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Veterinary Medicines and Product Data Management

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Nobivac L4 (EMEA/V/C/002010)

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

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Introduction

An application for the granting of a Community marketing authorisation of Nobivac L4 has been submitted to the Agency on 10 December 2010 by Intervet International BV in accordance with Regulation (EC) No. 726/2004.

The CVMP had accepted on 14-16 September 2010 that the product was eligible for the submission of a dossier for granting of a Community marketing authorisation via the centralised procedure under Article 3.2 (a) of Regulation (EC) No. 726/2004 as it contains a new active substance.

Nobivac L4 is an inactivated bacterial vaccine presented as a suspension for injection without adjuvant in single dose (1 dose) or multidose (10 doses) containers. The route of administration is subcutaneous use.

Nobivac L4 contains inactivated 4 *Leptospira* strains.

These are:

- *L. interrogans* serogroup Canicola serovar Portland-vere 3550-7100 U[1]
- *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni 290-1000 U[1]
- *L. interrogans* serogroup Australis serovar Bratislava 500-1700 U[1]
- *L. kirschneri* serogroup Grippotyphosa serovar Dadas 650-1300 U[1]

[1] Antigenic mass ELISA units.

The product is indicated for active immunisation of dogs to reduce infection and/or urinary excretion caused by *Leptospira* strains.

The CVMP adopted an opinion and CVMP assessment report on 16 May2012.

On 16 July 2012 the European Commission adopted a Commission Decision for this application.

Part 1 - Administrative particulars

Manufacturer of the active substance and responsible for batch release:

Intervet International BV
Wim de Körverstraat 35
5831 AN Boxmeer
Netherlands

Manufacturing authorisation issued on 31 August 2010 by the Dutch Minister for Agriculture, Nature and Food Quality.

The European Medicines Agency has reviewed the manufacturer information contained in the application form (Module 1) and available from the EEA National Competent Authorities and determined that all relevant sites underwent GMP inspections by EEA/MRA authorities with a satisfactory outcome within the last 3 years. Hence, no GMP inspections were deemed necessary within the scope of this MAA evaluation procedure.

Valid manufacturing licences and GMP certificates for all sites are available.

The pharmacovigilance system as described by the applicant fulfils the requirements and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any suspected adverse reaction occurring either in the European Union or in a third country.

Overall conclusions on administrative particulars

The assessment of the administrative particulars (Part 1) has revealed no risk.

Part 2 - Quality

Composition

Nobivac L4 is an inactivated bacterial vaccine presented as a liquid suspension for injection without adjuvant. It contains as active substances 3550 to 7100 U (antigenic mass ELISA units) of *Leptospira Canicola*, 290 to 1000 U of *Leptospira Icterohaemorrhagiae*, 500 to 1700 U of *Leptospira Australis* and 650 to 1300 U of *Leptospira Grippotyphosa*. Thiomersal is added as preservative and 0.01 M phosphate buffered saline (PBS) is used as diluent.

Container

European Pharmacopoeia (Ph. Eur.) Type I glass vials are filled with 1 dose or 10 doses of the vaccine, closed with a halogenobutyl rubber stopper and sealed with an aluminium cap.

Development pharmaceutics

Vaccination is the most effective method for prevention of leptospirosis in dogs. The commercially available vaccines up to now protect against serogroups Canicola and Icterohaemorrhagiae. These 2 serogroups have been decreasing in total number of infections, but other serogroups that infect dogs such as Australis and Grippotyphosa have increased.

There is consequently a need for a new vaccine against leptospirosis in dogs. The development of the vaccine Nobivac L4 was based on the preceding vaccine Nobivac Lepto. This vaccine is an inactivated vaccine without adjuvant that contains *Leptospira interrogans* serogroup Canicola serovar Portland-vere and *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni strains.

Nobivac L4 contains strains of the aforementioned serogroups Icterohaemorrhagiae and Canicola, supplemented with 2 new strains, i.e. a strain of serogroup Australis and a strain of serogroup Grippotyphosa.

Method of manufacture

The manufacturing process is straightforward and includes: preparation of medium and inocula, inoculation of the fermenter and growth of culture, termination of the cultivation and inactivation by β-propiolactone (BPL), concentration and purification by filtration, blending of the antigens with PBS diluent and thiomersal and aseptic filling and closing of the vials. Each antigen is purified and concentrated. Homogeneity of the blend is ensured by visual check of appearance of the solution.

Control of starting materials

Active substance

The 4 antigens comprised in the vaccine (i.e. Canicola, Ictero, Australis and Grippo) are sufficiently described with regard to their origin, isolation and history. The control testing on the bacterial seeds is performed in accordance with the relevant guidelines. This control testing is considered satisfactory and purity of the seed materials is sufficiently justified with regard to the risk of contamination of materials from pathogens of the species of origin and the risk for the target species.

The stability of these antigens was not addressed separately. Stability claim for the active substances is based on potency results of a final Nobivac L4 vaccine batch formulated with three-year-old antigens. In view of the data available on leptospirosis vaccines, the CVMP concluded that this approach is considered acceptable since stability results of this vaccine batch up to 39 months are presented with the recommendation to monitor the stability of a second vaccine batch manufactured with aged antigens to support of the claimed shelf-life of 3 years at 2-8 °C for the antigens.

Excipients

The starting materials used for the manufacture of the vaccine are sufficiently controlled by their specifications and Certificates of Analysis are provided for each material. Apart from the bacterial seeds, bovine serum albumin (BSA) is the only material of biological origin. Validation of the inactivation process (by heat and pH 5 treatments) is provided in the format of literature reference, data from suppliers on the validity of the manufacturing process to inactivate/remove viral extraneous agents, a risk assessment by the applicant for use of BSA in the production of Leptospira antigens and validation of the BPL inactivation treatment of the antigens contributing to the viral safety of BSA. For the materials of non-biological origin (i.e. BPL, Ellinghausen-McCullough-Johnson–Harris (EMJH) medium, Modified Hartmann's solution and PBS), acceptable sterilisation procedures are applied with appropriate bioburden limits where relevant.

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

All starting materials of ruminant origin and master seeds comply with the EU requirements, Ph. Eur. monograph 5.2.8 and guidelines EMEA/410/01-Rev.2 "Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" and EMEA/CVMP/019/01 "Position paper on the assessment of the risk of transmission of animal spongiform encephalopathy agents by master seed materials used in the production of veterinary vaccines".

A TSE risk assessment is provided related to the use of BSA in the manufacturing process and related to the bacterial seeds. A certificate of suitability by the European Directorate for the Quality of Medicines is provided for BSA. The seed materials of the 4 *Leptospira* antigens are considered in the risk assessment with regard to their origin and time of first isolation, source of isolation (human or non-ruminant), method of preparation of the seed materials (using EMJH medium containing BSA) and the recipient species of the vaccine (dogs, known to pose a species barrier to TSE infectivity).

The information provided is considered sufficient and in accordance with the TSE Note for Guidance (EMEA/410/01-Rev.2).

It can be concluded that the TSE risk for the use of Nobivac L4 vaccine is negligible.

Control tests during production

The control tests performed during production are described and include sterility of the medium, purity check, test on inactivation and antigen content and sterility determination of the inactivated antigens. Determination of the bacterial cell count during production has been established in accordance with the maximum valid titre prior to inactivation as demonstrated during inactivation kinetics validation studies. The sterility test by direct inoculation has been validated according to the Ph. Eur. requirements for all inactivated antigens.

Control tests on the finished product

The description of the methods used for the control of the finished product (i.e. appearance, pH, osmolarity, final inspection, identity and potency test by ELISA, thiomersal content, safety test, sterility and inactivation) and the specifications were provided. The analytical methods are considered validated and the specifications proposed at release are appropriate to control the quality of the finished product.

With regard to the potency of the vaccine, the applicant developed an *in vitro* antigenic mass ELISA to determine the antigen content in the active substance batches and final vaccine batches, as well as to establish the potency. The validation of the ELISA test shows an intrinsic variability, but the test is considered suitable for its intended use. The applicant proposed a strategy for the stability monitoring and replacement procedures for the reference standard, internal standard and coating monoclonal antibodies. However, the rationale for using t- and F-test as statistical tools to detect significant differences between old and new materials is disputable. For this reason, in case the existing reference would be replaced by a new reference, the applicant is recommended to ensure that the confidence interval of the first 10 results of the new reference are within the limits established based on the first 10 results of the existing reference. As for the revised release limits for the potency test, they are considered justified in relation to the principle of demonstrating the difference between standard and sub-standard vaccine batches. Although a correlation between the in vivo immunogenicity and the in vitro antigenic mass ELISA units is not directly addressed, it has been shown that minimum potent batches are efficacious and maximum potent batches are safe. Nevertheless, the CVMP recommended to re-examine the suitability of the acceptance limits of the potency test when data for 20 vaccine batches will be available.

The results provided on antigen batches are considered relevant to demonstrate batch-to-batch consistency at production scale, however the CVMP recommended that consistency data from three (pilot) production scale size batches should be provided once vaccine production has started and three vaccine batches have been produced.

Stability

Real-time stability data are provided for the final vaccine and three vaccine batches are tested for appearance, antigenic mass, pH and thiomersal content after 0, 9, 15, 21, 27 and 39 months of storage at 2-8 °C. Sterility was tested at the beginning and end of shelf-life. Based upon the results, a shelf-life claim of 3 years at 2-8 °C is supported but the CVMP recommended to monitor the stability of an additional pilot / production scale size batch in the stability program. In addition, in-use shelf-life of 10 hours at room temperature is also demonstrated.

Overall conclusions on quality

The manufacturing process is well described and complies with the relevant regulatory requirements. The inactivation procedure is adequately validated and the control method for the inactivation is sufficiently sensitive.

The 4 antigens comprised in the vaccine (i.e. Canicola, Ictero, Australis and Grippo) are adequately described with regard to their origin, isolation and history. The control testing on the bacterial seeds is performed in accordance with the relevant guidelines. This control testing is considered satisfactory and purity of the seed materials is sufficiently justified with regard to the risk of contamination of materials from pathogens of the species of origin and the risk for the target species.

The starting materials of biological origin pose a negligible risk with respect to the transmission of TSE and other extraneous agents. This is acceptable.

The control tests performed during production are adequately described and include sterility of the medium, purity check, test on inactivation and antigen content and sterility determination of the inactivated antigens.

The description of the methods used for the control of the finished product and the specifications are adequate. The analytical methods are considered validated and the specifications proposed at release are appropriate to control the quality of the finished product.

The antigen content determination (antigenic mass ELISA) is acceptable and ensures that correct amounts of antigen are added to the final product.

The final product controls for Nobivac L4 are in acceptable range for this type of vaccine.

The stability data provided justify a shelf-life of 36 months for the final product.

Nobivac L4 is stable when stored for 3 days at 30 °C and the properties of the vaccine are not affected by piercing of the stopper which justifies an in-use stability of 10 hours and transport at ambient temperature.

Overall, the manufacturing process is described in sufficient detail to give confidence that the manufacture will yield a safe, effective and stable immunological product.

The quality of Nobivac L4 can be considered to be adequately demonstrated.

Part 3 – Safety

Laboratory tests

The laboratory safety trials were carried out in the target species dogs, in young and pregnant animals. All were performed using the Nobivac L4 vaccine simultaneously with Nobivac DHPPi. Additionally, high phosphate Nobivac Rabies vaccine was administered concurrently or simultaneously. Nobivac KC was also administered in the repeated overdose in pups. During the field trial young and adults, including pregnant dogs were used. Also in this case Nobivac L4 was used simultaneously with Nobivac DHPPi, with exception of pups of 6 weeks were Nobivac Puppy DP was given simultaneously.

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

In the safety study in pups the administration of repeated single doses as well as repeated overdose was addressed.

For the single dose administrations 12 pups were vaccinated simultaneously with Nobivac DHPPi and Nobivac L4 and concurrently with Nobivac Rabies HP at 6 weeks of age. A second vaccination at 8 weeks contained DHPPi only and in the third and fourth vaccination at 10 and 12 weeks Nobivac DHPPi, Nobivac L4 and Nobivac Rabies HP were administered simultaneously.

Mild increase in temperature could be observed between 4 hours to 4 days after administration. Faeces with red coloration were observed on one occasion after first vaccination.

Local reactions present in all animals consisted of diffuse, soft or hard swellings up to 2 cm lasting for 14 days after the first vaccination where Nobivac Rabies HP had been given concurrently. A painful reaction was observed in 1 animal. Repeated administration of Nobivac L4 simultaneously with Nobivac

DHPPi and Nobivac Rabies HP resulted in soft or hard and in some cases painful swelling with a size up to 5 cm and lasting more than 28 days.

In repeated dose studies an overdose of Nobivac L4 was administered simultaneously with 20 doses Nobivac DHPPi and concurrently with an overdose Nobivac Rabies HP and a single dose Nobivac KC to 12 pups of 6 weeks of age. A booster of the same dose of Nobivac DHPPi, Nobivac L4 and Nobivac Rabies HP as simultaneous administration and a single dose of Nobivac KC was given at 12 weeks of age. Also in this case mild temperature reactions were observed. General reactions included faeces with red coloration on 2 consecutive days after first vaccination and blood in the cage on one occasion after first vaccination.

Following first vaccination soft or hard swellings up to 5 cm and lasting more than 41 days could be observed in both vaccination sites. Second vaccination resulted in soft or hard and occasionally painful local reactions, persisting more than 28 days.

Examination of reproductive performance

In the GLP safety study in bitches 3 groups of bitches, each in a different stage of pregnancy, were administered an overdose Nobivac L4 with 20 doses Nobivac DHPPi. An overdose Nobivac Rabies HP was applied concurrently. No large changes in temperature or systemic reactions to vaccination were observed in any of the bitches. One bitch displayed diarrhoea and vomiting 3 days after vaccination, but delivered normally with healthy pups.

For the site injected with Nobivac DHPPi + Nobivac L4, local reactions could be hard to soft, with a size up to 4 cm and lasting until day 20. Local reactions due to Nobivac Rabies HP were diffuse soft or hard maximally ranging up to 3 cm and persisting to at least 28 days. None of the vaccination sites were painful.

All bitches delivered at term. Stillborns in 5 bitches and 1 weak pup were recorded. Stillbirth was due to asphyxia during the birth process. The overall percentage of stillborn pups was in the same range as the average percentage of stillborn in the SPF breeding colony.

Pregnant bitches were vaccinated once. While the current Ph. Eur. monograph recommends to apply the schedule of vaccination (2 doses 4 weeks apart) in pregnant bitches this is acceptable as in the currently proposed revision of the Ph. Eur. monograph for inactivated canine leptospirosis the requirement for safety testing in pregnant bitches is removed on the basis that it is now accepted there is no particular risk associated with vaccination against leptospirosis during pregnancy. It can be therefore concluded that there is no concern for the safety of pregnant bitches.

Examination of immunological functions

Concerning the effect on immunological functions the immunological response to canine distemper virus, canine adenovirus-2 (CAV2), canine parvovirus and canine parainfluenza virus and *B. bronchiseptica* after vaccination with Nobivac DHPPi and Nobivac KC was investigated in 2 efficacy studies. Simultaneous administration of Nobivac L4 with Nobivac DHPPi did not show interference.

Special requirements for live vaccines

As Nobivac L4 is an inactivated vaccine, the special requirements for live vaccines were not discussed.

Study of residues

The target species is dogs. There is therefore no consumer safety risk.

Interactions

Interactions were not discussed. However, in both laboratory safety studies Nobivac DHPPi was given simultaneously and Nobivac Rabies HP vaccine was given concurrently and/or simultaneously. Nobivac KC was only given in the overdose study for pups. Also in the field trial Nobivac DHPPi was given simultaneously. The simultaneous use of Nobivac L4 with Nobivac DHPPi gave acceptable adverse reactions.

In general, the administration of Nobivac L4 + Nobivac DHPPi resulted in local reactions that were slightly more severe than described in the SPC for Nobivac DHPPi. However, because it is anticipated that the user will consult the relevant product literature of both of the vaccines to be used at the same time, reactions will be no worse than those described on the Nobivac L4 SPC (as these descriptions were based on studies where Nobivac L4 was simultaneously used with Nobivac DHPPi). Concurrent use of Nobivac L4 + Nobivac KC did not result in adverse reactions worse than described for Nobivac KC alone.

Field studies

Safety under field conditions was studied in a randomized, positive controlled, double blinded GCP compliant clinical study over different kennels. As well pups of 6 to 8 weeks old, pregnant bitches as other adult dogs of different breeds, were included in the study.

Nobivac Lepto was chosen as the positive control vaccine.

Pups were vaccinated twice: the first vaccination with either the product or the positive control at 6-8 weeks of age was administered simultaneously with Nobivac Puppy DP and the second two weeks later simultaneously with Nobivac DHPPi. Pregnant bitches and other dogs were given a single vaccination together with Nobivac DHPPi.

The majority of the pups showed no abnormalities in general health after vaccination. Some pups were less active and/or had a reduced appetite, but returned to normal within 1 to 2 days after vaccination. Two pups died 2 days after vaccination, but no cause of death could be found.

After vaccination mild increase in temperature could be observed but with no significant difference between the treatment groups after both vaccinations.

After first vaccination almost all pups vaccinated with either Nobivac Lepto or Nobivac L4 displayed local reactions up to a maximum size of 3.5 cm and lasting about 14 days. Painful reactions were reported shortly after vaccination in some dogs. Similar reactions were detected after the second vaccination

In other dogs and pregnant bitches mostly no abnormalities were observed in general health or feed intake after vaccination. One pregnant bitch died, but no post mortem examination was performed. For rectal temperatures, no significant differences were found between the treatment groups.

Local reactions in 18% of both treatment groups were found in other dogs and pregnant bitches. For Nobivac L4 reactions were ≤2cm and had disappeared after 5 days. Similar reactions were reported in Nobivac Lepto. Painful reactions were observed in some animals.

The outcome of pregnancy was similar for both groups, as well in the number of live born pups as the number of stillborns.

The safety of Nobivac L4 has been investigated in all instances under conditions when used simultaneously and/or concurrently with other vaccines of the Nobivac range. Thus, the applicant has implemented a worst-case scenario for investigation of safety of Nobivac L4.

The CVMP considered the study design acceptable and based on the results concluded that the safety in the target species in the minimum age recommended for vaccination and in pregnant animals has been demonstrated.

User safety

A user risk assessment has been provided for Nobivac L4 including the elements: hazard identification and characterisation, exposure assessment, risk characterisation, risk management and risk communication.

Given that:

- There are no live components to the vaccine, the excipients are innocuous and there is no adjuvant which may precipitate a reaction if accidentally self-injected.
- The risk of skin exposure and accidental self-administration is very low due to the fact that the vaccine is administered by trained professionals.
- The consequences of accidental exposure (inactivated vaccine, standard excipients, no adjuvant) are considered to be negligible.

The CVMP concluded that Nobivac L4 poses a negligible risk to the user.

Environmental risk assessment

Nobivac L4 is an inactivated bacterial vaccine. The vaccine is to be administered subcutaneously to dogs. Freedom from live organisms is guaranteed by the manufacturing process (GMP). The vaccine is filled into glass vials, and packaging is conventional. Disposal of unused vaccine should be carried out according to local requirements. The risk of possible ecological effects of the inactivated agents, the adjuvant or excipients, is considered negligible. All hazards identified have a negligible likelihood to occur and therefore assessment of the consequences is not necessary. The level of risk is assessed as negligible, and therefore no Phase II Assessment is necessary.

Overall conclusion on safety

In pups injection of Nobivac L4 combined with Nobivac DHPPi induces mild increase in body temperature. Local reactions consist in diffuse, soft or hard swelling and pain at injection site. Swellings lasted about 14 days or over 28 days when also Nobivac Rabies HP had been given simultaneously.

Overdose in pups induced a mild increase in body temperature. At injection site soft or hard, occasionally painful swelling could be observed.

Overdose in pregnant bitches induced mild fluctuations in body temperature. Local reactions consisted of soft to hard swellings which could last up to 20 days.

In pregnant bitches and adult dogs a single injection resulted in soft or hard swellings up to 5 days at injection site in the field.

There are no consumer safety concerns.

The risk to the user and to the environment is negligible.

Overall the CVMP concluded that based on the safety data provided in this dossier the safety profile for Nobivac L4 is acceptable.

Part 4 – Efficacy

Laboratory trials

Leptospira interrogans serogroup Canicola serovar Canicola

Immediate efficacy was tested in 24 dogs of about 6 weeks of age at the start of the study. Vaccinations at the age of 6 and 10 weeks were performed in 8 pups with the full dose and in pups with ¼ dose of Nobivac L4 and 8 pups served as controls. After challenge with serovar Canicola less clinical sings were observed in the vaccinated groups than in the control group, where 1 dog died. In contrast to the control group, no thrombocytopenia was observed in the vaccinated groups. The number of days of infection in blood and urine/kidney was statistically significantly higher in the control group than in the vaccinated groups. The number of dogs positive for infection and renal infection due to leptospirosis, was again statistically significantly higher in the control group than in the Nobivac L4 vaccinated groups. Microscopic Agglutination Test (MAT) titres after vaccination were higher in the full dose Nobivac L4 group than the 25% dose Nobivac L4 group. After challenge, a steep increase in serogroup Canicola MAT titres was observed in the control group and a slight increase in the vaccinated groups. The study demonstrated an onset of immunity at 3 weeks and efficacy in pups from 6 week old and vaccinated twice with a 4 week interval for the serovar Canicola. Since mild clinical signs could be observed in some animals in the vaccinated group, the claim of "reduction of infection and urinary excretion caused by the serovar Canicola" was retained.

To test the influence of maternal derived antibodies (MDA) 15 pups received 2 days prior to vaccination intravenously serum from a serum pool in order to induce artificial antibody positive pups. Seven pups were vaccinated at 6 and 10 weeks with Nobivac L4 only and 8 pups served as control. After challenge with serovar Canicola no clinical signs were observed in the vaccinated group, while signs of disease and thrombocytopenia were present in the control group. Three control pups were euthanized. The number of days of infection in blood and urine/kidney was statistically significantly higher in the control group than in the test group. The number of dogs positive for infection or renal infection, was again statistically significantly higher in the control group than in the test group. Serological response 24 hours after serum administration showed no or low MAT antibody levels against the 4 antigens of the vaccine in the animals from the vaccinated group. This result was stated to be in accordance with the MAT antibody levels measured in pups born from vaccinated dams in earlier experiments and are therefore representative for antibody levels in pups from vaccinated dams. After vaccination, the serological response in the vaccinated group was within the normal range. After challenge, the animals of the test group showed a serogroup Canicola MAT titre similar to that after vaccination. A steep increase in serogroup Canicola MAT titres was found post-challenge. Efficacy in pups from 6 week old and vaccinated twice with a 4 week interval for the serovar Canicola was supported and the study supports the absence of MDA interference on vaccination in case of average MDA levels.

Further justification concerning the MDA model was provided and the model itself could be considered valid for the investigation of MDAs interference.

In order to demonstrate the presence of interfering Canicola antibodies in the MDA study, an additional passive immunization study in hamsters was provided. Serum samples from dogs negative in the MAT were administered to 5 hamsters followed by a challenge with a Canicola challenge strain. Protection was 80% in this group and 100 % in the positive control group (MAT +). Challenge induced 100% mortality in the negative control group.

However, as the expected average MAT titres in puppies representing a worst case level could not be attained, the influence of high levels of MDA could not be answered for. As a consequence of the

presence of MDA levels the primary vaccination in puppies should be postponed so that the first and second injections are not given before 9 weeks and 13 weeks of age respectively, where it is expected that MDA levels for leptospirosis have decreased sufficiently.

For duration of protection 9 pups were vaccinated at 6 and 10 weeks and challenged 12 months after 2nd vaccination with serovar Canicola. A control of 9 pups was included. A statistical difference for body temperature between groups was recorded. Thrombocytopenia was not detected in the vaccinated group. The number of days of infection in blood was not statistically significantly different between the groups. These results would be due to age-resistance. The number of days of infection in urine/kidney was statistically significantly higher in the control group than in the vaccinated group. Also the number of dogs positive for infection or renal infections due to leptospirosis was statistically significantly different. The duration of immunity of 1 year is supported considering the claim of reduction of infection and urinary excretion of *Leptospira interrogans* serogroup Canicola serovar Canicola".

As a whole the claim of "reduction of infection and urinary excretion caused by the serovar Canicola" is considered supported.

***Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni**

Immediate efficacy was tested in 21 dogs of about 6 weeks. Vaccinations at the age of 6 and 10 weeks were performed in 7 pups with the full dose and in 7 pups with ¼ dose of Nobivac L4 and 7 pups served as control. After challenge with serovar Copenhageni the control group displayed wasting and pale conjunctivae in 2 dogs and a statistical significant lower thrombocyte counts on day 3 in comparison to the vaccinated groups. A statistically significantly higher number of days of infection in blood and urine/kidney were calculated in the control group over the Nobivac L4 vaccinated groups. The number of positive dogs in the control group was statistically significantly higher than the full dose group for infection and than both vaccinated groups for renal infection. The full dose vaccine induced higher MAT titres than the 25% dose vaccine for all active components except for serogroup Icterohaemorrhagiae because no antibodies were detected in both vaccinated groups. After challenge, an increase in serogroup Icterohaemorrhagiae MAT titres was observed in all groups. The study demonstrated an onset of immunity of 3 weeks and efficacy in pups from 6 week old and vaccinated twice with a 4 week interval for the serovar Copenhageni.

The artificial MDA procedure 2 day prior to vaccination was applied on 15 pups to evaluate the influence of maternal antibodies. Seven pups were vaccinated at 6 and 10 weeks with Nobivac L4 only and 8 pups served as controls. None of the vaccinated dogs showed signs of disease after challenge with serovar Copenhageni. Clinical signs and thrombocytopenia were present in the control group. The number of days of infection in blood and urine/kidney and the number of positive dogs for infection and renal infections were statistically significantly higher in the control group than in the vaccinated group. No infection in blood, urine and kidney and no abnormalities in the kidneys were detected in any of the vaccinated animals. Serological response 24 hours after serum administration showed no or low MAT antibody levels against the 4 antigens of the vaccine in the animals from the vaccinated group. After vaccination, a serological response in the vaccinated group was observed. After challenge, an increase in serogroup Icterohaemorrhagiae MAT titres was observed in both groups. The results for serovar Copenhageni in the control group were below the requirements of validation of the Ph. Eur Monograph. Nevertheless, an onset of immunity of 3 weeks and efficacy in pups from 6 week old and vaccinated twice with a 4 week interval for the serovar Copenhageni was supported and the study seems to support the absence of MDA interference on vaccination. Further justification on the model used to generate artificial MDA was provided.

For duration of immunity challenge with serovar Copenhageni in 18 dogs was performed 1 year after 2nd vaccination. Transient diarrhoea in 1 dog and ocular mucopurulent discharge in 3 dogs of the

control groups were seen. High body temperatures were measured in both groups during the entire study. No other signs of disease or haematological differences between both groups were reported. The number of days of infection in blood or urine/kidney was not statistically significantly different between the groups. The number of dogs positive for infection and renal infection was statistically significantly higher in the control group than in the test group. These results would be due to age-resistance. Low MAT titers were observed in both groups before challenge and a serological response was observed in both groups. The duration of immunity of 12 months is supported.

As a whole the claim of "reduction of infection and urinary excretion caused by the serovar Copenhageni" can be considered supported.

***Leptospira kirschneri* serogroup Grippothyphosa serovar Bananal/Liangguang**

Immediate efficacy was tested in 24 dogs of about 6 weeks. Vaccinations at the age of 6 and 10 weeks were performed in 8 pups with the full dose and in 8 pups with ¼ dose of Nobivac L4 and 8 pups served as control. Wasting, kyphosis and vomiting were observed in the control group, but not in the vaccinated group. No thrombocytopenia was reported in the vaccinated groups. In contrast, there were 3 thrombocytopenic pups in the control group. A statistically significantly higher number of days of infection in blood and urine/kidney was calculated in the control group over the vaccinated groups. The number of dogs positive for infection and renal infection, was again statistically significantly higher in the control group than in the vaccinated groups. For both vaccinated groups no challenge organisms could be detected in the blood, urine and kidney. No abnormalities were detected in the kidneys in any of the animals, including the control group. The full dose vaccine induced higher MAT titres than the 25% dose vaccine for all active components. After challenge, the Nobivac L4 vaccinated groups showed a higher MAT titre against serogroup Grippotyphosa when compared with the post-vaccination titres. The study demonstrated an onset of immunity at 3 weeks and efficacy in pups from 6 week old and vaccinated twice with a 4 week interval for the serovar Bananal/Liangguang.

The artificial MDA procedure 2 day prior to vaccination was applied on 16 pups to evaluate the influence of maternal antibodies. After challenge with serovar Bananal/Liangguang, the vaccinated pups did not show any sign of disease. One control dog was euthanized. Thrombocytopenia was observed in the control group. The number of days of infection in blood was statistically significantly different between groups, but was not different for renal infection. Also the number of dogs positive for infection was statistically significantly higher in the control group than in the test group, but no difference was observed for renal infection. Serological response 24 hours after serum administration showed no or low MAT antibody levels against the 4 antigens of the vaccine in the animals from the vaccinated group. After vaccination, a serological response in the vaccinated group was observed. After challenge, an increase in MAT titres was observed in both groups. Since no effect on urine/kidney infection could be demonstrated, the absence of interference of MDA on vaccination was considered not fully proven.

It is noted that although renal infection was established in only 4/8 control dogs, 0/8 vaccinated dogs and 8/8 control dogs were deemed positive for infection. As discussed previously for the MDA studies with serovars Canicola and Copenhageni, the study is relevant in supporting the efficacy of vaccination against the Bananal/Liangguang serovar.

One year after 2nd vaccination 18 pups were challenged with serovar Bananal/Liangguang. Only some mild ocular symptoms were observed in both groups. Thrombocyte counts were inconclusive. The number of days of infection in blood and number of positive dogs for infection was statistically significantly different between groups, but was not different for renal infection. From none of the dogs any of the urine or kidney cultures was positive in the duration of immunity study. Before challenge both groups had no or very low MAT titers and post challenge the serological responses for serovar

Bananal/ Liangguang were similar. The duration of immunity of 1 year is considered sufficiently supported in relation to the claim reduction of infection and urinary excretion.

As a whole the claim of "reduction of infection and urinary excretion caused by the serovar Bananal/ Liangguang" can be considered supported.

***Leptospira interrogans* serogroup Australis serovar Bratislava**

For immediate efficacy, vaccinations at the age of 6 and 10 weeks were administered in pups with the full dose and in 8 pups with ¼ dose of Nobivac L4 and 8 pups served as controls. After challenge with serovar Bratislava, no signs of disease or differences for the thrombocyte counts between the different groups were observed. Total leucocyte counts increased significantly in the full dose group. The number of days of infection in blood and number of positive dogs for infection was statistically significantly different between groups, but was not for renal infection. The full dose vaccine induced higher MAT titres than the 25% dose vaccine. Low MAT titers for the serovar Australis were present in the control group pre-challenge. An increase in titers was detected in all groups after challenge.

Although a beneficial effect of vaccination against urinary excretion or renal infection was not observed, overall there was a reduction in the parameter 'infection' between vaccinated vs control dogs. Therefore, the onset of immunity at 3 weeks was considered demonstrated for the serovar Bratislava.

To evaluate the influence of maternal antibodies 16 pups received serum pool serum 2 days prior to vaccination. None of the vaccinated dogs showed signs of disease after challenge with serovar Bratislava. Wasting in 1 dog and thrombocytopenia were present in the control group. The number of days of infection in blood and number of positive dogs for infection was statistically significantly different between groups, but was not different for renal infection. Minor changes to the liver were detected in both groups. Serological response 24 hours after serum administration showed no or low MAT antibody levels against the 4 antigens of the vaccine in the animals from the vaccinated group. For serovar Australis most dogs were seropositive. An increase in serogroup Australis MAT titres was found postchallenge, especially in the control group. No effect on urine/kidney infection could be demonstrated.

This study shows that the presence of passively-transferred antibodies raised against the Bratislava serovar do not interfere with the response to vaccination, albeit a relatively weak claim in line with the results obtained in the onset of protection study. Passive transfer of MDAs (Bratislava component only) did appear to be successful prior to vaccination showing that the presence of MDAs does not hamper the immunological response to the Bratislava component of the vaccine.

For duration of immunity double challenge with serovar Bratislava in 18 dogs was performed 1 year after 2nd vaccination. Double challenge was performed to assure a good challenge effect in 1-year old dogs which are expected to have an age-dependent increased resistance against leptospirosis. There was only a statistical significant difference for the number of dogs positive for infection between groups. Although four control dogs were regarded as positive for renal infection and all the vaccinated dogs were negative, this difference was not statistically different. Based on the results on infection, the duration of immunity of 12 months was considered supported.

As a whole the claim of "reduction of infection caused by *Leptospira interrogans* serogroup Australis serovar Bratislava" can be considered supported. However, the claim of reduction of urinary excretion was not justified.

***Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae**

No challenge studies were performed in the target animals for this serovar. The applicant conducted the following experiments on the basis that *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae and *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni are very closely related.

Passive immunization in 6 groups of hamsters against the monoclonal antibody (mab) directed against serovar Copenhageni was tested. Three groups received a dose of a relevant monoclonal antibody (mab) and 3 groups served as control. Different doses of serovar Icterohaemorrhagiae were administered. In contrast to the control groups, all animals in the mab-treated groups remained culture-negative. In the highest dose-group, there was no mortality in the mab-treated group whereas all control animals died. The study can only be considered indicative and implies that the mab used also protects hamsters from renal infection with a strain of L. interrogans serogroup Ictero serovar Ictero and mortality due to infection with this serovar at high challenge titers.

MAT cross-reactivity between serovars Icterohaemorrhagiae and Copenhageni was examined with sera from vaccinated dogs and non-vaccinated but challenged dogs. Measurement of the MAT titres in sera against different strains demonstrated no relevant differences between serovars Icterohaemorrhagiae and Copenhageni in the sera of challenged dogs. The MAT titers of sera of vaccinated dogs were slightly lower for serovar Icterohaemorraghiae strain in comparison to the serovar Copenhageni strains. The study indicates the close-relatedness of the strains.

Close relatedness within serogroups Icterohaemorrhagiae strains were tested against a panel of 18 serogroup Icterohaemorrhagiae specific mabs. Serovars Copenhageni and Icterohaemorrhagiae shared 14 out 18 agglutination reactions with mabs.

Further support was provided in an additional vaccination/challenge study in hamsters and a growth inhibition test (GIT).

In the hamster test at total of 20 hamsters were vaccinated or left unvaccinated. Challenge with different quantities of a virulent culture of serovar Ictero induced 80-100% mortality in the unvaccinated hamsters and at least 80% survival in the vaccinated groups.

In the GIT two-fold serial dilutions of test sera of dogs 2 weeks after re-vaccination and positive and negative control serum were prepared and inoculated with a standard amount of bacteria of the relevant serovars of Leptospira. Although there was variability in titres between dogs, in all sera significant neutralizing activity against serovar Copenhageni (vaccine strain), serovar Copenhageni (challenge strain) and serovar Ictero were present. Similar neutralizing activity between strains was observed.

These studies were performed as challenge studies in hamsters. Since no challenge studies have been performed in dogs, but close relatedness has been shown with strains shown as protective, the statement that there is an expected protection against serovar Icterohaemorrhagiae was included under section 5.1 of the SPC.

***Leptospira kirschneri* serogroup Grippothyphosa serovar Grippothyphosa**

No challenge studies were performed in the target animals for this serovar. The applicants conducted other experiments considering that *Leptospira kirschneri* serogroup Grippotyphosa serovar Dadas and *Leptospira kirschneri* serogroup Grippotyphosa serovar Grippotyphosa are very closely related.

Reference to a cross-protection study for serovar Grippothyphosa in hamsters was made. As the challenge strain used was not virulent, no conclusion could be drawn.

MAT cross-reactivity between serovars Dadas and Grippotyphosa was examined with sera from vaccinated dogs and non-vaccinated but challenged dogs. Measurement of the MAT titres in sera against different strains demonstrated no relevant differences between serovars Dadas and Grippotyphosa in the sera of challenged dogs. The provided study indicates the close-relatedness of the strains.

Close relatedness within serogroups Grippotyphosa strains were tested against a panel of 13 serogroup Grippotyphosa specific Mabs. Serovars Grippotyphosa and Dadas shared 10 out 13 agglutination reactions with mabs and close relatedness seems to be proven.

Further support was provided in an additional vaccination/challenge study in hamsters and a growth inhibition test (GIT). However, also in this case the hamster study was inconclusive, as the Grippo strain was not lethal to hamsters.

The GIT showed that although there was variability in titre between dogs, in all sera significant neutralizing activity against serovar Dadas, serovar Bananal/Liangguang and server Grippo was present. In most dogs titres of antibodies inducing growth inhibition of the serovar Grippo were higher than of both other strains.

Since no challenge studies have been performed in dogs, but since close relatedness has been shown with strains shown as protective, the statement that there is an expected protection against serovar Grippothyphosa was included under section 5.1 of the SPC.

To evaluate the effect of re-vaccination MAT titers after primary vaccination and re-vaccination after 1 year were compared in 2 trials with 16 dogs. In both studies single dose re-vaccination with Nobivac L4, one year after the primary vaccination course, was sufficient to boost serum MAT titres against all serogroups to similar or higher levels than those measured after primary vaccination. The annual revaccination in relation to the claims made can be considered supported since MAT titres are indicative of protection.

Field trials

A combined field safety/efficacy study was performed on five sites in the Netherlands following a randomised, blinded and controlled design and dogs of various breeds of different ages were vaccinated with Nobivac L4.

On each site, dogs were divided into three categories: pup, pregnant bitch or "other" (male dogs and non pregnant bitches) and subsequently randomly assigned to one of the two treatment groups: the Nobivac L4 group or the control group (vaccinated with Nobivac Lepto). The interval between the vaccinations was 2 weeks.

For pups the antibody response to the Australis and Grippothyphosa antigen were significantly higher for Nobivac L4 as compared to animals of the Nobivac Lepto group. No difference in response was found for the Canicola and Icteohaemorrhagiae antigen. Similar results were observed in the other dogs.

However, seroconversion against serogroup Icterohaemorrhagiae after vaccination in pups was only observed in 9% of the Nobivac L4 vaccinated pups and 30% of the Nobivac Lepto group.

In conclusion the study showed comparable serological response for Canicola and Icteroheamorrhagiae in both groups as well in pups as in other dogs. The low seroconversion for serovagroup Icterohaemorrhagiae in pups may indicate an interference of MDA.

The analysis of efficacy in the field trial was based on serological analysis. As a correlation has not been established between serology and protection, the data is supportive in nature. Furthermore, some clarification is requested regarding the serological analysis.

The impact of low seroconversion was further investigated in 2 hamster tests.

In the first test one group was injected with pooled serum of Nobivac L4 vaccinated pups from the field study and one group served as control. After challenge with serovar Copenhageni all serum treated hamster survived and all control hamster died.

In the second test three groups of hamsters were treated with serum pool of vaccinated pups from the field study before vaccination, serum pool of non-vaccinated dogs challenged with serovar Copenhageni, serum pool of non-vaccinated dogs before challenge with serovar Copenhageni and one group served as negative control. All hamster of the negative control group died to challenge with serovar Copenhageni while 100% survival was observed in hamsters treated with sera of non-vaccinated dogs. Also serum of non-vaccinated dogs induced protection in 40% of the animals suggesting protection via MDAs.

Overall, it was demonstrated by these hamster passive protection experiments that vaccination of the pups with Nobivac L4 in the field had induced a protective humoral immunity against a virulent strain of *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni which could not be determined by MAT. This also suggests other mechanisms of protection.

The study provides support for the notion that protection in dogs would be achieved without measurable antibody responses post-vaccination, but nevertheless the data are indicative in nature. Furthermore, a degree of protection achieved via the presence of MDAs could not be completely ruled out.

Considering the possible influence of high level MDA, the second vaccination of the primary vaccination in puppies should be given at 10 weeks at the earliest.

Some data was provided concerning interference of the CPi component by injection, which might be acceptable to accept the simultaneous use of Nobivac L4 with the CPi component by injection.

Overall conclusion on efficacy

Based on the efficacy data of this dossier, efficacy against the components *Leptospira interrogans* serogroup Canicola serovar Canicola, *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni, *Leptospira interrogans* serogroup Australis serovar Bratislava and *Leptospira kirschneri* serogroup Grippotyphosa serovar Bananal/Liangguang is considered demonstrated.

Concerning *L. interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae *and L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, in vitro and in vivo data in non-target species suggests that the vaccine may provide a degree of cross-protection against these components.

Part 5 – Benefit risk assessment

Nobivac L4 (L4) is a tetravalent inactivated vaccine containing whole cell concentrate of *Leptospira interrogans* serogroup Canicola serovar Portland-Vere, *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni , *Leptospira interrogans* serogroup Australis serovar Bratislava and *Leptospira kirschneri* serogroup Grippotyphosa serovar Dadas without adjuvant. The indication for use is "For active immunisation of dogs against:

- *Leptospira interrogans* serogroup Canicola serovar Canicola to reduce infection and urinary excretion;

- *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni to reduce infection and urinary excretion;

- *Leptospira interrogans* serogroup Australis serovar Bratislava to reduce infection;

- *Leptospira kirschneri* serogroup Grippotyphosa serovar Bananal/Liangguang to reduce infection and urinary excretion."

One dose of vaccine is administered twice subcutaneously with a 4 week interval. Onset of immunity in 6-week-old pups is observed from 3 weeks after the last injection. Annual booster vaccination is recommended.

Benefit assessment

Direct benefits

Reduction of infection and urinary excretion against the components *Leptospira interrogans* serogroup Canicola serovar Canicola, *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni and *Leptospira kirschneri* serogroup Grippotyphosa serovar Bananal/Liangguang and reduction of infection against *Leptospira interrogans* serogroup Australis serovar Bratislava.

Indirect or additional benefits

Reduction of infection might decrease the amounts of *Leptospira* shed and as such reduce the zoonotic risk.

Concerning *L. interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae and *L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, *in vitro* and *in vivo* data in non-target species suggests that the vaccine may provide a degree of cross-protection against these components.

Risk assessment

Overall, the manufacturing process is described in sufficient detail to give confidence that the manufacture will yield a safe, effective and stable immunological product.

The quality of Nobivac L4 can be considered to be adequately demonstrated. Nevertheless, the CVMP issued five quality recommendations for the post-authorisation phase:

- The suitability of the acceptance limits of the potency test should be re-examined when data for 20 vaccine batches will become available.
- For the stability monitoring and replacement procedures for the reference standard, internal standard and coating monoclonal antibodies, the applicant is recommended to ensure that the confidence interval of the first 10 results of the new reference are within the limits established based on the first 10 results of the existing reference.
- The batch protocols for the first 3 production batches post-authorization should be provided to the CVMP.
- The applicant is recommended to monitor the stability of a second vaccine batch manufactured with aged antigens to support of the claimed shelf-life of 3 years at 2-8 °C for the antigens.
- The stability of an additional pilot / production scale size vaccine batch should be monitored to support of the claimed shelf-life of 3 years at 2-8 °C for the vaccine.

In pups injection of Nobivac L4 combined with Nobivac DHPPi induces mild increase in body temperature. Local reactions consist in diffuse, soft or hard swelling and occasionally pain at injection

site. Swellings lasted about 14 days or over 28 days when also Nobivac Rabies HP had been given simultaneously.

Overdose in pups induced a mild increase in body temperature. At injection site soft or hard, occasionally painful swelling could be observed. Overdose in pregnant bitches induced mild fluctuations in body temperature. Local reactions consisted of soft to hard swellings which could last up to 20 days. In pregnant bitches and adult dogs a single injection resulted in soft or hard swellings up to 5 days at injection site in the field.

There are no consumer safety concerns.

The risk to the user and to the environment is negligible.

Overall the CVMP concluded that based on the safety data provided in this dossier the safety profile for Nobivac L4 is acceptable.

Evaluation of the benefit risk balance

The quality of the product is acceptable and well defined outstanding issues are addressed through recommendations to the applicant. The product is well tolerated in dogs and presents a low risk for users and the environment. Efficacy against the components *Leptospira interrogans* serogroup Canicola serovar Canicola, *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni, *Leptospira interrogans* serogroup Australis serovar Bratislava and *Leptospira kirschneri* serogroup Grippotyphosa serovar Bananal/Liangguang was demonstrated. Furthermore, in vitro and in vivo data in non-target species suggests that the vaccine may provide a degree of cross-protection against *L. interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae and *L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa.

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

Conclusion on benefit risk balance

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP considers that the application for Nobivac L4 is approvable.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that, the quality, safety and efficacy of Nobivac L4 can be considered to be in accordance with the requirements of Council Directive 2001/82/EC, as amended and that the benefit-risk balance is favourable.