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CVMP assessment report
Nobivac Myxo-RHD (EMEA/V/C/2004)

Common name: Live myxoma vectored RHD virus strain 009

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

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1. Summary of the dossier

Introduction

An application for the granting of a Community marketing authorisation for Nobivac Myxo-RHD has been submitted to the European Medicines Agency on 2 February 2010 by Intervet International BV in accordance with Regulation (EC) No. 726/2004. Further to the submission of a letter of intent by Intervet International BV on 15 September 2009 the CVMP accepted on 14 October 2009 that Nobivac Myxo-RHD was eligible under Article 3(1) of Regulation (EC) No 726/2004 which refers to veterinary medicinal products developed by means of biotechnological processes such as recombinant DNA technology. Nobivac Myxo-RHD is indicated for a minor species (rabbits) and has been classified as a product intended for a minor use minor species (MUMS)/limited market by the CVMP. The Guideline on Data Requirements for Immunological Veterinary Medicinal Products Intended for Minor Use or Minor Species/Limited Markets (EMEA/CVMP/IWP/123243/2006-Rev.1) is applicable.

Nobivac Myxo-RHD is a vaccine that contains live myxoma vectored RHD virus strain 009. It is an immunological medicinal product ATCvet code: QI08AD. It is available as a lyophilisate (freeze-dried substance) and a solvent that are made up into a suspension for injection. Nobivac Myxo-RHD is used in rabbits from five weeks of age onwards to reduce mortality and clinical signs of myxomatosis (skin tumours caused by the myxoma virus) and to prevent death due to rabbit haemorrhagic disease (RHD), a disease resulting in blood clot formation caused by the RHD virus.

Myxomatosis is a virus disease of the European rabbit (*Oryctolagus cuniculus*) caused by the myxoma virus, a member of the *Poxviridae*. It affects rabbits of all ages. There are two clinical forms of the disease:

1) The classical form is observed mainly in pet rabbits, on rural farms and in wild rabbits (a reservoir of the virus) where biting insects can cause indirect transmission of the disease. Close contact with an infected rabbit can cause transmission the disease as well. The disease has a short incubation period, is easy to diagnose based on typical clinical signs, and causes a high level of mortality.

2) During the last decades a respiratory form of myxoma virus infection has been observed in industrial units. This respiratory form is observed in industrial units all year round, and is induced by milder attenuated strains of virus. It can be subclinical and is more difficult to diagnose.

Traditionally, myxoma virus infections were predominantly spread by biting arthropods or, in exceptional cases, by direct contact. After the introduction of a small dose of virus into the skin by the biting arthropod, the rabbit developed clinical signs of myxomatosis characterised by lesions which are widely distributed over the body and are particularly obvious on the ears. Then the eyelids become thickened and are usually completely closed by the ninth day, followed by oedematous swelling of the head, base of the ears, perineum and the scrotum in male rabbits which becomes pronounced in the later stages of the disease. Virulent strains can kill a rabbit within 10-15 days. Myxomatosis is an OIE notifiable disease.

Rabbit haemorrhagic disease is a virus disease of the European rabbit (*Oryctolagus cuniculus*) caused by a member of the family of *Caliciviridae*. RHD spreads very rapidly and infection can occur by nasal, conjunctival and oral routes. The disease can be transmitted directly and indirectly (e.g. equipment, cages, humans, birds, insects).

The disease is characterised by high mortality in adult rabbits with few signs prior to sudden death (nervous signs and death within 48-96h). Typical lesions are: hepatocellular degeneration, splenic enlargement and diffuse haemorrhages in many different organs. Infection occurs in rabbits of all ages but clinical disease is observed only in animals over 8-10 weeks of age. The mechanism of resistance to clinical disease in young animals is still unclear and is probably correlated to the white blood cell population present in these young animals. Rabbit haemorrhagic disease is an OIE notifiable disease.

The vaccine strain is a myxoma virus expressing the capsid protein VP60 of rabbit haemorrhagic disease virus (RHDV). It is grown in a cell culture system using a continuous cell line of rabbit kidney cells. The vaccine is presented as single dose and 50 dose lyophilisate with accompanying solvent, available in 1 ml, 10 ml and 50 ml vials. The single dose can be used immediately after reconstitution of the freeze-dried vaccine with the solvent. For the administration of the multi-dose-preparation an automatic vaccination device is foreseen.

The benefits of Nobivac Myxo-RHD are that RHD protection could be demonstrated when serologically naïve and seropositive (MDA) rabbits were vaccinated. With regard to the efficacy against myxoma virus infections, reduction of mortality and clinical signs could be demonstrated. The vaccine does not contain any adjuvant or preservative. The most common side effects are a transient increase in temperature and a small non-painful swelling at the injection site which will resolve within 3 weeks.

The approved indication is: *For active immunisation of rabbits from 5 weeks of age onwards to reduce mortality and clinical signs of myxomatosis and to prevent mortality due to rabbit haemorrhagic disease.* Subcutaneous vaccination of one dose is regarded sufficient to induce protection. Annual revaccination is recommended. The duration of immunity is one year against both diseases.

Part 1 - Administrative particulars

Manufacturing Authorisations and Inspection Status

Copies of manufacturing authorisations for all production sites located in the EU were provided. Satisfactory certificates of GMP compliance were provided. Inspections of the manufacturing sites have been conducted at regular intervals. Any additional inspection was not considered necessary.

Pharmacovigilance system

A satisfactory detailed description of the pharmacovigilance system is provided. It was accepted that the applicant has in place a pharmacovigilance system that will allow it to meet its pharmacovigilance responsibilities as prescribed in the legislation.

GMO

As this vaccine contains a GMO according to Directive 2001/18/EC as amended, it falls under the scope of this Directive. A full set of data was provided and assessed in accordance with the requirements of Directive 2001/18/EC.

2. Quality assessment

Composition

Composition per vial (either 1 or 50 doses) of freeze-dried vaccine

Active substance - Live myxoma vectored RHD virus strain 009 ≥ $10^{3.0}$ FFU (Focus Forming Units) /dose and ≤ $10^{6.1}$ FFU/dose.

The vaccine also contains hydrolysed gelatin, pancreatic digest of casein, sorbitol, disodium phosphate dihydrate, potassium dihydrogen phosphate and water for injections) where compliance with the relevant Ph.Eur. or USP monograph has been demonstrated.

Container

Glass vials: Hydrolytical class type I glass (Ph. Eur. 3.2.1) for the freeze-dried vaccine and single 1 dose and 10-dose presentation of the solvent.

PET vials for the 50-dose presentation of the solvent, made of polyethylene terephthalate, in compliance with the specific monograph of PET vials intended for packaging liquid oral dosage forms as described in the USP.

Stoppers: Halogenobutyl rubber stopper (Ph. Eur. 3.2.10) and Aluminium capsules

The sterilisation process of the glass vials occurs under GMP-conditions in compliance with Ph. Eur. 5.1.1.

Development Pharmaceutics

Currently licensed vaccines against myxomatosis consist of live attenuated strains of myxoma virus or live strains of Shope fibroma virus. Shope fibroma virus is mainly used to vaccinate young rabbits for which the homologous myxoma virus vaccine strains are harmful. The duration of immunity of the conventional vaccines against myxomatosis is 4-6 months.

Currently licensed vaccines against rabbit haemorrhagic disease virus (RHDV) are inactivated and contain an adjuvant. RHDV does not replicate in cell cultures. Thus, the vaccine virus used must be prepared *in vivo*. Rabbits are infected with virulent RHDV. The virus is extracted from their livers after these animals have succumbed to the infection.

Contrary to these monovalent vaccines, Nobivac Myxo-RHD contains the RHDV component produced *in vitro.* The particularity of this GMO is that the myxoma virus acts as vector for the immunogenic capsid protein VP60 of RHDV and stimulates immunity against both myxoma virus and rabbit haemorrhagic disease infections. The myxoma virus itself is lacking two virulence genes where the insertion of VP60 RHDV was made. Thus, the deletion of these virulence genes is expected to increase the innocuousness in young rabbits compared to other vaccines against myxomatosis and to afford a one shot vaccination scheme in conjunction with a one-year duration of immunity.

According to the guideline EMEA/CVMP/IWP/123243/2006 on "Data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets" both myxomatosis and rabbit haemorrhagic disease are indicated as products intended for minor uses/limited markets. As this vaccine contains a GMO according to Directive 2001/18/EC as amended, a full set of data was provided.

The solvent used is identical with the solvent used for Intervet's range of live injectable vaccines for companion animals. The choice was made to use the same solvent for reconstitution of Nobivac Myxo-

RHD. In addition, there is good experience with this type of solvent in rabbits in the UK where a myxomatosis vaccine containing a live Shope fibroma virus strain is currently authorised.

As indicated already above, the vaccine is presented in single and 50-dose lyophilisate presentations with accompanying solvent for reconstitution. The solvent is presented in 1 ml, 10 ml, and 50 ml volumes. The single dose can be used immediately after reconstitution of the freeze-dried vaccine with 1 ml of the solvent. Taking into account the needs of the commercial rabbit market, the dose volume is limited to 0.2 ml for the multidose presentations. The 10 ml and 50 ml vial of solvent allows group sizes of 50 and 250 animals respectively to be vaccinated with a 0.2 ml dose volume using the same automatic vaccination equipment.

Safety and efficacy data created under controlled conditions are available with the 1 ml volume and the 0.2 ml volume. The most important advantages of Nobivac Myxo-RHD are i) no target animals are used for the production of the RHDV component, ii) freedom from any adjuvant and preservative, iii) safe for use in rabbits from 5 weeks of age and iv) no excretion of the vaccine strain to other unvaccinated target or non-target species.

As regards the propagated one-shot-vaccination-scheme in conjunction with a one-year-duration-of-immunity, the onset of immunity is fixed at 3 weeks against both rabbit haemorrhagic disease and myxomatosis.

A modification of the final product test on the exclusion of RHDV as prescribed in the myxomatosis Ph. Eur. monograph has been accepted. The Ph. Eur. monograph requires that for extraneous agents testing freedom from RHDV is demonstrated by serology. However, the myxoma virus RHDV vectored vaccine strain would also induce antibodies against RHDV. Thus, the test requirements would not be met. Therefore, rabbits being used for the batch safety test will also be monitored for signs of RHD during the 14 days observation period. Whereas RHDV cannot be grown *in vitro*, RHDV infections are lethal to rabbits (within one week after infection the animals may suddenly die, usually without showing clinical signs). Post-mortem investigation of the batch safety animals that died during the observation period can demonstrate presence of the virus in case of any accidental contact with pathogen RHDV. Rabbits at an age >10 weeks can be used; then they have lost their 'natural resistance' to RHDV.

Method of manufacture

Myxoma vectored RHDV is grown on rabbit kidney cells. After harvesting and treatment of the harvest by centrifugation and sonication, the bulk material is stored frozen until further processing. Tests on virus content and sterility are carried out before the bulk material is stored. After finalisation of the lyophilisation process including sealing of the vials, a variety of tests are performed such as virus titration, sterility, identity, residual moisture.

The components of the solvent are mixed. The bulk solvent for the 10 ml and 50 ml presentations is autoclaved. 1 ml glass-vials are heat sterilised. Filling and closing includes a random test for filling volume. The solvent is examined for content, appearance, identity.

Several satisfactory validation studies were performed.

Control of starting materials

Starting materials listed in a pharmacopoeia

The starting materials are compliant with the Ph. Eur. The starting materials of biological origin comply with either the Ph. Eur. or the USP. The serum Guideline EMEA/CVMP/743/00-rev.2 was also taken into consideration. The impact of this guideline on the control of bovine sera is properly addressed.

Starting materials not listed in a pharmacopoeia

Myxoma vectored RHD virus strain 009

Information on source, passage history, and preparation of the Master Seed is available to the greatest possible extent.

With regard to the controls carried out on Master and Working Seed, relevant Ph. Eur. monographs were taken into account as well as Note for Guidance III/3427/93 on extraneous agents to be tested for in relation to the general and species specific guidelines on production and control of mammalian veterinary vaccines and the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMEA/CVMP/IWP/123243/2004).

Sterility was tested according to Ph. Eur. 2.6.1, mycoplasmas according to Ph. Eur. 2.6.7. Tests for the exclusion of extraneous agents were performed in accordance with the recommendations of the guideline.

Identification and characteristics of the vaccine strain are determined. Essential test methods such as product identification are validated.

Genetic engineering

The cloning and construction process is described in detail; standardised methods were used.

The stability of the myxoma vectored RHD virus has been shown.

Rabbit kidney cells - Source, passage history, preparation and description of Master Cell Stock

The vaccine strain can be grown to sufficient titres using this cell line.

The master cell stock was tested according to the current legislation.

With regard to the controls carried out on Master Cell Stock, relevant Ph. Eur. monographs were taken into account as well as Note for Guidance III/3427/93 on extraneous agents to be tested for in relation to the general and species specific guidelines on production and control of mammalian veterinary vaccines and the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMEA/CVMP/IWP/123243/2004).

Sterility was tested according to Ph. Eur. 2.6.1, mycoplasmas according to Ph. Eur. 2.6.7. Tests for the exclusion of extraneous agents were performed in accordance with the recommendations of the Guideline on 'General requirements for the production and control of live mammalian bacterial and viral vaccines for veterinary use'.

A test on tumorigenicity was not performed. This is justified.

Identification of the species and karyology were performed in accordance with the Guideline and details were provided.

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

The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

Control tests during production

Sterility control and virus titration are carried out on myxoma vectored RHD virus prior to freeze-drying. During the filling process for the lyophilisation procedure a random test for filling volume is carried out. The solvent is tested for filling volume.

Control tests on the finished product

The results of the analysis of three consecutive production runs of both the single dose and multidose freeze-dried vaccine and for five series of three consecutive production batches of solvent taking into account the different production sites and presentations were presented which comply with the required specification.

All tests details were provided containing frequency, function, brief description, release requirements and further details relating to validation reports, if applicable.

Since the active substance is presented in a freeze-dried form, the general characteristics of the finished product focus on the solvent. Tests are performed on contents on average, appearance, colour, clarity, and pH-value. Whenever possible, relevant Ph. Eur. monographs were taken into consideration.

The lyophilisate is tested for identification of the active substance. The respective validation report was provided. The method is suitable for the identification of the active substance. The batch potency test is carried out determining the quantity of infectious Myxo-RHDV by titration in rabbit kidney cells. The validation report provided is complete with regard to robustness, inter- and intra-assay precision, the suitability of the method is proven. As regards the test to ensure freedom from mycoplasmas, a complete report is provided in conformity with the relevant Ph. Eur. monographs. The combined safety and extraneous agents test is acceptable. Sterility tests are carried out. Residual moisture is determined by coulometric titration.

Batch-to-batch consistency was demonstrated for 3 batches of single dose presentation and 3 batches of multi-dose presentation including batch protocols for the single-dose and multi-dose solvent for each production site. The specifications proposed are appropriate to control the quality of the finished product.

Stability

Stability of the bulk antigen

The bulk antigen can be stored up to 24 months.

Stability of the finished product

No loss of virus titre can be observed during the storage period up to 27 months at 2-8°C. The residual moisture remains also constant. The vaccine can be stored up to 24 months at 2-8°C.

Stability during transport

The vaccine can be transported at ambient temperatures.

Stability of the reconstituted product

The vaccine is stable for at least 4 hours after reconstitution.

Storage condition and shelf life of the solvent

50 ml PET vials: The results were indicative for a storage option up to 48 months at room temperature. However, it appeared that the solvent of the 50 ml PET vials evaporated; there was a loss of 0.8 ml per year when the vials were stored at 25°C. In consequence, the shelf life of the 50 ml PET presentations is limited to 2 years at 2-8°C.

Glass vials for the single dose and 10 dose presentations can be stored up to 48 months at ambient temperature.

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

In addition to the requirements of Directive 2001/82/EC as amended, Annex III A of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms applies to live recombinant vector vaccines. Due to the nature of Annex III A (Information required in notifications concerning releases of GMOs other than higher plants) not all the points included can be addressed.

The vaccine strain is a myxoma virus expressing the capsid protein of RHDV. It is grown in rabbit kidney cells. The insertion was made by replacement of two virulent genes of a myxoma virus strain by the RHDV VP60 gene. Therefore, the pathogenicity of the myxoma gene deleted virus is further diminished compared with the attenuated parent strain.

Information required in notifications concerning releases of genetically modified organisms other than higher plants (Annex IIIA Directive 2001/18/EC)

As regards the assessment in accordance with Directive 2001/18/EC, all points to be considered for a live recombinant vector vaccine have been satisfactorily addressed. The parental strain and the capsid protein of RHDV as well as the cloning vector are sufficiently described.

The genetic stability of the vaccine strain can be assumed. The characterisation of the GMO is sufficiently described. The GMO poses no enhanced risk for target and non-target animals, environment and humans since the vaccine virus replicates at the most at the injection site in the scruff of the neck and the probability that a recently vaccinated rabbit is bitten by an arthropod and virus is transferred to another susceptible organism is considered to be negligible.

Overall conclusions on quality

Quantitative and qualitative particulars of the constituents are indicated. The manufacturing method including the construction and related tests of the GMO such as identity etc. is sufficiently described. With regard to the starting materials of biological origin, details on the seed material have been provided. All starting materials listed in a pharmacopoeia are detailed including also some starting materials of animal origin. Information on their function, species of origin and treatment before use is given and adequately validated.

Essential information on the development, construction and control of the GMO is provided. Detailed descriptions of the parent strain, the cell line, the insert, the construction of the expression vector are provided. Related controls are provided; their validity has been demonstrated or is justified.

Seed materials and other starting material of animal origin relevant for the transmission of TSE comply with the Note for Guidance EMEA/410/01-rev2 and the corresponding Ph. Eur. monograph.

In-process- and final product control tests guarantee the production of safe and efficacious batches of consistent quality.

The quality of the product has been demonstrated.

3. Safety assessment

Nobivac Myxo-RHD is a live vaccine containing a vectored myxoma virus expressing the capsid protein gene VP60 of RHDV. As a consequence rabbits are immunised against both myxoma virus and rabbit haemorrhagic disease virus. The main benefit of this technique is that the RHDV component can be grown *in vitro* instead of using live rabbits.

The vaccine is presented in freeze-dried form to be reconstituted in an accompanying solvent and is intended for rabbits from 5 weeks of age onwards, including pregnant does. The maximum dose is $10^{6.1}$ FFU of live myxoma vectored RHD virus strain 009. The volume of one dose is 1.0 ml (single dose vaccine) or 0.2 ml (multidose vaccine).

Myxomatosis and rabbit haemorrhagic disease (RHD) are listed in Table 2 of Guideline EMEA/CVMP/IWP/123243/1006 (minor use or minor species/limited markets). Therefore Nobivac Myxo-RHD is considered as a MUMS product. As this vaccine contains a GMO according to Directive 2001/18/EC it completely falls under the scope of this Directive. A full set of data was provided. It would be acceptable to fulfil only parts of the requirements in the case that data had been gained with similar GMO constructs already authorised. That means all laboratory studies shall be carried out in accordance with GLP using batches with maximum virus titre.

Laboratory tests

A GLP Compliance Statement is included in every study.

Safety of the administration of one dose

No isolated study with one dose has been performed. Within many efficacy studies (1 dose of minimum or standard titre) a corresponding monitoring after the vaccination was performed. The findings were comparable to the observations from the safety studies.

Safety of one administration of an overdose

A study was performed in SPF rabbits at five weeks of age. The rabbits received an overdose (10 times the maximum virus titre) by SC route of administration followed by a second injection of one dose with a maximal virus titre 3 weeks later. A control group received solvent only. After the administration a slight increase in temperature was observed for a few days in some animals. The majority of vaccinates showed soft swellings of the draining lymph node lasting for two or three days. Some exhibited a swelling (1-2 cm in diameter for two days) at the site of injection. This occurred after the one dose injection as well. As an additional finding it was recorded that the virus did not spread to the control group because they did not seroconvert to myxomavirus and RHDV. Another overdose (10 times the maximum virus titre) followed by one dose safety study was performed in young Netherlands dwarf rabbits, which are considered as the most sensitive category of target species. The use of Nobivac Myxo-RHD in dwarf rabbits can be regarded as safe.

Safety of the repeated administration of one dose

No study has been performed. This issue is considered to be covered by the overdose study followed by a second injection 3 weeks later.

Examination of reproductive performance

Young SPF does were vaccinated with an overdose (10 times the maximum virus titre) 6 days after mating. At 20 days after mating, the animals received a second vaccination with an intermediate virus dose. Both vaccinations were performed by SC route. The kindling results were normal for does of the first breeding season. No differences could be observed between the vaccinated and control groups. Does and offspring appeared normal and healthy. Some does of the vaccinated group did not come to parturition. No abortion was observed within these rabbits. Does are able to resorb the embryos in stress situation at the early stage of pregnancy. Although the average fertility rate was within the normal limits, it is not clear whether mating was unsuccessful in some does of the vaccinated group or whether a loss of pregnancy occurred after vaccine administration.

The vaccine was well tolerated; the observation results were comparable to the results of the overdose study in rabbits at 5 weeks of age. The body temperature was recorded. Slight differences in the core temperature were observed. No safety study on the reproductive performance has been conducted in male rabbits (bucks). Therefore, the vaccination of breeding bucks is not recommended.

Examination of immunological functions

The applicant stated that the vaccine does not adversely affect the immunological functions. No study has been performed in this regard.

Special requirements for live vaccines

- Spread of the vaccine strain

In nearly all safety and efficacy studies unvaccinated control groups were housed together with the vaccinates. The controls did not seroconvert to myxoma virus and RHDV. No vector arthropods occurred in the housing areas that have an important relevance for the spread of myxomatotosis infection. No rabbit showed generalised signs of myxomatosis after the vaccination; results of the "dissemination in the body studies" clearly demonstrate that after subcutaneous vaccination the vaccine virus can be isolated from the skin of the administration region and the draining lymph node of this region only. Therefore shedding of virus is unlikely.

Additional studies have been performed in mice, chickens and European brown hares for which myxomatosis infection is a known potential risk as well. No signs of myxomatosis were detected within the vaccination groups of mice, one-day-old chickens and hares. No seroconversion or any signs of myxomatosis infection could be detected in the co-housed control group of the same species.

- Dissemination in the vaccinated animal

Rabbits were inoculated with one dose of a test batch with the maximum virus titre by the SC route. After five days the rabbits were killed and samples were taken from skin at the injection site, eyelids, lymph nodes and several organs from the intestine. A virus recovery was successful in the skin tissue of the injection site and the corresponding draining lymph node. It can be concluded that no dissemination of vaccine virus in the body occurs.

- Reversion to virulence of attenuated vaccines

Rabbits were vaccinated and the vaccine virus was re-isolated and administered to other rabbits. A final safety study in rabbits was performed with vaccine virus of the 6th rabbit passage. No increase in virulence was observed in the vaccination group which received the 6th passage compared to another group which received the original vaccine batch.

- Biological properties of the vaccine strain

The biological properties of the vaccine strain are linked to the reversion to virulence study and are described in the analytical part of the documentation in detail.

- Recombination or genomic reassortment of the strains

The test vaccine was mixed with a dose of a licensed myxomatosis vaccine. The mixed suspension was administered intradermally to a group of rabbits. A second group received the test vaccine by the same route. The co-administration of the recombinant myxomatosis vaccine did not result in any clinical signs typical for myxomatosis.

In vivo and *in vitro* studies were additionally performed to get an insight in the potential of recombination or genomic reassortment of the vaccine strain. The nucleotide sequences for the respective Master Seeds and the re-isolated viruses were compared and found to be identical.

Study of residues

The product is a live vaccine, thus no MRL is necessary and it does not contain any adjuvant or preservative. The excipients included in the product are either included in Table 1 of the Annex to Regulation (EC) 37/2010 or are considered as not falling within the scope of Regulation (EC) 470/2009. CVMP concluded that the consumption of products derived from rabbits vaccinated with Nobivac Myxo-RHD presents no risk for human health. Consequently, the withdrawal period is set at zero days.

Interactions

Compatibility with other vaccines is not claimed. Therefore no studies have been made.

Field studies

Two field studies in pregnant does and two field studies with rabbits of 5 weeks of age were performed (offspring from the studies with pregnant does). All received one dose with standard virus titre by the subcutaneous route. The vaccine was safe in all vaccinates.

Slight temperature increases and soft swellings at the injection sites, which sometimes occurred in the second week after vaccination and disappeared completely within a few days were observed in the vaccinates. The regional lymph nodes were not examined after vaccination. Regarding the reproduction performance the results were comparable between vaccinated and control groups and in line with the expected breeding results of the New Zealand rabbits. In one of the groups of does vaccinated on day 13 of pregnancy a few animals did not come to parturition although the animals were palpated for pregnancy before vaccination was performed. Post-mortem investigation confirmed a uterus infection in those animals. A mix-up between uterus enlargement caused by pregnancy and by uterus infection is possible. Nevertheless, a vaccine-related loss of pregnancy cannot clearly be excluded as this phenomenon did occur in the groups of does vaccinated up to day 13 of pregnancy only. This result confirmed the findings from the laboratory study in pregnant does that were vaccinated on days 6 and 20 of pregnancy. The SPC section 4.7 states that vaccination is not recommended during the first 14 days of pregnancy as studies were not conclusive.

User safety

Myxomatosis and RHD are not considered as zoonoses. No adjuvant or preservative is present in the vaccine. Except for injuries from needles and damaged primary packages, there is no risk for the user.

Environmental risk assessment

The environmental risk assessment and the assessment required for veterinary medicinal products containing or consisting of GMOs cover more or less identical issues. The issues covered by Note for Guidance EMEA/CVMP/074/95 are also reflected in the evaluation according to Directive 2001/18/EC.

Phase 1 assessment

Hazard identification

Any hazard was evaluated by a) examination of the capacity of the myxoma vectored RHD vaccine virus strain to transmit to non-target species, b) the potential of shedding, c) the capacity to survive, to establish and to disseminate, d) the potential pathogenicity to other organisms, e) potential of other effects of live vaccine strain, f) and g) toxic effects. Generally, the respective detection methods and their suitability is demonstrated.

The composition of the product without any adjuvant or preservative only containing well known excipients gives no reason to suspect any toxic effect in the vaccinated animal or by excreted metabolites.

Assessment of Likelihood

Referring to the hazard identification above, there are no potential risks either for the wider as well as for the local environment.

Assessment of the consequence of a hazard occurring

Already the probability that a hazard occurs is considered negligible. Therefore, the consequence of a hazard is negligible.

Assessment of level of risk

Taking all the risk factors into consideration the assessment of the level of risk for Nobivac Myxo-RHD can be considered as negligible. The second phase evaluation is not considered necessary.

In conclusion:

The environmental risk was assessed following the recommendations of Note for Guidance EMEA/CVMP/074/95. The first phase of the assessment is provided outlining that the potential exposure of the environment to the product and the level of risk associated with it is negligible. Special attention was paid to the target animal species and in-contact target or non-target animals, the method of administration and the potential of excretion and the likelihood to what extent the vaccine harbours a related risk. The conclusion of this first phase is that there is no potential exposure of the environment to the product. No second assessment phase is necessary.

Overall conclusion on safety

Vaccinated rabbits (compared to controls) were used to demonstrate the overall safety profile of the current vaccine. Limited observations were reported concerning the clinical monitoring of the animals, including a poor statistical analysis initially submitted of the only systemic safety parameter taken into account (i.e. the increase of rectal temperature). However, an updated statistical analysis of the rectal

temperatures was submitted during assessment. Some deviations from the requirements of Ph. Eur. or GLP were observed. The safety profile of Nobivac Myxo-RHD vaccine was demonstrated according to current legislation.

Nobivac Myxo-RHD is regarded as safe for rabbits from the age of 5 weeks. Small swellings at the injection site and soft swellings of the regional lymph node in case of accidental injection of an overdose must be expected. Often a slight increase in temperature occurs for a few days after vaccination. The potential for any adverse effects following the administration of the vaccine under the recommended conditions of use is adequately reflected in the relevant section of the SPC.

Studies involving the use of the vaccine during early pregnancy were inconclusive. In pregnant does vaccinated before day 14 of pregnancy sometimes a loss of pregnancy was observed (it is unclear if the does were not pregnant or if a loss of pregnancy occurred after the vaccination) in laboratory and field studies. Therefore the vaccination of does before day 14 of pregnancy is not recommended.

Since no safety study on the reproductive performance has been conducted in male rabbits (bucks), the vaccination of breeding bucks is not recommended.

Residue aspects were addressed adequately and a withdrawal period of zero days set.

The vaccine strain does not disseminate in the body and is not spread to the environment. Neither an indication of increase in virulence after 6 rabbit passages nor of reassortment of the vaccine strain were detected. From the studies performed it can be concluded that the vaccine is safe in European brown hares and other mammalians and chickens as well.

The first phase of the environmental risk assessment which was performed as recommended in the Note for Guidance EMEA/CVMP/074/95 and concluded that the product will not pose an unacceptable risk to the environment. No second assessment phase is necessary.

The potential exposure of the environment to the product and the level of risk associated with it is negligible. The composition of the product without any adjuvant or preservative and only containing well known excipients gives no reason to suspect any toxic effect in the vaccinated animal or by excreted metabolites. Special attention was paid to the target animal species and in-contact target or non-target animals, the method of administration and the potential of excretion and the likelihood to what extent the vaccine harbours a related risk. There is no potential exposure of the environment to the product.

4. Efficacy assessment

Introduction and general requirements

Nobivac Myxo-RHD is a live vaccine containing a vectored myxoma virus for rabbits to immunise against both myxoma virus and rabbit haemorrhagic disease virus. The main benefit of this technique is that the RHDV component can be grown *in vitro* instead of using live rabbits.

The vaccine is presented in freeze-dried form with accompanying solvent and is intended for rabbits from 5 weeks of age onwards. The minimum dose is $10^{3.0}$ FFU of live myxoma vectored RHD virus strain 009. The volume of one dose is 1.0 ml (single dose presentation) or 0.2 ml (multidose presentation).

Ph. Eur. monograph 1943 for live myxomatosis vaccines and Ph. Eur. monograph 2325 for RHD inactivated vaccines, even if not fully appropriate, were used as a guide for efficacy taking into consideration that the product falls under the scope of the MUMS guideline.

Tests were carried out in young and adult animals, both in laboratory and in field conditions. Unvaccinated animals served as controls. The influence of maternally derived antibodies has been studied.

Laboratory trials

Laboratory challenge studies have been performed to confirm the onset and duration of immunity for protection against myxomatosis and RHD.

Rabbits of 5 weeks of age serologically naïve against myxomatosis and RHDV were used in the onset and 12-months-duration-of-immunity studies. They were vaccinated with a test batch with a minimum virus titre.

Onset of immunity

Myxomatosis

The myxomatosis challenge was performed 3 weeks after the vaccination. Many animals showed an increase in body temperature and 18% showed swellings at the injection site of the challenge virus. One rabbit developed some small swellings on the ear and one above the eye which all formed scabs. These scabs resolved within 2 weeks. No typical signs of myxomatosis according to Ph. Eur. monograph 1943 were observed in this animal. This result is in line with the claim asked for, namely reduction of mortality and clinical signs of myxomatosis. However, the monograph for myxomatosis live vaccines requires that only 10 % of the rabbits may show typical signs of myxomatosis after the challenge. Therefore the observations regarding the protection rate are borderline.

RHDV

The onset of immunity studies regarding RHDV component were performed with rabbits of 11 weeks of age.

In total, two challenge studies were performed. To exclude the 'natural immunity' against RHDV in young rabbits, older animals were used. The start of one study was at the age of 12 weeks. The challenge was performed 13 days after the vaccination. The second study started with rabbits that were 11 weeks old at the vaccination date. The challenge was performed 3 weeks later. None of the vaccinated rabbits showed signs of illness while all controls died after the challenge. Thus, the onset of immunity for the RHDV fraction was clearly demonstrated 3 weeks after vaccination. Since the myxomatosis challenge was also performed 3 weeks after the vaccination, the onset of immunity is set at 3 weeks for the vaccine Nobivac Myxo-RHD.

Duration of immunity

Myxomatosis

After the first myxomatosis challenge that was performed 12 months post vaccination 40% of vaccinates developed mild signs of myxomatosis, but the general appearance remained normal throughout the observation period. All controls developed severe clinical signs myxomatosis after the challenge and had to be euthanased or died. Although this is in line with the claim for myxomatosis of Nobivac Myxo-RHD, the requirements for a full protection claim as indicated in the monograph were not fulfilled.

A second 12-month myxomatosis challenge study was submitted. All controls developed myxomatosis after the challenge and had to be euthanased. 75% of the vaccinated animals were fully protected. Mild signs of myxomatosis were observed in 25% of the vaccinates. This result is in line with the

myxomatosis claim of Nobivac Myxo-RHD (reduction of mortality and clinical signs of myxomatosis). One year duration of immunity has been demonstrated.

RHDV

An initial RHDV challenge study was performed 110 days after vaccination. Full protection was achieved. To prove a one year protection, an RHD challenge was performed 13 months after vaccination. Full protection was demonstrated.

Another "one-year" RHDV challenge study was performed 500 days after the vaccination. All vaccinates survived the challenge 50% of the controls survived the challenge, some of them without any signs of illness. Since too many controls survived the challenge, this is a clear signal that the challenge was too weak. Therefore the challenge is judged as not valid. It was argued that this was the result of the farm rabbit strain used in this study which was different compared to other studies. An external expert of the applicant confirmed that there is a difference in sensitivity to RHDV between rabbit strains. Due to the serological data obtained approximately one year after the vaccination, a one-year-protection can be expected.

Thus, duration of immunity of one year has been demonstrated.

Impact of maternally derived antibodies

An additional challenge study against myxomatosis was performed in rabbits which had persisting maternally derived antibodies at the vaccination date. Sufficient protection was demonstrated at the date of challenge performed 3 weeks after the vaccination. The serological response to RHDV in this study was sufficient. Protection against RHDV is confirmed.

Myxomavirus challenge mimicking natural infection

Additional challenge studies against myxomatosis were performed in seronegative farm rabbits. The challenge was performed by infected seeder rabbits which were placed into the groups 3 weeks after vaccination. A sufficient protection against myxomatosis was demonstrated in the vaccination group.

Within the 6-months challenge - also performed by infected seeder rabbits - the requirements for full protection of the myxomatosis monograph were not strictly fulfilled. However, the requirement for a reduced protection regarding myxomatosis can be regarded as fulfilled. One control rabbit showed a transient myxomatosis antibody titre prior to challenge. The reason is unclear. Adequate information was given regarding the challenge strains used. Within the myxomatosis challenge studies, the clinical observation after challenge was scored. Finally the scoring points obtained from the control group were compared to the results obtained from vaccinates.

Response to booster vaccination

A study demonstrating that a clear increase of antibody titres could be detected within a group which was revaccinated after one year under the persistence of myxomatosis antibodies was provided. The antibody levels obtained after the primary vaccination could not fully be reached again. This was mainly observed for RHDV. The revaccination performed 4 months after primary vaccination under high levels of myxomatosis antibodies did not induce any further antibody increase against both vaccine components.

Field trials

Serological efficacy studies in pregnant does and their offspring were performed in the EU.

For does that were never vaccinated before, a sufficient antibody development could be demonstrated against myxomatosis and RHDV. In case of animals that were already vaccinated with an other

myxomatosis vaccine before (but not against RHD), a booster effect against myxomatosis could be detected but the response to RHDV was poor and several myxomatosis pre-vaccinated does did not seroconvert to RHDV after the vaccination.

Part of the offspring was included in challenge studies. At the vaccination date of 5 weeks only a few rabbits showed traces of persisting maternally derived antibodies. Myxomatosis challenge was performed 5 weeks later; the RHD challenge was performed 12 weeks later. The mortality rates in the vaccination groups after the challenges were reduced.

Overall conclusion on efficacy

Protection against myxomatosis could be demonstrated in line with the proposed claim (reduction of mortality and clinical signs).

Protection against RHDV infections (prevention of mortality) has been demonstrated after the vaccination of serologically naïve and seropositive (MDA) rabbits.

In cases where the existence of myxomatosis antibodies (persisting from previous myxomatosis vaccinations) was seen the vaccination is not able to induce sufficient protection against RHDV in RHDV naïve rabbits. This issue is addressed in SPC section 4.5 (Special precautions for use).

However, an adequate booster against RHDV is possible under the persistence of myxomatosis antibody titres.

The duration of immunity against RHDV over one year is acceptable.
The onset of immunity of three weeks has been confirmed.

At the minimum vaccination age of 5 weeks it must be expected that not all rabbits are free of maternally derived antibodies against myxomatosis. However, the data support that the vaccine is capable breaking through residual maternally derived antibodies that may be present under field conditions at 5 weeks of age.

5. Benefit/risk balance

Part 5 – Benefit risk assessment

Introduction

Nobivac Myxo-RHD is a lyophilised live vaccine without any adjuvant or preservative, containing at least 3.0 log 10 FFU (Focus Forming Units), but not more than 6.1 log 10 FFU of myxoma vectored rabbit haemorrhagic disease virus strain 009 per dose as active ingredient. Nobivac Myxo-RHD is indicated for active immunisation of rabbits from five weeks of age via one injection by the subcutaneous route to reduce mortality and clinical signs of the classical form of myxomatosis and to prevent mortality due to rabbit haemorrhagic disease. Annual revaccination is recommended.

The vaccine strain is a myxoma virus expressing the capsid protein VP60 of rabbit haemorrhagic disease virus (RHDV). It is grown in a cell culture system using a continuous cell line of rabbit kidney cells.

Benefit assessment

Direct therapeutic benefit

Protection against RHDV infections has been demonstrated.

With regard to the efficacy against myxoma virus infections, reduction of mortality and clinical signs has been demonstrated.

Additional benefits

- Not using target animals for the production of the vaccine antigen RHDV component. Instead, production *in vitro.*
- No need for the addition of any adjuvant and preservative.
- Easier vaccination schedule for industrial rabbit farms.
- Safe for use in rabbits from 5 weeks of age.
- No excretion of the vaccine strain to other unvaccinated target or non-target species.

Risk assessment

In case of vaccination of myxoma virus antibody positive rabbits, protection was not fully achieved (see animals taken from the field studies for challenge and serology studies of RHDV antibody development). This issue is addressed in the product literature (SPC section 4.5).

Injection of Nobivac Myxo-RHD may induce small, soft, painless swellings at the injection site which completely disappear within 14 days. Transient increase in temperature of up to 1-2° C may occur.

An additional study has been conducted in young rabbits of dwarf breed that are known as the most sensitive category of the target animal. Therefore dwarf rabbits can be vaccinated.

Studies involving the use of the vaccine during early pregnancy were inconclusive, therefore vaccination of does before day 14 of pregnancy is not recommended.

Since no safety study on the reproductive performance has been conducted in male rabbits (bucks), the vaccination of breeding bucks is not recommended.

There are no user and consumer safety issues.

As regards the assessment in accordance with Directive 2001/18/EC, all points to be considered for a live recombinant vector vaccine have been addressed. The GMO poses no enhanced risk for target and non-target animals, environment and humans since the vaccine virus replicates at the most at the injection site in the scruff of the neck and the probability that a recently vaccinated rabbit is bitten by an arthropod and virus is transferred to another susceptible organism is considered to be negligible.

Evaluation of the benefit risk balance

Nobivac Myxo-RHD has been shown to have a positive benefit-risk balance except for pregnant rabbits (approximately 14th day of pregnancy) and bucks. Annual revaccination is justified. The vaccine is well tolerated by the target animals and presents negligible risk for users and the environment.

The formulation, manufacture and control of Nobivac Myxo-RHD are sufficiently well described.

Based on the original and amended data presented, it is concluded that the quality, safety and efficacy of Nobivac Myxo-RHD are in accordance with the requirements of Directive 2001/82/EC as amended and Directive 2001/18/EEC. The MUMS guideline was taken into consideration whenever appropriate. The benefit-risk balance is considered positive.

Conclusion

Based on the original and complementary data presented, the Committee for Medicinal Products for Veterinary Use concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Directive 2001/82/EC as amended.