Agencia Española de Medicamentos y Productos Sanitarios (AEMPS)
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Spain
(Reference Member State)

MUTUAL RECOGNITION PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

CZV AVIAN TUBERCULIN PPD

MODULE 1

PRODUCT SUMMARY

EU Procedure number	ES/V/0119/001/MR
Name, strength and pharmaceutical form	**CZV Avian Tuberculin PPD**, 25000 IU/ml, Solution for injection
Applicant	CZ VETERINARIA S.A. La Relva s/n 3600 Porriño (Pontevedra). SPAIN
Active substance(s)	Purified protein derivative of *Mycobacterium avium* subs. *avium*, strain D4 ER
ATC Vetcode	QI02AR02
Target species	Bovine
Indication for use	For use in bovine animals from 6 weeks of age or older where, as a consequence of exposure to slow growing mycobacteria in the environment, cross sensitisation to bovine tuberculin is suspected.

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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	23/05/2007
Date product first authorised in the Reference Member State (MRP only)	18/09/1997 (Updated in November 2006)
Concerned Member States for original procedure	IE, EL, FR, PT and UK.

I. SCIENTIFIC OVERVIEW

CZV avian Tuberculin PPD is a purified protein derivative (PPD) of *Mycobacterium avium* strain D4 ER, in injectable solution, to be administered to bovine. The product is used for "in vivo" diagnosis.

The product complies with the requirements indicated in the applicable legislation, as Commission Regulation (EC) 1226/2002 and European Pharmacopoeia monograph 01/2005: 0535 (amended in supplement 5.8, monograph 04/2007: 0535).

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: (per ml)

Active substances:

Purified protein derivative from culture of *Mycobacterium avium*, subsp. a*vium* strain D4 ER 25 000 IU

Excipients

Phenol
Glycerol
Ponceau red (E124)
Phosphate buffered saline (sodium chloride, disodium phosphate and potassium phosphate)

The containers are glass vials Type I, 6cc, containing 50 doses (5 mL), with butyl rubber stopper and aluminium seal. The specifications and sterilisation are detailed and conform to the regulation.

The production process and controls follows the specifications of European Pharmacopoeia and OIE Manual. The process has been properly validated. The inactivation process and the detection limit of the control of inactivation are correctly validated. Other validation studies presented in the dossier are: Antimicrobial preservative efficacy, sterility test, protein nitrogen determination, phenol determination of tuberculin and potency validation

B. Method of Preparation of the Product

The avian tuberculin purified protein derivative CZV Avian Tuberculin PPD is a preparation obtained from products of culture and lyses of *Mycobacterium avium* subs. *avium*, strain D4 ER that are heat-treated.

The bacterial strain of choice is the one recommended by European legislation and OIE Manual. The production process and controls follows the specifications of European Pharmacopoeia and OIE Manual. The process has been properly validated, including a study of inactivation kinetics.
As a summary, the tuberculin is obtained from water-soluble fractions prepared by inactivation under steam flow of *Mycobacterium avium* cultures, and subsequent filtering. The active fraction of the filtrate (mainly proteins), is isolated by precipitation, washed and resuspended. The formulation of the final product is based on protein concentration and made by blending each of the components at an adequate rate. The stated potency for a dose of avian tuberculin is 2.500 IU (0.1 mL). Phenol is added as an antimicrobial preservative, glycerol is added as stabiliser and Pounceau red (E124) is added as colouring matter (to allow the user to differentiate between avian and bovine tuberculin in the comparative intradermal tuberculin test).

As indicated in Ph. Eur. monograph 01/2005:0503, an identification test is performed, in compliance with the requirements established.

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, also in accordance with the European Pharmacopoeia and relevant European guidelines.

C. Control of Starting Materials

The active substance of the product is purified protein derivative from culture of *Mycobacterium avium*, subsp. *avium* strain D4 ER. The active substances is established as described in the European legislation, and manufactured in accordance with the principles of good manufacturing practice.
The master and working seeds have been produced according to the Seed Lot System as described in the relevant guidelines.

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

The other starting material of biological origin is Potato, used as component of culture media. The 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.

Starting materials of non-biological origin used in production comply with the European Pharmacopoeia monographs where these exist. The applicant presents certificates of analyses of the starting materials showing that all the products are in compliance with relevant Pharmacopoeia monograph in force.

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.

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

The applicant presents a TSE risk assessment. The applicant has sent a TSE declaration of compliance together with format table.

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 the control tests applied during the production process for six batches of antigen are included in the dossier These data show consistency between the batches, and all results obtained from the control tests are within the specified limits.

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:

Control tests on the bulk product: Appearance, absence of acid-fast bacilli, absence of irritant properties, determination of pH, sterility, phenol content, protein concentration,

Control test of finished product: Appearance, sensitizing effect, toxicity, batch potency test, sterility, content and presentation.

The controls applied are appropriate and follow the specifications of the Ph. Eur. for control tests of the finished product for avian PPD tuberculin (monograph 535), as well as the specifications of the OIE for this product.

The results from the control tests carried out on six consecutive production batches are presented. All the controls performed comply with the acceptance criteria established, and the data demonstrate the consistence of the production process

G. Stability

Antigen stability: Satisfactory data have been provided to justify a shelf life of 12 months at 28°C for the storage of the concentrate PPD avian tuberculin until blending.

Finished product: Satisfactory results have been presented from two stability studies to justify a shelf life of 24 months at 2-8°C.

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

H. Genetically Modified Organisms

Not applicable.

J. Other Information

Shelf life: 2 years
In-use shelf life: Use immediately once the vial is opened.

Storage conditions: Store and transport between +2 □C – +8 □C protected from light. Do not freeze

SAFETY ASSESSMENT

The applicant presents the following studies and bibliographical data to support the safety of the administration of the product.
As presented in the SPC, the indications for use of the product is the diagnosis of bovine tuberculosis by means of the intradermal comparative tests which implies one injection of avian PPD tuberculin and one injection of bovine PPD tuberculin, given simultaneously.

In the studies presented the preparation was administered to cattle of 6-10 months old (in EU regulations is stated that cattle may be subjected to the tuberculin test from the age of 6 weeks). Unless not all the safety experiments have been performed in animals of the minimum recommended age, it is unlikely that, in view of the type of preparation, such animals will react differently in respect of safety than older animals. The pharmacovigilance data (discussed later) also support this.

The batch used in the studies had a potency of 2430 IU/dose which is equivalent to the potency guaranteed until the end of shelf life. It is of interest to note that the potency required by the Commission Regulation (EC) No. 1226/2002 is 2000 IU/ dose. This means that the product has been tested at a potency level above the minimum level specified by the Regulation.
The laboratory and field studies have been conducted on the safety and efficacy of the product in bovine and details of batches used in these studies were provided.

Laboratory trials

Safety of the administration of one dose:
The safety of the administration of a single dose was not demonstrated in laboratory tests, but other studies presented support the safety of the administration of one dose:

- The safety of the administration of an overdose have been demonstrated (see the following point)

- Pharmacovigilance data

As presented by the applicant the product has been used extensively in the field since 1997. A high number of doses have been used in Spain. In the Periodic Safety Update Report covering the period of September 1997- October 2001 states that 2.750.000 doses were used in this period and no adverse reactions were reported, and also the PSUR covering the period of October 2001 and August 2002 shows that during that period 701.000 doses were used. And no adverse reactions were reported.

These data supports the conclusion that the administration of a single dose is safe.

Safety of the administration of an overdose
The safety of the administration of an overdose is demonstrated. 8 Friesian cattle (6-10 months, free from tuberculosis and paratuberculosis) were intradermally injected with a double dose of the product on the right side of neck area and the same volume of PBS in the left side. The animals were injected with 0.2 ml avian tuberculin.

They were observed for the occurrence of systemic and local reactions during a period of 14

days. The body temperature was measured before injection, 4 hours after injection and daily for 4 days after injection.

The degree of local reaction at the injection sites was measured at different moments after injection. No systemic or local reactions were observed. No significant difference in skin thickness was present between the injections sites.

From the results obtained, is concluded that the administration of an overdose is adequately demonstrated.

Safety of the repeated administration of one dose

The safety of repeated administration has been demonstrated.

The study was performed as a continuation to the study of safety of one the administration of an overdose (previous one), using the same cattle as used in the previous study. The cattle received an overdose (0.2 ml) of avian PPD tuberculin, and were intradermally injected 48 days later with one dose (0.1ml) of the same product.
The experimental procedure was similar to that described before, and the same batch was used.

No systemic (including fever) or local reactions were observed 14 days post-injection. During the study no adverse reactions due to tuberculin inoculation were observed.
The safety of repeated administration was demonstrated in animals that had previously been injected with a double dose of the product; this makes the test more severe than required.
As a conclusion the safety of repeated administration of the product is considered proved.

Examination of reproductive performance

The applicant presents bibliographical studies to support this section.
There is no information in literature that the preparation will have a negative effect on reproductive performance.

Examination of immunological functions

The tuberculin PPD is an allergen used in the intradermal tuberculin test to detect tuberculoses animals. The intradermal administration of tuberculin induces a delayed hypersensitivity response in the infected animal that may be estimated 72 hours post-inoculation. The inoculated PPD is processed by antigen presenting cells and recognized by the sensitized T-cells in the infected animals, these cells release lymphokines that results in a delayed hypersensitivity response as skin thickening (swelling) at the injection site.

Studies and bibliography are presented

The immunological response is described and the studies presented are adequate to demonstrate the immunological response induced by the product.

Residues

The study for residues is not relevant because the active ingredient is a biological product. It

falls outside the scope of Council Regulation No, 2377/90. The other substances present as excipients are listed on Annex II of that regulation and so no MRL needs to be established.

Field studies

No specific field trials to determine the safety of the preparation have been performed.

However the safety of the preparation under field conditions can be concluded from the Pharmacovigilance data and also from the field efficacy study (included in part IV). The applicant also presents bibliographical studies. These studies have not been performed with the applicant's product, but due to the standardization of tuberculins, the results have been considered as additional data demonstrate the safety of the product.

Ecotoxicity

The risk to the environment of the preparation is negligible. The dose of the preparation that is administered is extremely small and none of its components is excreted into the environment. A summary is presented about this point.

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

CLINICAL ASSESSMENT (EFFICACY)

IV.B Clinical Studies (Immunologicals) Laboratory Trials

A laboratory trial was performed. The experiment was primarily intended to determine the safety and efficacy of an inactivated vaccine against bovine paratuberculosis. In the study also efficacy of the administration of CZV Avian and Bovine Tuberculin PPD were studied. Only the data relevant for the evaluation of the avian PPD will be summarised and evaluated in this report.

10 male cattle free of tuberculosis and paratuberculosis were vaccinated with an inactivated vaccine against paratuberculosis at the age of 2,5-3 months. A control group of 8 animals was injected with saline solution.
Two months later 8 animals of the vaccinated group and 6 of the control group were administered orally a suspension of live virulent *Mycobacterium avium spp. paratuberculosis* at six different occasions with an interval of 2 days.

The cattle from which they were born were tested for the absence of paratuberculosis by Intradermal tuberculin test (ITT), interferon assay, ELISA test, agar gel diffusion and faecal examination. All tests were negative.

The animals were examined for allergic reactions both with bovine and avian tuberculin at day

of vaccination and 45, 150 and 330 days later. The evaluation of the results of the Comparative Intradermal test was done as stated in the SPC and in accordance with Commission Regulation (EC) No 1226/2002.

At day 0, none of the animals developed a reaction neither against avian nor against bovine PPD, indicating that they were free of tuberculosis. At days 45, 150 and 330 all vaccinated animals has reactions to avian tuberculin (3-26 mm), but were negative to ITT comparative test (reaction to bovine tuberculin was equal to or less than reaction to avian tuberculin) at 48 and 72 hours. Regarding the unvaccinated animals, the response to both tuberculins remained negative at day 45 post-vaccination. Nevertheless on day 150 after vaccination, a reaction against both tuberculins was observed in the group "not vaccinated, infected animals" and again the response was stronger to avian tuberculin.
The animals of the non vaccinated non infected group always gave a negative result to both avian and bovine PPD tuberculins.

Based on the results obtained, the specificity and sensitivity of the product in the comparative ITT test is proven. Also many scientific publications support this.

Field Trials

The specificity and sensitivity of CZV Avian Tuberculin PPD was examined in a **field trial.** The trial was initially performed to demonstrate the safety and efficacy an inactivated vaccine in the control of bovine paratuberculosis.
The trial was conducted on two farms known to be free of tuberculosis but not free of paratuberculosis (to study the safety and efficacy of an inactivated paratuberculosis vaccine). The animals were regularly examined for paratuberculosis with the Intradermal Comparative Test. The antibody response was studied with the ELISA test and the cellular response by measuring the gamma interferon.

Herd nº 1. A group of 296 cattle were used of which 239 were vaccinated with inactivated paratuberculosis vaccines and 57 kept as controls. They were all subject to the Intradermal Comparative Test before and at 2 different moments after vaccination.

Herd nº2 A group of 392 cattle were used of which 217 were vaccinated with inactivated paratuberculosis vaccine and 67 kept as controls. They were all subject to the Intradermal Comparative Test before and after vaccination.

In herd nº1, the response to avian tuberculin in the vaccinated groups was found positive in 83.5-95.6% of the animals, whereas the response to bovine tuberculin was negative. In herd nº2, the percentage of vaccinated animals positive to avian tuberculin was 86-97.6%, and the response to bovine tuberculin was negative.

The results show that paratuberculosis vaccination did not result in the development of positive reactions against the bovine PPD indicating that the avian PPD is specific.

Spanish report (Appendix 6)
The Spanish authorities have provided a summary of the use of bovine and avian tuberculin produced by CZV between 1998 and 2007. In 2006, 6 627,000 doses of bovine tuberculin

and 989 000 doses of avian tuberculin were used in the National programme. In the last 5 years, the animal incidence of bovine tuberculosis declined from 1.38% to 0.3% and the herd prevalence declined from 2.9% to 1.52%. These results indicate a positive effect of the use of these tuberculins. To estimate the sensitivity and the specificity of the comparative tuberculin test under field conditions, the Spanish authorities have provided the results obtained in a region of low prevalence (Canary Islands). The herd prevalence is 0.36% and the animal incidence is 0.20%. On 1385 herds tested, 16 gave a positive result to the comparative tuberculin test. The post mortem examination (lesions and positive PCR/culture) confirmed 5 herds infected and 11 as uninfected. The apparent sensitivity and the negative predictive value of the test at the herd level are near 100% and the specificity is near 99.20%.

Bibliographical study: Aranaz et al., 2006. (Appendix 9). Assessment of diagnostic tools for eradication of bovine tuberculosis in cattle co-infected with *M. bovis* and *M. avium subsp. paratuberculosis* (Vet. Res. 37:593-606).
A cattle herd (301 animals) with dual infection (bovine tuberculosis and paratuberculosis) was followed during 3.5 years. A testing (comparative tuberculin test with CZV tuberculin combination, the IFN-□□assay and serology of paratuberculosis) was repeated 8 times over the period. Animals which reacted to the IDTB test and a group of other selected animals were slaughtered in abattoirs. A total of 131 animals were slaughtered (44 were positive for the comparative IDT test and 87 were negative) and bacteriological culture was used as golden standard for the determination of the true status of the animals. The comparative tuberculin test detected 65.2% (sensitivity determined on the 23 *Mycobacterium bovis* -culture positive animals) of the *Mycobacterium bovis* culture-positive cattle. The specificity was 73.2% (26.8% of false positive reactors on the 108 culture negative animals). This sensitivity figure has a limited value since only animals positive to comparative test were taken into account meanwhile inconclusive animals were not included. Even more the epidemiological situation of the herd should be considered an extreme case and therefore sensitivity is probably underestimated.

The applicant also presents other Bibliographical studies. These studies have not been performed with the applicant's product, but due to the standardization of tuberculins, the results have been considered as additional data demonstrate the efficacy of the product.

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

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