5 June 2014
EMA/321742/2014
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Versican Plus Pi/L4R (EMEA/V/C/003682/0000)

Common name: canine parainfluenza virus (live attenuated), canine leptospirosis and rabies (inactivated) vaccine

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

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Introduction

On 26 June 2013 the applicant Zoetis Belgium s.a. submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Versican Plus Pi/L4R 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 14 June 2012 as the product contains a new active substance. The rapporteur appointed was E. Werner and co-rapporteur G. Kulcsár.

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

This vaccine is indicated for the immunisation of healthy puppies and dogs against parainfluenza, rabies and leptospirosis.

The route of administration is subcutaneous use.

On 5 June 2014, the CVMP adopted an opinion and CVMP assessment report.

On 31 July 2014, the European Commission adopted a Commission Decision granting a marketing authorisation for this veterinary medicinal product.

Scientific advice

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system which fulfils the requirements of Directive 2001/82/EC. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the European Union (EU) or in a third country.

Manufacturing authorisations and inspection status

Antigen production, in-process testing, formulation, primary and secondary packaging, release testing and batch release takes place at

BIOVETA, a. s.
Komenského 212
683 23 Ivanovice na Hané
CZECH REPUBLIC

A valid manufacturing authorisation was presented in the dossier (dated 14 December 2011).

A valid good manufacturing practice (GMP) certificate for the Bioveta site dated 7 May 2012 was submitted.

Overall conclusions on administrative particulars

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

Part 2 – Quality

Composition

Versican Plus Pi/L4R contains per 1 ml dose:

Lyophilisate (live attenuated):	
Canine parainfluenza type 2 virus, strain CPiV-2 Bio 15	$10^{3.1}$ – $10^{5.1}$ $TCID_{50}$
Solvent (inactivated):	
Leptospira interrogans serogroup Icterohaemorrhagiae, serovar Icterohaemorrhagiae, strain MSLB 1089	ALR ** titre ≥ 1:51
Leptospira interrogans serogroup Canicola, serovar Canicola, strain MSLB 1090	ALR ** titre ≥ 1:51
Leptospira kirschneri serogroup Grippotyphosa, serovar Grippotyphosa, strain MSLB 1091	ALR ** titre ≥ 1:40
Leptospira interrogans serogroup Australis, serovar Bratislava, strain MSLB 1088	ALR ** titre ≥ 1:51
Rabies virus, strain SAD Vnukovo-3	≥ 2.0 IU***.

$TCID_{50}$ is the quantity of the virus that will produce a cytopathic effect in 50% of the cultures inoculated.

** Antibody micro agglutination-lytic reaction.
*** International units.

Aluminium hydroxide gel is used as an adjuvant.

Container

Each fraction of the vaccine is filled into Type I glass vials. The vials are tested in accordance with European Pharmacopoeia (Ph. Eur.) monograph 3.2.1. The glass vials for the freeze-dried fraction are closed with 13 mm bromobutyl rubber stoppers, and the glass vials for the liquid fraction are closed with 13 mm chlorobutyl rubber stoppers. The rubber stoppers are tested in accordance with Ph. Eur. monograph 3.2.9.

The rubber stoppers are sealed with a 13 mm flip off aluminium cap. Corresponding certificates of analysis are provided. The outer packaging will be a transparent plastic box containing 25 or 50 vials of 1 dose of the lyophilisate and 25 or 50 type vials of solvent accordingly.

Development pharmaceutics

The Versican Plus Pi/L4R has been developed for the prevention of infectious disease caused by canine parainfluenza virus (live components) and against leptospirosis and rabies (inactivated components). The product was based on the preceding vaccine Versican DHPPi/L3R. This vaccine contains canine distemper virus (CDV), canine adenovirus type 2 (CAV-2), canine parvovirus type 2 (CPV-2) and canine parainfluenza virus (CPiV) and three leptospiras: *Leptospira interrogans* serovar Canicola, *L. interrogans* serovar Icterohaemorrhagiae, *Leptospira kirschneri* serovar Grippotyphosa and rabies virus. The Versican Plus Pi/L4R contains one additional leptospira strain from serogroup Australis serovar Bratislava.

Method of manufacture

The manufacturing procedure is adequately described in detail to give sufficient confidence that the product will be safe, effective and stable.

The manufacturing process includes: Preparation of medium and inocula, inoculation of the cell line or growth media and growth of culture, termination of the cultivation. For the antigens for liquid fraction another procedures are described as inactivation and concentration/purification. Each leptospira antigen is purified and concentrated. The liquid fraction is blended with adjuvant (aluminium hydroxide gel).

Control of starting materials

Active substance

All 6 antigens comprised in the vaccine (i.e. CPiV-2, four leptospira antigens and rabies virus) are sufficiently described with regard to their origin, isolation and history. The control testing on the viral and bacterial seeds is performed in accordance with the relevant guidelines. This control testing is considered satisfactory and purity of the seed materials is sufficiently justified with regard to the risk of contamination of the materials from pathogens of the species of origin and the risk for the target species.

Excipients

Certificates of analysis of starting materials listed in Ph. Eur. monographs were provided and are satisfactory.

Up-to-date European Directorate for the Quality of Medicines and HealthCare (EDQM) certificates and/or certificates of analysis for substances of biological origin used during production were provided. Certificates of analysis of the starting materials of non-biological origin were provided and are satisfactory. Details of in-house preparation of media were provided.

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

A detailed list of materials of animal origin included in the scope of the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products and of materials from animals other than those included in the scope of the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) was provided in the dossier together with a transmissible spongiform encephalopathy (TSE) risk assessment. The substances comply with the TSE Note for guidance and Commission Directive 1999/104/EC.

The TSE risk assessment is provided related to the use of the bovine serum in the manufacturing process. The material is sourced only from countries classified by geographical bovine spongiform encephalopathy (BSE) risk as either highly unlikely or unlikely but not excluded category. Therefore it can be concluded that the risk of TSE contamination from bovine serum is negligible.

The seed materials are considered in the risk assessment with regard to their origin (non-ruminant), method of preparation and recipient species of the vaccine (dogs, known as not susceptible to TSE)

The CVMP concluded that the TSE risk has been adequately addressed and is considered negligible.

Control tests during production

Freeze-dried fraction:

The control tests during production include sterility, absence of mycoplasma, virus titre, pH value and sterility after blending. The in-process tests are deemed to be sufficient to control all the critical steps in the manufacture.

Liquid fraction:

The following tests are performed for rabies virus: cell count (before virus inoculation), virus titre (after virus harvest), rabies virus inactivation, pH, sterility, glycoprotein content (after inactivation), sterility (after concentration).

The following tests are performed for *Leptospira* components: growth, purity (during cultivation), identity, cell count (before inactivation), *Leptospira* inactivation, sterility (after inactivation), serum absence, sterility and formaldehyde (after concentration and purification).

After vaccine blending the bulk is tested for aluminium content, pH and sterility.

The in-process tests are deemed to be sufficient to control all the critical steps in the manufacture.

However, as regards in-process tests of the rabies virus antigen, for future batches the applicant is recommended to implement an in process test to determine the glycoprotein value after concentration by enzyme linked immunosorbent assay (ELISA).

Control tests on the finished product

The description of the methods used for the control of the finished product and the specifications are provided:

Freeze-dried fraction:

The control tests of the finished freeze-dried fraction include appearance, sterility, extraneous agents, absence of mycoplasma, virus identity and titre, residual humidity and vacuum.

Liquid fraction:

The control tests of the finished liquid fraction include appearance, sterility, air tightness, extractable volume, *Leptospira* identity and potency, rabies virus identity and potency.

Reconstituted vaccine:

The control tests of the reconstituted vaccine include appearance and pH determination.

A detailed overview of all in-process and finished product tests of the vaccine Versican Plus Pi/L4R has been submitted.

The finished product tests are considered adequate to control the quality of the finished product.

Stability

The proposed shelf life of the vaccine Versican Plus Pi/L4R is 2 years when stored at 2–8 °C.

In order to support the proposed shelf life of the finished product three batches of each final fraction of the vaccine Versican Plus DHPPi/L4R (largest combination of the Versican Plus range of vaccines) were manufactured in accordance with the proposed method. Vials from each batch were stored over a

period of 27 months and tested at regular intervals at 0, 3, 6, 12, 18, 24 and 27 months in accordance with finished product specifications. In addition, at 0, 24 and 27 months of storage samples from each batch were reconstituted and tested for appearance, pH and sterility in accordance with the finished product specifications.

The proposed shelf life of 2 years can be accepted.

However, the final report on the antigen shelf life including the inactivated antigen stability data will still need to be submitted as soon as it is available. A relevant recommendation has been included in the report.

Overall conclusions on quality

The analytical part of the dossier is detailed and clearly states the production and control of this immunological veterinary medicinal product and demonstrates that it complies with the requirements of Directive 2001/82/EC.

All necessary information concerning qualitative and quantitative composition is submitted.

The choice of the vaccine strains and the adjuvant has been satisfactorily addressed and reference to the relevance of each strain to current epidemiological conditions is also provided.

The manufacturing process of the vaccine has been described in detail for the virus and for the *Leptospira* components. Regarding the starting materials all necessary information has been provided.

The TSE risk assessment provided by the applicant clearly demonstrates that the TSE risk of this product is negligible. Compliance with the corresponding Note for guidance is demonstrated.

Controls during manufacture and tests on the finished product are suitable to guarantee the compliance with the quality parameter mentioned. Test methods have been described and corresponding validation studies have been performed.

Batch to batch consistency has been demonstrated and a detailed overview of all in-process and finished product tests of the vaccine Versican Plus Pi/L4R has been provided. Based on the data provided, the proposed shelf life for the finished product of 2 years can be accepted. The CVMP recommended that the final study results should be provided when available in order to confirm final results for the antigen shelf life.

Based on the review of the data on quality, the manufacture and control of Versican Plus Pi/L4R are considered acceptable.

In addition, the applicant is recommended to provide the following information post-authorisation:

1. The antigen stability study is due to complete by the end of 2014. The final study report should be submitted during the first quarter in 2015.
2. Regarding the *Leptospira* components, for the first 10 batches produced for commercial release, the individual results of the tested rabbits including the results for all control sera (negative, hyperimmune and positive standard sera) should be submitted along with the batch release protocols.
3. Regarding the rabies component, the applicant is recommended to implement an ELISA method for determination of the glycoprotein value after concentration prior to marketing of the product.

Part 3 – Safety

Versican Plus Pi/L4R is a combined live virus and inactivated virus/bacterial vaccine indicated for the immunisation of healthy puppies and dogs against canine parainfluenza, rabies and leptospirosis.

The applicant presented laboratory vaccination studies (safety of a single and repeated dose, overdose, clearance, shed and spread of the vaccine, increase in virulence) and two field studies to support the safety of this vaccine.

Laboratory tests

Methods and corresponding validations for the serological and viral isolation tests used in the clinical studies were provided.

The safety of a single and repeated dose was demonstrated using the largest combination of the Versican Plus range of vaccines, Versican Plus DHPPi/L4R. All components of Versican Plus DHPPi/L4R are identical to those contained in Versican Plus Pi/L4R with a couple of components differing. This is in compliance with CVMP note for guidance: requirements for combined veterinary vaccines (CVMP/IWP/52/97) and also the CVMP Guideline on the requirements for combined vaccines and associations of immunological veterinary medicinal products (IVMPs) (EMA/CVMP/IWP/594618/2010).

Safety of the administration of one dose

The safety of a single dose was assessed together with the safety of repeated administration of a single dose using the fully-valent vaccine Versican Plus DHPPi/L4R.

Sixteen 6 week old puppies, free of antibodies against CDV, CAV-1, CAV-2, CPV, CPiV, *Leptospira* and rabies virus were vaccinated subcutaneously four times at an interval of 14 days, thus the vaccination scheme applied in the study differed from the vaccination scheme in accordance with the summary of product characteristics (SPC) which recommends as basic vaccination two doses 3–4 weeks apart from 6 weeks of age. The vaccination titre per dose (1 ml) correlates with the maximum proposed titres given in the SPC. All animals were observed at defined points in time for signs of abnormal local reactions including heat, pain, erythema and swelling, and systemic reactions including rectal temperatures.

During the study no systemic reactions were observed. At the injection sites, soft painless swellings were found. The maximum diameter after the first administrations was 25 mm. The maximum duration for until disappearance was 17 days. The rectal temperatures of all animals remained within the physiological range after each administration. In general, it can be concluded that the administration of one dose of Versican Plus DHPPi/L4R (or Pi/L4R respectively) containing maximum potency of antigens by the recommended route was safe for puppies of 6 weeks of age.

Safety of one administration of an overdose

Overdose safety study of the vaccine with the DHPPi component (Versican Plus DHPPi) in minimum age pups

This study was performed in compliance with good laboratory practice (GLP) and in accordance with Annex I of Directive 2001/82/EC and International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline GL44 on target animal safety for veterinary live and inactivated vaccines and the Ph. Eur. general monograph on safety.

Sixteen 6 week old puppies free of antibodies against CDV, CAV-1, CAV-2, CPV and CPiV were vaccinated subcutaneously with a ten-fold maximum dose. All animals were observed at defined points in time for signs of abnormal local reactions including heat, pain, erythema and swelling, and systemic reactions including rectal temperatures. During the study no systemic reactions were observed. Immediately after the vaccine administration, four out of eight animals showed evidence of pain at the injection site (scratching at injection site, vocalising). The signs of pain persisted for 10 seconds to 1 minute. At the injection sites, soft, painless swellings were found, which resolved within one day.

The rectal temperatures of all animals remained within the physiological range after each administration. White blood cell counts showed that no animal developed leukopenia.

In this study, only the live virus components CDV, CAV-2, CPV-2b and CPiV, diluted in water for injection instead of the actual diluent, were investigated. This was justified in a sufficient manner. Further overdose testing is no longer required for inactivated vaccines.

Master seed studies

Irreversibility of attenuation of vaccine strain – canine parainfluenza virus (CPiV)

This study is discussed and presented in detail under point “Increase in virulence – CPiV strain”. The CPiV strain was generally classified as safe since no adverse effects were observed in further studies, e.g. in the overdose study where the ten-fold dose of the maximum titre was administered.

Safety of the repeated administration of one dose

For details of the study design and the results see the above section on “Safety of the administration of one dose”.

The repeated administration of a single dose was presented and assessed together with the safety of a single dose. Four repeated administrations were included in the design to take account of the number of administrations for primary vaccination (2–3) and the first re-vaccination (1).

For primary vaccination two doses are recommended.

During the study no systemic reactions were observed. At the injection sites, soft painless swellings were found. After 4 injections a maximum of 30 mm in diameter was observed. The maximum duration for disappearance was 17 days.

The applicant chose to administer the single doses for this study four times with a 14-day interval between the administrations. While this vaccination schedule differs from the recommended SPC interval of 3–4 weeks, it does constitute a worse case and additionally is in line with VICH GL44 which permits shortening the intervals between administrations for repeat dose studies to at least 14 days.

Overall, it can be concluded that the administration of the repeated dose of Versican Plus DHPPi/L4R (or Pi/L4R respectively) containing maximum potency of antigens by the recommended route was safe for puppies of 6 weeks of age.

Examination of reproductive performance

No studies have been performed on reproductive safety and a warning in this respect is included in the SPC.

The Ph. Eur. monograph 5.2.6 on requirements for examination of reproductive performance of males and non-pregnant female dogs was also considered. This requirement is applicable if the vaccine contains organisms which are known as reproductive pathogens.

CPiV (the live viral component of Versican Plus Pi/L4R) is not generally associated with any pathological effects in the reproductive tract of the male or the non-pregnant female. The other components of the vaccine are inactivated viral and bacterial antigens, the aluminium hydroxide gel (adjuvant) and some inert excipients, which again are not generally associated with any pathological effects in the reproductive tract of the male or the non-pregnant female. The CVMP therefore concluded that there is no justification for requesting further examinations of reproductive performance. However, as no data regarding safety aspects of vaccine administration to pregnant or lactating dogs are available a warning is included in the SPC section 4.7: "Therefore the use is not recommended during pregnancy and lactation."

Examination of immunological functions

Ph. Eur. monograph 5.2.6 gives the following guidance concerning investigation of adverse effects on immunological functions: where the product might adversely affect the immune response of the animal to which the product is administered or of its progeny, suitable tests on the immunological functions are carried out.

None of the components of Versican Plus Pi/L4R is known to have any adverse effects on the immune functions of infected animals. CPiV is already known as component of Versican Plus DHPPi/L3R. No adverse effects are known for this antigen.

No studies have been performed on the concurrent use of any other non-Versican Plus vaccine. This fact is covered in section 4.8 of the SPC as follows:

"No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. 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 by the veterinarian".

Special requirements for live vaccines

Spread of the vaccine strain

Canine parainfluenza virus type 2 (CPiV-2)

The objective of the study was to demonstrate potential spread of CPiV-2 vaccine from vaccinated animals to in-contact naïve animals by evaluating the seroconversion of the control animals.

Five puppies were once vaccinated subcutaneously and five control puppies were kept in contact with the vaccinated animals.

General health observations were carried out daily until the end of the study on Day 84. No abnormal health observations were made. Blood samples were collected from all puppies at defined points in time. The virus neutralising antibodies to CPiV-2 were analysed. No organ samples were taken. There was an increase in neutralising antibodies to CPiV-2 in the five vaccinated animals. There were no detectable CPiV-2 neutralising antibodies in the control animals at any of the points in time of the test. The study results show that the vaccine CPiV-2 strain, when administered by the recommended route and titre, is not capable to spread from vaccinated dogs to in-contact control animals and induce an active infection.

Dissemination in the vaccinated animals

As stated by the applicant a separate study to investigate the dissemination of the CPiV vaccine strain has not been undertaken. CPiV is not a recognised zoonosis, hence the applicant believes that under the terms of Ph. Eur. monograph 5.2.6 it is not necessary to undertake a study of dissemination of this vaccine strain in the body. Furthermore, according to the publications CPiV is known to affect only the surface epithelium of the respiratory tract and not to cause a systemic infection.

While the applicant has not performed a separate study to investigate the dissemination of the CPiV vaccine strain reference is made to study "Irreversibility of Attenuation of Vaccine Strain – Canine parainfluenza virus" and Safety Study "CPiV strain: Spread of the vaccine strain".

In study on irreversibility of attenuation nasal swabs were taken after vaccination and isolated vaccine virus was passaged 5 times. Data show that this virus disseminates to the nasal mucosa and is excreted between approximately from 6 to 9 days after vaccination. No further secretion samples, e.g. faeces, were taken and tested. Spread of virus cannot be excluded. The CVMP therefore concluded that virus dissemination has been shown and shedding is likely to take place. Nevertheless, due to the low pathogenicity of these strains, it is not necessary to keep vaccinated dogs separated from non-vaccinated dogs.

In study on the potential spread of the vaccine virus strain CPiV-2 from vaccinated animals to in-contact animals by evaluating the seroconversion of the control animals to CPiV-2, an increase of neutralising antibodies to CPiV-2 in the vaccinated animals could be observed, but there was no increase in neutralising antibodies in the control animals.

Please see assessment under the corresponding studies.

Reversion to virulence of attenuated vaccines

In 2001 the vaccine Versican DHPPi/L3R was evaluated at national level. The strain is identical with the CPiV strain of the new Versican Plus Pi/L4R vaccine.

Step 1: Passage procedure:
Irreversibility of attenuation of the CPiV vaccine strain was evaluated by sequential passaging of the CPiV in twelve puppies. Nasal swabs were collected and tested for presence of the virus. Nasal swabs with the maximum amount of virus were selected and 1 ml of the suspension was administered intranasally to two other puppies for the first passage. The above operation was carried out five additional times and the presence of virus was verified at each passage.

Step 2: Subsequent safety evaluation:
CPiV positive passage material from the last two puppies (terminal passage) was administered intranasally to five puppies (Group 2). In addition, the un-passaged CPiV was directly vaccinated subcutaneously to another five puppies (Group 1).

No local or systemic adverse reactions were observed in any of the study animals during the whole study. The rectal temperatures recorded during the safety study remained below 39 °C in all animals. The presence of CPiV was verified in all passages. CPiV was isolated from Day 6 to Day 9 after inoculation.

In the subsequent safety evaluation, no neutralising antibodies to CPiV were detected before inoculation. Twenty-one days after inoculation, the titres in all animals were increased.

This study shows no indication of increased virulence of the vaccine CPiV and is in compliance with Ph. Eur. monograph 1955.

Biological properties of the vaccine strain

The intrinsic biological properties of the live virus vaccine strain has been adequately characterised by the information provided and the safety and efficacy data supplied. A summary regarding the characterisation of the live virus strain and the stability of the attenuation was provided, which emphasizes the stability of the attenuation of the strain. No increase of virulence was observed.

The CVMP therefore concluded that adequate information was provided on the biological properties of the vaccine strain.

Recombination or genomic re-assortment of the strains

The genetic stability of the vaccine strain of Versican Plus Pi/L4R has been demonstrated. The vaccine strain has demonstrated to be consistent and stable by *in vitro* passaging. Further the strain is attenuated and of low virulence.

Canine parainfluenza virus is a paramyxovirus. Being a single stranded RNA virus, it is therefore not readily amenable to either recombination or re-assortment.

Study of residues

Versican Plus Pi/L4R is a vaccine which is indicated solely for use in dogs. Therefore, the consideration of residues is not applicable.

Interactions

No studies have been performed to test the effect of the vaccine on concurrent use of any other vaccine. Therefore section 4.8 of the SPC includes the common statement to this effect.

Field studies

One multi-centre study was performed to evaluate safety and efficacy of the vaccine Versican Plus DHPPi/L4R in comparison to a positive control group. The findings are also applicable to the smaller fall-out product Versican Plus Pi/L4R. Three cohorts were analysed after vaccination. No abnormal general physical conditions were recorded. Regarding rectal temperatures no abnormalities could be identified. Examinations of the injection site show swellings with a maximum diameter of 35 mm. The maximum duration of swellings following treatment lasted 18 days.

After vaccination signs of lethargy (reduced liveliness), vomiting, diarrhoea and anorexia were observed in some dogs. These observations were reflected in the SPC.

A further field study was performed with a batch without the rabies component. Examinations of the injection site show swellings with a maximum diameter of 38 mm. The maximum duration of swelling following treatment lasted 20 days. No abnormal general physical conditions were recorded. Regarding rectal temperatures no abnormalities could be identified. These findings further supported the acceptable safety profile for this particular vaccine as well as the whole vaccine range.

User safety

The applicant provided a user risk assessment compliant with the CVMP Guideline on user safety for immunological veterinary medicinal products (EMEA/CVMP/IWP/54533/2006).

The following possible risks were discussed: self-administration, skin contamination, breaking of a glass vial and toxic or infectious ingredients.

Versican Plus Pi/L4R can be considered as presenting no particular risk to humans as many of its components are not harmful for humans and the other components have been shown to be not infectious for humans. Additionally the product must be administered by competent end users, i.e. a skilled veterinarian or a trained person under the supervision of a veterinarian.

In the SPC appropriate warnings are included concerning handling of the vaccine or in case of accidental self-injection.

The CVMP therefore concluded that the user safety for this product is acceptable when used as recommended in the SPC.

Environmental risk assessment

An environmental risk assessment (ERA) in compliance with the CVMP Note for guidance on environmental risk assessment of immunological veterinary medicinal products (EMEA/CVMP/074/95) was provided.

Phase I assessment

1. Hazard identification

- The vaccine is composed of 6 well characterised antigens and a well-known adjuvant.
- The stability of the attenuation of the CPiV strain was demonstrated.
- Spread of virus cannot be excluded, but since an appropriate warning is included in the SPC the risk of virus spreading to other susceptible species can be regarded as negligible.

2. Exposure to hazard

The product is manufactured in tightly closed vials. A small volume is parentally and individually administered to dogs (subcutaneously) by a qualified person. The potential exposure to a hazard is therefore considered adequately controlled and negligible.

Based on the data provided, the ERA can stop at Phase I. The product is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

Laboratory studies

The administration of one dose of Versican Plus DHPPi/L4R containing maximum potency of antigens by the recommended route was found to be safe for puppies of 6 weeks of age.

An overdose study with the live virus components (lyophilisate) showed an acceptable safety profile for these components. One study of a repeated dose has been performed. Overall, it can be concluded that the administration of repeated doses of Versican Plus DHPPi/L4R containing maximum potency of antigens by the recommended route was found to be safe for puppies of 6 weeks of age.

No studies have been performed on reproductive safety and a warning sentence that the use is not recommended during pregnancy and lactation is included in the SPC.

No studies have been performed to test the effect of the vaccine on the immune system. None of the components of Versican Plus Pi/L4R is known to have any adverse effects on the immune functions of infected animals. CPiV is already known as component of Versican DHPPi/L3R. No adverse effects are known for this antigen.

No studies have been performed on the concurrent use of any other vaccine not being part of the Versican Plus range of vaccines. This fact is addressed in section 4.8 of the SPC.

Spread of the vaccine strain

There was an increase in the neutralising antibodies to CPiV-2 in the vaccinated animals. There were no detectable neutralising antibodies to CPiV-2 in the control animals at any of the testing time points. No organ samples were taken.

Dissemination in the vaccinated animal

No investigation on the dissemination of the CPiV vaccine strain has been undertaken. Reference is made to the Spread study and the Irreversibility of Attenuation of Vaccine study. Data show that this virus disseminates to the nasal mucosa and is excreted after vaccination. Furthermore, neutralising antibodies to CPiV-2 in the vaccinated animals could be observed, but not in the control animals.

Reversion to virulence of attenuated vaccines

In a first passage procedure the virus was passaged several times in dogs. In a second evaluation step animals were inoculated with this passaged virus intranasal. The presence of CPiV was verified in all passages. Neutralizing antibodies to CPiV were detected in all animals. No indication of reversion to virulence during the passages was found.

Field studies

One multi-centre study was performed to evaluate safety and efficacy of the vaccine Versican Plus DHPPi/L4R in comparison to a positive control group. The findings are also applicable to the smaller fall-out product Versican Plus Pi/L4R. Three cohorts were analysed after vaccination. No abnormal general physical conditions were recorded. Regarding rectal temperatures no abnormalities could be identified. Examinations of the injection site show swellings with maximum diameter of 35 mm. The maximum duration of swelling following treatment lasted 18 days.

After vaccination signs of lethargy (reduced liveliness), vomiting, diarrhoea and anorexia were observed in some dogs and these observations are reflected in the SPC.

User safety

In the SPC appropriate warnings are included concerning handling of the vaccine or in case of accidental self-injection. Furthermore it is stated that the vaccine contains no ingredients that are toxic or infectious to humans. The user safety for this product is acceptable when used as recommended in the SPC.

Environmental risk assessment

Based on the data provided, the ERA can stop at Phase I. The product is not expected to pose a risk for the environment when used according to the SPC.

Part 4 – Efficacy

Introduction and general requirements

Versican Plus Pi/L4R is a multivalent live virus and inactivated viral and bacterial vaccine which is indicated for the immunisation of healthy puppies from 8–9 weeks of age and dogs against canine parainfluenza, rabies and leptospirosis. The live virus component of the vaccine (canine parainfluenza virus (CPiV)) is presented in freeze-dried form in a vial to be reconstituted with a vial of the inactivated components (rabies virus, *Leptospira* Bratislava, *Leptospira* Canicola, *Leptospira* Grippotyphosa and *Leptospira* Icterohaemorrhagiae) presented in liquid form. The liquid fraction also contains an adjuvant (aluminium hydroxide).

Laboratory vaccination/challenge studies (establishment of the minimum protective dose, onset of immunity, duration of immunity) and a field study were provided to support the efficacy claims.

Laboratory trials

The challenge trials were performed with batches of minimum protective doses.

Establishment of a challenge model

Efficacy of all components of Versican Plus Pi/L4R was assessed by challenges with heterologous challenge strains according to component-specific monographs. Certificates for the challenge strains were provided.

Determination of the vaccine dose

Minimum immunisation doses for viral components of Versican Plus Pi/L4R

The minimum immunisation doses (MIDs) for the Versican Plus Pi/L4R viral components CPiV and rabies virus were selected based on previous experiences with these strains. The CPiV component is approved as component of Versican DHPPi/L3R. The rabies component is approved in most EU countries in monovalent form as Vanguard R and in polyvalent form as Versican DHPPi/L3R.

Minimum immunisation doses for *Leptospira* components of Versican Plus Pi/L4R

Three possible MIDs (10^7, $5x10^7$ and 10^8 organisms/ml pre-inactivation) were selected for the *Leptospira* components *L.* Bratislava*, L.* Canicola, *L.* Grippotyphosa and *L.* Icterohaemorrhagiae based on previous data and experiences with the approved canine vaccine Versican DHPPi/L3R.

The results and conclusions of these studies confirmed that each *Leptospira* serovar in combination with the other vaccine components at an MID of $5x10^7$ organisms/ml protected minimum age dogs against clinical signs, systemic infection, and renal infection and excretion of *Leptospira*. No significant differences were found between formulations with or without adjuvant or rabies virus.

MIDs for all components were confirmed in onset of immunity studies described in Part 4.B.3.

Onset of immunity

Onset of immunity (OOI) has been demonstrated with challenge studies according to the relevant Ph. Eur. monographs.

CPiV:

Fifteen 6-week old dogs (10 with 5 control dogs), tested seronegative against CPiV were administered the vaccine Versican Plus DHPPi/L4R subcutaneously. They were challenged intranasally with the challenge strain CPiV D008 at Day 21 after vaccination. After challenge the vaccinated group showed no clinical signs and no increase of rectal temperature. There was a further increase of antibody titres against CPiV.

On virus isolation no CPiV-excretion was found prior to challenge whereas 8/10 vaccinated dogs excreted CPiV for 1–4 days starting from Day 2 until Day 6 after challenge. The duration of virus excretion was found to be significantly lower than in the controls.

As regards immunogenicity Ph. Eur. monograph 1955 states: "The vaccine complies with the test if the scores for coughing or virus excretion for the vaccinated dogs are significantly lower than in the controls." In this study no coughing could be observed in the control group.

This clinical sign is not induced in the challenge model used by the applicant. Signs following experimental infection with CPiV are known to be very mild and clinical signs observed in the field are mostly due to concurrent (secondary bacterial) infection. A more severe challenge model could only be achieved in dogs from the Bioveta SPF colony which is free of respiratory pathogens by introducing a second pathogen (e.g. *Bordetella bronchiseptica*), but this may interfere with the assessment of the efficacy against CPiV. As the monograph states that scores for *either* coughing or virus excretion need to be significantly lower than in controls the CVMP concluded that this wording implies that only one of the two options (i.e. coughing or virus excretion) needed to be fulfilled for the vaccine to comply with the test.

However, taking into consideration that the challenge was very mild, it cannot be excluded that in case of a severe challenge even the vaccinated animals would have developed mild clinical signs. Therefore more detailed information as regards the claim for CPiV, i.e. "to prevent clinical signs (nasal and ocular discharge)" has been added to the SPC.

Rabies virus:

The 1-year study investigating DOI against rabies was a negatively controlled, blinded laboratory study to evaluate the protective duration of efficacy of the rabies component. In this study, 30 seronegative dogs, 12 to 13 weeks of age, were vaccinated once with Versican Plus DHPPi/L4R containing the rabies component at minimum potency and maximum passage that will be present in a batch of commercial vaccine. As part of this study antibody titres against rabies virus were determined at multiple time points post-vaccination and pre-challenge. Time points 3 to 35 days after vaccination were the most relevant to assess onset of immunity for the rabies component of Versican Plus DHPPi/L4R (respectively Pi/L4R). Pre-vaccination titres of all animals were ≤ 0.04 IU/ml.

Ph. Eur. monograph 04/2008:0451 for inactive rabies vaccines states that the mean antibody titre against rabies virus in 20 animals must not be less than 0.5 IU/ml and no more than 10% of the animals have titres less than 0.1 IU/ml to show satisfactory protective immunogenicity of an inactivated rabies vaccine serologically. Based on the data from the duration of immunity study with Versican Plus DHPPi/L4R these conditions are fulfilled 12 days after vaccination indicating that the onset of immunity is 12 days for the rabies component of Versican Plus DHPPi/L4R (respectively Pi/L4R).

Additionally to the study described above, preliminary data from two 3-year rabies duration of immunity studies, which are still ongoing, have been provided to support the 2-week onset of immunity for rabies. Both 3-year DOI studies are negatively controlled, blinded, laboratory studies to evaluate the protective duration of efficacy of the rabies component. In these studies, a total of 60

seronegative dogs, 12 to 13 weeks of age, were vaccinated once with Versican Plus DHPPi/L4R containing the rabies component at minimum titre and maximum passage that will be present in a batch of commercial vaccine. As part of these studies antibody titres against rabies virus were determined at multiple time points post-vaccination and pre-challenge. Time points 3 to 39 days after vaccination were the most relevant to assess OOI. Pre-vaccination titres of all animals were ≤ 0.04 IU/ml.

The serology results of these two studies support a 2-week OOI. Fifteen and 14 days after vaccination, respectively, mean antibody titres against rabies virus were ≥ 0.5 IU/ml and no more than 10% of the animals had titres less than 0.1 IU/ml indicating satisfactory protective immunogenicity of the rabies component according to Ph. Eur. monograph 0451.

Therefore, it was concluded that based on the serological data from the three DOI studies including 90 dogs, one vaccination with Versican Plus DHPPi/L4R at 12–13 weeks of age has an OOI of two weeks for the rabies component.

Leptospira

The study design for each *Leptospira* component was as follows. Twelve 6-week-old dogs (6 with 6 control dogs), tested seronegative against the principle serovars of *Leptospira,* were subcutaneously administered the vaccine Versican Plus DHPPi/L4R. They were challenged by conjunctival and by intraperitoneal route. After challenge they were observed for clinical signs, measurement of rectal temperature, blood samples for serology, for haematology, biochemistry and isolation of the challenge organism, urine samples for isolation of the challenge organism. Twenty-eight days after challenge they were euthanized and the liver and kidneys examined macroscopically and microscopically and tested for the presence of the challenge organism.

Results:

L. Bratislava

All six vaccinated animals seroconverted. After challenge, only control animals showed typical clinical signs of canine leptospirosis such as apathy (5 out of 6 animals), mild to moderate anorexia (6/6), fever (3/6), dehydration (4/6), conjunctivitis (2/6), diarrhoea (1/6) and jaundice (2/6) starting four days after challenge and persisting in one animal until 28 days.

No noteworthy changes in biochemical and haematological parameters were observed.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals.

Macroscopic and microscopic examination of liver and kidney samples showed more pathological changes of greater severity in organs from control than from vaccinated animals.

For the post-challenge, the difference between vaccinated and control animals was significant regarding the total clinical score, the number of days that the challenge organism was detected in blood and the number of liver and kidney samples in which the organism was detected.

L. Canicola

All six vaccinated animals seroconverted. After challenge, only control animals showed typical clinical signs of canine leptospirosis such as apathy (5 out of 6 animals), mild to moderate anorexia (6/6), fever (4/6), dehydration (5/6), conjunctivitis (4/6), diarrhoea (2/6) and jaundice (2/6) starting two days after challenge and persisting until Day 25.

No noteworthy changes in biochemical and haematological parameters were observed.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals.

Macroscopic and microscopic examination of liver and kidney samples showed more pathological changes of greater severity in organs from control than from vaccinated animals.

For the post-challenge, the difference between vaccinated and control animals was significant regarding the total clinical score, the number of days that the challenge organism was detected in blood and the number of liver and kidney samples in which the organism was detected.

L. Grippotyphosa

All six vaccinated animals seroconverted. After challenge, only control animals showed typical clinical signs of canine leptospirosis such as apathy (3 out of 6 animals), mild to moderate anorexia (5/6), fever (4/6), dehydration (3/6), conjunctivitis (2/6) and jaundice (4/6) starting three days after challenge and persisting until Day 18.

No noteworthy changes in biochemical and haematological parameters were observed.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals.

Macroscopic and microscopic examination of liver and kidney samples showed more pathological changes of greater severity in organs from control than from vaccinated animals.

For the post-challenge, the difference between vaccinated and control animals was significant regarding the total clinical score, the number of days that the challenge organisms were detected in blood and the number of liver and kidney samples in which the organism was detected.

L. Icterohaemorrhagiae

All six vaccinated animals seroconverted. After challenge, only control animals showed typical clinical signs of canine leptospirosis such as apathy (6 out of 6 animals), mild to moderate anorexia (6/6), fever (4/6), dehydration (5/6), conjunctivitis (4/6) and jaundice (4/6) starting four days after challenge and persisting until Day 28.

Increases in creatinine (1 out of 6 control animals), AST (3/6), ALT (3/6) and ALP (2/6) and increase of ALT in two vaccinates were detected but the difference was not significant.

Increases in WBC above the upper limit of the physiological range were detected in four control animals but in none of the vaccinated dogs.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals.

Macroscopic and microscopic examination of liver and kidney samples showed more pathological changes of greater severity in organs from control than from vaccinated animals.

For the post-challenge, the difference between vaccinated and control animals was significant regarding the total clinical score, the mean total haematological score, the number of days that the challenge organism was detected in blood and the number of liver and kidney samples in which the organism was detected.

Conclusion:

Twenty-five days after vaccination of naïve dogs from six weeks of age according to the vaccination scheme with batches of Versican Plus DHPPi/L4R of minimum potency, the animals were protected

against challenge with heterologous *Leptospira* strains that resulted in typical signs of leptospirosis in control animals. These findings are also applicable to Versican Plus Pi/L4R.

Influence of maternal antibodies on the efficacy of the vaccine

The influence of maternally derived antibodies (MDA) against CPiV, rabies and *Leptospira* was investigated in laboratory and field studies.The data show that MDA against *Leptospira* could not be detected in puppies from four weeks of age and that the interference of MDA against CPiV does not play a considerable role. Therefore, a corresponding warning in the SPC is unnecessary.

As regards rabies virus, a small number of puppies (4/44) showed antibodies against rabies virus before their first vaccination (field study). Although the influence of MDA against rabies virus does not seem to play an important role, it should be noted that in the field study 8% of the animals did not seroconvert after vaccination and more than 25% of the animals did not reach 0.5 IU/ml. This should be taken into consideration when vaccinating young dogs against rabies for the first time.

Duration of immunity (DOI)

Ten laboratory challenge studies in dogs were performed to demonstrate 1-year DOI for the CPiV, rabies virus and *Leptospira* components of Versican Plus Pi/L4R.

CPiV

Sixteen 6–7 week old dogs (10 with 6 control dogs), tested seronegative against CPiV were administered the vaccine Versican Plus DHPPi/L4R subcutaneously at Day 0 and 21. They were challenged intranasally with the challenge strain CPiV D008 at 12 months after vaccination.

As controls were co-housed with vaccinated animals and therefore served as sentinels, it can be concluded that no concurrent infections with CPiV boosted the immunity in vaccinated animals before challenge.

After challenge the vaccinated group showed no clinical signs and no increase of rectal temperature.

Virus excretion: 70% started excreting CPiV from Day 2 until Day 4 after challenge (duration 1–5 days). The number of days of viral shedding was significantly higher in control than in vaccinated animals.

In the control group 67% showed respiratory signs (mild or moderate nasal discharge, mild or moderate ocular discharge) 3–7 days after challenge. Additionally, sneezing was seen for two days in one of the four control animals with discharge. No coughing was observed. There was no increase in rectal temperature and no virus excretion before challenge. 100% excreted virus starting from 2 to 3 days after challenge. There was a further increase of CPiV antibody titres. On virus isolation no CPiV-excretion was found prior to challenge whereas 8/10 vaccinated dogs excreted CPiV for 1–4 days starting from 2 days until 6 days after challenge. The duration of virus excretion was found to be significantly lower than in the controls.

As regards immunogenicity Ph. Eur. monograph 1955 states: “The vaccine complies with the test if the scores for coughing or virus excretion for the vaccinated dogs are significantly lower than in the controls.” However, in this study no coughing could be observed in the control group.

This clinical sign is not induced in the challenge model used by the applicant. Signs following experimental infection with CPiV are known to be very mild and clinical signs observed in the field are mostly due to concurrent (secondary bacterial) infection. A more severe challenge model could only be

achieved in dogs from the Bioveta SPF colony which is free of respiratory pathogens by introducing a second pathogen (e.g. *Bordetella bronchiseptica*), but this may interfere with the assessment of the efficacy against CPiV. As the monograph states that scores for *either* coughing or virus excretion need to be significantly lower than in controls the CVMP concluded that this wording implies that only one of the two options (i.e. coughing or virus excretion) needed to be fulfilled for the vaccine to comply with the test.

However, taking into consideration that the challenge was very mild, it cannot be excluded that in case of a severe challenge even the vaccinated animals would have developed mild clinical signs. Therefore, more detailed information as regards the claim for CPiV, i.e. “to prevent clinical signs (nasal and ocular discharge)” has been added to the SPC.

Twelve months after the second vaccination the antibody titres have declined drastically (one dog 8, one dog 16, one dog 32, one dog 2 and 6 dogs < 2). However, the animals were protected against a challenge with a virulent CPiV challenge. Based on existing knowledge it can be assumed that in this case cellular immunity plays an important role in protection.

Rabies virus

Forty-two 9–10-week-old dogs, tested seronegative against rabies virus were split into two groups. Thirty dogs had a vaccination at Day 0 with Versican Plus DHPPi/L4 and at Day 21 with Versican Plus DHPPi/L4R.

Twelve control dogs: vaccination at Days 0 and 21 with Versican Plus DHPPi/L4. Four animals died and one animal was withdrawn during the study for reasons unrelated to vaccination. Twelve months after the 2nd vaccination (26 animals of the vaccinated group and 10 dogs of the control group) were challenged intramuscularly.

<u>After vaccinations:</u>

Clinical signs: 3 animals (0257, 0319, 0339) vaccinated first with Versican Plus DHPPi/L4 and revaccinated with Versican Plus DHPPi/L4R showed swellings for 4 days at the injection site following vaccinations with Versican Plus DHPPi/L4 (0257, 0319) and Versican Plus DHPPi/L4R (0319, 0339). One out of 12 control dogs developed a swelling for 4 days at the injection site following the first vaccination. Rectal temperature: no increases above the upper limit (39.5 °C).

A significant difference between vaccinates and controls was observed from 6 days post-vaccination until the day of challenge.

At the time of challenge, 14 out of 26 (54%) Versican Plus DHPPi/L4R vaccinated animals showed antibody titres against rabies virus of less than 0.5 IU/ml. Control animals remained seronegative for rabies virus until challenge.

<u>After challenge:</u>

Vaccinates: Clinical signs: none.

Virus isolation: no rabies virus was found.

Control group: Clinical signs: 8/10 control animals showed signs of the paralytic phase of rabies such as depression, coma, paresis, paralysis and were euthanised. One of these 8 died before it could be euthanised. Other typical signs of rabies seen in these animals were barking, irritability, restlessness, strabismus and/or seizures. One animal showed aggression and seizures, which are signs of rabies, but did not display signs of paralytic disease, before it was found dead. Another control animal did not show any abnormal clinical signs before it was found dead.

Virus isolation from brain tissue (fluorescent antibody test):10/10 controls were rabies virus positive.

This study fulfils the requirements of Ph. Eur. monograph 0451 and is considered acceptable. Duration of immunity for rabies virus for at least one year was demonstrated.

However, it should be noted that 12 months after one vaccination with Versican Plus DHPPi/L4R 14/26 (53.8%) dogs had titres below 0.5 IU/ml. It should be noted that inn case of antibody titres against rabies virus ranging from 0.1 to 0.5 IU/ml, the risk to develop rabies after contact with rabies challenge or wild-type virus is clearly higher. The probability of infection increases when travelling to high risk areas. Then additional rabies vaccinations or vaccination of dogs younger than 8 weeks might be necessary. Corresponding information has been included in the SPC.

Please refer also to the comments on the field study and the vaccination scheme.

Leptospira

The study design for each *Leptospira* component was as follows. Twelve 6-week-old dogs (6 with 6 control dogs), tested seronegative against the principle serovars of *Leptospira,* were subcutaneously administered the vaccine Versican Plus DHPPi/L4R at Day 0 and 21. They were challenged by conjunctival and by intraperitoneal route at 12 months after vaccination. After challenge they were observed for clinical signs, measurement of rectal temperature, blood samples for serology, for haematology, biochemistry and isolation of the challenge organism, urine samples for isolation of the challenge organism. Twenty-eight days after challenge they were euthanized and the liver and kidneys examined macroscopically and microscopically and tested for the presence of the challenge organism.

Results:

L. Bratislava

After challenge, five out of six control animals showed typical clinical signs of canine leptospirosis such as apathy (1 out of 6 animals), mild to moderate anorexia (2/6), dehydration (2/6) and conjunctivitis (4/6). Vaccinated animals did not show any abnormal clinical signs.

No noteworthy changes in biochemical and haematological parameters were observed.

Post-challenge *Leptospira* were re-isolated from the blood of all control animals (100%) and from urine, kidney and liver of fife out of six control animals (83.3%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals.

Macroscopic and microscopic examination of liver and kidney samples showed more gross pathological and more severe histopathological changes in organs from control than from vaccinated animals.

For the post-challenge, the difference between vaccinated and control animals was significant regarding the total clinical score, the number of days that the challenge organism was detected in blood and the number of liver and kidney samples in which the organism was detected.

It could be shown that Versican Plus DHPPi/L4R (respectively Pi/L4R) prevented clinical signs, infection and excretion caused by *L.* Bratislava following two administrations, in seronegative animals from six weeks of age for at least one year after completion of the basic immunisation.

L. Canicola

After challenge, three out of six control animals showed typical clinical signs of canine leptospirosis such as apathy (1 out of 6 animals), mild to moderate anorexia (2/6), fever (2/6) and dehydration (2/6).

Vaccinated animals did not show any abnormal clinical signs.

No noteworthy changes in biochemical parameters were observed. An increase in WBC count was detected in one vaccinated animal five days post-challenge. A decrease in thrombocyte numbers was seen in four out of six control and one vaccinated animal.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals except for one vaccinated animal that had positive blood samples three and five days after challenge.

Macroscopic and microscopic examination of liver and kidney samples showed more pathological changes of greater severity in organs from control than from vaccinated animals.

For the post-challenge the difference between vaccinated and control animals regarding the total clinical score was not significant. For the number of days that the challenge organisms were detected in blood and the number of liver and kidney samples in which the organism was detected the difference between vaccinated and control animals was significant.

It could be shown that Versican Plus DHPPi/L4R (respectively Pi/L4R) prevented excretion and clinical signs and reduced infection caused by *L.* Canicola following two administrations, in seronegative animals from six weeks of age for at least one year after completion of the basic immunisation.

L. Grippotyphosa

After challenge, four out of six control animals showed typical clinical signs of canine leptospirosis such as mild anorexia (1/6), fever (3/6), dehydration (1/6), conjunctivitis (2/6), diarrhoea (1/6) and jaundice (1/6).

Vaccinated animals did not show any abnormal clinical signs including fever after challenge except for one animal with jaundice. However, *Leptospira* could not be isolated from blood, urine or organs of this animal.

There were increases in the biochemical variable AST, ALT and creatinine detected in control and vaccinated animals pre- and post-challenge; but none of the increases observed pre- and post-challenge was considered to be clinically relevant.

Decreases in thrombocyte numbers were seen in 4 control animals; the decrease was noticeable in only one control animal reaching >50%. The animal had developed jaundice after challenge.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). In vaccinated animals, *Leptospira* were detected in the blood of two animals for one and two days post-challenge, respectively. The vaccinated animal with two positive blood samples post-challenge also tested positive for *Leptospira* in urine for one day 14 days post-challenge, and in kidney and liver.

Macroscopic and microscopic examination of liver and kidney samples showed more gross pathological and more severe histopathological changes in organs from control than from vaccinated animals.

The difference between vaccinated and control animals was significant regarding the number of days that the challenge organisms were detected in blood and the number of liver and kidney samples in which the organism was detected.

It could be shown that Versican Plus DHPPi/L4R (respectively Pi/L4R) prevented clinical signs and reduced excretion and infection caused by *L.* Grippotyphosa following two administrations, in seronegative animals from six weeks of age for at least one year after completion of the basic immunisation.

L. Icterohaemorrhagiae

After challenge, three out of six control animals showed typical clinical signs of canine leptospirosis such as apathy (2 out of 6 animals), mild to moderate anorexia (2/6) and fever (3/6).

Vaccinated animals neither showed abnormal clinical signs nor hyperthermia after challenge.

A more than 100% increase from the pre-challenge baseline value in ALP was noted in one control animal with clinical signs three days post-challenge. An increase in AST of > 25–50% was detected in the control animal with hyperthermia two days post-challenge. A decrease in thrombocyte numbers was seen in four out of six controls, including the three animals which showed clinical signs and hyperthermia post-challenge, and one vaccinated animal.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals except for two vaccinated animal that had positive blood samples three days after challenge.

Macroscopic and microscopic examination of liver and kidney samples showed more pathological changes of greater severity in organs from control than from vaccinated animals.

The difference between vaccinated and control animals was significant regarding the number of days that the challenge organisms were detected in blood and the number of liver and kidney samples in which the organism was detected.

It could be shown that Versican Plus DHPPi/L4R (respectively Pi/L4R) prevented excretion and clinical signs and reduced infection caused by *L.* Icterohaemorrhagiae following two administrations, in seronegative animals from six weeks of age for at least one year after completion of the basic immunisation.

Immunity after revaccination – response to booster (RTB)

To demonstrate protective immunity of the components of Versican Plus Pi/L4R following re-vaccination (annual booster) with a single dose 12 months after completion of the primary vaccination course laboratory response-to-booster (RTB) studies in dogs were performed. Protective immunity following an annual booster was demonstrated by challenge.

CPiV

Eighteen 6 weeks old dogs (12 with 6 control dogs) tested seronegative against CPiV were administered the vaccine Versican Plus DHPPi/L4R subcutaneously at Day 0 and 21 and one year after the 2nd vaccination. They were challenged intranasally with the CPiV D008 at Day 21 after the one year booster.

After challenge they were observed for clinical signs, measurement of rectal temperature, serology at Day 14 after challenge, nasal swabs from 2 to 10 days after challenge for virus isolation of the challenge organism.

As controls were co-housed with vaccinated animals and therefore served as sentinels, it can be concluded that no concurrent infections with CPiV boosted immunity in vaccinated animals before challenge.

After challenge the vaccinated group showed no clinical signs and no increase of rectal temperature. There was a further increase of antibody titres against CPiV.

The number of days of viral shedding was significantly lower in the vaccinates (42% excreted CPiV on a single day 3 days after challenge) than in the controls (83% excreted virus starting from 2 to 5 days after challenge).

As regards the CPiV component the applicant proposed the following claim: “prevent clinical signs and reduce viral excretion caused by canine parainfluenza virus”.

The challenge data clearly demonstrated that there were no clinical signs in the vaccinated group. However, taking into consideration that the challenge was very mild (no coughing, only mild nasal and ocular discharge in the control group) it cannot be excluded that in case of a severe challenge even the vaccinated animals would have developed mild clinical signs. Therefore, the claim for CPiV has been amended. More detailed information has been included: “to prevent clinical signs (nasal and ocular discharge)”.

As regards the conclusion that the minimum protective titre for CPiV is 1:32, it should be taken into consideration that it was only one animal which was protected by this titre. All other animals showed higher titres. From a statistical point of view this conclusion cannot be supported. Basing the minimum protective titre for CPiV on the titre of one animal alone cannot be accepted. As protection also depends on other factors (e.g. general health status, individual immune system, cellular immunity) it is basically very difficult to determine fixed protective titres for the antigens.

Twelve months after the second vaccination the antibody titres have declined drastically (one dog 32, three dogs 8, three dogs 4, three dogs 2 and two dogs < 2). However, after a single booster vaccination the titres increased again until challenge (the titres were comparable to those observed three weeks after primary vaccination course) and the animals were protected against a challenge with a virulent CPiV.

Leptospira

The study design for each *Leptospira* component was as follows. Twelve 6-week-old dogs (6 with 6 control dogs), tested seronegative against the principle serovars of *Leptospira*, were subcutaneously administered the vaccine Versican Plus DHPPi/L4R at Day 0 and 21 and one year (the control group received vaccination only at Day 0 and 21). They were challenged by conjunctival and by intraperitoneal route at 12 months after vaccination. After challenge they were observed for clinical signs, measurement of rectal temperature, blood samples for serology, for haematology, biochemistry and isolation of the challenge organism, urine samples for isolation of the challenge organism. Twenty-eight days after challenge they were euthanized and the liver and kidneys examined macroscopically and microscopically and tested for the presence of the challenge organism.

Results:

L. Bratislava

After challenge, four out of six control animals showed typical clinical signs of canine leptospirosis, such as anorexia (1 out of 6 animals), dehydration (3/6), conjunctivitis (1/6) and jaundice (1/6). In two control animals and in one vaccinated animal the body temperatures exceeded the upper physiological limit of 39.5 °C slightly. Apart from this, no abnormal clinical signs were found in vaccinated dogs.

No noteworthy changes in biochemical and haematological parameters were observed.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of five out of six control animals (83.3%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals.

Findings of macroscopic and microscopic examinations of liver and kidney samples were inconclusive.

Concerning the post-challenge, the difference between vaccinated and control animals was significant regarding the number of days that the challenge organism was detected in blood and urine and the number of liver and kidney samples in which the organism was detected.

It could be shown that Versican Plus DHPPi/L4R (respectively Pi/L4R) prevented clinical signs, infection and excretion caused by *L.* Bratislava following booster vaccination one year after primary vaccination to animals from six weeks of age.

L. Canicola

After challenge, four out of six control animals showed typical clinical signs of canine leptospirosis such as apathy (2 out of 6 animals), anorexia (4/6), conjunctivitis (3/6), jaundice (1/6) and dehydration (3/6). In two control animals the body temperatures exceeded the upper physiological limit of 39.5 °C.

In the vaccinated animals no abnormal clinical signs and temperature increases were observed.

No noteworthy changes in biochemical parameters were observed. An increase in WBC count was detected in all control animals and in four vaccinated animal after challenge. A decrease in thrombocyte numbers was seen in three out of six control animals and one vaccinated animal.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals.

Findings of macroscopic and microscopic examinations of liver and kidney samples were inconclusive.

Concerning the post-challenge, a significant difference was found between total clinical scores of male vaccinated and male control animals, but not between female animals because the two female control dogs did not show any abnormal clinical signs. The difference between vaccinated and control animals was significant as regards the number of days that the challenge organisms were detected in blood and urine and the number of liver and kidney samples in which the organism was detected.

It could be shown that Versican Plus DHPPi/L4R (respectively Pi/L4R) prevented clinical signs, excretion and infection caused by *L.* Canicola following booster vaccination one year after primary vaccination to animals from six weeks of age.

L. Grippotyphosa

After challenge, five out of six control animals showed typical clinical signs of canine leptospirosis such as anorexia (2 out of 6 animals), dehydration (4/6), vomiting (2/6), diarrhoea (1/6) and jaundice (1/6). Body temperatures exceeding the upper physiological limit of 39.5 °C were not observed in any control animal after challenge.

Vaccinated animals did not show any abnormal clinical signs including fever after challenge.

Except for an increase of AST in three out of six control animals and one vaccinate no clinically relevant post-challenge changes in any of the biochemical variables were seen in the other control animals and in the vaccinates.

No increases in WBC counts above the physiological range and no decreases in thrombocyte numbers were detected neither in control animals nor in vaccinated animals.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of five out of six control animals (83%).

No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals after challenge.

Findings of macroscopic and microscopic examinations of liver and kidney samples were inconclusive.

Concerning the post-challenge, a significant difference was found between total clinical scores of vaccinated and control animals. The difference between vaccinated and control animals was significant as regards the number of days that the challenge organisms were detected in blood and urine and the number of liver and kidney samples in which the organism was detected.

It could be shown that Versican Plus DHPPi/L4R (respectively Pi/L4R) prevented clinical signs, excretion and infection caused by *L.* Grippotyphosa following booster vaccination one year after primary vaccination to animals from six weeks of age.

L. Icterohaemorrhagiae

After challenge, five out of six control animals showed typical clinical signs of canine leptospirosis such as apathy (1 out of 6 animals), anorexia (2/6), dehydration (3/6), conjunctivitis (2/6) and jaundice (3/6). In two control animals the body temperatures exceeded the upper physiological limit of 39.5 °C.

Vaccinated animals neither showed abnormal clinical signs nor hyperthermia after challenge.

No noteworthy changes in biochemical and haematological parameters were observed.

Post-challenge *Leptospira* were re-isolated from the blood, urine, kidney and liver of all control animals (100%). No *Leptospira* were detected in the blood, urine, kidney or liver of vaccinated animals except for two vaccinated animals which had positive blood samples for one day two days after challenge.

Findings of macroscopic and microscopic examinations of liver and kidney samples were inconclusive.

Concerning the post-challenge, a significant difference was found between total clinical scores of vaccinated and control animals. The difference between vaccinated and control animals was significant as regards the number of days that the challenge organisms were detected in blood and urine and the number of liver and kidney samples in which the organism was detected.

It could be shown that Versican Plus DHPPi/L4R (respectively Pi/L4R) prevented excretion and clinical signs and reduced infection caused by *L.* Icterohaemorrhagiae following booster vaccination one year after primary vaccination to animals from six weeks of age.

Additional studies

Compatibility

No studies on immunological compatibility of Versican Plus Pi/L4R with other products were undertaken. Section 4.8 of the proposed SPC for Versican Plus Pi/L4R contains the following text:

"No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. 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 by the veterinarian."

In view of this text, data on the concurrent administration of Versican Plus Pi/L4R with other veterinary medicinal products are not required for this application. The justification and the proposed text of the SPC are acceptable.

Field trials

The applicant performed a multi-centre, positively controlled, randomised, blinded field study in two countries (France and Germany), in compliance with CVMP/VICH/595/98 "VICH Topic GL9 Step 7 - Guideline on Good Clinical Practices".

Field trials (cohort study 1, cohort study 2 and cohort study 3) were carried out in 3 centres in France (FR) and 3 centres in Germany (DE). A total of 128 dogs (FR 63, DE 65) were included in the field trials, i.e. 45 mixed bred and 83 pure bred dogs of 28 breeds including toy breeds, utility/hunting breeds and large breeds; 50 females, 23 neutered females, 41 males and 14 neutered males.

Cohorts were composed as follows:

- Cohort 1: 54 naïve dogs (FR 27, DE 27) with an age range of 8 weeks to 15 years. The dogs were administered two doses of vaccine (V1= Versican Plus DHPPi/L4; V2= Versican Plus DHPPi/L4R) 3–4 weeks apart followed by the owner observations;
- Cohort 2: 41 dogs (FR 21, DE 20) with an age range of 1 year to 11 years. The dogs were administered one annual booster vaccination (Versican Plus DHPPi/L4R), followed by the owner observations;
- Cohort 3: 33 naïve puppies (FR 15, DE 18) with an age range of 8 to 9 weeks. The dogs were administered two doses of vaccine (V1= Versican Plus DHPPi/L4; V2= Versican Plus DHPPi/L4R) 3–4 weeks apart, followed by observations through trained personnel.

For ethical reasons no unvaccinated dogs were included in the study and competitor vaccines were used in the controls for antibody comparison. Competitors vaccines used in France were Enduracell 7 and Enduracell 8 and in Germany were Vanguard 7 and Vanguard R.

Serological control tests were performed on cohort 1 and 3 before the first and the second vaccination (V1 and V2) (on the same day of vaccinations) and 21 days after the second vaccination (V2+21). Serological control tests were performed on cohort 2, before the annual booster vaccination (V1) (on the same day of vaccination) and 21 days after it (V1+21). Efficacy was assessed by measuring antibody responses and comparing titres before and after vaccination with Versican Plus DHPPi/L4R or the comparator vaccine. The antibody response by means of seroneutralisation (SN) test, fluorescent antibody virus neutralisation (FAVN) test and microscopic agglutination test (MAT) was categorised as follows:

- No increase.
- Increase 1: < 2-fold increase of CPiV antibodies (by SN)
  < 4-fold increase of rabies antibodies (by SN) and < 0.5 IU/ml (by FAVN)
  < 4-fold increase of *Leptospira* antibodies (by MAT)
- Increase 2: ≥ 2-fold increase of CPiV antibodies (by SN)
  ≥ 4-fold increase of rabies antibodies (by SN) and > 0.5 IU/ml (by FAVN)
  ≥ 4-fold increase of *Leptospira* antibodies (by MAT).

Only results relevant to the components of Versican Plus Pi/L4R are summarised below.

Results in naïve puppies

Forty-four dogs aged from 8 weeks to 6 months, (without a previous history of vaccination were selected from cohort 1 (11 dogs, 7 of which vaccinated with Versican Plus DHPPi/L4R and 4 with a

competitor vaccine) and cohort 3 (33 dogs vaccinated with Versican Plus DHPPi/L4R). Less than 10% of the puppies had MDA against rabies virus, *Leptospira* and CPiV at the time of the first vaccination. Serological results are reported hereafter:

Puppies without MDA:

- 100% of the puppies showed full serological response (Increase 2) against the live viral component CPiV, and the inactivated bacteria *L.* Canicola and *L.* Icterohaemorrhagiae
- 11% of the puppies did not respond to *L.* Grippotyphosa and *L.* Bratislava (no increase).
- 8% of the puppies did not respond to rabies virus; 19% of puppies did not reach 0.5 IU/ml (Increase 1).
- The proportions of puppies without MDA responding to Versican Plus DHPPi/L4R were greater and their responses generally higher than those following vaccination with comparator products.

Puppies with MDA:

- Serological results for CPiV, *L.* Bratislava and *L.* Grippotyphosa did not allow more general conclusions as there were only three MDA positive animals. MDA titres were moderately high before the first vaccination and remained stable (CPiV) or increased after the second vaccination (*L*. Bratislava and *L.* Grippotyphosa) indicating a response to primary immunisation.
- Only 1 dog showed full serological response (increase 2) against rabies.

Results in naïve dogs (adults and puppies)

Fifty-four unvaccinated dogs (cohort 1) divided in: 43 dogs over 6 months of age without a previous history of vaccination or with a previous history of vaccination that had lapsed by more than 14 months and 11 naïve puppies younger than 6 months, showed the following serological results:

Dogs without pre-existing antibodies:

- 100% showed full serological response (Increase 2) against *L*. Canicola and *L*. Icterohaemorrhagia.
- One dog did not respond to the CPiV component (no increase).
- On dog showed a very low serological response (Increase 1) against *L*. Bratislava.
- Three dogs showed an antibody titre increase against rabies virus that did not reach 0.5 IU/ml. Two were puppies and are discussed above. One was an adult and reached a titre of 0.39 IU/ml after immunisation which is considered protective.

Dogs with pre-existing antibodies:

- The proportion of dogs with pre-existing antibodies showed lower serological response (Increase 1 or no increase) if compared to dogs without pre-existing antibodies.

Results in previously vaccinated adult dogs

Forty-one dogs of more than 6 months of age, with a previous history of vaccination and requiring an annual booster (cohort 2), showed the following serological results:

Dogs without pre-existing antibodies:

- 100% showed full serological response (Increase 2) against CPiV, *L.* Canicola and *L.* Icterohaemorrhagiae.
- One dog showed a very low serological response (Increase 1) against *L*. Bratislava.

- Two dogs showed no increase serological response to *L*. Bratislava and *L*. Grippotyphosa.
- Two dogs showed low serological response (Increase 1) against rabies virus that did not reach 0.5 IU/ml.

Dogs with pre-existing antibodies:

- The proportion of dogs with pre-existing antibodies showed lower serological response (Increase 1 or no increase) if compared to dogs without pre-existing antibodies.

Conclusions

Evaluable serological data from 86 (out of 128) animals were generated. Since antibody titres from field and laboratory studies were determined using the same assay systems in the same laboratory, it was possible to directly compare field titres with minimum protective titres established in laboratory studies.

The applicant summarised all serological data irrespective of their antibody status pre-vaccination via descriptive statistics and compared the minimally induced antibody titre per antigen with the titre that was fixed as minimum protective titre in the challenge studies (16 for CPiV, 0.17 UI/ml for rabies, 16 for *L.* Bratislava and 32 for the other three *Leptospira*).

The percentages of dogs that were protected in the case of an infection are presented as follows.

For CPiV, 97% (36 out of 37) of the adult dogs from cohort 1 responded with titres ≥ 16 to primary immunisation (V1 + V2). In cohorts 2 and 3, respectively, 11% and 27% of dogs, the majority part of which were seronegative before vaccination, showed antibody titres between 2 and 16 after vaccination.

For rabies, 100% of dogs from cohort 2 were protected following an annual booster vaccination and all adult dogs from cohort 1 were protected following a primary immunisation with Versican Plus DHPPi/L4R. Due pre-existing MDA, 3% of the puppies from cohort 1 and 14% from cohort 3 did not respond with protective antibody levels against rabies.

For *Leptospira*, the majority of dogs in all cohorts responded to vaccination with Versican Plus DHPPi/L4R. Dogs from cohort 2 without pre-existing antibodies required a second vaccination to achieve full protection. In cohorts 1 and 3, a few dogs responded to vaccination with increases albeit below 16 for *L.* Bratislava and 32 for the other three *Leptospira*. As antibody titres against *Leptospira* do not greatly correlate with protection, animals that responded with an antibody titre increase to vaccination may still be protected. However, it is questionable whether dogs that did not show any increase following vaccination are protected.

From the CVMP point of view, the following critical issues were identified:

a) The youngest dog that received the rabies component was 11 weeks old.

b) The joint evaluation of sub-groups (cohort 1: dogs > 6 months subdivided into a) non-vaccinated, seronegative and b) vaccinated more than 14 months ago, seronegative) is not acceptable from an immunological point of view. Seronegative animals with a previous vaccination history, so-called primed animals, react immunologically differently compared to naïve seronegative animals.

c) As regards the rabies component, 10% did not seroconvert and 17% of the seronegative dogs did not reach 0.5 IU/ml after a single vaccination at 11 weeks. Since no efficacy (challenge) data from animals that remained seronegative after vaccination are available, protection against infection cannot be expected. Furthermore, as stated by the applicant, it is known that dogs with a poor immune response to vaccination against rabies after a single vaccination can be observed in the

field (especially dogs of large breeds were found to be poor responders to rabies vaccination). Therefore, the efficacy of a single vaccination against rabies is questionable.

Consequently, the immunisation scheme has been amended taking into account the results of the field trial.

It should be noted that 17 out of 33 seronegative dogs did also not seroconvert against CPiV after the first vaccination and 2 dogs even remained seronegative after the second vaccination.

The data show that the interference of MDA against rabies and *Leptospira* does not play a considerable role. Therefore, a corresponding warning in the SPC is unnecessary.

Leptospira: The CVMP could not fully agree with the applicant's conclusions regarding the minimum protective titres for the *Leptospira* components in puppies which were pre-challenged, at least 32 against *L.* Canicola, Grippotyphosa and Icterohaemorrhagiae and 16 against *L.* Bratislava, 3 weeks after the second vaccination with Versican Plus DHPPi/L4R. Minimum protective titres for the *Leptospira* components do not exist although these titres may still be associated with protection from clinical signs, infection and urinary excretion following challenge. Thus, the correlation between antibody titre and protection is tenuous and the proposed claims regarding the *Leptospira* components were revised.

Overall conclusion on efficacy

Versican Plus Pi/L4R is intended for use in dogs from 8–9 weeks of age.

The minimum protective dose is indicated below:

Component	Minimum potency/ Antigen content
Freeze-dried fraction (live attenuated):	
Canine parainfluenza type 2 virus, strain CPiV-2-Bio 15	$10^{3.1}$ $TCID_{50}$[1]
Liquid fraction (inactivated):	
Leptospira interrogans serovar Bratislava, strain MSLB 1088	GMT[2] ≥ 1:51 ALR[3]
Leptospira interrogans serovar Canicola, strain MSLB 1090	GMT ≥ 1:51 ALR
Leptospira kirschneri serovar Grippotyphosa, strain MSLB 1091	GMT ≥ 1:40 ALR
Leptospira interrogans serovar Icterohaemorrhagiae, strain MSLB 1089	GMT ≥ 1:51 ALR
Rabies virus, strain SAD Vnukovo-32	≥ 2.0 IU[4] 4.4 µg/ml[5]

1 $TCID_{50}$ is the quantity of the virus that will produce a cytopathic effect in 50% of the cultures inoculated
2 Geometric mean titre
3 Antibody agglutination-lytic reaction
4 International units
5 Glycoprotein content before blending

The proposed minimum titres for the virus components are acceptable.

Leptospira

Indication

The proposed claims regarding the *Leptospira* components are acceptable as follows:

- to prevent clinical signs, infection and urinary excretion caused by *L. interrogans* serogroup Australis serovar Bratislava,
- to prevent clinical signs and urinary excretion and reduce infection caused by *L. interrogans* serogroup Canicola serovar Canicola and *L. interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae,
- to prevent clinical signs and reduce infection and urinary excretion caused by *L. interrogans* serogroup Grippotyphosa serovar Grippotyphosa.

This could be shown for at least one year after completion of the basic immunisation.

Maternally derived antibodies (MDA)

Several studies have been performed to assess the possible influence of MDA on the antibody response to the antigens of Versican Plus Pi/L4R. The data show that MDA against *Leptospira* could not be detected in puppies from four weeks of age and the interference of MDA against rabies virus and CPiV does not play a considerable role. Therefore, a corresponding warning in the SPC is unnecessary.

The following vaccination scheme is justified:

Subcutaneous use.

Dosage and route of administration:

Aseptically reconstitute the lyophilisate with the solvent. Shake well and administer immediately the entire content (1 ml) of the reconstituted product.

Primary vaccination scheme:

Two doses of Versican Plus Pi/L4R 3–4 weeks apart from 8–9 weeks of age. The second dose should not be given before 12 weeks of age.

Rabies

The efficacy of the rabies fraction is proven after a single dose from 12 weeks of age in laboratory studies. Therefore, the first dose may be given using Versican Plus Pi/L4. In this case the second vaccination with Versican Plus Pi/L4R should not be given before 12 weeks. However, in field studies 10% of seronegative dogs did not show seroconversion (> 0.1 IU/ml) 3–4 weeks after single primary vaccination against rabies. Another 17% did not show the 0.5 IU/ml antibody titre against rabies virus required by some non-EU countries to travel in. In case of travelling to risk areas or for travel outside the EU veterinary surgeons may wish to use a two dose primary course including rabies or give an additional rabies vaccination after 12 weeks.

In case of need, dogs younger than 8 weeks can be vaccinated as safety of this product has been demonstrated in 6 weeks old dogs.

Re-vaccination scheme:

A single dose of Versican Plus Pi/L4R to be given annually.

Part 5 – Benefit-risk assessment

Introduction

Versican Plus Pi/L4R is a multivalent vaccine which is indicated for the immunisation of healthy puppies and dogs against canine parainfluenza, rabies and leptospirosis. The live virus component of the vaccine (canine parainfluenza virus (CPiV)) is presented in freeze-dried form in a vial to be reconstituted with a vial of the inactivated components (rabies virus, *Leptospira* Bratislava*, L.* Canicola, *L.* Grippotyphosa and *L.* Icterohaemorrhagiae) which are presented in liquid form. The liquid fraction also contains the adjuvant (aluminium hydroxide).

Versican Plus Pi/L4R is a fixed combination containing six active substances as detailed in the introduction.

The vaccine components are directed against canine infectious diseases present and widespread in most European countries. Versican Plus Pi/L4R is a fall-out formulation of Versican Plus DHPPi/L4R with fewer components to allow for choice of vaccination scheme based on risk. Canine parainfluenza virus (CPiV) infects the canine respiratory tract and on its own usually only cause mild disease. In combination with bacterial pathogens such as *Bordetella bronchiseptica*, however, they can result in kennel cough. In rare cases, severe bronchopneumonia may occur. Kennel cough is highly contagious and can persist for many weeks, and thus of great concern.

Rabies is a notifiable disease and a zoonosis which inevitably always results in death.

Leptospirosis is a zoonotic disease with a variable clinical presentation. Infections can be subclinical or cause acute to chronic renal or hepatic disease which may result in death. An acute icterohaemorrhagic form is known as Weill's disease. Subclinically infected dogs or dogs that survive acute disease may shed the organism via urine and thereby serve as reservoirs of infection for other dogs and even for humans. Historically, *L.* Canicola and *L.* Icterohaemorrhagiae were seen as the primary causative agents of canine leptospirosis and most canine *Leptospira* vaccines include only these two serovars. In recent years, however, *L.* Bratislava and *L.* Grippotyphosa have been identified with increasing frequency as pathogens of dogs in Europe. This has resulted in the inclusion of these two serovars in novel canine vaccines.

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

Benefit assessment

Direct therapeutic benefit

Controlled clinical trials demonstrated that the product is efficacious for the following indications:

Active immunisation of dogs from 8–9 weeks of age:

- to prevent clinical signs (nasal and ocular discharge) and reduce viral excretion caused by canine parainfluenza virus,
- to prevent clinical signs, infection and urinary excretion caused by *L. interrogans* serogroup Australis serovar Bratislava,

– to prevent clinical signs and urinary excretion and reduce infection caused by *L. interrogans* serogroup Canicola serovar Canicola and *L. interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae,

– to prevent clinical signs and reduce infection and urinary excretion caused by *L. interrogans* serogroup Grippotyphosa serovar Grippotyphosa,

– to prevent mortality, clinical signs and infection caused by rabies virus.

Onset of immunity has been demonstrated at:

- 2 weeks after a single vaccination from 12 weeks of age for rabies,
- 3 weeks after completion of the primary course for CPiV,
- 4 weeks after completion of the primary course for *Leptospira* components.

DOI has been demonstrated for at least one year after completion of the basic immunisation. DOI for rabies was demonstrated after one vaccination at 12 weeks of age.

Additional benefits

Using multivalent vaccines for dogs has some advantages for the animal related to animal welfare and the pet owner. Stress and pain for the animal are reduced as only one vaccine injection is required per visit to the veterinary practice and compliance by the pet owners is usually improved because the appointments at the veterinarian are reduced.

In addition, the use of this vaccine potentially reduces the need for antimicrobial treatment against *Leptospira* infections.

Risk assessment

Main potential risks:

Quality:

The formulation and manufacture of Versican Plus Pi/L4R is well described and specifications set will ensure that product of consistent quality will be produced.

The choice of the vaccine strains and the adjuvant has been satisfactorily addressed and reference to the relevance of each strain to current epidemiological conditions is also provided. The manufacturing process of the vaccine has been described in detail for the virus and for the *Leptospira* components. Regarding the starting materials all necessary information has been provided. The TSE risk of this product is negligible. Controls during manufacture and tests on the finished product should guarantee the compliance with the quality parameter mentioned. Test methods have been described and corresponding validation studies have been performed. Batch to batch consistency has been demonstrated and a detailed overview of all in-process and finished product tests of the vaccine Versican Plus Pi/L4R has been provided. The antigen stability study is due to complete by the end of 2014 and the final study report will be submitted in early 2015. The first 10 batches produced for commercial release will have the individual results for *Leptospira* of the tested rabbits submitted along with the batch release protocols. The final batch release protocol was provided regarding the rabies component, an ELISA method for determination of the glycoprotein value after concentration will be implemented prior to marketing of the product. With all these recommendations in place the quality of Versican Plus Pi/L4R is considered to be satisfactorily demonstrated.

Safety for the target animal

The administration of one dose and a repeated dose of Versican Plus Pi/L4R containing maximum potency of antigens by the recommended route was found to be safe for puppies of 6 weeks of age. An overdose study was performed with the live virus component (lyophilisate) showed an acceptable safety profile.

Several studies have been performed to assess the possible influence of MDA on the antibody response to the antigens of Versican Plus Pi/L4R. The presented laboratory and field studies clearly show that MDA against *Leptospira* could not be detected in puppies from four weeks of age and the interference of MDA against rabies virus and CPiV does not play a considerable role.

No studies have been performed on reproductive safety and a warning is included in the SPC. The use of the product is not recommended during pregnancy and lactation. No studies have been performed on the concurrent use of any other non-Versican Plus vaccine. This fact is covered in section 4.8 of the SPC. The possible spread and dissemination of the vaccine strain has been correctly reflected in the SPC and is acceptable. No indication of reversion to virulence during the passages was found. In the field study dogs showed signs of lethargy, vomiting, diarrhoea and anorexia after vaccination and these observations are reflected in the SPC.

User safety:

The user safety for this product is acceptable when used as recommended and taking into account the safety advice in the SPC.

Environmental risk assessment:

The product is not expected to pose any risk to the environment when used as recommended.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

Versican Plus Pi/L4R has been demonstrated to be efficacious for the active immunisation of dogs from 8–9 weeks of age:

- to prevent clinical signs (nasal and ocular discharge) and reduce viral excretion caused by canine parainfluenza virus,
- to prevent clinical signs, infection and urinary excretion caused by *L. interrogans* serogroup Australis serovar Bratislava,
- to prevent clinical signs and urinary excretion and reduce infection caused by *L. interrogans* serogroup Canicola serovar Canicola and *L. interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae,
- to prevent clinical signs and reduce infection and urinary excretion caused by *L. interrogans* serogroup Grippotyphosa serovar Grippotyphosa and
- to prevent mortality, clinical signs and infection caused by rabies virus.

Based on the laboratory and field studies the proposed vaccination scheme is acceptable. The product is well tolerated by the target animals and presents a low risk for users and the environment. Appropriate warnings have been included in the SPC and also in the product information.

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

Conclusion on benefit-risk balance

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

Conclusion

Based on the original and complementary data presented, the Committee for Medicinal Product for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of Versican Plus Pi/L4R were considered to be in accordance with the requirements of Directive 20010/82/EC.

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP recommended the granting of the marketing authorisation for Versican Plus Pi/L4R.