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SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for LETIFEND (EMA/V/C/003865/0000)

Common name: canine leishmaniasis vaccine (recombinant protein)

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



Introduction

On 14 October 2014, the applicant Laboratorios LETI, S.L.U. submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for LETIFEND through the centralised procedure, falling within Article 3(1) of Regulation (EC) No 726/2004 (biotechnological veterinary medicinal product).

The eligibility to the centralised procedure was agreed upon by the CVMP on 10 October 2013 as the product is developed by means of a biotechnological process. The rapporteur appointed was C. Muñoz and the co-rapporteur was J.-C. Rouby.

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

LETIFEND is a recombinant vaccine containing as the active substance a chimerical protein consisting of 5 antigenic fragments from four different *Leishmania infantum* proteins. The pharmaceutical form of the whole product is lyophilisate and solvent for solution for injection. The proposed route of administration is subcutaneous (SC) use. The target species is dogs.

The vaccine was proposed for the active immunisation of dogs from 6 months of age to reduce the risk of developing clinical leishmaniasis.

The vaccine is presented in a box containing 1, 4, 5, 10, 20, 25, 50 or 100 single doses of the lyophilisate in type I glass vials with bromobutyl stoppers and aluminium caps, and correspondingly 1, 4, 5, 10, 20, 25, 50 or 100 type I glass vials of solvent for solution for injection.

On 18 February 2016, the CVMP adopted an opinion and CVMP assessment report.

On 20 April 2016, the European Commission adopted a Commission Decision granting the marketing authorisation for LETIFEND.

Scientific advice

Scientific advice from the CVMP was provided on 16 September 2010 (EMA/411358/2010). The scientific advice pertained to regulatory, quality and efficacy aspects of the dossier. Adherence to the advice was generally maintained, however, a few considerations were not followed. The points concerned are addressed under the appropriate sections of this report. In summary, the parameters that deviated from the scientific advice caused some difficulties for the interpretation of some of the results, however the overall results are considered as valid.

MUMS/limited market status

A minor use minor species (MUMS)/limited market classification was established for this product by the CVMP, and the Committee confirmed at their 11 March 2010 meeting that, where appropriate, the data requirements in the appropriate CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMA/CVMP/IWP/123243/2006-Rev.2) would be applied when assessing the application. MUMS/limited market status was granted for the following reason: the CVMP classified LETIFEND as veterinary medicinal product for minor use in dogs.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (version 1.0 dated 22 May 2014) 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

The active substance of LETIFEND is manufactured by 3P BIOPHARMACEUTICALS SL, C/ Mocholi 2, Poligono Industrial Mocholi, Noáin, Navarra, 31110, Spain.

The manufacturer responsible for batch release is Laboratorios LETI, S.L.U., C/ Del Sol, 5, Poligono Industrial Norte, Tres Cantos, Madrid, 28760, Spain.

Good Manufacturing Practice (GMP) certificates were provided for all the sites involved in production and testing of product.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the GMP certification of the manufacturing sites were considered in line with legal requirements.

Part 2 - Quality

Composition

The active substance is a recombinant chimerical protein, "Protein Q" which consists of 5 antigenic fragments from four *Leishmania infantum* (zymodeme MON-1) proteins. Each (single dose) vial contains ≥ 36.7 ELISA units. No adjuvants are included in the composition. The active ingredient is lyophilised in a buffered medium containing arginine hydrochloride which helps to suppress the protein aggregation and preserve the protein stability. Other buffer components are boric acid and sodium chloride.

The solvent vials contain water for injections.

Containers

The freeze-dried fraction is presented in a 3 ml type I colourless clear glass vial sealed with a 13 mm bromobutyl type I rubber stopper and an aluminium cap.

The solvent is presented in a 3 ml type I colourless clear glass vial also sealed with a 13 mm bromobutyl type I rubber stopper and an aluminium cap.

The container and closures comply with the relevant monographs of the European Pharmacopoeia (Ph. Eur.).

Details of the validation of the dry heat method used to sterilise the vials was provided.

Development pharmaceuticals

The product was developed by selecting 5 antigenic fragments from four proteins from *Leishmania infantum* (zymodeme MON-1). The fragments were cloned into a commercial plasmid vector which was used to express the recombinant Protein Q in *E. coli*.

L. infantum MON-1 which was used for selecting the genes introduced by molecular biology techniques in the recombinant protein, is the most prevalent strain of the parasite in Europe and is considered relevant for the European epidemiological context. Several controls were implemented to identify the correct sequences and their location on the gene, as well as to test for the consistency of production and the selection of the master cell bank (MCB) and working cell bank (WCB).

Developmental studies were performed in order to obtain the final composition of the vaccine at the concentration which gave the best results in a comparative dose study.

A study was conducted to select the adjuvant. However, no adjuvant was eventually included as this avoided the occurrence of adverse reactions whilst maintaining the same, or a better, immune response as the formulations with adjuvants.

In the developmental studies the product concentration was based on total protein and eventually a fixed amount of protein was selected. This strength was used as the reference batch for the potency test; the chosen potency being based on the duration of immunity (DOI) study. This reference batch was used to establish the minimum titre (ELISA units) for the release of future batches.

The vaccination schedule was studied and a single dose of vaccine by SC route of administration was established as the basic vaccination scheme. The final product testing initially relied on determination of the total concentration of protein by bicinchoninic acid assay (using bovine serum albumin as a standard) and potency (by amino acid analysis). However, the initial method used was shown not to be accurate or repeatable. Following the CVMP Scientific Advice, indirect ELISA test was used which allowed for quantification of the active ingredient in the final vaccine. The ELISA method in combination with the identification tests (protein profile and identity using SDS page and western blot) enables full characterisation of the active ingredient in the finished product.

Method of manufacture

The vaccine is lyophilised in order to provide an adequate shelf life for the product.

The active ingredient is lyophilised in a buffered medium containing arginine hydrochloride which helps to suppress the protein aggregation and preserve the protein stability. Other buffer components are boric acid and sodium chloride.

The manufacture of Protein Q is well documented. The development of the recombinant method has been included and justified. The steps followed to produce the MCB and the WCB are included obtaining a seed lot system. This was fully tested and it is intended to be used for future commercial manufacture. Data on batch consistency has been provided.

Antigen production has been adequately described. After fermentation, Protein Q is purified by a series of downstream purification steps.

Blending details are also described. The final vaccine is blended by mixing a buffer (sodium chloride, boric acid and arginine hydrochloride) and Protein Q antigen bulk, and then is lyophilised. A blending target is based on the requirement for finished batches to pass the potency specification of ≥ 36.7 EU/dose.

Three batches of active ingredient produced consecutively to demonstrate the consistency of production.

The vials are freeze-dried. One batch of finished product was out of specification for relative humidity. The bulk antigen used to produce the same batch of finished product was also out of specification for conductivity. However, the batch of finished product out of specification showed efficacy on all of the other developmental tests therefore no concerns remain. The remaining batches of finished product produced from the same bulk antigen batch however complied with the specifications.

A detailed description of the lyophilisation process is included. The calculations for the estimated potency and intended potency for blending are provided. Bulk antigen is mixed with the buffer and lyophilised. The batch is then tested according to the finished product specifications.

Data on batch-to-batch tests has been provided to show the stability of the product.

Control of starting materials

Active substance

LETIFEND is a recombinant vaccine containing as the active substance a chimerical protein consisting of 5 antigenic fragments from four different *Leishmania infantum* proteins.

Active substance controls are described in the control of the manufacturing process. A flow chart including all production steps and controls in process was provided. Appropriate tests are carried out on the bulk antigen, including turbidity, degree of coloration, sequencing, peptide mapping, molecular mass, Protein Q global purity, host cells proteins, host cells residual DNA, residual urea, residual ampicillin, residuals, total protein content, potency, pH, conductivity, bioburden, and endotoxins. Results for these control tests have been provided.

In compliance with the Ph. Eur. monograph 0784 (products of recombinant DNA technology), the identity, purity and potency of the final active ingredient have been established on the basis of a wide range of chemical, physical, immunochemical and biological tests.

Testing of the MCB and the WCB were performed according to the requirements of Ph. Eur. 0062 "Vaccines for veterinary use", Ph. Eur. 0784 "Recombinant DNA technology, products of", and also the CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010).

The nature of the starting materials, the manufacturing process, the controls and treatments applied enable assurance of the sterility of the vaccine and the absence of the introduction of any extraneous agent.

Control tests on the MCB, WCB, antigen bulk and lyophilised product guarantee the consistency and homogeneity of the production.

Excipients

The final vaccine is blended by mixing the active substance with a buffer containing an aqueous solution of sodium chloride, boric acid and arginine hydrochloride. All the excipients comply with the relevant current specifications of the Ph. Eur.

No other substances are included in the final product.

Water for injections is used as the solvent.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

No materials derived from animal sources are used for the MCB, WCB or for the active ingredient manufacture, therefore all raw materials fall outside the scope of the Note for Guidance on minimising the risks of transmissible spongiform encephalopathy (TSE) agents via human and veterinary medicinal products (EMA/410/01 rev.3) and Commission Directive 1999/104/EEC. The product is therefore out of scope of the relevant Ph. Eur. monograph and the Note for guidance.

The overall transmissible spongiform encephalopathy (TSE) risk associated with this vaccine is considered negligible.

Control tests during production

The routine tests provided ensure the consistency and homogeneity of the vaccine production.

Routine control tests are carried out at various stages during production of the antigen.

For the production of the MCB and WCB several control tests are performed (cell growth, optical density, the biomass produces, the pH and the total protein weight).

The antigen production is controlled during several steps, and the control tests performed are: appearance (optical density, turbidity and degree of opalescence, and degree of colouration), identity (SDS-PAGE), identity by western blot, protein profile by SDS-PAGE, peptide mapping, potency by ELISA technique), purity, pH of every antigen bulk, and microbiological tests (bioburden, bacterial endotoxins).

Some additional controls were performed during the development of the product on the first produced batches however are not intended to be included as routine control tests. The developmental control tests are sequencing, the molecular mass determination, residual host cells proteins by western blot, peptide mapping and conductivity.

For the lyophilisation process, appropriate controls are carried out at various stages: UV absorbance, bioburden and filling controls (weight, pressure, temperature, time, cap check and vacuum).

All control test results during production were in conformance with the specifications.

A protein identity test by western blot (by SDS-PAGE) is carried out according specific standard operating procedures for the identification of Protein Q and Protein Q-related proteins on both the antigen bulk and the finished product. Protein bands are identified by used of monoclonal antibodies.

Control tests on the finished product

The description of the methods used for the control of the finished product and the specifications were provided.

The following tests are performed on the finished product: visual appearance, identity by western blot and by protein profile SDS-PAGE, potency by ELISA technique, physiochemical and microbiological tests.

The specifications proposed at batch release and at the end of shelf life are appropriate to control the quality of the finished product and guarantee the safety and efficacy of released batches of the product. The results of the analysis of six consecutive production runs of the freeze-dried vaccine and for two consecutive production batches of the solvent were presented and these comply with the required specification.

Following the scientific advice from the CVMP data from several batches were provided to show the consistency of scale up batches.

Stability

Stability of the bulk active ingredient was tested for up to 24 months at -20 °C.

Stability data for the finished product are provided for up to 27 months storage at temperatures between 2–8 °C and also at 25 °C. Although the results observed were similar for both temperatures, it was proposed to store the product at 2–8 °C and this is reflected in the SPC.

No in-use stability data have been generated and therefore a recommendation to use the product immediately after reconstitution is reflected in the SPC.

Overall conclusions on quality

The composition and characteristics of the vaccine were well documented. Scientific advice from the CVMP was obtained regarding the quality of the product and tests to be performed. Adherence to the advice was generally maintained and the overall results can be taken as valid.

The manufacturing process was satisfactorily described and validated.

Construction of Protein Q expressing cells is clearly described. Sequence testing, residues and further tests to assess for the identity, purity, integrity and stability of the obtained recombinant protein are performed. Developmental tests comply with the specifications except in one case. A failure with the conductivity test was shown and was out of specifications but new tests showed no more batches out of specification. The tests included are acceptable. All the starting materials listed in the Ph. Eur. are of a satisfactory quality.

No material of animal origin is used in the manufacture of the product.

The in-process tests are described satisfactorily.

Control tests on the finished product are described in satisfactory detail. Data provided indicate satisfactory batch-to-batch consistency.

Stability data for the antigen batches is demonstrated for up to 24 months of storage.

Stability data on the finished product demonstrate a shelf life 24 months when stored at 2–8 °C or at 25 °C.

Potency of the product has been adequately described. The finished product specification is 36.7 EU/dose or higher.

An updated batch protocol template for the vaccine, in line with the OCABR/OBPR format, was provided.

In addition, the applicant is recommended to provide the following information post-authorisation: full scale batch certificates.

Part 3 – Safety

Safety documentation

Three (3) safety studies (2 laboratory studies and 1 combined field trial for safety and efficacy) were

provided in accordance with the Ph. Eur. monograph 5.2.6 on Evaluation of safety of veterinary vaccines and immunosera and Directive 2009/9/EC. Laboratory studies were Good Laboratory Practice (GLP)-compliant whereas the field trial was Good Clinical Practice (GCP)-compliant.

A total of 158 vaccinated dogs were enrolled in the 3 safety studies. In the field study, 116 out of 120 vaccinated dogs were revaccinated 1 year after the first administration of the vaccine.

Laboratory tests

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

The safety of single and repeated SC administrations of the vaccine, and the administration of a single overdose, was evaluated in Beagle dogs of 6 to 7 months of age. Twenty four (24) dogs were enrolled in this study and divided in 3 groups of 8 dogs each. A group was treated with one dose of LETIFEND (0.5 ml) and two additional doses were given, each 14 days apart by SC injection. The second group was treated with a 2-fold overdose (1 ml) by SC injection, and the last group was left unvaccinated as a control (only a placebo was administered).

Animals were monitored for clinical signs and local reactions at the injection site twice a day for 14 days after each treatment. Rectal temperature and food consumption was measured daily and body weight was recorded weekly. In addition, histopathology of the injection site and prescapular lymph nodes was performed.

No systemic adverse reactions were observed.

Transient scratching was observed immediately after vaccination but for less than 4 hours. No difference with the control groups was observed.

In conclusion, the study demonstrated that subcutaneous administration of the vaccine to 6 month old dogs is safe and well tolerated.

A transient hardening at the injection site was observed after a repeated dose. However, as the vaccine is intended only for a single administration and hardenings were not observed with either single dose or 2-fold (overdose) treated animals, the CVMP did not consider this finding relevant or of concern.

A second study was performed to investigate both the evaluation of the immune response and efficacy of a single dose of the vaccine in dogs of the minimum age. Results from the study support the safety of one dose administration. Forty four (44) animals of 5 to 6 months of age were enrolled in the study. One dose of the vaccine, containing a relative potency (RP) ≥ 1 was administered subcutaneously to 22 dogs. A group of 22 dogs were administered a placebo control (buffer). No reactions were observed either after the single administration or after the booster vaccination in the 22 dogs that were experimentally challenged with *Leishmania*.

Local reactions were monitored for 4 days. Red spots were observed in both the control and vaccinated animals for up to 4 days. However, as these reactions were seen in both groups of animals (vaccinated and controls) they were not considered related to the product and this is considered acceptable.

In conclusion, results from laboratory studies showed that the administration of a single dose of LETIFEND to dogs of the minimum age is considered safe. Adverse reactions such as scratching at the injection site are adequately addressed in the SPC. Red spots observed after vaccination in one of the studies were not considered linked to the vaccine.

Examination of reproductive performance

No study has been carried out to assess the safety of the product in pregnant or lactating dogs. Therefore LETIFEND cannot be recommended for use in dogs during pregnancy and lactation and this is adequately addressed in the SPC.

Examination of immunological functions

No specific tests on immunological functions were carried out and this is considered acceptable considering that the vaccine does not contain any adjuvant or any live organism.

Special requirements for live vaccines

Not applicable.

Study of residues

Not applicable. The vaccine is intended for use in a non food-producing species.

Interactions

No interaction studies with other veterinary medicines have been carried out and a precautionary measure has been included in the SPC accordingly.

Field studies

According to the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMA/CVMP/IWP/123243/2006-Rev.2), field studies are not required if the safety of the product is already satisfactorily demonstrated by laboratory studies. A combined field study for safety and efficacy was submitted.

The safety investigation was conducted on a total of 120 animals aged from 6 months to 11 years. One (1) dose of the vaccine was administered and 1 year after the first administration a booster vaccination was carried out on 116 animals. The batch potency test results showed low titre after a re-test, however since the efficacy was demonstrated the safety results are also considered acceptable. The following examinations were carried out for local reactions at the injection site such as oedema, pain, skin induration and inflammation. Moreover observations of systemic reactions were performed including rectal temperature and observations of the integumentary, circulatory, respiratory, musculoskeletal, genitourinary, digestive, neural systems; as well as the appearance of the ears, eyes, lymph nodes and mucous membrane.

No general signs or local reactions at the injection site related to LETIFEND administration were observed in any dog at any time point after injection of the vaccine.

Nine (9) seropositive dogs were included in the study and no adverse reactions after the 2 vaccinations were observed over a 2 months observation period after the second vaccination.

In conclusion, it was demonstrated that the vaccine was well tolerated and no general or local adverse events were observed. Seronegative dogs revaccinated one year after the first administration of the product did not show any adverse reactions after a 2-month observation period.

Moreover, results from the efficacy part of the field study (described under part 4 of this document) support the safety of one administration of LETIFEND and of the booster vaccination after 1 year. In this

study, 215 vaccinated dogs were revaccinated after 1 year (day 365) and no adverse reactions were observed after one year from the revaccination (day 730).

User safety

A user safety risk assessment has been conducted in accordance with the CVMP guideline for user safety for immunological veterinary medicinal products (EMA/CVMP/IWP/54533/2006). There is no expected user safety concern. The vaccine, to be administered by SC injection, does not contain any organism (live or inactivated). Neither the active substance nor the excipients are expected to pose a risk for the user at the concentrations used in the product.

It is considered that the probability of accidental self-injection of this product is very low and that the probability that the user self-injects a whole vaccine dose is negligible. In addition, there are no potential hazards identified in the vaccine for the user, even if such exposure occurred.

The user safety for this product is acceptable when used as recommended and no specific warning is therefore needed in section 4.5 of the SPC.

Environmental risk assessment

An environmental risk assessment has been provided in accordance with the Note for Guidance on the environmental risk assessment of immunological veterinary medicinal products (EMA/CVMP/074/95).

Hazard identification:

- The vaccine is administered by the SC route to individual dogs.
- The active ingredient, Protein Q, is a recombinant protein purified from the bacterial cell culture in which it is produced, and according to Directive 2001/18/EC it does not fall under the definition of a genetically modified organism (GMO).

Exposure to hazard:

- None of the vaccine components are toxic to the environment. They are not metabolised or excreted.
- The quality of packaging materials complies with the relevant requirements of Ph. Eur.
- There is a very minimal risk of environmental exposure to the vaccine and the consequences would be negligible even if this did occur.

Based on the data provided the ERA can stop at Phase I. LETIFEND is expected to pose a negligible risk for the environment when used according to the SPC.

Environmental risk assessment for products containing or consisting of genetically modified organisms

Not applicable.

Overall conclusions on the safety documentation

The safety of the vaccine was investigated in 2 GLP-compliant laboratory safety studies and 1 GCP-compliant field study. The studies were performed in accordance with Ph. Eur. 5.2.6 and Directive 2009/9/EC.

Data from laboratory studies and from the field study allowed to conclude that the administration of one dose and the administration of the booster injection of LETIFEND to non-infected dogs from 5 months of age is well tolerated.

The safety of the vaccination and revaccination in infected dogs was demonstrated only for 2 months after re-vaccination and it is reflected in SPC for accuracy of information.

A very common adverse reaction is a transient scratching at the injection site which resolves spontaneously within 4 hours. The adverse reaction is appropriately reflected in section the SPC.

The safety of the product was not demonstrated in pregnant or lactating dogs and this is reflected in the SPC.

The user safety for this product is acceptable when used as recommended, and no specific warning is needed in the SPC.

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

Overall, data from the laboratory studies and from the field study indicate that the vaccine has an acceptable safety profile, and the most common adverse reaction is scratching at the injection site

Part 4 – Efficacy

Introduction and general requirements

Three (3) GLP-compliant laboratory studies (dose determination and onset of immunity (OOI), DOI at 12 and at 6 months post vaccination and a study on the immunological mechanism of action and efficacy of a single dose) and one GCP-compliant field study were provided to demonstrate the efficacy of the vaccine.

Analytical methods were used to establish the efficacy parameters. Scientific advice was not entirely followed as two studies had been initiated earlier. These concerned the diagnostic methods employed that differed between laboratory and field studies and concerning the timepoints for the study samples (which were not completely justified to cover all aspect of duration, consistency and intensity of parasitaemia in the field trial study). The parameters that were not followed made it more difficult to interpret some of the results but the overall results can however be taken as valid. Where appropriate, methods have been fully validated in line with Ph. Eur. 0062, VICH GL49 and VICH GL9. Method descriptions and validation reports were provided. In all efficacy studies the dogs were injected subcutaneously one dose of the product or one dose of placebo.

Efficacy studies have been conducted in accordance with Directive 2001/82/EC and the Ph. Eur. monographs: Vaccines for veterinary use (0062) and Evaluation of efficacy of veterinary vaccines and immunosera (5.2.7).

In the laboratory studies, the immune response was analysed and results showing the presence of antibodies against the vaccine antigen (ELISA IgG2 against Protein Q), the presence of soluble *Leishmania* antigen (ELISA IgG2 against SLA) and the total IgG have been provided. The cellular response has been analysed by delayed-type hypersensitivity (DTH). The analytical part of the studies was completed by showing a reduction in parasite burden.

The parasite burden was determined by culturing lymph nodes or bone marrow, by smears or by quantitative PCR (qPCR). The qPCR results showed high variability. Due to the sparse distribution of

parasites in all stages of the disease the occurrence of a negative culture and/or PCR results is to be expected and therefore individual negative results would not exclude that the animal can be infected.

In the field study, parasite detection was performed only in those dogs that were suspected of having developed leishmaniasis, based on their clinical signs or serology. At the end of the follow up period, one bone marrow sample was taken from every dog for parasite detection.

Laboratory trials

Challenge model

Three (3) laboratory trials were carried out to investigate the efficacy of the vaccine according to a challenge model established by the applicant. As leishmaniasis is a very difficult disease to reproduce and the clinical signs of infection can differ between animals, the challenge studies are considered as supportive of the efficacy field trial.

The challenge strain was obtained from an infected dog in Spain and classified as a zymodeme MON-1 (M/CAN/ES/96/BCN150/MON-1) which is responsible for most of the cases of visceral leishmaniasis in humans and for more than 85% of the cases of canine leishmaniasis in dogs. Dogs were infected with 500,000 promastigote forms in 0.5 ml saline buffer administered intravenously via the cephalic vein.

Naturally infected dogs develop clinical signs in a low percentage (10% to 12%) as shown in 2 published studies (D. Otranto et al Clin Vaccine Immunol. March 2009 vol. 16 no. 3, 337–343 and Dantas-Torres et al Vet. Parasitol. 140: 54–60) whereas challenged animals showed clinical signs in almost 100% of the placebo animals. Due to the difference between challenge infection and natural infection, laboratory studies are considered only as supportive to the field trial data. In addition, some differences could be observed comparing a natural infection with the present challenge model in a prepatent period. Differences were mainly in the number of promastigotes during the infection and in the number of infected animals. Challenge infected dogs developed the disease within 1 year. For naturally infected dogs the incubation time is variable.

Dogs were challenged 28 days after the administration of the vaccine and 6 and 12 months after vaccination. The vaccine dose and the OOI in one study, and DOI for 6 and for 12 months in another study, were investigated to support the efficacy claims.

In all studies the level of IgG2 was higher in vaccinated animals compared to control animals.

Vaccinated and placebo animals had an increase on DTH response. In one study a positive DTH reaction was detected in more vaccinated dogs (60 to 90%) than in the controls (50%). The maximum score (indicating a bigger response) was also greater in the vaccinated dogs than in the controls, however differences were not statistically significant in all studies.

OOI

A laboratory study was designed with the aim to determine the final dose of the vaccine and to establish the OOI. Fifty (50) Beagle dogs from 8 to 18 months of age were enrolled in the study. Forty (40) dogs were vaccinated subcutaneously with 4 different doses of vaccine (25 µg, 50 µg, 100 µg and 200 µg of Protein Q/dose) and 10 dogs remained as controls and a placebo was administered. All dogs were challenged, as described previously, 28 days after vaccination or after placebo administration.

Dogs were monitored using different diagnostic methods (SLA, IFAT, bone marrow/lymph node smears and clinical signs) for 1 year after vaccination (330 days post infection(dpi)) to monitor parasite infection and clinical signs of disease.

Results from the study with the selected dose for the product (50 µg of Protein Q/dose) showed a high antibody response (IgG2) to Protein Q at 14 days after vaccination. In conclusion, an immunological response is therefore observed at 14 days after administration of the product. However, this result cannot be linked to protection.

The final dose of 50 µg of Protein Q/dose was selected based on the following different factors:

- The immunological response, when it could be observed, showed statistically significant differences between placebo and vaccinated animals in the production of cellular and humoral response for the groups vaccinated with 25 µg and 100 µg of Protein Q/dose.
- Clinical signs (percentage of dogs with signs and the number of signs per dog): greatest reduction was seen in the groups vaccinated with 25 µg and 50 µg of Protein Q/dose.
- Histopathological lesions: the greatest reduction in the severity of the lesion was observed in the groups vaccinated with 100 µg and 200 µg of Protein Q/dose. The highest reduction in the number of pathological lesions was observed in the groups vaccinated with 50 µg and 100 µg of Protein Q/dose.
- Parasite burden: parasite burden was measured by direct count of the parasite of tissue smears (spleen and lymph node), by culture method on spleen and lymph node and by PCR on blood and liver samples. Results from spleen samples showed a statistical significant reduction in the parasite count in all vaccinated groups (25 µg, 50 µg, 100 µg and 200 µg of Protein Q/dose) compared to placebo dogs however, it could not be seen in other organs such as the liver or lymph nodes. PCR results showed a statistical significant reduction in parasite burden in blood in groups vaccinated with 25 µg and 50 µg of Protein Q/dose.

In relation to the OOI, results after challenge demonstrated an OOI at 28 days after vaccination.

DOI

A laboratory study was designed to investigate the DOI by evaluating the reduction of clinical signs in two groups of vaccinated dogs challenged at 12 months and at 6 months after vaccination. Thirty two (32) Beagle dogs from 14 to 18 months of age were used for each challenged group. The batch of vaccine used in this study was also used as a reference in the batch potency test.

Eight (8) vaccinated dogs and 8 control dogs were challenged at 6 months after the administration of the product and 8 vaccinated dogs and 8 control dogs were challenged at 12 months after the administration of the product. All dogs (vaccinated and controls) were observed for 1 year (360 dpi) for the evolution of clinical signs of leishmaniasis.

Results at necropsy showed that control dogs had significantly more clinical signs of infection when compared to vaccinated dogs ($p=0.0247$ in the 6 months challenge study and $p=0.0378$ in the 12 months challenge study).

Vaccinated animals showed an increased DTH response to *Leishmania* antigens compared to the control groups (71% versus 41%, respectively). However, there were no statistically significant differences in the 6 months challenge study. A significant difference in the size (mm) of the DTH response was observed in the vaccinated group compared to the control group at 48 hours ($p =0.012$) in the 12 months challenge study.

Some biopathological parameters (such as the anatomical pathology of the spleen, liver and kidney and histopathology of the spleen, liver, kidney and lymph node) were monitored during the study, and abnormalities were observed including abnormal blood counts and changes in the concentration of serum proteins. The results showed the efficacy of the vaccine, since the laboratory abnormalities of canine

leishmaniasis were clearly present in the group of control dogs, compared to very few abnormalities in vaccinated dogs.

In conclusion, the overall results showed efficacy by a reduction of clinical signs and symptoms (lymphadenomegalia, conjunctivitis, episcleritis, dermatitis, alopecia, body fat loss), by a reduction of IFAT titres, by a reduction of parasite burden and by a reduction of pathological abnormalities (lesions in spleen, liver and kidney) in the vaccinated groups after challenges at 6 or 12 months. Differences in DTH response were statistically significant comparing vaccinated animals versus control animals at 48 hours after vaccination in the group challenged at 12 months after vaccination.

Due to reduction of clinical signs and differences in DTH responses in vaccinated animals in the 12 months challenge study a DOI of 12 month has been established.

Immunological mechanism of action and efficacy

An additional study was designed to evaluate the immune response and efficacy of a single dose of vaccine in Beagle dogs experimentally challenged 28 days after vaccination.

Twenty two (22) dogs from 5.4 to 5.9 months of age were vaccinated subcutaneously with one dose of vaccine and 22 other dogs were used as controls and received a placebo.

The challenge material on this occasion was not sufficiently virulent to cause the expected progression of the clinical pathology during the 1-year observation period. Minor clinical signs due to the *Leishmania infantum* challenge were observed in both vaccinated and control groups.

Serological responses to the vaccine antigens increased after vaccination however did not interfere with the field detecting tools for *Leishmania* allowing for differential diagnosis of infected from vaccinated animals. Based on the tests used, there were no differences in the cellular response between the groups after vaccination or after challenge.

A beneficial effect was observed as a lower parasite burden was seen in vaccinated animals compared to placebo treated animals; however the results were not statistically significant.

In conclusion, results for humoral immune response (by IFAT) and secondary efficacy endpoints (biopathology and parasite burden) support the additional benefit of vaccination, in terms of attenuating the evolution of the active infection.

Influence of maternally derived antibody (MDA) on the efficacy of the vaccine

The efficacy of the vaccine in relation to MDA has not been investigated as dogs are vaccinated from 6 months of age and no remaining MDA are expected at that time. This is acceptable.

Field trials

One GCP-compliant field study, conducted partially in France and in Spain, was provided. The study was designed to investigate clinical efficacy and safety of the vaccine in dogs.

The primary parameter, which was taken into account to evaluate the vaccine efficacy, was the percentage of vaccinated dogs showing no development of leishmaniasis (vaccine success) against vaccinated dogs showing clinical signs of the disease (vaccine failure).

In total, 549 dogs were included in the study: 361 dogs of 35 different breeds and 188 crossbreed dogs.

Different breeds of dogs were randomly allocated into 2 experimental groups (control and vaccinated). No seropositive dogs were included in the efficacy part of the study and no clinical signs on the day of vaccination were observed except in 2 dogs that were excluded prior to start the study.

Two hundred seventy five (275) animals were vaccinated with LETIFEND and 274 dogs were used as controls and received placebo. Out of the 275 vaccinated animals, 215 dogs were given a booster vaccination one year after the first administration. The entire observation period was two years (730 days).

Confirmation of leishmaniasis was defined on the basis of the following criteria:

- in the presence of clinical signs, a positive soluble *Leishmania* antigen (SLA) and/or positive IFAT, and presence of *Leishmania* in bone marrow or lymph nodes
- in the absence of clinical signs, high positive IFAT results and presence of *Leishmania* in bone marrow or lymph nodes at the last time point.

To track the onset of clinical disease, the appearance of various clinical signs associated with leishmaniasis were evaluated periodically during the study. Clinical observations were performed including the assessment for the presence of some of the most common clinical manifestations of the disease.

Number of dogs with leishmaniasis showing clinical signs

At the end of the observation period (2 years after vaccination), results show that the total number of dogs confirmed with *Leishmania* in their lymphoid organs was lower in the vaccine group in comparison with the placebo group (9.4% of the total 155 booster vaccinated animals versus 16.1% of the total of 156 placebo animals, $p=0.0564$). Also, both clinical signs and the presence of the parasite in the lymphoid organs were significantly less after vaccination, which suggests that vaccination reduces the infection rates.

During the trial, 18 dogs presented clinical signs of leishmaniasis: 12 control dogs and 6 vaccinated dogs out of 429 animals that ended the study (549 dogs were recruited at the beginning of the study however 429 dogs survived). Moreover, additional 9 dogs, (7 from the placebo group and 2 from the vaccine group), presented clinical signs of leishmaniasis at the end of the observation period (2 years).

In total 19 leishmaniasis cases from the placebo group and 8 leishmaniasis cases in the vaccinated group were confirmed (by presence of clinical signs, by positive SLA and/or positive IFAT, and presence of *Leishmania* in the bone marrow or lymph nodes).

The analysis of lesions in organs showed that 89% of the control dogs with clinical signs of leishmaniasis (17 out of 19) had more than 1 organ affected and 50% of the vaccinated animals with clinical signs of leishmaniasis (4 out of 8 dogs) had only 1 organ affected. No subclinical cases were presented in this study.

These results showed that the incidence of the disease was significantly lower in the vaccinated group than in the placebo group after 2 years of observation.

Additional 7 dogs from the placebo group were suspected to be infected on the basis of 2 out of 3 positive criteria required to confirm a case of leishmaniasis (clinical signs of the disease, detection of the parasite in lymphoid organs and positive serological results). Since they were not fully confirmed, these data have been taken into account only as supportive in the overall conclusion. No further dogs from the vaccinated group were considered suspected to be infected.

Number of clinical signs of leishmaniasis per dog

At the end of the observation period a comparison between vaccinated dogs and control dogs was performed on the basis of the number of clinical signs per dog and their distribution (general/local signs or both) per dog with the aim of demonstrating the efficacy of LETIFEND in reducing clinical signs of leishmaniasis in vaccinated dogs.

In conclusion, results showed that the number and the intensity of clinical signs per animal and the number of animals showing clinical signs were less in vaccinated dogs when compared to controls.

The statistical analysis based on odds ratio from the field trial demonstrates that the probability of an exposed population developing the disease is lower in vaccinated than in unvaccinated animals.

In France, 11 vaccinated dogs showed clinical signs and 65 vaccinated dogs did not show any clinical sign. Thirteen (13) placebo dogs showed clinical signs and 67 placebo dogs did not. The difference between vaccinated and control dogs with clinical signs of leishmaniasis was not statistically significant ($p=0.7586$).

In Spain 11 vaccinated dogs showed clinical signs and 84 vaccinated dogs did not show any clinical signs. Forty eight (48) placebo dogs showed clinical signs and 58 placebo dogs did not. The difference between vaccinated and control dogs with clinical signs of leishmaniasis was statistically significant ($p=0.0000$).

Considering the whole study, a total of 22 vaccinated dogs showed clinical signs and 149 vaccinated dogs did not show any clinical signs. Sixty one (61) placebo dogs showed clinical signs and 125 placebo dogs did not. The difference between vaccinated and control dogs with clinical signs of leishmaniasis was statistically significant ($p=0.0000$).

Considering all results from the whole study, at the end of the observation period, dogs from the vaccinated group showed less clinical signs (22 out of 171 (12.9%)) than dogs from the placebo group (61 out of 186 (32.8%)). These results are statistically significant.

With regard to the distribution of clinical signs, dogs were divided in three categories on the basis of showing symptoms/clinical signs:

- General signs of the disease: weight loss, thinness, dullness, increased size of lymph nodes/ lymphadenitis, asthenia, tiredness, mucosal paleness
- Local signs (e.g. one organ system affected): for example, "blepharitis"
- General and local signs.

The clinical signs in dogs with confirmed clinical leishmaniasis were distributed as follows: in the group of the 19 control dogs, 4 dog presented only general signs, 1 dog showed only local signs only and 14 dogs presented both: local and general signs. In the group of the 8 vaccinated dogs, 2 dogs showed only general signs 3 dogs presented only local signs and 3 dogs showed local and general signs.

The results demonstrate that vaccinated dogs exhibited general signs of leishmaniasis (associated or not with local signs) less frequently than placebo dogs.

Mortality rates (none of them related to leishmaniasis) were evaluated and no statistically significant differences between vaccinated and control animals were observed.

In conclusion, a total of 19 leishmaniasis cases in the placebo group and 8 leishmaniasis cases in the vaccinated group were confirmed. The incidence of the disease was significantly lower in the vaccinated group than in the placebo group.

Protein Q vaccine was found to be 72% efficacious in the prevention of clinical cases of leishmaniasis. The likelihood that a dog vaccinated with LETIFEND develops a confirmed clinical case of leishmaniasis or clinical signs of the disease is respectively 5 and 9.8 time less than that for an unvaccinated dog. The likelihood that a vaccinated dog would show a positive result for the parasite in the lymphoid organs is 3.5 times less.

Overall conclusion on efficacy

The efficacy of the vaccine was investigated in 3 GLP-compliant laboratory studies and 1 GCP-compliant field study. The diagnostic methods (techniques and samples) used in the studies were not homogeneous. However, the overall results were comparable among studies and this is considered acceptable.

At present three laboratory methods are the most used in the diagnosis of leishmaniasis (SLA or IFAT or rk-39 rapid diagnostic test) and they do not react with the active substance of LETIFEND, allowing the differentiation between infected and vaccinated dogs.

The efficacy of the vaccine has been investigated in challenged dogs under laboratory conditions, using a challenge model which was considered acceptable. However, since this model was not able to simulate the full symptoms and lesions of the disease, results from laboratory studies can be considered only as supportive of the efficacy of the vaccine that was demonstrated in the field study.

Laboratory studies demonstrated the OOI at 28 days after vaccination and DOI of 1 year (12 months) after vaccination.

One laboratory study showed a significant difference in DTH response in the vaccinated dogs compared to control dogs which demonstrated that animals are in part protected against *Leishmania infantum*; however, the immune response observed would have required further demonstration that its origin was exclusively produced by the vaccination, and not by natural infection and data could not be taken into account.

From one of the laboratory studies provided no conclusions could be drawn because the scoring system to classify the disease was used different.

The efficacy of the vaccine in relation to MDA has not been investigated as dogs are vaccinated from 6 months of age and no remaining MDA are expected at that time. The vaccine is not recommended for use during pregnancy and lactation as no such study has been carried out and it is properly reflected in the SPC. As a result, any dams vaccinated more than one month prior to conceiving are unlikely to transmit significant levels of MDA to the progeny.

In the field study, a significant treatment difference was observed in clinical disease in vaccinated dogs (confirmed case of leishmaniasis) compared to unvaccinated dogs (confirmed case of leishmaniasis).

Overall, data demonstrate that the product is effective for the active immunisation of non-infected dogs from 6 months of age in reducing the risk of developing clinical leishmaniasis after infection (challenge or natural infection) with *Leishmania infantum*.

No conclusions can be drawn for the efficacy in seropositive dogs from the results of the field study (see safety part of the study which is described in Part 3 of this report), in which 9 seropositive dogs were included to demonstrate that the presence of antibodies against SLA (as a result of a previous leishmaniasis infection) does not interfere with the protective immune response to vaccination with LETIFEND. Therefore the indication is restricted to non-infected dogs. This is reflected in the SPC.

Part 5 – Benefit-risk assessment

Introduction

Leishmaniasis is an important disease in dogs that is endemic in the Mediterranean countries of Europe, the Middle East and many subtropical areas of the world. In the past decade, an increased incidence of canine leishmaniasis in endemic zones as well as spread of the infection to non-endemic areas of Europe

has been observed. Canines are the main reservoir for the parasites and play a relevant role in transmission to humans. The aetiological agent – *Leishmania infantum* – is transmitted by sandflies of the genus *Phlebotomus*.

In endemic areas dogs become exposed immediately. Evolution of the infection in dogs is then complex and unpredictable. Some will develop protective immunity, some remain asymptomatic after infection and may relapse later and others develop a clinical disease. It is considered that establishment of infection and development of the disease both depend on the host's immunological response and that once the parasite escapes immunity and is able to multiply, no clearance is possible anymore. Infection may evolve over a period of a few weeks to several months toward disease patterns that can be extremely variable and polymorphic, which makes it difficult to classify dogs within specific categories.

LETIFEND is a vaccine intended to reduce the risk of developing active infection and/or clinical disease after exposure to *Leishmania infantum* infection in non-infected dogs.

The active substance is a recombinant protein (Protein Q) produced by selection of 5 different fragment sequences from 4 different proteins of *Leishmania infantum* and is synthesized by *E. coli*. Protein Q is able to trigger immunity against 5 antigenic fragments contained in the parasite. Scientific advice was provided for the development of the product with regard to regulatory, quality and efficacy aspects. It was generally followed; the advice given on efficacy was not fully taken into account however conclusions are acceptable.

The product has been classified as MUMS/limited market and therefore reduced data requirements apply and have been considered in the assessment.

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

Benefit assessment

Direct therapeutic benefit

Well conducted laboratory trials including experimental challenge demonstrate that when administered to seronegative dogs the product is efficacious in increasing the cellular immune response. A treatment effect on both the number of clinical signs and lesions, and the number of symptomatic dogs could be observed in comparison to controls. The OOI has been established 28 days post vaccination and the DOI has been demonstrated for 1 year post vaccination.

Efficacy of the vaccine was demonstrated in one field study performed in endemic zones with high infection pressures over a 2 year period where animals were naturally exposed to *Leishmania infantum*. The vaccine reduced the severity of the disease, including clinical signs and parasite burden in spleen and lymph nodes. A reduction of the risk of developing an active infection could be evaluated.

The benefit of the vaccine has been adequately described in different epidemiological situations such as in endemic areas, in non-epidemic areas or in areas with a low infection pressure.

Despite the fact that complete protection against leishmaniasis or eradication of the disease cannot be achieved, this vaccine is able to reduce the risk of developing a clinical case of leishmaniasis due to *Leishmania infantum* in vaccinated animals from 6 months of age.

A longer prepatent period was demonstrated in infected vaccinated dogs and this is considered a direct benefit for those animals, which will result in a longer time period without clinical signs of the disease.

In summary, the benefit of the vaccine is the active immunisation of non-infected dogs from 6 months of age to reduce the risk of developing an active infection and/or clinical disease after exposure to *Leishmania infantum*.

Additional benefits

LETIFEND increases the range of available treatment options against leishmaniasis in dogs.

The vaccine has a long lasting effect (12 months) and it can be expected that the product may reduce the need for antimicrobial treatment in dogs against leishmaniasis.

Infected vaccinated dogs have a reduced probability of spreading the disease compared with non-vaccinated dogs due to the reduced parasite burden that has been demonstrated in vaccinated animals.

Risk assessment

Main potential risks have been identified as follows:

Quality:

The formulation and manufacture of the vaccine is well described and specifications set will ensure that product of consistent quality will be produced. The CVMP recommends the provision of the full scale batch certificates post-authorisation.

For the target animal:

The product is generally well tolerated in the target animal. Mild and transient reactions after vaccination cannot be excluded. Scratching at the injection site is very commonly observed after a single dose administration. Some hardenings are also observed at the injection site after repeated administration of the vaccine, however since only a single administration is recommended no warning is required on the SPC.

After vaccination dogs are not fully protected and may still become infected and develop clinical signs. Therefore a warning that vaccination should not prevent any measures taken to reduce exposure to sand flies has been added to the SPC.

Safety was investigated in re-vaccinated infected dogs for a period of 2 months resulting in no safety concern. The efficacy in infected dogs has not been demonstrated.

For the user:

The user safety for this product is acceptable when used as recommended.

For the environment:

The product is not expected to pose any risk to the environment when used according to the SPC.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

The product has been shown to have a positive benefit-risk balance overall.

The vaccine has been demonstrated to be efficacious for the indication for active immunisation of non-infected dogs from 6 months of age to reduce the risk of developing an active infection and/or clinical disease after exposure to *Leishmania infantum*.

The OOI has been established 28 days after vaccination and the DOI has been demonstrated 1 year after vaccination.

The formulation and manufacture of the vaccine is adequately described and set specifications will ensure that a finished product of consistent quality will be produced.

The product is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended and appropriate warnings have been included in the SPC.

Conclusion on benefit-risk balance

The overall benefit-risk evaluation for the product is deemed positive with a sufficiently clear and complete SPC and product literature.

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 LETIFEND is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

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