathogenicity of the strain.

Two studies were conducted to validate a challenge model in lactating cows. The first one was conducted in 2012 and was considered less suitable whereas an improved challenge model was established in a second study conducted in 2015.

In a first study the challenge strain was administered by intramammary (IMM) application of 5 ml of an inoculum with a high bacteria concentration to 3 lactating cows and an inoculum with a lower concentration to other 3 lactating cows.

The preparation of the challenge strain was sufficiently described and details on the origin were given. Data are provided on the characteristics of this strain, e.g. slime formation or toxin production.

The chosen challenge strain seems to be appropriate to induce an intramammary infection in lactating cows. The inoculum with the lower concentration was able to produce changes in rectal temperature, in local clinical signs, in SCC and in bacterial count in milk samples in a similar manner to that produced with the inoculum containing a higher logarithm concentration. Regarding the local clinical signs, alterations in the milk, decreased milk production and inflammation and hardening of the mammary glands could be observed from day 2 post-infection with both inoculum concentrations.

The challenge model developed in this first study was used in the safety and efficacy trial. In general, the efficacy results of this laboratory trial showed a trend towards efficacy but were not overall satisfactory. The administration of the challenge material deviates from the natural infection route of environmental pathogens. Subsequently, a very fast infection could be observed which might be too severe. Therefore, an experimental infection method closer to the natural infection has been tested in order to better mimic the natural conditions for infection.

In a second study the challenge strain was administered by dipping of the teats into a bacterial suspension. For this model three lactating cows were used. To a second group of three lactating cows the challenge strain was administered by IMM application of 1 ml of an inoculum.

Despite the high bacterial concentration in the challenge suspension used for dipping of the teats no bacteria could be isolated and almost no local clinical signs were recorded in challenged cows. No changes in milk production were observed and the challenge did not have any influence on rectal temperatures confirming that this challenge model was not able to induce clinical signs for mastitis.

The IMM administered inoculum was able to produce a clear rectal temperature decrease from day 1 to day 4 and to induce local clinical signs marked by an effect on the milk production and showing clear signs of moderate-slight mastitis from day 2 to day 5. In addition, the infected animals showed positive results in the California mastitis test, clinical signs of mastitis, a clear increase of SCC from

day 1 until the end of the study, and bacteria were isolated in all quarters of these cows. These results indicated that this second challenge model is able to produce an intramammary infection with clear signs of mastitis.

This challenge model was considered adequately validated and therefore appropriate for using in the efficacy trials.

Efficacy parameters and tests

The efficacy of UBAC vaccine against *S. uberis* mastitis in pregnant cows was based mainly on local clinical signs of mastitis (milk appearance and mammary gland abnormalities) and milk analysis (bacterial count, SCC) as primary outcomes.

As secondary outcomes rectal temperature, general clinical signs, milk production and antibodies against *S. uberis* in serum were monitored.

The standard description of the test methods was submitted separately or the descriptions were included in the study reports.

Efficacy documentation

Three studies were conducted to investigate the efficacy of the product, one laboratory study investigating the efficacy after challenge 15 days after the administration of the primary vaccination scheme (consisting of three administrations of one dose), one laboratory study investigating the onset of immunity (challenge about 35 days after two administration of one dose) as well as one multicentre field trial.

The vaccine was administered by the intramuscular route, as recommended. Laboratory studies were reported to be GLP compliant and carried out in target animals of the most sensitive category recommended for vaccination (heifers <24 months), using standard batches. All studies were also carried out to assess the safety of the product, as the antigen content is fixed and no separate studies with minimum or maximum potency were necessary.

The field trial was carried out according to GCP principles.

The following efficacy studies are performed:

Study title	Batch used
Assessment of efficacy of an inactivated vaccine (PB-123) in pregnant cows against challenge of *S. uberis*.	RP = 1.07 RP = 1.15
Study for the determination of the efficacy of the PB-123 vaccine in pregnant cows.	RP = 1
Safety and efficacy assessment of vaccine PB-123 in controlling *S. uberis* infections in dairy cows under field conditions.	RP = 1 RP = 1.06

Dose determination

The vaccine dose and the schedule of vaccination are determined in line with the applicant's knowledge and expertise regarding mastitis vaccines. The vaccination schedule is adjusted according to the dates of parturition of each reproductive cycle. The recommended basic vaccination scheme has been designed in order to confer protection in the periods of risk of *S. uberis* infection. This

includes three vaccine doses to be administered around 2 months and 3 weeks before the expected date of parturition and around 2 weeks after parturition. This basic vaccination scheme is to be repeated for each reproductive cycle.

Laboratory trials

In order to assess the efficacy of the vaccine in pregnant cows after administration of the primary vaccination scheme (consisting of three administrations of one dose) against challenge of *S. uberis* a safety and efficacy laboratory study was performed according to the requirements of Ph. Eur. 0062 and 5.2.7. The applicant used a sufficient number of animals of the most sensitive category (28 pregnant heifers), the recommended administration route (intramuscular) and the recommended application scheme (3 vaccinations) to investigate the efficacy of the product.

In this study 14 pregnant heifers received the vaccine and an appropriate control group (14 pregnant heifers) received a placebo (PBS). 15 days after the third vaccination (day 90 of the study) 13 vaccinated and 13 control animals were challenged by intramammary application of 5 ml of *S. uberis* challenge strain. Two animals were excluded from the challenge because of antibiotic treatment just before challenge.

All study animals were free or had a low titre of antibodies against *S. uberis*.

The animals were observed and examined daily starting 5 days before challenge up to 21 days post challenge. The efficacy was assessed by monitoring of following parameters: General clinical signs (scoring system), clinical signs of mastitis (assessment scoring system), assessment of milk appearance (visual and using California Mastitis Test, scoring system)], milk sample collection (to perform bacterial count (cfu/ml) and SCC (cells/ml)), milk production (on days -5, -2, -1, 0, 1 – 7, 8-20 and 21) and rectal temperature (on days -2, -1, 0, 1-7, 8-20 and 21). Blood samples were collected on day-1.

Statistical analyses were conducted with a level of significance (p) < 0.05 to detect significant differences between the groups.

The primary outcomes are the following:

Clinical signs of mastitis: Milk appearance was similar in both groups the first days after challenge. However, vaccinated animals showed less abnormalities than control animals, especially from D6 to D10, suggesting that vaccinated animals were able to overcome the infection sooner than control animals. In addition, the cumulative number of animals that did not show abnormalities in milk after challenge was higher in vaccinated animals compared to control animals from day 7 to the end of the study. Regarding the mammary gland percentage affected and score, significant differences were observed on day 7 post meridiem (p.m.), ($p<0.05$) and on day 10 ($p<0.10$), when vaccinated animals demonstrated a lower percentage affected than control animals. Despite these differences, no statistically significant differences were observed between groups when analysing the entire period.

Bacterial count: Because the average of the bacterial count of the control group during the first five days after challenge was greater than the average of the bacterial count of the vaccinated group during the same period, it can be concluded that the vaccination was able to reduce the bacterial count, especially during the first 5 days post-challenge.

SCC: Most samples obtained from day 1 p.m. to day 6 could not be analysed due to the bad quality of milk samples. Although no statistically significant differences between groups were observed from

day 6 to day 21, except for day 16, the average SCC of the control animals was higher compared to vaccinated animals, which suggests that the vaccinated animals were able to control the infection better than the control animals.

The secondary outcomes are the following:

General clinical signs and rectal temperature: All animals remained in good health throughout the challenge period, except for two control animals with a slight affection (score =1) on days 2 and 9.

In both groups an increase in rectal temperatures from day 1 p.m. to day 3 p.m. was observed, but without significant differences between both groups. This suggests that experimental infection had an influence on rectal temperatures 30 hours later and after 3-4 days, when the animals are able to control the bacteria proliferation in the mammary gland, body temperatures return to normal.

Antibodies against *S. uberis*: Compared to control animals, vaccinated animals showed statistically significant higher antibody titres after the 1st, 2nd and 3rd vaccination until the end of the trial.

Milk production: There were no statistically significant differences in milk production between groups. Nevertheless, it was observed that vaccinated animals showed a greater production compared to control animals. Furthermore, regarding the cumulative data, it was observed that the average milk loss of vaccinated animals during the 21 days after challenge was 69.7 litres, whereas for the control animals it was 86.4 litres.

In conclusion, although statistically significant differences between groups could not be recorded, results in vaccinated animals show a clear trend to overcome the infection better than the control animals. However, the study was only of supporting character because of use of a less suitable challenge model.

Onset of immunity

In order to establish the onset of immunity in pregnant cows after two administrations of one dose of UBAC, an efficacy laboratory study was performed. The third administration was replaced by the *S. uberis* challenge. The applicant uses a sufficient number of animals of the most sensitive category (32 pregnant heifers) and the recommended administration route (intramuscular).

In this study, 16 pregnant heifers received the vaccine and an appropriate control group (16 pregnant heifers) received a placebo (PBS). Thirty six days after the second vaccination (about 15 days after parturition), 13 vaccinated and 12 control animals were challenged by intramammary application of 1 ml of a *S. uberis* challenge strain. Seven animals were excluded from challenge for different reasons which are documented.

All study animals were free of antibodies against *S. uberis*.

The animals were observed and examined daily starting 5 days before challenge up to 21 days post challenge. The efficacy was assessed by monitoring of the following parameters: General clinical signs (scoring system), clinical signs of mastitis (milk appearance and mammary gland assessment [scoring system] and %), milk sample collection (to perform bacterial count (cfu/ml), SCC (cells/ml) and antibody estimation), milk production (on days -5, -2, -1, 0, 1-7, 8-20 and 21) and rectal temperature (on days -2, -1, 0, 1-7, 8-20 and 21). Blood samples were collected on day 0 and day 21.

Statistical analyses were conducted with a level of significance (p) < 0.05 to reveal significant differences between the groups.

The primary outcomes are the following:

Clinical signs of mastitis: After challenge, signs of clinical mastitis were recorded in all animals for the first 3 days of the study. From day 4, milk appearance and mammary gland abnormalities reduced in severity scoring in the vaccinated group, and results demonstrated that vaccinated animals suffered less damage in the mammary gland. Comparing the total average of all scores per group (3.20 for vaccinates versus 4.13 for controls) shows a reduction of 22.5% of clinical signs of mastitis in the vaccinated group.

Concerning the bacterial count, results demonstrated that the numbers were lower in the vaccinated group compared to the control group on most of the sampling time points of the study (25 out of 29). These differences were statistically significant at four of these time points. In addition, the percentage of animals with negative samples was 23.08% for the vaccinated group compared to 9.52% for the control group.

SCC: Both groups showed a similar increase in the SCC after challenge. Between day 2 and 4, slightly higher values were recorded in the vaccinated group at 4 sampling time points compared to the control group. After that, SCC started to decrease and the value was similar in both groups until day 15. In the last week of the study, the SCC of the vaccinated group was considerably lower compared to the control animals. In addition, during the last week of this study (from day 15 to day 21) the percentage of vaccinated animals with low SCC values (< 200,000 cells/ml) was significantly higher compared to the control group.

Based on the results of SCC and bacterial count at the end of the trial, the cure rate was calculated which was defined as inability to isolate *S. uberis* in milk and SCC lower than 200,000 cells/ml for two consecutive days after mastitis infection. The cure rate of the vaccinated animals was 2.3 times higher than for control animals (53.85% versus 25.00%).

The secondary outcomes are the following:

Rectal temperature: During the first 3 days post challenge both groups showed an initial increase. Afterwards, the average rectal temperature of vaccinated animals decreased faster to lower values than those of control animals and the control group demonstrated higher temperatures until day 5. Furthermore, the average of rectal temperature throughout the study was considerably lower in the vaccinated group compared to the control group.

Antibodies against *S. uberis*: After the 1st and 2nd vaccination a clear antibody response against *S. uberis* was established in sera samples of the vaccinated animals and the titres were significantly higher compared to the antibody titres of the control group. The antibody response against *S. uberis* in milk correlated with the results observed in sera: vaccinated animals had statistically significantly higher antibody titres compared to control animals before and after challenge.

With respect to the milk production: A substantial decrease was observed in the control group during the first 5 days after challenge, whereas the decrease in milk production in vaccinated animals was only recorded up to two days after challenge. The vaccinated animals recovered their normal milk production value 8 days after challenge whereas the control animals reached their normal milk value not until day 18 post challenge. Consequently, the average of cumulative milk losses during the trial was considerably higher in the control group compared to the vaccinated group. In total, the control animals had an estimated average loss of 51.7 litres per animal, whereas the vaccinated animals had an estimate average loss of only 6.5 litres of milk per animal.

It was concluded that for the proposed indication the onset of immunity approximately 1 month after the second dose has been demonstrated since reduction of the clinical signs of mastitis caused

by *S. uberis*, reduction of the severity of the infection in milk appearance and in mammary gland parenchyma, reduction of the bacterial count and SCC in milk, improvement of the mastitis cure rate, reduction of the rectal temperature affectation and minimisation of the decrease in milk production after intramammary infection with *S. uberis* could be demonstrated.

Duration of immunity

In order to assess the duration of immunity, a field trial was carried out. The results of this study are described, assessed and summarised under section Field trials.

The results of the field efficacy study presented support an induced immunity lasting 5 months of lactation.

Maternally derived antibodies (MDA)

The vaccine is intended for active immunisation of healthy cows and heifers in the last trimester of gestation. Therefore, the influence of maternally derived antibodies with regard to the efficacy of the vaccine is not applicable and thus no MDA studies were conducted.

Field trials

One multicentre randomised, double blinded, placebo controlled study was conducted to evaluate safety and efficacy of UBAC in controlling *S. uberis* infections in clinically healthy dairy cows (primiparous or multiparous) under field conditions.

The study was conducted on 6 commercial dairy farms located in Spain with historical records of *S. uberis* clinical mastitis and recently confirmed presence of *S. uberis* infection and did adhere to GCP. Randomisation was performed stratifying by farm and heifers/multiparous conditions. A randomisation list was provided by the statistician for each farm.

For the statistical analysis of the efficacy study variables the "Valid for Efficacy Population" dataset was established which includes all cows (303 in the placebo group and 277 in the UBAC group) that received all three doses of the product in accordance with the vaccination schedule and did not have severe protocol deviations that might interfere with the results.

Cows previously vaccinated against *S. uberis* and multiparous animals positive for *S. uberis* and/or *Staphylococcus aureus* and/or coliforms in milk or with a high SCC (>800,000 cells/ml) were excluded from the study.

A sufficient number of animals, the recommended dose (2 ml), the recommended administration route (intramuscular) and the recommended application scheme were used to investigate the efficacy of the product. Standard vaccine batches (RP=1) and (RP = 1.06) were administered.

The study was well designed and conducted. The efficacy parameters evaluated during the observation period of 21 weeks after parturition were divided into primary (incidence of new cases of *S. uberis* clinical mastitis) and secondary parameters (incidence of new cases of sub-clinical mastitis, severity of *S. uberis* intramammary infection, SCC and milk production (litres/day).

The primary outcomes are the following:

Incidence of new cases of *S. uberis* clinical mastitis: The incidence of new cases of *S. uberis* clinical mastitis in the group vaccinated with UBAC was 50% lower than the incidence in the placebo group

(6.1% versus 12.2%) which was statistically significantly different (p=0.012). Bearing in mind that some cows had suffered more than one episode of *S. uberis* clinical mastitis, the incidence of cows with clinical mastitis was 52.5% lower in the vaccinated group than those of the placebo group (4.7% versus 9.9%), with a statistical significance of p<0.017.

The secondary outcomes are the following:

Incidence of new cases of *S. uberis* subclinical mastitis: The incidence of new cases of *S. uberis* subclinical mastitis in the placebo group was higher than the incidence in the group vaccinated with UBAC. Specifically, 78 (25.7%) and 55 (19.9%) new cases of *S. uberis* subclinical mastitis have been reported in the placebo group and in the UBAC group, respectively. However, the difference between groups (24.1% less incidence in the UBAC group than in the placebo group) was not statistically significant. The average time (in days) elapsed between the calving date and the first *S. uberis* subclinical mastitis were calculated to be 58 vs 74 days in the placebo and in the UBAC groups, respectively. While there was a trend in favour of the vaccinated group, the difference was not statistically significant (p=0.094).

Severity of CMT scores among *S. uberis* subclinical mastitis cases: For the evaluation of this parameter only data from quarters with *S. uberis* positive milk samples were used. Particularly, mean California Mastitis Test (CMT) scores from all milk quarter samples that were *S. uberis* positive in each group were compared.

S. uberis positive quarter milk samples in the placebo group were overall given higher CMT scores than those in the group vaccinated with UBAC, the differences being statistically significant (p<0.003). The maximum CMT score of 4 was noticed in 39.6% of *S. uberis* positive quarter milk samples in the placebo group, whereas in the vaccinated group this occurred in only 23.5% of *S. uberis* positive quarter milk samples. In addition, the mean CMT score given to *S. uberis*+ quarter milk samples in the placebo group was statistically significantly higher (p=0.026) than in the UBAC group (2.91 versus 2.38).

SCC among subclinical mastitis cases: SCC is evaluated in all milk samples weekly collected by the investigators during milking, but only data from *S. uberis* positive milk samples have been used for the evaluation of this parameter. The mean SCC among subclinical mastitis cases was statistically significantly higher in the placebo group than in UBAC group (5303.65 cells x 10^3/ml versus 3289.91 cells x 10^3/ml; p<0.001).

Milk production (litres/day): The mean daily milk production during the study has been statistically significantly higher in the group vaccinated with UBAC than in the placebo group (38.3 versus 37.8 litres/day; p<0.003).

Antibiotic treatment: All *S. uberis* clinical mastitis episodes were treated with antibiotics. The total number of antibiotic doses administered to the animals was higher in the control than in the UBAC group (167 in the control and 74 in the UBAC group), respectively. In terms of mean number of doses per animal, a statistically significant reduction was observed in the UBAC group compared to the placebo group (0.27 vs 0.55 doses/animal, respectively; p=0.02).

In summary, vaccination of healthy cows and heifers with UBAC according to the recommended vaccination schedule provided statistically significant reductions of the incidences of *S. uberis* clinical mastitis during a total period of 21 weeks (5 months) after calving, of the SCC in cows with *S. uberis* subclinical mastitis and of milk production losses in farms with *S. uberis* mastitis. Although in terms of mean number of antibiotic doses received per animal a statistically significant reduction was shown, the reduction in use of therapeutic antibiotics was not included as a claim in the indication

because the antibiotic treatment protocols were not standardized for all farms. Thus, the comparison of treatment duration between groups does not yield reliable results. Regarding the incidence of new cases of *S. uberis* subclinical mastitis, the differences between groups were not statistically significant.

Overall conclusion on efficacy

Bovine mastitis is currently the pathological process that causes the greatest economic losses in dairy herds. Bacteria that commonly cause mastitis are generally classified as either contagious or environmental pathogens. The main groups of environmental pathogens known to cause mastitis are *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus dysgalactiae* and *S. uberis*. From these environmental pathogens, *S. uberis* has been reported to be the most prevalent. This was taken into account in the development of the vaccine UBAC.

The efficacy of UBAC has been investigated for the category of the target species for which the vaccine is recommended, healthy cows and heifers, by the recommended administration route (intramuscular injection in the neck muscles) and using the proposed schedule of administration (vaccination approximately 60 days before expected parturition date, a second dose at least 21 days before expected calving and a third dose about 15 days after calving).

The choice of the vaccine strain was made according to the current knowledge of the microorganisms and on the basis of epizootiological data. The clinical relevance of biofilms in intramammary infections, the mechanism of adhesion, the role of biofilm structural components, the relevance of different virulence-associated factors and the relevance of the vaccine strain in terms of the strain diversity have been discussed.

Furthermore, the challenge model and the challenge strain (after intramammary infection with *S. uberis*) used in the efficacy laboratory trials has been previously studied to assess their pathogenicity and consequently validate the performed trials. An overall characterisation of the challenge strain has been provided.

To demonstrate the efficacy in pregnant cows, two laboratory trials were conducted. In the 1st study the challenge was carried out 15 days after the 3rd vaccination. In this trial a clear trend has demonstrated that the elimination of the infection is more favourable in the group of cows that received the vaccine. However, the study was only of supporting character because of using a less than suitable challenge model. In the 2nd study the challenge was carried out on day 75 (about 15 days after parturition), in order to show the efficacy of UBAC at the period of maximum risk of bovine mastitis which is around the parturition period. The results of the study (reduction of clinical signs of mastitis caused by *S. uberis*, reduction of the severity of the infection in milk appearance and in mammary gland parenchyma, reduction of the bacterial count and SCC in milk, improvement of the mastitis cure rate, reduction of rectal temperature affectation and minimisation of the decrease in milk production) are reflected in an acceptable way in the SPC. The onset of immunity approximately 1 month (36 days) after the second dose, as proposed by the applicant, has been demonstrated.

Test descriptions and justification for diagnostic methods used have been provided.

The laboratory trials were complemented with a pivotal field trial. In the field trial the third dose of the proposed schedule of vaccination (at approximately 15 days after parturition, day 75) was administered in order to maintain the duration of the immunity induced by the 1st and 2nd vaccinations, and to sufficiently cover the whole period of maximum risk.

In the field trial the demonstration of protection has been performed using clinical parameters (incidence and severity of mastitis) and additional parameters such as milk production losses and number of used antibiotic doses on which the efficacy of the vaccine is based, and comparing the results of vaccinated and control animals.

Overall, a reduction of the incidence of clinical mastitis, a reduction of the severity of California Mastitis Test scores, a reduction of the severity of SCC and a reduction of milk production losses could be shown. In addition to the direct benefits a statistically significantly positive effect of the vaccine on the parameters "mean number of antibiotic doses per animal" was observed in the vaccine group compared to the placebo group. While interventions that lead to a reduction of antibiotic use are desirable, this effect will not be included as a claim in the indication because the treatment protocols were not standardized for all farms resulting in inadequate data.

The results of the field efficacy study presented support an induced immunity lasting the first 5 months of lactation.

Part 5 – Benefit-risk assessment

Introduction

UBAC is an inactivated subunit vaccine consisting of Biofilm Adhesion Component (BAC) including lipoteichoic acid (LTA) of *S. uberis* (strain 5616).

The product is intended for use in cattle (cows and heifers) to reduce the incidence of clinical mastitis caused by *S. uberis* and to reduce the SCC in milk from subclinically infected quarters.

The proposed effective dose of 2 ml, administered by deep intramuscular injection approximately 60 and 21 days before the expected parturition date and 15 days after calving has been confirmed.

The LTA from BAC of *S. uberis* is a new active substance not authorised for a veterinary medicinal product in the EU before.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

Benefit assessment

Direct therapeutic benefit

The benefit of UBAC is the reduction of the incidence of clinical intramammary infection caused by *S. uberis,* and to reduce the SCC in *S. uberis* positive quarter milk samples which was investigated in well-designed laboratory and field studies conducted to an acceptable standard. In addition, the reduction of milk production losses after *S. uberis* intramammary infections was shown in a multicentre field study.

The onset of immunity is approximately 1 month (36 days) after the second dose and the duration of immunity is approximately the first 5 months of lactation.

Additional benefits

S. uberis has been reported to be the most prevalent environmental pathogen causing mastitis.

UBAC is a vaccine aimed at reducing the use of therapeutic antibiotics in farms affected with clinical mastitis. Likewise, UBAC has a beneficial animal welfare potential by reducing the suffering of lactating cows due to severe clinical symptoms caused by udder infection.

Risk assessment

Quality:

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

Safety:

Risk for the target animal:

Administration of UBAC in accordance with SPC recommendations is generally well tolerated. Further, the vaccine is considered safe when used in a second vaccination cycle of three doses.

The main reported adverse reactions include local reactions at the injection site and transient temperature increase. Local swelling more than 5 cm in diameter at the injection site is a very common reaction after administration of the vaccine. This swelling will have disappeared or be clearly reduced in size by 17 days post vaccination. However, in some cases, swelling may persist for up to 4 weeks. In addition, a transient increase in rectal temperature (mean increase of 1 °C but may be up to 2 °C in individual animals) may very commonly occur in the first 24 hours after injection. The potential adverse effects of vaccination are reflected in the SPC.

No impact on reproductive performance or on milk production were observed after vaccination.

Risk for the user:

The user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice for products containing mineral oil is included in the SPC.

Risk for the environment:

UBAC is not expected to pose a risk for the environment when used according to the SPC recommendations.

Risk for the consumer:

All substances included in the composition of the vaccine are listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 or are considered as not falling within the scope of Council Regulation (EEC) No 470/2009. As numerical MRLs have not been set for any of the ingredients a withdrawal period of zero days can be established. UBAC is not expected to pose a risk for the consumer.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

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