22 March 2023
EMA/141924/2023
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Newflend ND H9 (EMEA/V/C/005860/0000)

Vaccine common name: Newcastle disease and Avian Influenza vaccine (live, recombinant)

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

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Introduction

The applicant Ceva-Phylaxia Co. Ltd submitted on 23 July 2021 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Newflend ND H9, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 17 February 2021 as Newflend ND H9 has been developed by means of a biotechnological process.

Newflend ND H9 is a concentrate and solvent for suspension for injection for chickens, containing cell-associated live recombinant turkey herpesvirus (rHVT/ND/H9), expressing the fusion protein of Newcastle disease virus and the hemagglutinin of low pathogenic avian influenza virus, subtype H9.

The concentrate is contained in glass ampoules containing 1000, 2000 and 4000 doses. The solvent is contained in plastic bags made of polyvinylchloride of 400, 800, 1000, 1200 and 1600 ml.

For the *in ovo* administration, one dose of 0.05 ml should be administered to 18-day-old chicken embryonated eggs.

Subcutaneous administration is intended for one-day-old chicken, one dose of 0.2 ml to be administered in the back of the neck.

The applicant applied for the following indications:

For the active immunisation of one-day-old chicks or 18-day-old chicken embryonated eggs:

- to reduce mortality, clinical signs, lesions and virus shedding caused by Newcastle disease virus (NDV);
- to reduce mortality in the most susceptible period, clinical signs, lesions, and virus shedding caused by H9 subtype of low pathogenic avian influenza virus (LPAIV-H9).

According to the current scientific knowledge, H9 subtype LPAIVs are not recognised to cause significant animal health concerns in Europe thanks to the high sanitary standards. In contrast, H9 subtype LPAI viruses cause serious animal health problems and associated economic losses in other regions, such as Middle East, Northern Africa and Asia due to the combination of less advanced biosecurity, husbandry and sanitary systems and the climatic conditions that enhance the spread of H9 LPAIV strains. The applicant would like to obtain and maintain an EU marketing authorisation for this new recombinant influenza vaccine in order to facilitate regulatory approvals in non-EU countries that rely upon a marketing authorisation in the country of origin and on the acceptance of European authorities' registration dossier assessment. Scientific advice was requested on the subject of using non-EU challenge strains in laboratory efficacy studies and the eligibility of the vaccine in Europe. Advice was given that "The use of non-European (i.e. Middle Eastern or African) challenge strains is acceptable in this particular case."

The rapporteur appointed is Jacqueline Poot and the co-rapporteur is Christine Miras.

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

On 22 March 2023, the CVMP adopted an opinion and CVMP assessment report.

On 16 May 2023, the European Commission adopted a Commission Decision granting the marketing authorisation for Newflend ND H9.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

The applicant received scientific advice from the CVMP on 8 November 2018. The scientific advice pertained to safety and efficacy development questions.

The applicant has generally followed the advice given.

MUMS/limited market status

Not applicable.

Multi-strain dossier

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (dated July 2017) which fulfils the requirements of Directive 2001/82/EC was provided. 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.

Manufacturing authorisations and inspection status

Active substance manufacturing and quality control, bulk product manufacturing and primary packaging as well as quality control, secondary packaging and batch release are to be performed at Ceva-Phylaxia Co. Ltd, Budapest, Hungary. At this facility bulk product manufacturing, primary and secondary packaging as well as quality control and batch release of the solvent may also be performed. Appropriate and valid authorisation has been granted after inspections by the competent authority of Hungary.

Bulk product manufacturing, primary and secondary packaging as well as quality control of the solvent may be performed at Infomed fluids SRL in Bucharest, Romania. The facility holds a relevant and valid GMP certificate received after inspection by the Romanian competent authorities.

Secondary packaging may be performed at CEVA Santé Animale, Libourne, France. This facility is suitably authorised as certified by the French competent authorities.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of the active substance and of the finished product manufacturing sites has been satisfactorily established and are in line with legal requirements.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The vaccine contains one active ingredient: a recombinant live herpesvirus of turkey (HVT, Marek's disease virus serotype 3) which was genetically modified to express the fusion (F) gene of Newcastle disease virus (NDV) and the haemagglutinin (HA) -encoding gene of low pathogenic avian influenza virus (LPAIV) H9N2. The vaccine virus is present at a concentration of 3,000 to 12,000 plaque forming units (PFU) per dose.

Other ingredients are Eagle's minimum essential medium (EMEM), L-glutamine, sodium bicarbonate, Hepes, bovine serum, dimethyl sulfoxide (DMSO) and water for injections.

The solvent is composed of sucrose, casein hydrolysate, sorbitol, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, phenol red and water for injections.

Container and closure

The vaccine is presented as a frozen suspension for injection in 2-ml flame sealed glass ampoules, containing 1,000, 2,000 or 4,000 doses. The solvent is presented in 400, 800, 1,000, 1,200 and 1,600 ml sealed plastic bags made of polyvinylchloride and fitted with injection and infusion ports.

The containers and closures are in compliance with the pharmacopoeial requirements, and their sterilisation is adequate. The pack sizes are consistent with the vaccination schedule and intended use.

Product development

The parent strain of the vaccine strain (the FC-126 strain of HVT) is a naturally occurring, non-pathogenic alphaherpesvirus that was originally isolated from domestic turkeys. For decades, and worldwide, HVT based vaccines have been used in chicken flocks to control Marek's disease (MD). Their use is safe since HVT strains are not known to cause disease in any species and do not replicate in mammals. The applicant does not seek a claim for efficacy against Marek's disease virus.

Newcastle disease is prevalent worldwide, outbreaks caused by virulent strains have severe consequences. Vaccination with live attenuated ND vaccines may cause clinical signs and reduced growth in vaccinated birds. The proposed rHVT vaccine avoids some limitations of the live ND vaccines since it is uniformly administered, safe and elicits a response that persists life-long. The relevance of the inserted F-gene to the NDV epidemiological situation has been justified.

Low pathogenic avian influenza (LPAI) caused by strains of the H9 subtype may cause respiratory signs and malaise in broilers and layers. LPAI-H9 does not cause significant animal health concerns in

Europe but it is a problem for poultry in the Middle East, Northern Africa and Asia. The applicant would like to obtain a marketing authorisation in Europe in order to allow marketing authorisation in countries that rely on acceptance by the EU authorities.

The vaccine strain was constructed using standard techniques for genetic manipulation. The entire HVT genome was cloned into bacterial vectors and fragments were mapped by restriction enzyme analysis. The NVD F-gene expression cassette, of which construction was sufficiently described, was placed between two open reading frames in the HVT genome by homologous recombination. After transfection of CEF cells a rHVT/ND virus clone was isolated by limiting dilution and plaque purification. A HA (H9) sequence was selected from a collection of 80 HA sequences from recent H9N2 strains. A phylogenetic tree was constructed, and the centre of tree (COT) sequence and the most recent common ancestor sequence (MRCA) were identified. A single consensus sequence (CS) was selected which was expected to provide cross-protectivity to most H9 strains. This was well described. The expression cassette was inserted into the rHVT/ND DNA by homologous recombination and after transfection of CEF cells a rHVT/ND/H9 clone was isolated by limiting dilution and plaque purification. The correct sequence of the inserted genes and the surrounding sequences were verified by sequence analysis of two regions covering each of the inserts and flanking regions. Genetic and phenotypic stability of the recombinant virus was determined. Because the vaccine contains only a haemagglutinin gene of AIV, serological tests detecting other AIV antigens (i.e. routine tests detecting antibodies to nucleoprotein and matrix antigens) can be used to differentiate vaccinated from infected birds.

While the vaccine was originally targeted at Middle Eastern countries only, it was decided during development to widen the registration to countries in Asia. Thus, in order not to exhaust the MSV stock, it was decided to extend the passage level of the finished product up to MSV+7. The efficacy of the vaccine was determined using batches at a passage level of MSV+7. The MSV and MSV+7 were found to be genetically identical.

The method of manufacture, formulation, packaging and storage is the same as for other live rHVT and Marek vaccines routinely manufactured by the applicant. All excipients are well-known pharmaceutical ingredients. The vaccine does not contain an adjuvant or preservative. Gentamicin is used in the cell culture medium and in cryoprotectant 1, but it is not considered as an antimicrobial preservative for the finished product by the applicant. Rather, it is considered a safeguard for the harvesting process which gives greater assurance for the quality of the product. This approach is similar to the situation when SPF eggs are being harvested. The applicant notes that this approach was accepted by regulatory authorities in the past.

Description of the manufacturing method

The manufacturing method can be considered standard for cell-associated vaccines and the same method is used by the manufacturer for MD and recombinant HVT vaccines.

Briefly, for the production of the cell suspension, chicken embryo fibroblast (CEF) cells are prepared from specific-pathogen free (SPF) embryonated hen’s eggs. Cells are planted and inoculated with working seed virus. After incubation, (virus-infected) cells are harvested, and the cell suspension is diluted and blended with the freeze-drying stabiliser solutions. The bulk vaccine is subsequently filled in ampoules which are then flame-sealed and deep frozen. The proposed batch size range is 1-45 litres; consistency batches validate this range. The maximum time of intermediate storage of sealed ampoules prior to freezing is 6 hours.

For production of the sterile solvent, ingredients are mixed and allowed to dissolve. The solvent is subsequently filled in plastic bags and terminally sterilised. The applicant claims maximum holding

times of 24 hours for the pre-filtration bulk and 24 hours for the filled bags pre-sterilisation, validation for this is to be provided post-authorisation (recommendation).

Production and control of starting materials

Starting materials listed in pharmacopoeias

Internal specifications and/or representative certificates of analysis (CoA) of all starting materials listed in pharmacopoeias were provided and all conform to Ph. Eur. required specifications.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

Master seed virus (MSV):

The MS was constructed using genetic manipulation. A seed-lot system was satisfactorily established for the active ingredient. Details of source, passage history, controls, storage conditions MSV and WSV were provided and are considered appropriate. Certificates of analysis for the MSV and WSV were provided.

Genetic stability of the rHVT/ND/H9 virus was tested by sequence analysis of MSV and MSV+7 insertion region. No alterations were observed between MSV, MSV+7 and the reference plasmids.

Phenotypic stability was tested by performing 7 consecutive passages on CEF cells: the virus was found to express both inserted genes at each passage. After the 7th passage the virus was able to induce an immune response with detectable antibodies against NDV and LPAIV. In the frame of the increase in virulence study, virus was passaged 4 times in chickens and found to be stable as both inserts were expressed at each passage (as detected in virus-infected CEF cells by immune staining).

Extraneous agents testing was performed, in accordance with Ph. Eur. 2.6.24 (general tests on embryonated hen eggs, chicken kidney cells and SPF chicks as well as specific tests for avian leucosis viruses, REV, CAV and turkey lymphoproliferative disease virus).

Working seed virus (WSV):

A certificate of analysis is provided for the WSV. The WSV was shown to be sterile and free of mycoplasma. Identity was confirmed by the presence of HVT virus and expression of F- and HA proteins.

Further starting materials of biological origin comply with the current regulatory texts related to Ph. Eur. monograph 5.2.8 "Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" and TSE Note for Guidance (EMEA/410/01-Rev.3).

Starting materials of non-biological origin

Example of certificate of analysis are provided for all starting material of non-biological origin indicating conformity to in-house specifications.

In-house preparation of media and solutions consisting of several components

The applicant has provided the composition, sterilisation method, storage conditions and shelf life for the solutions prepared in house. All the media and solutions are sterile filtered or aseptically prepared, this is considered adequate to control the risk of contamination.

Control tests during the manufacturing process

In general, adequate information of the in-process testing is provided. The following tests are performed on the vaccine: cell counting of CEF cells before planting, microscopic observation of cytopathic effect, cell count after harvest, microbiological tests after inoculation of cells and on the bulk vaccine, filling volume during filling of ampoules. Sealing of the ampoules is visually controlled.

During manufacture of the solvent, a test for pH is performed and filling volume is measured during filling.

The in-process tests and the set limits are considered to sufficiently control the manufacturing process of the vaccine and the solvent.

Control tests on the finished product

For the vaccine, tests are performed for appearance, pH, sterility, absence of mycoplasma, identity and virus titre (potency). No testing for extraneous agents is performed; a risk assessment is provided in accordance with Ph. Eur. 5.2.5., which is acceptable. In order to control the risk of contamination of the finished product with *Chlamydia spp.* a commercial PCR test is performed on the SPF eggs. If eggs are obtained from suppliers that perform testing for Chlamydia spp., this test is not required.

For the solvent, tests are performed for appearance, pH, osmolarity, sterility and extractable volume.

For all tests, specifications are provided and considered adequate to control the quality of the vaccine and the solvent. The methods used are mostly adequately described and appropriately validated.

Batch-to-batch consistency

Results of four finished product batches are provided. The first three are consecutive batches of 1,000, 2,000 and 4,000 doses. The 4,000 doses batch however, was filled in 5 ml rather than 2 ml (because of the required cell count). With further experience in formulation, the required amount of antigen could be filled in 2 ml and therefore results for an additional (later) batch of 4,000 doses in 2 ml are included. The four batches conformed to the requirements.

The results of finished product testing of 3 consecutive batches of each of the 5 presentations of solvent are provided. All batches conformed to the requirements.

The results presented for finished product and solvent batches support consistency of production.

Stability

Stability of the finished product

A 27-month stability study was performed for the finished product. Stability was tested on three consistency batches (1,000 and 2,000 doses in 2 ml and 4,000 doses in 5ml) and one additional batch

(4,000 doses, 2 ml). The virus titre, identity, appearance and pH were all stable throughout the study and the product was sterile at onset and after 27 months of storage in liquid nitrogen. A statistical analysis of the virus titre of all four batches showed no titre loss (ANOVA, linear regression, p=0.2488) over the storage period of 27 months. A shelf life of 24 months is proposed for the vaccine, which is considered adequately supported by the data presented.

For the solvent, stability data on three consecutive batches for each of the five presentations was collected. Tests were performed every 3 months for 30 months. The data show a trend for decreasing volume and increasing osmolality for all presentations. Nevertheless, all specifications were met throughout the study, but for pH at one timepoint for each of two batches (out of specification by 0.01). The applicant considers this a result of the accuracy of the potentiometric method and reasons that these batches were within pH specifications for all other timepoints and thus the stability of the solvent is not questioned; this is accepted. A shelf life of 30 months is proposed; this is accepted based on the data provided.

Stability of the reconstituted product/ in use stability

In order to study in-use stability, two batches (2,000 doses in 2 ml and 4,000 doses in 5 ml) were each reconstituted in solvent to mimic subcutaneous and *in ovo* application and kept at room temperature for 2 hours. Titre losses were observed in all dilutions, ranging from 25% to 8.5%. The applicant calculated an average titre loss (for both dilutions) of 15.7%. The average in-use titre loss is used to calculate the overage; this is acceptable.

New active substance (NAS) status

The applicant requested the active substance 'cell-associated live recombinant turkey herpesvirus, expressing the fusion protein of Newcastle disease virus and the hemagglutinin of low pathogenic avian influenza virus, subtype H9' contained in Newflend ND H9 to be considered a new active substance on the basis that no live recombinant turkey herpesvirus (rHVT) expressing NDV F protein and AIV H9 together has hitherto been registered in the European Union and that, similarly, an ATC Vet code for such a vaccine has not been assigned yet.

Based on the review of the data provided, the CVMP considered that the active substance contained in Newflend ND H9 is to be qualified as a new active substance considering that no veterinary medicinal product containing live recombinant turkey herpesvirus (rHVT) expressing NDV F protein and AIV H9 together has been registered in the European Union to date.

Overall conclusions on quality

Information on the development, manufacture and control of the active substance and the finished product has been presented in a satisfactory manner.

The production method, including appropriate in-process controls and quality control on the finished product together with control of the starting materials, ensure a consistent quality of batches of vaccine. The whole production process was evaluated at the lower to middle production scale range and shown to be consistent.

A risk assessment has been performed and appropriate procedures have been implemented to ensure the absence of extraneous agents in starting materials of animal origin and in the finished product. However, absence of *Chlamydia spp.* in the finished product must be assured. A TSE risk assessment is provided, the risk that the final product may transmit TSE to the target animal is considered negligible.

Results of the stability testing of the final product showed no relevant changes, in particular no loss in infectivity titre, during a 27-month storage period in liquid nitrogen. Stability data of reconstituted product shows that the vaccine titre decreases during the proposed 2 hours in-use shelf life; an overage is applied to account for these losses, this is acceptable.

In conclusion, the production process is adequately described and controls in place are appropriate to ensure the quality of the product at release and throughout the shelf life.

The applicant is given the following recommendation: the applicant is requested to provide the results of the validation of the maximum holding times (24 hours) for the pre-filtration bulk and for the filled bags pre-sterilisation.

Part 3 – Safety

Introduction and general requirements

Newflend ND H9 is indicated for active immunisation of chickens or embryonated chicken eggs against NDV and LPAIV H9N2. The vaccine is intended to be administered *in ovo* to 18-day-old embryonated chicken eggs or subcutaneously to one-day-old chickens. The volume of one dose is 0.2 ml for subcutaneous (s.c.) administration and 0.05 ml for *in ovo* vaccination. One dose contains at least 3,000 PFU but not more than 12,000 PFU rHVT/ND/H9 virus as active ingredient. Before use, the vaccine is diluted in the Cevac Solvent Poultry.

The parent strain of rHVT/ND/H9 vaccine (the FC-126 strain of the HVT virus) is a naturally occurring, non-pathogenic alphaherpesvirus. HVT strains are not known to cause disease in any species and do not replicate in mammals.

Some laboratory safety studies were performed using the MSV, in line with the requirement of Ph. Eur. 0589. In the majority of laboratory safety studies, MSV+2 was used which is acceptable since it is the least-attenuated passage to be used in production. The vaccine batches used in the combined safety-efficacy field trials were produced in accordance with the method described in part 2 of the dossier. These batches were at the maximum passage level of MSV+7.

Two commercial ELISA kits for detection of MDV and NDV antibodies in blood were used in the safety studies, these tests are considered fit-for-purpose.

The qPCR for quantitation of rHVT/ND-H9 in clinical samples (spleen and feather pulp), the qPCR for detection of HVT virus in clinical samples (feather pulp) and the black plaque assay used to titrate live rHVT/ND/H9 were all adequately validated.

Safety documentation

Ten safety studies were conducted to investigate the safety of the product. This included laboratory studies investigating the safety of the administration of a 10-fold overdose and two combined safety and efficacy field trials. The vaccine was administered by the *in ovo* and subcutaneous routes, as recommended. Laboratory studies were reported to be GLP compliant and carried out in embryonated eggs and in one-day-old chickens, using pilot batches containing virus of passage MSV+2. Production batches at MSV+7 were used in the field trials.

Studies applicable to GMO products were conducted to investigate the dissemination of a single dose of the vaccine strain, the spread from vaccinated animals to non-vaccinated contacts, the spreading to non-target species, safety in non-target species and reversion to virulence.

Study title
Overdose safety test of the MSV+2 of R062 vaccine (120,000 PFU) in SPF chickens after *in ovo* inoculation
Overdose safety test of the MSV+2 of R062 vaccine (120,000 PFU) in SPF chickens after subcutaneous inoculation
Shed, spread and dissemination test of the MSV+2 of R062 vaccine in SPF chickens after subcutaneous inoculation
Spread test of the MSV+2 of Newflend ND H9 vaccine from subcutaneously inoculated SPF chickens to non-treated turkeys
Foreign bird safety test of the MSV+2 of Newflend ND H9 vaccine in turkeys after subcutaneous inoculation
Foreign bird safety test of the MSV of R062 vaccine in pigeons after subcutaneous inoculation
Foreign bird safety test of the MSV of R062 vaccine in pheasants after subcutaneous inoculation
Increase in virulence test of the MSV of R062 vaccine in SPF chickens after *in ovo* inoculation
Field safety and efficacy trial of R062 vaccine in broiler chickens in Alsótold
Field safety and efficacy trial of R062 vaccine in broiler chickens in Bár

Laboratory tests

Safety of the administration of one dose

No study for the safety of administration of one dose was performed. Instead, overdose safety testing was performed.

Safety of one administration of an overdose

A GLP study was performed using three groups of 18-day-old embryonated SPF chicken eggs. Group 1 was inoculated *in ovo* with a ten-fold maximum dose of the vaccine strain at MSV+2. Group 2 was inoculated intraperitoneally (i.p.) with reference strain MD70 after hatching. Group 3 was inoculated *in ovo* with diluent only. After hatching, group 1 was observed daily until Day 123, group 2 until Day 73 and group 3 until Day 17. At one week of age the number of birds per group was reduced to 44. All birds were euthanised and necropsied at the end of the observation period, samples for histology were taken where needed for diagnosis. In group 1, 10 oviducts and 10 testes were sampled at necropsy.

Hatchability was not significantly different between the three groups (89%, 84% and 89% respectively). In groups 1 and 3, no birds showed clinical signs or died during the follow up period. In group 2, 43 birds were MD positive, and 37 birds died during the observation period, indicating susceptibility of the flock to MDV. No histological lesions were observed in the reproductive organs of group 1.

The study was considered valid since hatchability was >80% for all groups, no chickens died in the week after hatching, no clinical signs of intercurrent diseases were observed and not fewer than 70% of bird in group 2 showed clinical signs or lesion of Marek's disease. The vaccine virus complied with

the test since a tenfold overdose induced no signs or lesions of Marek's disease and no histological lesions were observed in oviduct or testis samples.

A second GLP study was performed in two groups of 44-day-old SPF chicks. Group 1 vaccinated s.c. with a tenfold maximum dose of the vaccine strain, group 2 received diluent only. Group 1 was observed daily until Day 120, group 2 until Day 14. Injection sites in group 1 were examined daily for 14 days. All chickens were necropsied at the end of the observation period, samples for histology were taken where needed for diagnosis. In group 1, 10 oviducts and 10 testes were sampled at necropsy (day 120).

No injection site reactions were observed. No birds showed clinical signs or died during the follow up period in either group. No histological lesions were observed in the reproductive organs of group 1.

The study was considered valid since no clinical signs of intercurrent diseases were observed and no birds died. The vaccine virus complied with the test since a tenfold overdose induced no signs or lesions of Marek's disease and no histological lesions were observed in oviduct or testis samples.

On the basis of the results no safety concerns arose following the administration of an overdose 10 times higher than the recommended maximum dose through either of the recommended routes to chickens of the minimum recommended age.

Safety of the repeated administration of one dose

The vaccine is intended for single lifetime use. No study was performed to test the safety of repeated administration, this is acceptable.

Examination of reproductive performance

Newflend ND H9 vaccine is not recommended for use in breeding birds. However, the safety of the vaccine for the reproductive organs was evaluated in the frame of the two overdose safety studies. In the frame of these trials ovary and testis samples were taken and investigated macroscopically and also evaluated by histology. All samples were free of any lesions compatible with Marek's disease. It can be concluded that the vaccine has no negative impact on reproductive performance of the chickens when used as directed. A warning sentence is included in the SPC not to use the product in birds in lay and within 4 weeks before the start of the laying period.

Examination of immunological functions

No studies were conducted to investigate the effects of the product on immunological functions. The parent HVT virus is non-pathogenic it is not known to be immunosuppressive in chickens. According to the safety studies conducted with this vaccine, the genetic modification did not result in any change of the safety profile of the virus.

Special requirements for live vaccines

Spread of the vaccine strain

The spread of the vaccine strain from vaccinated to unvaccinated chickens and to turkeys was investigated in 2 studies. The safety of the vaccine strain for non-target species turkeys, pigeons and pheasants was investigated in 3 studies.

One study was performed using two groups of 40-day-old SPF chickens, group 1 was vaccinated with the vaccine strain at MSV+2 while group 2 remained as non-vaccinated in-contact birds. Groups were kept in the same room, but physically separated for 120 days and observed daily. Chicks were taken from both groups for sampling of internal organs and feathers. PCR was performed on samples of spleen, liver, kidney and feather tip to determine dissemination, shedding and spreading. Samples of litter and dust were tested for virus presence.

No birds showed clinical signs or macroscopic abnormalities at necropsy. In group 1, 100% of samples was PCR positive on Day 7 (spleen, liver, kidney, feather tip), this percentage gradually decreased but virus was still readily detectable on day 35. Feather tip samples were positive up to Day 35, but not thereafter. Environmental samples were negative throughout the study. Samples taken from in-contact birds were all negative.

It was concluded that the study was valid. Whereas the virus was shown to disseminate and was shed, no evidence of spreading to in-contact chickens was obtained.

In another study, spread to non-vaccinated turkeys was investigated. Briefly, two groups of 30 day-old SPF chicks and two groups of 30 commercial day-old turkey chicks were included in the study. One group of chicks was inoculated with the vaccine strain at MSV+2 while the second group was inoculated with the HVT Fc-126 parent strain. A group of turkeys was kept in the same airspace but physically separated with each group of chicks. Birds were observed daily until Day 63. Turkey hatch mates were tested for anti-MDV maternally derived antibodies (MDA). At regular intervals turkeys were removed from both groups, necropsy was performed, and spleen and feather tips were sampled for PCR.

No clinical signs or MDV specific mortality was observed in any of the groups. Turkeys were negative for MDV MDA. PCR samples (feather tip and spleen) for turkeys in contact with vaccinated chickens were negative on Day 35 and 42 but positive from Day 49. Turkeys in contact with HVT-inoculated chickens were mostly PCR positive from Day 35 onward.

It was concluded that the study was valid. The vaccine virus was shown to spread from vaccinated chickens to in-contact turkeys at a rate similar (albeit slightly slower) to that of the HVT parent strain.

One study was performed using two groups of day-old commercial turkeys, one group was vaccinated with a tenfold maximum dose of MSV+2, while a second group remained as non-vaccinated in-contact birds. Groups were comingled (same airspace, physically separated) for 70 days, birds were observed daily and body weights of 10 male and 10 female (marked) turkeys from each group were measured weekly. Hatch mate turkeys were blood sampled on Day 0 to determine anti-MDV antibodies. Vaccinated birds were sampled, and in-contact birds were necropsied at regular intervals. PCR was performed on samples of spleen and feather tip. No clinical signs or MDV specific mortality was observed in either of the groups. MDV MDA was not detected. Body weight gain was not significantly different between Groups. Feather tip samples in the vaccinated group were generally negative with the exception of one sample on day 21. Spleen samples from in contact birds were positive from Day 35 onward.

It was concluded that the study was valid. The vaccine virus at maximum dose was shown to be safe for day-old turkeys. Vaccinated birds shed the virus and the virus was shown to spread from vaccinated turkeys to in-contact turkeys. The vaccine was safe for in-contact turkeys.

Another study was performed using 3.5-month-old commercial pigeons. One group of 20 birds was vaccinated with a 6.7-fold overdose of the vaccine strain while a second group of 40 birds was kept as unvaccinated in-contact birds (same airspace). Birds were observed daily until day 70. Vaccinated birds were sampled, and in-contact birds were necropsied at regular intervals. PCR was performed on

samples of spleen and feather tip. No mortality or clinical signs attributable to the vaccine were observed. Virus shedding was not observed. Virus spread was not observed.

It was concluded that the study was valid. The vaccine virus was found safe for pigeons. The vaccine was not found to shed from vaccinated pigeons and no spread to non-vaccinated pigeons was observed.

A last study was performed in 16-day-old pheasants; one group of 20 birds was vaccinated with a 6.7 fold overdose of the vaccine strain while a second group of 40 birds was kept as unvaccinated in-contact birds (same airspace). Birds were observed daily until day 70. Vaccinated birds were sampled, and in-contact birds were necropsied at regular intervals. PCR was performed on samples of spleen and feather tip. No mortality or clinical signs related to vaccination were observed in either group. Virus shedding was not observed. Virus spread was not observed.

It was concluded that the study was valid. The vaccine virus was found safe for pheasants. The vaccine virus was not found to shed from vaccinated pheasants and no spread to non-vaccinated pheasants was observed.

All studies were appropriately designed and executed to an appropriate standard. The vaccine disseminates in the vaccinates and is shed. The vaccine was shown to spread to in-contact turkeys. The vaccine at ten-fold maximum dose was found to be safe for one-day-old turkeys. The vaccine virus can spread between turkeys. A warning sentence is proposed in the SPC, which is acceptable in principle: "The vaccine strain was shown to be excreted by chickens and there was a slow spread to turkeys which was not detectable at 42 days but was detectable after 49 days of a contact study. Safety trials have shown that the excreted vaccine strain is not harmful in turkeys. However, appropriate veterinary and husbandry measures such as cleaning and disinfection procedures should be taken to avoid spread of the vaccine strain to turkeys."

Pigeons and pheasants showed no evidence of infection after administration of an overdose of the vaccine strain.

Dissemination in the vaccinated animal

Dissemination of the vaccine strain in the vaccinated target animal was investigated in the second study summarised above. It can be concluded that the virus disseminates to internal organs where it could be detected for at least 35 days. The dissemination pattern and persistence of the vaccine strain in the internal organs is not unexpected based on the known properties of the parent HVT virus strain.

Reversion to virulence of attenuated vaccines

In one study, a group of 45 18-day-old embryonated SPF chicken eggs were inoculated with a single near-maximum dose of MSV. Subsequently, 4 passages were performed each using 30-day-old SPF chicks (inoculated i.p. with white blood cells suspension from the previous passage birds). These birds were observed for 6-7 days. Finally, 55 18-day-old embryonated SPF chicken eggs were inoculated with the 5th passage material, containing 11.95 PFU/0.1 ml. These birds were observed for 120 days.

Hatching rate was 100% for the *in ovo* inoculated birds in the 1st passage and 95% for the *in ovo* inoculated birds in the 5th passage. Virus was detected after each passage with virus titres decreasing gradually. In none of the birds, clinical signs or pathology attributable to the vaccine were observed.

It was concluded that the study was valid as no signs of intercurrent disease were observed and less than 10% of birds died in the week after hatching. The vaccine complied with the test as no chicken in

group 1 showed notable signs of MD or died from causes attributable to the vaccine. No indication of increase in virulence was found after *in ovo* inoculation with the last passage (MSV+5).

The study was appropriately designed and executed to an appropriate standard. The results of the study are considered to adequately support the phenotypic stability of the vaccine strain.

Biological properties of the vaccine strain

As this vaccine is a GMO, the properties of the vaccine strain and its nature are fully outlined in Part 3E.

Recombination or genomic reassortment of the strains

The chance of *in vivo* recombination between rHVT/ND/H9 and MDV field strains was considered. The exchange of genetic material would be possible only in case the same host cells become infected with more than one type of virus at the same time. There is evidence that suggests that although infection of the same cells with different herpesviruses is possible, it is rare due to a phenomenon called superinfection inhibition which leaves only a very limited time (1-4 hours) for a cell to become infected with different herpesviruses. This is also supported by the fact that the common vaccination involves mixing of HVT (serotype 3), SB1 (serotype 2), and Rispens (serotype 1) vaccine viruses, which, to date, has not led to the generation of recombinant strains.

Since HVT replicates its DNA in the cell nucleus, recombination events with viruses replicating RNA (i.e. NDV) in the cell cytoplasm are highly unlikely due to the different locations of genetic material and the different types of genetic material.

Genomic reassortment is exclusive to segmented RNA viruses, the genome of HVT is not segmented and thus reassortment cannot occur.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guideline EMEA/CVMP/IWP/54533/2006 (and EMEA/CVMP/543/03-Rev.1).

Hazards identified were exposure to the live virus and the excipients, to the residual antibiotics (i.e. gentamicin) and hazards associated with the handling of liquid nitrogen tanks and frozen glass vials when manipulating the veterinary medicinal product.

The vaccine is not infectious to any mammalian species and the excipients are substances commonly used in other vaccines; none of them present a safety concern. Therefore, it is concluded that the exposure entails no consequences other than the possible mechanical damage due to self-injection.

The residual antibiotic (gentamicin) was calculated to be 0.15 µg/dose according to a 'worst case scenario'; this would result in a maximum residue of 0.0065 µg/kg egg. Gentamicin sulphate is used in human medicine for a variety of systemic and dermatological conditions. Sensitivity reactions are more commonly associated with allergic contact dermatitis. It was concluded that there is no risk for the user from the presence of residual gentamicin.

Finally, because the product is kept frozen in liquid nitrogen, a user safety recommendation for risk management is proposed in the SPC to wear personal protective equipment during handling.

Study of residues

No studies of residues were performed. This is considered acceptable.

MRLs

The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

The excipients, listed in section 6.1 of the SPC are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

Gentamicin used in the manufacturing process is present at low residual levels (not more than 0.15 µg/dose) in the finished product which is not considered to constitute a risk to the consumer.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The applicant has not provided data investigating interactions of the vaccine with other veterinary immunological products and therefore proposes to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.'

Field studies

Two combined field safety and efficacy studies were conducted in two different commercial broiler farms located in Hungary. These birds were seropositive for MD and ND on the day of vaccination but seronegative for H9. The studies were performed under GCP. Only the safety aspects of the studies are considered in this chapter.

Study CLI-146-2020 Field safety and efficacy trial of R062 vaccine in broiler chickens in Alsótold	
Objectives	Field safety and efficacy
Study sites	Commercial hatchery in Hernád and animal facility of Lab-Nyúl Ltd. in Alsótold, Hungary (CRO).
Study design	Two test groups and one control group of 324 eggs were included. The study was performed under GCP and partially blinded (with respect to clinical observations). Eggs were assigned to the groups as they came to hand. Animals were moved from the hatchery to Lab-Nyúl on the day of hatching and placed in separate housing per group. Local reactions were monitored in 20 birds in group 2 between Day 3 and 17. Mortality, feed intake and clinical status were recorded daily. Body weight was determined weekly for 20 birds/group. Necropsy

	and blood sampling was performed on 10 birds/group on days 24 and 42. On day 45 birds from group 2 (n=25) and group 3 (n=23) were transported to a different facility for challenge. The study ended on Day 48, with necropsy and weighing of all animals. Any animals found dead during the study were necropsied. Blood samples were taken from 12 hatch mates on Day 3, to determine MDA to MDV and NDV.
Compliance	Study was performed under GCP
Animals	Ross 308 commercial broilers: 18-days-old embryonated eggs
Eligibility criteria	Embryonated eggs were included. After hatching, weak or deformed birds were excluded.
Test product	R062 vaccine; 2,000 doses/5 ml; MSV+7; 4.05 x 10^6 PFU/ml equivalent to 10,125 PFU/dose, Solvent (400 ml/bag). Group1: 1 dose (in 0.05 ml), *in ovo*, Group 2: 1 dose (in 0.2 ml), *s.c.*
Control product	Cevac Vitapest L Group 3: 1 dose, via spray (Day 3) and drinking water (Day 24)
Vaccination scheme	Group 1: 18th day of incubation (Day 0) – 324 eggs Group 2: after hatching (Day 3), at Lab-Nyúl – 303 birds Group 3: after hatching (Day 3) and on Day 24, at Lab-Nyúl.- 257 birds All chicks were vaccinated with commercial vaccines against IBV (Day 3, Cevac IBird) and IBDV (Day 21).
Safety end points	Clinical signs, production performance (weight gain, feed conversion, mortality), hatching, local reactions.
Statistical method	Birds were kept in three separate houses under identical conditions.
Results	
Outcomes-Safety observations	Hatching ratio was identical in the three groups at 95.06%. After transport (in paper boxes) and vaccination of group 2, it was found that 67 birds in group 1 had died due to suffocation/crowding: 235 birds remained. Nineteen birds in group 3 were excluded after vaccination due to suffocation and 25 birds were kept separate as a control for challenge: 257 birds remained in group 3. All 303 birds in group 2 (being removed from the boxes) remained. Local reaction observations revealed no abnormalities. At necropsy on Day 17, no macroscopic local abnormalities were found. No clinical signs of MD, ND or LPAI-H9 or any other disease were observed during the trial. Total mortality during follow-up was 3.40% in group 1, 3.63% in group 2 and 0.39% in group 3: the difference was statistically significant for groups 1 and 2 vs group 3. In the first week mortality was mostly due to hatching weakness (0.85%, 0.33% and 0.004% in the three groups respectively). In the following period death was mostly attributed to runting-stunting syndrome, hydropericard, pneumonia and air sacculitis. Body weights were significantly higher in groups 1 and 2 compared to group 3 for the period D3-D45 (p=0.0000 for G1 vs G3 and for G2 vs G3). On day 49 bodyweight was significantly higher for G1 (p=0.014) compared to G3 but the difference between G2 and G3 was not significant. Vaccinated groups had numerically higher FCR results than the CVP vaccinated one (G1: 1.64, G2: 1.98, G3: 1.46). The higher value for FCR in G2 is due to removal of (more) birds during

	the study. The European Production Efficiency Factor was 448, 360 and 502 respectively for the three groups. The EPEF is considered numerically similar for G1 and G3 but lower in G2 (due to distorted FCR calculations). Nevertheless, EPEF values in all groups are considered good.
Adverse events	No adverse events occurred.
Discussion	
Discussion/ conclusions further to assessment	The trial was valid as no serious technical disturbances or concurrent diseases occurred during the study. The IVP is considered safe since: No vaccine related symptoms were registered during the study period in either group. Hatchability was not affected by vaccination. The significantly higher mortality in the IVP vaccinated groups is considered to be coincidental and not a safety concern. The mortality ratios of G1 and G2 represent normal values obtained under field conditions (mortality ratio <4%). Necropsy revealed no abnormalities related to vaccination. The bodyweights were significantly higher in the vaccinated groups while (in group 1) the FCR/EPEF was somewhat lower compared to controls: all values were in the normal range. The results give no indication of safety issues under field conditions of use, when the vaccine was applied via either of the proposed routes in commercial broilers.

Study CLI-149-2020 Field safety and efficacy trial of R062 vaccine in broiler chickens in Bár	
Objectives	Field safety and efficacy
Study sites	Prophyl Ltd. Bár, Hungary (CRO).
Study design	Two groups of 300 commercial day-old broilers were included. The study was performed under GCP and partially blinded (clinical observations). Birds were assigned to the groups as they came to hand. Animals were moved from the hatchery to the CRO facilities on the day of hatching and placed in separate housing/group. Local reactions were observed in 20 birds in G1 between Day 0 and 14. Necropsy of 20 birds in G1 for local reactions on D14. Mortality, feed intake and clinical status were recorded daily. Body weight was determined weekly for 20 birds/group. Necropsy and blood sampling was performed on 20 birds/group on days 21 and 42. On day 40 birds from group 1 (n=23) were transported to a different facility for challenge. The study ended on Day 42, with necropsy and weighing of all animals. Any animals found dead during the study were necropsied as well. Blood samples were taken from 20 hatch mates on Day 0, to determine MDA to MDV and NDV.
Compliance	Study was performed under GCP
Animals	Ross 308 commercial broilers: day-old chicks
Eligibility criteria	Normal, healthy day-old chicks were included

Test product	R062 vaccine; 4.96 x 6log10 PFU/ml; Solvent. Group 1: 1 dose (in 0.2 ml), *s.c.* Cevac Vitapest L Group 2: 1 dose, via spray (Day 0) and drinking water (Day 21)
Control product	
Vaccination scheme	Group 1: Day 0 (Day of age) Group 2: Day 0 (spray) and Day 21 (drinking water) All chicks were vaccinated with a commercial vaccine against IBV (Day 0, Cevac IBird).
Safety end points	Daily mortality up to Day 14, weight gain. Secondary parameters: local reactions, production performance (feed conversion, feed consumption, EPEF)
Statistical method	Birds were kept in two separate houses under identical conditions.
Results	
Outcomes-Safety observations	Local reactions were found in three animals on Days 1-3 (less than 1cm diameter, painful or warm, duration: unknown). At necropsy on Day 14, no macroscopic local abnormalities were found. No clinical signs of MD, ND or LPAI-H9 or any other disease were observed during the trial. Total mortality was 3.00% in group 1, 3.67% in group 2. The difference was not statistically significant. In the first week mortality was mostly due to hatching weakness (2.67% in both groups). In the following period death was mostly attributed to the fast-growing characteristic of the breed. Body weights not significantly different throughout the study or at necropsy. Vaccinated groups had numerically lower FCR results than the CVP vaccinated one (G1: 2.29, G2: 2.52). The EPEF was 198 in group 1 and 178 in group 2, which are considered similar.
Adverse events	No adverse events occurred.
Discussion	
Discussion/conclusions further to assessment	The trial was valid as no serious technical disturbances or concurrent diseases occurred during the study. The IVP is considered safe since:. Mortality was not significantly different between the groups. Body weight was not significantly different between the groups. Minor local reactions were observed during the first three days post vaccination (incidence: 5%). Considering that neither in the other field study (CLI-146-2020), nor in any of the laboratory GLP safety studies any signs of local reaction at the injection site could be seen, it is the Applicant's position that these local reactions were only unluckily events and no unfavourable side effects of the vaccination. Production parameters (FCR, EPEF) were similar for both groups. The results give no indication of safety issues under field conditions of use, when the vaccine was applied via the s.c. route in commercial broilers.

These studies were appropriately designed and executed to an acceptable standard (GCP). The husbandry practises in these small-scale studies cannot be considered to be fully comparable to those for commercial farms. However, the birds were from a commercial hatchery and the *in ovo* vaccination was performed in the hatchery. The potential differences in husbandry conditions in the small scale holding compared to commercial broiler houses are not considered to be of such extent that this would affect the relevance of safety outcomes. With respect to the local reactions observed after s.c. vaccination, it is very unfortunate that random animals were taken for evaluation and that it is therefore not clear whether the local reactions were due to vaccination and how long such reactions persisted. However, the applicant has provided a further justification as to why these reactions could be disregarded: vaccines with the same composition are on the market for several years (Vectormune ND, Cevac MD HVT, Cevac MD Rispens) and no cases of local reactions after proper administration have been reported. Together with the results of the GLP laboratory safety (overdose) studies, this can be accepted as sufficient support for the safety of the vaccine, also with regard to the absence of local reactions. Thus, it can be concluded that the results of this field safety evaluation of the vaccine support the results of the laboratory safety studies.

Environmental risk assessment

The assessment of the potential risk to the environment of the use of the vaccine was carried out as requested in Annex II section D of Directive 2001/18/EC.

Spread of the vaccine strain to unvaccinated chickens was not observed in the studies but spreading to turkeys and between turkeys was observed. Vaccination or infection of turkeys was shown not to lead to clinical signs of disease and is therefore not considered a hazard. A warning sentence is included in the SPC to mitigate the risk of spreading to turkeys. The HVT FC-126 parent virus host range is limited to avian species. HVT virus is non-pathogenic for the target species and other avian non-target species. The genetic modification did not increase the risk for the transfer of genetic material, the insert is genetically stable, and the phenotypic characteristics of the GMO are stable as well. The parent HVT virus is distributed worldwide and as such the rHVT/ND/H9 virus does not represent a new burden to the environment since it has similar safety characteristics as the parent HVT strain. None of the inserted genetic materials used during the genetic modifications codes for a product that may be toxic or allergenic. 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 using automatic syringes and/or *in ovo*-injector machines, which minimises the probability of the product to contaminate the environment. The quantity of vaccine per animal used is very low and it is applied once in the lifetime of the chickens.

Based on the data provided the ERA can stop at Phase I. Newflend ND H9 is not expected to pose a risk for the environment when used according to the SPC.

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

This product falls within the scope of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. Detailed information on the possible risks for humans and for the environment has been provided. The assessment of the potential risk to the environment of the use of R062 vaccine was carried out following the guidance presented in the Notice to Applicants

(guidance on environmental risk assessment for veterinary medicinal products consisting of or containing genetically modified organisms [GMOs] as or in products, March 2006).

A technical dossier containing the information required by Annex IIIA of Directive 2001/18/EC is provided in part 3E. This includes permission for use in controlled laboratory conditions and a copy of a written consent for release of R062 vaccine in two field studies, issued by the Hungarian authorities. Information on the origins, method of recombination, genetic stability, biological properties and genomic sequence of the vaccine strain as well as on sensitivity and specificity of detection methods is provided in this part. An assessment of risks based on the detailed information provided for the genetically modified organism was performed.

In summary, the vaccine strain was generated by homologous recombination using bacterial vectors with large overlapping sub-genomic sequences. No signs of any unknown sequences at the insertion sites were detected. An *in vitro* study for genetic stability of vaccine cultured to MSV+7 did not show any tendency for genetic instability. Phenotypic stability was confirmed *in vivo* for MSV+5 and *in vitro* (black plaque assay for expression of F and HA proteins) for all passages between MSV and MSV+7.

Based on the properties of the parent HVT strain, which is not capable of replication in humans or other mammals, and the results of safety studies in chickens and other birds which support that the parent HVT apathogenic nature was not changed during the genetic modification, it is concluded that there are no animal health concerns.

Since no impact on animal health, human health or the environment could be identified that may be caused by the GMO if it reached the environment, the overall level of risk to the environment of the use of the GMO is negligible.

Overall conclusions on the safety documentation

The safety of a tenfold overdose of Newflend ND H9 when administered *in ovo* to 18-day-old embryonated chicken eggs or subcutaneously to one-day-old chickens was investigated in accordance with the regulatory requirements. Based on the results obtained, it can be concluded that the vaccine is safe for the target animal when used in accordance with the recommended schedule and via the recommended routes.

Examination of the reproductive performance was not performed. However, no indications were found that the vaccine carries a risk for the reproductive tract and there is no need to exclude replacement layers and breeders from the indication. The SPC contains a warning not to use the product in birds in lay and within 4 weeks before the start of the laying period.

No study was done to examine possible consequences of vaccination on immunological functions. This is acceptable based on the biological properties of the parent HVT virus. There is no indication that the vaccine virus exerts a specific negative effect on immunological functions of the host.

Studies were conducted to establish the potential for spread and dissemination of the vaccine strain. No evidence of spread to unvaccinated chickens was obtained. However, shedding of the vaccine cannot be excluded based on PCR positive feather tip samples. Also, spread of the vaccine strain from vaccinated chickens to turkeys was observed as well as spread from vaccinated to unvaccinated turkeys. The vaccine was found to be safe (apathogenic) for turkeys. No infection or spread was observed in pigeons or in pheasants. An appropriate warning is included in the SPC which should limit the risk of spread from vaccinated chickens to turkeys.

Reversion to virulence of the strain was investigated in accordance with the regulatory requirements and no indication of reversion to virulence was found. The vaccine strain was found to be genetically

and phenotypically stable after *in vitro* passages up to MSV+7. The chances of recombination or reassortment of the rHVT/ND/H9 vaccine strain were considered and found to be negligible.

Based on the assessment presented, the product does not pose an unacceptable risk to the user when used in accordance with the SPC. Appropriate warnings for the user concerning the handling of ampoules stored in liquid nitrogen have been included in the product literature.

Based on the MRL status of the components of the vaccine and the solvent, no withdrawal period is required, a zero-day withdrawal period is therefore acceptable.

No studies were performed to investigate compatibility with other products and an appropriate warning to this extent is included in the SPC.

Two small-scale combined safety and efficacy field studies were performed, using commercial broiler chicks and embryonated eggs. Vaccination was shown to be generally safe, with no effects on hatchability, production parameters, mortality and general health. In one study some local reactions were observed, however these are considered chance observations. Although chicks were not housed in commercial farms and the numbers of birds included in the studies were relatively small, the studies are considered sufficiently representative of the field situation. The results of these safety investigations of vaccination under field conditions support the results of the laboratory safety studies.

An environmental risk assessment was provided, in accordance with the requirements. No risk to the environment was identified. A risk assessment for GMO products was provided and no risk to the environment was identified. The vaccine virus was shown to be genetically and phenotypically stable in both *in vitro* and *in vivo* studies.

Part 4 – Efficacy

Introduction and general requirements

The vaccine is intended for single lifetime application, *in ov*o to 18-day-old embryonated eggs or subcutaneous to one-day-old chickens. The vaccine is claimed to reduce mortality, clinical signs, lesions and virus shedding caused by Newcastle disease virus (NDV) and to reduce clinical signs, lesions, and virus shedding caused by H9 subtype of low pathogenic avian influenza virus (LPAIV-H9). The onset of immunity is claimed at 3 weeks for NDV and 4 weeks for LPAIV-H9. The duration of immunity is claimed to be at least 9 weeks for both NDV and LPAIV-H9.

The applicant is not seeking a claim for efficacy against Marek's disease, although the vaccine is based on a recombinant HVT virus. In the countries where the vaccine is to be used, a claim against Marek's disease is not deemed necessary by the applicant. Scientific advice was given concerning this issue, CVMP concluded that studies concerning the efficacy against MDV would not be requested if no claim against MDV is made in the SPC.

LPAIV-H9 has been reported only in some sporadic cases within the European Union. The applicant would like to obtain and maintain an EU marketing authorisation for this new recombinant influenza vaccine in order to facilitate regulatory approvals in non-EU countries that rely upon a marketing authorisation in the country of origin and on the acceptance of European authorities' registration dossier assessment. Scientific advice was given concerning the eligibility of performing the laboratory efficacy studies with a LPAIV-H9 challenge strain that is not relevant in Europe and through this question, the eligibility of the vaccine in Europe. CVMP concluded that the use of non-European (i.e. Middle Eastern or African) challenge strains is acceptable in this particular case.

Studies were performed in accordance with requirements as laid down in Directive 2001/82/EEC (as amended by 2004/28/EC and Directive 2009/9/EC) and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7. (Evaluation of efficacy of veterinary vaccines) as well as monograph 0062 (Vaccines for veterinary use) and monograph 0450 (Newcastle disease vaccine (live)). The vaccine virus does not fall strictly into the scope of Ph. Eur. 0450 monograph, however the evaluation criteria for the vaccine to pass the efficacy requirements of this monograph were respected as much as possible.

Challenge model:

Newcastle disease

The challenge strain used in all the laboratory efficacy trials was NDV Herts 33/56 which is the reference strain of the Ph. Eur. monograph 0450.

Low pathogenic avian influenza virus H9N2

The challenge strain (D3398) used in all the laboratory studies is a low pathogenic avian influenza virus subtype H9N2. The strain was originally isolated from broilers in Morocco. A challenge dose calibration study was performed to identify the lowest titre of the challenge virus that could result in the typical characteristics signs of LPAIV infection. Intranasal application of two different doses of challenge virus resulted in infection (100%), mild to severe respiratory clinical signs (90%) and pathology (100%) in the majority of birds. A scoring system for clinical and pathological changes was validated.

Efficacy parameters and tests:

Two commercial ELISA kits for detection of MDV and NDV antibodies in blood and a commercial NDV qPCR test (detecting wild type NDV) were used in the studies. All three kits include positive and negative controls with each test and are considered fit-for-purpose.

An in-house haemagglutination inhibition test (HI) was developed to demonstrate antibodies against LPAIV-H9 in blood samples. The test was appropriately validated.

A qPCR for detection of LPAI H9N2 MA/18 challenge virus in clinical samples was developed in-house. The method was appropriately validated.

A qPCR for detection of MDV-1 virus in clinical samples (blood, organs) was developed in-house. The method was appropriately validated.

Study title
ND efficacy (3 weeks onset of immunity) test of R62 vaccine in SPF chickens after *in ovo* vaccination (2000 PFU)
ND efficacy (3 weeks onset of immunity) test of R62 vaccine in SPF chickens after subcutaneous vaccination (2000 PFU)
efficacy (4 weeks onset of immunity) test of R062 vaccine in SPF chickens after *in ovo* and subcutaneous vaccination
Keeping study of broiler chickens vaccinated with R062 vaccine by the *in ovo* route
Keeping study of broiler chickens vaccinated with R062 vaccine by the subcutaneous route
H9 efficacy (9 weeks duration of immunity) test of R062 vaccine in SPF chickens after *in ovo* and

Study title
subcutaneous vaccination
ND efficacy (9 weeks duration of immunity) test of R062 vaccine in broiler chickens after *in ovo* and subcutaneous vaccination
H9 efficacy test of R062 vaccine in H9-MDA positive broiler chickens at 5 weeks of age after *in ovo* and subcutaneous vaccination
Field safety and efficacy trial of R062 vaccine in broiler chickens in Alsótold
Field safety and efficacy trial of R062 vaccine in broiler chickens in Bár
ND onset of immunity efficacy test of R062 vaccine in broiler chickens after subcutaneous vaccination
ND onset of immunity efficacy test of R062 vaccine in broiler chickens after in ovo vaccination

Laboratory trials

Onset of immunity

NDV

Two studies were performed to determine the onset of immunity against NDV. In one study, 18-day-old chicken embryonated SPF eggs were vaccinated with a below minimum dose of vaccine (2,000 PFU) while another group of eggs remained untreated. Challenge was performed with NDV Herts 33/56 at day 24 post vaccination (p.v.). Vaccination did not affect hatching ratio. In the control group, all 12 birds died within 3 days of challenge. In the vaccinated group, 2 out of 22 birds showed ND signs and died, resulting in a protection percentage of 90.9%. The second study involved one-day-old SPF chicks: one group was vaccinated subcutaneously with a below minimum dose of vaccine (2,000 PFU) and one group remained unvaccinated. Challenge was performed with ND Herts 33/56 on day 21 p.v.. None of the 22 vaccinated birds showed signs or died of ND whereas all 12 control birds died due to ND resulting in a protection percentage of 100%.

The two studies support the claimed onset of immunity at 3 weeks of age, with a reduction of clinical signs, mortality and lesions. Data on virus shedding were not collected.

With the responses to questions, the applicant presented the reports of two studies in support of the OOI for reduction of NDV virus shedding. Both studies were performed in commercial broilers, one study in day-old chicks while in the other study 18-day-old embryonated eggs were vaccinated with minimum doses of the vaccine. Non-vaccinated broilers and SPF birds were included as controls. After challenge at 4 weeks of age, all control birds died while 86.7% protection was observed in subcutaneously vaccinated birds and 83.3% after *in ovo* vaccination. Significant reduction of NDV shedding, as observed by PCR of oropharyngeal and cloacal swabs, was found in the vaccinated groups in both studies. The two studies support a claim for reduction of NDV shedding, with an onset of immunity of 4 weeks of age.

LPAIV H9N2

One study was performed to determine the onset of immunity against LPAIV H9. In groups 1 and 2, SPF eggs and one-day-old chicks were vaccinated with a minimum dose (3,000 PFU) of vaccine and an

additional group (3) was kept as non-vaccinated controls. On day 31 (28 days of age), 25 birds in each group were challenged with LPAIV H9N2. Clinical scoring was performed for depression, breathing pattern and lacrimation. Oro-pharyngeal swabs were taken at day 34, 36 and 38 and/or on the day of death. Necropsy was performed on dead and euthanised animals, scoring of air sac lesions was performed. Histology and PCR of trachea and lung samples was performed. The clinical scores were significantly lower in the vaccinated groups. Mortality was reduced in the vaccinated groups. Air sac lesions as well as histological scores (trachea and lung) were significantly lower in the vaccinated groups. Virus (PCR copy number) in lung samples was significantly lower in the vaccinated groups but trachea samples showed no significant difference. Virus shedding (PCR in oropharyngeal swabs) was significantly lower in vaccinated groups.

The study supports the claimed onset of immunity against LPAIV H9N2 at 4 weeks of age, with a reduction of clinical signs, lesions and virus shedding.

Duration of immunity

NDV

A study investigating the duration of immunity against NDV was performed in commercial broilers. Broiler chicks from a flock with high maternal antibody levels against NDV were included in the study at an age of 68 days. Two groups previously vaccinated, *in ovo* (18-day-old embryonated eggs) or s.c. at one day of age, with a minimum dose (3,000 PFU) of vaccine were included. In addition, a group of non-vaccinated hatch mates and a group of 5-week-old non-vaccinated SPF birds were included. All birds were challenged with NDV Herts 33/56 and observed for 14 days. Oro-pharyngeal and cloacal swabs were taken on Day 1, 2, 3, 4, and 5 from 10 animals in groups 1, 2 and 3 for qPCR to detect ND virus. All of the SPF birds died within 4 days of challenge. The unvaccinated broilers all had typical signs of ND and 75% mortality. In the *in ovo* vaccinated group, 2/22 birds had signs of ND resulting in a protection of 90.9%. In the subcutaneous vaccinated group, protection was 100%. Challenge virus was not detected in any of the swab samples taken from vaccinates. In the unvaccinated broilers sample positivity ranged between 20 and 100%.

The study supports the claimed duration of immunity of 9 weeks since the vaccine at minimum dose and applied via either *in ovo* or s.c. routes was shown to provide protection against virulent NDV challenge in NDV MDA+ broiler chickens at 67 days of age. A reduction of clinical signs, mortality, lesions and virus shedding was achieved.

LPAIV H9N2

One study was performed to study the duration of immunity against LPAIV-H9. Briefly, 18-day-old embryonated eggs and one-day-old SPF chicks were vaccinated with a minimum dose of vaccine (3,000 PFU). An additional group remained unvaccinated. At day 67 (9 weeks of age) all birds were challenged with H9N2 virus. Birds were observed daily and scored for altered behaviour and respiratory signs. Oro-pharyngeal swabs were taken from all birds on D70, 72 and 74. Birds were euthanised on D74 and necropsy was performed; lesion scoring was performed for air sacs, samples of trachea and lung were taken for PCR and histological scoring. Clinical scores were significantly lower in vaccinated groups, mortality was numerically lower. Air sac lesions as well as histological scoring of trachea and lungs were statistically lower in vaccinates. Virus load was significantly lower in the trachea of the *in ovo*-vaccinated groups but not in the other samples. Virus copy numbers (but not percentage positive samples) were significantly lower in oro-pharyngeal swabs of the vaccinates.

The study supports the claim for reduction of clinical signs, lesions and virus shedding with a duration of immunity of 9 weeks.

Maternally derived antibodies (MDA)

A study investigating the duration of immunity against NDV in NDV MDA+ commercial broilers was provided and is described above. Although a group of MDA- vaccinated birds was not included in the study for comparison, the results fully comply with the efficacy criteria of Ph. Eur. 0450 and thus it may be concluded that the study supports the efficacy of the vaccine in (NDV) MDA+ birds.

A further study was performed in commercial broilers derived from a flock vaccinated with an inactivated vaccine against LPAIV-H9. A group of chicken embryonated eggs and a group of one-day-old chicks were vaccinated with a minimum dose of vaccine a further group of chicks remained unvaccinated. Challenge with H9N2 virus was performed on day 35 of age. Hatch mates were 80% seropositive to H9 and 100% to ND. After vaccination LPAI-H9 antibodies were detected in all birds in both vaccinated groups. Average bodyweight gain after challenge was significantly higher in both vaccinated groups. Clinical scores, air sac lesions and pathological scores were significantly lower in vaccinates. No vaccinates died versus 2/15 controls. Virus copy number and percentage positive samples of the lungs were significantly lower in vaccinates, virus in samples of trachea was not significantly different. Virus copy number in oro-pharyngeal and conjunctival swabs was significantly lower in vaccinates at most timepoints. Vaccination resulted in reduction of clinical signs, lesions and virus shedding and weight gain was significantly higher in vaccinates. Thus, the efficacy of the vaccine in LPAIV-H9 MDA+ birds was supported by the study.

The level of MDA against MDV in the commercial birds used in the efficacy studies was present at a level relevant to what is generally found in the field. This is considered to comprise adequate support for use in MDV MDA+ birds.

Interactions

The applicant has not provided data investigating interactions of the vaccine with other veterinary immunological products and therefore proposes to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.' The standard warning on concurrent use is considered adequate.

Field trials

Two combined safety and efficacy field trials were performed. The general design of the studies is described in the chapter on safety. Additional laboratory (challenge) studies were performed using animals taken from the field trials.

In the field trial in Alsótold (Hungary), 18-day-old commercial broiler eggs were vaccinated *in ovo*, a second group of birds were vaccinated s.c. at one day of age. A third group of birds were vaccinated with Cevac Vitapest, a live attenuated vaccine against ND, by coarse spray (CVP). All three groups received spray vaccination against infectious bronchitis (Cevac IBird) on D3. No clinical signs of or mortality due to MD, ND or LPAI-H9 or any other disease were observed during the field trial. Based on the totality of data, the presence of MDV-1, NDV and H9-LPAIV could be excluded.

Twenty-five birds were selected prior IVP and CVP vaccination to be involved in a complementary ND efficacy study:
The study included three groups: s.c. vaccinated broiler chicks (group 1), non-vaccinated broiler chicks (group 2), both taken from the field study, at 6 weeks of age and one group of 10 non-vaccinated 4-

week-old SPF chickens (group 3). All birds were challenged with NDV Herts 33/56 and observed for 14 days. Oropharyngeal and cloacal swabs were taken and analysed by qPCR for NDV.
All birds in group 1 survived without clinical signs. In group 2, all birds died between 3 and 5 days post challenge. In group 3, all birds died between days 3 and 4 post challenge. PCR of oropharyngeal and cloacal swabs showed no NDV in group 1 birds whereas the samples in group 2 were 100% positive from day 2 post challenge.

It was concluded that s.c. vaccination of broilers under field conditions conferred protection against virulent NDV challenge (reduction of clinical signs, mortality and lesions) and significantly reduced virus shedding.

In the field study in Bár (Hungary), one group of one-day-old birds were vaccinated with one commercial dose of vaccine (IVP) s.c. A second group was vaccinated with one commercial dose of Cevac Vitapest L (CVP) by coarse spray. Both groups received spray vaccination against infectious bronchitis (Cevac IBird) on D3. Blood samples were taken on Day 0 (20 hatch mates), D21 and D42 for NDV and LPAIV-H9 serology. No clinical signs of or mortality due to MD, ND or LPAI-H9 or any other disease were observed during the field trial. Based on the total data, field exposure to MDV-1, NDV and LPAIV-H9 could be excluded.

Thirty-five birds were selected prior IVP and CVP vaccination to be involved in a complementary ND efficacy study:

The study included three groups: s.c. vaccinated broiler chicks (group 1), non-vaccinated broiler chicks (group 2), both taken from the field study, 40-day-old and one group of 10 non-vaccinated 23-day-old SPF chickens (group 3). All birds were challenged with NDV Herts 33/56 and observed for 14 days. Oropharyngeal and cloacal swabs were taken and analysed by qPCR for NDV.
In the vaccinated group two birds were diagnosed with ND. In the control group all birds died from ND. All SPF birds died due to ND. PCR of oropharyngeal and cloacal swabs showed NDV in 7 of 20 samples from group 1 birds on day 5 post challenge. Samples in group 2 were 100% positive from day 2 post challenge. The protection percentage was 91.3%.
It was concluded that s.c. vaccination of broilers under field conditions conferred protection (91.3%) against virulent NDV challenge (reduction of clinical signs, mortality and lesions) and reduced virus shedding.

The applicant has provided three laboratory challenge studies in support of the efficacy against LPAIV H9N2 which have been performed with the same challenge strain. In the absence of evidence of efficacy against field challenges, these studies cannot support a claimed broad efficacy against LPAI H9. The applicant has provided additional serological data to support cross-protective efficacy. Sera collected at day 65 post vaccination from SPF birds vaccinated with a minimum dose via *in ovo* and subcutaneous routes were tested against the Moroccan virus used in the challenge studies (GI lineage) as well as a second GI lineage virus from the Middle East and two Y280 lineage virus isolates (origin not disclosed). These two lineages comprise an important part of the H9N2 isolates to date. The sera were shown to meet the minimum HI titres for clinical protection as suggested in the OIE manual (for conventional vaccines) against four different influenza isolates from two different lineages. The data provided thus support the assumption that cross protection can be induced by vaccination with a live vaccine containing a 'consensus sequence' HA gene. The analysis of data on the related vaccine Vectormune AI provides additional support for the notion that live recombinant vaccines induce protective cellular and mucosal responses on top of the humoral responses. In conclusion, the totality of data presented provides adequate support for the claimed protection against low pathogenic H9 subtype avian influenza strains.

Overall conclusion on efficacy

Based on the laboratory and field efficacy data, it can be concluded that the vaccine is efficacious against NDV when applied via either *in ovo* or subcutaneous routes, at the minimum dose. A reduction of clinical signs and lesions caused by Newcastle disease virus was found in the laboratory and field (challenge) studies, with an onset of immunity of 3 weeks and a duration of immunity of 9 weeks. The claimed 9-week DOI is supported by data from a study in broilers, which can be accepted since the controls were sufficiently susceptible to the infection to allow appropriate analysis of protection. The claimed reduction of NDV virus shedding is supported by the results of the DOI study in broilers and the complementary challenge studies of field-vaccinated birds where statistically significant reduction of virus detection in oro-pharyngeal and cloacal swab samples was found. Two additional laboratory studies in broilers support the claimed reduction of virus shedding with an onset of immunity of 4 weeks of age.

Based on the laboratory efficacy data, it can be concluded that the vaccine is efficacious against the LPAIV H9N2 challenge strain originating from Morocco. The claimed reduction of clinical signs, lesions and virus shedding with an onset of immunity of 4 weeks and a duration of 9 weeks is supported by the data from both the OOI and DOI study.

The applicant has provided additional serological data to support cross-protective efficacy. Sera collected at day 65 post vaccination from SPF birds vaccinated with a minimum dose via *in ovo* and subcutaneous routes were tested against the Moroccan virus used in the challenge studies (GI lineage) as well as a second GI lineage virus from the Middle East and two Y280 lineage virus isolates (origin not disclosed). These two lineages comprise an important part of the H9N2 isolates to date. The sera were shown to meet the minimum HI titres for clinical protection as suggested in the WOAH manual (for conventional vaccines) against four different influenza isolates from two different lineages. The data provided thus support the assumption that cross protection can be induced by vaccination with a live vaccine containing a 'consensus sequence' HA gene. The analysis of data on the related vaccine Vectormune AI provides additional support for the notion that live recombinant vaccines induce protective cellular and mucosal responses on top of the humoral responses. In conclusion, the totality of data presented provides adequate support for the claimed protection against Low pathogenic H9 subtype avian influenza strains.

The vaccine was shown to confer adequate protection in the presence of MDA against NDV and LPAIV-H9N2.

Part 5 – Benefit-risk assessment

Introduction

NEWFLEND ND H9 is a bivalent, cell-associated, live recombinant vector vaccine for chickens for *in ovo* or subcutaneous injection.

The vaccine contains one active ingredient: the recombinant live herpesvirus of turkey (HVT, Marek's disease virus serotype 3) which was genetically modified to express the Fusion (F) gene of Newcastle disease virus (NDV) and the haemagglutinin (HA) -encoding gene of low pathogenic avian influenza virus (LPAIV) H9N2.

The vaccine is presented as a frozen suspension for injection in flame-sealed ampoules, containing 1,000, 2,000 or 4,000 doses. The vaccine is to be reconstituted before use in a sterile diluent to either 0.05 ml/dose for *in ovo* use or 0.2 ml/dose for subcutaneous use.

The application has been submitted in accordance with art. 12(3) of Directive 2001/82/EC and concerns a full application.

Benefit assessment

Direct therapeutic benefit

The proposed benefit of Newflend ND H9 is its efficacy against Newcastle disease and disease caused by low pathogenic avian influenza H9 subtype strains, which was investigated in a number of well-designed laboratory and field studies conducted to an acceptable standard.

Additional benefits

Being a bivalent vaccine, Newflend ND H9 reduces the handling of chicks. The method of application ensures a good coverage of the flock.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has generally been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

Risk for the target animal:

The safety of rHVT/ND/H9 vaccine strain in chickens vaccinated *in ovo* or subcutaneously at the youngest recommended age was confirmed in a GLP safety study. No adverse effects were observed in any of the birds administered a ten-fold overdose of the maximum recommended treatment dose.

Risk for the user:

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Safety advice concerning the handling of ampoules stored in liquid nitrogen is included in the SPC.

Risk for the environment:

Newflend ND H9 is not expected to pose a risk for the environment when used according to the SPC recommendations. However, the vaccine strain may spread to unvaccinated birds, in particular turkeys, and specific measures to mitigate the risk of transmission are described in the SPC.

Risk for the consumer:

Newflend ND H9 is not considered to pose a risk to the consumer when used according to the SPC recommendations. The withdrawal period has been set to zero days.

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 user and environment 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 the active immunisation of one-day-old chicks or 18-day-old chicken embryonated eggs:

- to reduce clinical signs, lesions and virus shedding caused by Newcastle disease virus (NDV);
- to reduce clinical signs, lesions, and virus shedding caused by H9 subtype of low pathogenic avian influenza virus (LPAIV-H9).

The formulation and manufacture of Newflend ND H9 is well described, and specifications set will ensure that product of consistent quality will be produced.

It is well tolerated by the target animals and presents a low risk for users and the environment and appropriate warnings have been included in the SPC.

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 Newflend ND H9 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). The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned product.

In addition, based on the review of data provided, the CVMP considers that the active substance contained in Newflend ND H9 - cell-associated live recombinant turkey herpesvirus (rHVT/ND/H9), expressing the fusion protein of Newcastle disease virus and the hemagglutinin of low pathogenic avian influenza virus, subtype H9 - is to be qualified as a new active substance considering that no veterinary medicinal product containing live recombinant turkey herpesvirus (rHVT) expressing NDV F protein and AIV H9 together has been registered in the European Union to date.

Finally, the CVMP recommended the applicant to provide the results of the studies to validate the maximum holding times (24 hours) for the pre-filtration bulk and for the filled bags pre-sterilisation.