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DEPARTAMENTO DE
MEDICAMENTOS
VETERINARIOS

Agencia Española de Medicamentos y Productos Sanitarios

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28022 – Madrid
España
(Reference Member State)

DECENTRALISED PROCEDURE

FINAL PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

VIMCO Emulsion for injection for ewe and goat

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MODULE 1

PRODUCT SUMMARY

EU Procedure number	ES/V/0209/001/DC
Name, strength and pharmaceutical form	VIMCO, *Staphylococcus aureus* inactivated, expressing Biofilm strain SP 140 ≥ 8.98 SaCC * * *Staphylococcus aureus* Cell Count in log_{10}. Emulsion for injection for ewe and goat
Applicant	Laboratorios Hipra, S.A. Avda. la Selva, 135 17170 Amer (Girona) Spain
Active substance(s)	*Staphylococcus aureus* inactivated, expressing Biofilm strain SP 140
ATC Vet code	QI03AB and QI04AB
Target species	Ewe and goat
Indication for use	For active immunisation of healthy sheep with mastitis problems, to reduce the incidence of sub-clinical mastitis (reduction of udder lesions, somatic cell count and *S. aureus* count) caused by *Staphylococcus aureus*. For active immunisation of healthy goats with mastitis problems, to reduce the incidence of sub-clinical mastitis caused by *Staphylococcus aureus* and Coagulase-Negative Staphylococci; when clinical mastitis caused by Coagulase-Negative Staphylococci however occurs, the severity of clinical signs (udder and milk aspect) is reduced. The onset of immunity and the duration of immunity have not been established.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Medicines Agencies website (http://www.hma.eu).

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Decentralised application in accordance with Article 12.3 of Directive 2001/82/EC as amended.
Date of completion of the original decentralised procedure	Day 210: 23/04/2014
Date product first authorised in the Reference Member State (MRP only)	N/A
Concerned Member States for original procedure	EL, FR, IT, PT

I. SCIENTIFIC OVERVIEW

***For public assessment reports for the first authorisation in a range**:*

Staphylococcus aureus (*S. aureus*) is recognised as the main contagious pathogen in bovine mastitis. The strain included in the Vimco vaccine is based on the presence of the Slime Associated Antigenic Complex (SAAC), which is an exopolysaccharide. This is an important virulence factor implicated in the adhesion of the bacteria to the epithelium of the mammary mucous. The induction of antislime antibodies will help the minor colonisation and subsequent multiplication of S. aureus in the glandular epithelium.

The product is produced and controlled using validated methods and tests, which ensure the consistency of the product released on the market.
It has been shown that the product can be safely used in the target species.
The product is safe for the user, the consumer of foodstuffs from treated animals and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC.
The efficacy of the product was demonstrated according to the claims made in the SPC.
The overall risk/benefit analysis is in favour of granting a marketing authorisation.

II. QUALITY ASPECTS

A. Composition

The product contains *Staphylococcus aureus* inactivated, expressing Biofilm strain SP 140 ≥ 8.98 SaCC (*Staphylococcus aureus* Cell Count in $\log_{10}$) and Benzyl alcohol as preservative.

The container/closure system is composed of 10 ml, 50 ml and 100 ml Type I colourless glass vials, closed with rubber stoppers (bromobutyl) and aluminium caps. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the adjuvant (Liquid paraffin), vaccine strain (SP 140), formulation, inactivating agent (Formaldehyde) and presence of preservative are justified. The quantitative composition has been well defined.

The inactivation process and the detection limit of the control of inactivation are correctly validated.

The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

The production process for the vaccine antigen is based on a "seed lot system" as described in Ph. Eur. monograph no. 62 (Vaccines for veterinary use).

The method of production of this active ingredient is an innovative method, which is carried out in such way that a high content of SAAC is induced. For this purpose, firstly a higher Slime producing strain of *S. aureus* was selected from different SP *S. aureus* strains. Secondly, a culture medium and culture conditions were set up in order to guarantee a good expression of SAAC.

The strain of *S. aureus* is propagated in a scale-up system. The culture of the fermentor is inactivated and afterwards the antigen is concentrated by centrifugation. The inactivation procedure is adequately validated by appropriate inactivation kinetic study. The concentrated antigen is stored at +2 °C - +8 °C for a maximum period of 12 months until it is used for blending purposes. The bulk of active ingredient is blended with other components to an emulsion, filled in defined containers, labelled and packed to obtain the finished product. The volume of antigen to be added in order to obtain the target concentration of 1 x 10^{10} microorganisms of the antigen per dose of 2 ml is calculated on the basis of concentration of total bacteria determined after the concentration step which follows the inactivation step.

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site.

Process validation data on the product have been presented in accordance with the relevant European guidelines.

C. Control of Starting Materials

Active substances

The original strain of *Staphylococcus aureus* CP8 was obtained from the isolate collection from DIAGNOS, the Diagnostic Centre of LABORATORIOS HIPRA, S.A.

This strain was characterized as a phenotype producer of Slime (SP) by means of immunoelectrophoresis (IEP) and the Congo Red test. It was also determined by immunoelectrophoresis (IEP) as a strain belonging to Capsular Polysaccharide 8 (CP8).

Information relating to the vaccine strain *S. aureus* CP8, their origin, characterisation, passage history, preparation and storage conditions has been provided. Seed lot systems have been followed. Identity and purity of MSB and WSB have been confirmed by morphology, growth characteristic and biochemical analysis. Serotyping will be introduced as a routine control in any new *S. aureus* working seed.

Excipients

Starting materials listed in a pharmacopoeia are sodium alginate, calcium chloride dehydrate, liquid paraffin, benzyl alcohol, Polysorbate 80, sorbitan oleate, sodium hydroxide, glucose monohydrate, formaldehyde solution (35%), sodium chloride, potassium chloride, disodium phosphate dodecahydrate, gelatine, sucrose, povidone, monosodium glutamate, water purified, water, highly purified and water for injections.

All starting materials are referred to Ph. Eur. with the exception of monosodium glutamate for which references are made to the US Pharmacopoeia (USP). Defined specifications are provided in the Certificates of Analysis (CoA). The Certificates of Analysis comply with the related monograph and results match every specification.

The following in-house media are used: freeze-drying excipient, TSB-G medium, CB120 culture medium, PBS solution, sodium hydroxide solution and antifoam solution. The composition, preparation and sterilisation are adequately described. Sterility control is performed by direct inoculation. Shelf live and storage conditions are well defined and validated.

The master and working seeds have been produced according to the Seed Lot System as described in the relevant guideline.

D. Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

Scientific data have been provided and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products and Directive 2001/82/EC has been satisfactorily demonstrated.

E. Control tests during production

The tests performed during production (Gram stain, viability/ purity, identity, count of viable bacteria, count of total bacteria, inactivation, pH, and sterility) are described and the results of 3 consecutive runs, conforming to the specifications, are provided.

Detailed information of the methods, their frequency, their function and their specifications are included in the dossier. Maximum pre-inactivation specifications (viable count according to the inactivation kinetic studies) are established.

The following methods are adequately validated:

- Concentration of viable bacteria
- Concentration of total bacteria
- SAAC concentration
- Test for complete inactivation.

F. Control Tests on the Finished Product

The tests performed on the final product conform to the relevant requirements; any deviation from these requirements is justified. The tests include in particular appearance, viscosity, identification and quantification of the preservative, pH, volume control, residual formaldehyde, conditioning, sterility, determination of endotoxins, safety test, and batch potency test.

Satisfactory validation data for the analytical methods have been provided. The following methods are adequately validated:
- Identification and quantification of the preservative
- Residual formaldehyde
- Sterility test
- Determination of endotoxins
- Batch potency tests.

The demonstration of the batch to batch consistency is based on the results of two industrial batches of Vimco produced according to the method described in the dossier. Other supportive data provided confirm the consistency of the production process.

G. Stability

Two studies are included in the dossier to demonstrate the stability of the antigen before blending for 12 months at +2 to +8 ºC and the stability of the final product for 18 months.

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life when stored under the approved conditions.

The proposed in-use shelf-life of 10 hours after first opening of the bottle was considered sufficiently substantiated by appropriate data (microbial safety, sterility and potency results).

III. SAFETY ASSESSMENT

The safety section is covered for all aspect of the vaccine safety and for the two target species, pregnant goats and ewes.

The studies presented in the safety part were satisfactorily described. The Applicant conducted adequate laboratory studies and one field study to assess the safety of a single, double and repeat single dose after intramuscular administration.

Laboratory trials

The safety of the administration of one dose, an overdose and the repeated administration of one dose in the target animal is demonstrated in two studies. The investigation was performed according to the recommendations of Directive 2001/82/EC as amended and the relevant guidelines.

A third study intended mainly to test the efficacy of the product is included because the safety for the animals was also checked.

Safety of the administration of one dose
Safety of one administration of an overdose
Safety of the repeated administration of one dose

In one study, administration of one overdose of Vimco to one group of 6 six pregnant goats were used to check the safety of an overdose. Then two single doses were administered later to test the safety for a repeated administration of the vaccine.

The parameters local reactions, general clinical signs, rectal temperature, serological responses, evolution of milk production and evolution of pregnancy, and new born calves were examined.

There was a temperature increase for 50% of the animals. Such a temperature increase did not affect the general health status of the animals. No animals showed a temperature rise greater than 2ºC.

For the general clinical signs, no systemic reactions attributable to the vaccine overdose and/or the repeated single doses were observed in any of the goats included in the study.

As for the local clinical signs, only a hard spot in the skin around the inoculation site less than 2 cm in diameter was observed in five goats after the administration of the vaccine overdose, six goats after the administration of the first single dose and in four goats after the administration of the second single dose. This finding was considered not clinically significant when it was not followed by evident signs of inflammation.

In a second study, safety of the administration of one overdose and the safety of the repeated administration of one dose was assessed in seven pregnant ewes by administering one double dose (4 ml) and two single vaccine doses: the basic vaccination scheme consists of the administration of two vaccine doses separated by 3 weeks in a way that the second dose was administered two weeks before parturition. A

third vaccination (single dose of 2 ml) was administered one week before the expected date of parturition.

The parameters local reactions, general clinical signs, rectal temperature, serological responses, evolution of milk production and evolution of pregnancy, and new born calves were examined.

Rectal temperature values higher than 40.0 ºC were reported in two ewes 2 hours after the administration of the vaccine overdose that lasted for 48 hours in one case and up to 4 days in the other case. No values higher than 40.0 ºC were reported after the administration of the first and second single doses. The average values only exceed 1.5 ºC after the administration of the vaccine overdose in two cases.

For the general clinical signs, no systemic reactions attributable to the vaccine overdose and/or the repeated single doses were observed in any of the ewes included in the study.

The local clinical signs showed only a hard spot in the skin around the inoculation site of 2-5 cm in diameter in one ewe after the administration of the vaccine overdose (ewe ref. 5) and one local nodule smaller than 2 cm in diameter was observed in two ewes as well. Slight local swelling was also reported in one ewe after the administration of the vaccine overdose. No local reactions were reported in any ewe after the administration of the vaccine single doses.

Effects on reproductive performance were examined: Both studies examined the evolution of pregnancy, the evolution of new born calves and the evolution of milk production. No reproductive troubles such as abortion or dystocia were reported.

All the ewes lambed healthy lambs that suckled normally during three days after birth. The weight at birth was considered as normal for this species. All the lambs remained healthy throughout the observation period of ten days post-parturition. This means the reproductive data shows no anormalities.

There are no data suggesting that this product might adversely affect the immune system of the vaccinated animal or its progeny therefore a specific study was not carried out.

The vaccine is inactivated and thus the specific tests to be performed for live vaccines are not applicable.

All substances included in the composition of this vaccine are listed in Annex 1 of Commission Regulation (EU) 37/2010 (not being necessary to establish MRLs), therefore no studies on residues have been performed.
Based on this information, no withdrawal period is proposed.

No specific assessment of the interaction of this product with other medicinal product was made. Therefore, an appropriate warning in the SPC is included.

Field studies

One study was performed for the evaluation of the safety and efficacy of the inactivated Vimco vaccine.

The animal serological status was not considered as a criterion of inclusion or exclusion, since the purpose of the study was to mimic real field conditions. The vaccination scheme was one administration at Day 0, around 5 weeks before the expected parturition date and a second dose at Day 21, corresponding to 2 weeks before the expected parturition date.

Parameters recorded for safety: The assessment of the safety parameters in ewes has been carried out in two farms. The vaccinated group was composed of 19 ewes and the control group of 21 ewes.
The parameters local reactions, general clinical signs (such as apathy, anorexia, reluctance to move, depression, etc) and adverse events, rectal temperature, effects on reproductive parameters and milk production (as secondary safety parameter) were examined.

Slight body temperature increments have been reported in the vaccinated group of goats. Such increments were higher than 1.5 ºC in two goats at 24 hours and in one goat 48 hours after the administration of the first vaccine dose respectively. The body temperature increments reported after the administration of the second dose were higher than 1.0 ºC in just two animals 48 hours after treatment. No cases of body temperature increment higher than 2ºC were reported in any case.

Local reactions at the inoculation site were observed in 85.7% of the vaccinated goats at different times of the observation period, whereas only 30.4% of the control group did so. The local reactions observed were limited to local swelling of less than 2 cm in most cases and of 2-5 cm in nine animals, all of them never lasting longer than 14 days. Only five vaccinated goats showed a local swelling higher than 5 cm in diameter that lasted up to 48 hours in just one case.

Neither the Vimco vaccinated ewes/goats nor the control ones showed any adverse event attributable to the vaccination. None of the vaccinated ewes/goats with Vimco showed anomalous general clinical signs after vaccination.

Slight body temperature increments have been reported in the vaccinated group of ewes and goats. No increases over 2ºC have been recorded at any time.

No ewe and no goat from the Vimco group showed abortion, dystocia or malformations at lambing.

User safety

VIMCO is an inactivated vaccine.
The raw materials used to prepare active ingredient and vaccine comply with the relevant Ph. Eur. monographs (where applicable) and are carefully controlled to prevent contamination with other infectious agents. The adjuvant comprises liquid paraffin. The excipients are sorbitan monooleate, Polysorbate 80, sodium alginate, calcium chloride dehydrate, benzyl alcohol, simeticone and water for injections. All components are included in Annex 1 of Commission Regulation (EU) 37/2010.

Liquid paraffin is a mineral oil but its concentration in the vaccine is very low compared to other oily vaccines. It is known that, in case of accidental self-injection, an oily adjuvant might cause local tissue irritation and lesions to the person administering the

vaccine. For that reason, the applicant has included in the SPC (section 4.5.) an advice to the user and to the physician in case of accidental injection/ self injection.

Benzyl alcohol is used as preservative with a quantity of 20 mg per dose in order to limit risks of product contamination after first use. It is used extensively as an antimicrobial preservative in a wide range of cosmetics and pharmaceutical formulations, including oral and parenteral preparations. Therefore, the preservative is not expected to represent a hazard to the user.

Sorbitan monooleate and Polysorbate 80 (authorised food additive-E433) are emulsifiers which promote the dispersion of watery droplets of antigen throughout the oil. The use of these emulsifiers does not represent a toxicity hazard to the user.

Sodium alginate is a gelification polysaccharide extracted from giant brown seaweed that precipitates in presence of calcium chloride. Simeticone is a commonly used antifoam, water for injections is the dilution vehicle of the vaccine and also commonly used in medicinal products for parenteral administration. Their utilisation does not represent a risk.

The conclusion that no specific risk associated with the use of this vaccine is identified is supported.

Ecotoxicity

An ecotoxicity Phase I study was performed, which demonstrated the product as having an estimated risk for the environment as effectively zero. Therefore, a Phase II ecotoxicity study was considered unnecessary.

IV. CLINICAL ASSESSMENT (EFFICACY)

Introduction and General Requirements

The efficacy against mastitis in pregnant ewes after the administration of the regimen of vaccination of the VIMCO vaccine has been demonstrated in the laboratory trial by means of a challenge. Concerning goats, the suitability of the established regimen of vaccination has been demonstrated under field studies.

The regimen of vaccination established for the vaccine VIMCO consists of a basic vaccination scheme (The first vaccination about 5 weeks before lambing/kidding and the second vaccination 21 days later, about 2 weeks before lambing/kidding). No re-vaccination is included and the basic vaccination scheme is to be repeated on each reproductive cycle.

The onset of immunity of four weeks after parturition has been established in base of the results obtained in the efficacy laboratory test demonstrated by the inoculation of a challenge strain around four weeks after lambing.

Laboratory Trials

The study design was established in compliance with the requirements stated in the European pharmacopoeia monograph 04/2008:50207 “Evaluation of efficacy of veterinary vaccines and immunosera; and monograph no. 04/2013;0062 “Vaccines for Veterinary Use”.

This study has been carried out under a randomised and blinded basis in 18 pregnant ewes. Two groups of pregnant ewes were included in the study design. The challenge strain was isolated from one ewe that had subclinical mastitis in a region of Spain.

The animals were not vaccinated against *Staphylococcus aureus* and seronegative or with low titres of antibodies against slime of *Staphylococcus aureus* at the time of inoculation.

The results of this laboratory trial showed reduction in the time of the abnormalities in milk for vaccinated ewes. The severity of the abnormalities was found not to be statistically significant.

No significant differences on general clinical signs between treatment groups were reported at any time.

A reduction of the time of colonization of *S. aureus* in vaccinated animals was found statistically significant. From day 7 after challenge, most of the vaccinated animals had healthy udders values and still showed significant differences with the control animals about the SCC (Somatic Cell Count) values.

Field Trials

A multicentric trial, in accordance with a protocol study carried out on different production farms and in compliance with the Good Clinical Practices (GCP) was

conducted. The breeds, farms and management systems were representative of the situation of other countries in which it is also intended to obtain the marketing authorisation of this vaccine.

The vaccine was administered in the target species, in pregnant ewes and goats, by the recommended route of administration (intramuscular) and according to the vaccination program.

The vaccinated group was compared to a negative control group, vaccinated with PBS (placebo).

The efficacy parameters studied under field conditions were:
- Efficacy of the vaccine in ewes:
  - Reduction in the incidence of intra-mammary infection
  - Reduction of the severity of the symptoms caused by intra-mammary infections.
  - Cure rate.
  - Reduction in the Somatic Cell Count (SCC).
  - Mastitis treatments with pharmacological products.
  - Rate of specific anti-slime antibodies of Staphylococcus aureus in blood serum.

- Efficacy of the vaccine in goats:
  - Reduction in the incidence of intra-mammary infection,
  - Reduction of the severity of the symptoms caused by intra-mammary infections.
  - Cure rate.
  - Mastitis treatments with pharmacological products.
  - Rate of specific anti-slime antibodies of Staphylococcus aureus in blood serum.

Ewes showed no differences in the number of intra-mammary infections with clinical or subclinical manifestations due to CNS between treatments groups. And the low number of cases of intra-mammary infection due to Staphylococcus aureus made impossible to evaluate the incidence of this infection. A laboratory trial was developed to show the efficacy in pregnant ewes.

Goats showed a reduction in some of the parameters observed. Incidence of intra-mammary infection after vaccination was reduced in vaccinated animals compared with controls; it was found a statistically significant reduction of the incidence for both S. aureus and CNS in a subclinical mastitis.

No conclusions on a reduction of the symptoms for clinical mastitis caused by *S. aureus* can be drawn as no cases of clinical mastitis with *S. aureus* were obtained for these evaluations.

About the cure rate also statistically significant differences can be found for intramammary infections caused by CNS. No statistically significant differences can be found for intramammary infections with *S. aureus* alone, only combined with the results for CNS infections significant differences can be obtained.

V . OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the risk benefit profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

Vimco is intended for immunisation of healthy sheep with mastitis problems to reduce the incidence of sub-clinical mastitis (reduction of udder lesions, somatic cell count and S. aureus count) caused by Staphylococcus aureus. And it is intended for active immunisation of healthy goats with mastitis problems, to reduce the incidence of sub-clinical mastitis caused by Staphylococcus aureus and Coagulase-Negative Staphylococci; when clinical mastitis caused by Coagulase-Negative Staphylococci however occurs, the severity of clinical signs (udder and milk aspect) is reduced.

The risk assessment is based on the estimated risks to target and non-target animals, users, consumers of animal derived food and to the environment.

The Vimco vaccine is in the interests of animal health at the Community level because mastitis is the most significant cause of economic loss to the dairy industry.

The choice of the vaccine components, the pharmaceutical form, the proposed formulation and the production process gives a vaccine that complies with all the quality specifications requested.

Based on a Phase I environmental risk assessment it can be concluded that the vaccine represents a negligible risk to the environment.

The vaccine does not contain any ingredients that are likely to pose a risk for consumers of milk and meat.

The vaccine contains liquid paraffin, a mineral oil as adjuvant but the concentration is low. It is known that, in case of accidental self-injection, an oily adjuvant might cause local tissue irritation and lesions to the person administering the vaccine.

The risk of the use of the vaccine VIMCO for the immunised animal can be evaluated as minimal. Only a transient increase in body temperature in the first 3 days for ewes and up to 48 hours for goats after immunisation may occur and a slight to moderate transient local reactions (swellings up to 5 cm) that didn't last more than 14 days.

For the user, special safety precautions (risk management measure) are mentioned in the product information as the vaccine contains mineral oil as adjuvant and it is known that, in case of an accidental self-injection, local tissue irritation and lesions to the person administering the vaccine can occur.

The benefits of the vaccine as stated above have been sufficiently substantiated. The risks identified for the target species, the user and the environment are considered acceptable. Therefore, the overall benefit-risk balance is considered as favourable.

Preventive measures other than immunisation for mastitis control that should be applied, within a good management program, include: (a) a clean, stress-free

environment (b) proper maintenance and operation of milking equipment; (c) good milking procedures including teat dipping; (d) a dry cow treatment program and culling chronic cows when necessary; and (e) a program for monitoring the health status of udders.

Based on the original and complementary data presented it can be concluded that the quality, safety and efficacy of VIMCO were considered to be in accordance with the requirements of Directive 2001/82/EC, as amended, and that the benefit-risk balance was favourable.

MODULE 4

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the veterinary Heads of Agencies website (www.hma.eu).

This section contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

None