10 November 2022
EMA/892443/2022
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Neoleish (EMEA/V/C/005538/0000)

Vaccine common name: Canine leishmaniasis vaccine (recombinant DNA plasmid)

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Introduction

The applicant CZ VETERINARIA, S.A. submitted on 29 September 2020 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Neoleish, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 23 January 2020 as Neoleish has been developed by recombinant DNA technology.

At the time of submission, the applicant applied for the following indication:

For the active immunisation of dogs from 6 months of age to reduce the risk to develop an active infection and clinical disease, and to reduce parasite burden in bone marrow and blood, after contact with Leishmania infantum.

The efficacy of the vaccine was demonstrated in a field study where dogs were naturally exposed to Leishmania infantum in zones with high infection pressure over a two-year period.

In laboratory studies including experimental challenge with Leishmania infantum, the vaccine reduced the severity of the disease, including clinical signs and parasite burden in bone marrow, spleen and lymph nodes.

Onset of immunity: 2 weeks after the primary vaccination course.

Duration of immunity: 12 months after the primary vaccination course.

The active substance of Neoleish is a plasmid DNA containing the sequence expressing the LACK protein of *Leishmania infantum*. The target species is dogs. The product is intended for administration by nasal use.

Neoleish solution for nasal spray contains, as controlled in the finished product, 212.5 to 250 µg micrograms of supercoiled DNA plasmid per dose containing the sequence expressing the LACK protein from *Leishmania infantum.* The product is presented in packs with 1 glass vial containing 1 dose of 1 ml.

The applicant was registered as an SME pursuant to the definition set out in Commission Recommendation 2003/361/EC at time of submission of the dossier but is not anymore an SME.

The rapporteur appointed is Christine Miras and the co-rapporteur is Merete Blixenkrone-Møller.

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

On 10 November 2022, the CVMP adopted an opinion and CVMP assessment report.

On 20 December 2022, the European Commission adopted a Commission Decision granting the marketing authorisation for Neoleish.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

The applicant requested eligibility of this application for MUMS/limited market by the CVMP, and the Committee confirmed that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) would be applied when assessing the application. MUMS/limited market status was granted on 19 April 2018 as the indication for the active immunisation of *Leishmania* negative dogs from 6 months of age, to reduce the parasite load and clinical signs after contact with *Leishmania infantum* in dogs is considered a minor use.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant provided a detailed description of the pharmacovigilance system (v6 – February 2013) which fulfils the requirements of Directive 2001/82/EC. 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 Union or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the active substance and final product takes place in CZ VETERINARIA, S.A. La Relva – Torneiros s/n, 36410 Porriño, Spain in the EU. This site is also responsible for packaging and batch release.

The site has a manufacturing authorisation by the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) and a GMP certificate issued following an inspection is provided and it is satisfactory.

Overall conclusions on administrative particulars

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

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The finished product is presented as 1 ml solution of a DNA plasmid containing the sequence of the LACK protein from *Leishmania infantum* (formulation at 250 micrograms per dose) for intranasal administration to dogs. No adjuvants are included in the composition and the vaccine is formulated with phosphate buffered saline (potassium dihydrogen phosphate, disodium phosphate anhydrous, sodium chloride and water for injection).

Container and closure

The vaccine is available in monodose presentation.

The vaccine is filled as one dose (1.1 – 1.2 ml) into 3 ml type I glass vials previously sterilised by heat treatment and closed with autoclaved perforable butyl rubber stoppers and aluminium seal.

The containers and closures are in compliance with the European pharmacopoeia requirements and their sterilisation is adequate.

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

Product development

The vaccine is a solution of the pPAL-LACK plasmid obtained from the purification of the culture of transformed *E. coli* pPAL-LACK. The plasmid was created by insertion of the LACK gene (homologous to Leishmania's activated protein kinase C receptor) into a commercial plasmid and after replacement of antibiotic resistance genes with an alternative non-antibiotic resistance marker.

The development of a DNA vaccine to be administered by the intranasal route has been justified by the applicant on a general and theoretical way from an immunological point of view. The selection of the LACK antigen is also justified as the LACK protein is expressed in promastigotes and amastigotes forms of different Leishmania species, it is highly conserved and preliminary published protection results, in different models, confirmed the interest of this protein as a vaccine antigen. Whole elements contained in the final plasmid are described and their presence and properties defined and justified.

The vaccine is formulated in phosphate buffer saline with well-known pharmaceutical ingredients in compliance with Ph. Eur. standards. The list of excipients is included in section 6.1 of the SPC. The formulation of the vaccine is targeted on a fixed DNA content of 250 µg/ml.

Most of the clinical studies presented in the dossier were conducted with vaccine batches either experimental or produced according to a preliminary non-optimised strategy. Data have been provided allowing to conclude that the optimisation of the process aimed to increase plasmid yields and improved consistency of the production, and do not affect the quality of the active ingredient. Specifications set for the controls of the finished product (controls of the active substance and impurities) have been justified based on clinical data.

Description of the manufacturing method

- Culture phase (upstream phase of the process)

Seed cultures are prepared by incubation of culture medium with a bactericidal and antifungal substance inoculated with thawed working seed. Production cultures are prepared by inoculating seed cultures into production fermenter containing culture medium and a bactericidal and antifungal substance followed by incubation. The obtained culture is concentrated by successive washing and centrifugation steps. The concentrated cell mass may be stored or further processed.

- Purification phase (downstream phase of the process)

Bacterial cultures are subjected to lysis and different purification steps to obtain the plasmid. The clarified culture is diluted and injected into chromatography column. The fraction containing plasmid DNA is collected. The DNA is identified and quantified. The fraction is then concentrated and diafiltered and may be stored.

Appropriate in-process control testing with relevant specifications is in place to assess homogeneity of the production. Relevant validation reports have been provided.

The vaccine is formulated by addition of phosphate buffer saline to the plasmid solution to reach the target concentration of 250 µg/ml of total DNA before filling (1.1 to 1.2 ml per vaccine vial).

The validation of the removal or reduction of the bioburden, the impurities (host DNA, RNA and proteins) and the endotoxin levels have been provided and these validations are considered adequate.

Production and control of starting materials

The composition of the product is presented in section 6.1 of the SPC.

Starting materials listed in pharmacopoeias

Certificates of analysis have been provided for all substances listed in pharmacopoeias and used during the manufacturing process. These certificates conform to specifications in the European Pharmacopoeia monographs.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

Active substance

The plasmid is obtained after cultivation of *E. coli* strain transformed with the plasmid pPAL-LACK. The bacteria are managed with master and working seeds which are correctly identified and have been controlled.

Substances of biological origin

Yeast extract and tryptone are the only raw materials of biological origin used during the manufacturing process. They are included in the culture media. Certificates of analysis are provided and these raw materials are accepted for use for production of the active substance.

Transmissible spongiform encephalopathy (TSE) risk assessment

An assessment of risk of transmission of TSE agents through the strain is provided according to the position paper EMEA/CVMP/019/01 and Ph. Eur. 5.2.8. "Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products". This assessment takes into account the source of the bacterium used, the nature of the raw materials of biological origin and the manufacturing process control measures. It is concluded that the risk of transmitting TSE infectivity through the use of this vaccine is negligible.

Viral risk assessment

Only tryptone for which viral risk assessment is appropriate is used during the manufacturing process. Taking into account the treatments applied and the validations provided, the risk of viral transmission is considered negligible.

The vaccine complies with the current regulatory texts related to Ph. Eur. monograph 5.2.5 on management of extraneous agents in the vaccines.

Starting materials of non-biological origin

In house preparation of media and solutions consisting of several components

The components and complex media used at the different steps in the manufacturing process (culture media, washing buffers, elution media) are described (qualitative and quantitative composition) and, when relevant, appropriate certificates of analysis are provided. Information on the storage conditions, the controls and the sterilisation treatments are provided.

Control tests during the manufacturing process

During production, the culture is controlled for optical density, purity, viable count, quantity of extracted DNA, plasmid yield and identity of the plasmid. These in-process controls have been set to monitor and control all critical steps during the manufacturing process. During the purification phase, the controls are: optical density, pH, conductivity, identification of the pPAL-LACK plasmid by electrophoresis, quantification using spectrophotometry, and on active substance bulks: appearance, sterility, identification and quantification of supercoiled form.

Control tests on the finished product

The description of the methods used for the control of the finished product, their validations and their specifications are provided. The controls include the general characteristics (filling volume, appearance, pH), the characterisation of the active substance (DNA quantification, biological activity, identification and percentage and quantity of supercoiled isoforms) and sterility and purity tests (sterility, bacterial endotoxins, residual bacterial DNA, residual bacterial RNA and residual proteins).

The proposed specifications for impurities (bacterial endotoxins, residual bacterial DNA, residual bacterial RNA and residual proteins) reflect results observed for most of the produced batches and are justified based on the results obtained for batches used in pivotal studies to support safety and efficacy. The proposed specifications set for the active substance content as percentage of supercoiled isoforms (ie $\geqslant$ 85% of scDNA in the finished product, corresponding to $\geqslant$ 212.5 µg/ml of scDNA) is based on the vaccine batches used in the clinical studies supportive of efficacy and is considered justified.

The controls aimed to characterise the active substance rely on the conjunction of many parameters: a total DNA quantification, confirmation of the presence of the plasmid, biological activity (expression in cells of the protein encoded by the genetic insert without expression level), percentage of supercoiled isoforms (active form of the plasmid) and identification by Next Generation Sequencing (NGS).

Batch-to-batch consistency

The applicant presented final product data for the manufacture of seven recent final product batches derived from two culture batches produced according to the optimised production process. The results for the different controls are consistent and conform to the release specifications.

Stability

Stability of the bulk active substance

A maximal storage of 12 months of the plasmid at -15 to -30°C is possible before formulation.

Samples of 5 batches of antigens stored at -15 to -30°C for 23 to 40 months were analysed for percentage of supercoiled DNA content. The identity was confirmed by NGS after storage at +2 to +8°C for 19-36 months. Overall, stability of the plasmid at -15 to -30°C is supported.

The applicant committed to finalise stability study of the plasmid at -15 to -30°C for 12 months and provide the results. This is acceptable and will be handled as a recommendation.

Stability of the finished product

Initially, 2-8 °C were proposed for long-term storage, however adequate stability could not be demonstrated at these conditions. Therefore, in order to guarantee the quantity of active substance in the finished product during the storage and based on observed stability of the scDNA at -15 to -30°C,

the recommendations for storage was changed to -15 to -30°C for up to 24 months that may include a period of 1 month at 2-8°C after thawing. The specification for active substance at the end of storage is the same as at release (ie ⩾85% of scDNA ie ⩾ 212.5 µg/ml of scDNA) which is the content supported by efficacy data.

The applicant committed to provide additional data to support one-month storage at +2 to +8°C after thawing and complete report on stability at -15 to -30°C followed by a period of at least 1 month at +2 to +8°C after thawing, for a maximum of 24 months storage. This will be handled as a recommendation.

Overall conclusions on quality

Neoleish is a DNA-based vaccine formulated to contain a plasmid containing the sequence of *Leishmania infantum* LACK protein (pPAL-LACK) in phosphate buffered saline. It is presented as a solution to be administered to dog via intranasal route. One 1-ml dose will contain 250 micrograms of total DNA in phosphate buffered saline. There is no adjuvant nor preservative.

The qualitative and quantitative particulars of the vaccine and the containers are described adequately. The necessary certificates are provided.

In the section product development, sufficient information on the development of the vaccine is provided.

The manufacturing process includes a culture phase of the *E. coli* strain containing the plasmid followed by a purification phase (lysis of the bacterial culture and different purification steps to obtain the plasmid). Appropriate in-process control testing with relevant specifications is in place to assess homogeneity of the production. Relevant validation reports have been provided.

The starting materials comply with the provisions of Ph. Eur. and the TSE risk assessment is adequate.

Seven recent final product batches derived from two culture batches produced according to the proposed production process have been presented. The results for the different controls are consistent and conform to the release specifications.

Proposed release and shelf-life limits are justified based on clinical data. Storage at -15 to -30°C for a maximum of 24 months that may include a period of 1 month at +2 to +8°C after thawing will ensure stability of the vaccine and in particular active substance content.

Part 3 – Safety

Introduction and general requirements

The active substance is a plasmid DNA containing the sequence expressing the LACK protein of *Leishmania infantum* of Neoleish. A full safety file in accordance with Article 12(3)(j) has been provided.

The studies presented aim to investigate the safety of the vaccine.

The safety studies were conducted according to European Directive 2001/82/EC (as amended by 2004/28/EC and Directive 2009/9/EC), the European Pharmacopoeia (Ph. Eur.) chapter 5.2.6 "Evaluation of safety of veterinary vaccines and immunosera" and guideline EMEA/CVMP/IWP/123243/2006-Rev.2 "Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market.

Safety documentation

Six studies were conducted to investigate the safety of the product: five in laboratory and one in field conditions. The pivotal study focuses on the safety of an overdose and biodistribution of the plasmid after vaccination. The vaccine was administered by the intranasal route, as recommended, using two vaccine administrations of 0.5 ml in each nostril for each vaccination. The laboratory studies were reported to be Good Laboratory Practice (GLP) compliant and carried out in seronegative dogs whereas field trial was Good Clinical Practice (GCP) compliant.

Study title
Safety of the vaccine – 2 doses 14 days apart
Safety of the vaccine - 2 or 3 doses 14 days apart
Safety of the vaccine – 2 doses 14 days apart +/- a booster on D180
Safety of an overdose – biodistribution
Safety of an overdose and repeated dose – 2 doses followed by 1 dose 14 days later
Safety of the vaccine in field conditions

Laboratory tests

Safety of the administration of one dose & repeated administration of 1 dose

The first study was considered for information only as it was performed in dogs older than the minimal age claimed in the product information with experimental vaccine batch.

Two laboratory studies were performed on groups of ten seronegative dogs of minimal age (6 months) receiving 2 or 3 doses of vaccine 14 days apart or 2 doses 14 days apart followed by a booster vaccination after 6 months. In these studies, the dogs were followed during 14 days for adverse reactions, including rectal temperature and recording of local and general reactions. During this investigation period, no adverse effects have been evidenced.

Safety of one administration of an overdose & distribution of the plasmid in vaccinated animal

One pivotal safety study was performed. It includes vaccination of 16 seronegative dogs of minimal age with an overdose of vaccine (corresponding to 10 times the standard dose) followed by a second dose (with maximal quantity) 14 days later. This study is valuable and consists in a worst-case scenario to evaluate the safety of the vaccine according to the recommended vaccination schedule. Four dogs were sacrificed 24 hours after the first vaccine dose; four were sacrificed 24 hours after the second dose; four were sacrificed 14 days after the second dose and the four remaining were sacrificed 77 days after the second dose.

The clinical investigation after vaccination demonstrates a transient increase of temperature in 2 dogs after administration of the second dose. No haematological or biochemical alterations were observed. At necropsy, the single observation is lymphoid hyperplasia in the drainage nodes at the administration site indicative of an immune response. Gliosis was observed in the frontal lobe of the brain in one dog out of four vaccinated dogs investigated 14 days after second vaccination. This observation together with possibility of retrograde biodistribution to the CNS were further analysed. Causal association between vaccination and gliosis cannot be concluded but remains plausible (anatomical connection,

connection in time, potential pharmacological explanation). The applicant has committed to carry out a close monitoring for Pharmacovigilance Signal Detection of the VeDDRA System Organ Class (SOC) Term: "neurological disorders" every 3 months for the first 2 years after placing the product in the market.

The presence of plasmid was investigated in several organs in the sacrificed dogs. In positive samples, quantification of the plasmid was performed. Assessment of the plasmid integration was also conducted.

The biodistribution investigation shows that the plasmid can be found (5.68×10^4 to 1.71×10^6 copies per µg host DNA) in liver, kidney, nasal mucosal, submandibular lymphatic nodes and brain of some dogs between 24 hours to 28 days after vaccination

After 91 days, no plasmid was detected in any organs. A study on plasmid expression was conducted and confirms that a transient mRNA expression of the LACK gene is observed in the tested organs, consistent with the results of biodistribution and persistence study, and it disappeared after 91 days. As expected, an expression similar to that of the constitutive gene is observed in the nasal mucosa and the local draining lymph nodes, shortly after vaccination.

The risk of integration of the plasmid into the dog genome has been addressed in this experimental study. A state of the art method was developed to extract and separate plasmid DNA from genomic DNA. High molecular weight genomic DNA samples were then tested for presence of plasmid sequence, using qPCR assays. A Quantitative Risk Assessment (QRA) was conducted considering the worst hypothetical scenario. The QRA found the risk to be negligible.

Another study was performed involving 8 vaccinated dogs receiving an overdose of vaccine (2 doses) followed by an additional dose 14 days later and 8 controls. The dogs were followed during 14 days for adverse reactions. Investigations also include determination of daily weight gain and haematological and biochemical evaluation performed on blood and serum samples taken 14 and 28 days after vaccination. No adverse effect was evidenced in this study which also aimed to validate the endotoxin content that is contained in the vaccine and set specification for endotoxins content in the finished product.

Examination of reproductive performance

The biodistribution study reveals that no plasmid was detected in the reproductive organs in any animal at any time points, suggesting absence of toxicity on reproduction. In the absence of specific studies on the safety of the vaccination during pregnancy, the product information specifies that the safety of the veterinary medicinal product has not been established during pregnancy.

Examination of immunological functions

Efficacy studies address the immune response following vaccination and demonstrate the induction of cellular response and transient humoral response, confirming the expression of the plasmid in vaccinated dogs. The risk of induction of autoimmune response against dsDNA in vaccinated dogs is considered low based on the bibliographic information available. The sera of the vaccinated dogs have been analysed and the results support the absence of an increase of antibodies against dsDNA after vaccination.

User safety

The applicant provided a risk analysis for user concluding that the risk is minimal as the vaccine contains a plasmid constructed in such a way that it does not replicate in eukaryotic cells and it is a molecule easily degraded in the environment. The vaccine is administered by intranasal route and a statement on safe

administration of the product has been included in the product information.

The product information recommends wearing personal protective equipment (gloves, surgical mask and safety glasses) during the handling of the vaccine and during vaccination which allows to minimise the risks of inhalation and the contacts of the mucosal surface with the vaccine (minimising exposure to the vaccine and potential immunisation after repeated contacts).

Study of residues

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

Interactions

The applicant has not provided data investigating the interactions of the vaccine with other veterinary immunological products and therefore proposes to include a relevant statement in Section 4.8 of the Summary of product characteristics (SPC). This is considered acceptable.

Field studies[1]

The applicant performed one field study including 361 outdoor dogs from 8 kennels located in 3 sites in canine leishmaniosis epidemiologically active areas. This study allows to assess the safety and efficacy of the vaccination. The dogs were older than 6 months of age, of different breeds, weights, from both sexes, not exposed and not infected to leishmaniosis. One hundred and eighty-one dogs were vaccinated and 180 kept as controls. The dogs received a primo-vaccination of 2 doses, 14 days apart, and a booster every 6 months during 2 years.

The dogs were observed for adverse reactions with particular attention to the observation of anaphylactic shock, systemic reactions (rectal temperature, anorexia, depression and behavioural changes) and local reactions. No animal showed any symptoms of shock, no local or systemic disorders were observed, confirming the observations in laboratory studies that the vaccination is well tolerated. In females treated during a pregnancy (9 vaccinates and 12 controls – a posteriori analysis of effects not foreseen in the study protocol), no effect of the vaccination on gestation or offspring was observed. Nevertheless, as the number of pregnant animals was low, the vaccination did not specifically focus on the most sensitive period and the parameters evaluated were limited (litter size and abortions with no investigation of the presence of the plasmid in milk or in puppies), it is considered that safety of the vaccine has not been established during pregnancy and this is adequately reflected in the product information.

Environmental risk assessment

An environmental risk assessment, taking into account the nature of the plasmid, the risk of contact for in-contact animals or humans and the risk for the environment, has been provided, which allows to conclude that the risk to the environment is considered negligible.

Overall conclusions on the safety documentation

Neoleish is a DNA vaccine to be administered via intranasal route in dogs from 6 months of age as 2 vaccine administrations, 14 days apart, followed by a revaccination every 6 months as proposed after assessment procedure. The applicant has conducted five laboratory studies and one field study, involving 242 vaccinated dogs, to investigate the safety of the vaccine. The data currently available indicate that Neoleish vaccine is well tolerated in dogs. No adverse reactions were observed during the

[1] If relevant for safety.

14 days following vaccination. Only transient increase of temperature was reported after administration of an overdose followed by the administration of one dose. This has been adequately reflected in the Product information. Limited data are available on safety during pregnancy and this is adequately stated in the relevant section of the SPC.

The pivotal safety study is a study performed in 16 dogs investigating the safety of an overdose (about 10 times the standard formulation followed by a second administration 14 day after the first administration), the biodistribution and excretion of the plasmid and assessing the risk of integration.

The data confirmed that the biodistribution of the plasmid is limited (mainly local) and transient. An expected, transient expression of the plasmid, consistent with the biodistribution data, is observed. After 91 days, no plasmid nor expression is found in any tested organ. The risk of integration of the plasmid in the dog genome has been assessed and was negligible.

In a laboratory safety study small foci of periventricular gliosis without associated necrosis were observed in the brain tissue in one animal amongst the 16 vaccinated. Among the 242 vaccinated dogs included in all studies, which for many of them included a 2 years observation period, no clinical signs related to gliosis such as cognitive dysfunction were observed. To get further data on the possible association between vaccination and gliosis, a post-marketing pharmacovigilance focus with monitoring for neurological disorders as signal detection for the first 2 years after market, will be implemented. This will be handled as a recommendation.

The vaccine is not expected to pose any risk to the user and also to the environment when used as recommended.

Part 4 – Efficacy

Introduction and general requirements

The applicant initially claimed for an indication for the active immunisation of dogs from 6 months of age, to reduce the risk to develop an active infection and clinical disease and to reduce parasite burden in bone marrow and blood after contact with *Leishmania infantum*. The vaccination scheme, as specified in the product information, consists of 2 administrations of 1 dose (1ml) of vaccine by the intranasal route, 14 days apart; the 1-ml dose being administered as 0.5 ml in each nostril. Immunity is intended to be established 58 days after primary vaccination course and to last for 6 months. The efficacy studies were conducted according to European Directive 2001/82/EC (as amended by 2004/28/EC and Directive 2009/9/EC), and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7 "Evaluation of efficacy of veterinary vaccines and immunosera" and guideline EMEA/CVMP/IWP/123243/2006-Rev.2" Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market.

Challenge model:

Three different challenge strains have been used by the applicant. All of them have been isolated from diseased dogs and classified as zymodeme MON-1 and genotype A which is the genotype predominant in the Mediterranean basin. The challenges were conducted by intravenous administration, via cephalic veins, of high amounts of promastigote form of the parasite. Although not fully representative of the natural infection, such an experimental challenge model has been described in literature for canine leishmaniosis. The efficacy data obtained from these experimental challenge models in the laboratory, despite being often subjected to high variations in outcome, are considered nevertheless as supportive.

Efficacy parameters and tests:

Several analytical methods to assess immunogenicity of the vaccine have been used in the studies and were appropriate. These methods have been validated in line with Ph. Eur. 0062 "Vaccines for veterinary use" and relevant VICH guidelines. The method descriptions and validation reports were provided in the dossier.

These methods aim to evaluate cellular immunity (peripheral blood mononuclear cells, tumour necrosis factor alpha (TNFα), interferon gamma (IFNɣ) and interleukin 10 (IL-10)) as well as humoral response (IgG1, IgG2 and total IgG).

After the challenge (laboratory studies) or after the natural exposure to *Leishmania infantum* (field study), the primary parameters considered for the assessment of the vaccine efficacy were: presence of clinical signs characteristic of leishmaniosis and parasite load in bone marrow, peripheral blood and in target organs as spleen, liver and lymph node.

Efficacy documentation

Six studies were conducted to investigate the efficacy of the product and these included five laboratory studies and one field trial.

Laboratory trials

The studies presented investigated the safety and efficacy of the vaccination.

Immunogenicity assessment

Two laboratory studies focused on the assessment of immunological parameters after vaccination of 20 dogs as recommended in the SPC.

In one study, ten seronegative dogs of 6 months of age received 2 vaccine doses 14 days apart (group GV3) and 10 dogs receiving placebo (GCP5) were kept as controls. In another study, ten seronegative dogs of 6 months of age received 2 doses of vaccine 14 day apart, and a booster vaccination 6 months later and a third group of 10 dogs receiving placebo was kept as controls. In these studies, the immunogenicity of the vaccine was investigated through observation of cellular [lymphoproliferation tests, expression of IFN-ɣ, IL-10 as well as specific antibody response (IgG1, IgG2 and total IgG)].

The large individual variability and the low specificity observed in the immunological parameters evaluated make the results difficult to interpret in terms of expected efficacy. No consistent immune profile after vaccination could be evidenced.

Challenge studies

Onset of immunity

In one study, five dogs, 24 to 27 months old, were vaccinated with 2 doses of an experimental vaccine 14 days apart and 5 dogs, kept as controls, received a placebo. These dogs were subjected to a challenge 58 days after the second vaccine dose. This study is considered as supportive information only as it was performed in dogs older than the minimal age vaccinated with an experimental vaccine batch.

Onset of immunity has been set based on the pivotal efficacy field trial.

Duration of immunity

Dogs vaccinated in the first laboratory study described above (groups GV3 and GCP5), were subjected to challenge 6 months after vaccination and dogs vaccinated in the second study described above were

subjected to challenge 1 year after vaccination. The corresponding efficacy data are reported respectively in two different studies.

In these studies, lower parasite load in bone marrow and tissues (significant difference in 1 study) and a significant decrease of severity of clinical signs of Leishmaniosis were observed in vaccinated dogs after at least 6 months after challenge compared to controls.

A duration of immunity of 6 months has been set for the vaccine based on the vaccine scheme used in the pivotal efficacy field trial which includes revaccination every 6 months after primary course.

Maternally derived antibodies (MDA)

No specific study was conducted. Considering the age at vaccination (6 months) and considering that all efficacy studies include vaccination of seronegative dogs, a specific statement on this point has been included in the product literature.

Field trials

Considering the questionable relevance of the challenge models and the laboratory studies to support efficacy of leishmania vaccines, the key trial for this dossier is the field trial, in which vaccinated dogs are naturally exposed to leishmania infection.

Objectives	Efficacy of the vaccination in active epidemiological areas of canine leishmaniosis.
Study design	Double-blinded trial with homogenous randomised groups receiving vaccination or kept as controls.
Study sites	In Spain. Sites with active infection (presence of vectors *Phlebotomus perniciosus* and/or *Phlebotomus ariasi* during 2 seasons of exposition and seroprevalence in dogs above 8%).
Compliance with regulatory guidelines	GCP.
Animals	361 dogs from 8 kennels located in 3 sites (names A, B and C) – outdoor kennels mainly dedicated to hunting activities – dogs older than 6 months of different breeds, weights, both sexes, not exposed (seronegative/ELISA), not infected (negative qPCR in bone marrow) and non-symptomatic. 181 vaccinated dogs (60 per area). 180 control dogs (60 per area).
Interventions: Vaccine	Neoleish
Control product/ Placebo	2 batches produced using non optimised preliminary process and 1 batch produced according to the optimised process described in the dossier. Vaccines are administered via intranasal route. All treatments that may interfere with the trial were forbidden.
Vaccination scheme	2 doses (1 dose consisting in 0.5 ml in each nostril) 14 days apart at Day 0 and Day 14 and re-vaccination every 6 months during 2

	years.
Follow-up	Dogs were naturally exposed and followed during 2 consecutive transmission seasons. Individual assessment of clinical signs every 6 months during the first year and every 3 months during the second year – attribution of a clinical score based on signs typical of canine leishmaniosis. Assessment of cellular and humoral response from 15 vaccinated and 13 controls at regular timepoints. Detection of the parasite in blood and bone marrow (Day 0, Day 28, Day 194, Day 374, Day 554, Day 644 and Day 734).
Efficacy assessment	Primary efficacy endpoint was defined as reduction in the number of infected animals in the vaccinated group, reduction of the parasite load and/or reduction in the number of symptomatic infected animals.
Statistical analysis	Analysis conducted for each area separately as well as for cumulative data. 2 tailed tests with significance level $P<0.05$. Status compared using Fisher's exact test or Chi-square test – in case of significant difference, determination of odds ratio. For continuous variables variance analysis was performed. At the end of the study efficacy assessment, including withdrawn animals (last observation carried forward).

The follow-up included detection of the leishmania in blood and bone marrow and recording of clinical signs every 6 months during the first year and then every 3 months, and serological diagnosis.

This allowed to define and follow the infection status of the dogs with regard to leishmaniosis and categorise dogs as not infected/leishmania free, subpatently infected (low burden of parasite in bone marrow below diagnostic threshold, absence of parasite in blood, negative serology, no clinical signs) or with active infection (presence of the parasite in bone marrow higher than positive threshold, absence or presence of parasite in blood, absence or presence of serological response) whether asymptomatic or symptomatic (clinical score above 8 composed by at least 3 or more clinical signs indicative of leishmaniosis and 2 or more biochemical deviations).

The 3 epidemiological areas differed on infection pressure and only one site presented higher infection pressure up to 40%.

Two years after vaccination, few dogs presented a clinical leishmaniosis (5 vaccinates and 14 controls). A significant decrease of parasite load was observed in bone marrow (3.4 times less) and blood (30 times less) in vaccinated dogs but the severity of the disease did not differ significantly.

Calculation of odds ratio allows to conclude that vaccinated dogs have around 2 times decreased odds (expressed as 2 times less risk in the product information) to develop active infection and 3 times decreased odds (expressed as 3 times less risk in the product information) to develop clinical signs and 3.5 decreased odds (expressed as 3.5 times less risk) of having detectable parasites in blood than non-vaccinated dogs.

Onset of immunity has been established 58 days after the primary vaccination course and duration of immunity 6 months after the primary vaccination course.

Overall conclusion on efficacy

The vaccine is intended for the active immunisation of Leishmania negative dogs from 6 months of age to reduce the risk to develop an active infection and/or clinical disease, after exposure to *Leishmania infantum* after 2 vaccine administrations, 14 days apart, followed by a revaccination every 6 months.

The studies presented confirmed the difficulty to assess the efficacy of a vaccine against a parasitic disease with heterogeneous evolution and manifestation. Therefore, the laboratory studies are not considered conclusive but can be considered as supportive data to reinforce the results of the field trial. The assessment of the immunological parameters demonstrated the effect of the vaccine in inducing an immune response, but these observations are of limited value with regard to demonstration of efficacy.

In laboratory studies including experimental challenge 6 months after vaccination with *Leishmania infantum*, the vaccine reduced the severity of the disease, including clinical signs and parasite burden in bone marrow, spleen and lymph nodes.

Key demonstration of efficacy relies on a 2-year duration study involving 181 vaccinated dogs and 180 controls with natural exposure (zones with high infection pressure) to the parasite, which occurs from 2 months after vaccination. The benefit of the vaccination is evidenced after 2 years in reducing parasite load in bone marrow, in blood and limiting progression to active infection, and reduce progression from asymptomatic disease to symptomatic disease.

Part 5 – Benefit-risk assessment

Introduction

Neoleish is a new vaccine against *Leishmania infantum.* The active substance is a DNA plasmid – pPAL-LACK - which encodes LACK protein *(*Leishmania homologue of activated C kinase receptor) from *Leishmania infantum*. The vaccine is presented as a solution for intranasal administration. The dose is 1 ml containing a target amount of 250 micrograms of DNA and should be administered via intranasal route (0.5 ml per nostril) to dogs from 6 months of age. It is intended to reduce the risk of developing active infection and/or clinical disease after exposure to *Leishmania infantum* infection in non-infected dogs.

The product is a new immunological product and has been classified as MUMS/limited market and therefore appropriate reduced data requirements can apply, according to EMA/CVMP/IWP/123243/2006-Rev.3 "Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market".

Benefit assessment

Direct therapeutic benefit

The vaccine is intended to stimulate the immune system and elicit a protective immune response against *Leishmania infantum* but the mechanism underlying the disease and protection after infection are not well known yet.

Two relevant controlled clinical trials including challenge have been performed. These support the activity of the vaccine and the benefit of it within the limitations on the representativeness of the challenge models for leishmaniosis.

In a field trial conducted in dogs naturally exposed to sandflies and Leishmania in endemic zones over a 2-years period, vaccination has been shown to significantly reduce the probability to develop asymptomatic or symptomatic disease.

Vaccination could be regarded as a complementary tool in addition to the other usual preventive measures against leishmaniosis such as insecticide collars.

Additional benefits

Vaccination does not interfere with serological diagnostic tools for leishmaniosis. Infected dogs can be distinguished from vaccinated animals and be identified in the population.

The vaccine increases the range of available treatment options against leishmaniosis in dogs.

The reduction of the number of dogs presenting parasitemia and parasite burden in blood (30 times less) and in bone marrow (3.4 times less) could contribute to the decrease of infectiousness of the dogs to sandflies and the decrease of the transmission of the infection. Nevertheless, the biological relevance of this observation at a large scale and the role in protection for the public health remain to be established. No conclusion on this aspect could be drawn from the data in the dossier.

Risk assessment

Potential risks have been identified as follows:

For the target animal:

As causal association between vaccination and a small foci of periventricular gliosis without associated necrosis (1 case observed in 1 out 16 dogs vaccinated) cannot be concluded; a post-marketing pharmacovigilance focus with monitoring for neurological disorders as signal detection for the first 2 years after market will be implemented.

The vaccine contains a plasmid constructed in such a way that it does not replicate in eukaryotic cells and it is a molecule easily degraded in the environment.

The user safety of the product is acceptable when used as recommended.

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

Risk management or mitigation measures

Statement on safe administration of the product has been included in the product information to wear personal protective equipment (gloves, mask and glasses) during the handling of the vaccine and vaccination to minimise the risks of inhalation and the contacts of the mucosal surface with the vaccine. SPC also mentions that vaccinated dogs may excrete the plasmid contained in the vaccine for 15 days following vaccination and that contact with faeces should be avoided during this period.

Evaluation of the benefit-risk balance

Neoleish has been demonstrated to be efficacious 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 efficacy of the vaccine was demonstrated in a field study where dogs were naturally exposed to *Leishmania infantum* in zones with high infection pressure over a two-year period.

In laboratory studies including experimental challenge with *Leishmania infantum*, the vaccine reduced the severity of the disease, including clinical signs and parasite burden in bone marrow, spleen and lymph nodes.

OOI has been established 58 days after primary vaccination course (2 doses 2 weeks apart) and DOI has been demonstrated 6 months after primary vaccination course. Single revaccination every 6 months is recommended.

The manufacture, formulation and controls of Neoleish are adequately described and supported by clinical data.

With regard to the safety of vaccination, the data available demonstrate that the vaccine appears to be well tolerated by dogs and no post-vaccination adverse reactions were observed. As causal association between vaccination and small foci of gliosis cannot be concluded; a post-marketing pharmacovigilance focus on adverse reactions based on neurological disorders during 2 years will be applied.

The product presents an acceptable risk for users and environment when used as recommended and appropriate warnings have been included in the SPC.

Conclusion on benefit-risk balance

Based on the data presented to date, the overall benefit-risk balance is considered positive.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Veterinary Medicinal Products (CVMP) concluded that the application for Neoleish 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 by majority decision that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.