8 May 2014
EMA/284173/2014
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Versican Plus DHPPi (EMEA/V/C/003679/0000)

Common name: canine distemper, canine adenovirus, canine parvovirosis and canine parainfluenza virus vaccine (live attenuated)

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

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Introduction

On 29 May 2013 the applicant Zoetis Belgium s.a. submitted an application for marketing authorisation to the European Medicines Agency (the Agency) for Versican Plus DHPPi, 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 canine distemper, adenovirus hepatitis, adenovirus respiratory disease, parvovirosis and parainfluenza.

The route of administration is subcutaneous use.

On 8 May 2014, the CVMP adopted an opinion and CVMP assessment report.

On 4 July 2014 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 DHPPi contains per 1 ml dose:

Lyophilisate (live attenuated):

Canine distemper virus, strain CDV Bio 11/A,	$10^{3.1} – 10^{5.1}$ $TCID_{50}$
Canine adenovirus type 2, strain CAV-2 Bio 13,	$10^{3.6} – 10^{5.3}$ $TCID_{50}$
Canine parvovirus type 2b, strain CPV-2b Bio 12/B,	$10^{4.3} – 10^{6.6}$ $TCID_{50}$
Canine parainfluenza type 2 virus, strain CPiV-2 Bio 15,	$10^{3.1} – 10^{5.1}$ $TCID_{50}$

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

Solvent
Water for injection 1 ml.

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 DHPPi has been developed for the prevention of infectious diseases caused by canine distemper virus, canine parvovirus, canine adenovirus and canine parainfluenza virus. This vaccine contains canine distemper virus (CDV), canine adenovirus type 2 (CAV-2), canine parvovirus type 2 (CPV-2) and canine parainfluenza virus (CPiV). The Versican Plus DHPPi contains a new strain of canine parvovirus (2b).

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.

Liquid fraction:

Water for injections (WFI) is manufactured from purified water by distillation through a pump.

After distillation the WFI is drawn up to a sterile homogenisation vessel. Then it is filled into storage bottles and sterilised at a temperature of 121 °C for 15 minutneutralies.

The vials are stored at a temperature to 25 °C until labelling and packaging (max. 60 months).

Samples are taken for final product testing.

The consistency of the production is demonstrated on three consecutive batches, the manufacturing procedure is adequately described in detail.

Control of starting materials

Active substance

All 4 antigens comprised in the vaccine (i.e. CDV, CAV, CPV-2b, CPiV-2) 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, cell count, identity (CPV-2b), 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:

No in-process control tests are performed on the diluent (water for injection).

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, bacterial endotoxin, acidity or alkalinity, conductivity, oxidisable substances, chlorides, nitrates, sulfates, ammonium, calcium and magnesium, residue on evaporation.

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 DHPPi 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 DHPPi 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 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 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 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 DHPPi 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 DHPPi 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. The final batch release protocol of the filled batch (batch number 015820) should be provided by the end of March 2014.

Part 3 – Safety

Versican Plus DHPPi is a combined live virus vaccine indicated for the immunisation of healthy puppies and dogs against canine distemper, adenovirus hepatitis, adenovirus respiratory disease, parvovirosis and parainfluenza.

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 were demonstrated using the largest combination of the Versican Plus range of vaccines, Versican Plus DHPPi/L4R, which includes in addition inactivated rabies and *Leptospira* components. All live virus components of Versican Plus DHPPi/L4R are identical to those

contained in Versican Plus DHPPi. This is in compliance with CVMP note for guidance: requirements for combined veterinary vaccines (CVMP/IWP/52/97) and the CVMP Guideline on the requirements for combined vaccines and associations of immunological veterinary medicinal products (IVMPs) (EMA/CVMP/IWP/594618/2010).

Versican Plus DHPPi/L4R contains an adjuvant (aluminium hydroxide) which does therefore probably not benefit the safety profile of Versican Plus DHPPi without adjuvant. In order to avoid animal use for additional safety studies with Versican Plus DHPPi, the applicant is prepared to accept this disadvantage. This is supported by the CVMP.

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 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 tenfold 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, the live virus components CDV, CAV-2, CPV-2b and CPiV, diluted in water for injection were investigated.

Master seed studies

Safety testing of the canine distemper virus (CDV)

Canine distemper master seed was tested in the target species of animals according to Ph. Eur. monograph 0448 “Canine distemper vaccine (live)” – Section 2-3-1 "Safety" and based on the results, the CDV strain was considered to have met the Ph. Eur. requirements. The study proved the safety of the administration of ten-fold maximum via the subcutaneous route to 6 week old puppies.

Irreversibility of attenuation - canine adenovirus type 2

This study is discussed and presented in detail under “Increase in virulence – CAV-2 strain”. The CAV-2 strain was generally classified as safe since no adverse effects were observed in further studies.

Safety testing of the canine parvovirus (CPV-2v)

Canine parvovirus master seed in first passage was tested in the target species according to Ph. Eur. monograph 0964 “Canine parvovirosis vaccine (live)” – Section 2.3-1-1 “Safety” and Section 2-3-1-2 “Effect on the thymus”. Based on these results the CPV-2b strain was considered to have met the requirements of the Ph. Eur. Therefore, the study proves the safety of the administration of $10^{8.5}$ $TCID_{50}$/dose via the subcutaneous route to 4 week old puppies.

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, but occasionally three doses may be required in areas of high risk of infection with CPV or CDV.

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 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.

CDV, CAV-2, CPV and CPiV (the live viral components of Versican Plus DHPPi) 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.

Except for the canine parvovirus (CPV) component, none of the components of Versican Plus DHPPi are known to have any adverse effects on the immune functions of infected animals. CDV, CPiV and CAV-2 are already known as components of Versican DHPPi/L3R. No adverse effects are known for these antigens.

Wild-type strains of CPV can cause leucopoenia and thymic damage. Ph. Eur. monograph 0964 includes some specific requirements concerning the safety testing of live CPV vaccines, namely investigations of the effects of a 10-fold overdose on white blood cell count and on thymic histology. In safety study 9b data on this issue are presented and discussed. No signs of leucopoenia or hypoplasia of thymus were found. The study demonstrates that this vaccine strain does not have any adverse effects on the immunological functions.

This vaccine can be safely combined with products of the Versican Plus L4 and Vanguard R.

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

Specific requirements for live vaccines – canine parvovirus (CPV-2b)

This study evaluates the dissemination, shedding and spread of the modified live CPV-2b vaccine strain of the Versican Plus DHPPi/L4R vaccine.

Six dogs were vaccinated once subcutaneously with CPV-2b vaccine strain. Six unvaccinated dogs were kept as in-contact controls and housed together with the vaccinated dogs. The six control animals were

the moved and housed together with a new group of six control dogs. The details of group allocation provided were adequate.

The animals were examined for abnormal clinical observations or systemic reactions; rectal temperatures were recorded and the injection site was measured. No abnormalities or deviations were observed.

All vaccinated dogs and five animals of control group 1 had seroconverted as well as all dogs of control group 2.

Faecal samples for detection of vaccine virus by reverse transcription polymerase chain reaction (RT-PCR) were taken at defined time points. CPV could be detected in samples from vaccinated animals and animals of control group 1 and 2. The vaccine virus was found to be shed by faeces from all vaccinated and control animals. No quantitative virus titre determination was performed.

Organ samples were taken from the vaccinated animals to detect vaccine virus. The vaccine virus disseminated to thymus, spleen and mesenteric lymph nodes of the vaccinated animals. No information on or evaluation of the dissemination in the control group animals were provided.

The live attenuated vaccine virus strain CPV-2b may be shed by vaccinated animals for a number of days following vaccination and passed on to non-vaccinated dogs. The results show that the CPV-2b vaccine strain spreads from vaccinated puppies to other non-vaccinated dogs. Dogs infected through contact with vaccinated animals were able to act as a source of infection by spreading CPV-2b to a second contact group. Antibodies were detected in all animals (except for 1) of all groups. None of the dogs, which were infected with vaccine strain CPV-2b, developed any abnormal clinical signs. An appropriate statement is given in the SPC under section 4.5.

The applicant did not provide other information on the spread to other possibly susceptible non-target species. According to publications it is however known that CPV-2b can infect cats and cause parvovirus disease. Even though the vaccine strain is attenuated, it is to be expected that cats may become infected with the vaccine virus from vaccinated dogs since dogs and cats commonly have close contact. Several species of the family Canidae have been reported in publications to be susceptible as well and infection of wildlife carnivores by domestic cats may be feasible. Therefore, transmission of vaccine virus from vaccinated dogs in the wildlife population cannot definitely be excluded. Furthermore, due to the high tenacity and low infection dose of parvoviruses, direct contact between individuals may not be necessary to transmit the virus to other susceptible species.

In the absence of data on the pathogenicity of the vaccine strain for domestic cats and other carnivores, a warning has been included in the SPC under section 4.5 “Special precautions for use” to reflect the need to separate vaccinated dogs from other canine and feline species after vaccination.

Specific requirements for live vaccines – canine distemper virus (CDV)

This study was intended to assess the safety of canine distemper master seed virus in terms of its spread and dissemination after administration by the subcutaneous route to animals of six weeks of age.

Six unvaccinated dogs were kept as in-contact controls, housed together with vaccinated dogs. The six control animals were then moved and housed together with a new group of six control dogs. All animals showed normal general health for the duration of the study. No adverse reactions were observed in the vaccinated animals. Temperatures of all animals remained within the physiological norm. Serology (serum neutralization test) to CDV was assessed. All vaccinated dogs had seroconverted, whereas all controls remained seronegative. In blood samples CDV was detected in one vaccinated animal. No virus was detected in samples from the other five vaccinated animals or any control animal. No CDV was detected in nasal swabs, samples of nasal secretions, urine, faeces and saliva by RT-PCR.

CDV was detected in lung lymph nodes of one vaccinated animal only. Based on the negative serology and virus isolation results of the first control group and the negative serology results of the second control group, secretion samples (nasal, faecal, urine and oral) of control group 2 animals were not tested for the presence of CDV. The provided studies demonstrate that no CDV is excreted. The vaccine strain Onderstepoort is known to be well attenuated. Therefore, the probability of transmission to non-target species is deemed to be negligible. No specific warning in SPC was considered necessary.

The study results show that the vaccine CDV strain, when administered by the recommended route, is not capable to spread or disseminate from vaccinated to unvaccinated puppies.

Canine adenovirus type 2 (CAV-2)

The objective of the study was to demonstrate potential spread of canine adenovirus type 2 vaccine strain (CAV-2) from vaccinated animals to in-contact naïve animals by evaluating the seroconversion of the control animals.

Five puppies were once vaccinated subcutaneously with CAV-2 vaccine strain. Five control puppies were kept in contact with the vaccinated animals. General health observations were carried out daily. Blood samples were collected from all puppies pre-vaccination and at defined points in time. The virus neutralising antibodies to CAV-2 were analysed.

There was an increase in the neutralising antibodies to CAV-2 in the 5 vaccinated animals. There were no detectable neutralising antibodies to CAV-2 in the control animals. No secretion samples were taken to demonstrate the presence or absence of virus.

If seroconversion was not found in the co-housed control animals, it was concluded that infection by secretion must have been absent or of very low titre. The CAV-2 component of the vaccine in question corresponds to the CAV-2 strain of the product Versican DHPPi/L3R already authorised in the EU via the decentralised procedure. For the latter vaccine the SPC contains a warning with regard to potential virus excretion lasting several days after vaccination.

The study results showed that the vaccine CAV-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 and therefore, section 4.5 of the SPC includes a statement reflecting the findings:

"The live virus vaccine strains CAV-2, CPiV and CPV-2b may be shed by vaccinated dogs for a number of days following vaccination. Due to the low pathogenicity of CPV-2b-strain, it is not necessary to keep vaccinated dogs separated from non-vaccinated dogs.

The vaccine virus strain CPV-2b has not been tested in domestic cats and other carnivores (except dogs) that are known to be susceptible to canine parvoviruses. Therefore vaccinated dogs should be separated from other canine and feline species after vaccination."

Canine parainfluenza virus 2 (CPiV-2)

The objective of the study was to demonstrate potential spread of CPiV-2 vaccine strain 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 neutralising antibodies to CPiV-2 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

Canine distemper virus

Dissemination of the CDV vaccine strain was investigated together with shedding and spread in Safety Study 4. Furthermore, the virus isolation data from the CDV reversion to virulence study Safety Study 3, discussed below, provides some information on the dissemination of the CDV vaccine strain.

Specific requirements for live vaccines – Canine distemper virus (CDV)

This study was already discussed and the design presented under point “Spread of the vaccine strain”.

Six unvaccinated dogs were kept as in-contact controls, housed together with vaccinated dogs, the six control animals were then moved and housed together with a new group of six unvaccinated control dogs. Nasal swab samples were collected from all animals. Samples of faeces, urine, oral and nasal secretions were taken from all vaccinated animals. Vaccinated animals were euthanized on study Day 14 and samples of brain, nasal mucosa, tonsils, thymus, spleen and the lungs and their local lymph nodes were collected.

In this study the CDV was found to disseminate to blood and mediastinal lymph nodes in a minority of vaccinated animals when administered to seronegative dogs. No dissemination to the brain, nasal mucosa, tonsils, thymus, spleen or lungs was detected. No CDV was detected in nasal swabs, samples of nasal secretions, urine, faeces and saliva.

The study results show that the vaccine CDV strain, when administered by the recommended route, is not capable to spread or disseminate from vaccinated to unvaccinated puppies.

Safety testing of the Canine distemper virus (CDV) – Lack of increase in virulence

This study is discussed and the design presented under point “Reversion to virulence of attenuated vaccines”.

The virus was passaged in two series. CDV could be detected by polymerase chain reaction (PCR) in pooled samples from nasal mucosa, tonsils, thymus, spleen, lungs and mediastinal lymph nodes of animals 5–8 days after inoculation with CDV. As virus could not be recovered from the 1st passage animals, the first set of passages was terminated and a second set of passages started. No vaccine virus was detected in any samples by cultivation in cell lines.

Canine adenovirus type 2

Dissemination of canine adenovirus type 2

The objective of the study was to investigate the presence of the canine adenovirus 2 (CAV-2) vaccine strain in the organs of vaccinated animals by isolation of the vaccine virus on cell cultures.

The dissemination of CAV-2 was evaluated following administration of the CAV-2 vaccine strain. Four puppies were once vaccinated subcutaneously.

CAV-2 was isolated from the mediastinal, cervical, mesenteric and axillary lymph nodes. No vaccine virus was found in nasal mucous membrane, larynx mucous membrane, tonsils, lungs, spleen, liver and kidney. Only 4 animals were used for this study. This seems to be acceptable because the CAV-2 Reversion to Virulence Study – Irreversibility of Attenuation - canine adenovirus type 2 provides some more information on dissemination in tissues. These data show that after administration of a high titre of the CAV-2 to 6/7-week old dogs vaccine virus was isolated by cell culture from the larynx mucosa, tonsils,

lungs, and cervical and axillary lymph nodes, but not from the nasal mucosa, spleen, liver, kidney, mesenteric or mediastinal lymph nodes.

No abnormal health observations were made.

No secretion samples were taken to demonstrate the presence or absence of vaccine virus as required by Ph. Eur. monograph 5.2.6, Section 1-8-2 “Dissemination in vaccinated animals”. The SPC section 4.5 was therefore updated as explained above:

"The live virus vaccine strains CAV-2, CPiV and CPV-2b may be shed by vaccinated dogs for a number of days following vaccination. Due to the low pathogenicity of CPV-2b-strain, it is not necessary to keep vaccinated dogs separated from non-vaccinated dogs.

The vaccine virus strain CPV-2b has not been tested in domestic cats and other carnivores (except dogs) that are known to be susceptible to canine parvoviruses. Therefore vaccinated dogs should be separated from other canine and feline species after vaccination."

Canine parvovirus

Data relevant to dissemination of the CPV-2b strain were investigated in the Shed & Spread study. The virus isolation data from the CPV-2b reversion to virulence study Safety Study, discussed in part “Increase in virulence” provide some information on the dissemination and excretion of the CPV-2b vaccine strain.

Specific requirements for live vaccines – canine parvovirus (CPV-2b)

This study is discussed and the design presented under point “Spread of the vaccine strain”.

Six dogs were vaccinated with the CPV-2b. Six unvaccinated dogs were kept as in-contact controls, housed together with the vaccinated dogs. The six control animals were then housed together with a new group of control dogs.

All vaccinated and control animals excreted vaccine virus in faeces.

As demonstrated by examination of the organs the vaccine virus disseminated to thymus, spleen and mesenteric lymph nodes of the vaccinated animals.

Please see evaluation under “Spread of the vaccine strain”.

Safety testing of the canine parvovirus – Irreversibility of Attenuation

This study is discussed and the design presented under point “Increase in virulence – CPV-2b strain”.

In a first step, 2 dogs were once vaccinated subcutaneously with the CPV-2b strain. Faecal samples were collected and vaccine virus was isolated. These virus samples were administered oronasally to another two dogs. In total the vaccine virus was passaged through 5 pairs of animals.

In a second step, 8 dogs were inoculated oronasally with passaged virus (derived from the final passage of the first step). Eight dogs were vaccinated with un-passaged virus.

During the passages excretion of vaccine virus in faeces could be shown. No further secretion samples, e.g. oral or nasal secretion were taken and tested.

Please see evaluation under “Increase for virulence”.

Canine parainfluenza virus

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 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

Canine distemper virus

The increase in virulence of canine distemper master seed virus has to be evaluated according to Ph. Eur. monograph 0448 on canine distemper vaccine (live) – section 2-3-2 Increase in virulence.

In a first series of passages 2 dogs were vaccinated subcutaneously with a high dose of the CDV strain. As vaccine virus could not be recovered from the first passage animals the first set of passages was terminated and a second set of passages was started. For a second passage again two dogs were inoculated subcutaneously.

The study design was changed from those for the first set of passages to increase the probability of re-isolating vaccine virus. Apart from that the study procedure was the same.

There were no abnormal clinical observations or systemic reactions. No increase in body temperature was observed after the administrations of the tested material and/or organ suspension. No local reactions were recorded after CDV vaccine strain administration. No vaccine virus was isolated from the organ cultures when tested in cell culture. However, the semi-quantitative RT-PCR assay confirmed presence of virus in organ suspensions from both animals administrated the maximum vaccine titre.

The amount of CDV administered corresponds to approximately ten-times the assumed maximum dose for the commercial vaccine. According to the SPC the maximum titre for CDV is $10^{5.1}$ $TCID_{50}$. Ph. Eur. monograph 0448 does not require tenfold of the maximum titre for the reversion to virulence study.

The results show that the virus could not be recovered at the first passage level in two series of passages. However, the Ph. Eur. monograph requires administering a quantity of the vaccine virus that will allow recovery of the virus however the strain is attenuated such that this was not possible.

Canine adenovirus type 2

This study was performed in 1996 and was part of the application for the vaccine Versican DHPPi/L3R to confirm the irreversibility of attenuation of the CAV-2 vaccine strain. The strain is identical with the CAV-2 strain of the new Versican Plus DHPPi/L4R vaccine.

Step 1: Passage procedure:
The CAV-2 vaccine strain was passaged sequentially in twelve 6 to 7 week old puppies free from antibodies against CAV-2. The first two puppies were administered subcutaneously CAV-2. Five to six days post-inoculation the 2 dogs were euthanized and tissue samples were collected and examined for the presence of virus. Positive samples were pooled and 1 ml of the suspension was administered intranasally to two other puppies for the first passage. The above operation was carried out four additional times and the presence of virus was verified at each passage.

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

All ten animals were blood sampled at defined points in time for determination of CAV-2 specific antibodies. General health observations were made and rectal temperatures were measured. In the subsequent safety evaluation (Step 2), no neutralising antibodies to CAV-2 were detected before inoculation in any of the animals. Fourteen days after inoculation, titres in all animals were increased at least fourfold. These results confirm an immune response to CAV-2 antigen following inoculation. No canine adenovirus could be isolated from the tested tissues 14 days after inoculation. This study shows no indication of increased virulence of the vaccine CAV-2 and is in compliance with Ph. Eur. monograph 1951.

Canine parvovirus

The increase in virulence of canine parvovirus master seed virus has to be evaluated according to Ph. Eur. monograph 0964 Canine parvovirus vaccine (live) – section 2-3-2 Increase in virulence.

Step 1: Passage procedure:
Five MSV passages were performed in this study. In the first passage, two dogs were vaccinated subcutaneously with CPV-2b. The administered vaccine virus titre was at least ten times the maximum virus titre that can be expected in a dose of commercial vaccine.

Samples of faeces were collected at defined points in time. The sample with the highest virus titre was used for the inoculation of the next two animals. Samples of thymus from both animals inoculated with test material were collected after euthanasia.

Two new dogs were inoculated oronasally with 1 ml of the suspension of canine parvovirus recovered from the faecal sample from the first couple of animals. Samples of faeces were collected. Samples positive for virus presence were pooled and used for inoculation of further two animals, which were used for the third virus passage. 1ml of the pooled faecal sample from the previous two animals was administered oronasally. Two further passages, fourth and fifth virus passage, were performed on a couple of animals respectively. Samples of the fifth passage positive for virus presence were pooled and used for the inoculation of eight animals for a sixth passage and a final comparison of the maximal attenuated virus passage with the initial material.

Step 2: Subsequent safety evaluation:
Sixteen animals were used for a final safety evaluation. One ml of the pooled faecal suspension from the

last passage (fifth) was administered oronasally to each of eight animals and the first passage from MSV was administered subcutaneously to each of another eight animals.

The results show that animals administered with un-passaged and first passage suspension showed seroconversion, other groups remained seronegative. Leucopenia was not observed after MSV administration.

During passage procedure excretion of virus in faeces was shown by haemagglutination (HA) test and isolation in sensitive cell cultures in animals inoculated with first passage only. In all other groups of animals the excretion of virus was shown by a PCR method.

During the final safety evaluation the excretion of virus in faeces was shown in animals administered CPV-2b by HA and PCR method. No excreted virus was detected in animals inoculated with high passage CPV, neither by HA test nor PCR. No further secretion samples, e.g. oral or nasal secretion were taken and tested. Excretion of CPV vaccine strains was only demonstrated in faeces as this is known to be the main route of excretion. A special warning is already implemented in the SPC.

The study results implicate that the master seed of canine parvovirus, when administered by the recommended route, does not increase in virulence when passaged in puppies.

Canine parainfluenza virus

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 DHPPi 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 strains have been adequately characterised by the information provided and the safety and efficacy data supplied. A summary regarding the characterisation of the live virus strains and the stability of the attenuation was provided, which emphasizes the stability of the attenuation of each 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 each of the vaccine strains of Versican Plus DHPPi has been demonstrated. Each of the vaccine strains has been demonstrated to be consistent and stable by *in vitro* passaging. Further the strains are attenuated and of low virulence.

The potential for recombination or genomic re-assortment for each of the vaccine viruses is as follows:

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

CAV-2: Canine adenovirus is a double stranded DNA virus and is certainly potentially capable of recombination with field strains. In practice, however, this is unlikely to occur in the host.

CPV: Canine parvovirus is a single-stranded DNA virus that replicates in the nucleus of the host cell. Both recombination and re-assortment are therefore theoretically possible.

CPiV: 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 DHPPi 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 products of the Versican Plus L4 and Vanguard R.

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:

"No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product other than Vanguard R and Versican Plus L4. 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."

Field studies

One multinational study was performed to evaluate safety and efficacy of the vaccine in comparison to a positive control group. 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. 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 DHPPi 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 well characterised antigens.
- The stability of the attenuation of each 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 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 DHPPi 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. Except for the CPV component, none of the components of Versican Plus DHPPi is known to have any adverse effects on the immune functions of infected animals. CDV, CPiV and CAV-2 are already known as components of Versican DHPPi/L3R. No adverse effects are known for these antigens.

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

Spread of the vaccine strain

CPV-2b strain: The virus was found to be shed by faeces in all animals, vaccinated and controls. The virus disseminated to thymus, spleen and mesenteric lymph nodes of the vaccinated animals. No evaluation of the spread to other possibly susceptible non-target species was provided.

CDV strain: There was an increase in the neutralising antibodies to CDV in the vaccinated animals. There were no detectable neutralising antibodies to CDV in the control animals. CDV was detected in the lung lymph nodes of one vaccinated animal by PCR. The control animals were negative.

The study results show that the vaccine CDV strain is not capable to spread from vaccinated to unvaccinated puppies.

CAV-2 strain: There was an increase in the neutralising antibodies to CAV-2 in the vaccinated animals. There were no detectable neutralising antibodies to CAV-2 in the control animals at any of the testing time points. No secretion samples were taken to demonstrate the presence or absence of virus.

CPiV-2 strain: There was an increase in 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

CPV-2b strain: Dissemination of the vaccine strain was investigated together in the shedding and spreading study and in the reversion to virulence study. Both studies demonstrate that all vaccinated and control animals excreted virus in faeces. No further secretion samples, e.g. oral or nasal secretion were taken and tested.

CDV strain: Dissemination of the CDV vaccine strain was investigated together in the shedding and spreading study. CDV was found to disseminate to blood and mediastinal lymph nodes in a minority of vaccinated animals. No dissemination to the brain, nasal mucosa, tonsils, thymus, spleen or lungs was detected. No CDV was detected in nasal swabs, samples of nasal secretions, urine, faeces and saliva.

CAV-2 strain: Tissue samples of vaccinated puppies were collected and prepared for passaging on cell culture. In this study CAV-2 was isolated from the mediastinal, cervical, mesenteric and axillary lymph nodes. No virus was found nasal mucous membrane, larynx mucous membrane, tonsils, lungs, spleen, liver and kidney. No secretion samples were taken to demonstrate the presence or absence of virus.

CPiV-2 strain: 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

CPV-2b strain: In a first passage procedure the virus was passaged several time in dogs. In a second evaluation step, animals were inoculated with this passaged virus oronasally. No excretion of virus in faeces was detected in animals inoculated with the last passage of virus, neither by HA test nor PCR. No seroconversion could be shown in this group. The study results implicate that the master seed of canine parvovirus, when administered by the recommended route, does not increase in virulence when passaged in puppies.

CDV strain: As vaccine virus could not be recovered from the 1st passage animals (two animals) the first set of passages was terminated and second set of passages started with two animals. This is acceptable because further studies are available which demonstrate a negligible risk of reversion to virulence.

CAV-2 strain: In a first passage procedure the virus was passaged several times in dogs. In a second evaluation step animals, which were inoculated with this passaged virus intranasally, show an increase of antibody titres. No canine adenovirus could be isolated from the tested tissues from these animals. This study shows no indication of increased virulence of the vaccine CAV-2.

CPiV-2 strain: 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 in comparison to a positive control group. 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 DHPPi is a multivalent live virus vaccine indicated for the immunisation of healthy puppies from six weeks of age and dogs against canine distemper, adenovirus hepatitis, adenovirus respiratory disease, parvovirosis and parainfluenza. The vaccine was developed as part of a larger combination (Versican Plus DHPPi/L4R) consisting of live virus components (canine distemper virus (CDV), canine adenovirus type 2 (CAV-2), canine parvovirus (CPV), canine parainfluenzavirus (CPi)) presented in

freeze-dried form in a vial to be reconstituted with a vial of the inactivated components (rabies virus, *Leptospira* Canicola, *Leptospira* Icterohaemorrhagiae, *Leptospira* Bratislava and *Leptospira* Grippotyphosa) presented in liquid form. The adjuvant of the liquid fraction is aluminium hydroxide. Therefore, many studies presented have been conducted with the larger combination. According to the CVMP guideline on multi-component vaccines these can be used to fully support the safety and efficacy of the smaller fall-out combinations.

The live virus components of Versican Plus DHPPi (canine distemper virus (CDV), canine adenovirus type 2 (CAV-2), canine parvovirus type 2b (CPV-2b) and canine parainfluenzavirus (CPiV)) are presented in freeze-dried form in a vial to be reconstituted with a vial of the diluent (water for injection). The vaccine Versican Plus DHPPi itself does not contain any adjuvant.

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 DHPPi 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

The minimum immunisation doses (MIDs) for the Versican Plus DHPPi viral components CDV, CAV-2, CPV and CPiV were selected based on previous experiences with these strains. The CAV-2 and CPiV components are approved as components of Versican DHPPi/L3R. The CDV component of Versican Plus DHPPi is a new master seed of the CDV component approved in Versican DHPPi/L3R. The CPV-2b component of Versican Plus DHPPi is a new master seed not included in any vaccine approved in the EU. The MID for this component was selected based on previous experiences with polyvalent vaccines for dogs.

Onset of immunity

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

CDV:

Seven 6-week old dogs (5 with 2 control dogs), tested seronegative against CDV were administered the vaccine Versican Plus DHPPi/L4R subcutaneously. They were challenged intravenously with the canine distemper challenge virus CHSV Snyder Hill at Day 21 after vaccination. After challenge the vaccinated group showed no clinical signs of distemper. There was no increase in the rectal temperature and a further increase in antibody titres while the control group showed clinical signs of distemper and was euthanized for humane reasons.

While six dogs are not a large number they are considered adequate and representative in this specific situation of the tight correlation between antibody titre and protection for this particular antigen. Based on the data provided titres of 1:2 or 1:4 were considered indicative of protection against CDV. It should be noted that this conclusion cannot be generalised because no standardised tests exist for determination

of antibodies against CDV (like it is established for rabies virus) making it almost impossible to define "protective antibody titres against CDV" because the antigen titres will vary dependent on the individual laboratory.

CAV-1:

Seven 6-week old dogs (5 with 2 control dogs), tested seronegative against CAV-1 and CAV-2 were administered the vaccine Versican Plus DHPPi/L4R subcutaneously. They were challenged intravenously with the challenge strain CAV-1 Mirandola at Day 21 after vaccination. After challenge the vaccinated group showed no clinical signs of canine hepatitis but one dog developed a slight increase of rectal temperature (39.5 °C) above the upper physiological limit of 39.5 °C at Day 6 after challenge. There was a further increase in antibody titres while the control group showed clinical signs of canine hepatitis and was euthanized for humane reasons.
This study is considered valid because it fulfils the requirements of Ph. Eur. monograph 1951. The claims stated for this antigen are supported by this study.

CAV-2:

Twenty 6-week old dogs (10 with 10 control dogs), tested seronegative against CAV-2 were administered the vaccine Versican Plus DHPPi/L4R subcutaneously. They were challenged intranasally with the challenge strain CAV-2 Manhattan 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 in antibody titres against CAV-2 and on virus isolation 5/10 dogs excreted CAV-2 for 1–3 days from Day 3 to Day 6 after challenge. The duration of virus excretion was significantly lower than in the controls. This study complies with the requirements of Ph. Eur. monograph 1951 and is considered acceptable. The claims stated for this antigen are supported by this study.

CPV-2b:

Seven 6-week old dogs (5 with 2 control dogs), tested seronegative against CPV (haemagglutination inhibition (HAI) and virus neutralisation (VN)), were administered the vaccine Versican Plus DHPPi/L4R subcutaneously. They were challenged oronasally with the challenge strain CPV-2b strain 212/98 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 in antibody titres against CPV-2b while the WBC count demonstrated no leukopenia.

On virus isolation and HA 3/5 dogs excreted virus on one day between 3 and 5 days after challenge. This was less than 1/100 of the geometric mean of the maximum titres found in control animals by HA. No isolation of infectious CPV-2b was found in cell culture.

This study complies with the requirements of Ph. Eur. monograph 0964 and is considered acceptable. The claims stated for this antigen are supported by this study.

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 to 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.

Equivalence study:

The objective of this study was the evaluation of the serological and clinical responses to non-adjuvanted Versican Plus DHPPi and Versican Plus Pi fall-out products of the Versican Plus DHPPi/L4R vaccine range after primary vaccination and by challenge with a virulent, heterologous strain of canine parainfluenza virus.

Twenty-five 6-week old dogs (10 dogs vaccinated with Versican Plus DHPPi, 10 dogs vaccinated with Versican Plus Pi and 5 control dogs), tested seronegative against CPiV and were administered the vaccines subcutaneously at Day 0 and Day 21. They were challenged intranasally with the CPiV D008 at Day 21 after the 2nd 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 in both groups of vaccinated animals.

Virus excretion through the nasal tissue was determined using a virus isolation test. Results showed that the number of animals shading the virus was lower and number of days of viral shedding was significantly lower in the Versican Plus DHPPi vaccinates and in the Versican Plus Pi vaccinates than in the controls.

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

The challenge data clearly demonstrate 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, a rewording containing more detailed information as regards the claim for CPiV - i.e. “to prevent clinical signs (nasal and ocular discharge)” - has been added.

As regards the conclusion that the minimum protective titre for CPiV is 1:16, 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.

As part of this study, the immunogenicity of the other viral vaccine components was assessed by serology. The serological responses against CDV, CAV-1, CAV-2 and CPV following primary vaccination with the non-adjuvanted Versican Plus DHPPi and Versican Plus Pi fall-out products of Versican Plus DHPPi/L4R vaccine were evaluated and visually compared to the results of historical data from studies with adjuvanted Versican Plus DHPPi/L4R. If the 95% confidence intervals of the geometric mean titres of the non-adjuvanted components of the fall-out products in this study overlap the 95% confidence intervals of the geometric means of the adjuvanted components of Versian Plus DHPPi/L4R in historic studies on at least one time point after primary immunisation, responses were considered to be equivalent.

CDV serology results:

At the time of the second vaccination all animals had seroconverted. However, after the second vaccination the titres increased which demonstrates the booster effect of this vaccination.

CAV-1 and CAV-2 serology results:

At the time of the second vaccination all animals had seroconverted. However, after the second vaccination the titres increased which demonstrates the booster effect of this vaccination.

CPV serology results:

At the time of the second vaccination all animals had seroconverted. However, after the second vaccination the titres increased which demonstrates the booster effect of this vaccination.

Assessment of the equivalence of the serological data:

Based on the results of this assessment it can be concluded that the CDV, CAV, CPV and CPiV-2 components used in the Versican Plus range vaccines protect against canine distemper, adenovirus-induced diseases, parvovirosis and canine parainfluenza irrespective of whether they are adjuvanted or non-adjuvanted (95% confidence interval of geometric mean antibody titres against CDV, CAV-1, CAV-2, CPV-2 and CPiV-2 of seronegative, vaccinated dogs). This was considered acceptable.

Influence of maternal antibodies on the efficacy of the vaccine

The presented studies clearly show the influence of maternally derived antibodies (MDA) regarding CDV, CPV and CAV antigens. While MDA negative dogs seroconvert after the first vaccination and are boostered after the second vaccination, MDA positive dogs show a titre decrease until Days 28–35. Seroconversion is observed from Days 35–42 onwards. Although the animals were protected against challenges with virulent CDV, CPV and CAV-1 strains, the possible interference of MDA should always be taken into consideration when vaccinating very young puppies. The studies demonstrate the importance of the second vaccination as part of the primary vaccination: animals with MDA do not seroconvert after the first vaccination but after the second one. As immunological responses to CDV, CPV and CAV-2 may be delayed due to MDA the vaccination scheme for young dogs - especially for puppies at 6 weeks of age - should be planned carefully. This has been reflected in the SPC.

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

Duration of immunity (DOI)

Ten laboratory challenge studies in dogs were performed to demonstrate 1-year duration of immunity for the CDV, CAV-2, CPV and CPiV components of Versican Plus DHPPi.

CDV:

Nine 6-week-old dogs (6 with 3 control dogs), tested seronegative against CDV were administered the vaccine Versican Plus DHPPi/L4R subcutaneously at Day 0 and Day 21. They were challenged intravenously with the canine distemper challenge virus CHSV Snyder Hill at 12 months after vaccination.

After challenge the vaccinated group showed no clinical signs of distemper, no increase in rectal temperature above the upper limit (39.5 °C) and a satisfactory anamnestic antibody response while the control group showed clinical signs of distemper and was euthanized for humane reasons.

CAV-1:

Nine 6-week old dogs (6 with 3 control dogs), tested seronegative against CAV-1 and CAV-2 were administered the vaccine Versican Plus DHPPi/L4R subcutaneously at Day 0 and Day 21. They were challenged intravenously with the challenge strain CAV-1 Mirandola.

After challenge the vaccinated group showed no clinical signs of canine hepatitis and a satisfactory anamnestic antibody response.

This study fulfils the requirements of Ph. Eur. monograph 1951 and is considered acceptable. Duration of immunity for at least one year was demonstrated for CAV-1.

CAV-2:

Twenty 6-week old dogs (10 with 10 control dogs), tested seronegative against CAV-1 and CAV-2 were administered the vaccine Versican Plus DHPPi/L4R subcutaneously at Day 0 and Day 21. They were challenged intranasally with the challenge strain CAV-2 Manhattan 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 CAV-2 boosted the immunity in vaccinated animals before challenge.

After challenge the vaccinated group showed no clinical signs of canine hepatitis and a satisfactory anamnestic antibody response. The mean total score was significantly lower in vaccinated animals than in the controls.
Virus isolation: 3/10 vaccinated animals excreted CAV-2 for one day either 2 or 3 days after challenge. Duration of virus excretion: the number of days was significantly lower in vaccinated animals than in the controls.

In the control group 4/10 dogs showed mild nasal discharge and mild ocular discharge starting 2 days after challenge and persisting for up to 4 days until 6 days after challenge. The mean total score of abnormal clinical observations was significantly higher in control than in vaccinated animals. Virus isolation: 10/10 controls excreted CAV-2 for 2–4 days starting 2 until 5 days after challenge. Duration of virus excretion: the number of days was significantly higher in the controls than in the vaccinates. The area under the curve of CAV-2 titres was significantly higher in control than in vaccinated animals.

This study fulfils the requirements of Ph. Eur. monograph 1951 and is considered acceptable. Duration of immunity for at least one year was demonstrated for CAV-2.

CPV-2b:

Seven 6-week old dogs (5 with 2 control dogs), tested seronegative against CPV (HAI and VN) were administered the vaccine Versican Plus DHPPi/L4R subcutaneously at Day 0 and Day 21. They were challenged oronasally with the challenge strain CPV-2b strain 212/98 at 12 months after vaccination.

Control animals remained seronegative until challenge. As they were housed together with vaccinated animals, these results confirm that vaccine induced immunity to CPV was not further boosted through concurrent infections with field strains during the study.

After challenge the vaccinated group showed no clinical signs and no increase of rectal temperature. Virus isolation: by HA, 3/5 animals showed CPV excretion for 2 days between 5 and 10 days after challenge. This was less than 1/100 of the geometric mean of the maximum titres found in control animals by HA. By cell culture, 0/5 vaccinated animals excreted infectious CPV.

The control group showed no abnormal clinical signs, there was hyperthermia in 1/2 controls 10 and 11 days after challenge. WBC count: leukopenia - white blood cell numbers decreased more than 50% compared to pre-challenge mean values (leukopenia) in 2/2 controls. Virus isolation: by HA, 2/2 controls showed CPV excretion starting 5 days and peaking from 7 to 10 days after challenge. By cell culture, 2/2 controls started to excrete CPV 5 days and peaked 7 days post-challenge.

This study fulfils the requirements of Ph. Eur. monograph 0964 and is considered acceptable. Duration of immunity for at least one year was demonstrated for CPV-2b.

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 Day 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 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 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

Immunity after revaccination – response to booster (RTB)

To demonstrate protective immunity of the components of Versican Plus DHPPi/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. For the CPiV and *Leptospira* components of Versican Plus DHPPi/L4R, protective immunity following an annual booster was demonstrated by challenge. For the CDV, CAV-2, CPV components, protective immunity following an annual booster was demonstrated serologically by comparing antibody titres in response to the annual booster with those after primary vaccination.

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 Day 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 CPiV antibody titres.

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 proposes the following claim: “prevent clinical signs and reduce viral excretion caused by canine parainfluenza virus”.

The challenge data clearly demonstrate 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.

As part of this study, the immunogenicity of the other viral vaccine components was assessed by serology following a booster vaccination one year after the primary immunisation of minimum age dogs.

The antibody titres against CDV of vaccinated animals 12 months after the second vaccination have declined drastically (two dogs 32, two dogs 8, four dogs 4, two dogs 2 and two dogs < 2). However, after a single booster vaccination the titres increased again and were comparable to those observed three weeks after primary vaccination course.

The antibody titres against CAV-1 and CAV-2 of vaccinated animals 12 months after the second vaccination have declined. After a single booster vaccination the titres increased again. They were comparable (even higher) to those observed three weeks after primary vaccination course. However, it should be noted that in this study the titres were lower compared to those of the DOI studies presented in the submitted dossier.

The antibody titres against CPV of vaccinated animals 12 months after the second vaccination have declined slightly. After a single booster vaccination the titres increased again and were comparable to those observed three weeks after primary vaccination course.

Additional studies

Compatibility

Versican Plus DHPPi is compatible with Versican Plus L4 and Vanguard R. As mentioned in Part 3.A, the SPC will include an option to use Versican Plus DHPPi in combination with these vaccines. The manufacturing processes of Vanguard R are basically identical to those of the rabies component of Versican Plus DHPPi/L4R apart from a difference in the timing of the concentration step. The applicant plans to submit a variation to change the manufacturing processes of Vanguard R to be identical to those for Versican Plus so that rabies antigen bulk for both products is manufactured identically. This means that it will only be the number of active ingredients which will be different between Versican Plus DHPPi/L4R and Versican Plus L4 or Vanguard R, and consequently there is no reason to believe that a combination of Versican Plus DHPPi and Vanguard R or Versican Plus L4 could result in any safety issues. No compatibility studies of Versican Plus DHPPi with other products were undertaken.

Section 4.8 of the SPC 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 Vanguard R and Versican Plus L4. 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 DHPPi 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 was categorised as follows:

- No increase.
- Increase 1: < 2-fold increase of CDV, CAV-1, CAV-2, CPV CPiV antibodies (SN).
- Increase 2: ≥ 2-fold increase of CDV, CAV-1, CAV-2, CPV CPiV antibodies (SN).

Only results relevant to the components of Versican Plus DHPPi 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 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 CDV, CAV-2, CPV, CPiV,

- 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:

– 100% of the puppies showed full response (Increase 2) to primary immunisation (V1+V2) against CAV-1 (n=5) and CAV-2 (n=8).

– 20% of puppies did not show an antibody increase against CDV (n=1) and CPV (n=5):

  CDV: The animal had an antibody titre of 4 before the first vaccination, was seronegative (< 2) before the second vaccination and had a titre of 4 after the second vaccination, showing that MDA interfered with the first, but not the second vaccination.

  CPV: In three puppies MDA interfered with responses after both vaccinations leaving the puppies without any protective antibody levels (< 2) after primary immunisation.

  The other two pups had higher than average MDA titres before the first vaccination which decreased to levels that allowed them to respond to the second vaccination.

Serological results for CPiV, did not allow more general conclusions as there was only one MDA positive animal. MDA titres were moderately high before the first vaccination and remained stable (CPiV) after the second vaccination indicating a response to primary immunisation.

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) after primary immunisation (V1+V2).
- One dog did not respond to the CPiV component (no increase).

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 CDV, CAV-1, CAV-2, CPV and CPiV.

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).

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

Against 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.

The results of the field study show that vaccination with Versican Plus DHPPi/L4R induced antibody titres that can be considered protective against CDV, CAV-1 and CAV-2 in 100% of the dogs.

Against CPV, 100% of the adult dogs were protected following an annual booster vaccination (cohort 2) and all adult dogs of cohort 1 were protected following a primary immunisation. Puppies did not all respond with protective antibody levels against CPV (cohorts 1 and 3) because of pre-existing MDA.

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

a) 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.

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

Furthermore, from a statistical point of view, the CVMP could not agree with the applicant's conclusions regarding the protective titres for CDV and CAV-1. It was only one animal in each challenge study which was protected by these low titres. As protection also depends on other factors (e.g. general health status, individual immune system, cellular immunity) it is very difficult to determine fixed protective titres for the antigens. In addition, it should be noted that there do not exist standardised tests for determination of antibodies against CDV and CAV-1 (like it is established for rabies virus). Therefore, it is almost impossible to define "protective antibody titres against CDV or CAV-1" because the antigen titres will vary dependent on the individual laboratory.

The presented field study clearly shows the influence of MDA regarding CDV, CPV and CAV antigens. While the MDA negative dogs mostly seroconvert after the first vaccination and are boostered after the second vaccination, MDA positive dogs react after the second vaccination and in general have lower titres than those without MDs. The study demonstrates again the importance of the second vaccination as part of the primary vaccination scheme. As immunological responses to CDV, CPV and CAV-2 may be delayed due to MDA, the vaccination scheme for young dogs should be planned carefully. This has been reflected in the SPC.

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.

Overall conclusion on efficacy

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

The minimum protective dose is indicated below:

Component	Minimum potency/ Antigen content
Freeze-dried fraction (live attenuated)	
Canine distemper virus, strain Bio 11/A	$10^{3.1}$ $TCID_{50}$
Canine adenovirus type 2, strain CAV-2-Bio 13	$10^{3.6}$ $TCID_{50}$
Canine parvovirus type 2b, strain CPV-Bio 12/B	$10^{4.3}$ $TCID_{50}$
Canine parainfluenza type 2 virus, strain CPiV-2-Bio 15	$10^{3.1}$ $TCID_{50}$
Liquid fraction	
Water for injection	

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

The proposed minimum titres for the virus components are acceptable.

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 DHPPi. Challenge studies have been conducted to demonstrate that the vaccine is protective against virulent challenge in the presence of MDA against CDV, CAV and CPV at levels equal to or higher than those likely to be encountered under field conditions. The presented laboratory and field studies clearly show the influence of MDA regarding CDV, CPV and CAV antigens. While the MDA negative dogs mostly seroconvert after the first vaccination and are boostered after the second vaccination MDA positive dogs react after the second vaccination and, in general, have lower titres than those without MDA. These studies demonstrate the importance of the second vaccination as part of the primary vaccination because animals with MDA do not seroconvert after the first vaccination but after the second one. As immunological responses to CDV, CPV and CAV-2 may be delayed due to MDA the vaccination scheme for young dogs - especially for puppies at 6 weeks of age - should be planned carefully. This has been reflected in section 4.4 of the SPC:

“Immunological responses to the CDV, CAV and CPV components of the vaccine may be delayed due to maternally derived antibody interference. However, the vaccine has been proven to be protective against virulent challenge in the presence of maternally derived antibodies against CDV, CAV and CPV at levels equal or higher to those likely to be encountered under field conditions. In situations where very high maternally derived antibody levels are expected, the vaccination protocol should be planned accordingly”.

The following vaccination scheme in line with the other vaccines of the Versican Plus range is proposed:

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 DHPPi 3–4 weeks apart from 6 weeks of age.

Leptospira

If protection against *Leptospira* is required dogs can be vaccinated with two doses of Versican Plus DHPPi mixed with Versican Plus L4 3–4 weeks apart from 6 weeks of age:

The content of a single vial of Versican Plus DHPPi should be reconstituted with the content of a single vial of Versican Plus L4 in place of the diluent. Once mixed, the content of the vial should appear as clear

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

Rabies:

If protection against rabies is required:

First dose: Versican Plus DHPPi from 8–9 weeks of age.

Second dose: Versican Plus DHPPi mixed with Vanguard R 3–4 weeks later but not before 12 weeks of age.

The content of a single vial of Versican Plus DHPPi should be reconstituted with the content of a single vial of Vanguard R in place of the diluent. Once mixed, the content of the vial should appear as pink/red or yellowish colour with slight opalescence. The mixed vaccines should be injected immediately via the subcutaneous route.

The efficacy of the rabies fraction is proven after a single dose from 12 weeks of age in laboratory studies. 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 has been demonstrated in 6 weeks old dogs.

Re-vaccination scheme: A single dose of Versican Plus DHPPi to be given annually.

Part 5 – Benefit-risk assessment

Introduction

Versican Plus DHPPi is a multivalent live virus vaccine which is indicated for the immunisation of healthy puppies and dogs against canine distemper (CDV), adenovirus hepatitis adenovirus respiratory disease(CAV-2),, parvovirosis(CPV) and parainfluenza (CPiV). The vaccine is presented in freeze-dried form in a vial to be reconstituted with a vial of the diluent (water for injections).

Versican Plus DHPPi is a fixed combination containing four known 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 DHPPi is a fall-out formulation of Versican Plus DHPPi/L4 with fewer components to allow for choice of vaccination scheme based on risk. CDV, CPV and CAV-2 are commonly included in canine vaccines to protect against distemper, parvovirosis and adenovirus type 1 hepatitis which are serious illnesses and often fatal in young dogs. CAV-2 and CPiV infect the canine respiratory tract and on their 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.

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 mortality and clinical signs caused by canine distemper virus,
- to prevent mortality and clinical signs caused by canine adenovirus type 1,
- to prevent clinical signs and reduce viral excretion caused by canine adenovirus type 2,
- to prevent clinical signs, leukopenia and viral excretion caused by canine parvovirus and
- to prevent clinical signs (nasal and ocular discharge) and reduce viral excretion caused by canine parainfluenza virus.

Onset of immunity has been demonstrated at:

- 3 weeks after the first vaccination for CDV, CAV, CPV.
- 3 weeks after completion of the primary course for CPiV.

DOI has been demonstrated for at least one year after primary vaccination course for all components of Versican Plus DHPPi.

Additional benefits

Using multivalent vaccines for dogs has 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.

Risk assessment

Main potential risks:

Quality:

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

The choice of the vaccine strains 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 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 DHPPi has been provided. The quality of Versican Plus DHPPi is considered to be satisfactorily demonstrated.

Safety for the target animal:

The administration of one dose and a repeated dose of Versican Plus DHPPi 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 also performed with the live virus components (lyophilisate) and 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 DHPPi. The presented laboratory and field studies clearly show the influence of MDA regarding CDV, CPV and CAV antigens.

It has been demonstrated that immunological responses to the CDV, CAV and CPV components of the vaccine may be delayed due to MDA interference. However, the vaccine has been proven to be protective against virulent challenge in the presence of MDA against CDV, CAV and CPV at levels equal or higher to those likely to be encountered under field conditions. The studies demonstrate the importance of the second vaccination as part of the primary vaccination because animals with MDA do not seroconvert after the first vaccination but after the second one. Therefore, the vaccination scheme for young dogs should be planned carefully.

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 vaccine except Versican Plus L4 and Vanguard R. This fact is covered in section 4.8 of the SPC. The possible spread and dissemination of the vaccine strains has been correctly reflected in the SPC. 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 DHPPi has been demonstrated to be efficacious for the active immunisation of dogs from 6 weeks of age:

- to prevent mortality and clinical signs caused by canine distemper virus,
- to prevent mortality and clinical signs caused by canine adenovirus type 1,
- to prevent clinical signs and reduce viral excretion caused by canine adenovirus type 2,
- to prevent clinical signs, leukopenia and viral excretion caused by canine parvovirus,
- to prevent clinical signs (nasal and ocular discharge) and reduce viral excretion caused by canine parainfluenza 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 of findings in the laboratory and field trials 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 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 DHPPi 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 DHPPi.