10 June 2014
EMA/316861/2014
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Versican Plus L4 (EMEA/V/C/003680/0000)

Common name: canine leptospirosis vaccine (inactivated)

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 marketing authorisation to the European Medicines Agency (the Agency) for Versican Plus L4, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (new active substance).

The eligibility to the centralised procedure was agreed upon by the CVMP on 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 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 L4 contains per 1 ml dose:

Liquid fraction (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

* Antibody micro agglutination-lytic reaction.

Aluminium hydroxide gel is used as an adjuvant.

Container

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 vaccine 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 (1 ml) of the vaccine.

Development pharmaceutics

The Versican Plus L4 has been developed for the prevention of infectious diseases caused by leptospiras. The product was based on the preceding vaccine Versican DHPPi/L3R. This vaccine contains three leptospiras*: Leptospira interrogans* serovar Canicola, *L. interrogans* serovar Icterohaemorrhagiae and *Leptospira kirschneri* serovar Grippotyphosa. The Versican Plus DHPPi/L4R contains a new strain of canine parvovirus (2b) and 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 inactivation and concentration/purification of the antigens. Each leptospira antigen bulk is processed separately. The liquid fraction is blended with adjuvant (aluminium hydroxide gel).

Control of starting materials

Active substance

All 4 antigens comprised in the vaccine (i.e. *Leptospira interrogans* serovars Icterohaemorrhagiae, Canicola, Bratislava and *Leptospira kirschneri* serovar Grippotyphosa antigens) are sufficiently described with regard to their origin, isolation and history. The control testing on the 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

The control tests during production include 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.

Control tests on the finished product

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

The control tests of the finished liquid fraction include appearance, sterility, air tightness, extractable volume, *Leptospira* identity and potency. A detailed overview of all in-process and finished product tests of the vaccine Versican Plus L4 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 L4 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.

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 *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 L4 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 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 L4 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.

Part 3 – Safety

Versican Plus L4 is a multivalent inactivated bacterial vaccine indicated for the immunisation of healthy puppies and dogs against leptospirosis.

The applicant presented laboratory vaccination studies (safety of a single and repeated dose, overdose) 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 were demonstrated using the largest combination of the Versican Plus range of vaccines, Versican Plus DHPPi/L4R, which also includes canine distemper virus (CDV), canine adenovirus type 2 (CAV2), canine parvovirus (CPV) and an inactivated rabies component. All components of Versican Plus DHPPi/L4R are identical to those contained in Versican Plus L4 with only the number of component 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 L4 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

The demonstration of the safety of an overdose is not required for inactivated vaccines anymore according to Directive 2009/9/EC amending Directive 2001/82/EC.

Thus, the following statement is inserted in section 4.10 of the SPC:

"No data are available on the safety of an overdose."

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. 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 L4 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.

The components of the vaccine are inactivated bacterial antigens (*Leptospira*), 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 L4 is known to have any adverse effects on the immune functions of infected animals.

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 other than Versican Plus DHPPi and Versican Plus Pi. 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

Since all components of Versican Plus L4 are inactivated, the special requirements for live vaccines (Ph. Eur. monograph 5.2.6) are not applicable to Versican Plus L4.

Study of residues

Versican Plus L4 is a vaccine which is indicated solely for use in dogs. Therefore, the consideration of residues is not applicable.

Interactions

This vaccine can be safely combined with Versican Plus DHPPi or Versican Plus Pi.

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 multinational 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 L4. 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 L4 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 4 well characterised antigens and a well-known adjuvant.

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 (respectively L4) containing maximum potency of antigens by the recommended route was found to be safe for puppies of 6 weeks of age.

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 (respectively L4) 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 L4 is known to have any adverse effects on the immune functions of infected animals.

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.

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 L4. 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 L4 is a multivalent inactivated bacterial fall-out product of the Versican Plus range of vaccines and is indicated for the immunisation of healthy puppies from six weeks of age and dogs against leptospirosis. The inactivated bacterial components (*Leptospira* Bratislava*, Leptospira* Canicola, *Leptospira* Grippotyphosa and *Leptospira* Icterohaemorrhagiae) are 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 L4 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

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.

Onset of immunity

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

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 L4.

Influence of maternal antibodies on the efficacy of the vaccine

The influence of maternally derived antibodies (MDA) against *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. Therefore, a corresponding warning in the SPC is unnecessary.

Duration of immunity (DOI)

Four laboratory challenge studies in dogs were performed to demonstrate 1-year duration of immunity for the *Leptospira* components of Versican Plus L4.

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, 5 out of 6 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 5 out of 6 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 L4) 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, 3 out of 6 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 L4) 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, 4 out of 6 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 L4) 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, 3 out of 6 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 L4) 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)

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 later (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 L4) 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 L4) 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 L4) 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 L4) 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

Versican Plus L4 is compatible with vaccines of the Versican Plus range. Versican Plus L4 can be used in combination with other products of the Versican Plus range of vaccines. The manufacturing processes of Versican Plus L4 are identical to those of Versican Plus DHPPi/L4R and its fall-out products apart from a difference in the number of active components. Consequently there is no reason to believe that vaccination with Versican Plus L4 preceded or followed by other products of the Versican Plus range of vaccines could result in any additional efficacy issues.

No studies on immunological compatibility of Versican Plus L4 with other products were undertaken. Section 4.8 of the proposed SPC for Versican Plus L4 contains the following text:

"No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product other than Versican Plus DHPPi and Versican Plus Pi. 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 L4 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: < 4-fold increase of *Leptospira* antibodies (MAT)
- Increase 2: ≥ 4-fold increase of *Leptospira* antibodies (MAT).

Only results relevant to the components of Versican Plus L4 are summarised below.

It was noteworthy that antibodies against *L*. Bratislava (in 14 dogs) and *L*. Grippotyphosa (in 6 dogs) were detected in cohort 1 before vaccination and this could have affected the results.

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). MDA against *Leptospira* (before the first vaccination) were detected only in 9% (4 out of 44) of the puppies. Serological results are reported hereafter:

Puppies without MDA:

- 100% of the puppies showed full serological response (Increase 2) against *L*. Canicola and *L*. Icterohaemorrhagiae.
- 11% of the puppies did not respond to *L*. Grippotyphosa and *L*. Bratislava.

Puppies with MDA:

- Serological results against *L*. Grippotyphosa and *L*. Bratislava and *L*. Icterohaemorrhagiae did not allow general conclusions as there was only a very limited number of MDA positive animals. MDA titres were moderately high before the first vaccination or increased after the second vaccination (*L*. Bratislava and *L*. Grippotyphosa) indicating that they responded to primary immunisation.

Results in naïve dogs (adults and puppies)

Fifty-four non vaccinated 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% of the dogs showed full serological response (Increase 2) against *L*. Canicola and *L*. Icterohaemorrhagia.
- One dog showed a very low serological response (Increase 1) against *L*. Bratislava.

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% of the dogs showed full serological response (Increase 2) against *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.

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 *L.* Bratislava and 32 for the other three *Leptospira*).

For *Leptospira*, the majority of dogs in all cohorts responded to vaccination with Versican Plus DHPPi/L4R. Dogs from cohort 2 dogs without pre-existing antibodies required a second vaccination to achieve full protection. In cohorts 1 and 3, a few dogs that 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.

Overall summary and comments:

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

a) None of the field trial animals was at minimum age (6 weeks). The youngest were 8 weeks that received the vaccine without the rabies component.

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.

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

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/L4(R). 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

Target species

Versican Plus L4 is intended for use in dogs from six weeks of age.

The minimum protective dose is indicated below:

Component	Minimum potency/ Antigen content
Leptospira interrogans serovar Bratislava, strain MSLB 1088	GMT[1] ≥ 1:51 ALR[2]
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

[1] Geometric mean titre
[2] Antibody agglutination-lytic reaction

The proposed minimum titres are acceptable.

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 and
- 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 L4. The data show that MDA against *Leptospira* could not be detected in puppies from four weeks of age. Therefore, a corresponding warning in the SPC is unnecessary.

The following vaccination scheme is justified:

Subcutaneous use.

Dose and route of administration:

Shake well and administer immediately the entire content (1 ml) of the product.

Primary vaccination scheme: two doses of Versican Plus L4 3–4 weeks apart from 6 weeks of age.

Vaccination against distemper, adenovirosis, parvovirosis and parainfluenza virus (DHPPi):

If protection against DHPPi or parainfluenza virus is required, dogs can be vaccinated with two doses of Versican Plus DHPPi or Versican Plus Pi mixed with Versican Plus L4 3–4 weeks apart from 6 weeks of age.

The contents of a single vial of Versican Plus DHPPi or Versican Plus Pi should be reconstituted with the contents of a single vial of Versican Plus L4 (instead of the solvent). Once mixed, the contents of the

vial should appear a whitish to yellowish colour with a slight opalescence. The mixed vaccines should be injected immediately via the subcutaneous route.

Revaccination scheme: a single dose of Versican Plus L4 to be given annually.

Part 5 – Benefit-risk assessment

Introduction

Versican Plus L4 is a multivalent vaccine which is indicated for the immunisation of healthy puppies and dogs against leptospirosis. The inactivated components (*Leptospira* Bratislava*, L.* Canicola, *L.* Grippotyphosa and *L.* Icterohaemorrhagiae) are presented in liquid form. The vaccine also contains the adjuvant (aluminium hydroxide).

Versican Plus L4 is a fixed combination containing four 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 L4 is a fall-out formulation of Versican Plus DHPPi/L4R with fewer components to allow for choice of vaccination scheme based on risk.

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 six weeks of age:

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

Onset of immunity has been demonstrated at 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.

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 reduces the need for antimicrobial treatment against *Leptospira* infections.

Risk assessment

Main potential risks:

Quality:

The formulation and manufacture of Versican Plus L4 is well described and specifications set will ensure that a 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 *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 L4 has been provided. Some data is needed for finalisation of reports to fully consolidate the risk assessment. 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 were submitted with the individual results for *Leptospira* of the tested rabbits submitted along with the batch release protocols. With all these recommendations in place the quality of Versican Plus L4 is considered to be satisfactorily demonstrated.

Safety for the target animal:

The administration of one dose and a repeated dose of Versican Plus L4 containing maximum potency of antigens by the recommended route was found to be safe for puppies of 6 weeks of age.

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

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. 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 L4 has been demonstrated to be efficacious for the active immunisation of dogs from 6 weeks of age

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

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 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 L4 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 L4.