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Productos Sanitarios (AEMPS)**
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(Reference Member State)

MUTUAL RECOGNITION PROCEDURE

**DRAFT PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY
MEDICINAL PRODUCT**

CEVAC CLOSTRIDIUM OVINO



MODULE 1

PRODUCT SUMMARY

EU Procedure number	ES/V/0120/001/MR
Name, strength and pharmaceutical form	CEVAC Clostridium Ovino Panclostil S (EL) Coglavax S (IT) ≥ 1.1 IU/ml (<i>Clostridium perfringens</i> type A) ≥ 10 IU/ml (<i>Clostridium perfringens</i> type C) ≥ 5 IU/ml (<i>Clostridium perfringens</i> type D) ≥ 2.5 IU/ml (<i>Clostridium septicum</i>) ≥ 3.5 IU/ml (<i>Clostridium novyi</i> type B) ≥ 2.5 IU/ml (<i>Clostridium tetani</i>) 100 % of protection in mice (<i>Clostridium sordellii</i>) At least 90 % of protection in guinea pigs (<i>Clostridium chauvoei</i>) Suspension for injection
Applicant	CEVA Sante-Animale (Ceva-Phylaxia)
Active substance(s)	Alpha toxoid of <i>Clostridium perfringens</i> type A Beta toxoid of <i>Clostridium perfringens</i> type C Epsilon toxoid of <i>Clostridium perfringens</i> type D Toxoid of <i>Clostridium septicum</i> Alpha toxoid of <i>Clostridium novyi</i> type B Toxoid of <i>Clostridium tetani</i> Toxoid of <i>Clostridium sordellii</i> Anaculture of <i>Clostridium chauvoei</i>
ATC Vetcode	QI04AB01
Target species	Sheep
Indication for use	Active Immunization against enterotoxaemia due to <i>C. perfringens</i> type A, B, C and D, and <i>Clostridium sordellii</i> and clostridial infections due to <i>C. novyi</i> type B, <i>septicum</i> , <i>chauvoei</i> and <i>tetani</i> .



MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Medicines Agencies (veterinary) (HMA(v) website) (www.HEVRA.org).



MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Mutual Recognition application in accordance with Article 12 of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	25/07/2007
Date product first authorised in the Reference Member State	07/09/2004
Concerned Member States for original procedure	Italy Greece

III. I. SCIENTIFIC OVERVIEW

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 slight reactions observed are indicated in the SPC>.

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.

IV. II. QUALITY ASPECTS

A. Composition

The product contains:

Active substances:

Alpha toxoid of <i>Clostridium perfringens</i> type A	≥ 1.1 IU/ml
Beta toxoid of <i>Clostridium perfringens</i> type C	≥ 10.0 IU/ml
Epsilon toxoid of <i>Clostridium perfringens</i> type D	≥ 5.0 IU/ml
Toxoid of <i>Clostridium novyi</i> type B	≥ 3.5 IU/ml
Toxoid of <i>Clostridium septicum</i>	≥ 2.5 IU/ml



Toxoid of <i>Clostridium tetani</i>	≥ 2.5 IU/ml
Toxoid of <i>Clostridium sordellii</i>	100 % protection in mice
Anaculture of <i>Clostridium chauvoei</i>	≥ 90 % protection in guinea pigs

Adjuvant:

Aluminium hydroxide as $\text{Al}(\text{OH})_3$ (adjuvant)	5.19 mg
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Excipients

Trometamol
Maleic acid
Sodium chloride
Free formaldehyde
Water for injection

The containers are plastic bottles of 50 ml, 100 ml and 250 ml for 25, 50 and 125 doses respectively. The bottles are of low density polyethylene, with grey brombutyl stoppers and sealed with aluminium caps. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the Aluminium hydroxide adjuvant, vaccine strain, formulation, inactivating agent, presence/absence of preservative are justified.

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 product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site, and in accordance with the European Pharmacopoeia and relevant European guidelines.

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

C. Control of Starting Materials

The active substances are Alpha toxoid of *Clostridium perfringens* type A, Beta toxoid of *Clostridium perfringens* type C, Epsilon toxoid of *Clostridium perfringens* type, Toxoid of *Clostridium novyi* type B, Toxoid of *Clostridium septicum*, Toxoid of *Clostridium tetani*, Toxoid of *Clostridium sordellii*, Anaculture of *Clostridium chauvoei*. These active substances are an established active substance described in the European. The active substances are manufactured in accordance with the principles of good manufacturing practice.



The active substances specifications are considered adequate to control the quality of the material. Batch analytical data demonstrating compliance with this specification have been provided.

Starting materials of non-biological origin used in production comply with the European Pharmacopoeia monographs where these exist. For the substances where there is not such requirement, the company has identified the source of the substance, explained how its quality is controlled and provided relevant certificates of analysis.

Biological starting materials used are in compliance with the relevant Ph. Eur. Monographs and guidelines and are appropriately screened for the absence of extraneous agents according to the Ph. Eur. Guidelines; any deviation was adequately justified.

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 and/or certificates of suitability issued by the EDQM 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 has been satisfactorily demonstrated.

E. Control tests during production

The tests performed during production are described and the results of 3 consecutive runs, conforming to the specifications, are provided.

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, extractable volume, physico-chemical controls (pH, Aluminium hydroxide, formaldehyde content), sterility control (absence of clostridia), safety control and potency control.

The demonstration of the batch to batch consistency is based on the results of 3 batches produced according to the method described in the dossier. Other supportive data provided confirm the consistency of the production process.

G. Stability

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.

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.



H. Genetically Modified Organisms

Not applicable.

J. Other Information

Shelf life: 18 months

In-use shelf life: After puncture of the stopper, use immediately

Storage conditions:

The product must be stored between +2°C and +8°C protected from light.

Do not freeze.

V. SAFETY ASSESSMENT

The laboratory and field studies have been conducted on the safety and efficacy of the product in sheep and details of batches used in these studies were provided.

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 laboratory studies. All safety tests carried out in a controlled environment or in the field included untreated control animals. Laboratory studies were performed on lambs of young age (2 and 8 weeks old) and on pregnant ewes. The field trials were performed on the target species on pregnant ewes, lambs of 2 and 8 weeks of age.

The investigation was performed according to the recommendations of Directive 2001/82/EC as amended and the relevant guidelines.

The adverse effects seen following the administration of one dose are a mild local reaction at the injection site, and swelling or a firm nodule that appears 2-5 days after administration. This nodule reaches a maximum of 18-22 mm around 5 to 7 days after vaccination. It is painless and disappears without need for treatment after 20-30 days. These observations are reflected in the SPC.

The adverse effects seen following the **administration of an overdose** in different sensitive categories (pregnant ewes, 2 and 8 week-old lambs) have shown that a mild local reaction at the injection site is expected. Swelling or a firm nodule appears 2-6 days after administration, reaching a maximum of 23-27 mm around 5 to 8 days after vaccination. It is painless and disappears without need for treatment after 20-30 days. Also these observations are reflected in the SPC.

Effects on **reproductive performance** were examined. Reproductive parameters were monitored in the safety study of administration of an overdose in pregnant ewes. Duration of



pregnancy, date and individual conditions of lambing, number of dead and living lambs were not affected by the effect of vaccination.

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.

About **residues** studies, the adjuvant and excipients used are Aluminium-hydroxide, Formaldehyde and Thiomersal. Aluminium hydroxide does not have a potential risk for humans and is considered safe for all food producing species. Formaldehyde remains below the highest amount authorised by the European Pharmacopoeia and it is recognised as safe for all food producing species. Thiomersal, the amount remains below 0.01% which is recognised also as safe for all food producing species.

Therefore, for this instance no MRL needs to be established and the withdrawal period is 0 days.

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

Two field studies were performed, one study that show the safety under field conditions in 8 weeks old lambs and in pregnant ewes, and other to demonstrate the safety of the administration of the vaccine in 2 weeks old lambs under field conditions, according to the recommended vaccination program.

Field studies were performed in 3 different sites for pregnant ewes, 3 sites for 8-week-old lambs and 2 separate sites for 2-week-old lambs. Different breeds of animals were used for each field trial and all trials were performed in accordance with the principles of “*good clinical practice*”.

In both trials, groups of 15 animals were treated.

Clinical examination was performed on the day of first vaccination (D1) and D2, D3, D4, D5 and then after 1,2 and 3 weeks. And on the day of 2nd vaccination (D29), D30, D31, D32, D33, and after 1,2 and 3 weeks. Parameters recorded for local signs were pain at the injection sites, swelling, oedema, indurations and other signs (daily). Parameters for general tolerance were behaviour after administration, appetite (daily), rectal temperatures (according to schedule of clinical examinations), individual body weight (at D1 and every 7 day until the end of the study).

In 8 week old lambs, the results showed only small nodules at the inoculation site lasted approximately 2 to 4 weeks post inoculation, when animals are vaccinated with each of the three vaccines, No palpable lesions were observed in any of the groups 4 weeks after injection. No local signs were observed in the control group.

In 2 week old lambs, the results were similar to these observed in the 8 week old lambs. The animals showed small nodules at three to five days post injection and lasted approximately 2 to 4 weeks post.



With regards to general tolerance, in both cases (2 and 8 week old lambs) no systemic side effects were noted during the study and no mortality that may be related to vaccination was observed in any group.

In pregnant ewes, the local findings were similar to those observed in lambs. With regards to general tolerance, in site 1 and 2 no systemic side effects were noted during the study and no mortality that may be related to vaccination was observed in any group. In site 3, no systemic side effects were noted during the study but one animal was found dead on day 47 of the study. This case was classified as a fatal lambing accident unrelated to the vaccination. No other mortality occurred during the study.

It may be concluded from these results that *Cevac Clostridium Ovino* was well tolerated in 2 and 8 week old lambs and in pregnant ewes. Local findings are consistent with those reported in laboratory studies and identical to those observed after the inoculation of the two reference vaccines.

Ecotoxicity

The applicant provided a first phase environmental risk assessment in compliance with the relevant guideline which showed that no further assessment is required further assessment was required. The assessment concluded that the vaccine does not constitute an environment hazard. No warnings regarding environmental exposure from the use of *Cevac Clostridium Ovino* are therefore required.

Warnings and precautions as listed on the product literature are adequate to ensure safety to the environment when the product is used as directed.

VI. CLINICAL ASSESSMENT (EFFICACY)

IV.B Clinical Studies (Immunologicals)

Laboratory Trials

Clostridial toxins are the main virulence factors, responsible for *Clostridium* diseases and the protection is achieved by vaccination-induced toxin neutralizing antibodies. The protection depends on the level of toxin-specific neutralizing antibodies and the amount of toxin delivered into the infection site, what has been well-documented in experimental animals, including rabbits and sheep.

The applicant has presented an extensive study of the literature to establish the minimum protective levels of antibodies against clostridial toxins in sheep sera. This data have been considered as references in the efficacy trials in sheep after vaccination with CEVAC CLOSTRIDIUM Ovino

Three immunological methods that have been used in these trials for checking the potency on the target species are the following:



-Toxin Neutralisation Test (TNT): For *Clostridium perfringens* C, *Clostridium septicum*, *Clostridium novyi* and *Clostridium tetani*. No specific monograph for *Clostridium perfringens* type A and *Clostridium sordellii* is described in Ph. Eur. A validation of a serological test of a *C. perfringens* type A according to the Ph. Eur and a challenge test on mice for *C. sordellii* based on the work published by Haas *et al.* has been performed.

For *C. chauvoei*, the challenge described in the Ph. Eur. monograph 0361 is performed on guinea pigs.

-Sero-neutralization test according to 9 CFR

-ELISA (Enzyme Linked Immuno-Sorbent Assay): These tests were used for measuring the antitoxin titres for the valences of *C. perfringens* type C and D. An ELISA was developed also for detecting the antibodies against *C. chauvoei*.

The efficacy of the product has been demonstrated in laboratory studies in a controlled environment in accordance with the relevant requirements which show that efficacy is supported by data from routine batch potency testing.

Cevac Clostridium Ovino was tested on lambs and pregnant ewes that are the two most important categories of sheep recommended for vaccination.

Two laboratory studies were conducted to show the potency and kinetics of different Clostridial antibody production before and after a booster, in 8 weeks old Hungarian lambs immunized with *Cevac Clostridium Ovino* vaccine. One study was performed in 8 week old lambs born from non-immunized ewes. The second study was performed in 8 weeks old lambs born from ewes that had been immunized with the same vaccine previously.

In both cases, animals were vaccinated at week 8 (D0), 12 (D28) and 20 (D84) of age. Blood samples were taken from each lamb before the first vaccination (D7), two weeks after the second injection (D42), and at six more time points (D7, D28, D42, D55, D69, D84, D98, D112 and D125). The evaluation of potency was performed in mice by Toxin Neutralisation Tests (TNT) for *C. perfringens* A, *C. perfringens* C, *C. perfringens* D, *C. septicum*, *C. novyi* and *C. tetani*. Moreover, ELISA was used in addition to the TNT, for measuring the antitoxin titres for the valences of *C. perfringens* C and D as markers. The ELISA was performed on pooled and individual samples too. For *C. sordellii*, as there is no specific Eur Ph. monograph a 9CFR method was adapted by CEVAC. The test was also performed on pooled sera.

The results from the first study support the immunization scheme proposed by the applicant. Animals will be immunized at 8 weeks of age. The second vaccination will be administered 4 weeks after the first vaccination (before the decrease of the antibody levels), and booster 2-4 weeks before any risk situation to ensure the establishment of a proper immune response.

The results from the second study showed that when the vaccine *Cevac Clostridium Ovino* is given to lambs of 8 weeks of age born from ewes that had been immunized with the same vaccine previously, during this time the antibodies titres remain higher than the minimum protective values.

The immunization scheme proposed by the applicant is fully justified and is considered acceptable.



Another laboratory study was conducted to show the potency and kinetics of different clostridial antibody production in 2-weeks-old Hungarian lambs immunised with Coglavax S (CEVAC Clostridium Ovino) vaccine before and after a booster. The study was performed in 2 weeks old lambs born from non-immunized ewes.

In this case the immunization scheme proposed by the applicant is considered overall acceptable and supported by the data obtained in this study.

Finally, another study that compares the potency of three polyvalent Clostridial Vaccines in pregnant Hungarian ewes and their newborn lambs was conducted. This study was focused mainly on transmission of passive immunity. In this case, the two aims for the vaccination were protection for pregnant ewes and colostral immunity afforded to their new born lambs.

The vaccine was administered twice at 4 weeks interval. The second vaccination was administered 2 to 7 weeks before the expected lambing. Blood samples were taken before the first immunization, 2, 5 and 7 weeks after second immunization and at the day of lambing. Blood and colostrum samples were taken at the day of lambing, and in one-day old lambs blood samples were taken after they received the colostrums.

The results showed that the proposed vaccination scheme, 2 injections of vaccine when the second vaccination is given 2-5 weeks before the expected lambing fully is justified by the data obtained.

Field Trials

The applicant has conducted field studies which provided information on the efficacy of the vaccine and the duration of immunity.

One field study was performed in which the potency of three polyvalent Clostridial vaccines in 8 weeks old French lambs born from immunized and non-immunized ewes was assessed.

The results showed that all the lambs vaccinated with *Cevac Clostridium Ovino* developed higher antitoxin levels for all the components compared to the minimum protective levels found in the literature. For *C. perfringes* C and D the highest antitoxin levels were developed in lambs immunized with *Cevac Clostridium Ovino*.

The same study was used to determine the potency of the vaccine under field conditions, in ewes by analysing the production of neutralising antibodies to different valences of three polyvalent clostridial vaccines. In this case, the vaccines were administered from 6 to 2 weeks before the expected lambing date. Six ewes per group were chosen randomly from 15 vaccinated animals and blood samples were taken from them before the first immunisation (D0) and two weeks after the second immunisation (D42).

The results showed under field conditions a significant immune response during 2 weeks after second vaccination in pregnant ewes.

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.



MODULE 4

VII. 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.HEVRA.org).

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