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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Vectormune ND (EMEA/V/C/003829/0000)

Common name: Newcastle disease and Marek's disease vaccine (live recombinant)

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

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Introduction

On 17 April 2014, the applicant CEVA-Phylaxia Veterinary Biologicals Co. Ltd. submitted an application for a marketing authorisation to the European Medicines Agency (The Agency) for Vectormune ND, under Article 3(1) of Regulation (EC) No 726/2004 (veterinary medicinal product developed by means of a biotechnological process).

The eligibility to the centralized procedure was agreed upon by the CVMP on 27 June 2013 as the product is produced by means of a biotechnological process (Article 3(1) of Regulation (EC) No 726/2004). The rapporteur appointed was F. Klein and the co-rapporteur was E. Werner.

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

Vectormune ND is a live virus vaccine intended for active immunisation of 18-day-old embryonated chicken eggs or one-day-old broiler chickens to reduce mortality and clinical signs caused by Newcastle disease virus (NDV) and to reduce mortality, clinical signs and lesions caused by Marek's disease virus (MDV).

It contains as active substance a live recombinant serotype 3 herpesvirus of turkey (HVT) including the inserted fusion protein (F) gene of a lentogen strain of a NDV. Each dose contains from 2,500 to 8,000 plaque forming units (PFU).

Vectormune ND is a suspension after reconstitution of the frozen cell-associated vaccine strain (rHVT/ND) in sterile vaccine solvent. The inoculation volume is 0.05 ml for in ovo (IO) application and 0.2 ml for subcutaneous (SC) injection.

The product is presented in 2 ml pre-labelled hydrolytic type 1 glass ampoule of 1,000, 2,000 or 4,000 doses in pack sizes of 5 ampoules per cane and the solvent is presented in polyvinylchloride (PVC) infusion bags of 200 ml, 400 ml, 800 ml.

On 9 July 2015, the CVMP adopted an opinion and CVMP assessment report.

On 8 September 2015, the European Commission adopted a Commission Decision granting the marketing authorisation for Vectormune ND.

Scientific advice

The applicant received scientific advice from the CVMP on 15 September 2011 (SA-095-11-I; EMA/CVMP/453569/2011). The scientific advice accepted the applicant's proposal to not establish duration of immunity for the Marek's disease (MD) component of the vaccine.

Nevertheless, it was expected that the applicant would present some evidence on the duration of protection provided by the vaccine during the risk period, according to the target species and production cycle.

These recommendations were followed by the applicant.

MUMS limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided an acceptable detailed description of the pharmacovigilance system (DDPS) (dated 1 April 2014) which fulfils the requirements of Directive 2001/82/EC, as amended. 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 Community or in a third country. There are no outstanding issue.

Manufacturing authorisations and inspection status

CEVA-Phylaxia (Budapest, Hungary), undertakes the manufacturing, secondary packaging and the batch release as well. CEVA SANTE ANIMAL (Libourne, France), responsible for the packaging as a secondary packaging site, both of them have been recently inspected by European Union (EU) competent authorities (2012 and 2013) and were found to be Good Manufacturing Practice (GMP) compliant. GMP certificates were provided. No additional GMP inspections are deemed necessary at the current stage.

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

Vectormune ND is a bivalent, cell-associated, live recombinant vector vaccine (rHVT/ND) intended for the active immunization of chickens, against Newcastle disease (ND) and MD. The active ingredient is a recombinant strain of the live turkey herpesvirus serotype 3 (FC-126 HVT) genetically modified by including the F gene of a lentogenic NDV (NDV strain D26). The vaccine is administered by IO or SC injection after reconstitution in sterile vaccine solvent.

Excipients include cryoprotectant 1 (Marek's EMEM, L glutamine, sodium bicarbonate, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), bovine serum, water for injection) and cryoprotectant 2 with dimethyl sulphoxide (DMSO) (Marek's EMEM, L glutamine, sodium bicarbonate, HEPES), and water for injection. No adjuvants are added. Gentamicin is present in cryoprotectant 1, which is used in the final stage of harvesting. Gentamicin is also used in the cell culture medium. Since the residual gentamicin content in the final vaccine will be not more than 150 ng per dose, therefore no pharmacological activity can be expected, the proposed use of gentamicin is acceptable.

The solvent contains sucrose, casein hydrolysate, sorbitol, dipotassium hydrogen phosphate and potassium dihidrogen phosphate, phenol red and water for injection. No adjuvants or preservatives are added.

Container

The vaccine is filled into 2 ml pre-labelled hydrolytic type I glass ampoules, which are flame sealed providing an airtight closure. The containers are compliant to the European Pharmacopoeia (Ph. Eur.). The chosen containers are suitable for storage in liquid nitrogen and are typical to ampoules used for the storage of cell-associated vaccines against MD. The solvent is presented in polyvinylchloride infusion bags containing 200 ml, 400 ml or 800 ml solvent.

Development pharmaceutics

The vaccine is a bivalent, cell-associated, live recombinant vector vaccine that contains one active ingredient: a modified live turkey herpesvirus (rHVT/ND). It was constructed using the naturally occurring, non-pathogenic FC-126 HVT vaccine strain as a vector, genetically modified to express the immunogenic F of NDV.

The vaccine is presented as a suspension for injection in flame sealed ampoules, to be reconstituted before use in a sterile solvent.

The parental FC-126 HVT strain was purchased and the inserted gene originated from a lentogenic NDV strain D26, also called the Sato strain (Nagai et al., 1980). F gene from the NDV strain D26 was inserted in the HVT genome via homologous recombination. The correct sequence of F gene product was verified after insertion by Western blot, Southern blot and PCR specific to the F gene and the flanking region.

The vaccine strain (rHVT/ND) is propagated in chicken embryo fibroblast (CEF) cell culture produced using specific pathogen free (SPF) chicken embryos. At harvest trypsin of animal or vegetable origin (irradiated) is used to detach the cells from the culture vessels. No adjuvant is contained in the vaccine. The virus suspension is buffered to ensure consistent pH. As the finished product is a cell-associated virus stored in liquid nitrogen, cryoprotectants are used. Gentamicin is added to cryoprotectant 1, which is used in the final stage of the harvesting step. The finished product therefore contains a low residual level of this antibiotic substance (not more than 150 ng per dose) and this is acceptable.

Phenolsulfonphthalein (phenol red) is added to the solvent as a pH indicator.

The maximum titre to be applied for each batch of the vaccine at release is based on results from the overdose safety studies showing that 80,000 PFU/dose was safe: the maximum titre limit is defined as 8,000 PFU/dose.

The establishment of the minimum protecting dose is based on the immunogenicity studies. According to the efficacy studies, 2,500 PFU/dose of vaccine applied to the target species by the IO or the SC route was demonstrated to be efficacious by challenge studies in chicks.

Method of manufacture

The production process was carried out under aseptic conditions, using sterile materials, containers and equipment. SPF chicken eggs are used to produce CEF for virus propagation. Cells are counted, planted and incubated. The working seed virus (WSV) is thawed and diluted in a growth media; CEF cultures are inoculated and incubated. When monolayer cells show the characteristic cytopathic effect, the cells are harvested. Cell concentration is adjusted by addition of cryoprotectant 1 and cryoprotectant 2 to prepare the final vaccine bulk. The final bulk vaccine is prepared based on

validated manufacturing process.

The bulk vaccine is filled into sterilised pre-labelled glass ampoules using a filling-sealing machine. The filled ampoules are sealed and subsequently frozen and placed into liquid nitrogen for storage.

Batches of finished product are made based on the seed lot system from the master seed virus (MSV).

Data of process validation was provided. Process parameters and acceptance criteria have been sufficiently described and documented and are acceptable.

The manufacturing process was shown to be capable to produce final vaccine of sufficient quality in a consistent way. The potency test method was properly validated.

Solvent components are: sucrose casein hydrolysate, sorbitol, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, phenol red, water for injection. The solvent is dispensed into final containers (PCV bags) using a hand fill or an automatic filling and sealing machine. The finished product is tested for appearance, pH, osmolarity and sterility.

Control of starting materials

Active substance

The generation of the MSV and WSV including all the control testing that has been performed on the MSV/WSV has been properly described. Sequencing analysis confirmed genetic stability of the recombinant virus. Furthermore the absence of wild type HVT in the MSV was demonstrated.

Excipients

All starting materials listed in a pharmacopoeia are compliant with the respective Ph. Eur. monographs and supported by appropriate certificates of analysis: SPF chicken's eggs (Ph. Eur. 5.2.2), D glucose monohydrate (Ph. Eur. 0177) or D glucose anhydrate (Ph. Eur. 0178), sodium bicarbonate (Ph. Eur. 0195), gentamicin sulphate (Ph. Eur 0331), potassium chloride (Ph. Eur. 0177), sodium chloride (Ph. Eur. 0193), DMSO (Ph. Eur. 0763) and water for injection (Ph. Eur. 0169).

Starting materials of biological origin not listed in a pharmacopoeia include: the MSV, the WSV, SPF hen's eggs (Ph. Eur. 5.2.2.), porcine trypsin, bovine serum (fetal, calf or newborn), tryptose broth (porcine tissue, bovine milk) and casein hydrolysate (stabilizer).

Starting materials of non-biological origin not listed in a pharmacopoeia include: Eagle's minimum essential medium (for cell culture), L-glutamine (cell culture additive) and HEPES (buffer).

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

A transmissible spongiform encephalopathy (TSE) risk assessment was made in accordance with the current note for guidance (NfG) on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01 Rev. 3).

During the construction and passages of the genetically modified organism (GMO) no materials

having TSE infectivity risk were used, except for propagation in CEF cell culture media containing bovine serum. During early passages of the recombinant virus only bovine serum was used sourced from countries with negligible or controlled TSE risk. It is also to note that the CEF culture is a cell system which is not susceptible to ruminant prion infection. During establishment of the EU MSV bovine serum certified by the EDQM having no TSE risk was used. Furthermore, the product casein hydrolysate (excipient) uses the bovine milk derived casein starting material. Information was provided by the applicant on starting materials of animal origin. Representative certificates of suitability are provided for the different bovine sera. All materials used in the process are in compliance with the NfG on TSE/BSE risk (EMEA/410/01 Rev. 3) and there is no evidence for any risk that the seed material would be contaminated with TSE agents. It is concluded that the risk of transmitting TSE through the use of this vaccine is negligible.

Control tests during production

In-process controls during the manufacturing of the vaccine include cell counting at the cell planting stage and after cell harvesting as well as testing fill volume during the filling of the cell suspension in ampoules. All test methods were sufficiently described. A pH measurement after dissolution of the solvent components is included as in-process controls.

Control tests on the finished product

The following control tests were performed on the finished product (cell suspension and solvent):

- Cell suspension: Appearance, pH, sterility, mycoplasma, identity, potency, extraneous agents and safety.
- Solvent: Appearance, pH, sterility, osmolarity and fill volume.

The specifications proposed are appropriate to control the quality of the finished product. All release test methods were properly validated.

Batch analysis data were provided for cell suspension and the solvent, demonstrating compliance to the specifications.

Stability

For the cryoprotectant solutions shelf lives are assigned which have been validated in the consistency lots. Batches of the vaccine (cell suspension for injection) stored in the final container and under the recommended conditions (in liquid nitrogen) are being tested for real time stability. Stability data up to 27 months were provided. Parameters at all-time points met the specifications. The test results indicate that the virus titres remain constant during the shelf life; no decline in potency is observed. These data support a shelf life of 2 years. The in use stability of the vaccine was demonstrated for both the IO and the SC administration in compliance with CVMP Guideline on data requirements to support in-use stability claims for veterinary vaccines (EMA/CVMP/IWP/250147/2008). The in use stability of the vaccines is guaranteed at room temperature within 2 hours from reconstitution. This is reflected in the Summary of Product Characteristics (SPC).

Stability data were provided for batches of 200, 400 and 800 ml presentations of the sterile solvent. The appearance, pH, osmolarity and the sterility were measured. Results are available up to 24

months; all results are compliant to the specifications. Based on the stability data provided, the proposed shelf life for the solvent of 2 years at room temperature is acceptable.

Overall conclusions on quality

The composition and characteristics of the vaccine were sufficiently described. Detailed information has been provided on the starting materials, including the preparation and characterisation of the master virus seed. The manufacturing process was described and properly validated. The process was shown to be capable to produce final vaccine of sufficient quality in a consistent way. In-process controls tests and release tests on final product are properly described and are considered sufficient to control the quality of the vaccine.

The validation of the potency assay was described in detail and adequately performed.

The main risks concerning TSE are considered negligible.

Batch release and stability data showed consistency and compliance of the product's quality attributes with the specifications.

The results from the stability studies support the proposed shelf life for the vaccine suspension (2 years) and the solvent (2 years).

For the time being, only the 200, 400 and 800 ml presentations of the solvent completed a full stability program and will be included in the marketing authorisation application (MAA).

In conclusion, the production and quality control of Vectormune ND are adequately described and comply with the respective legal requirements including the TSE risk assessment.

Part 3 – Safety

Vectormune ND is a cell-associated live viral vaccine intended for a single administration to 18-day-old broiler embryonated eggs by IO administration or to one-day old broiler chicks by the SC route. The vaccine is a suspension for injection containing a live recombinant virus (rHVT/ND) derived from the modified live turkey herpesvirus (HVT) by insertion of F gene from a lentogenic NDV.

Safety documentation

The safety profile of the parental strain after insertion of the F gene of NDV-26 lentogen strain was studied in 6 laboratory and 2 field studies and was supported by pharmacovigilance data gathered outside Europe and data from laboratory efficacy studies. The laboratory studies were conducted in compliance with Ph. Eur. monograph 0589 for MD (live) as well as general requirements of the Ph. Eur. general chapter 5.2.6 Evaluation of safety of veterinary vaccines and immunosera and in Directive 2001/82/EC. According to special requirements for live vaccines, studies were provided on the spread of the vaccine strain from vaccinated chickens to contact target species and non-target species, as well as the spread from non-target species to non-target species. Studies were provided also on dissemination in the vaccinated animals, on reversion to virulence, on biological properties and recombination or genomic re-assortment of the vaccine strains.

Laboratory tests

Safety studies were reported in compliance with Good Laboratory Practice (GLP) standards.

Safety of the administration of one dose

A study demonstrating the safety of one dose was not provided. This is acceptable since the vaccine met the safety criteria required by Marek's disease vaccine (live) Ph. Eur. monograph 0589 in the following overdose studies.

Safety of one administration of an overdose

The safety of a 10-fold overdose of Vectormune ND administered by both indicated routes of administration (IO and SC) was assessed in 2 studies. The studies were compliant with requirements of Ph. Eur. 5.2.6 Evaluation of safety of veterinary vaccines and immunosera, CVMP Guideline on live recombinant vector vaccines for veterinary use (EMEA/CVMP/004/04), Directive 2001/82/EC as amended and CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010).

In the first study, 50 eighteen-day-old SPF embryonated chicken eggs and 40 SPF one-day-old chicks were administered a 10-fold overdose of the vaccine strain at the lowest passage (MSV) respectively by IO and by SC route and monitored for 123 days. No clinical signs and no macroscopic lesions of MD after necropsy were observed in both groups. Results showed that vaccination did not affect hatchability (hatchability rate was 96%), or mortality within 7 days post vaccination. The study met the safety criteria required by Ph. Eur. 0589 on Marek's disease vaccine (live), as more than 70% of positive control birds died of MD and no clinical sign or lesion of MD was reported in the vaccinated group.

In conclusion, the administration of a 10-fold overdose of Vectormune ND MSV is considered safe in 18-day embryonated chicken eggs by IO administration and in one-day-old chicks by SC administration.

A second study was conducted on 60 eighteen-day-old SPF embryonated chicken eggs treated with 10-fold overdose of Vectormune ND (80,000 PFU/0,05ml/egg) by IO route and 50 one-day-old SPF chicks treated with 10-fold overdose of Vectormune ND (80,000 PFU/0.2ml/animal) by SC route. Fifty (50) animals were kept unvaccinated as control group. The animals were monitored daily, for 3 weeks, for clinical signs, behaviour and mortality. Body weight was also recorded in all birds weekly. Chickens vaccinated by SC route were examined for local injection site reactions.

Results showed that the IO administration of the vaccine did not adversely affect the hatching rate of vaccinated chickens compared to controls. The weight gain of vaccinated birds (by both administration routes) was similar to the controls. No lesions were observed at the injection site at necropsy.

Due to the short time of observation of this study in comparison to the normal course of MD, results from this study can only be considered supportive.

Safety of the repeated administration of one dose

Data on the repeated administration of Vectormune ND were not provided because the product is intended to be administered only once. This is in accordance with Directive 2001/82/EC.

Examination of reproductive performance

Vectormune ND is not recommended for use in breeding birds. The vaccine is indicated for use in broiler chickens only. No study was provided on the examination of reproductive performance. This approach is considered acceptable.

Examination of immunological functions

The parent strain HVT is non-pathogenic and it is not known to be immunosuppressive in chickens. In addition, NDV is not known to negatively influence immunological functions in chickens. According to the safety studies conducted on this vaccine, the genetic modification did not result in any change of the safety profile of the recombinant virus. No specific study was provided on examination of immunological functions. Taking into account these arguments, the CVMP concluded that this omission can be accepted.

Special requirements for live vaccines

Spread of the vaccine strain

A 10-fold overdose of Vectormune ND was used to demonstrate potential spread of the vaccine strain (rHVT/ND) from vaccinated chickens to contact naïve chickens (target species) and also to several other non-target species: i.e. turkeys, natural host of the parental strain (Witter, 1970), ducks, quails, guinea fowls, pheasants and pigeons. With exception of ducks, all the other species are supposed to be susceptible to MDV (Schat, 2008).

Spread from target species to target species

In the first study provided the potential spread of rHVT/ND strain to target species was investigated on a group of 40 one-day-old naïve chicks housed together with a group of 40 one-day-old SPF chicks treated by SC route with a 10-fold overdose of Vectormune ND (80.000 PFU/0.2ml/animal). Spread of rHVT/ND strain from vaccinated chickens to naïve contact chickens was monitored by vaccine strain detection (by PCR) in spleen samples, feather quills and white blood cells (WBC) collected from unvaccinated contact chickens. Cloacal and oropharyngeal swabs were also collected and tested from 5 birds. Observations were carried out for 6 weeks.

Spreading of the vaccine strain was also investigated in a group of 40 18-day-old naïve embryonated chicken eggs put in contact with a group of 50 eighteen-day-old embryonated chicken eggs treated with a 10-fold overdose of Vectormune ND by IO (80,000 PFU/0.1ml/egg). Spreading of rHVT/ND vaccine virus from vaccinated to naïve animals was monitored for 6 weeks by PCR detection in spleen samples and WBC. Cloacal and oropharyngeal swabs were also collected and tested from 5 birds.

All vaccinated animals presented viraemia and samples form the spleen and feather quills were positive to the rHVT/ND strain by PCR. Viral excretion from vaccinated animals was limited to 1 out of

10 cloacal swabs (1/10 PCR positive). Oropharyngeal swabs were all negative. Vaccine virus was not detected by PCR in the spleen or WBC from the unvaccinated contact birds.

In conclusion, the study demonstrates that when Vectormune ND is administered by both recommended administration routes (IO and SC) the vaccine strain does not spread from vaccinated chickens to in contact unvaccinated chickens.

Spread from target species to non-target species (turkeys)

The potential spread of the vaccine strain from vaccinated chickens to contact turkeys was investigated since turkeys, from which the parental HVT was isolated, are supposed to be the most susceptible species to rHVT/ND strain. Twenty-five (25) naïve one-day-old turkeys were housed together with 25 vaccinated chickens treated with a 10-fold dose (80,000 PFU in 0.2 ml) of vaccinated with a 10-fold overdose of Vectormune ND by SC route. Observations were carried out for 6 weeks. Spleen samples were collected and tested during the observation period from 5 turkeys at week 5 and 6. No clinical signs of MD were observed in turkeys during the whole observation period. Detection of rHVT/ND genome by PCR was negative in all spleen samples collected on week 5 and positive in all samples on week 6. Parental strain was detected in 3 out of 5 samples (60%) on week 5. Albeit all contact turkeys resulted rHVT/ND positive, at the necropsy none of them showed MD sign or lesions. A few animals showed white spots on the liver however not related to the vaccine virus.

In conclusion, this study confirms that the vaccine virus strain spread from vaccinated chickens to unvaccinated contact turkeys. This is reflected in the SPC. Moreover the vaccine strain showed a lower capacity of spreading in birds than the parental strain.

Spread from non-target species to non-target species

The potential spread of the vaccine strain (rHVT/ND p26 EU) among non-target species sharing the same ecosystem of chickens was examined by direct administration of the vaccine to non-target species of concern in compliance with CVMP Guideline on live recombinant vector vaccines for veterinary use (EMEA/CVMP/004/04-FINAL).

The safety of one administration and the potential spread of the vaccine strain and of the parental strain were investigated in 6 non-target species: turkey, ducks, quails, guinea fowls, pheasants and pigeons, to contact naïve birds of the same species. All studies were designed as follows.

Twenty (20) non-target naïve birds (group 1) were treated with a 10-fold overdose of rHVT/ND by SC route and housed together with 20 contact birds of the same species (group 3).

Twenty (20) non-target naïve birds (of the same species of group 1 and 3) were administered a 10-fold overdose of parental virus strain (HVTFc126) by SC route (group 2) and housed together with 20 contacts birds of the same species (group 4).

Observations were carried out during 5 weeks. Weight gain and local reactions of the vaccinated animals were monitored. After necropsy, microscopical investigation of the injection sites and of any lesion was carried out. Spread of the vaccine strain and of the parental strains from vaccinated birds to naïve contact birds was monitored by vaccine strain PCR detection in spleen samples and WBC collected from unvaccinated contact birds. Results and conclusions are presented divided by species in the following sections.

Results in turkeys

The study was conducted on 27-day-old turkeys. No clinical signs of MD and a normal weight gain were observed in all vaccinated turkeys (group 1, 2). Microscopic lesions were seen at the injection

site in 3 out of 20 vaccinated turkeys (group 2) showing a slight focal perivascular lymphocytic infiltration and 5 in group 1. Spleen samples from 100% contact turkeys (group 3 and 4) showed positive PCR results. The vaccine strains spread to contact turkeys (groups 3 and 4) however none presented clinical signs of MD.

In conclusion, a 10-fold overdose was demonstrated to be safe in turkeys (group 1). Results showed that rHVT/ND strains can spread from vaccinated turkeys to unvaccinated turkeys.

Results in ducks

The study was conducted on 35-day-old ducks. In the vaccinated animals, no reaction at the injection sites and no clinical signs of MD were observed and the weight growth was normal (group 1, 2). All spleen samples collected from the contact control groups (groups 3 and 4) were negative by PCR.

In conclusion, a 10-fold overdose was demonstrated to be safe in ducks (group 1). The lack of spread capacity of rHVT/ND virus from vaccinated to unvaccinated ducks was demonstrated.

Results in quails

The study was conducted on 60-day-old quails. A microscopic reaction at the injection site at day 35 after vaccination was observed in 1 out of 20 birds. No clinical signs of MD were observed and there was no difference in average weight between vaccinated groups (1 and 2) and control (3 and 4) at the end of the observation period. No vaccine virus genome was detected by PCR in 100% of the contact quails (groups 3 and 4).

This safety profile was corroborated in a study carried out in the United States of America (US) in 6-week-old birds.

In conclusion, a 10-fold overdose was demonstrated to be safe in quails (group 1). The lack of potential spread of the vaccine strain from vaccinated to unvaccinated quails was reasonably demonstrated.

Results in guinea fowl

The study was conducted on 115-day-old guinea fowls. Observations concerning weight growth were unreliable due to lack of homogeneity of the groups at the beginning of the study. No local reactions at the injection site at day 35 after vaccination and no clinical sign of MD was observed (groups 1 and 2). No vaccine virus gene was detected by PCR in 100% of the contact guinea fowls. In conclusion, a 10-fold overdose was demonstrated to be safe in vaccinated guinea fowls. The lack of potential spread of the vaccine strain from vaccinated to unvaccinated guinea fowls was demonstrated.

Results in commercial pheasants

The study was conducted on 221-day-old commercial pheasants. In the vaccinated animals (groups 1 and 2) no macroscopical local reactions at the injection site at day 35 after vaccination and no clinical sign of MD was observed. However, focal perivascular lymphocytic infiltrations in 3 cases in group 2 were found. Weight growth of vaccinated animals showed no differences to the control group. No vaccine virus genome was detected by PCR in 100% of contact pheasants.

In conclusion, a 10-fold overdose was demonstrated to be safe in vaccinated pheasants. The lack of spread capacity of the vaccine strain from vaccinated to unvaccinated pheasants was demonstrated. Moreover the vaccine strain caused fewer local reactions.

Results in commercial domestic pigeons

The study was conducted on domestic pigeons from 6 months to 3 years of age. Eight (8) out of 20 vaccinated animals (group 1, 2) showed local reactions at the injection site at day 35 after vaccination. Vaccinated pigeons did not show MD clinical sign and weight growth was comparable to the control group. No vaccine virus genome was detected by PCR in 100% of contact pigeons (group 3, 4).

In conclusion, a 10-fold overdose was demonstrated to be safe in vaccinated pigeons. The lack of spread capacity of the vaccine strain from vaccinated to unvaccinated pigeons was demonstrated.

Dissemination in the vaccinated animal

The vaccine strain dissemination into chickens was evaluated in SPF 1-day-old chicks after administration of the vaccine by IO (50 birds) and by SC (40 birds). Data were compared to results from a study on the dissemination of the parental strain (HVTFc126). Detection of both viral strains was performed by PCR on organ samples (i.e. spleen, lung, liver, kidney, bursa, feather quills) and in WBCs, collected from 5 birds necropsied on days 7, 14, 21, 28 and 42 and on cloacal and oropharyngeal swabs sampled from 5 birds on days 28 and 42. The vaccine strain was detected by PCR 6 weeks after vaccination from all samples except from the oropharyngeal swabs as did the parental strain. As demonstrated also in a previous shedding study, the virus in emunctory organs of vaccinated chickens was still infectious in unvaccinated contact turkeys. Results show that dissemination of the vaccine strain was comparable to the dissemination of the parental strain, and it can be concluded that the insertion of the F gene from NDV did not modify the tropism of the parental virus strain.

Reversion to virulence of attenuated vaccines

The potential of the vaccine strain to revert to virulence and the safety of an overdose administration in chickens was examined in a laboratory study conducted in accordance with Ph. Eur. 5.2.6. In the study a 10-fold overdose of MSV was inoculated by the IO route into 18-day-old embryonated chicken eggs. After 7 days of incubation, blood samples were taken and a WBC suspension was prepared from pooled material and inoculated to one-day-old chicks. The inocula were prepared in accordance with Ph. Eur. 0589 but not in compliance with VICH GL41 which requires that inocula should be prepared from the most likely source of spread for MDV that is feather dust however it is considered acceptable. Five (5) passages were done in one-day-old SPF chicks. In each passage the inoculum was titrated on duplicate CEF tissue culture plates and confirmed by PCR detection of HVT L78 gene and NDV F gene. No significant change in the titre of the virus recovered from the WBC during the sequential passages was reported. It can be concluded that vaccine properties remained stable.

The overdose safety and the residual pathogenicity of Vectormune ND administered by OI and SC route in SPF chickens were investigated in a second study.

A comparison was performed between the residual pathogenicity of the vaccine strain after 5 intraperitoneal (IP) passages in chickens (MSV +5), the MSV (the most attenuated vaccine passage). Serotype 1 virulent MD strain (vMDV) was used as positive control (MD70). Three (3) groups of 40 one-day-old chicks were administered by SC respectively with a 10-fold overdose of MSV (80,000 PFU/bird), an underdosed MSV+5 (with a back-titer of 7.2 PFU/ml) and a 10-fold overdose of vMDV (150 PFU/bird).

Three (3) groups of 50, eighteen-day-old embryonated SPC chicken eggs were administered by IO with the same treatment mentioned above.

After 7 days of observation the MSV and MSV+5 groups of one-day-old chicks showed no clinical signs and mortality was lower than 10% in each group, while the chickens in the control group died or showed macroscopic lesions associated with MDV. The weight was not monitored.

Results from the both groups of 18-day-old embryonated SPC chicken eggs treated with MSV and MSV+5 showed hatchability above 80%. Even if MSV+5 was underdosed, no increased virulence would be suspected because rHVT/ND appeared stable over 5 IP passages, by showing the same titre, and by showing to be safe in chickens.

In conclusion, no reversion to virulence was demonstrated as the vaccine remained stable after 5 IP passages in chickens.

Biological properties of the vaccine strain

The rHVT/ND vaccine is a FC-126 HVT strain modified by the insertion of the F gene from the lentogenic D26 strain of NDV, FC-126 HVT strain is a naturally apathogenic strain largely used in vaccines since the 1980. F gene has been included in GMO vaccines authorized in the US.

Results from in-vivo studies have shown that, rHVT/ND replicates less in chickens than the parent strain (FC-126 HVT). In fact, a lower number of birds excreted rHVT/ND in comparison to those excreting FC-126 HVT by cloacae and oropharynx and spread it to contact turkey 35 days after exposure (0 vs 60%). In addition, in pheasants rHVT/ND did not elicit perivascular lymphocytic infiltration at the injection site, indicative of less viral replication. Since no HVT infected group was included in the efficacy studies no information can be drawn from the efficacy studies. In conclusion, a body of clues showed that the rHVT/ND strain would replicate less in chickens than the parental strain (FC-126 HVT).

Recombination or genomic reassortment of the strains

Recombination between rHVT/NDV and field NDV strains is highly unlikely due to different locations and different types of genetic material: HVT (DNA) is replicating in the nucleus while NDV (RNA) replicates in the cytoplasm.

An in vivo recombination between rHVT/NDV and field HVT or MDV viruses is theoretically possible but is deemed very unlikely. Moreover, superinfection inhibition limits the frequency of recombination between 2 herpesviruses. In published literature (Hirai et al. 1990) an in-vitro recombination between avian herpesviruses (serotype 1 and serotype 2) was reported which was demonstrated to be not repeatable. In addition, despite vaccinations combining serotypes 1 and 2 or 1 and 3 have been extensively used in the field (since the 1980s) recombination between virus vaccines was never reported in scientific literature.

Study of residues

Not required.

The active ingredient being a substance of biological origin intended to produce active immunity does not fall within the scope of Regulation (EC) No. 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin.

Phenol red is residual and considered not pharmacologically active at the dose applied.

The other components of the vaccine are either listed in table 1 of the annex of Commission Regulation No. 37/2010 or considered as not falling within the scope of Regulation (EC) No. 470/2009 when used as in this product.

The gentamicin used in the manufacturing process is present at low residual levels (not more than 150 ng per dose) in the finished medicinal product and it is considered acceptable as stated earlier.

The withdrawal period is set at zero days.

Interactions

No interaction studies have been conducted.

Field studies

Two (2) Good Clinical Practice (GCP) compliant field studies were conducted in Hungary on conventional broiler chickens. In the studies a comparison between chickens treated with Vectormune ND (4000 PFU/bird) and chickens treated with a comparator vaccine against ND (Cevac Vitapest L) were done. Broiler chickens were positive to anti-ND maternal derived antibodies (MDA+) and were vaccinated against IBV and IBDV as well. In each study, both administration routes (SC and IO) were investigated in a 3 arms design including about 20000 chickens in each group. No MD or ND outbreak occurred during the studies. Body weight gain was recorded. In the first study the production efficiency factor (PEF) which is an integrative parameter, was used as the primary safety end point (PEF= [(vitality x mean weight in qram at slauqhter/age in davs) x100]/[feed conversion ratio]). PEF was slightly higher in Vectormune ND groups than in the control group, as were feed consumption and feed conversion ratio. No statistical difference of the overall weight growth was detected between groups.

In addition, mortality was lower in all groups vaccinated with Vectormune ND apart from one. No ND or MD lesions were reported by post-mortem examinations performed on the carcass losses occurring at 6 time points (D10, D17, D25, D31, D38 and D50) and also at the end of the observation period (day 45) by necropsy and on the groups at slaughter (composed by 50 birds each). Hatching rate reached 89% in all groups. No local reaction was determined in the group vaccinated by SC route.

The second study corroborated the conclusions of the first study.

In conclusion, Vectormune ND was shown to be safe by both recommended administration routes in both field trials.

User safety

A user safety assessment was provided in line with the CVMP Guideline on user safety for immunological veterinary medicinal products (EMEA/CVMP/IWP/54533/2006).

Vectormune ND is a cell-associated live vaccine which contains the recombinant turkey herpesvirus strain rHVT/ND. Avian herpesviruses are not known to be a hazard to humans. The insertion of the protein F gene of ND virus did not alter the biological properties of the strain. The live vaccine strain does not replicate in mammalian cells and is not pathogenic in any species investigated in this dossier.

The risk for the user to be exposed to self-injection of the vaccine is considered to be possible only when the vaccine is used by the SC route.

The risk of an ampoule exploding when transferred from liquid nitrogen for thawing is estimated as very low and the consequence of potential cuts by the glass of the ampoule are estimated as medium (could lead to e.g. skin cut). The overall risk is therefore considered to be acceptable.

The potential risk for the user to be exposed to allergic reactions due to acceptable traces of antibiotic (gentamicin) and due to egg proteins during handling of the vaccine ampoule (by skin contact) or as a result of self-administration have been evaluated and considered acceptable. The safety warnings are reflected in the SPC.

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

Environmental risk assessment

An assessment of the potential risk to the environment from use of Vectormune ND following the CVMP note for guidance on environmental risk assessment for immunological veterinary medicinal products (EMEA/CVMP/074/95) was provided.

The environmental risk potential is considered negligible on the basis of the following points:

The vaccine strain capacity to spread from broiler to other avian species has been demonstrated to be negligible. In case other avian species are infected with the vaccine strain, the consequences are negligible as the vaccine virus is apathogenic to avian species. Therefore, spreading of the vaccine strain to non-target species, if occurs at all, does not represent a hazard.

Taking into consideration that HVT is endemic and ubiquitous in domestic poultry producing areas and due to the similarity of characteristics of the vaccine HVT strain, rHVT/ND, and the parental HVT strain, Vectormune ND vaccine strain does not represent a new burden to the environment.

The HVT virus is very sensitive to common disinfectants and survival and dispersal into the environment when common cleaning disinfection procedures usually used in poultry production are carried out is very unlikely.

None of the inserted genetic materials used during the genetic modifications codes for a product that may be toxic or allergen. None of the excipients in the vaccine represent any hazard to the target species, nor to the environment. Components of the vaccine are commonly used in numerous immunological veterinary medicinal products, they comply with current requirements. Their use is safe and does not represent any hazard to the environment. No toxic metabolites are known.

The product is applied parenterally by automatic syringes/in ovo-injector machines, which minimises the probability of the product to contaminate the environment and the quantity of vaccine per animal used is very low, it is applied once in the lifetime of a broiler chicken.

Taking all the risk factors into consideration, the level of risk to the environment of Vectormune ND can be considered as negligible. Therefore, second phase evaluation is considered unnecessary.

Based on the data provided, the environmental risk assessment can stop at Phase I. Vectormune ND is not expected to pose a risk to the environment when used according to the SPC.

Environmental risk assessment for products containing or consisting of genetically modified organisms (GMO)

Satisfactory information on the parent strain as well as on the genetically modified virus (including details on the construction of the recombinant virus) has been provided. A risk assessment was made with relation to possible release of the GMO into the environment.

The risk of in-vivo recombination between rHVT/NDV and field NDV strains and other viruses was considered. Since HVT replicates its DNA in the cell nucleus, and the NDV replicates its RNA in the cell cytoplasma, recombination events are highly unlikely due to the different locations of genetic material and the different types of genetic material. The chance of an in vivo recombination between rHVT/NDV and field HVT or MDV viruses is theoretically possible. Common vaccination practices in Europe and in the US involve the mixture of HVT (serotype 3), SB1 (serotype 2), and Rispens (serotype 1) vaccines. However, to date recombination of the vaccines strains caused by the concurrent application of any of these vaccines or with field MDV viruses (serotype 1) have not been reported. The exchange of genetic material would be theoretically possible with other HVT or MDV strains, in cases where the same host cells became infected with more than 1 type of virus at the same time. Although infection of the same cells with different herpesviruses is possible, it is rare due to superinfection inhibition (the prevention of infection of already infected cells by other viral particles of the same viral species). Due to superinfection inhibition there is only a very limited time for a cell to become infected with different herpesviruses. This significantly reduces the risk of transfer of genetic material.

Comparative safety studies performed in chickens and in other 6 avian non-target species demonstrated that the vaccine strain's characteristics, such as the apathogenic nature, the spreading potential and the dissemination capability remained the same of the parental strain (FC-126 HVT). It was observed that turkeys are the most susceptible species, but the virus can propagate in chickens, pheasants and quail too. Studies showed that the vaccine virus can spread from chickens to turkeys after 6 weeks of contact exposure. No viral spread was observed from vaccinated chickens to naïve chickens during 6 weeks observation period post vaccination.

No evidence exists that the parental strain FC-126 HVT or the recombinant rHVT/NDV is capable of replication in human, mammalian or plant cells. The long established use of FC-126 HVT strain and the data acquired for the GMO during the safety studies in animals and in mammalian cell cultures demonstrate that there are no human or animal health concerns with the use of the GMO as a vaccine for chickens.

The vaccine virus is a cell-associated virus. Infectivity is lost when the cells die. The virus is not able to survive in the environment.

Sufficient information was provided on the possible environmental risk with relation to the genetically modified rHVT/ND vaccine virus. It can be concluded that the risk emerging from the use of the rHVT/ND vaccine virus is negligible for humans and has to be considered as very low for the environment. Moreover, the proposed vaccine Vectormune ND vaccine is considered compliant with Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

The CVMP concluded that, taken together, any risk emerging from the use of the Vectormune ND vaccine virus is negligible for humans and for the environment.

Overall conclusions on the safety documentation

Vectormune ND is a live recombinant vaccine derived from a MD vaccine strain (FC-126 HVT), which is known to be apathogenic in animals from a long time. The F gene of NDV was inserted into the FC-126 HVT strain. A similar vaccine, containing the same recombinant vaccine strain but different MSV, has been authorised in North America. Several billions of doses have been used without reporting of adverse events.

The safety of the vaccine was investigated in 6 Ph. Eur. compliant laboratory studies in chickens and in further 6 studies in non-target species. Two (2) field studies conducted according to GCP requirements were also provided.

Safety of Vectormune ND was demonstrated in five 10-fold overdose laboratory studies in the target species, in which no clinical signs or lesions of MD and of ND were observed in 120 day observation period. No local reaction was observed after SC vaccine administration. The field studies supported the similar safety profiles for both recommended administration routes. Safety of the repeated administration was not investigated as the vaccination scheme stipulates only one administration at one day of age, and this is acceptable. Accidental double vaccination on the same day might be possible but would be a lesser burden than a 10-fold vaccination which proved to be safe.

Safety of a 10-fold overdose of Vectormune ND was also demonstrated in 6 avian non-target species (turkey, duck, quail, guinea fowl, pheasant and pigeon). The vaccine strain in comparison with the parental strain showed a decrease in the capacity of replication (lower dissemination in birds, fewer local reactions). Dissemination of the vaccine virus strain was shown in WBC, spleen and the feather quills. The vaccine strain tropism was confirmed to be similar to the parental strain.The vaccine strain can be shed from vaccinated chickens and spreads to naïve contact turkeys. Spread to contact chickens cannot be excluded, and appropriate care should be taken to separate vaccinated from non-vaccinated chickens. An appropriate warning is included in the SPC. Spread to other avian non-target species could not be detected.

It was also demonstrated that the safety profile of rHVT/ND in the investigated species turkey, duck, quail, guinea fowl, pheasant and pigeon was maintained similar to the parental HVT, which was used as control in most of the safety studies.

Recombination with other vaccine viruses (e.g. vMDV) is not expected when used in the field, especially as Vectormune ND is not claimed to be used in combination with another vaccine.

A user risk assessment compliant with the CVMP Guideline on user safety for immunological veterinary medicinal product (EMEA/CVMP/IWP/54533/2006) was provided. The user safety has been adequately addressed. Appropriate warnings are included in the SPC.

Based on the data provided, the environmental risk assessment can stop at Phase I. Vectormune ND is not expected to pose a risk to the environment when used according to the SPC.

The environmental risk has been sufficiently addressed and it can be concluded that the risk of rHVT/ND vaccine virus is negligible for humans and has to be considered as very low for the environment in compliance with Directive 2001/18/EC requirements. An assessment of the potential risk to the environment from use of Vectormune ND in compliance with the CVMP note for guidance on the environmental risk assessment of immunological veterinary medicinal products (EMEA/CVMP/074/95) was provided.

Residue studies are not required. The withdrawal period is set at zero days.

Overall it is concluded that, the safety of the vaccine has been satisfactorily demonstrated.

Part 4 – Efficacy

Introduction and general requirements

Efficacy of Vectormune ND against both MD and ND was investigated. The vaccine was claimed to reduce mortality and clinical signs caused by ND virus and to reduce mortality, clinical signs and lesions caused by MD virus further vaccination of 18-day-old embryonated chicken eggs or one-day-old chicks.

Since rHVT/ND is a recombinant vaccine derived from a well-used HVT vaccine strain, specific attention is given whether insertion would have an impact on HVT protection.
Studies were designed in compliance with requirements of Ph. Eur. monograph 0589 on Marek's disease vaccine (live), Ph. Eur. monograph 0450 on Newcastle disease vaccine (live). IO administration was performed by a calibrated Egginject EG12 device. ND challenge was carried out with the Herts 33/56 strain as recommended by Ph. Eur.

MD challenge was performed with a Hungarian strain (MD70) the virulence of which was considered intermediate (vMDV). No HVT control group was used. Therefore no information was produced whether insertion of the F gene from NDV modified the well-known HVT efficacy profile. However, no negative effects or lack of efficacy of the recombinant strain could be observed in any of the studies and the GMO is therefore considered safe and efficacious.

OOI for ND and MD and duration of ND immunity were investigated. The DOI against MD was not evaluated because the target species (broilers chicken) is not reared long enough to develop clinical signs and immunity against MD. Five (5) studies were carried out in chickens with maternal derived antibodies (MDA+) to investigate the possibility of interference of MDA with ND vaccination.

Laboratory trials

Onset of ND immunity

A Ph. Eur. compliant study was conducted aiming to investigate the OOI of Vectormune ND against ND in SPF chickens after SC vaccination.

Ninety-five (95) one-day-old SPF chicks were involved in this study. Fifty-five (55) were vaccinated (1,000 PFU/0.2 ml/dose) and 40 were kept as unvaccinated controls.

At 3 weeks post vaccination all chickens were intramuscularly (IM) challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/animal) and observed for 2 weeks post challenge. Mortality was 100% in the control group within 5 days post challenge, while none of the vaccinated chicken showed any clinical signs of ND or mortality.

In conclusion, the OOI against ND after SC vaccination in one-day-old chicks was demonstrated at 3 weeks post vaccination.

A second Ph. Eur. compliant study was conducted to investigate OOI of Vectormune ND against ND in SPF chickens after IO vaccination.

Sixty-eight (68) 18-day-old embryonated SPF chicken eggs were involved in this study. Fifty-two (52) were vaccinated (1,500 PFU/0.05 ml/egg) and 16 were kept as unvaccinated controls. Twenty-four (24) days post vaccination the chickens were IM challenged with the Herts 33/56 NDV strain (5$\log_{10}$ EID50/0.2 ml/animal) and observed for 2 weeks post challenge. In the control group, the

relative percentage of unprotected birds was 100% (mortality of all the control chickens) while 97.8% of the vaccinated group was protected (51/52 chickens).

In conclusion, the OOI against ND after IO vaccination of 18-day-old embryonated SPF chicken eggs was demonstrated at 3 weeks of age (21 days after hatching).

Onset of MD immunity

A Ph. Eur. compliant study was conducted aiming to investigate OOI of Vectormune ND against MD in SPF chickens after SC vaccination.

Ninety-seven (97) one-day-old SPF chicks were involved in this study. Forty-seven (47) were vaccinated (1,000 PFU/0.2 ml/dose) and 50 animals were kept as unvaccinated controls. One week post vaccination the chickens were challenged with MD70 MDV strain IP (161 PFU/0.2 ml/dose) and observed for 10 weeks post challenge. In the unvaccinated control group, the percentage of protection was 8.2% while the percentage of protection in the vaccinated group was 80.4%.

In conclusion, the OOI against MD after SC vaccination in one-day-old chicks was demonstrated at 1 week post vaccination.

A second Ph. Eur. compliant study was aiming to investigate OOI of Vectormune ND against MD in SPF chickens by IO vaccination.

Sixty-four (64) 18-day-old embryonated SPF chicken eggs were involved in this study. Thirty-two (32) animals were vaccinated (2,500 PFU/0.05ml/egg) and 32 were kept as unvaccinated controls.

Nine (9) days post vaccination (7 days after hatching) the chickens were challenged with MD70 MDV strain IP (50 PFU/0,2ml/animal) and observed for 10 weeks post challenge. In the control group, the relative percentage of unprotected birds was 87.1% while the relative percentage of protection was 85.2% in the vaccinated group.

In conclusion, the OOI against MD after IO vaccination of 18-day-old embryonated SPF chicken eggs was demonstrated at 1 week of age (7 days after hatching).

Maternal derived antibody interference

The efficacy of Vectormune ND against ND and MD in MDA positive (MDA+) chickens was investigated respectively in 6 and 4 laboratory studies. The design of these studies was compliant with Ph. Eur.

Newcastle disease

Seventy-two (72) one-day-old chicks were involved in this study. The anti-NDV MDA levels of the flock were confirmed before vaccination (mean titre of 7.9 $\log_2$ HI). Thirty (30) one-day-old MDA+ broilers were vaccinated by SC (1,500 PFU/0.2 ml/dose), 30 one-day-old MDA+ broilers were kept as unvaccinated controls and further 12 one-day-old SPF chicks were used as challenge controls. The chickens were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2ml/animal) 3 weeks post vaccination and observed for 2 weeks post challenge.

Results showed the rate of protection was 0% in SPF controls and 53% in MDA+ unvaccinated controls and 90% in MDA+ vaccinated chickens.

In conclusion, in the presence of anti–NDV MDA, the OOI against ND in SC vaccinated one-day-old broilers was confirmed at 3 weeks.

Seventy (70) 18-day-old embryonated broiler eggs were vaccinated (2,500 PFU/0,05 ml/egg) by IO route, 40 one-day old MDA+ broilers were kept as unvaccinated controls and further 12 one-day-old SPF chicks were used as challenge controls. The anti-NDV MDA levels of the flock were confirmed before vaccination (mean titre of 7.9 $\log_2$ HI). The chickens were challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/animal) IM 3 weeks post vaccination and observed for 2 weeks post challenge. Results showed that the rate of protection was 0% in SPF controls and 53% in MDA+ unvaccinated controls and 87% in MDA+ vaccinated chickens.

In conclusion, in the presence of anti-NDV MDA, the OOI against ND in vaccinated 18-day-old embryonated broiler eggs was confirmed at 3 weeks of age (24 days after vaccination; 21 days after hatch).

Flock A

Fifty-five (55) one-day-old chicks were involved in this study. The anti-NDV MDA levels were confirmed before vaccination (mean titre of 4.5 $\log_2$ HI). Twenty-two (22) one-day-old MDA+ broilers were vaccinated by SC (2,500 PFU/0.2 ml/dose), 22 one-day-old MDA+ broilers were kept as unvaccinated controls and further 12 one-day-old SPF chicks were used as challenge controls. The chickens were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/animal) 3 weeks post vaccination and observed for 2 weeks post challenge.

Results showed that the rate of protection was 0% in SPF controls and MDA+ unvaccinated controls and 84% MDA+ vaccinated chickens.

In conclusion, in the presence of anti–NDV MDA, the OOI against ND in SC vaccinated one-day-old broilers was confirmed at 3 weeks of age.

Flock A

Fifty-five (55) 18-day-old embryonated chicken eggs were involved in this study. The anti-NDV MDA levels of the flocks were confirmed before vaccination (mean titre of 4.5 $\log_2$ HI). Twenty-two (22) 18-day-old embryonated broiler eggs were vaccinated by IO (2,500 PFU/0.05 ml/egg), 22 eighteen-day-old embryonated broiler eggs were kept as unvaccinated controls and further 11 eighteen-day-old embryonated SPF chicken eggs were used as challenge controls. The chickens were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/e) 3 weeks after hatching (day 24 post vaccination) and observed for 2 weeks post challenge.

Results showed that the rate of protection was 0% in SPF controls and MDA+ unvaccinated controls and 90% in MDA+ vaccinated chickens.

In conclusion, in the presence of anti-NDV MDA, the OOI against ND in IO vaccinated 18-day-old embryonated broiler eggs was confirmed at 3 weeks of age (24 days after vaccination; 21 days after hatch).

Flock B

Fifty-five (55) one-day-old chicks were involved in this study. The anti-NDV MDA levels were confirmed before vaccination (titre of 5.2 $\log_2$ HI). Twenty-two (22) one-day-old MDA+ broilers were vaccinated by SC (2,500 PFU/0.2 ml/dose), 22 one-day-old MDA+ broilers were kept as unvaccinated controls and further 11 one-day-old SPF chicks were used as challenge controls. The chickens were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/animal) 3 weeks post vaccination. The observation period was of 2 weeks post challenge

Results showed that, the rate of protection was 0% in SPF controls and MDA+ unvaccinated controls and 95.2% in MDA+ vaccinated chickens.

In conclusion, in the presence of anti–NDV MDA, the OOI against ND in SC vaccinated one-day-old broilers was confirmed at 3 weeks of age.

Flock B

Fifty-five (55) 18-day-old embryonated chicken eggs were involved in this study. The anti-NDV MDA levels were confirmed before vaccination (titre of 5.2 $\log_2$ HI). Twenty-two (22) 18-day-old embryonated broiler eggs were vaccinated by IO (2,500 PFU/0.05 ml/egg), 22 eighteen-day-old embryonated broiler eggs were kept as unvaccinated controls and further 12 eighteen-day-old embryonated SPF chicken eggs were used as challenge controls. The chickens were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/egg) 3 weeks after hatching (day 24 post vaccination) and observed for 2 weeks post challenge.

Results showed that the rate of protection was 0% in SPF controls and MDA+ unvaccinated controls and 95% in MDA+ vaccinated chickens.

In conclusion, in the presence of anti-NDV MDA, the OOI against ND in IO vaccinated 18-day-old embryonated broiler eggs was confirmed at 3 weeks of age (24 days after vaccination; 21 days after hatch.)

Marek's Disease

Flock A

Ninety-six (96) one-day-old chicks were involved in the Ph. Eur. compliant study. Thirty-two (32) one-day-old MDA+ broilers were vaccinated by SC (2,500 PFU/0.2 ml/dose), 32 one-day-old MDA+ broilers were kept as unvaccinated controls and further 32 one-day-old SPF chicks were used as challenge controls. The chickens were challenged with MD70/13-4 SV4 PBMC MDV strain IP (50 PFU/0.2 ml/animal) 9 days post vaccination and observed for 10 weeks post challenge.

Results showed the rate of protection was 3% in SPF controls and 13% in conventional unvaccinated controls and 93% in conventional chickens.

In conclusion, in the presence of anti–MDV MDA the OOI at 9 days after the vaccination for the protection against MD was confirmed.

Flock A

Ninety-six (96) 18-day-old embryonated chicken eggs were involved in this Ph. Eur. compliant study. Thirty-two (32) 18-day-old embryonated broiler eggs were vaccinated by IO (2,500 PFU/0.05 ml/egg), 32 eighteen-day-old embryonated broiler eggs were kept as unvaccinated controls and further 32 eighteen-day-old embryonated SPF chicken eggs were used as challenge controls. The chickens were challenged with MD70/13-4 SV4 PBMC MDV strain IP (50 PFU/0.2 ml/animal) 9 days after hatching observed for 10 weeks post challenge.

Results showed that the rate of protection was 13% in SPF controls and conventional unvaccinated controls and 90.3% in conventional vaccinated chickens.

In conclusion, in the presence of anti–MDV MDA the OOI at 9 days after the vaccination for the protection against MD was confirmed.

Flock B

Ninety-six (96) one-day-old chicks were involved in this Ph. Eur. compliant study. Thirty-two (32) one-day-old MDA+ broilers were vaccinated by SC (2,500 PFU/0.2 ml/ dose), 32 one-day-old MDA+ broilers were kept as unvaccinated controls and further 32 one-day-old SPF chicks were used as challenge controls. The chickens were challenged with MD70/13-4 SV4 PBMC MDV strain IP (50 PFU/0.2 ml/animal) 9 days post vaccination observed for 10 weeks post challenge.

Results showed the rate of protection was 3% in SPF controls and 10% in conventional unvaccinated controls and 97% in conventional vaccinated chickens.

In conclusion, in the presence of anti–MDV MDA the OOI at 9 days after the vaccination for the protection against MD was confirmed.

Flock B

Ninety-six (96) one-day-old chicks were involved in this study. Thirty-two (32) one-day-old MDA+ broilers were vaccinated by SC (2,500 PFU/0.2 ml/ dose), 32 one-day-old MDA+ broilers were kept as unvaccinated controls and further 32 one-day-old SPF chicks were used as challenge controls. The chickens were challenged with MD70/13-4 SV4 PBMC MDV strain IP (50 PFU/0.2 ml/animal) 9 days post vaccination observed for 10 weeks post challenge.

Results showed the rate of protection was 13% in SPF controls and in conventional unvaccinated controls and 74.2% in MDA+ vaccinated chickens which led to a reasonable protection rate of above 70%.

In conclusion, Vectormune ND provided a reasonable protection against MD in broiler chickens as all vaccinates had a higher protection than controls.

Duration of ND immunity

A Ph. Eur. compliant study was conducted to investigate the DOI of Vectormune ND against ND in broilers 6 weeks post vaccination by SC route.

Thirty-three (33) one-day-old chicks were involved in this study. Twenty-two (22) one-day-old MDA+ broilers were vaccinated by SC route (2,500 PFU/0.2 ml/ dose), and 11 one-day-old SPF chicks were used as challenge controls. The anti-NDV MDA levels of the broilers were confirmed before vaccination (mean titre of 5.2 $\log_2$ HI). All chickens were challenged with Herts 33/56 NDV strain IM (5 $\log_{10}$ EID50/0.2 ml/animal) 6 weeks post vaccination and observed for 2 weeks post challenge.

Results showed 100% mortality in the control group within 5 days post challenge w while 100% of the vaccinated chickens survived without showing any ND clinical sign.

In conclusion, the DOI in one-day-old MDA+ chicks against ND was demonstrated at 6 weeks post vaccination by SC route.

A second Ph. Eur. compliant study was conducted to investigate the DOI of Vectormune ND against ND in broilers 6 weeks post vaccination by IO route.

Thirty-three (33) 18-day-old embryonated chicken eggs were involved in this study. Twenty-two (22) 18-day-old embryonated broilers eggs were vaccinates by SC (2,500 PFU/0.05 ml/ egg), and 11 eighteen-day-old embryonated SPF chicken unvaccinated eggs were used as challenge controls. The anti-NDV MDA levels of the broilers were confirmed before vaccination (titre of 5.2 $\log_2$ HI). All chickens were challenged with Herts 33/56 NDV strain IM (5 $\log_{10}$ EID50/0.2 ml/animal) 6 weeks post hatching and observed for 2 weeks post challenge.

Results showed 100% mortality in the control groups within 5 days post challenge while 100% of the vaccinated chickens survived without showing any ND clinical sign.

In conclusion, DOI after IO vaccination in MDA+ 18-day-old embryonated broilers eggs was demonstrated at 6 weeks after hatching.

A third Ph. Eur. compliant study was conducted aiming to determine the DOI of Vectormune ND against ND in broiler chickens 9 weeks post vaccination by SC route.

Fifty one (51) one-day-old chicks were involved in this study. The anti-NDV MDA levels were confirmed before vaccination (mean titre of 6.5 $\log_2$ HI). Twenty-one (21) one-day-old MDA+ broilers were vaccinated by SC (2,500 PFU/0.2 ml/dose), 21 one-day-old MDA+ broilers were kept as unvaccinated controls and further 9 one-day-old unvaccinated SPF chicks were used as challenge controls. The chickens were challenged with Herts 33/56 NDV strain IM (5 $\log_{10}$ EID50/0.2 ml/animal) 9 weeks post vaccination and observed for 2 weeks post challenge.

Results showed 100% mortality in the control groups within 5 days post challenge while 100% of the vaccinated chickens survived without showing any ND clinical sign.

In conclusion, DOI in one-day-old chicks was demonstrated at 9 weeks post vaccination by IO route.

A fourth Ph. Eur. compliant study was conducted aiming to determine the DOI of Vectormune ND against ND in broiler chickens 9 weeks post vaccination by IO route.

Fifty one (51) 18-day-old embryonated chicken eggs were involved in this study. The anti-NDV MDA levels were confirmed before vaccination (mean titre of 6.5 $\log_2$ HI). Twenty-one (21) 18-day-old embryonated broilers eggs were vaccinated by SC (2,500 PFU/0.05 ml/egg), 21 eighteen-day-old unvaccinated embryonated broilers eggs (vaccine controls) and 9 eighteen-day-old embryonated SPF chicken unvaccinated eggs (challenge controls). The chickens were IM challenged with of Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/animal) 9 weeks post vaccination and observed for 2 weeks post challenge.

Results showed that mortality was 100% in the control groups within 5 days of challenge while 100% of the vaccinated chickens survived without showing any ND clinical sign.

In conclusion, DOI after IO vaccination in 18-day-old embryonated broilers eggs was demonstrated at 9 weeks after hatching.

Conclusion of laboratory trials

The OOI of Vectormune ND against ND was determined in white leghorn SPF chickens vaccinated by both routes of administration. All IO vaccinated birds except one were protected against a ND after challenge while controls presented a 100% of mortality.

The relative protection against MD challenge was higher than the threshold of 80% set by the Ph. Eur. 0589 (81% for the SC vaccinated group and 85% for the IO vaccinated group).

In broilers (chickens with ND maternal derived antibodies) the results were similar. In a flock composed by 308 Ross chickens with MDA titres of 7.9 (mean value) by means of haemagglutination inhibition (HI) test, 90% were protected against NDV challenge after SC vaccination and 87% after IO vaccination. The result was reverse in another flock (A) with a mean value of MDA HI titres of 4.5 $\log_2$; 84% of the chickens were protected after SC vaccination and 95% after IO administration of the vaccine. In a third flock (B) with a mean value of MDA HI titres of 5.2 $\log_2$, the protection was slightly higher (95% of protected chickens by both routes administration).

Regarding efficacy of Vectormune ND against MD, the Ph. Eur. 0589 criterion was met in flock A (relative protection of 93 chickens from the SC group and 89 chickens from the IO group) as well as in the SC group of flock B (relative protection of 96). In the IO group of flock B the criteria were not met (relative protection of 70). These birds were not SPF and specific applicant's criteria set for broiler was met. Generally, MDA titres against MD ranged from 56 to 122 UI/ml albeit their interference with cell-associated MD vaccines is known to be rare and moderate.

The OOI against ND and MD after IO and SC vaccination was demonstrated at 21 days and at 7 days after hatching, respectively.

The duration of ND immunity was challenged at 6 and 9 week-old in conventional birds (mean values of MDA HI titres of 5.2 $\log_2$ and 6.5 $\log_2$). Whatever vaccination routes, the birds were fully protected.

Field trials

Two (2) field trials involving about 120.000 broiler chickens has performed in Hungary. During the field studies, no natural outbreaks of MD and ND occurred and some of the chickens were relocated and challenged in the laboratory, in accordance with Ph. Eur. in order to demonstrate the protection after vaccination under field conditions.

Field study

In the first field study 21,120 eighteen-day-old embryonated broiler eggs were vaccinated (4,000 PFU/0.05 ml/egg) by IO with an automatic egg injector (group 1); 21,120 eighteen-day-old embryonated broiler eggs were vaccinated by IO (group 2); 21,120 eighteen-day-old embryonated broiler eggs were treated by IO with a sterile solvent (group 3); further 21,120 eighteen-day-old embryonated broiler eggs were left as unvaccinated (group 4). The anti-NDV MDA levels were confirmed randomly after hatching.

After hatching (day 3) proportions of the one-day-old chicks from the 4 groups were allocated separately in the farm: 18,800 one-day-old broiler chicks from group 1; 18,916 one-day-old broiler chicks from group 2; 18,800 one-day-old broiler chicks from group 3 were vaccinated by coarse spray with CEVAC Vitapest L (comparator vaccine) and 18,832 one-day-old broiler chicks from group 4 were subcutaneously vaccinated with a commercial batch of Vectormune ND (4,000 PFU/0.02 ml/dose).

Moreover, at the time of the OOI, further proportions from the mentioned groups of chickens were reallocated and challenged in the laboratory. Four (4) studies are described hereafter: in 2 studies, animals were challenged with NDV and in other 2 studies, animals were challenged with MDV.

ND challenge

The objective of the study was to investigate the efficacy of Vectormune ND against ND in 5-week-old broilers originating from a field trial, after SC vaccination.

Fifty-nine (59) 5-week-old broilers were reallocated in the laboratory. Twenty-two (22) 5-week-old MDA+ broilers from the SC vaccinated broilers at day one (group 4), 22 five-week-old MDA+ unvaccinated broilers (vaccine controls) and 22 one-day-old unvaccinated SPF chicks (challenge controls) were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/animal). The anti-NDV MDA levels were confirmed before challenge (mean titre of 6.2 $\log_2$ HI 3 days after hatching). The observation period was of 2 weeks post challenge. Results were compliant to the Ph.Eur. and

showed the rate of protection was 0% in SPF controls and in MDA+ unvaccinated controls and 81% in MDA+ vaccinated chickens.

The vaccinates were significantly more protected than MDA controls. In conclusion Vectormune ND provided protection against MD in broiler chickens vaccinated in the field.

The objective of this Ph. Eur. compliant study was to investigate the efficacy of Vectormune ND vaccine against ND in 5-week-old broiler originating from a field trial, after IO vaccination.

Fifty seven (57) 5-week-old broilers were reallocated in the laboratory. Twenty (20) 5-week-old MDA+ broiler from IO vaccinated embryonated broiler eggs (group 1), 22 5-week-old MDA+ unvaccinated broilers (vaccine controls) and 15 one-day-old unvaccinated SPF chicks (challenge controls) were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50). The anti-NDV MDA levels were confirmed before challenge (titre of 6.2 $\log_2$ HI 3 days after hatching). The observation period was of 2 weeks post challenge.

Results were compliant to the Ph. Eur. and showed the rate of protection was 0% in SPF controls and in MDA+ unvaccinated controls and 90.9 % in MDA+ vaccinated chickens.

The vaccinates were significantly more protected than MDA controls. In conclusion Vectormune ND provided protection against MD in broiler chickens vaccinated in the field.

MD challenge

The objective of this Ph. Eur. study was to investigate the efficacy of Vectormune ND against MD in broilers originating from a field trial after subcutaneous vaccination.

Ninety-four (94) 9-day-old broilers were reallocated in the laboratory. Thirty (30) one-day-old MDA+ broilers from the subcutaneously vaccinated broilers at day one (group 4), 32 nine-day-old MDA+ unvaccinated broilers (vaccine controls) and 32 one-day-old unvaccinated SPF chicks (challenge controls) were challenged with MD70/13-4 SV4 PBMC MDV strain IP (25 PFU/0.2 ml/animal) 9 days post vaccination. The observation period was of 10 weeks post challenge.

Results showed the rate of protection was 12% in SPF controls, 9% in MDA+ unvaccinated controls and 90% in MDA+ vaccinated chickens.

The results of the study are not completely Ph. Eur compliant however, due to the short life time of broiler birds and their lower susceptibility for MD in comparison to layer type and SPF birds the study is considered acceptable. In conclusion, Vectormune ND provided protection against MD in broiler chickens. The vaccinates were significantly more protected than MDA controls.

The objective of this Ph. Eur. compliant study was to investigate the efficacy of Vectormune ND against MD in broilers originating from a field trial after IO vaccination.

Ninety-six (96) 9-day-old broilers were reallocated in the laboratory. Thirty-two (32) one-day-old MDA+ broilers from IO vaccinated embryonated broiler eggs (group 1), 32 nine-day-old MDA+ unvaccinated broilers (vaccine controls) and 32 one-day-old unvaccinated SPF chicks (challenge controls) were challenged with MD70/13-4 SV4 PBMC MDV strain IP (25 PFU/0.2 ml/animal) 9 days post vaccination and observed for 10 weeks post challenge.

Results showed the rate of protection was 9% in SPF controls, 12% in MDA+ unvaccinated controls and 88% in MDA+ vaccinated chickens.

The vaccinates were significantly more protected than MDA controls. In conclusion, Vectormune ND provided protection against MD in broiler chickens vaccinated in the field.

Field study

In the second field study 16.800 eighteen-day-old embryonated broiler eggs were vaccinated (4,000 PFU/0.05 ml/egg) by IO by automatic vaccinator (group 1A), 20,866 eighteen-day-old embryonated broiler eggs were treated IO with a sterile solvent (group 2), further 21,120 eighteen-day-old embryonated broiler eggs were left as unvaccinated controls (group 1B).

The anti-NDV MDA levels were confirmed randomly after hatching.

Three (3) days after hatching one-day-old chicks were allocated separately in the farm: 14,600 one-day-old broiler chicks from group 2 were vaccinated by coarse spray with CEVAC Vitapest L (comparator vaccine), and 14,286 one-day-old broiler chicks from group 2B were SC vaccinated with a commercial batch of Vectormune ND (4,000 PFU/0.02 ml/dose) and one-day-old chicks from group 1A.

Proportions from the mentioned groups of chickens were reallocated and challenged in laboratory at the time of the OOI studies. The 4 laboratory challenged studies are described hereafter: in 2 studies, animals were challenged by NDV and in other 2 studies, animals were challenged by MDV.

ND challenge

The objective of this Ph. Eur. compliant study was to investigate the efficacy of Vectormune ND against ND in 4-week-old broilers originating from a field trial, after SC vaccination.

Sixty-three (63) 4-week-old broilers were reallocated in the laboratory. Twenty-one (21) 4-week-old MDA+ broilers from the subcutaneously vaccinated broilers at day one (group 1B), 21 four-week-old MDA+ unvaccinated broilers (vaccine controls) and 21 one-day-old unvaccinated SPF chicks (challenge controls) were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/animal). The anti-NDV MDA levels were confirmed randomly before challenge (titre of 6.2 $\log_2$ HI 3 days after hatching). The observation period was of 2 weeks post challenge.

Results showed that the rate of protection was 0% in SPF controls and 9.5% in MDA+ unvaccinated controls and 95% in MDA+ vaccinated chickens.

In conclusion, Vectormune ND provided protection against ND in broiler chickens vaccinated in the field.

The objective of this study was to investigate the efficacy of Vectormune ND against ND in 4-week-old broilers originating from a field trial, after IO vaccination.

Fifty-seven (57) 4-week-old broilers were reallocated in the laboratory. Twenty-one (21) 4-week-old MDA+ broilers from IO vaccinated embryonated broiler eggs (group 1A), 21 four-week-old MDA+ unvaccinated broilers (vaccine controls) and 15 one-day-old unvaccinated SPF chicks (challenge controls) were IM challenged with Herts 33/56 NDV strain (5 $\log_{10}$ EID50/0.2 ml/animal). The anti-NDV MDA levels were confirmed before challenge (titre of 6.2 $\log_2$ HI 3 days after hatching). The observation period was of 2 weeks post challenge. Results showed that the rate of protection was 0% in SPF controls and 9.5% in MDA+ unvaccinated controls and 85.7% in MDA+ vaccinated chickens.

In conclusion, even if not completely compliant with Ph. Eur. as the monograph prescribes SPF chickens, the study is acceptable as field birds were challenged and showed a high level of protection.

MD challenge

The objective of this Ph. Eur. compliant study was to investigate the efficacy of Vectormune ND against MD in broilers originating from a field trial after SC vaccination.

Sixty-six (66) 9-day-old broilers were reallocated in the laboratory. Thirty-three (33) 9-day-old MDA+ broilers from the SC vaccinated broilers at one day (group 1B), 33 four-week-old MDA+ unvaccinated broilers (vaccine controls) were challenged with MD70/13-4 SV4 PBMC MDV strain IP (25 PFU/0.2 ml/animal). The observation period was of 10 weeks post challenge. Results showed that the rate of protection was 12% in MDA+ unvaccinated controls and 82% in MDA+ vaccinated chickens.

In conclusion Vectormune ND provided protection against ND in broiler chickens vaccinated in the field.

The objective of this Ph. Eur. compliant study was to examine the efficacy of Vectormune ND against MD in broilers originating from a field trial after IO vaccination.

Sixty-six (66) 9-day-old broilers were reallocated in the laboratory. Thirty-three (33) 9-day-old broilers MDA+ broilers from IO vaccinated embryonated broiler eggs (group 1A), 33 nine-week-old MDA+ unvaccinated broilers (vaccine controls) were challenged (25 PFU/0.2 ml/animal) with MD70/13-4 SV4 PBMC MDV strain IP (25 PFU/0.2 ml/animal). The observation period was of 10 weeks post challenge. Results showed that the rate of protection was 9% in MDA+ unvaccinated controls and 85% in MDA+ vaccinated chickens.

In conclusion, Vectormune ND provided protection against ND in broiler chickens vaccinated in the field.

Conclusion of field trials

Protection against MD of the 33 birds of each vaccine group ranged from 82% to 90%, which is above the Ph. Eur. 0589 threshold.

Protection of 21-22 birds against ND ranged from 82% to 95%. Two out of four (2/4) groups were just below the 90% of protection threshold recommended by Ph. Eur. 0450 for SPF birds, however the study is acceptable as the 90% threshold needs to be only strictly fulfilled in SPF birds and the respective studies are field trials. The achieved protection rates are satisfactory.

Overall conclusion on efficacy

The efficacy of the vaccine was demonstrated in 18 laboratory studies. In 2 field studies animals were not exposed to NDV or MDV field strain. The laboratory studies were designed in line with Ph. Eur. monograph 0589 on Marek's disease vaccine (live) and Ph. Eur. monograph 0450 on Newcastle disease vaccine (live). No comparison was done with the parental strain. Both IO and SC routes of administration were investigated. The protection against MD was challenged by a Hungarian strain and against ND by the viscerotropic velogenic strain recommended by Ph. Eur. 0450.

Study for the OOI in SPF chickens was compliant with ND and MD Ph. Eur.

The onset of ND immunity was supported by 3 other Ph. Eur. compliant studies in chickens with high MDA titres (4.5 to 7.9) (2 studies for the SC route and 3 studies for the IO route) and by 2 additional studies in birds from the field where in 10 out of 14 groups the protection was higher than the threshold set by Ph. Eur. OOI was established against NDV infection at 3 weeks of age.

The onset of MD immunity was corroborated by 2 other laboratory studies in conventional chickens (2 studies for the SC route and 2 studies for the IO route) and 2 other ones in birds from the field studies which complied with Ph. Eur. criterion in 7 out of 8 groups. OOI was established against MDV infection at 9 days after vaccination.

The 9-week duration of ND immunity was demonstrated in a Ph. Eur. compliant study on chickens with 6.5 MDA HI titres. No experimental data was provided for MD duration of immunity because the target birds, broilers, are not reared long enough to develop clinical signs and immunity against MD. Nevertheless data from the literature support a lifelong immunity provided by the parental HVT and evidence from the dossier has backed up that Vectormune ND behaves as its parental HVT.

Although no direct comparison was undertaken, the efficacy was similar in birds with or without MDA. Furthermore, there was no difference between groups vaccinated by the IO or by the SC route.

Consistent with the above results, for both diseases the proposed indications are reduction of mortality, clinical signs and in addition for MD, lesions.

Pharmacovigilance data from non-EU countries were provided for the use of a similar vaccine, indicating extensive use of the vaccine in the field without reports of lack of efficacy.

Part 5 – Benefit-risk assessment

Introduction

Vectormune ND is a frozen, cell-associated, live recombinant vaccine derived from the FC-126 herpesvirus of turkey (HVT) strain which has been used for long time as MD vaccine strain, with the insertion of the F gene of NDV to form the recombinant virus strain rHVT/ND. The product is stored in liquid nitrogen.

The vaccine is intended for active immunisation of 18-day-old chicken embryonated eggs or one-day-old chicks to reduce mortality and clinical signs caused by NDV and to reduce mortality, clinical signs and lesions caused by MDV. These diseases are registered on the OIE list of diseases, infections and infestations owing to the threat they pose to animal health.

The dossier was submitted in line with requirements of Article 12(3) of Directive 2001/82/EC.

Benefit assessment

Direct therapeutic benefit

In laboratory and field studies the vaccine was shown to be efficacious for the active immunisation of 18-day-old embryonated chicken eggs or one-day-old chicks to reduce mortality and clinical signs caused by NDV and to reduce mortality, clinical signs and lesions caused by MDV.

The efficacy of the vaccine was adequately confirmed in the presence of MDA.

OOI was established against NDV infection at 3 weeks of age and DOI was established for 9 weeks after vaccination.

OOI was established against MDV infection at 9 days after vaccination. No data are provided for the DOI against MDV infection and this is acceptable as the HVT virus produces a persistent infection

providing a lifelong immunisation.

Additional benefits

The Vectormune ND vaccine strain did not show any residual virulence in the vaccinated chickens, as usually have been shown by other live ND vaccines as reported in literature (Alexander 2008).

Risk assessment

Main potential risks:

Quality:

The formulation and manufacture of Vectormune ND is well described and specifications set will ensure that a product of consistent quality will be produced provided that conditions are fulfilled.

For the target species:

The product is generally well tolerated in the target animal. No adverse reactions were observed after administration of Vectormune ND. The vaccine was not detected but is based on an apathogenic strain, which is shown to be safe for chickens. Spread to contact chickens cannot be excluded, and appropriate care should be taken to separate vaccinated from non-vaccinated chickens. An appropriate warning is included in the SPC.

For the user:

The user safety for this product is acceptable when used as recommended and taking into account the safety advice and also the special precautions for handling nitrogen stored products listed in the SPC and package leaflet.

For the environment:

The vaccine virus is shed by the feather epithelium and the infected dander can persist in the environment.

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

Recombination of rHVT/ND vaccine strain with other vaccine viruses (e.g. vMDV) is not expected when used in the field, especially as Vectormune ND is not claimed to be used in combination with another vaccine. In conclusion the risk of the recombinant rHVT/ND vaccine strain is negligible for humans and has to be considered as very low for the environment in compliance with Directive 2001/18/EC.

For the consumer:

Residue studies are not required. The withdrawal period is set at zero days.

Specific potential risks:

Safety studies conducted in 6 avian non-target species demonstrated that Vectormune ND is safe in turkeys (natural host of the parental strain), ducks, quails, guinea fowls, pheasants and pigeons. The vaccine strain can be shed from vaccinated chickens and spreads to naïve contact turkeys. Spread to other avian non-target species could not be detected.

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, environment and consumer and to provide advice on how to prevent or reduce these risks.

Evaluation of the benefit-risk balance

The product has been shown to be efficacious for the indication for active immunisation of 18-day-old embryonated chicken eggs and one-day-old chicks to reduce mortality and clinical signs caused by ND virus and to reduce mortality, clinical signs and lesions caused by MD.

The formulation and manufacture of Vectormune ND is adequately described and set specifications will ensure that a finished product of consistent quality will be produced. Vectormune ND is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended and appropriate warnings have been included in the SPC. The withdrawal period is set at zero days.

Conclusion on benefit-risk balance

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

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Vectormune ND is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP recommends the granting of the marketing authorisation for Vectormune ND.