15 February 2023
EMA/85879/2023
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Eurican L4 (EMEA/V/C/005944/0000)

Vaccine common name: Canine leptospirosis vaccine (inactivated)

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 Boehringer Ingelheim Vetmedica GmbH submitted on 1 October 2021 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Eurican L4, through the centralised procedure under Article 3(2)a of Regulation (EC) No 726/2004 (new active substance).

The eligibility to the centralised procedure was agreed upon by the CVMP on 12 May 2021 as Eurican L4 contains a new active substance, which was not authorised in the Union on the date of entry into force of Regulation (EC) No 726/2004.

Eurican L4 is a suspension for injection for subcutaneous use in dogs.

The applicant applied for the following indications:

Active immunisation of dogs to prevent or reduce mortality, clinical signs, infection, bacterial excretion, renal carriage and renal lesions caused by:

- *Leptospira interrogans* serogroup Canicola serovar Canicola,

- *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae,

- *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni,

- *Leptospira kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, and

- *Leptospira interrogans* serogroup Australis serovar Bratislava.

Serogroup / Serovar	**Indication**					
	Mortality	**Clinical signs**	**Infection**	**Bacterial excretion**	**Renal carriage**	**Renal lesions**
Canicola / Canicola	Prevention*	Prevention*	Reduction	Reduction	Reduction	Reduction
Icterohaemorrhagiae / Icterohaemorrhagiae	Prevention	Prevention	Reduction	Reduction	Reduction	Reduction
Icterohaemorrhagiae / Copenhageni	Prevention**	Prevention**	Prevention**	Prevention**	Prevention**	Prevention**
Grippotyphosa / Grippotyphosa	Prevention***	Prevention	Reduction	Reduction	Reduction	Reduction
Australis / Bratislava	Prevention	Prevention	Prevention	Prevention	Prevention	Prevention

* For *Leptospira interrogans* serovar Canicola, no mortality and clinical signs occurred during challenge experiment for duration of immunity.

** For Leptospira *interrogans serovar* Copenhageni the duration of immunity was not established.

*** For *Leptospira kirschneri serovar* Grippotyphosa, no mortality occurred during challenge experiment for duration of immunity.

Onset of immunity: 2 weeks after the second injection of the primary vaccination course for all strains.

Duration of immunity: at least one year after the second injection of the primary vaccination course for all strains.

The active substances of Eurican L4 are four inactivated *Leptospira interrogans* of different serovars. The target species is dog. The product is intended for administration by subcutaneous use.

Eurican L4 is presented in plastic boxes containing 10 or 50 glass vials containing 1 ml suspension.

One dose of Eurican L4 (1 ml) contains:

Inactivated *Leptospira interrogans* serogroup and serovar Canicola, strain 16070: activity acc. to Ph. Eur.447*

Inactivated *Leptospira interrogans* serogroup and serovar Icterohaemorrhagiae, strain 16069: activity acc. to Ph. Eur.447*

Inactivated *Leptospira interrogans* serogroup and serovar Grippotyphosa*,* strain Grippo Mal 1540: activity acc. to Ph. Eur.447*

Inactivated *Leptospira interrogans* serogroup *Australis* and serovar Bratislava*,* strain 16785: activity acc. to Ph. Eur.447*

* ≥80% protection in hamsters

The applicant considers justified to claim one of the vaccine strains as a new active substance and the CVMP considered it acceptable.

The rapporteur appointed is Dr Gábor Kulcsár and the co-rapporteur is Dr Jeremiah Gabriel Beechinor.

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

On 15 February 2023, the CVMP adopted an opinion and CVMP assessment report.

On 31 March 2023, the European Commission adopted a Commission Decision granting the marketing authorisation for Eurican L4.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (dated 22 Jan 2021), 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 Union or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the final product takes place in the European Union at two sites of Boehringer Ingelheim Animal Health France SCS, located in Saint Priest (formulation, primary packaging) respectively and Lentilly (primary and secondary packaging), France. The manufacturing of the

active substances takes place at the Boehringer Ingelheim Animal Health France SCS site in Saint Priest, France. Quality control of the active substances and finished product testing takes place at the Boehringer Ingelheim Animal Health France SCS site in Saint Priest respectively and in Saint Vulbas, France (testing using animals). The manufacturer responsible for batch release is Boehringer Ingelheim Animal Health France SCS, Saint Priest, France.

A manufacturing authorisation has been granted for all manufacturing sites by the competent authority of France.

GMP certificates, which confirm the date of the last inspections and show that all sites are authorised for the manufacture and batch release of such veterinary dosage forms, have been issued by the competent authority of France. The initially submitted GMP certificate for Boehringer Ingelheim Animal Health France, Chemin de Cruzols, 69210 Lentilly, has an expiration date on 20 Oct 2022. However, a GMP inspection has been performed on the 5th to 7th of July 2022 and the corresponding GMP certificate has been provided.

A GMP declaration for the active substances manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit by the manufacturing site responsible for batch release.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements. Two minor issues regarding the EVVET registration and the CV of the QPPV have been clarified.

The GMP status of the active substances and of the finished product manufacturing sites has been satisfactorily established and is 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

Eurican L4 is presented as a liquid suspension for injection (1ml/dose) containing *Leptospira (L.) interrogans* serogroup Canicola serovar Canicola (*L.* Canicola), *L. interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae (*L.* Icterohaemorrhagiae), *L. interrogans* serogroup and serovar Grippotyphosa (*L.* Grippotyphosa) and *L. interrogans* serogroup Australis serovar Bratislava (*L.* Australis) with the quantity of >80% protection in hamster potency test according to European Pharmacopoeia (Ph. Eur.) 0447 for each component. The product contains no adjuvant. Potassium chloride, sodium chloride, potassium dihydrogen phosphate and disodium phosphate dihydrate are included as excipients, as well as water for injections. The vaccine does not contain a preservative in compliance with Ph. Eur. 0062 (single dose liquid preparation).

Container and closure

The product is filled into type I neutral glass vials containing one dose. Each vial is closed with a chlorobutyl rubber stopper and sealed with an aluminium cap.

The certificates of analysis for the type I glass vials and for the elastomer closure and a representative conformity certificate for the aluminium caps are provided in Part 2.C. The glass containers and rubber stoppers are in compliance with Ph. Eur. chapters 3.2.1 and 3.2.9. The sterilisation processes applied are suitable to achieve the appropriate sterility assurance level with respect to the risk of contamination due to container materials. The pack /container sizes are consistent with the vaccination schedule and intended use.

Product development

The applicant has provided adequate information on the choice of the antigen, the quantification method, the inactivation and formulation, the choice of the pharmaceutical form, containers and storage conditions, which are considered satisfactorily justified.

Reasonable justification is given regarding the suitability of the chosen vaccine strains.

L. Canicola and *L.* Icterohaemorrhagiae strains have been used since the early 90's. More recently (in 2015), Boehringer Ingelheim placed on the market a canine leptospirosis vaccine (Eurican Lmulti) containing the same strains with the addition of *L.* Grippotyphosa strain. This update on the vaccine composition was driven by the need to adapt the strains in the vaccine to the evolving epidemiology of canine leptospirosis in the EU. *L.* Australis has also been identified as another major circulating serogroup as described in Ellis *et al*., 2010, leading to the development of this new vaccine combining the previous three *Leptospira* strains used in Eurican Lmulti with the new *L.* Australis strain.

L. Canicola and *L.* Icterohaemorrhagiae strains were both supplied by the reference laboratory of Weybridge (Great Britain). *L.* Grippotyphosa was supplied by the American Type Culture Collection (ATTC). *L.* Australis was isolated from an infected dog at the National Veterinary School VetAgro Sup (Marcy l'Etoile, France). The bacterial strains used have been satisfactorily characterised by direct observation, serum agglutination, pulsed field electrophoresis and/or other nucleic acids identification methods such as VNTR as described in part 2.C.

The vaccine contains no adjuvant. All other excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The proposed acceptance limit for residual thiomersal at 0.06 mg/ml is considered suitable.

The antigen amounts are selected in such a way that a suitable immunological response is elicited with minimal or no local reactivity at the site of injection. The final formulation of Eurican L4 has been set in view of the efficacy, safety and serological results obtained during the assessment of different formulations.

The formulation of batches used during clinical studies is the same as that intended for marketing.

The potency test chosen for routine batch release is the hamster potency test as described as one of the possible options in the current Ph. Eur. monograph 0447. The applicant, as mentioned above, has a previously authorised product, Eurican Lmulti, for which Eurican L4 has three of the same antigens. This product was authorised in 2015 by the decentralised procedure and at that time, the applicant committed to move to an in vitro potency test (ELISA) which they had developed and validated. The applicant proposed to switch to the ELISA test following the release of a number of batches using both test methods for comparative purposes.

The applicant provided an update and clarified that, while for the decentralised authorised product the variation to switch to an ELISA potency test is on going, for Eurican L4 it will be submitted after the testing of the first commercial batch and not later than 9 months after the conclusion of the

marketing authorisation procedure. However, this variation will be to replace the in vivo test only for the same three strains also included in the decentralised authorised product (*L.* Canicola*, L.* Icterohaemorrhagiae*, L.* Grippotyphosa). For the fourth active ingredient (*L. australis*), a timeline of maximum 5 years is foreseen before the variation will be submitted.

Description of the manufacturing method

The production process of the inactivated antigens *L.* Canicola, *L.* Grippotyphosa, *L*. Icterohaemorrhagiae and *L*. Australis is considered a standard manufacture for bacterial vaccines using a seed lot system. The manufacturing process consists after an initial revification culture of the seed bacteria of several bacterial culture passages followed by inactivation with thiomersal and a final concentration of the antigens. The inactivation method is validated in line with Ph. Eur., the maximum pre-inactivation titres of *Leptospira* per ml for each antigen harvest are described in the validation studies and the antigen turbidity (formazine turbidity units - FTU) specification are stated for the in-process control for each antigen. The correlation between the bacterial concentration expressed in FTU and the number of live *Leptospira* organisms per ml has been demonstrated.

To produce the finished product, the antigens are blended with the excipient and filled in sterilised glass vials which are then immediately closed.

The production processes are described sufficiently. The applicant has also provided a flowchart with all tests performed at each manufacturing step. Major steps of the manufacturing process for the antigens have been validated by performing three manufacturing runs at commercial scale and data regarding antigen batch size at different production steps are available for all antigens. The results of the critical process parameters and those of the in-process controls comply with the acceptance ranges and indicate consistent manufacture of the antigen so far.

Validation reports on validation of the quantification method of live *Leptospira*, the *Leptospira* identification method, the technique of turbidity measure, the inactivation kinetics, on the limit of detection for the inactivation control test and the bacterial and fungal sterility test have been provided. The complete production information of the batches used in the studies have been submitted.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Starting materials listed in the Ph. Eur. are agar, dimethyl sulfoxide (DMSO), ester of fatty acids and ethoxylated polyols (polysorbate 80), purified water, thiomersal and water for injection. Sufficient information is provided with regard to the starting materials listed in the Ph. Eur. In-house specifications and/or representative certificates of analysis (CoA) have been provided and all conform to the required specifications.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

Starting materials of biological origin, which are not listed in the Ph. Eur., are *L.* Canicola, strain 16070*, L.* Icterohaemorrhagiae, strain 16069*, L.* Grippotyphosa, strain Grippo Mal 1540, *L.* Australis, strain 16785, bovine albumin fraction V and calf serum used during cryopreservation for two of the *Leptospira* strains MSB. For the active ingredients, a seed lot system was satisfactorily established in line with Ph. Eur. 0062. Details of source, passage history, preparation, controls,

storage conditions and certificates of analysis for the master seed bacteria (MSB) and working seed bacteria (WSB) have been provided and are considered appropriate in line with Ph. Eur. requirements. Purity of the seed materials and of the materials of animal origin used for their preparation has been justified and is considered acceptable in line with Ph. Eur 5.2.5.

The only substance of bovine origin described in the dossier is bovine albumin fraction V, used during antigen propagation, is sourced from bovine blood plasma derivative from cattle born and bred in BSE-free regions. Satisfactory certificates of analysis were provided and all CEP certificates provided are currently valid. A risk assessment of potential contamination of the bovine albumin fraction V with various extraneous agents and the results of validation of the capacity of the BSA manufacturing process to eliminate or reduce the potential contamination with extraneous agents using a range of model viruses were provided. The risk assessment provided considers both bovine (species of origin) and canine (target species) extraneous agents, in line with the Ph. Eur. 5.2.5. Information on the suitability of the test methods to detect the relevant extraneous agents and the limits of detection has been provided.

Based on the risk assessment provided, the risk for TSE transmission via the seed materials is considered negligible.

Starting materials of non-biological origin

Certificates of analysis have been provided for ammonia solution concentrated, sodium hydroxide solution and PBS without calcium and magnesium with reports of the results of routine control tests, which is considered satisfactory. The sodium hydroxide solution is prepared from starting materials complying with Ph. Eur. 0677 (sodium hydroxide) and Ph. Eur. 0169 (water for injections). The ammonia solution concentrated is used in small quantities for pH adjustment and therefore, poses no risk concerning chemical impurities. Identification and assay quality control tests for ammonia solution performed according to the compendial Ph. Eur. methods ensure an appropriate level of quality of the finished product.

In-house preparation of media and solutions consisting of several components

Information regarding the qualitative and quantitative composition of the culture medium is provided in the dossier. All media components are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk of contamination.

Control tests during the manufacturing process

During the manufacture of the antigens, the following tests are carried out: bacterial concentration (turbidity), inactivation and bacterial and fungal sterility. Test descriptions and the limits of acceptance for the in-process controls tests have been presented as well as the validation studies for the relevant test methods. To confirm the purity, a direct microscopic examination of the *Leptospira* culture to check the characteristic morphology / viability of leptospiresis conducted at the end of each bacterial culture passage after revification of the bacterial seeds. The applicant has provided further information on how the identity of each of the antigen is confirmed after harvesting of the culture. The established in-process tests are considered to be sufficient to control all critical steps in the manufacturing of the antigens.

Control tests on the finished product

Finished product controls performed on the bulk vaccine are pH value, hamster potency test,

thiomersal limit test and bacterial and fungal sterility. The filled product will be controlled for appearance and correct volume. A justification for omission of testing of the finished product for freedom from extraneous agents in line with Ph. Eur. 0062 is provided.

An in vivo potency test in hamsters is performed for each antigen contained in the vaccine in accordance with Ph. Eur. monograph 447. The applicant stated that the replacement of the hamster challenge test by an in vitro ELISA test will be performed, by variation, after the procedure is finalised.

Batch-to-batch consistency

The applicant has provided the production parameters including the details of each bacterial culture passage, the harvest, the inactivation, the concentration and the storage condition for three consecutive antigen batches of each of the four *Leptospira* active ingredients. During the manufacture of the antigens, the following tests were carried out: sterility, bacterial concentration before and after the concentration step and the proof of inactivation.

The batch-to-batch reproducibility of the antigens was shown by compliance with the specifications. The in-process tests are deemed to be sufficient to control all the critical steps in the manufacturing process. The size of the antigen batches used, the details and specifications of the tests performed were provided with the batch release protocols (BRP).

The applicant has presented finished product test data for the manufacture of 3 consecutive finished product batches, one with the standard batch size of bulk vaccine and two with the minimum batch size, indicating a consistent composition of the finished product in a quantitative and qualitative manner. The same final batches were also included in the stability studies.

The following tests are carried out on the finished product: appearance, pH value, volume, hamster potency test, thiomersal detection and bacterial and fungal sterility. The batch-to-batch reproducibility of the batches was shown by compliance with the specification of the test methods and results were satisfactory.

Stability

The antigen suspension can be stored at 2-8 °C for a maximum of 24 months until being further processed. The stability study was carried out on three batches for each of the four antigens stored for 27 months at a temperature of +5 ± 3 °C. Stability (demonstrated by testing the parameters bacterial and fungal sterility and hamster potency test) was shown for the *L*. Canicola, *L*. Grippotyphosa, *L*. Icterohaemorrhagiae and *L*. Australis antigen batches. Data on the stability of the final bulk have been provided with three final product batches that have been produced with antigens stored at +5 ± 3 °C for 3-24 months before blending. For the three batches, data demonstrating stability up to 27 months have been provided. This supports the proposed antigen shelf-life in addition to the results of the stability study of the individual active ingredients.

Real-time stability data of three standard size batches of final product (two batches of minimum size and one batch of medium size) were provided up to 27 months. The batches of Eurican L4 can be considered as representative of those proposed for marketing.

Test results are acceptable over time, within the inherent variability of the tests. The results obtained show that Eurican L4 keeps its quality specifications within the established limits for at least 27 months. Based on the data provided a shelf life of 24 months can be considered as demonstrated for Eurican L4. In-use shelf life data has not been provided as the vaccine vials contains one dose and are intended to be used immediately after first opening.

Overall conclusions on quality

Overall, a comprehensive description of the development of the product, the manufacture and validation of the production process has been provided. The composition of the vaccine has been sufficiently described. The applicant considers justified to claim one of the vaccine strains as a new active substance and the CVMP considered it acceptable.

The production process of the antigens is considered as standard manufacture for bacterial vaccines and is described adequately in the essential parts.

The complete production information of the batches used in the studies have been submitted.

The starting materials have been properly described. For the starting materials of biological origin, fully assurance has been provided that there is no potential risk caused by extraneous agents. For the bovine serum albumin fraction V, a risk assessment in line with the Ph. Eur. 5.2.5 is provided. Data on the suitability of the test methods to detect the relevant extraneous agents and the limits of detection have been provided and are satisfactory. The risk that the final product may transmit TSE to the target animal is negligible.

The in-process tests are considered to be sufficient to control the critical steps in the manufacturing process. Validation studies have been provided for all key tests. Purity and identity are verified by a direct microscopic examination of the *Leptospira* culture at each passage after revification of the Working Seed stock.

The finished product controls performed on Eurican L4 are described in detail, validation studies have been provided and appropriate specifications are proposed. The applicant proposes the use of the hamster potency test for quality control and release of batches which is considered questioned in the light of the 3Rs concept. As the applicant stated that the replacement of the hamster challenge test will be performed by variation within 9 months after the end of the procedure, this is considered acceptable. The first variation will aim to replace the invivo test for *L*. Canicola, *L*. Icterohaemorrhagiae and *L.* Grippotyphosa antigens. Within 5 years, a variation will also be submitted for the *L.* Australis antigen.

Three batches of each of the four active ingredients and three batches of the final product at production scale were provided to show batch-to-batch consistency. An appropriate justification that these batches are suitable to demonstrate batch-to-batch reproducibility of the antigen batches and the finish product was given.

Stability data have been provided for storage of the antigen bulk before formulation and the finished product. The applicant targets a final shelf life of 24 months for the four antigens which is considered supported.

Full stability data are available for up to 27 months for three final product batches and data have been provided to show that the product meets the proposed specifications when formulated with aged antigen stored for 3–24 months. Based on the data provided, a shelf life of 24 months can be considered demonstrated for Eurican L4.

Based on the review of the data on manufacture and control of Eurican L4, the quality is considered acceptable.

In addition, the applicant is recommended to submit a variation, post-authorisation, to replace the hamster in vivo test for *L.* Canicola, *L.* Icterohaemorrhagiae and *L.* Grippotyphosa antigens within 9 months after the end of this procedure and, within 5 years, a variation to replace it for *L.* Australis.

Part 3 – Safety

Introduction and general requirements

Eurican L4 is an inactivated vaccine indicated for the immunisation of healthy puppies from 7 weeks of age against *L.* Canicola, *L.* Icterohaemorrhagiae, *L.* Grippotyphosa and *L.* Australis infections. The product can be mixed with Boehringer Ingelheim's vaccines Eurican DAPPi or Eurican DAP (freeze-dried attenuated canine distemper virus, adenovirus type 2 and parvovirus associated or not with parainfluenza type 2 as active ingredients) to confer protection against often occurring canine viral diseases.

If necessary, from the age of 12 weeks, Boehringer Ingelheim's inactivated and adjuvanted rabies vaccine Rabisin can be concomitantly administered via the subcutaneous route, albeit at a separate injection site.

Eurican L4 contains the same active ingredients and excipients as Eurican Lmulti (containing three *Leptospira* strains as antigens), which is authorised nationally in a number of EU Member States, plus a fourth, new, inactivated active ingredient: *L.* Australis antigen. In all safety studies presented for Eurican L4, the animals were administered Eurican L4 mixed with Eurican DAPPi (this mix is termed DAPPi-L4 in the present report). The dossier also contains supportive studies carried out in dogs vaccinated with the Eurican Lmulti vaccine mixed with the Eurican DAPPi vaccine (this mix is termed DAPPi-L3 in the present report). These latter study reports were already provided for the national authorisation of the Eurican Lmulti.

In order to demonstrate the safety of Eurican L4, tests were carried out both in the laboratory and in the field, using the recommended route of administration, i.e. subcutaneous administration. Safety tests were either carried out in young dogs at the minimum recommended age or in adult animals.

The requirements of Directive 2001/82/EC or of the Ph. Eur. General Text 5.2.6 have been fulfilled, either through the performance of required tests required or by providing appropriate scientific justification. Also, the requirements of the specific *Leptospira* Ph. Eur. monograph (canine leptospirosis vaccine [inactivate] 0447) and the CVMP "Guideline on combined vaccines" (EMA/CVMP/IWP/594618/2010) as well as recommendations outlined in VICH GL44 ("Target animal safety for veterinary live and inactivated vaccines") have been considered.

Safety documentation

The following table summarises the design of the trials and antigen contents of the products used in the individual studies:

Trial	Injected dose								Type and number of animals used
	FTU/dose				log10CCID50/dose				
	Lc	Li	Lg	La	CDV	CAV_2	CPV	Pi_2	
LABORATORY TESTS									
Safety of the administration of one dose									
1	190	210	400	210	6.0	6.3	7.1	7.3	14 SPF puppies aged 7 weeks (8 vaccinates and 6 controls)
Safety of one administration of an overdose									
1	380	420	800	420	7.0	7.3	8.1	8.3	14 SPF puppies aged 7 weeks (8 vaccinates and 6 controls)
Safety of the repeated administration of one dose									
1	190	210	400	210	6.0	6.3	7.1	7.3	14 SPF puppies aged 7 weeks (8 vaccinates and 6 controls)
Examination of reproductive performances									
2*	190	210	400	/	6.1	6.4	7.5	7.7	20 conventional pregnant bitches
Interactions									
3**	160	180	370	180	5.5	5.7	6.3	6.1	10 SPF puppies aged 7 weeks
FIELD STUDIES									
4	160	180	370	180	5.6	5.5	6.4	6.2	265 conventional puppies aged 18.8 weeks on average (193 vaccinated with Eurican L4) 293 conventional dogs aged 5.9 years on average (213 vaccinated with Eurican L4)
5*	160	180	370	/	5.4	5.1	6.0	5.9	113 conventional puppies aged 11.2 weeks on average
6*	160	180	370	/	5.4	5.1	6.0	5.9	93 conventional puppies aged 10.7 weeks on average
7*	160	180	370	/	5.4	5.1	6.0	5.9	22 conventional puppies aged 8.5 weeks on average
8*	160	180	370	/	5.4	5.1	6.0	5.9	172 conventional dogs aged 5.6 years on average
9*	160	180	370	/	5.4	5.1	6.0	5.9	108 conventional dogs aged 5.8 years on average
10*	160	180	370	/	5.4	5.1	6.0	5.9	18 pregnant bitches

Lc: *Leptospira* Canicola Li: *Leptospira* Icterohaemorrhagiae

Lg: *Leptospira* Grippotyphosa La: *Leptospira* Australis

CDV: canine distemper virus CAV_2: canine adenovirus type 2

CPV: canine parvovirus Pi2: canine parainfluenza type2

* Studies already provided for the national authorisation of Eurican Lmulti

** Study investigating the concomitant injection of a standard dose of Rabisin

Laboratory tests

Safety of the administration of one dose

No specific study on the safety of the administration of one dose is included in the application, but the applicant refers to the study demonstrating safety of the administration of one overdose and the safety of the repeated administration of one dose. This is in accordance with Directive 2001/82/EC as amended and is considered acceptable by the rapporteurs.

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

In this GLP-compliant standard laboratory safety study, fourteen 7-week-old SPF puppies were randomized into Group A (containing 8 puppies) vaccinated with Eurican DAPPi mixed with Eurican L4 and Group B (containing 6 puppies) control animals vaccinated with vaccine diluent and stabiliser. The inclusion of only 6 control animals instead of the required 8 animals can be accepted. On day 0, dogs in Group A received a 10x overdose of Eurican DAPPi mixed with a 2x overdose of Eurican L4. On day 28, dogs in Group A received a 1x maximum dose of Eurican DAPPi mixed with a 1x maximum dose of Eurican L4. Thus, the safety of the recommended vaccination schedule was evaluated under worst case conditions with two injections 4 weeks apart. One additional dose of Eurican DAP vaccine was given on day 43. Repeated injection with Eurican DAPPi mixed with Eurican L4 (1x maximum dose of each) was performed on day 60. The safety was assessed by clinical examination, including examination of the injection site and measurement of rectal temperature on the day before treatment, just before treatment and 4-6 hours after treatment, then daily for 14 days. Animals were weighed before each treatment. No general clinical signs were recorded, with the exception of an infection with *Giardia duodenalis*, which was not treated and not considered by the applicant to have affected the results of the study. All dogs remained in good general condition throughout the study and gradually gained weight. The maximum rectal temperature measured in vaccinated animals was 39.2°C in two puppies for one day. No pruritus was observed. Following vaccination on day 0, all vaccinated puppies experienced local reactions consisting of swelling (8/8 dogs) and transient pain at palpation (7/8 dogs), the latter of which was experienced mostly 4-6 hours after overdose. No pain after injection was recorded in the control group. In the vaccinated group, after overdose, the overall duration of swelling ranged from 2 to 21 days. Oedema or cutaneous thickening was observed in all animals on the clinical examination performed 4-6 hours after injection and lasted between 2 and 12 days, turning into nodule between day 5 and day 11 at the injection site of 5 animals (the other 3 dogs exhibited no nodule).

After one dose (day 28), additional dose (of DAP at day 46) and repeated dose (day 60), only oedema or cutaneous thickening was recorded (no nodule), which lasted between 1 to 7 days.

In the control group, if at all, local reactions in a form of oedema or cutaneous thickening mostly 4-6 hours after the injection were observed.

Safety of an overdose, one dose and repeated dose of Eurican L4 mixed with Eurican DAPPi in puppies was satisfactory with no general reactions, and transient local reactions consisting mostly of small swellings, which lasted up to 21 days after overdose and up to 7 days after one dose and repeated dose, respectively. The maximum temperature increase post-vaccination, and the maximum size and duration of the swellings after one dose, overdose and repeated dose as well as the frequency have been correctly included in the adequate sections of the PI.

Examination of reproductive performance

No specific studies were performed on reproductive performance with Eurican L4.

The reproductive safety has been demonstrated for Eurican Lmulti (containing only three *Leptospira* strains) in pregnant bitches, both under laboratory and field conditions, through the administration of the combined vaccine Eurican DAPPi-Lmulti at 4-week intervals during the second and third stages of pregnancy. A maximum dose was used in the laboratory study and a standard dose in the field study.

In the laboratory study, twenty pregnant bitches were randomized into Group A (10 animals), which were vaccinated with Eurican DAPPi-Lmulti and Group B (10 animals) which comprised unvaccinated control animals. The vaccination scheme was followed with two injections 4 weeks apart (day 0 [second pregnancy stage] and day 28 [third pregnancy stage]). The safety was assessed by clinical examination including examination of the injection site and the measurement of rectal temperature on the day before treatment, just before treatment and 4-6 hours after treatment, then daily for 5 days and on day 14, 21 and 42 (for control animals also on day 28 and day 33). After whelping, each bitch and its offspring were monitored for 6 weeks. At day 42, a motor activity test was performed. No general clinical signs were observed in pregnant bitches after the two injections of Eurican DAPPi-Lmulti vaccine. Local reactions at the injection site were observed up to 3 days after the first injection and up to 2 days after the second injection. The main sign was oedema, which was only palpable the day after injection. No nodules were observed. No significant difference was found between the vaccinated group and the unvaccinated control group regarding the adverse effects on the pregnancy. Dystocia was similar in the two groups and the number of stillborn puppies was not significantly different. The most frequent cause was thereby an inappropriate behaviour of the bitch. Neither abortions nor malformations in the living puppies were observed.

No significant difference was found between the vaccinated group and the unvaccinated control group regarding the adverse effects on the offspring. Mortality (death within 48 hours post-whelping), number of weaned puppies and evolution of bodyweight were similar in the two groups. Examination of the motor activity at 6 weeks of age showed no abnormalities in puppies of both groups.

Clarifications on why the bitches were not vaccinated in the 1st trimester, have been provided. Safety of a maximum dose Eurican DAPPi-Lmulti in pregnant bitches and their offspring was satisfactorily demonstrated with no abnormal local or systemic reactions observed in the bitches, during their pregnancy and in the offspring. However, it was raised as a concern that vaccinated bitches (both in the laboratory and in the field studies) were vaccinated only in the second and third trimester of pregnancy. The applicant was requested to justify how it could be considered that safety in the first trimester was supported. In their response, it was noted that pregnancy in a bitch cannot be confirmed by ultrasound until the beginning of the second phase, and therefore if vaccination was to be conducted in the first trimester and later confirmed as not pregnant, the root cause could not be determined (i.e., unsuccessful mating vs negative impact of vaccination). The applicant also justified that the applicant's trivalent and bivalent leptospirosis vaccines have been safely used during pregnancy (without a restriction on the stage at which vaccination may be conducted) for a substantial length of time without safety concerns arising via pharmacovigilance. Consequently, it would not be unreasonable to expect that Eurican Lmulti will have been used (safely) in all trimesters of pregnancy. The justification provided by the applicant was accepted.

However, considering that the data provided to support use during pregnancy of Eurican L4 was generated in studies conducted with the trivalent leptospirosis vaccine, Eurican Lmulti, it was raised

as a concern that it cannot be excluded that the addition of *L.* Australis in Eurican L4 could alter the safety profile in pregnant bitches. The applicant provided justification to support that the inclusion of one additional inactivated serovar would not be expected to adversely affect safety of use during pregnancy. It was highlighted that the total antigenic content, based on FTU/dose, of Eurican Lmulti (800 FTU/dose when combining *L.* Canicola*, L.* Icterohaemorrhagiae*, L.* Grippotyphosa) was approximately 90% of the total antigenic content of Eurical L4 (890 FTU dose when combining *L.* Canicola*, L.* Icterohaemorrhagiae*, L.* Grippotyphosa and *L.* Australis) in terms of total amount of inactivated leptospira serovars. It was argued that qualitatively, an inactivated *Leptospira interrogans* bacteria serogroup australis is not known to cause particular safety concerns with respect to pregnancy. Overall, it was accepted that the safety data generated with Eurican Lmulti was relevant to support the safety of use during pregnancy of Eurican L4. Section 3.7 of the SPC states that safety data are available with the 3-component leptospirosis vaccine Eurican Lmulti, and reflects that no safety data for the additional inactivated serovar *L. australis* are available.

Examination of immunological functions

Eurican L4 is an inactivated, non-adjuvanted canine leptospirosis vaccine. It is not expected to have a deleterious effect on the immune system of the target species. Therefore, no study was performed to evaluate the impact on immunological functions. This approach is accepted and it is further noted that the studies conducted to investigate the compatible use with Eurican DAPPi supported that there was no adverse impact on immunological function following vaccination with Eurican L4.

Special requirements for live vaccines

Not applicable as Eurican L4 is an inactivated vaccine.

User safety

A user risk assessment has been provided in accordance with the guidance set out in EMEA/CVMP/IWP/54533/2006 (Guideline on user safety for immunological veterinary medicinal products). Given the nature of the vaccine and its mode of administration, the risk to the user is considered to be low. The vaccine contains inactivated bacteria and it is not adjuvanted, therefore accidental exposure to the vaccine is not expected to be harmful to humans. Therefore, no special precautions are necessary to be taken by the person administering the veterinary medicinal product to animals.

Study of residues

Not applicable, since Eurican L4 is not intended for use in food-producing species.

Interactions

Under *Interactions (section 3.8)*, the applicant proposes additional information concerning interactions with two of its other already authorised vaccines: Eurican DAP and Eurican DAPPi with Eurican L4 as a solvent.

This claim (safety of the association of Eurican L4 with Eurican DAPPi and Eurican DAP) is supported by one laboratory study (the pivotal laboratory safety study discussed previously) with a maximum dose of *Leptospira* components and one field study including dogs of the minimum age (7 weeks). The requirements of the CVMP "Guideline on combined vaccines" (EMA/CVMP/IWP/594618/2010) regarding association by mixing of IVMPs are fulfilled. This also affects the mixing of Eurican L4

with DAP, where only live canine parainfluenza type 2 is missing. Several field trials with Eurican DAPPi-Lmulti (containing only three *Leptospira* strains) showed similar adverse events occurring at equivalent frequencies. The statement in the SPC section 3.8 *Interaction with other medicinal products and other forms of interaction* that "safety and efficacy data are available, which demonstrate that this vaccine can be mixed with Eurican DAP or Eurican DAPPi" is justified, which also applies to the claim "safety and efficacy data are available, which demonstrate that this vaccine can be administered on the same day but not mixed with Rabisin in dogs from 12 weeks of age". This was tested in one compatibility study where Eurican DAPPi diluted with Eurican L4 and Rabisin were applied at different injection sites.

Ten 7-week-old SPF puppies were vaccinated on day 0 with Eurican DAPPi diluted with Eurican L4 and on day 28 concomitantly with Eurican DAPPi diluted with Eurican L4 and Rabisin at two separate injection sites. The dogs then received a booster injection with Eurican DAPPi diluted with Eurican L4 associated to Rabisin (different injection sites) about one year after the primary vaccination. For the safety assessment, the dogs were monitored daily for general and local signs after the vaccination performed on day 28 and until clinical signs disappeared.

After concomitant administration of Eurican DAPPi + Eurican L4 and Rabisin on day 28 and for the annual booster, no general reactions were recorded. All dogs remained in good general condition without any hyperthermia. Following the first administration, the only clinical sign recorded was oedema at the injection sites in 8 dogs out of 10. This local reaction lasted up to 6 days and was always inferior to 5 mm.

The observations in this study were comparable to the known safety profiles for the concomitant administration of both Rabisin and Eurican DAPPi. The safety profile of Eurican L4 has been investigated in the pivotal laboratory safety study when used mixed with Eurican DAPPi, and therefore the adverse reactions in section 3.6 and information in section 3.10 of the SPC pertain to the safety of Eurican L4 when used mixed with Eurican DAPPi, and it is accepted that the safety profile of Eurican L4 has therefore been investigated under worst case conditions (i.e. in association with a multivalent live viral vaccine, or in association with a multivalent viral vaccine and an adjuvanted Rabies vaccine).

The reactions following the simultaneous vaccination with Eurican DAPPi + Eurican L4 and Rabisin were similar in nature, severity and duration to the reactions caused by Eurican DAPPi or Rabisin used alone. These data show that Eurican L4 can be used alone or associated to Eurican DAPPi (mixed and administered at the same site) and /or associated to Rabisin (not mixed, but injected at the same time at a different site to that of the site of injection of Eurican L4 or Eurican DAPPi + Eurican L4).

Field studies

The safety of the administration of Eurican L4 mixed with Eurican DAPPi was demonstrated at different sites in France after a primary course of two injections at a 4-week interval or a booster injection in previously vaccinated dogs.

Five hundred fifty eight (558) dogs were included and vaccinated with either the test vaccine, Eurican L4 mixed with Eurican DAPPi, or a reference vaccine authorised since 2014 in EU (Versican Plus DHPPi/L4, from Zoetis). Two hundred sixty five (265) puppies received the primary vaccination (193 received the test vaccine and 72 the reference vaccine) and 293 fully primed dogs received an annual booster injection (213 animals received the test vaccine and 80 the reference vaccine).

The primary vaccination consisted of two injections, 4 weeks apart, at a minimum age of 7 weeks. Immediate local and general safety was assessed after both injections by the investigators. Delayed

local reaction and general safety were assessed based on owners' reports and investigators' notifications, up to 2 weeks after the second injection.

The booster vaccination consisted of a single injection to animals fully primed against all components. Immediate local reaction and general safety were assessed after the injection, by the investigators. As for primary vaccination, delayed local reaction and general safety were assessed based on owners' reports and investigators' notifications, up to 2 weeks after the injection.

Comparison of safety between the test and reference vaccines was based on a non-inferiority test applied to the rate of animals that experienced at least one delayed local adverse event. Secondary variables were based on the same test applied to the rate of other types of reaction (delayed local and general, immediate local and general). The approach of the applicant is noted, however the objective of the investigation of safety under field conditions is to investigate the safety profile of the test vaccine under field conditions, rather than to establish a cut-off threshold for an acceptable safety profile based on a nominal increase of adverse reactions compared to an authorised IVMP. Consequently, the use of a non-inferiority approach to support safety is, in the opinion of the CVMP, questionable. Regarding blinding of the study, it is noted that the Investigator knew the treatment group of each animal, as only dogs from group B (test group) were bled. It is stated that the Investigator was asked to keep the owner blinded until the end of the study. Given that the study investigator was not blinded to treatment allocation, assessment bias of safety parameters cannot be excluded. However, notwithstanding this point, both test and reference vaccines were well tolerated since only moderate and transient adverse events were reported. The overall rate of animals with at least one adverse event reported during the monitoring period was similar between the two groups, with 57/406 (14.0%) in the test group and 22/152 (14.5%) in the reference group. When focusing on events qualified as probably or possibly related to the vaccination by the investigators, this rate falls to 44/406 (10.8%) in the test group and 17/152 (11.2%) in the reference group.

The adverse events most frequently observed following the first or second injection of the primary vaccination cycle were (in decreasing order of frequency): pain at the injection site, lethargy, diarrhoea, swelling/oedema/nodule, and pruritus at the injection site. The rate of adverse events decreased considerably after the second injection compared to the first. Globally, these events remained at low frequencies and were mild and transient.

The adverse events most frequently observed following booster vaccination were (in decreasing order of frequency): swelling/oedema/nodule, lethargy, emesis, pruritus at the injection site and diarrhoea. Globally, these events remained at low frequencies and were mild and transient.

Local and general safety of Eurican L4 mixed with Eurican DAPPi, was confirmed as satisfactory under field conditions, whether used for primary vaccination of puppies from 7 weeks of age or for booster in already primed dogs. Moreover, it was claimed to be at least as good as that of an authorised competitor vaccine comprising the same components.

In addition to this field trial, five studies (3 in puppies and 2 in adult dogs) previously run to evaluate the safety of both primary vaccination and booster vaccination with Eurican Lmulti (containing only three *Leptospira* strains) are also considered as supportive for the safety of Eurican L4. Indeed, as both products have the same composition except the additional inactivated *L.* Australis antigen and both are used under the same conditions (same age and scheme of administration), they are expected to have similar safety profiles. The information collected in these trials allowed side-by-side comparison of the adverse reactions caused by Eurican DAPPi-Lmulti and Eurican DAPPi-L4. Three studies on primary vaccination in puppies and two studies on booster in adults have been presented. The applicant compares the adverse events of Eurican Lmulti with

Eurican L4 (mixed with Eurican DAPPi) to support the relevance and equivalence of data for the safety assessment.

This comparison was used to define the adverse reactions in section 3.6 of the SPC of Eurican L4 with the exception of muscle weakness and polydipsia, which had a low occurrence rate in Eurican Lmulti and were not observed in the field trial of Eurican L4. Hair wrap was only observed in one case of Eurican L4 vaccination and has also not been included in the SPC.

The first supportive field study in 171 puppies with 11.2 weeks of age (average) was to confirm the safety of the primary vaccination with Eurican DAPPi-Lmulti vaccine (containing *Leptospira* strains Icterohaemorrhagiae, Canicola and Grippotyphosa). The safety evaluation was based on the monitoring of immediate and delayed local and general adverse reactions in comparison with an already authorised competitor vaccine (Canigen CHPPi/L from Virbac, including *L.* Canicola and *L.* Icterohaemorrhagiae as *Leptospira* strains).

One hundred thirteen (113) puppies were vaccinated by subcutaneous route with Eurican DAPPi-Lmulti vaccine and 58 puppies with Canigen CHPPi/L (the reference vaccine). The dogs were monitored for immediate adverse reactions for approximately 15 minutes post-injection and for delayed adverse reactions for approximately 28 days after the first injection and 14 days after the second injection.

No statistically significant difference was observed between treatments regarding the immediate general reactions. Two puppies vaccinated with Eurican DAPPi-Lmulti vaccine presented only emesis. This adverse reaction, which did not require any treatment, was considered to be stress related (handling, transport to vet practice). One puppy vaccinated with Canigen CHPPi/L had an anaphylactic shock with circulatory signs within 15 minutes after the first injection. This puppy recovered without any treatment.

Delayed general reactions were not significantly different between groups. Both treatments induced moderate and transient reactions that did not seriously affect the general condition of the animals. In details, these reactions were lethargy (9.8% of puppies vaccinated with Eurican DAPPi-Lmulti vaccine and 10.5% of puppies vaccinated with Canigen CHPPi/L), digestive disorders (4.5% of puppies vaccinated with Eurican DAPPi-Lmulti vaccine and 1.8% of puppies vaccinated with Canigen CHPPi/L) and anorexia (1.8% of puppies from each group). Most delayed general reactions (95% in Eurican DAPPi-Lmulti vaccine group and 89% in Canigen CHPPi/L group) lasted not more than 2 days and did not need any treatment. Only 2 puppies (one from each group) had lethargy for 6 or 7 days.

No statistically significant difference regarding immediate local reactions was observed between the two treatments. These reactions consisted of transient pruritus and/or pain in dogs vaccinated with Eurican DAPPi-Lmulti vaccine. They lasted a few minutes and disappeared without treatment.

Delayed local reactions were not significantly different between groups. Most reactions were mild and transient, lasted a few days and did not require any treatment. The most frequent delayed local reactions observed were pain and pruritus in puppies vaccinated with Eurican DAPPi-Lmulti vaccine (no persistent swelling reaction or nodule observed), and pain and swelling in puppies vaccinated with Canigen CHPPi/L.

The second supportive field study in 93 puppies aged 10.7 weeks of age (average) was to confirm the safety of two injections with Eurican DAPPi-Lmulti vaccine (4 weeks apart). The safety evaluation was based on the monitoring of immediate (15 minutes after each injection) and delayed (28 days after the first injection and 14 days after the second injection) local and general adverse reactions.

No immediate local or general reactions were observed.

Delayed general reactions were moderate and transient (lethargy), which did not seriously affect the general condition of the animals. They affected 4 puppies (4.4%) and did not need any treatment. Few delayed local reactions were observed in 4 puppies (4.4%). These reactions lasted up to 2 days and disappeared without any treatment.

The third supportive field study in 33 puppies aged 8.5 weeks of age (average) was to confirm the safety of two injections with Eurican DAPPi-Lmulti vaccine (4 weeks apart). The safety evaluation was based on the monitoring of immediate (15 minutes after each injection) and delayed (28 days after the first injection and 14 days after the second injection including a clinical examination within 4 days after each injection) local and general adverse reactions in comparison with an already authorised competitor vaccine (Canigen CHPPi/L from Virbac, including *Leptospira* Canicola and *Leptospira* Icterohaemorrhagiae as *Leptospira* strains). A total of 22 dogs were vaccinated with Eurican DAPPi-Lmulti vaccine and 11 dogs with Canigen CHPPi/L.

No immediate general reactions and no delayed local reactions were observed in this trial.

No statistically significant difference was observed between treatments for the immediate local reactions. Only one puppy from each group experienced injection site pain and pruritus. These reactions were transient and did not need any treatment.

Delayed general reactions were also not significantly different between groups. Four puppies vaccinated with Eurican DAPPi-Lmulti vaccine presented mild anorexia or hyperthermia without any impact on general condition. One puppy vaccinated with Canigen CHPPi/L presented lethargy, anorexia and diarrhoea on Day 6 after the first vaccination. Despite treatment, no improvement was observed and the puppy died from septicaemia 5 days after first occurrence of the clinical signs.

All reactions (except those of the dead puppy vaccinated with Canigen CHPPi/L) were transient and none of the animals required any treatment.

In conclusion, Eurican DAPPi-Lmulti vaccine, administered to puppies as first and second vaccination under field conditions, was well tolerated. In the two studies comparing the safety with a reference vaccine, the safety was similar to that of the already authorised Canigen CHPPi/L with no significant difference in the reactions observed between the two products.

The first supportive field study performed in 258 adult dogs aged 5.6 years (average) was to confirm the safety of a booster vaccination with Eurican DAPPi-Lmulti vaccine (172 dogs in total were treated with the latter, while 86 animals received an already authorized reference vaccine containing *L. canicola* and *L. icterohaemorrhagiae* as *Leptospira* components.). The safety evaluation was based on the monitoring of immediate (15 minutes after each injection) and delayed (14 days after injection) local and general adverse reactions in comparison with an already authorised competitor vaccine (Canigen CHPPi/L from Virbac, including *L.* Canicola and *L.* Icterohaemorrhagiae as *Leptospira* strains).

No statistically significant difference regarding immediate general reactions was observed between treatments. No anaphylactic shock with circulatory signs was observed immediately after the vaccination. Only one dog vaccinated with Eurican DAPPi-Lmulti vaccine presented a vomiting not associated with other symptoms. This adverse reaction was probably related to stress (handling, transport to vet practice) rather than to the vaccine itself. It did not require any treatment.

Delayed general reactions were also not significantly different between groups. It can be noticed that both treatments induced moderate reactions, which did not seriously affect the general

condition of the animals. In both groups, most of these reactions were lethargy. Digestive disorders and other isolated symptoms (hyperthermia, wheezy breathing, musculoskeletal disorders or polydipsia) were also reported (vomiting or diarrhoea were reported in 1.7% of dogs vaccinated with Eurican DAPPi-Lmulti vaccine and 2.3% of dogs vaccinated with Canigen CHPPi/L).

Other isolated symptoms were reported in 2.9% of dogs vaccinated with Eurican DAPPi-Lmulti vaccine and in 1.2% of dogs vaccinated with Canigen CHPPi/L. Most delayed general reaction (92% in the Eurican Lmulti group and 88% in the reference group) lasted 2 days or less and did not need any treatment. One of the 2 dogs injected with Eurican DAPPi-Lmulti vaccine with delayed general reactions lasting more than 2 days presented left hind limb lameness (following trauma) observed from day 6 post vaccination. The other dog presented an anaphylactic shock with cutaneous signs characterized by oedema (lips and forelimbs) lasting 2 days and urticaria (itching, redness and/or pustules on belly or flanks) lasting 7 days. It fully recovered 7 days after vaccination. No statistically significant difference regarding immediate local reactions was observed between the two treatments. These reactions were transient injection site pruritus and/or pain in dogs vaccinated with Eurican DAPPi-Lmulti vaccine. They lasted a few minutes and disappeared without treatment.

Delayed local reactions were not significantly different between groups. Most reactions lasted few days and none of them required any treatment. Injection site pain was observed in one dog in each group. The most frequent local reaction observed was swelling at a similar level in the two groups. All but one swelling reactions were noticed during the first days following the vaccination and disappeared within one day after vaccination with Eurican DAPPi-Lmulti vaccine. One swelling reaction after vaccination with Eurican DAPPi-Lmulti vaccine, which had not been noticed by the owner, was however palpated by the investigator during the last clinical examination 14 days after vaccination. Swelling reaction disappeared after up to 7 days in dogs vaccinated with Canigen CHPPi/L. Importantly, all delayed local reactions observed in dogs vaccinated with Eurican DAPPi-Lmulti vaccine were mild and transient and no persistent injection site oedema was observed.

The second supportive field study in 108 adult dogs aged 5.8 years (average) was to confirm the safety of a booster vaccination with Eurican DAPPi-Lmulti vaccine. The safety evaluation was based on the monitoring of immediate (15 minutes after each injection) and delayed (14 days after injection) local and general adverse reactions after treatment with DAPPi-Lmulti. No immediate local or general reactions were observed in this trial. Delayed general reactions (mostly lethargy) were moderate and transient and did not seriously affect the general condition of the animals. They affected 2 dogs (1.9%) and did not need any treatment. The only delayed local reaction observed was injection site pain in 2 dogs (1.9%). This reaction lasted a few hours and disappeared without any treatment.

In conclusion, Eurican DAPPi-Lmulti vaccine, administered to adult dogs as booster vaccination under field conditions, was well tolerated. In the study with an already authorised competitor vaccine, the safety was similar to that of marketed Canigen CHPPi/L with no significant difference in the reactions observed with the two products.

Finally, a field trial was carried out to demonstrate the safe use of Eurican Lmulti vaccine in pregnant bitches. The aim of the study was to confirm the safety of vaccination with Eurican DAPPi-Lmulti vaccine in pregnant bitches under field conditions, with a focus on the monitoring of immediate and delayed local and general adverse reactions, and on the monitoring of whelping and offspring until 6 weeks of age. A comparison with an already authorised competitor vaccine (Nobivac CHPPi-L from Intervet) has been provided.

A total of 18 pregnant bitches (at either the 2nd or 3rd trimester of pregnancy) were vaccinated with Eurican DAPPi-Lmulti vaccine and 9 pregnant bitches with Nobivac CHPPi-L. All vaccines were injected by subcutaneous route. Pregnant bitches were monitored for immediate adverse reactions for approximately 15 minutes post-injection and for delayed adverse reactions for approximately 14 days after the injection.

After whelping, each bitch and its offspring were regularly monitored until about 6 weeks after whelping. At this time, a motor activity test was carried out on each puppy.

No general or local reactions were observed in pregnant bitches after vaccination whatever the vaccine. All pregnant bitches remained in good general condition.

Regarding the adverse effects on the pregnancy, no significant difference was found between the group vaccinated with Eurican DAPPi-Lmulti vaccine and the group vaccinated with Nobivac CHPPi-L. Neither the occurrence of dystocia nor the number of stillborn puppies were significantly different between groups.

Neither abortions nor malformations in the living puppies were observed.

Regarding the adverse effects on the offspring, no significant difference was found between the group vaccinated with Eurican DAPPi-Lmulti vaccine and the group vaccinated with Nobivac CHPPi-L. Mortinatality (death within 48 hours post whelping) and number of weaned puppies were similar in the two groups. The motor activity test at 6 weeks of age showed no abnormalities irrespective of group.

Safety of Eurican DAPPi-Lmulti vaccine in pregnant bitches and their offspring was satisfactory under field conditions since no pregnant bitch showed any local or systemic reactions and no adverse effects on the pregnancy or the offspring were observed. By extension to the vaccine Eurican L4 it is postulated by the applicant, that this vaccine is also considered as safe for use in pregnant bitches, but, as discussed under 'Examination of reproductive performance', the lack of data concerning the use in pregnant bitches of Eurican L4, which contains an additional inactivated leptospira serovar, *Leptospira* australis is appropriately reflected in the SPC. It has been justified by the applicant, that the vaccinated bitches (in the laboratory and in the field study) were only in the 2nd and 3rd trimester of pregnancy, please refer to 'Examination of reproductive performance'.

Environmental risk assessment

An environmental risk assessment was carried out in accordance with the "Note for guidance on the environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95).

The active ingredients of Eurican L4 are inactivated bacteria, which are not expected to interact with ecosystems. Therefore, and in line with the above-mentioned guidance (EMEA/CVMP/074/95), no specific hazard was identified with regard to the environment.

The product is presented in tightly closed single-dose vials. It is parenterally administered to individual dogs via the subcutaneous route by a qualified person in a small volume (1 ml per animal). Safe disposal of used containers or containers with expired shelf life will generally be ensured, as the vaccine will be administered by or under the supervision of a veterinary surgeon.

The veterinary medicinal product will only be used in non-food-producing animals.

The other ingredients in the product (excipients like water, common salt ions like sodium, potassium, chloride and phosphate) do also not pose a threat to the environment at the concentration used in this product.

In accordance with Annex I to the Note for Guidance *"Environmental risk assessment for immunological veterinary medicinal products"* (EMEA/CVMP/074/95), and as a result of previous assessments and characteristics of the product, and given that the likelihood of hazards occurring is low and the consequences of a hazard occurring are negligible, the level of risk to the environment is considered to be "effectively zero" (using the matrix approach). Eurican L4 is thus not expected to pose a risk to the environment when used as recommended in the product information.

Overall conclusions on the safety documentation

The safety of Eurican L4 was investigated in one laboratory and in one field trial in puppies and adult dogs. One supportive laboratory study conducted with Eurican Lmulti (containing only three *Leptospira* strains) in pregnant bitches has been provided. In addition, five field studies (three in puppies, two in adult dogs and one in pregnant bitches) using Eurican Lmulti have been presented. The studies were carried out with the most sensitive category of target animals, i.e. 7-week-old pups, and with adult dogs for booster and reproductive performance studies. In the laboratory studies, batches containing the maximum release titre and - for compatibility - also standard batches, were used. For the overdose study, a batch with double dose of inactivated antigen was used. In the field trials, standard batches were used.

Based on the results it was concluded that the safety of the vaccine in target animals when the vaccine is administered according to the recommended schedule and via the recommended route is acceptable, in general. The vaccine is considered safe regarding the administration of one dose, double overdose and repeated administration of one dose as no general reactions and only transient local reactions (swellings) were recorded. Local reactions are adequately mentioned in the SPC.

Reproduction safety was not investigated for Eurican L4. Nevertheless, data are available with the 3-component leptospirosis vaccine Eurican Lmulti showing that the vaccine is safe in pregnant bitches. The applicant has justified that the data generated with Eurican L4 may be considered relevant to support the safety of use of Eurican L4 during pregnancy, however information is included in section 3.7 of the SPC to reflect that no data are available during pregnancy for Eurican L4, which contains an additional inactivated serovar, *L.* Australis.

The product is not expected to adversely affect the immune response of the target animals or of their progeny, and therefore no suitable tests on the immunological functions were carried out.

A user safety assessment in line with the relevant guidance has been presented. Based on this assessment, the potential health risk of the product to the user is considered to be low and acceptable when used in accordance with the SPC. There are no risks identified for the user.

No residues studies were performed, as Eurican L4 is not intended for use in food-producing species.

The interaction of Eurican L4 with Eurican DAPPi and Eurican DAP was recorded in one laboratory and one field study. For completeness, the association of Eurican Lmulti and Eurican L with Eurican DAPPi and Eurican DAP had been well established in several studies in the past. The interaction of Eurican L4 with Eurican DAPPi and Rabisin following simultaneous vaccination were similar in nature, severity and duration to the reactions caused by each of the vaccines when used independently. These data show that Eurican L4 can be used alone or associated to Eurican DAPPi (mixed and administered at the same site) and /or associated to Rabisin (not mixed, but injected at the same time at a different site to that of the site of injection of Eurican L4 or Eurican DAPPi + Eurican L4). In one combined safety and efficacy GCP-compliant, positively-controlled field study, local and general safety of Eurican L4 mixed with Eurican DAPPi, was confirmed as satisfactory

under field conditions, whether used for primary vaccination in puppies from 7 weeks of age or for booster vaccination in already primed dogs.

Six multicentric studies were performed with Eurican Lmulti mixed with Eurican DAPPi to evaluate primovaccination, annual booster and effects on pregnant bitches. The adverse reactions observed in the field were not different from the reactions observed in the laboratory studies. These studies are considered supportive of the safety profile of Eurican L4 given that Eurican L4 differs with respect to Eurican Lmulti by the addition of *L. interrogans* serogroup Australis serovar Bratislava.

An environmental risk assessment in line with the relevant guidance has been presented. It can be concluded that Eurican L4 is not expected to pose a risk for the environment when used according to the SPC.

The vaccine was administered by the subcutaneous route as recommended. Laboratory studies were reported to be compliant with GLP. Studies were carried out in the target species of the minimum age recommended for vaccination.

Part 4 – Efficacy

Introduction and general requirements

Eurican L4 is a multivalent, non-adjuvanted, inactivated bacterial vaccine against leptospirosis for healthy dogs from 7 weeks of age. The inactivated bacterial components (*L*. Canicola, *L*. Grippotyphosa, *L*. Icterohaemorrhagiae, *L*. Australis) are presented in liquid form.

The composition of Eurican L4 is shown below:

Ingredients	***Quantity per 1 ml dose***	***Function***
Inactivated *Leptospira interrogans* serogroup and serovar Canicola, strain 16070	Activity acc. to Ph. Eur. 0447*	Antigen
Inactivated *Leptospira interrogans* serogroup and serovar Icterohaemorrhagiae, strain 16069	Activity acc. to Ph. Eur. 0447*	Antigen
Inactivated *Leptospira interrogans* serogroup and serovar Grippotyphosa, strain Grippo Mal 1540	Activity acc. to Ph. Eur. 0447*	Antigen
Inactivated *Leptospira interrogans* serogroup Australis and serovar Bratislava, strain 16785	Activity acc. to Ph. Eur. 0447*	Antigen

* ≥80% protection in hamsters

The originally proposed relevant SPC claims were:

3.2 Indications for use, specifying the target species

"Active immunisation of dogs from 7 weeks of age to prevent or reduce mortality, clinical signs, infection, bacterial excretion, renal carriage and renal lesions caused by:
- *Leptospira interrogans* serogroup Canicola serovar Canicola,

- *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae,

- *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni,

- *Leptospira kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, and

- *Leptospira interrogans* serogroup Australis serovar Bratislava.

Serogroup / Serovar	Indication					
	Mortality	**Clinical signs**	**Infection**	**Bacterial excretion**	**Renal carriage**	**Renal lesions**
Canicola / Canicola	Prevention*	Prevention*	Reduction	Reduction	Reduction	Reduction
Icterohaemorrhagiae / Icterohaemorrhagiae	Prevention	Prevention	Reduction	Reduction	Reduction	Reduction
Icterohaemorrhagiae / Copenhageni	Prevention**	Prevention**	Prevention**	Prevention**	Prevention**	Prevention**
Grippotyphosa / Grippotyphosa	Prevention***	Prevention	Reduction	Reduction	Reduction	Reduction
Australis / Bratislava	Prevention	Prevention	Prevention	Prevention	Prevention	Prevention

* For *Leptospira interrogans* serovar Canicola *Leptospira interrogans* serovar Icterohaemorrhagiae and *Leptospira kirschneri* serovar Grippotyphosa the prevention of mortality and clinical signs was not demonstrated for duration of immunity.
** For *Leptospira interrogans* serovar Copenhageni the duration of immunity was not established.
*** For *Leptospira kirschneri* serovar Grippotyphosa, no mortality occurred during challenge experiment for duration of immunity.

Onset of immunity: 2 weeks after the second injection of the primary vaccination course for all strains.

Duration of immunity: at least one year after the second injection of the primary vaccination course for all strains."

3.8 Interaction with other medicinal products and other forms of interaction

"Safety and efficacy data are available which demonstrate that this vaccine can be mixed with Boehringer Ingelheim live attenuated vaccines against distemper, adenovirosis, parvovirosis and parainfluenza type 2 respiratory infections.

Safety and efficacy data are available which demonstrate that this vaccine can be administered on the same day as, but not mixed Boehringer Ingelheim with, rabies vaccine in dogs from 12 weeks of age. Efficacy of the vaccine for protection against the Copenhageni serovar has not been investigated after use with Boehringer Ingelheim rabies vaccine on the same day.

No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product except the products mentioned above. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis."

3.9 Administration routes and dosage

"When Eurican L4 is used alone, inject a 1 ml dose subcutaneously.

When Eurican L4 is used as a diluent of a freeze-dried vaccine against distemper, adenovirosis, parvovirosis and parainfluenza type 2, aseptically reconstitute the contents of the lyophilisate with the Eurican L4 vaccine suspension. Mix well before use. The entire contents of the reconstituted vial should be administered as a single dose.

The following schedule should be followed:

Primary vaccination: Two injections separated by an interval of 4 weeks from 7 weeks of age.

Revaccination: Administer one dose 12 months after completion of the primary vaccination course. Dogs should be revaccinated with a single booster dose on an annual basis."

5.1 Major incompatibilities

"Do not mix with any other veterinary medicinal product except those listed in section 4.8."

Efficacy was demonstrated in compliance with the 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. as well as specific monographs applicable to the product. In particular, the following were taken into account:

-Ph. Eur. 0062: Vaccines for veterinary use

-Ph. Eur. 5.2.7: Evaluation of efficacy of veterinary vaccines and immunosera

-Ph. Eur. 0447: Canine leptospirosis vaccine (inactivated)

-Guideline EMA/CVMP/IWP/594618/2010: Guideline on the requirements for combined vaccines and associations of immunological veterinary medicinal products (IVMPs)

The product inoculated for the efficacy tests was the Eurican DAPPi-L4 vaccine (i.e. the vaccine suspension obtained by mixing Eurican L4 (suspension for injection) and Eurican DAPPi (lyophilisate for suspension for injection)). Eurican DAPPi is a freeze-dried live vaccine containing modified live canine distemper virus, canine adenovirus type 2, canine parvovirus and canine parainfluenza virus type 2, and freeze-drying excipient.

This is justified to demonstrate the efficacy of Eurican L4 in accordance with the guideline on combined vaccines and associations (EMA/CVMP/IWP/594618/2010), as it is a vaccine containing a smaller combination of the active substances and excipient compared to the associated vaccines suspension. The applicant claims that this approach considers a worst case for any potential interference and allows to demonstrate the compatibility of Eurican L4 and Eurican DAPPi. This reduces the burden of challenge studies in laboratory animals, in accordance with the 3Rs principles, as the same studies do not have to be duplicated.

For the same reasons, in most of the laboratory studies performed to demonstrate the efficacy of Eurican L4, the Boehringer Ingelheim's inactivated and adjuvanted vaccine against rabies (Rabisin) was associated (injected at the same time at a separate injection site).

As the composition of Eurican L4 is the same as that of Eurican Lmulti, except for the new *Leptospira* strain added, the applicant has included in this dossier several studies performed in dogs vaccinated with the latter. These studies are presented as additional supporting data. In these earlier studies, where dogs were vaccinated with the association of Eurican Lmulti vaccine and Eurican DAPPi vaccine (mixed), the Eurican Lmulti vaccine is named L3 vaccine and Eurican DAPPi vaccine is named Eurican DAPPi2 vaccine. The association of Eurican Lmulti vaccine mixed with Eurican DAPPi vaccine is referred to as Eurican DAPPi2-L3. These studies have already been evaluated by the French competent authority, as France was acting as RMS in decentralised procedures for the authorisation of Eurican DAPPi2-L3 and Eurican L3, but they have not been evaluated by the CVMP. It is noted that whilst the approach of the applicant to investigate the safety of Eurican L4 in combination with Eurican DAPPi is acceptable (worst case scenario for the potential for adverse reactions to occur), in order to accept the same approach for the efficacy studies, in accordance with the GL on the Requirements for combined vaccines and associations of immunological veterinary medicinal products (IVMPs) (EMA/CVMP/IWP/594618/2010), *the*

supporting data must take into account that the associated administration of two or more IVMPs may cause an interaction leading to either a diminished or increased immunological response to individual components, compared to when each IVMP is administered alone. The applicant has not specifically addressed this point. However, given that the lack of interference is accepted for the 3-component Eurican L3 vaccine in combination with Eurican DAPPi and Rabisin, it is not considered that the addition of one more inactivated leptospiral antigen in Eurican L4 would cause an interaction leading to a diminished or increased response to individual components. Therefore, the approach to demonstrate the efficacy of Eurican L4 when mixed with Eurican DAPPi can be accepted (and when administered at a separate site but at the same time as Rabisin).

The batches used in the efficacy studies were manufactured according to the process described in Part 2 of this dossier for Eurican L4. Batches of Eurican DAPPi and Rabisin were manufactured according to the process described in the manufacturing authorisation dossiers of these products.

Eurican L4 is an inactivated vaccine formulated to contain a fixed quantity of antigen per dose. Nevertheless, the majority of the studies used batches formulated with a lesser amount of each active ingredient (minimum dose) compared to standard batches. The quantities of *Leptospira* in these batches were:

- 130 (mini) or 160 (standard) FTU/dose for the inactivated *Leptospira* Canicola serovar,
- 150 (mini) or 180 (standard) FTU/dose for the inactivated *Leptospira* Icterohaemorrhagiae serovar,
- 340 (mini) or 370 (standard) FTU/dose for the inactivated *Leptospira* Grippotyphosa serovar,
- 150 (mini) or 180 (standard) FTU/dose for the inactivated *Leptospira* Australis serovar.

FTU: Formazine Turbidity Unit

The pivotal onset of immunity and duration of immunity studies for *L. interrogans* Canicola, *L. interrogans* Icterohaemorrhagiae, *L. interrogans* Grippotyphosa and *L. interrogans* Australis /Bratislava were conducted with the minimum proposed dose of the respective antigens.

Regarding the modified live viral components in Eurican DAPPi mixed with Eurican L4 in laboratory studies, the batches were at standard dose unless specified. Batches containing the minimum viral titre for a viral component were used in studies whose purpose was to demonstrate the efficacy of this component, as follows:

- Minimum 4.0 $\log_{10}CCID_{50}$/dose for the attenuated canine distemper virus antigen,
- Minimum 2.5 $\log_{10}CCID_{50}$/dose for the attenuated canine adenovirus type 2 antigen,
- Minimum 4.9 $\log_{10}CCID_{50}$/dose for the attenuated canine parvovirus antigen,
- Minimum 4.7 $\log_{10}CCID_{50}$/dose for the attenuated canine parainfluenza type 2 antigen.

Standard batches of Rabisin were used in all studies.

Challenge model

Efficacy of all components of Eurican L4 was assessed by challenges with heterologous challenge strains. The origin of challenge strains (infected dogs) has been adequately described. Certificates of analysis were provided for each challenge strain.

The Leptospira strains used for challenge are genetically representative of a given serogroup and serovar with no particular geographical variation.

All challenge strains have been identified by the reference Leptospira laboratory at Pasteur Institute (Paris). The typing of the challenge strains allowed to confirm that they are genetically close to the

reference strains belonging to these epidemiologically relevant serogroups.

For *L*. Icterohaemorrhagiae, *L*. Canicola and *L*. Grippotyphosa the challenge model was essentially the same as previously shown to provide adequate severity during the development of Eurican Lmulti. Only a few modifications were made to the production of the challenge stocks, i.e. for animal welfare reasons.

The *L*. Australis challenge strain has been recently isolated. The validation of the challenge model was performed prior to the vaccination challenge studies. Different challenge doses were tested in puppies and adult dogs to choose the most appropriate one. The challenge validation criteria were achieved with all doses. The higher dose was selected, as it would ensure a more robust challenge model and the dose was comparable with the other leptospira challenge models used.

Efficacy parameters and tests

Efficacy documentation

Sixteen studies were conducted to investigate the efficacy of the product, including 15 laboratory studies (two of the studies were divided into two parts, vaccination and challenge phases, identified with different study numbers) and 1 field trial. Twenty-one additional studies conducted with the product Eurican Lmulti were included, as supportive data. Laboratory studies were well documented and carried out in dogs of the minimum age recommended for vaccination, using batches manufactured according to the process described in Part 2 of this dossier for Eurican L4. Batches of Eurican DAPPi and Rabisin were manufactured according to the process described in the manufacturing authorisation dossier of these products.

Laboratory trials

Dose determination

No dose determination study was carried out for *Leptospira* Australis. The same efficacy profile as the historical strains was expected and, therefore, the standard dose of 180 FTU/dose was targeted. The efficacy of this dose was checked in a proof-of-concept study in the early stage of development. The selected minimum dose has been confirmed as being efficacious for the claimed indications in the OOI and DOI studies.

The minimum protective dose for *Leptospira* Canicola component in Eurican L4 was selected to be the same as in Eurican Lmulti vaccine, and it is 130 FTU / dose.

The minimum protective dose for *Leptospira* Icterohaemorrhagiae component in Eurican L4 was selected to be the same as in Eurican Lmulti vaccine, and it is 150 FTU / dose.

The minimum protective dose for *Leptospira* Grippotyphosa component in Eurican L4 was defined to be the same as Eurican Lmulti vaccine, and it is 340 FTU / dose.

Onset of immunity

For the demonstration of the immunogenicity, two studies are presented for each serovar. Five trials were carried out to demonstrate the protection provided by Eurican L4 vaccine against *L. interrogans* serovar and serogroup Canicola (*L.* Canicola), *L. interrogans* serovar and serogroup Icterohaemorrhagiae (*L*. Icterohaemorrhagiae), *L. kirschneri* serovar and serogroup Grippotyphosa (*L.* Grippotyphosa) and *L. interrogans* serovar australis serogroup bratislava (*L.* australis). In addition to these studies, the reports of three trials demonstrating the immunogenicity of Eurican DAPPi-Lmulti vaccine are provided as supportive information.

The study design for each *Leptospira* component was as follows in "Efficacy of a minimum dose" studies with Eurican L4:

Animals	Twelve conventional Beagle puppies aged between 7 (6) and 9 weeks were randomly allocated to two groups of 6 animals.
Vaccine	Eurican L4 *L*. Bratislava potency: 150 FTU/dose *L*. Canicola potency: 130 FTU/dose *L*. Grippotyphosa potency: 340 FTU/dose *L*. Icterohaemorrhagiae potency: 150 FTU/dose
Administration route	Subcutaneous
Vaccine scheme	Vaccinates: vaccination on D0 and D28 with Eurican DAPPi-L4 (+ Rabisin on D28) Controls: vaccination on Days 0 and 28 with Eurican DAPPi (+ Rabisin on D28)
Challenge strains	*L. interrogans* serovar Bratislava 14 500 16803-B of 04/11/14 *L. interrogans* serovar Canicola 07 500 16671-B of 15/09/07 *L. kirschneri* serovar Grippotyphosa 06 500 16660-B of 15/10/15 *L. interrogans* serovar Icterohaemorrhagiae 07 500 16664 2p hamster of 15/12/13
Challenge scheme	Two weeks after the 2nd vaccination both groups were challenged intraperitoneally
Follow-up	After vaccination: Observation for clinical signs, measurement of rectal temperatures and weight, blood samples for serology After challenge: Observation for clinical signs, measurement of rectal temperature and weight, blood samples for serology, for haematology, biochemistry and detection of the challenge organism by qPCR, urine samples for detection of the challenge organism by qPCR After euthanasia twenty-eight days after challenge Liver and kidney samples were collected for detection of *Leptospira* by qPCR (kidneys) and for histological analysis (kidneys and liver).

Results

***Leptospira* Australis**

All animals remained healthy during the vaccine phase. All vaccinated animals seroconverted during the vaccine phase with a titre ≥ 0.38 $\log_{10}OD_{50}$ at the time of the challenge (mean = 0.51 $\log_{10}OD_{50}$).

Challenge was validated since five of the six controls were infected. Between days 4 and 5 post-challenge, they developed severe leptospirosis (alteration of general condition, digestive signs and cutaneo-mucosal signs associated with alteration of biological parameters) leading to euthanasia for ethical reasons or death. These controls had positive blood and kidney samples by qPCR, and kidney and liver lesions. Four of them had also positive urine samples by qPCR (one animal had only one urine sample and this sample was negative).

Despite the severity of the challenge with mortality in the control group, no animals were infected in the vaccinated group. They remained healthy throughout the post-challenge monitoring period. No *Leptospira* was detected in blood, urine or kidney, and no kidney lesions were recorded. Four vaccinated animals had alteration of two or three hepatic parameters. The applicant commented that histology examination of the liver in two of these dogs were not evocative of leptospirosis infection but suggestive of a preexisting hepatic condition. The other two dogs had hepatic parameters close to the limit before challenge took place and the moderate increase after challenge was not indicative of acute infection. As these four animals did not have associated clinical signs, hepatic lesions or impact on weight gain, increase of the hepatic parameters in these four vaccinates is considered not meaningful.

The primary vaccination program with Eurican DAPPi-L4 vaccine induced seroconversion of all vaccinated animals and supported the prevention of mortality, clinical signs, infection, bacterial excretion, renal carriage and kidney lesions against *Leptospira* Australis/Bratislava challenge two weeks after the primary vaccination. The proposed claims for *L*. Australis/Bratislava at OOI are considered to have been satisfactorily supported.

***Leptospira* Canicola**

All animals remained healthy during the vaccine phase. All vaccinated animals seroconverted during the vaccine phase with three animals with a titre ≥ 0.51 $\log_{10}OD_{50}$ at the time of the second vaccine injection and all animals with a titre ≥ 0.80 $\log_{10}OD_{50}$ at the time of the challenge (mean = 1.71 $\log_{10}OD_{50}$).

Challenge was validated, as five out of the six controls were infected. Between days 4 and 6 post-challenge, two controls developed severe leptospirosis (prostration, dehydration, digestive signs and cutaneo-mucosal signs associated with alteration of biological parameters) leading to euthanasia for ethical reasons and one control died after having presented apathy and digestive signs associated with alteration of biological parameters. The same three controls had moderate to severe alteration of biological parameters, positive blood and kidney samples by qPCR, and severe kidney and liver lesions. Two of them had also positive urine samples by qPCR (the animal found dead was in anuria and no urine was sampled). Two controls which survived until the end of the study and remained healthy had positive urine sample and one of them had also positive kidney samples and kidney lesions.

Despite the severity of the challenge with mortality in the control group, in the vaccinated group, no animals were infected. They all remained healthy throughout the post-challenge monitoring period. No *Leptospira* was detected in blood, urine or kidney, and no kidney lesions were recorded.

The primary vaccination program with DAPPi-L4 vaccine induced seroconversion of all vaccinated animals and supported the prevention of mortality and clinical signs, and the reduction of infection, bacterial excretion, renal carriage and kidney lesions against *Leptospira* Canicola/Canicola challenge two weeks after the primary vaccination. The proposed claims for *L*. Canicola/Canicola at OOI are considered to have been satisfactorily supported.

***Leptospira* Icterohaemorrhagiae**

All animals remained healthy during the vaccine phase. All vaccinated animals seroconverted during the vaccine phase with a titre ≥ 0.52 $\log_{10}OD_{50}$ at the time of the second vaccine injection and ≥ 1.39 $\log_{10}OD_{50}$ at the time of the challenge (mean = 1.53 $\log_{10}OD_{50}$).

Challenge was validated as, between days 5 and 6 post-challenge, all controls were infected and developed severe leptospirosis (alteration of general condition, digestive signs and cutaneo-mucosal signs associated to alteration of biological parameters) leading to euthanasia for ethical reasons or death. These controls had positive blood and kidney samples by qPCR, and kidney and liver lesions. Five of them had also one positive urine sample by qPCR (one animal had only one urine sample and this sample was negative).

Despite the severity of the challenge with mortality in the control group, in the vaccinated group, no animals were infected. They all remained healthy throughout the post-challenge monitoring period. No *Leptospira* was detected in blood, urine or kidney, and no kidney and liver lesions were observed.

The primary vaccination program with DAPPi-L4 vaccine induced seroconversion of all vaccinated animals and supported the prevention of mortality and clinical signs, and the reduction of infection, bacterial excretion, renal carriage and kidney lesions against *Leptospira*

Icterohaemorrhagiae/Icterohaemorrhagiae challenge two weeks after the primary vaccination. The proposed claims for *L*. Icterohaemorrhagiae/Icterohaemorrhagiae at OOI are considered to have been satisfactorily supported.

***Leptospira* Grippotyphosa**

All animals remained healthy during the vaccine phase. All vaccinated animals seroconverted during the vaccine phase with a titre ≥ 0.57 $\log_{10}OD_{50}$ at the time of the second vaccine injection and all animals with a titre ≥1.50 $\log_{10}OD_{50}$ at the time of the challenge (mean = 1.70 $\log_{10}OD_{50}$).

Challenge was validated as all controls became infected. Between days 4 and 5 post-challenge, three controls developed severe leptospirosis (prostration, dehydration, cutaneo-mucosal signs associated to loss of weight and alteration of biological parameters) leading to euthanasia for ethical reasons. These animals had positive blood, urine and kidney samples by qPCR, and severe kidney and liver lesions. The three controls which survived until the end of the study and remained healthy (one animal had mild and transient clinical signs for two day) had positive blood, urine and kidney samples associated with mild to moderate kidney lesions and were therefore considered as infected.

In the vaccinated group, one animal was infected with *Leptospira* detected in urine and one kidney. The five other animals remained all healthy throughout the post-challenge monitoring period. No *Leptospira* was detected in blood, urine or kidney, and no kidney lesions were recorded.

The primary vaccination program with DAPPi-L4 vaccine induced seroconversion of all vaccinated animals and supported the prevention of mortality and clinical signs, and reduction of infection and kidney lesions, and reduced bacterial excretion and renal carriage against *Leptospira* Grippotyphosa/Grippotyphosa challenge two weeks after the primary vaccination. The proposed claims for *L.* Grippotyphosa/Grippotyphosa at OOI are considered to have been satisfactorily supported.

In addition to these studies an "Efficacy of a lower dose" study was also carried out with *Leptospira* Australis.

The objective of the study was to assess the immediate efficacy of Eurican L4 vaccine (two injections 4 weeks apart) diluted at 1/4 or 1/10 in puppies when challenged with virulent *Leptospira* Australis/Bratislava two weeks after the second vaccine injection.

Eighteen conventional Beagle puppies, aged between 7 and 9 weeks, were randomly allocated to three groups of 6 animals each. One group was vaccinated with Eurican L4 vaccine at 1/4 dose and one group with Eurican L4 vaccine at 1/10 dose on D0 and D28. The control group was vaccinated with Eurican DAPPi vaccine on D0 and D28, and one dose of Rabisin on D28 in a separate site from Eurican DAPPi vaccine. Two weeks after the 2nd vaccine injection, all animals were intraperitoneally challenged with a virulent suspension of *L*. Australis/Bratislava.

Following challenge, all animals were examined daily to detect signs of leptospirosis and weighed regularly. Blood and urine were regularly sampled for blood biochemistry and platelets count (blood), or detection of pathogenic *Leptospira* by qPCR (blood and urine). At the time of death or euthanasia (during or at the end of the study), animals were necropsied, and kidneys and liver were sampled for detection of pathogenic *Leptospira* by qPCR (kidneys) or histological analysis (kidneys and liver). All animals remained healthy during the vaccine phase.

Challenge was validated since five of the six controls were infected. Between days 4 and 5 post-challenge, they developed severe leptospirosis (alteration of general condition, digestive signs and cutaneo-mucosal signs associated to alteration of biological parameters) leading to euthanasia for ethical reasons or death. These controls had positive blood and kidney samples by qPCR, and kidney

and liver lesions. Four of them had also positive urine samples by qPCR (one animal euthanised on day 5 post-challenge had only one urine sample before and this sample was negative).

Despite the severity of the challenge with mortality in the control group, no animals were infected in the vaccinated groups. They remained healthy throughout the post-challenge monitoring period except one animal vaccinated with 1/4 dose. This animal experienced ocular and nasal discharge from day 2 to day 7 post challenge and lost weight within 4 days post-challenge. Apathy was also observed in this animal on day 17 post-challenge. According to the applicant prevention of mortality and clinical signs were demonstrated in vaccinated groups compared to control group.

Four dogs vaccinated with 1/4 dose had alteration of two or three hepatic parameters. Two of them had also urea slightly above threshold once. Three dogs vaccinated with 1/10 dose had alteration of urea and AST once. Reduction of biological parameters disorders was demonstrated in vaccinated group compared to control group.

No *Leptospira* was detected in blood (only one positive sample in one animal of 1/10 dose vaccinated group), urine or kidney of vaccinated animals, and no kidney lesions were recorded.

The applicant claims that the primary vaccination program with Eurican L4 vaccine at 1/4 or 1/10 dose prevented mortality, clinical signs, infection, bacterial excretion, renal carriage and kidney lesions against *Leptospira* Australis/Bratislava challenge two weeks after the primary vaccination. However, it is noted that one animal vaccinated with ¼ dose experienced clinical signs in the post-challenge phase, and one animal vaccinated with 1/10 dose was reported with leptospiraemia, and therefore the applicant's conclusions that a ¼ dose or a 1/10 dose prevented clinical signs and infection, respectively, are not fully supported. However, this was a supportive study and the efficacy of the proposed minimum antigen content of *L*. Australis per dose has been satisfactorily supported in the pivotal OOI study.

Three other studies were carried out previously with Eurican Lmulti and were introduced as supportive data.

***Leptospira* Canicola**

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine 4 weeks later conferred a full protection against signs of disease, infection and urinary tract infection and excretion against *L.* Canicola challenge two weeks after the primary vaccination.

***Leptospira* Icterohaemorrhagiae**

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine 4 weeks later conferred a full protection against signs of disease, infection and urinary tract infection and excretion against *L.* Icterohaemorrhagiae challenge two weeks after the primary vaccination.

***Leptospira* Grippotyphosa**

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R

vaccine 4 weeks later conferred a full protection against signs of disease, infection and urinary tract infection and excretion against *L.* Grippotyphosa challenge two weeks after the primary vaccination.

Cross protection against Leptospira Copenhageni

Vaccine *Leptospira* strains have been shown to afford cross-protection against *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni in a vaccination-challenge study carried out in accordance with Ph. Eur. monograph 0447 and provided in the claim extension procedure of Eurican DAPPi-Lmulti in 2017.

Fourteen 8- to 9-week old puppies, seronegative for *Leptospira*, were randomised into one vaccinated and one control group of 7 animals each. The animals received one dose of Eurican DAPPi-Lmulti (vaccinated) or DAPPi (control) on D0 and one dose on D28 by subcutaneous route. Two weeks after the second vaccination, all animals were challenged by intraperitoneal route (IP) with a virulent suspension of *L*. Copenhageni.

The challenge was validated since no *Leptospira* could be detected in blood and urine samples of any dog prior to the challenge and all control dogs were infected (with at least 2 positive blood samples on at least 2 different days). Five out of the 7 controls presented also mild to severe clinical signs (icterus, dehydration, diarrhoea, loss of weight, alteration of haematological and biochemical parameters) leading to euthanasia of 3 animals. One animal died just before euthanasia could be performed, 9 days post challenge.

Between day 5 and day 28 post challenge, *Leptospira* DNA was detected in urine, for 1 to 6 timepoints, in all 7 controls (on day 28 post challenge, all surviving animals had detectable *Leptospira* DNA in the urine). *Leptospira* DNA was detected in the blood, for time points 2-5, in all 7 controls.

Kidney samples showed renal lesions and *Leptospira* DNA was detected in at least one kidney in all the 7 controls.

All the vaccinated dogs remained in good general condition without any clinical signs or hyperthermia throughout the challenge monitoring. No *Leptospira* was detected in blood, urine or kidney from vaccinates. No vaccinates showed renal lesions.

The global scores were significantly lower in the vaccinated group than in the control group for the following parameters:

- The global clinical score,

- The global haematological and biochemical score.

The frequency of animals with leptospiraemia and the number of days with leptospiraemia were significantly lower in the vaccinated group than in the control group.

The frequency of animals with leptospiruria and the number of days with leptospiruria were significantly lower in the vaccinated group than in the control group.

The frequency of animals with *Leptospira* in the kidney was significantly lower in the vaccinated group than in the control group.

The kidney histopathology score was significantly lower in the vaccinated group than in the control group.

The primary vaccination of 8- to 9-week old puppies with Eurican DAPPi-Lmulti vaccine conferred a full protection against a *L.* Copenhageni challenge (carried out two weeks after the primary

vaccination) with prevention of clinical signs of disease (including mortality), infection, bacterial excretion, renal carriage and renal lesions. The efficacy and cross protection of the vaccine DAPPi-Lmulti against *L*. Copenhageni was successfully proven. These data are considered relevant to evaluate the efficacy of Eurican L4 against serovar Copenhageni, because it is not anticipated that the addition of an extra leptospiral component, *L.* Australis/Bratislava, in Eurican L4 will have a negative impact on the cross-protection. This approach of the applicant is acceptable and there is no scientific reason to question the relevance of the data.

In all immediate efficacy challenge studies, challenge was performed 14 days after the second dose of the primary vaccination. Based on the results of the studies, 14 days of onset of immunity was established, which is accepted.

As the duration of immunity was not established for *L*. Copenhageni, the information concerning protection at onset of immunity for this serovar was moved from section 3.2 of the SPC to section 4.

Duration of immunity

Four trials were carried out to demonstrate the duration of immunity provided by Eurican L4 vaccine (mixed with Eurican DAPPi) against *L.* Canicola, *L.* Icterohaemorrhagiae, *L.* Grippotyphosa and *L.* Australis.

In addition, the results of three studies demonstrating the duration of immunity of Eurican DAPPi-Lmulti vaccine are provided as supportive information. These studies are relevant to the evaluation of the efficacy of the product as Eurican L4 and Eurican Lmulti contain the same active and inactive ingredients in the same quantities, excepting the additional *L.* Australis strain in Eurican L4.

In studies concerning Eurican Lmulti, the dogs were subjected to non-lethal viral challenge by viruses such as Pi2 and CAV2 prior to Leptospira challenge. The vaccination phase and each challenge phase have been described in separate reports. This sequential challenge design was applied whenever possible to reduce the number of dogs used. A sufficient recovery period was set up between challenges to allow recovery of the challenged animals.

The study design for each *Leptospira* component was as follows in Eurican L4 studies:

Animals	Fourteen conventional Beagle puppies aged between 7 and 10 weeks were randomly allocated to two groups of 7 animals. In the specific study for *L.* Canicola, sixteen puppies were allocated into one group of 9 vaccinated animals and one group of 7 controls.
Vaccine	Eurican L4 *L*. Australis potency: 150 FTU/dose *L*. Canicola potency: 130 FTU/dose *L*. Grippotyphosa potency: 340 FTU/dose *L*. Icterohaemorrhagiae potency: 150 FTU/dose
Administration route	Subcutaneous
Vaccine scheme	Vaccinates: vaccination on D0 and D28 with Eurican DAPPi-L4 (+ Rabisin on D28) Controls: vaccination at Days 0 and 28 with Eurican DAPPi
Challenge strains	*L. interrogans* serovar Bratislava 14 500 16803-B of 04/11/14 *L. interrogans* serovar Canicola 07 500 16671-B of 15/09/07 *L. kirschneri* serovar Grippotyphosa 06 500 16660-B of 15/10/15 *L. interrogans* serovar Icterohaemorrhagiae 07 500 16664 2p hamster of 15/12/13
Challenge scheme	About one year after the 2nd vaccination both groups were challenged intraperitoneally
Follow-up	After vaccination:

	Observation for clinical signs, measurement of rectal temperatures, blood samples for serology After challenge: Observation for clinical signs, measurement of rectal temperature, blood samples for serology, for haematology, biochemistry and detection of the challenge organism by qPCR, urine samples for detection of the challenge organism by qPCR After euthanasia twenty-eight days after challenge Liver and kidney samples were collected for detection of *Leptospira* by qPCR (kidneys) and for histological analysis (kidneys and liver).

Results

***Leptospira* Australis**

Only two vaccinates seroconverted during the vaccine phase on D42 and D56. At the time of the challenge, anti- *L.* Australis antibodies were not detected in any animals.

Challenge was validated since 86% controls (6/7) were infected and four animals had to be euthanised due to severe leptospirosis (alteration of general condition, dehydration, digestive signs and loss of weight associated with cutaneo-mucosal signs in three animals). Severe alterations of most of the biological parameters were observed in the four euthanised controls. All controls had at least two positive blood samples. Five controls had at least one positive urine sample, positive kidney samples and mild or severe kidney lesions. One control had 2 positive urine samples and severe kidney lesions but no positive kidney samples. Liver lesions were observed in the four controls euthanised before the end of post-challenge monitoring period.

Despite the severity of the challenge, with mortality in the control group, all vaccinated animals remained healthy throughout all the post-challenge period. Slight dehydration and/or diarrhoea was observed on one or two occasions in three animals but considered unrelated to challenge. No *Leptospira* was detected in blood, urine or kidney, and no kidney or liver lesions were recorded.

The four euthanised controls had alteration of at least four biological parameters. One other control had only a single day with alteration of two biological parameters and the last two controls had no alteration of biological parameters.

In the vaccinated group, all biological parameters in all animals remained within normal range or only one parameter on each time point was above threshold, except in one animal with two biological parameters altered on T5.

Prevention of disorders in biological parameters was demonstrated in vaccinated group compared to control group.

All controls had at least two positive blood samples. All vaccinates remained negative after challenge.

Prevention of infection was demonstrated in vaccinated group compared to control group.

It is accepted that the primary vaccination program with the Eurican DAPPi-L4 vaccine **prevents** mortality, clinical signs, infection, bacterial excretion, renal carriage and kidney lesions against *L.* Australis/Bratislava challenge one year after the primary vaccination.

***Leptospira* Canicola**

Before challenge, all animals were seronegative for *L.* Canicola (titre below positivity threshold of 0.30 $\log_{10}OD_{50}$). After the challenge, all animals had high titre (≥2.06 $\log_{10}OD_{50}$).

No clinical signs were observed in any of the groups. Transient hyperthermia was recorded in four vaccinates and two controls (knowing that for two vaccinates and one control, hyperthermia was present before challenge).

Variation of body weight was observed in both groups in a similar way with a variation of weight that did not exceed 6% between two consecutive recordings (except once in three vaccinates and twice in one control). As these animals are adult dogs, such slight variation of body weight has no biological significance.

Since there was no mortality and no clinical signs recorded in this study, prevention or reduction of mortality or clinical signs was not demonstrated in vaccinated group compared to control group.

Four controls and five vaccinates had transient alteration of some biological parameters. The three other controls and the four other vaccinates had no alteration of biological parameters. Reduction of biological parameters disorders was not demonstrated in vaccinated group compared to control group.

All controls except one, which had only one positive sample, had three or five positive blood samples. All vaccinates except one, which had five positive samples, had one and two positive blood samples. Reduction of infection was demonstrated in vaccinated group compared to control group.

Five controls had at least two positive urine samples, at least one positive kidney and mild to moderate kidney lesions. The two other controls had no positive kidney samples and no kidney lesions (one dog had a single positive urine sample).

Only three vaccinates had positive urine samples, associated with one positive kidney in two animals, and with mild renal lesions in one kidney for one animal only. Two vaccinates had only mild renal lesions in one kidney with no positive urine sample. The four other vaccinates had no positive urine samples, no positive kidney samples and no renal lesions. Reduction of bacterial excretion was demonstrated in vaccinated group compared to control group. Reduction of renal carriage and reduction of renal lesions were demonstrated in vaccinated group compared to control group.

No liver lesions were observed in any of the groups.

Challenge was validated since more than 80% controls were infected (six animals had at least three positive blood samples and one positive urine sample). Only one control animal was not infected with only one positive blood sample.

It is accepted that the primary vaccination program with Eurican DAPPi-L4 vaccine **reduces** infection, bacterial excretion, renal carriage and kidney lesions against *L.* Canicola/Canicola challenge about 1 year after the primary vaccination, but no mortality and clinical signs were recorded in this study. Prevention or reduction of mortality and clinical signs were not demonstrated in vaccinated group compared to control group. This is clearly indicated in section 4.2 of the SPC.

***Leptospira* Icterohaemorrhagiae**

Before challenge, all controls and one vaccinate were seronegative for *L.* Icterohaemorrhagiae (titre <0.48 $\log_{10}OD_{50}$), and six vaccinates had anti- *L.* icterohaemorrhagiae antibodies (≥ 0.54 $\log_{10}OD_{50}$).

After the challenge, all animals had high titre in both groups (≥ 1.76 $\log_{10}OD_{50}$).

Clinical signs were observed only in one control animal which presented alteration of general condition, dehydration, severe vomiting, bloody diarrhoea and icterus leading to euthanasia on day 5 post-challenge. Three controls (including the one euthanised on day 5 post-challenge) and two vaccinates had transient alteration of some biological parameters.

The challenge was validated since all controls were infected with one to three positive blood samples by qPCR, two to seven positive urine samples, at least one positive kidney sample and mild to severe kidney lesions. In comparison, three vaccinates were infected with one positive blood sample, 5 or 6 positive urine samples, positive kidney samples and mild kidney lesions in two animals, and 2 positive urine samples in one animal. Two vaccinates had only one positive urine sample and two vaccinates had only negative samples and no kidney lesions. Liver lesions were observed only in the euthanised control dog.

It is accepted that the primary vaccination program with DAPPi-L4 vaccine **reduces** infection, bacterial excretion, renal carriage and kidney lesions against *L.* Icterohaemorrhagiae/Icterohaemorrhagiae challenge about 1 year after the primary vaccination, but no mortality was observed in this study. Clinical signs were recorded only in one control animal. Prevention or reduction of mortality and clinical signs were not demonstrated in vaccinated group compared to control group. As this is appropriately reflected in the SPC, the duration of immunity of one year can be accepted for *L*. Icterohaemorrhagiae/Icterohaemorrhagiae.

***Leptospira* Grippotyphosa**

Even though few clinical signs were observed in any of the groups and transient alteration of some biological parameters was observed in both groups, the challenge was validated since all controls were infected, with at least five positive urine samples and at least one positive kidney. Mild to severe renal lesions were observed in six controls. In the vaccinated group, two animals were infected. One animal had 2 positive blood samples, 5 positive urine samples, positive kidneys with moderate kidney lesions, and another animal had one positive urine sample and one positive kidney. One animal had no positive samples and no kidney lesions, four animals had only one positive blood sample or one positive urine sample (associated to one positive kidney in one animal) or one positive kidney sample. Liver lesions were observed in three controls.

It is accepted that the primary vaccination program with Eurican DAPPi-L4 vaccine induced seroconversion of all vaccinated animals and supported the reduction of infection, bacterial excretion, renal carriage and kidney lesions against *L.* Grippotyphosa/Grippotyphosa challenge one year after the primary vaccination. As no mortality and few clinical signs were recorded, reduction was not statistically demonstrated in vaccinates when compared to the controls for these two parameters. As this is appropriately reflected in the SPC, the duration of immunity of one year can be accepted for *L*. Grippotyphosa/Grippotyphosa.

Three other studies were carried out previously with Eurican Lmulti and were introduced as supportive data.

***Leptospira* Canicola**

The primary vaccination with DAPPi2-Lmulti vaccine including a first injection at 7 to 9 weeks of age followed by a second injection 4 weeks later confers a full prevention of clinical signs as well as a reduction of infection, excretion and renal carriage against *L.* Canicola challenge about one year after the primary vaccination.

***Leptospira* Icterohaemorrhagiae**

The primary vaccination with DAPPi2-Lmulti vaccine including a first injection at 7 to 9 weeks of age followed by a second injection 4 weeks later confers a protection against signs of disease, infection, and urinary tract infection and excretion against *L.* Icterohaemorrhagiae challenge about one year after the primary vaccination. The antigen content of *L.* Icterohaemorrhagiae in the Eurican Lmulti batch in this study was 140 FTU/dose (that is, slightly lower than that proposed as the minimum dose for inclusion in Eurican L4, 150 FTU dose). It is noted that although a prevention of mortality

and clinical signs was not demonstrated in the corresponding Eurican L4 *L.* Icterohaemorrhagiae DOI study due to an assumed less severe challenge, in this study a beneficial effect of vaccination with respect to mortality / clinical signs was observed; all controls exhibited mild to severe clinical signs (dehydration, alteration of body condition, icterus or digestive disorders) for 1 to 8 days, 3 control animals were euthanised due to the severity of the clinical signs. In contrast, clinical signs or mortality was not reported in vaccinates.

***Leptospira* Grippotyphosa**

The primary vaccination with DAPPi2-Lmulti vaccine including a first injection at 7 to 9 weeks of age followed by a second injection 4 weeks later conferred a reduction of signs of disease, infection, renal carriage and urinary excretion against *L.* Grippotyphosa challenge about one year after the primary vaccination.

Efficacy of the booster injection

To demonstrate the immunity of the components of Eurican L4 following re-vaccination (annual booster) with a single dose, 12 months after completion of the primary vaccination course laboratory response-to-booster study in dogs was performed.

Ten 7-week-old SPF puppies were involved in the study. Eurican L4 was mixed with Eurican DAPPi and Rabisin was also administered concomitantly at the second vaccination of the primary vaccination and at the booster. To prove that a single dose of the vaccine is sufficient for the annual booster, the serological response of the vaccinated dogs was evaluated. No challenge was carried out, however the serology allowed to demonstrate a quick increase of leptospira antibodies as satisfactory as the one seen after a second primary course injection. The applicant concluded that the single annual re-vaccination was able to boost the immune response for all the four *Leptospira* components. While significant increase of the ELISA titre after the booster was observed in all dogs in case of *L*. Grippotyphosa and in 80% of the dogs in case of *L.* Australis, no increase was observed in any dogs in case of *L*. Canicola and only one animal seroconverted after booster in case of *L*. Icterohaemorrhagiae. The lack of increase of antibodies in these dogs was attributed to issues with the ELISA method. Therefore, the applicant used the MAT method to determine the increase of the titre, however due to technical reasons most of the sera prior to administration of the booster had a non-interpretable reading. Notwithstanding this, high, anti-*L*. Canicola and anti-*L*. Icterohaemorrhagiae titres were detected in 8 and 7 dogs, respectively, after booster.

The results of a previous study (2014) investigating the booster effect after Eurican DAPPi2-Lmulti vaccine are also shown as supportive data. This study is considered as relevant because both products have the same composition in active and inactive ingredients, excepting the additional *L.* Australis component in Eurican L4. Annual booster with Eurican DAPPi2-L3 vaccine was stated to be able to boost the immune response in dogs previously undergoing primary vaccination with the same vaccine, however this was based on the evaluation of serology only.

The applicant was requested to justify the approach used to support the efficacy of the booster dose. In their response, it was discussed that although leptospira antibodies are unlikely to be the only protective mechanism as they wane quickly, they are known to play a role in protection, and they are considered as good markers of vaccine uptake when seroconversion takes place. Furthermore, it was uncertain if a demonstration by challenge would provide more conclusive results than serology in this context, considering that control dogs at this age were more resistant to challenge.

It was accepted that although the efficacy of the booster dose was not demonstrated by challenge, the rationale for using the serological response as an indicator of the immune response to booster was justified, given that the susceptibility of adult dogs to challenge was shown to decrease in the

duration of immunity studies, and that conducting a challenge experiment to demonstrate the efficacy of the booster dose may not have yielded meaningful results, and should be avoided in this case taking into account the 3R's principles. In the case of immunity against leptospirosis, the antibody response may be variable, and it was shown that dogs may be protected in the absence of detectable antibody levels (e.g. in the dose finding study for *L*. Australis). Overall, it was shown in the studies provided in the dossier that the administration of a single booster dose resulted in seroconversion for each of the leptospira components, and whilst not a definitive marker of protection, serology can be accepted as a sufficient indicator of protection for the evaluation of the response to booster.

Maternally derived antibodies (MDA)

No dedicated studies were carried out to investigate the influence of maternally derived antibodies on the efficacy of the vaccine. The applicant refers to scientific publications and to the practical experience of over 30 years of using similar inactivated leptospirosis vaccines in the field (such as Eurican L or Eurican Lmulti). Based on these, they concluded that the interference of maternal antibodies with the efficacy of vaccination against leptospirosis was not an identified concern.

The data collected during laboratory trials shown in the dossier further confirm that maternal antibodies would wane before 7 weeks of age. A large number of conventional puppies were tested before vaccination for the presence of *Leptospira* antibodies by MAT and LPS-antigen ELISA assays. The puppies aged between 6 and 10 weeks at the time of testing were all negative for *Leptospira* antibodies.

As *Leptospira* maternal antibodies wane before 7 weeks of age, no specific precaution is required when vaccinating at this age puppies born from vaccinated bitches.

The approach of the applicant is acceptable. The vaccine can be used in puppies born from vaccinated bitches from 7 weeks of age without any specific precaution.

Additional studies

Compatibility with Eurican DAPPi (DAP) vaccine

Eurican L4 can be used as a solvent to reconstitute Boehringer Ingelheim vaccines containing canine distemper virus, canine adenovirus type 2 and canine parvovirus associated or not with canine parainfluenza virus type 2 (Eurican DAP and Eurican DAPPi vaccines). The mixed vaccine suspension is used to vaccinate dogs according to the same recommendations as Eurican L4 and Eurican DAPPi when used separately.

The applicant has performed different studies to demonstrate the compatibility of Eurican L4 with the Eurican DAPPi and Rabisin vaccines. These data also support the compatibility of Eurican L4 and Eurican DAP (referred to as DAP-L4) in accordance with the Note for guidance regarding combined veterinary vaccines (CVMP/IWP/52/97-Final) and the Guideline on the combined vaccines and associations (EMA/CVMP/IWP/594618/2010), as the DAPPi-L4 and DAP-L4 vaccine associations contain the same active and inactive ingredients, except that Eurican DAP does not contain a canine parainfluenza type 2 active ingredient.

The immediate efficacy and duration of immunity afforded by the association of Eurican Lmulti vaccine with Eurican DAPPi vaccine have been demonstrated by virulent challenges in accordance with Directive 2001/82/EC and with the relevant Ph. Eur. monographs. The study reports have already been provided in Eurican DAPPi-Lmulti vaccine dossier. These results are considered as relevant and supportive of the compatibility between Eurican DAPPi and Eurican L4 because the composition of Eurican L4 and Eurican

Lmulti are identical except for the addition of a new *L.* Australis component. Therefore, the applicant also submitted these study reports in the Eurican L4 dossier.

As the compatibility of *Leptospira* vaccine Eurican Lmulti with Eurican DAPPi is already well established by challenge, serology was used to confirm the compatibility of Eurican L4 with Eurican DAPPi in order to avoid unnecessary distress caused by challenge in experimental animals in agreement with 3Rs principles. This is in line with the guideline on combined vaccines and associations (EMA/CVMP/IWP/594618/2010) regarding the use of marker parameters to replace virulent challenge.

As there is no obvious and well-established correlation between clinical protection and serological response to vaccination for canine parainfluenza virus type 2, the efficacy of the association between Eurican L4 and Eurican DAPPi was confirmed by virulent challenge for this component.

Compatibility demonstrated by virulent challenge

Several studies were previously conducted during development of Eurican Lmulti combined with Eurican DAPPi to demonstrate by challenge the compatibility of both vaccines and these studies are considered as supportive for association of Eurican L4 with Eurican DAPPi.

Canine distemper virus

Two studies have demonstrated the immediate efficacy and duration of immunity of the live attenuated canine distemper active ingredient in Eurican DAPPi mixed with Eurican Lmulti. In both studies, the canine distemper component was set to the minimum viral titre (4.0 $\log_{10}CCID_{50}$/mL). The mixed vaccine suspension was administered by subcutaneous route according to recommendations: two injections at 4-week interval.

The objective of one fo the study was to assess the immediate efficacy of Eurican DAPPi2-Lmulti/R and DAPPi2-Lmulti vaccines in dogs when challenged with virulent canine distemper virus (CDV). Puppies without any anti-CDV antibodies were vaccinated at the age of 7 to 9 weeks with an injection of DAPPi2-Lmulti vaccine and again at the age of 11 to 13 weeks with an injection of DAPPi2-Lmulti or DAPPi2-Lmulti/R vaccine.

Fifteen 7- to 9-week old puppies, seronegative for CDV, were randomised into two vaccinated groups of 6 animals and one control group of 3 animals. The vaccinated animals received one dose of Eurican DAPPi2- Lmulti vaccine on D0 and one dose of DAPPi2-Lmulti or DAPPi2-Lmulti/R vaccine on D28. The 3 animals from the control group were not vaccinated. All animals were challenged by intravenous route with a virulent strain of CDV on D42.

Blood samples were regularly taken to monitor the antibody response against CDV.

A daily clinical examination including rectal temperature monitoring was carried out on all animals for 21 days post-challenge. Dogs were weighed just before the challenge and twice a week after the challenge.

The challenge was validated since all the control dogs had to be euthanised due to canine distemper severe signs on day 6 or 10 post-challenge.

All vaccinated dogs remained in good health with no severe clinical signs throughout the challenge phase. They also gained weight between the beginning and the end of the monitoring period.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine 4 weeks later conferred a good protection against canine distemper challenge two weeks after the primary vaccination.

The study is fully compliant with the requirements of the Ph.Eur. monograph 0448 - Canine distemper vaccine (live) - except for the challenge performed earlier (14 days instead of 21 days after the last injection of the vaccine), which represents a worst case for the assessment of the vaccine. The onset of immunity for the distemper strain to prevent mortality and clinical signs was thus set at 2 weeks.

The objective of the second study was to assess the 1-year duration of immunity of Eurican DAPPi2-Lmulti vaccine in dogs when challenged with virulent canine distemper virus (CDV). The puppies were vaccinated with 2 injections of Eurican DAPPi2-Lmulti vaccine (the first given at the age of 7 to 9 weeks and the second at the age of 11 to 13 weeks) then challenged about 1 year after the second injection.

Eleven 7- to 9-week old puppies were randomised into one vaccinated group of 7 animals and one control group of 4 animals. The vaccinated animals received one dose of Eurican DAPPi2-Lmulti vaccine on D0 and on D28. The animals from the control group were not vaccinated against distemper. All animals were challenged by intravenous route with a virulent CDV strain about 1 year after the second injection.

Blood samples were regularly taken to monitor the antibody response against CDV.

A daily clinical examination, including rectal temperature monitoring, was carried out on all animals for 21 days post-challenge. Dogs were weighed just before the challenge and twice a week after the challenge.

The challenge was validated since all control dogs showed typical signs of canine distemper (especially nervous signs) and had to be euthanised between days 6 and 9 post-challenge for ethical reasons.

All vaccinated dogs remained in good health with no clinical signs of distemper throughout the challenge phase.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of DAPPi2-Lmulti vaccine 4 weeks later conferred a good protection against distemper challenge about 1 year after the primary vaccination.

This study fully supports a duration of immunity of at least 1 year for the distemper component of the Eurican DAPPi2-Lmulti vaccine to prevent mortality and clinical signs caused by distemper virus.

Canine adenovirus type-1

Two studies have demonstrated the immediate efficacy and duration of immunity of the live attenuated canine adenovirus active ingredient in Eurican DAPPi mixed with Eurican Lmulti against canine contagious hepatitis.

In these studies, the canine adenovirus type 2 component was set to the minimum viral titre (2.5 $log_{10}CCID_{50}$/mL). The mixed vaccine suspension was administered by subcutaneous route according to recommendations: two injections at 4-week interval.

The objective of one of the study was to assess the immediate efficacy of Eurican DAPPi2-Lmulti/R and Eurican DAPPi2-Lmulti vaccines in dogs when challenged with virulent canine adenovirus type 1 (CAV1). Puppies were vaccinated at the age of 7 to 9 weeks with an injection of Eurican DAPPi2-Lmulti vaccine and again at the age of 11 to 13 weeks with an injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine. The challenge was carried out 2 weeks after the second vaccination.

Fifteen 7- to 9-week old SPF puppies were randomised into 2 vaccinated groups of 6 animals and one control group of 3 animals. The vaccinated animals received one dose of Eurican DAPPi2-Lmulti vaccine on D0 and one dose of Eurican DAPPi2-Lmulti/R (group A) or Eurican DAPPi2-Lmulti (group B) vaccine on D28. Animals from control group C were not vaccinated. Two weeks after the second vaccination, all animals were challenged by intravenous route with a virulent strain of CAV1.

Blood samples were regularly taken to monitor the antibody response against CAV.

A daily clinical examination, including rectal temperature monitoring, was performed on all animals for 21 days post-challenge. Dogs were weighed just before the challenge and once a week after the challenge.

The challenge was validated since all the control dogs died or had to be euthanised due to canine adenovirosis on day 3 or 4 post-challenge.

All vaccinated dogs remained in good health with no clinical signs of the disease after challenge.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine 4 weeks later conferred a good protection against a canine adenovirus type 1 challenge two weeks after vaccination.

The study was fully compliant with the Ph. Eur. monograph 1951 (Canine adenovirus vaccine (live)), except for the challenge performed earlier (14 days instead of 21 days after the last injection of the vaccine), which represents a worst case for the assessment of the vaccine. The onset of immunity for the CAV strain to prevent mortality and clinical signs linked to canine contagious hepatitis was thus set at 2 weeks.

The objective of the second study was to assess the 1-year duration of immunity of Eurican DAPPi2-Lmulti vaccine in dogs when challenged with virulent canine adenovirus type 1 virus (CAV1 - canine contagious hepatitis). The puppies were subcutaneously vaccinated at the age of 7 to 9 weeks with an injection of Eurican DAPPi2-Lmulti vaccine and 4 weeks apart with a second injection of Eurican DAPPi2-Lmulti vaccine. The challenge was carried out about 1 year after the second injection.

Eleven 7- to 9-week old puppies were randomly allocated to one vaccinated group of 7 animals and one control group of 4 animals. The vaccinated animals received one dose of Eurican DAPPi2-Lmulti vaccine on D0 and on D28. The animals from the control group were not vaccinated against CAV. All animals were challenged by intravenous route with a virulent CAV1 strain about 1 year after the second injection.

Blood samples were regularly taken to monitor the antibody response against CAV.

A daily clinical examination, including rectal temperature monitoring, was carried out on all animals for 21 days post-challenge. Dogs were weighed just before the challenge and once a week after the challenge.

The challenge was validated since all control dogs died from canine contagious hepatitis on day 2 post-challenge.

All vaccinated dogs remained in good health with no clinical signs of canine contagious hepatitis throughout the challenge phase.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of DAPPi2-Lmulti vaccine 4 weeks later confers a good protection against CAV1 challenge about 1 year after the primary vaccination.

This study fully supports a duration of immunity of at least 1 year for the canine adenovirus component of the Eurican DAPPi2-Lmulti vaccine to prevent mortality and clinical signs linked to canine contagious hepatitis.

Canine adenovirus type-2

Two studies have demonstrated the immediate efficacy and duration of immunity of the live attenuated canine adenovirus active ingredient in Eurican DAPPi mixed with Eurican Lmulti against canine infectious laryngotracheitis.

In these studies, the canine adenovirus type 2 component was set to the minimum viral titre (2.5 $log_{10}CCID_{50}$/mL). The mixed vaccine suspension was administered by subcutaneous route according to recommendations: two injections at 4-week interval.

The objective of one of the study was to assess the immediate efficacy of Eurican DAPPi2-Lmulti vaccine in puppies when challenged with virulent canine adenovirus type 2 (CAV2). The puppies were subcutaneously vaccinated at the age of 7 to 9 weeks with an injection of Eurican DAPPi2-Lmulti vaccine and again at the age of 11 to 13 weeks with another injection of Eurican DAPPi2-Lmulti vaccine. The challenge was carried 2 weeks after the second vaccination.

Twenty-two 7- to 9-week old puppies, seronegative for CAV2, were randomised into two groups of 11 animals. All the animals from one group were vaccinated and all the animals from the other group remained unvaccinated and served as controls. Animals from both groups were challenged by intranasal and intratracheal route with a virulent strain of CAV2.

A daily clinical examination, including rectal temperature monitoring, was carried out on all animals for 10 days post-challenge. Dogs were weighed just before the challenge and at the end of the challenge monitoring period. Nasal swabs for CAV2 isolation were taken prior to the challenge and daily from day 2 to day 10 post-challenge. In addition, a blood sample was taken approximately every 2 weeks to monitor the antibody response against CAV.

The challenge was validated since all unvaccinated control dogs excreted the challenge virus and some of them showed clinical signs.

All vaccinated dogs remained in good health with no clinical signs throughout the post-challenge monitoring period. In comparison, three unvaccinated control dogs showed serous nasal discharge or cough. No loss of weight was observed in any of the groups. A slight hyperthermia was only observed in 2 control animals at one or two occasions. Despite the trend, the global clinical score was not significantly different between both groups.

Few viruses were detected in vaccinates following challenge, whereas virus excretion was high and lasted for more than 6 days in controls: this difference was highly significant.

Regarding the clinical signs, it is underlined that there are difficulties in inducing consistent and serious clinical signs during laboratory challenge with CAV2. This is linked to the pathogenicity of the virus, which is one of the causes of the infectious tracheobronchitis in dogs, also known as kennel cough. This disease is multifactorial and results from the combination of several aetiological agents which increase the severity of the infection.

Thus, reproducing the infection of this multifactorial disease in laboratory is difficult when using only one of the agents. Clinical signs are then limited in severity as there are no secondary infections such as *Bordetella* or canine parainfluenza virus and consequently no complications can occur. Nevertheless, laboratory experimental conditions developed by the applicant were able to reproduce a CAV2 subclinical infection with some mild clinical signs and a consistent virus excretion, allowing

the analysis of the onset and duration of immunity associated to the vaccine. Obtaining a statistical difference in clinical signs would have required a significantly higher number of dogs.

In this trial, no vaccinated dogs showed clinical signs, whereas three control dogs presented serous nasal discharge or slight cough on one or two occasions. It is acknowledged that the statistical analysis does not show a significant difference between the vaccinated and control groups with regard to the clinical signs in this study for the reasons detailed above, but the trend observed between both groups is considered as fully biologically significant, as vaccinated dogs were fully protected.

The primary vaccination program including a first injection of DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti vaccine 4 weeks later conferred a good protection against clinical disease and virus shedding in CAV2 challenge two weeks after vaccination.

The study was fully compliant with Ph. Eur. monograph 1951, except for the challenge performed earlier (14 days instead of 21 days after the last injection of the vaccine), which represents a worst case for the assessment of the vaccine. The onset of immunity for the CAV strain to reduce clinical signs and reduce viral excretion during respiratory disease caused by type 2 canine adenovirus was thus set at 2 weeks.

The objective of the the second study was to assess the 1-year duration of immunity of DAPPi2-Lmulti vaccine in dogs when challenged with virulent canine adenovirus type 2 (CAV2). The puppies were vaccinated at the age of 7 to 9 weeks with an injection of Eurican DAPPi2-Lmulti vaccine and 4 weeks apart with a second injection of Eurican DAPPi2-Lmulti vaccine. The challenge was carried out about 1 year after the second injection.

Twenty-three 7- to 9-week old SPF puppies were allocated into one group of 12 animals vaccinated with Eurican DAPPi2-Lmulti vaccine and one group of 11 animals as unvaccinated controls. Animals from both groups were challenged by intranasal and intratracheal route with a virulent strain of CAV2.

A daily clinical examination, including rectal temperature monitoring, was carried out on all animals for 10 days post-challenge. Dogs were weighed just before the challenge and on 3, 6 and 10 days after the challenge. Nasal swabs for CAV2 isolation were taken prior to the challenge and from day 2 to day 10 post-challenge, to determine nasal virus shedding. In addition, a blood sample was taken prior to the challenge and at the end of the challenge to monitor the antibody response against CAV.

The challenge was validated since all the control dogs excreted the virus nasally.

All vaccinated dogs remained in good health with no clinical signs throughout all of the challenge phase (except one dog which had a serious ocular discharge for 3 days). By comparison, 2 control dogs had nasal discharge and one of them had a purulent nasal discharge. No significant difference in clinical signs was underlined between control and vaccinated groups. However, during a CAV2 challenge in adult dogs, few clinical symptoms are expected.

Virus shedding was significantly reduced in vaccinates when compared to controls that had a higher and longer shedding. It is important to note that 2 vaccinates did not excrete any virus throughout the whole of post-challenge monitoring.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age, followed by a second injection of Eurican DAPPi2-Lmulti vaccine 4 weeks later conferred a significant reduction of virus excretion in a CAV2 challenge about 1 year after the primary vaccination.

Canine parvovirus

Three studies have demonstrated the immediate efficacy and duration of immunity of the live attenuated canine parvovirus active ingredient in Eurican DAPPi mixed with Eurican Lmulti.

In these studies, the canine parvovirus component was set to the minimum viral titre (4.9 $log_{10}CCID_{50}$/mL). The mixed vaccine suspension was administered by subcutaneous route according to recommendations: two injections at 4-week interval.

The objective of one of the study was to assess the immediate efficacy of a single injection of Eurican DAPPi2-Lmulti/R or Eurican DAPPi2-Lmulti vaccines in dogs when challenged with virulent canine parvovirus (CPV). The puppies were vaccinated at the age of 7 to 9 weeks, then challenged 2 weeks after.

Twelve 7- to 9-week old SPF puppies were randomised into 2 groups of 5 animals (treated groups) and one group of 2 animals (control group). On D0, the animals from the treated groups received one dose of Eurican DAPPi2-Lmulti/R or Eurican DAPPi2-Lmulti vaccine, respectively. The 2 animals from control group were not vaccinated. All animals were challenged by oronasal route with a virulent CPV strain on D14.

Blood sample was taken to monitor the antibody response against CPV (before vaccination, before challenge and on the day of euthanasia after challenge).

A daily clinical examination, including rectal temperature monitoring, was carried out on all animals for 14 days post-challenge. Dogs were weighed just before the challenge and twice a week after the challenge. Rectal swabs and blood sample on EDTA tubes were regularly taken after challenge to monitor CPV excretion in faeces and leucocytes count, respectively.

The challenge was validated since the 2 control dogs showed typical signs of canine parvovirosis, leucopenia and CPV excretion in faeces (one of them had to be euthanised on day 5 post-challenge for ethical reasons). Histopathological examination of thymus (both animals) and intestines (only the dog euthanised on day 5 post-challenge) showed lesions that confirmed parvovirosis in control animals.

All vaccinated dogs remained in good health with no clinical signs of parvovirosis and no parvovirus excretion in faeces throughout the challenge phase. They also gained weight throughout the monitoring period.

Histopathological examination of thymus (sampled at the end of monitoring) was normal in all vaccinated animals.

The single injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine at 7 to 9 weeks of age conferred a good protection against a parvovirus challenge two weeks after one single vaccination.

The objective of the second study was to assess the immediate efficacy of Eurican DAPPi2-Lmulti/R and DAPPi2-Lmulti vaccines in conventional dogs when challenged with virulent canine parvovirus virus (CPV). The puppies were vaccinated at the age of 7 to 9 weeks with an injection of Eurican DAPPi2-Lmulti vaccine and again at the age of 11 to 13 weeks with an injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine. The challenge was carried out 2 weeks after the second injection.

Fifteen 7- to 9-week old puppies were randomised into 2 vaccinated groups of 6 animals and one control group of 3 animals. The vaccinated animals received one dose of DAPPi2-Lmulti vaccine on D0 and one dose of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine on D28. The 3 animals

from the control group were not vaccinated. All animals were challenged by oronasal route with a virulent CPV strain on D42.

Blood samples were regularly taken to monitor the antibody response against CPV.

A daily clinical examination including rectal temperature monitoring was carried out on all animals for 14 days post-challenge. Dogs were weighed just before the challenge and twice a week after the challenge. Rectal swabs and blood sample were regularly taken after challenge to monitor CPV excretion in faeces and leucocytes count, respectively.

The challenge was validated since all the control dogs had to be euthanised or died due to canine parvovirosis on day 6 post-challenge.

All vaccinated dogs, except one, remained in good health, with no clinical signs of parvovirosis and no parvovirus excretion in faeces throughout the challenge phase. They also gained weight between the beginning and the end of the monitoring period. One vaccinated dog died on day 6 post-challenge due to parvovirosis.

This dog was a non-responder that remained seronegative after both vaccine injections.

Eight out of the 11 surviving vaccinates had maternally derived antibodies at the time of first vaccine injection but, after completion of the 2nd vaccine injection, all animals seroconverted and were protected against virulent challenge.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti or Eurican DAPPi2-Lmulti/R vaccine 4 weeks later confers a good protection against parvovirus challenge two weeks after the primary vaccination, even in dogs with maternally derived antibodies at the time of the 1st injection. It has to be noted that one non-responder dog, which is an unexpected and rare event regarding anti-CPV vaccination, was not protected.

The objective of the last study was to assess the 1-year duration of immunity of DAPPi2-Lmulti vaccine in dogs when challenged with virulent canine parvovirus (CPV). The puppies were vaccinated with 2 injections of DAPPi2-Lmulti vaccine (the first given at the age of 7 to 9 weeks and the second at the age of 11 to 13 weeks) then challenged about 1 year after the second injection.

Eleven 7- to 9-week old puppies were randomised into one vaccinated group of 7 animals and one control group of 4 animals. The vaccinated animals received one dose of Eurican DAPPi2-Lmulti vaccine on D0 and on D28. The animals from the control group were not vaccinated. All animals were challenged by oronasal route with a virulent CPV strain about 1 year after the second injection.

Blood samples were regularly taken to monitor the antibody response against CPV.

A daily clinical examination including rectal temperature monitoring was carried out on all animals for 14 days post-challenge. Dogs were weighed just before the challenge and twice a week after the challenge.

Rectal swabs and blood sample on EDTA tubes were regularly taken after challenge to monitor CPV excretion in faeces and leucocytes count, respectively.

The challenge was validated since all control dogs showed typical signs of canine parvovirosis and CPV excretion in faeces. One of them was found dead on day 5 post-challenge and another one had to be euthanised on day 7 post-challenge for ethical reasons. Histopathological examination of intestines and mesenteric lymph nodes (both animals) and thymus (only the dog euthanised on day 7 post-challenge) showed lesions that confirmed parvovirosis.

All vaccinated dogs remained in good health with no clinical signs of parvovirosis and no parvovirus excretion in faeces throughout the challenge phase.

Four out of the 7 vaccinates had maternally derived antibodies at the time of first vaccine injection but, after completion of the 2nd vaccine injection, all animals seroconverted and were protected against virulent challenge.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti vaccine 4 weeks later confers a good protection against parvovirus type 2b challenge about 1 year after the primary vaccination, even in puppies with maternally derived antibodies at the time of the 1st injection.

This study supports a duration of immunity of at least 1 year for the parvovirus component of the Eurican DAPPi2-Lmulti vaccine to prevent mortality, clinical signs and viral excretion caused by parvovirus.

Canine parainfluenza virus type-2

Two studies demonstrate the immediate efficacy and duration of immunity of the live attenuated canine parainfluenza type 2 active ingredient in Eurican DAPPi mixed with Eurican Lmulti.

An additional study was carried out to demonstrate the immediate efficacy against challenge of this component when mixing Eurican DAPPi and Eurican L4. This study was carried out because unlike core viral components (CDV, CAV, CPV), there is no strongly established connection between antibody response and protection for canine parainfluenza type 2.

In these studies, the canine parainfluenza type 2 component is set to the minimum viral titre (4.7 $log_{10}CCID_{50}$/mL) or a lesser titre. The mixed vaccine suspension is administered by subcutaneous route according to recommendations: two injections at 4-week interval.

The objective of the first study was to assess the immediate efficacy of Eurican DAPPi-L4 vaccine against a virulent canine parainfluenza virus (CPiV) challenge. Puppies aged between 7 and 8 weeks received two injections of vaccine 4 weeks apart. They were then challenged two weeks after the second injection of vaccine.

Fifteen SPF Beagle puppies, aged between 6 and 8 weeks, were randomly allocated to one group of 10 vaccinated animals and one group of 5 control animals. Animals of vaccinated group were vaccinated with one dose of Eurican DAPPi-L4 on D0 and D28, and one dose of Rabisin on D28 in a site different from that of Eurican DAPPi-L4. Controls were not vaccinated. Two weeks after the second vaccine injection, all animals were challenged with a virulent CPiV suspension by intranasal / intratracheal route.

Following challenge, all animals were monitored daily for CPiV clinical signs and nasal swabs were collected daily from day 2 to day 10 post-challenge to monitor CPiV shedding.

All animals remained healthy during the vaccine phase. All vaccinates seroconverted during the vaccine phase with all dogs with a titre ≥ 2.55 $log_{10}OD_{50}$ just before challenge (mean titre > 3.31 $log_{10}OD_{50}$).

The challenge was validated since all controls showed CPiV shedding in nasal swabs for 6 days during the challenge period. No controls showed clinical signs after challenge. All vaccinates remained in good general condition without any clinical sign except rare cough once for 2 vaccinates during the post-challenge study course. As no clinical sign was observed in controls, no protection against the disease could be demonstrated in the study.

Virus shedding was significantly different between groups, with a lower and shorter excretion in vaccinates (virus only detected in 4 of the 9 dogs until day 6 post challenge) compared to controls (virus detected in all controls until day 7 post challenge). Vaccination provided strong protection against virus shedding. It is considered that the data presented are in accordance with the immunogenicity requirements of Ph. Eur. 1955 (Canine parainfluenza virus vaccine (live)), given that the test was valid and that the study complied with the requirement that a statistically significant difference in viral excretion (reduction) was demonstrated in the vaccinated group compared to the control group. Therefore, it is accepted that at OOI there is no adverse impact on the response to CPiV vaccination when Eurican DAPPi is administered mixed with Eurican L4.

The primary vaccination program with Eurican DAPPi2 mixed with Eurican L4 vaccine induced the seroconversion of all vaccinated animals and conferred a strong protection against virus shedding in CPiV challenge 2 weeks after the second vaccination. This is consistent with prior studies evaluating the protection afforded in dogs vaccinated with Eurican DAPPi2, and Eurican DAPPi2 mixed with Eurican Lmulti vaccine, as detailed below.

The objective of the second study was to assess the immediate efficacy of Eurican DAPPi2-Lmulti vaccine in puppies when challenged with virulent canine parainfluenza virus type 2 (CPiV2). The puppies were subcutaneously vaccinated at the age of 7 to 9 weeks with an injection of Eurican DAPPi2-Lmulti vaccine and 4 weeks apart with a second injection of Eurican DAPPi2-Lmulti vaccine. The challenge was carried out 2 weeks after the second vaccination.

Twenty-two 7- to 9-week old SPF puppies were randomised into two groups of 11 animals. All the animals from one group were vaccinated and all the animals from the other group remained unvaccinated and served as controls. Animals from both groups were challenged by intranasal and intratracheal route with a virulent strain of CPiV2.

A daily clinical examination, including rectal temperature monitoring, was carried out on all animals before vaccinations, before the challenge and for 14 days post-challenge. Dogs were weighed before vaccinations, before challenge and at the end of the challenge monitoring period. Nasal swabs for CPiV2 isolation were taken prior to the challenge and daily from day 2 to day 10 post-challenge.

In addition, a blood sample was taken approximately every 2 weeks to monitor the antibody response against CPiV2.

The challenge was validated since all the control dogs nasally excreted virus. In 5 of these animals, excretion was associated with clinical signs such as ocular discharge and cough.

All dogs were seronegative to canine parainfluenza virus at the beginning of the animal phase. After the first vaccination, all vaccinates except 2 dogs developed antibodies. About 2 weeks after the second vaccination, all vaccinates except one dog had a high level of anti-CPiV2 antibodies. Antibody titre rose after the challenge in all vaccinates. The controls remained seronegative until the challenge and developed antibodies against canine parainfluenza virus after inoculation of the challenge suspension.

All vaccinated dogs remained in good health with no clinical signs throughout all of the challenge phase. In comparison, 3 controls presented serous ocular discharge or slight cough on 1 to 3 occasions and 2 controls had a slight hyperthermia on one occasion. Like CAV2, this disease is multifactorial and results from the combination of several aetiological agents which increase the severity of the infection. No significant difference in clinical signs was underlined between controls and vaccinates. However, again, important fact is that no vaccinates exhibited any clinical signs: vaccination protected against clinical signs of CPi2.

Virus excretion was significantly different between groups, with a lower and shorter excretion in vaccinates compared to controls.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 7 to 9 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti vaccine 4 weeks later conferred a good protection against clinical disease and virus shedding in CPiV2 challenge 2 weeks after the second vaccination.

The objective of the last study was to assess the 1-year duration of immunity of Eurican DAPPi2-Lmulti in puppies when challenged with virulent canine parainfluenza virus type 2 (CPiV2). The puppies were vaccinated at the age of 8 weeks with an injection of Eurican DAPPi2-Lmulti vaccine and 4 weeks apart with a second injection of Eurican DAPPi2-Lmulti vaccine. The challenge was carried out about 1 year after the second injection.

Twenty-one 7- to 9-week old SPF puppies were allocated into one group of 10 animals vaccinated with Eurican DAPPi2-Lmulti vaccine and one group of 11 animals as unvaccinated controls. Animals from both groups were challenged by intranasal and intra-tracheal route with a virulent strain of CPiV2.

A daily clinical examination including rectal temperature monitoring was carried out on all animals for 14 days post-challenge. Dogs were weighed just before the challenge and on day 14 post-challenge. Nasal swabs for CPiV2 isolation were taken prior to the challenge and from day 2 to day 10 post-challenge to determine nasal virus shedding. In addition, a blood sample was taken prior to the challenge and at the end of the challenge to monitor the antibody response against CPiV2.

The challenge was validated since all the control dogs excreted the virus nasally.

All vaccinated dogs remained in good health with no clinical signs throughout all of the challenge phase (except one dog which had cough/sneeze on one day). By comparison, 4 control dogs had a purulent ocular discharge up to 4 days and one of these animals also had cough/sneeze for 3 days. No clear significant difference (only a trend) in clinical signs was underlined between control and vaccinated groups. However, during a CPiV2 challenge in adult dogs, few clinical symptoms are expected.

Virus shedding was significantly reduced (intensity and duration) in vaccinates when compared to controls.

The primary vaccination program including a first injection of Eurican DAPPi2-Lmulti vaccine at 8 weeks of age followed by a second injection of Eurican DAPPi2-Lmulti vaccine 4 weeks later conferred a significant reduction of virus excretion in a CPiV2 challenge about 1 year after the primary vaccination.

Compatibility demonstrated by serology

There is a well-established correlation between canine distemper virus and canine adenovirus seroneutralising antibodies, and canine parvovirus haemagglutination inhibiting antibodies (HIA) with the level of protection afforded by attenuated live vaccines containing these components.

This correlation has been demonstrated in dogs vaccinated with the associated vaccine Eurican DAPPi- Lmulti and subjected to virulent challenge, already included in Eurican DAPPi-Lmulti dossier. For the titration of antibodies against CPV, an ELISA method was used instead of the HIA test. This method detects functional seroneutralising antibodies targeting the VP2 protein and showcases the same correlation with protection as the detection of HIA.

The antibody titres recorded in vaccinated dogs prior to virulent challenge from these studies were used to define a minimum protective threshold as the lowest antibody titre ensuring complete protection against mortality and/or clinical signs after challenge. It must be noted that this approach allowed to define a "lowest antibody titre observed in protected animals", rather than a "minimum protection threshold" in the sense that vaccinated animals with lower antibody levels may be protected but were not observed in these studies.

These lowest antibody titres observed in protected animals before challenge are summarised in the table below, indicating the reference of the study they are based on. The lowest titres observed in protected dogs across studies are chosen as the reference value to compare the antibody response to.

Antibodies against (unit)	**Study No.**	**Antibody assay**	**Lowest antibody titre observed in protected animals** (log10SN50)
CDV (log10SN50)	09.0470.R (OOI) 10.0125.R (DOI)	Virus neutralisation	1.20 (OOI) 1.37 (DOI)
CAV (log10SN50)	10.0021.R (OOI) 08.0246.R (DOI)	Virus neutralisation	2.56 (OOI) 3.20 (DOI)
CPV (log10OD50)	09.0521.R (OOI) 09.0471.R (DOI)	ELISA	3.95 (OOI) 4.85 (DOI)

These serological thresholds were defined based on protection afforded by the association of Eurican DAPPi and Eurican Lmulti vaccine, but they are considered as relevant to evaluate the protection of the association between Eurican DAPPi and Eurican L4 vaccines because:

- The antibody responses against these live viral components are considered as good correlates of protection, independent on the vaccine environment they are presented in,
- Eurican Lmulti and Eurican L4 have the same composition excepted the addition of *L.* Australis active ingredient, manufactured according to a similar process to the other *Leptospira* components in the vaccine. In view of such relatedness, it is considered acceptable to apply the reference serological values established for Eurican DAPPi-Lmulti to Eurican DAPPi-L4 as well.

Regarding canine parainfluenza type 2 virus, although a significant and robust seroconversion is observed after vaccination, the correlation between seroneutralising antibodies and protection is not well established. Consequently, a serological threshold to achieve protection is not proposed, and the compatibility of the Eurican DAPPi-L4 association was demonstrated by virulent challenge for this component. Nevertheless, the antibody titres against canine parainfluenza virus type 2 can be used as a marker of immunogenicity (or surrogate, according to the definition of Plotkin, 2008). As such, the canine parainfluenza antibody response is relevant and can be used to compare the outcome of vaccination in different settings. In the context of this documentation, such comparison can be made between the responses in laboratory trials and field trials, or between different vaccine associations containing the same canine parainfluenza active ingredient. This approach was used to confirm the duration of immunity of canine parainfluenza virus in the associated Eurican DAPPi-L4 vaccine.

Two studies were carried out to demonstrate the efficacy of Eurican DAPPi by serology when mixed with Eurican L4. Vaccination was performed as recommended (2 subcutaneous injections at 4-week interval), using batches of Eurican DAPPi vaccine containing the minimum viral titre for each component evaluated in the study (or close to the minimum for canine parainfluenza virus type 2). Eurican L4 vaccine contained the minimum amount of each *Leptospira* antigen.

The aim of one of the studies was to assess the compatibility between Eurican DAPPi, Eurican L4 and Rabisin. Assessment was based on serological results against viral components (CAV2, CPV, CPiV and rabies virus) through one year post primary vaccination.

Forty SPF puppies aged between 7 and 9 weeks were randomly allocated to 4 groups of 10 animals each and vaccinated according to the table:

Group	Vaccination on D0	Vaccination on D28	Clinical examination/rectal temperature/weighting	Blood sampling on dry tubes for serology
A (9 dogs)	Eurican DAPPi-L4	Eurican DAPPi-L4 + Rabisin	D0, D28, D42, D56, D112, D202, D290, D395	D0, D28, D42, D56/D57, D112, D202, D290, D395
B (10 dogs)	Eurican DAPPi	Eurican DAPPi + Rabisin		
C (10 dogs)	Eurican DAPPi	Eurican DAPPi		
D (10 dogs)	-	Rabisin		

Mean serological response to the CAV, CPV and CPiV components of Eurican DAPPi-L4 vaccine was similar between dogs vaccinated concomitantly with Eurican DAPPi-L4 and Rabisin (full combination), and dogs vaccinated with Eurican DAPPi vaccine associated to Rabisin or injected alone.

At peak of serological response (two to four weeks after primary vaccination), serological response against the viral components of Eurican DAPPi-L4 vaccine (CAV, CPV and CPiV) was proven to be statistically non-inferior in dogs vaccinated with Eurican DAPPi-L4 and Rabisin when compared to that in dogs vaccinated with Eurican DAPPi vaccine associated or not to Rabisin.

One year after the primary vaccination, even if serological response against the viral components of Eurican DAPPi-L4 vaccine (CAV, CPV and CPiV) was not proven to be statistically non-inferior in dogs vaccinated with Eurican DAPPi-L4 and Rabisin when compared to that in dogs in the two other groups, titres for all antigens were sufficiently high in all animals to assess the one-year duration of immunity.

Compatibility between Eurican DAPPi-L4 vaccine and Rabisin when injected in two different sites was fully demonstrated regarding serological response against CAV, CPV and CPiV components of the two vaccines.

The aim of the other study was to assess the compatibility between Eurican DAPPi-L4 vaccine and Rabisin when injected in two different sites. Assessment was based on serological results against canine distemper virus (CDV) through one-year post-primary vaccination.

Fourteen conventional puppies aged between 7 and 9 weeks were used in this study. They were randomly allocated to 2 groups of 7 animals each and vaccinated as indicated below.

Group	Vaccination on D0	Vaccination on D28	Clinical examination/rectal temperature/weighting	Blood sampling on dry tubes for serology
A (7 dogs)	Eurican DAPPi-L4	Eurican DAPPi-L4 + Rabisin	D0, D28, D42, D56, D86, D112, D142, D171, D203, D232, D262, D291, D319, D352, D380	D0, D28, D42, D56, D112, D202, D292, D395
B (7 dogs)	Eurican DAPPi	Eurican DAPPi		

All dogs remained in good general condition throughout the study.

Serological response to the CDV component of Eurican DAPPi-L4 vaccine was similar between dogs vaccinated with Eurican DAPPi vaccine only and dogs vaccinated with Eurican DAPPi-L4 vaccine and Rabisin. At every sampling time after injection (from response peak to one year), mean titre was

inferior in dogs vaccinated with Eurican DAPPi-L4 vaccine and Rabisin when compared to dogs vaccinated with Eurican DAPPi vaccine only. However, the mean titre difference between groups was limited, ranging between 0.07 and 0.46 $\log_{10}SN_{50}$ which is less than one dilution step in the antibody titration method. However, statistical significance could not be achieved for non-inferiority although the difference between mean titres of both groups was not higher than the acceptable threshold (0.3 $\log_{10}SN_{50}$, i.e. the minimal dilution step) throughout the study except at one year (0.46).

All individual titres were above the reference antibody titre previously defined (1.20 $\log_{10}SN_{50}$) at response peak (between 14 and 28 days after primary vaccination) which underlines a satisfactory vaccine intake whatever the group.

At one-year post primary vaccination, minimum titre and maximum loss of titre were the same in both groups. All dogs but one were still above 1.20 $\log_{10}SN_{50}$ (one dog titre was 1.12 $\log_{10}SN_{50}$). Therefore, the serological titres at one year were sufficient in dogs vaccinated with Eurican DAPPi-L4 vaccine and Rabisin to support the duration of immunity of CDV component.

Compatibility between Eurican DAPPi-L4 vaccine and Rabisin when injected in 2 different sites was demonstrated regarding serological response against CDV component of Eurican DAPPi vaccine.

The two previous comparative serological studies showed that the association of Eurican L4 with Eurican DAPPi has no significant impact on the profile of the antibody response against canine distemper virus, canine adenovirus and canine parvovirus. Despite not always being able to demonstrate a non-inferiority of the Eurican DAPPi-L4 vaccine response compared to the Eurican DAPPI vaccine response, the mean titres were very similar between groups.

Importantly, when comparing the individual antibody levels in vaccinated dogs to the titres known to afford clinical protection against challenge, all the dogs had greater titres around the peak response and greater or close to it at one year after the second vaccine injection. For canine parainfluenza virus, the immediate efficacy of the Eurican DAPPi-L4 associated vaccine was confirmed by challenge. In addition, the antibody kinetics in dogs vaccinated with the associated Eurican DAPPI-L4 vaccine was remarkably similar to that in dogs vaccinated with Eurican DAPPi (non-inferior at the peak, and with a trend towards statistical non-inferiority at one year).

Compatibility with Rabisin vaccine (efficacy against rabies virus)

Eurican L4 can be injected at the same time as (not mixed with) Boehringer Ingelheim inactivated and adjuvanted vaccine against rabies (Rabisin) from the age of 12 weeks, which is the minimum age to vaccinate dogs with Rabisin. To evaluate the efficacy of Rabisin, the applicant used serology and other biological markers to demonstrate the compatibility of Eurican L4 with Rabisin in order to avoid unnecessary suffering caused by rabies challenge in experimental animals (in accordance with 3Rs principles). The rabies virus neutralising antibodies were determined and compared between dogs receiving the association (Eurican DAPPi-L4 and Rabisin) and dogs injected with Rabisin alone. Comparison was performed at the peak of the antibody response and at around the date when a booster would be performed (i.e. around one year after the primary course). To complement this analysis, several other biological markers of the humoral and cell-mediated immune response were also evaluated, which had been identified in prior work as relevant to evaluate the response to vaccination with Rabisin (Chapat, 2017). The design choice was also made knowing that:

- The compatibility between Eurican Lmulti and Rabisin has already been demonstrated using this approach and the possibility to inject both at the same time (and separate sites) is included in the SPC of each product,
- Eurican Lmulti and Eurican L4 have the same composition except for the additional *L.* Australis active ingredient in Eurican L4.

The approach is in accordance with the guideline on combined vaccines and associations (EMA/CVMP/IWP/594618/2010) regarding the use of marker parameters as a substitute for virulent challenge. Indeed, the rabies virus neutralising (VN) antibodies response is a well-established correlate for the protection afforded by inactivated rabies vaccines. It is also notable that VN antibodies are acceptable for evaluation of the efficacy of rabies vaccines in species other than cats and dogs, according to Ph. Eur. 0451 - Rabies vaccine (inactivated). Finally, similar studies (serology and biological markers) were previously carried out to demonstrate the efficacy of the association between Eurican Lmulti and Rabisin and have been evaluated during the marketing authorisation procedure of Eurican Lmulti. They are considered relevant because Eurican L4 and Eurican Lmulti have the same composition except for the additional *L.* Australis active ingredient.

The aim of one of the studies was to assess the compatibility between Eurican DAPPi, L4 and Rabisin vaccines.

The study design and the result related to CAV, CPV and CPiV components have already been introduced (see the paragraph "Compatibility with Eurican DAPPi vaccine").

The mean serological response against rabies in dogs vaccinated with Eurican DAPPi-L4 and Rabisin had a similar profile to that in dogs vaccinated with Eurican DAPPi vaccine associated to Rabisin or Rabisin alone but with lower values, mainly due to the presence of two dogs with low serological titres. At response peak, even if non-inferiority of anti-rabies serological response in dogs vaccinated with Eurican DAPPi-L4 and Rabisin was not statistically demonstrated when compared to response in dogs vaccinated with Rabisin associated or not to Eurican DAPPi vaccine, all animals remained above the protective threshold of 0.5 IU/mL. This means that dogs vaccinated with Eurican DAPPi-L4 and Rabisin had a strong priming of the immune system and were considered to be protected at that time.

One year after the primary vaccination, non-inferiority of serological rabies response in dogs vaccinated with full combination was also not statistically demonstrated when compared to the response in the two other groups. Seven of the 9 animals vaccinated with full combination had a rabies titre above the protective threshold of 0.5 IU/mL. This means that these dogs were considered to be protected at that time. Two dogs had a titre lower than 0.5 IU/mL but were nevertheless seropositive (0.17 and 0.22 IU/mL). It must be noted that a titre below 0.5 IU/mL one year after the vaccination with Rabisin is not unusual even when Rabisin is injected alone.

Compatibility between Eurican DAPPi-L4 vaccine and Rabisin when injected in two different sites was fully demonstrated regarding serological response against rabies components of the two vaccines.

The objective of the other study was to assess, through immune fingerprint (IFP) approach, the compatibility between Eurican DAPPi-L4 and Rabisin when injected at two different injection sites in puppies, focusing particularly on the possible interference of Eurican DAPPi-L4 on Rabisin vaccine efficacy. The analysis of the immune response specific to rabies antigen was performed with the final goal to assess any impact of the concomitant injection of the other antigens on the efficacy of Rabisin vaccines through the immune fingerprint approach.

As such, the immune responses elicited by rabies vaccine were followed after Rabisin injection with or without Eurican DAPPi or Eurican DAPPi-L4 vaccination 28 days before.

From D28 onwards, several humoral and cellular parameters (rabies antibodies by VNT, total IgG and IgG subclasses, memory B cells, plasma cells, cytokines profile and IFNγ-secreting cells) were monitored at different time points after Rabisin vaccination.

For this evaluation, four groups (A, B, C, D) of 10 dogs in each group, were vaccinated at D0 and D28 according to the combinations described in the table below.

Group	Vaccination on D0	Vaccination on D28	Blood sampling	
			Sera	Whole blood on sodium heparin
A (9 dogs)	Eurican DAPPi-L4	Eurican DAPPi-L4 + Rabisin	D0, D28, D42, D56	D31, D35, D42, D56
B (10 dogs)	Eurican DAPPi	Eurican DAPPi + Rabisin		
C (10 dogs)	Eurican DAPPi	Eurican DAPPi		
D (10 dogs)	-	Rabisin		

A whole set of humoral and cellular immune parameters was quantified in each vaccinated group (specific anti-rabies antibody responses, rabies-specific T-lymphocyte responses detected upon *ex vivo* re-stimulated canine peripheral blood mononuclear cells (PBMCs) and rabies-specific B-lymphocyte response monitoring).

The Eurican DAPPi vaccine (group C) served as negative control for anti-rabies immune responses and was used to select the specific immune markers relevant of Rabisin vaccination.

Then, the anti-rabies immune responses were evaluated in absence or presence of Eurican DAPPi, or of Eurican DAPPi-L4.

The results were firstly compared individually with statistical analysis of the individual immune markers and further analysed through an Exploratory Factor Analysis (EFA) to integrate the full immune responses against rabies.

This later EFA was used to investigate the lack of interference after concomitant injection of Eurican DAPPi-L4 or Eurican DAPPi with inactivated rabies vaccine.

The results showed no significant difference of the immediate immune response "fingerprint" between the group vaccinated with Eurican DAPPi-L4 and Rabisin and the groups vaccinated with Eurican DAPPi and Rabisin or Rabisin alone. These investigations support that the Eurican DAPPi-L4 vaccine does not meaningfully interfere with the mounting of the immune response against rabies when associated with Rabisin, and thus can be administered concomitantly.

The aim of another study was to assess the compatibility between Eurican DAPPi2-Lmulti vaccine and Rabisin when injected in two different sites, and the safety of the association. Only efficacy results are detailed below.

Assessment was based on serological results against viral components (CDV, CAV, CPV, CPi2 and/or rabies virus) through one year post-primary vaccination (comparison of serological response induced by Eurican DAPPi2-Lmulti vaccine and Rabisin when injected alone or in combination).

Forty-two SPF puppies aged between 7 and 9 weeks participated in this study. They were randomly allocated to 3 groups of 14 animals each and vaccinated as indicated below.

Group	Vaccination on D0	Vaccination on D28	Blood sampling on dry tubes
A	Eurican DAPPi2-Lmulti	Eurican DAPPi2-Lmulti + Rabisin	D0, D14, D28, D42, D55/56, D70, D98, D147, D210, D301, D391/392
B	Eurican DAPPi2-Lmulti	Eurican DAPPi2-Lmulti	
C	-	Rabisin	

All dogs were regularly blood-sampled to monitor the serological response against the viral components of the two vaccines (CDV, CAV, CPV, CPi2 and rabies virus) from the 1st injection of

Eurican DAPPi2-Lmulti vaccine to about 1 year after the concomitant injections of Eurican DAPPi2-Lmulti vaccine and Rabisin.

Serological response against the viral components of Eurican DAPPi2-Lmulti vaccine (CDV, CAV, CPV and CPiV2) and Rabisin (rabies virus) was proven to be significantly non-inferior in dogs vaccinated with Eurican DAPPi2- Lmulti and Rabisin when compared to that in dogs vaccinated with only one of the two vaccines.

Compatibility between Eurican DAPPi2-Lmulti vaccine and Rabisin when injected in two different sites was fully demonstrated regarding serological response against the viral components of the two vaccines.

The objective of one of the studies was to assess the compatibility between Eurican DAPPi2-Lmulti and Rabisin when injected at two different injection sites in puppies. The immune responses elicited by the different components were followed after vaccination, focusing on several cellular and humoral parameters observed after injection (antibodies, memory B cells, cytokines in PBMCs, plasma cells and IFN-γ secreting cells).

The analysis of the immune parameters induced by both vaccines was performed with the final goal to have a general picture against each valence and assess any impact of the concomitant injection on the efficacy of the vaccines.

The experimental design was as follows:

Group	**Vaccination on D0**	**Vaccination on D28**	**Blood sampling** **Sera** **Whole blood on sodium heparin**
A (14 dogs)	Eurican DAPPi2-L3	Eurican DAPPi2-L3 + Rabisin	D31, D315 D42, D56, D70
B (14 dogs)	Eurican DAPPi2-L3	Eurican DAPPi2-L3	
C (14 dogs)	-	Rabisin	
D (2 dogs)	-	-	

Per strain, a whole set of immune parameters was tested in each group and the relevant specific parameters were further selected. This was represented in a radar chart.

The global analysis focused then only on those selected parameters. This was performed through a principal component analysis (PCA) that summarises at one glance the full immune response per strain.

This analysis was validated since it allows to distinguish the specific response to the different components and the impact on each other. In addition, this assessment was used to support the lack of interference between Eurican DAPPi2-Lmulti and Rabisin as detailed below.

The rabies immune response monitored after vaccination was first assessed. It showed no significant difference between Eurican DAPPi2-Lmulti associated with or without Rabisin for almost all parameters, except for IL-10 release and IFN-γ-secreting cells. In both cases, the biological significance appeared low. Indeed, the quantity of IFN-γ secreted displayed no difference when the Rabisin vaccine was co-injected with the combination. Moreover, despite the role played by IL-10 in the regulation of the immune response, the latter was not affected by the difference of the secretion of this cytokine in group A. The similarity between the Rabisin vaccine response associated or not with the combination was therefore demonstrated.

Comparably, the immune response towards the CPV and CAV antigens showed a highly similar profile in both Eurican DAPPi2-Lmulti alone or co-administered with Rabisin. No interference could be detected.

The analysis of the immunogenicity of CDV antigen was reduced compared to the other strains due to the limited number of tools. Nevertheless, a high similarity of responses between groups vaccinated with Eurican DAPPi2-Lmulti with or without Rabisin was obvious in the radar chart.

Thus, whatever the strain studied, the concomitant injection of Rabisin with Eurican DAPPi2-Lmulti showed no significant impact on the broad set of tested parameters of the humoral and cellular immune responses when compared to each of the vaccines injected alone, further demonstrating compatibility of the association of vaccines.

Field trials

The applicant performed a multi-centred, positively controlled, field safety and efficacy study in compliance with CVMP/VICH/595/98. A total of 558 dogs were included to be vaccinated with either the test vaccine or a reference, which was an already marketed vaccine (Versican Plus DHPPi/L4 from Zoetis). Two different subpopulations were concerned: 265 puppies coming to the practice for their primary vaccination (193 receiving the test vaccine and 72 the reference vaccine) and 293 fully primed dogs coming to the practice for an annual booster injection (213 receiving the test vaccine and 80 the reference vaccine).

The age, size and gender of the animals were appropriate for the experiment.

Assessment of efficacy was based on the antibody response against *L*. Canicola, *L*. Icterohaemorrhagiae, *L.* Grippotyphosa and *L.* Australis, as well as the attenuated live viruses (canine distemper virus, canine adenovirus type 2, canine parvovirus and canine parainfluenza virus) in the associated Eurican DAPPi vaccine.

The data show that most of the animals in primary vaccination with Eurican DAPPi-L4 vaccine developed rapid and satisfactory seroconversion.

The proportion of seropositive animals prior to the first injection of primary vaccination was:

against *L*. Icterohaemorrhagiae (Li): 6.8%; *L*. Canicola (Lc): 21.9%; *L.* Grippotyphosa (Lg): 22%; *L.* Australis (La): 0%.

The individual results of these seropositives showed a significant increase in titres on days D28 and D42. Consequently, the applicant's conclusion that maternal antibodies do not affect the development of the immune response after 7 weeks of age is correct.

The booster vaccination significantly reduced the number of seronegative animals.

Efficacy was evaluated by serological response only, but there is no practical alternative to serology for such trial. Demonstrating the efficacy of the vaccine against natural infections could not be done with reasonable number of dogs and within reasonable timeframe.

Athough serology is not a complete surrogate for protection, it allows to assess the vaccine response in field conditions, and the results in this trial compare favourably with Eurican Lmulti in this respect. Please also refer to comments raised under 'Efficacy of the booster injection'.

Overall conclusion on efficacy

The applicant adequately demonstrated the efficacy of the vaccine. Sixteen studies were conducted

to investigate the efficacy of the product, including 15 laboratory studies (two of the studies were divided into two parts, vaccination and challenge phases, identified with different study numbers) and one field trial. Twenty-one additional studies, generated with the product Eurican Lmulti which contains the same *Leptospira* strains as Eurican L4, except for the new *L.* Australis component, were included as supportive data.

Target species

Eurican L4 is intended for use in dogs from seven weeks of age.

One dose (1 mL) of suspension contains

Ingredients	***Quantity per 1 ml dose***
Inactivated *Leptospira interrogans* serogroup and serovar Canicola, strain 16070	Activity acc. to Ph. Eur. 0447*
Inactivated *Leptospira interrogans* serogroup and serovar Icterohaemorrhagiae, strain 16069	Activity acc. to Ph. Eur. 0447*
Inactivated *Leptospira interrogans* serogroup and serovar Grippotyphosa, strain Grippo Mal 1540	Activity acc. to Ph. Eur. 0447*
Inactivated *Leptospira interrogans* serogroup Australis and serovar Bratislava, strain 16785	Activity acc. to Ph. Eur. 0447*

* ≥80% protection in hamsters

The demonstrated SPC claims, slightly different from the one for which the applicant applied for are:
Active immunisation of dogs to prevent or reduce mortality, clinical signs, infection, bacterial excretion, renal carriage and renal lesions caused by:

- *Leptospira interrogans* serogroup Canicola serovar Canicola,
- *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae,
- *Leptospira kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, and- *Leptospira interrogans* serogroup Australis serovar Bratislava.

Serogroup / Serovar	**Indication**					
	Mortality	**Clinical signs**	**Infection**	**Bacterial excretion**	**Renal carriage**	**Renal lesions**
Canicola / Canicola	Prevention*	Prevention*	Reduction	Reduction	Reduction	Reduction
Icterohaemorrhagiae / Icterohaemorrhagiae	Prevention*	Prevention*	Reduction	Reduction	Reduction	Reduction
Grippotyphosa / Grippotyphosa	Prevention*	Prevention*	Reduction	Reduction	Reduction	Reduction
Australis / Bratislava	Prevention	Prevention	Prevention	Prevention	Prevention	Prevention

* For *Leptospira interrogans* serovar Canicola, *Leptospira interrogans* serovar Icterohaemorrhagiae and *Leptospira kirschneri* serovar Grippotyphosa the prevention of mortality and clinical signs was not demonstrated at the duration of immunity timepoint.

The applicant originally proposed that claims of prevention of mortality, clinical signs, infection, bacterial excretion, renal carriage and renal lesions for *L*. Copenhageni would be included in section 3.2 of the SPC; however, given the absence of information concerning the duration of immunity against this serovar, it was concluded that the efficacy information (limited to onset of immunity only) for *L*. Copenhageni should be included in section 4 of the SPC.

Onset of immunity
It is accepted that immunity has been demonstrated 2 weeks after completion of the primary vaccination course.

Duration of immunity
The one-year duration of immunity has been satisfactorily demonstrated. Concerning the *L*. Canicola, *L*. Icterohaemorrhagiae and *L*. Grippotyphosa components, the prevention of mortality or clinical signs was not demonstrated in the duration of immunity challenge experiments; this fact is adequately addressed in the SPC.

Maternally derived antibodies
No dedicated studies were carried out to investigate the influence of maternally derived antibodies on the efficacy of the vaccine.
As *Leptospira* maternal antibodies wane before 7 weeks of age, no specific precaution is required when vaccinating at this age puppies born from vaccinated bitches.

Vaccination scheme
Based on the laboratory and field studies the applicant proposes the following vaccination scheme:

Basic vaccination scheme
Two injections separated by an interval of 4 weeks from 7 weeks of age.

Revaccination scheme
One dose 12 months after completion of the primary vaccination course. Dogs should be revaccinated with a single booster dose on an annual basis.

The applicant investigated the efficacy of the booster dose on the basis of serology, and whilst serology is not definitively correlated with protection (dogs may be protected in the absence of detectable antibodies), the approach taken was scientifically justified and it was accepted that serology could be used as a sufficient indicator of protection taking into account the totality of data provided in the dossier.

Interaction with other medicinal products
Efficacy data demonstrated that Eurican L4 can be mixed with Boehringer Ingelheim live attenuated vaccines (Eurican DAP or Eurican DAPPi) against distemper, adenovirosis, parvovirosis and parainfluenza type 2 respiratory infections.
Efficacy data demonstrated that Eurican L4 can be administered on the same day, but not mixed with Boehringer Ingelheim rabies vaccine (Rabisin) in dogs from 12 weeks of age.

Part 5 – Benefit-risk assessment

Introduction

Eurican L4 is a liquid vaccine against canine leptospirosis containing the inactivated active ingredients *L.* Canicola, *L.* Icterohaemorrhagiae, *L.* Grippotyphosa and *L.* Australis.

The product is intended for use in dogs to prevent or reduce mortality, clinical signs, infection, bacterial excretion, renal carriage and renal lesions caused by *L*. *interrogans* serogroup Canicola serovar Canicola, *L*. *interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae, *L*. *kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, and *L*. *interrogans* serogroup Australis serovar Bratislava. A beneficial effect of vaccination for protection against *L*. *interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni was also demonstrated at the onset of immunity time point (but no duration of immunity was established for this serovar).

The proposed effective dose of 1 ml, administered by subcutaneous injection with two injections separated by an interval of 4 weeks from 7 weeks of age, has been confirmed.

The one-year duration of immunity has been satisfactorily demonstrated. Although not all claims could be adequately substantiated at the duration of immunity time point, this information is clearly reflected in the SPC.

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

Benefit assessment

Direct therapeutic benefit

Leptospirosis is a contagious disease of animals and man, caused by various immunologically distinct leptospiral serovars. Since all warm-blooded animals and humans are susceptible to Leptospira, this is considered a significant zoonosis. Dogs typically become infected by contact with leptospires in urine from reservoir hosts, or from urine-contaminated water or soil. Infections may be asymptomatic or cause various signs (fever, icterus, haemoglobinuria, renal failure, infertility, abortion and death). After acute infection, leptospira often localized in the kidneys or reproductive organs and are shed in the urine.

Historically, *L*. Canicola and *L*. Icterohaemorrhagiae were seen as the primary causative agents of canine leptospirosis and most canine Leptospira vaccines contained only these two serovars. In recent years, however, *L*. Grippotyphosa have been identified with increasing frequency as pathogens of dogs in Europe. This has resulted in the inclusion of this serovar in novel canine vaccines. *L*. Australis has also been identified as another major circulating serogroup as described in Ellis et al., 2010, leading to the development of new vaccines combining the previous three Leptospira strains with the new *L*. Australis strain. No cross protection exists between the different serotypes.

The benefit of Eurican L4 is the prevention or reduction of mortality, clinical signs, infection, bacterial excretion, renal carriage and renal lesions caused by four Leptospira serovars.

Additional benefits

The subcutaneous application is easy for the vet and reduces the stress for the animal.

The reduction of infection might decrease the amounts of Leptospira shed and, as such, reduce the zoonotic risk.

Using Eurican L4 as a diluent of Boehringer Ingelheim freeze-dried vaccine against canine distemper, adenovirosis and parainfluenza type 2 has advantages for the animals and the owners. It reduces the stress and pain for the animals and the number of visits of vets.

Immunisation of dogs reduces the Leptospira shedding into the environment and, in this way, reduces the risks of human infections.

The immunisation of dogs reduces the therapeutic use of antimicrobials.

Risk assessment

Quality:

The quality of the product is described in sufficient detail and is overall considered adequate.

Safety:

Risk for the target animal:

Administration of Eurican L4 in accordance with the SPC recommendations is generally well tolerated. Further, the vaccine is considered safe when used in a second vaccination cycle of one dose.

The main reported adverse reactions, as mentioned in SPC section 3.6, include local reactions at the injection site and transient temperature increase. Local swelling up to 5 cm (in one case 20 cm after 3rd injection) after repeated administration at the injection site is a very common reaction after administration of the vaccine. This swelling will have disappeared or be clearly reduced in size by 8 days post vaccination after overdose by 22 days. It is very commonly accompanied by transient pruritus, heat and pain at the injection site. Transient lethargy, anorexia and emesis may also commonly be observed.

Diarrhoea, muscle tremor, vocalisation, hyperthermia, tachycardia and tachypnoea may uncommonly be observed.

Hypersensitivity reactions (facial oedema, anaphylactic shock, urticaria) may rarely occur.

The potential adverse effects of vaccination are adequately reflected in the SPC.

No studies have been performed on reproductive performance with Eurican L4. Nevertheless, data are available with the 3-component leptospirosis vaccine Eurican Lmulti showing that vaccination of pregnant bitches is safe. This is reflected in section 3.7 of the SPC, and includes information to state that no safety data are available for Eurican L4, which contains an additional inactivated serovar, *L*. Australis

Risk for the user:

The user safety for this product is acceptable when used according to the SPC recommendations; no risks to the user have been identified. In the absence of identified risks, no specific user safety warnings are required.

Risk for the environment:

Eurican L4 is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

Risk for the consumer:

Not applicable since Eurican L4 is not intended for food producing species.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

Based on the data presented, 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 Eurican L4 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 veterinary medicinal product.