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Knowledge base for the assessment of environmental risks by the use of genetically modified virus-vectored vaccines for domesticated animals

Opinion of the Panel on Microbial Ecology of the Norwegian Scientific Committee for Food Safety

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Risk assessment on the Knowledge base for the assessment of environmental risks by the use of genetically modified virus-vectored vaccines for domesticated animals

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Assessed and approved

The opinion has been assessed and approved by the Panel on Microbial Ecology. Members of the panel are: Ida Skaar (chair), Tor Gjøen, Jacques Godfroid, Anders Jelmert, Jörn Klein Arinze Okoli, Arne Tronsmo, og Bjørnar Ytrehus.

(Panel members in alphabetical order after chair of the panel)

Acknowledgment

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The Norwegian Scientific Committee for Food Safety (VKM) was requested by the Norwegian Environment Agency in November 2015 to develop a knowledge base for assessment of the environmental risks related to the use of genetically modified (GM) virus vaccine vectors for vaccination of domesticated animals. The Agency requested that the task be conducted in the form of a desk study with the following mandate: (1) to provide a short description of GM virus vectors in use in veterinary vaccines; (2) summarize available information relevant to environmental risk assessment (ERA) of GM veterinary virus vaccines; and (3) identify environmental risk factors and knowledge gaps of relevance to ERA of GM virus vaccines within the Norwegian context. This report provides background for future environmental risk assessment of veterinary medicinal products containing or consisting of GMO for use in Norway.

VKM has appointed a working group consisting of members of the Panel on Microbial Ecology and the VKM secretariat to answer the request. The VKM Panel has reviewed and revised the draft prepared by the working group and finally approved the opinion.

This Report contains the findings of a desk study of current virus vectors used in GM virus vectored vaccines of domesticated livestock. A survey of the published literature for current knowledge in the area was undertaken with the aim of providing information relevant to the ERA of veterinary GM virus vectors (GM-VV). In identifying potential risk factors associated with vaccination of domesticated animals using GM-VV, focus was on the Norwegian environment, but relevant parallels were drawn from other European countries.

The European Commission directive 2001/18, that regulates the deliberate release of genetically modified organisms into the environment, the European Union Regulation for approval of medicinal products (726/2004/EU), and other relevant guidelines from the European Medicines Agency (EMA) on ERA of GMOs and live recombinant vector vaccines for veterinary use served as reference documents. Virus vaccine vectors, according to the Directive 2001/18/EC, are genetically modified if they were produced using techniques of recombinant gene technology. The environment, according to the Directive, constitutes all components of the ecosystem (excluding the vaccinated animals) that could be at risk of the deliberate use (or release) of veterinary GM-VV.

Enumeration of GM virus vectors was limited to those applied in domesticated livestock vaccination, but evaluation of relevant environmental risk factors of the GM vaccine vectors was extended to other animals, e.g. wild animals as well as to humans and microorganisms, as these are the at-risk non-target components of the environment. The Report, however, did not dwell extensively on risks to human health and non-domesticated animals. Nonetheless, occupational and non-occupational risk routes of handling GM-VV were briefly discussed, and parallels were drawn from the use of GM-VV to control rabies in wildlife.

The DNA virus genera of poxvirus, herpesvirus and adenovirus are the most commonly employed in GM vaccine vectors for domesticated animal vaccination. Most virus vector strains are specific to the animal species, but some have been used across species. For example, canarypox virus and human adenovirus serotype 5 (HAd5) vectors derived from the genera of pox- and adenoviruses respectively. Canarypox virus

has been used to vaccinate cat, horse, ferret, dog, sheep and rabbit; HAd5 has been used in field trial for vaccination of dog, fox, pig and cattle. Target veterinary diseases for vaccination are those for which there currently exist no efficient therapeutic and prophylactic measures. Animal health is the main driver that determines the choice of disease against which GM-VVs are produced, the most successful application of GM-VVs in the vaccination of domesticated animals being the control of avian diseases.

Hazards and potential risk to the environment are linked to shedding, survival and potential dissemination of the GM-VV. For example, as a consequence of delivery mechanism, GM-VVs have been delivered directly into the environment, in the case of the rabies GM vaccine bait used to control rabies in several parts of Europe. Although this is not applicable to the Norwegian mainland, relevant parallels can be drawn from these experiences. Studies on the GM rabies vaccines currently in use show that they are stable for few months in the environment, but residual pathogenicity cannot be ruled out entirely. In addition, successive selections from the original strain may produce hazardous and uncontrolled results, and variants may remain pathogenic both in target and non-target species.

Compared to other geographic regions, the Norwegian physical and veterinary environments are unique in many ways, especially in relation to climate, diversity of both macro- and microorganisms, farm and animal handling practices (e.g. animal species and population, confinement, distances of animal transportation, manure and carcass disposal, and government regulations) –factors that are relevant to ERA of GM-VV. Finally, some knowledge gaps and the limitations these could portend to ERA of GM-VV in Norway were highlighted in the Report in the following context:

- GM-VV are currently not used in livestock vaccination in Norway. Thus, information on the ERA of GM-VV within the Norwegian context could not be derived;

- Even in countries with experience of the use of GM-VV, ERA-relevant important information such as post-release data, is not publicly available;

- Climate change will impact some factors relevant for ERA of GM-VV, especially within the Norwegian context. The extent of such impact is currently unknown.

Key words: VKM, environmental risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency, desk study, genetically modified virus vectors, veterinary medicines, viral-vectored vaccines, domesticated animals

Sammendrag

I november 2015 ba Miljødirektoratet Vitenskapskomiteen for mattrygghet (VKM) om å utarbeide et kunnskapsgrunnlag for vurdering av miljørisiko ved bruk av genmodifiserte virus (GM-VV) til vaksinering av domestiserte dyr. Mandatet for kunnskapsgrunnlaget er:

1. Gi en kort beskrivelse av genmodifiserte virusvektorer som benyttes i veterinærmedisinske vaksiner

2. Oppsummere tilgjengelig informasjon av betydning for miljørisikovurdering av genmodifiserte virusvaksiner til veterinærmedisinsk bruk

3. Identifisere miljørisikofaktorer og eventuelle kunnskapshull av spesiell relevans for vurdering av genmodifiserte virusvaksiner under norske forhold

VKM utpekte en arbeidsgruppe med medlemmer fra Faggruppen for mikrobiell økologi. Faggruppen for mikrobiell økologi har gått gjennom og revidert arbeidsgruppens utkast og godkjent risikovurderingen.

Rapporten er en litteraturstudie som gir oversikt over publisert litteratur om virusvaksiner basert på genmodifisering (rekombinante vaksiner), som kan være aktuelle å bruke på domestiserte dyr. Litteraturen er gjennomgått for å vurdere miljørisiko knyttet til bruk av genmodifiserte rekombinante virusvaksiner (GM-VV) i veterinærmedisin. Vurderingen er forsøkt relatert til faktorer som er spesielt relevante for Norge, men erfaringer fra andre europeiske land er også inkludert.

De viktigste referansene har vært EU-direktiv 2001/18, som regulerer utsetting og bruk av levende genmodifiserte organismer, EUs forordning for godkjenning av legemidler (EU-forordning 726/2004), og relevante retningslinjer fra Det europeiske legemiddelbyrået (EMA). Miljø er definert i direktiv 2001/18/EC som: «alle deler av økosystemet, unntatt den som vaksineres».

Utvalget av vaksinetypene som er omtalt, er begrenset til de som er aktuelle for bruk i veterinærmedisin, men evaluering av risiko knyttet til dette omfatter også ville dyr, mennesker og mikroorganismer. Disse er ikke-målorganismer for legemiddelet, som kan tenkes å bli utsatt for risiko. Håndtering av genmodifiserte rekombinante virusvaksiner kan innebære både miljø- og helserisiko for yrkesutøvere, men hovedfokus i rapporten er på miljø, hvor bl.a. kontroll av rabies hos rev ved bruk av genmodifiserte rekombinante virusvaksiner er tatt med som et eksempel.

DNA-virus fra familiene kopper-, herpes- og adenovirus er de mest brukte i genmodifiserte rekombinante virusvaksiner for veterinærområdet. De fleste virusvektorer er spesifikke for sine respektive verter, men enkelte kan brukes i flere arter. To eksempler er canarypox og humant adenovirus type 5 (HAd5) vektorer som hører hjemme i disse gruppene (hhv kopper- og adenovirus). Canarypox har blitt brukt i vaksiner til katt, hest, ilder, hund, sau og kanin. HAd5 er brukt i feltforsøk med vaksiner mot sykdommer i hund, rev, gris og storfe. Vaksiner i dyr brukes når det ikke finnes andre effektive forebyggende tiltak eller behandlingsformer. Den viktigste driveren for hvilke genmodifiserte rekombinante virusvaksiner som utvikles er dyrehelse. Mest erfaring med slike vaksiner er fra kontroll av infeksjoner hos fjærfe.

Den største miljørisikoen knyttet til bruk av genmodifiserte rekombinante virusvaksiner er koblet til utslipp av infeksiøse virus, deres overlevelse og evne til å infisere andre dyr. Et godt eksempel er direkte tilføring av genmodifiserte rekombinante virusvaksiner til miljøet for å kontrollere rabies i dyr (f.eks. rev) som spiser åte innsatt med en replikerende vaksine. Selv om denne metoden ikke er brukt på fastlandet i Norge, kan man høste verdifull erfaring fra slike forsøk. Studier viser at denne vaksinen er relativt stabil i miljøet, men det kan ikke utelukkes at dyr i og utenfor målgruppen er blitt syke av vaksinen fordi det har oppstått nye varianter av viruset etter at det ble tilført miljøet.

Der hvor norske forhold skiller seg fra forholdene ellers i Europa og er relevante for genmodifiserte rekombinante virusvaksiner er dette trukket fram. Spesielt vil forskjeller i praksis knyttet til dyrehold (besetningsstørrelse, grade av bevegelsesfrihet, offentlige reguleringer) kunne være relevante for vurdering av miljørisiko knyttet til genmodifiserte rekombinante virusvaksiner. Rapporten peker også på kunnskapshull av spesiell relevans for vurdering av genmodifiserte virusvaksiner under norske forhold. Følgende spørsmål er diskutert i rapporten:

- Genmodifiserte rekombinante virusvaksiner er foreløpig ikke i bruk som vaksiner til domestiserte dyr i Norge. Følgelig vil det ikke være tilgjengelig erfaringer knyttet til miljørisiko ved bruk av disse under norske forhold

- I de land hvor genmodifiserte rekombinante virusvaksiner har vært i bruk i en tid, eksisterer det lite relevant informasjon om miljørisiko knyttet til bruken av disse (ikke registrerte uønskede hendelser)

- Klimaforandringer kan tenkes å spille en rolle for vurdering av miljørisiko ved bruk avgenmodifiserte rekombinante virusvaksiner. Omfanget av slik virkning er ikke kjent ennå.

Abbreviations and glossary

Abbreviations

Ab	Antibody
ALVAC	Canarypox virus
AMR	Antimicrobial resistance
BRSV	Bovine Respiratory Synctial Virus
BVD	Bovine virus diarrhoea
CAE	Caprin arthritis-encephalitis
CDV	Canine distemper virus
CPV	Canine parvovirus
CyHV-3	Cyprinid Herpes virus 3
EMA	European Medicines Agency
ERA	Environmental risk assessment
EU	European Union
FWPV	Fowl pox virus
GM	Genetically modified
GMOs	Genetically modified organisms

GM-VV	GM veterinary vector vaccines
HAd	Human adenovirus
HAd5	Human adenovirus serotype 5
HVT	Herpes virus of turkey
IBD	Infection bursal disease
ILTV	Infectious laryngotracheitis virus
ISAV	Infectious salmon anemia virus
IPNV	Infectious pancreatic disease virus
MVA	Modified Vaccinia virus Ankara
NYVAC	Vaccinia virus vaccine vector
ORV	Oral rabies vaccination
PIV-3	Parainfluenza virus type 3
RABORAL	Rabies vaccine
SAD	Street Alabama Dufferin
SPDV	Salmon pancreas disease virus
TK	Thymidine kinase

TLR	Toll-like receptors
VLP	Virus like particle
VP2 / VP5	Outer capsid protein
V-RG	Vaccinia rabies glycoprotein

Glossary

Adjuvant - A substance that enhances the immune response to an antigen.

Attenuation - The reduction in virulence for a given host, often as a result of continued growth of a microorganism in an artificial host or culture system or by genetic manipulation to remove virulent genes.

Defective virus replication - Incomplete virus replication, with production only of viral nucleic acid, proteins or non-infectious virus particles.

Horizontal gene transfer - The transmission of DNA between different genomes.

Mutation - An alteration in the genetic material (the genome) of a cell of a living organism or of a virus.

Plasmid - A small extrachromosomal piece of genetic material in bacterium, replicating autonomously in the cytoplasm.

Promoter - DNA sequence that defines the initiation of transcription of a gene by RNA polymerase.

Replication-competent viral vectors - Contain all necessary genes for virion synthesis, and continue to propagate themselves once infection occurs.

Replication-defective virus vectors - Viruses that have had the coding regions for the genes necessary for additional rounds of virion replication and packaging deleted, mutated or replaced with other genes.

Reversion of a mutation occurs when a second mutation restores the function that was lost as a result of the first mutation.

Viral shedding - The release of viral progeny from the infected host.

Toxoid - A toxin rendered harmless but still capable of acting as antigen.

Transgene - A gene or genetic material transferred from one organism to another.

Vector – A vehicle such as a virus or a plasmid, used to transfer genetic material to a target cell.

Vertical transmission - The transmission of infection directly from parent to offspring.

Viral replication - the process of intracellular viral multiplication.

Virion - The extracellular complete infective form of a virus, consisting of an RNA or DNA core, a protein coat, and sometimes an external envelope.

Virulence – The degree of pathogenicity.

Background as provided by the Norwegian Environment Agency

Medicinal products containing or consisting of genetically modified organisms (GMO) may be used to vaccinate humans or domesticated animals. Medicinal products are regulated in Norway in accordance with the medical product regulation, the Regulation (EC) 726/2004 and are assessed in a centralized procedure in EU. In addition, for medical products containing or consisting of GMO it is required to submit an environmental risk assessment (ERA) according to Directive 2001/18/EC, regulating environmental release of living GMO. The Norwegian Environment Agency are responsible for the environmental risk assessment according to this directive in Norway. Our comments concerning environmental risk of the medicinal products under Directive 2001/18/EC, are submitted to the European Medicines Agency (EMA) during the centralized procedure.

Today, only few such medical products are authorized in EU, however, several products are under development. Most of the medicinal products developed until now, are genetically modified virus for vaccination of human or domesticated animals. The Norwegian Environmental Agency consider it necessary to achieve more knowledge about virus that are often used in such vaccines, including the area and amount of use. It is necessary to have knowledge about the environment where such medicines are intended to be used. Therefore, the Environmental Agency request an overview of relevant literature and an assessment of whether there are particular environmental risk factors that we particularly should focus on under Norwegian conditions. This report will provide a background for future environmental risk assessment of veterinary medicinal products containing or consisting of GMO for use in Norway.

Terms of reference as provided by the Norwegian Environment Agency

The Norwegian Environment Agency requests VKM to;

1. provide an overview and short description of genetically modifies virus vectors used as vaccine in veterinarian medicines

2. Summarize available information of relevance for environmental risk assessment of genetically modified virus vaccines used as veterinarian medicines

3. Identify environmental risk factors and possible knowledge gaps of special relevance for the assessment of use of genetically modified virus vaccines under Norwegian conditions.

This report should be based on published scientific literature and other relevant information. The focus should primarily be on Norwegian condition, but if relevant other geographical areas may be included. Domesticated animals in this report should include livestock, fish and pets. The environmental risk factors discussed in this report should be based on the requirements of Directive 2001/18/EU and of relevant guidelines from the European Medicines Agency (EMA).

A particular product should not be discussed in this report but rather include general issues of importance for environmental risk assessment of genetically modified virus vaccines used in veterinarian medicine. This report should not include a discussion of issues related to risk to human health by the use of veterinarian medicines. This report should not include inactivated vaccines. Several medical products contain proteins produced by use of genetically modified micro- or macroorganisms or cultured cells. Such products are not part of this assignment, as they do not consist of GMO. Finally, the report should not include any assessment of the legal framework.

Assessment

1 Literature

1.1 Background literature provided by the Norwegian Environment Agency

Legislations
- Regulation 726/2004/EC
- Legemiddelforskriften (f.18.12.2009 nr.1839)
- Directive 2001/18/EC

Guidance documents from the European Medicines Agency (EMA)
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003806.pdf
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003805.pdf
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/08/WC500095721.pdf
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004590.pdf
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/06/WC500187744.pdf
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002680.pdf
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500003989.pdf
- http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003964.pdf

Relevant reports

- http://www.cogem.net/index.cfm/en/publications/publicatie/gm-vaccines-from-bench-to-bedside
- http://genok.no/wp-content/uploads/2015/11/16102015_Uncertainties_and_Knowledge_ gaps%20related_to_Environmetal_Risk_Assessment_of_GMOs.pdf
- http://genok.no/wp-content/uploads/2014/06/Climate_and_Virus_final_2102141.pdf
- Methodology for environmental risk assessments in medical and veterinary biotechnology, COGEM Report: CGM 2012-04
- Review of the environmental risks from marketing GM veterinary and human medicines, 2008 ATKINS

1.2 Literature searches

A review of genetically modified veterinary vaccine vectors (GM-VV) currently in use, GM-VV for experimental release (field trials), and GM-VV that are still under research but with great promise was conducted. For GM-VV that are currently in use, i.e. those in the market, information was derived from

official homepages of various agencies that are responsible for licensing of livestock vaccines; see References for links to the homepages.

The scope of the search for GM-VV currently in use was limited to the European Union (EU), the United States of America (USA), United Kingdom (UK), Canada, Australia, New Zealand and Brazil, as these are countries in the forefront of the use of GM virus vaccines for vaccination of domesticated animals. Thus, it was reasonable to assume that the GM-VV approved for use in these countries/regions would constitute the bulk of GM-VV currently in the market place. Additionally, information from homepages of Centre for Disease Control (CDC), Institute for International Cooperation in Animal Biologics, The World Organization for Animal Health (OIE), and International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products (VICH) were analysed to capture data that might be missing from the regional/countries homepages.

Regarding GM-VV that have undergone field trials, information was derived from the European Commission's homepage for deliberate release into the environment of other than plant GMOs, for any other purpose than placing on the market (experimental releases) - http://gmoinfo.jrc.ec.europa.eu/gmo_browse.aspx. The website provides details of GM-VV that have been deliberately released for experimental purposes (field trials) in the EU (including Norway and Iceland) after October 2002.

In order not to exclude experimentally tested but yet-to-be released GM-VV, PubMed and Vaxvec (http://www.violinet.org/vaxvec/), a vaccine vector database that stores information on various recombinant vaccine vectors and those experimentally verified vaccines that use these vectors were also queried.

Search results were analysed for those that were of relevance. Each working group member performed relevance screening independently. The reference lists in selected citations were further assessed to identify additional articles that were not identified by the initial searches.

2 Introduction

2.1 GM vaccines: motivation for GM virus vaccines compared to other vaccines

Vaccination is probably one of the most cost effective medical interventions in animal as well as human health. The complete eradication of cattle rinderpest in 2011 and smallpox in 1977 through vaccination programs is testament to the efficiency of this intervention (D'Amelio et al., 2015; de Swart et al., 2012). Extensive vaccination programs have reduced mortality and morbidity from many diseases of farmed, companion and sport animals.

Genetically modified (GM) vaccines derived from virus or virus-vectors of heterologous disease antigens are considered the gold standard for vaccinations against diseases that are difficult to treat or for which there exists no effective conventional vaccine. In domesticated animals, the fatal rabies disease is being successfully managed using GM-virus vectored vaccine. Viruses as vaccine vectors have the advantages that they can stimulate humoral, cellular and mucosal immune responses in the vaccinated hosts. They may also increase herd immunity by spread from vaccinated to naïve animals i.e. inadvertent vaccination (Graham et al., 2013).

Viral vaccines are divided into two main types: live or inactivated. Inactivated viral vaccines are based on whole or subcomponents (antigenic viral component, e.g. glycoprotein, proteins or peptides) of disease-causing viruses and are incapable of replication because they have been treated with heat, formalin or detergents. Live viral vaccines, which is the focus of this review, can further be grouped into replication defective (undergoes partial lifecycle) or replication competent (undergoes full lifecycle) in the host. The vaccine or vaccine vector, which for the purpose of this review is a virus, is termed genetically modified if recombinant gene technology has been used to create the vaccine or vector.

Both replication defective and replication competent viral vectors can infect, deliver and eventually express heterologous genes (transgenes) in infected host. However, replication defective, unlike replication competent virus vectors, do not produce infective progeny viruses because they do not undergo full replication cycle in the host. Thus, an attenuated live viral vaccine holds the capacity to undergo partial or complete lifecycle in the host after administration. This way, it establishes a mild form of infection and induces protective immune response against subsequent infections with the wild type variants of the same virus (Knipe and Howley, 2013), or protection against the heterologous disease depending on the inserted transgene.

Attenuation of the vaccine strains can be by use of recombinant gene technology to remove sequences responsible for hazardous properties, while keeping sequences important for gene delivery, functionality and effectiveness. As stated earlier, this results in a GM vaccine/vector. Other examples of attenuation, but which are not considered to be genetic modification include spontaneous mutations giving rise to naturally occurring vaccine strains (e.g. type 2 polio, adenovirus 4/7), laboratory selection by cultures in cells of unnatural hosts thereby inducing attenuating mutations(e.g. modified vaccinia virus Ankara (MVA) (Meyer et al., 1991).

In general, inactivated virus vaccines are considered safer than live virus vaccines because they contain no infectious material, but often provide less protective immunity mainly due to lack of an efficient CD8 CTL response (generation of cytotoxic T-cells with capacity to destroy virus infected host cells). The superior immunogenicity of live viral vaccines is a main driver for development of new vaccines based on GMO technology, even if risk may be higher. However, history holds several examples where vaccination with inactivated viral vaccines may augment disease at a later stage. One example is accentuated measles pneumonia in individuals previously immunized with an inactivated measles vaccine (Fulginiti et al., 1967). The explanation was later found to be inability of formalin fixed measles F-protein to induce hemolysis-inhibiting Abs, resulting in more severe disease when neutralizing anti HA antibodies had waned (Norrby et al., 1975). Similar potentiation of disease has been described for inactivated respiratory syncytial virus vaccine (Kim et al., 1969). Live viral vaccine, although more efficient than inactivated vaccines may inadvertently infect and cause unintended effect(s) in naïve animals. In addition, reversion to pathogenic forms may occur during production or in recipient animals.

Another type of viral vaccine introduced in recent years is the so-called virus like particle (VLP) vaccines. These are self-assembling viral capsid proteins expressed in yeast or insect cells with a native viral structure, but without an encapsulated viral genome, in many ways resembling defective genome-less sub-virion particles produced by many viruses during cell culture and natural infections (Whistler et al., 1996). Virus-like particles require adjuvants for delivery into the host cells and are formulated with agonists for pattern recognition receptors like TLR. Presently, VLPs are not classified as GM vaccines. Similarly, DNA vaccination based on bacteria-derived plasmid fall outside GM vaccines since plasmids are not organisms; thus, both VLP and plasmid vectors are not within the scope of this report.

2.2 Need/requirement for ERA

Vaccination of domesticated animals with GM vaccines including virus-derived GM veterinary vector vaccines (GM-VVs) - the virus may serve as a vector or in itself constitute the vaccine - is considered

deliberate release of GMOs into the environment. There are concerns over the environmental and health impacts of such deliberate release. Sources of concern include: GM virus vaccine/vector can be shed into the environment via the excreta of the GM-vaccinated animal; recombination between the vaccine vectors and resident wildtype as well as with related viruses in the environment leading to spread and dissemination of foreign genetic materials in the ecosystem; inadvertent transmission to naïve unvaccinated non-target animals as well as humans; may cause unwanted diseases in the non-target hosts. Such concerns motivate the need for risk assessments of GM vaccines before being placed on the market or approved for clinical studies. A robust ERA of GM-VVs requires knowledge of the characteristics of the GM virus (and its parental wild type), the environment of intended use as well as scope of use. The aim of an ERA of a deliberate release of a GMO is to identify and evaluate potential adverse effects on non-target animals, and the environment at large including human health under the conditions of the release, on a case-by-case basis (Bublot et al., 2010).

2.3 Short description of ERA and how it is performed

The placement in the market or clinical trials of GMOs, both for veterinary (in this case GM-VVs) and human use may be considered a deliberate release. In the EU this is governed by Directive 2001/18/EC and Regulation 726/2004/EU. Both documents stipulate that an ERA of a GMO is conducted before authorization for a deliberate release into the environment. The risk assessment methodology is similar in many legislative systems and comprises the following sequential steps: (1) hazard identification, (2) hazard characterization, (3) assessment of likelihood, (4) risk estimation, (5) evaluation of risk management options, (6) estimation of overall risk taking into consideration step 5 (Bublot et al., 2010; EC, 2006; EMEA, 2004; EMEA, 2005).

The ERA does not take the vaccinated animal into account, but takes into consideration the hazards such vaccination may constitute to non-target unvaccinated domesticated animals and the environment at large. The environment, according to the directive, constitutes the ecosystem including unvaccinated animals, humans, plants and microorganisms. The ERA also takes into account that the exposure of unvaccinated livestock and the environment will usually be significantly lower than the exposure animal. The ERA consists of identifying the characteristics of the GMO and its use, which have the potential to cause adverse effects for non-recipient animal and the environment. Thus, the ERA consists of identification and characterization of potential hazards and their probability of occurrence. In estimating the risks, presence of a live GM virus vector in a vaccine in itself does not always pose a risk to the environment or human health, but will depend on the characteristics of the vector, the intended application of the vaccine and the effectiveness of any management strategies that are applied. For example, a hazard such as the adverse

response of the recipient to vector (vector-induced immune response) is relevant as it is linked to the presence of the vector, rather than the expression of the transgenes (Atkins, 2008).

2.4 Objective & approach of this report

The objectives of the Report were (i) to provide an overview of the vectors used in GM-VVs and their areas of application emphasizing the Norwegian/EU context, and (ii) to identify knowledge gaps of relevance to the ERA of GM-VVs within Norwegian and European context. The scope of the study was limited to GM virus-derived vaccine vectors used in vaccination of domesticated animals, including GM-VV applied in the vaccination of diseases that are not of virus aetiology.

Virus-like particles and plasmid DNA are not regarded as GM vaccines. Neither are inactivated vaccines that contain proteins and other products that are produced with the aid of GM viruses/vectors, but do not contain the GM virus/vector as a part of the vaccine. Descriptions of the GM-VVs were not limited to those used only in Norway and the EU, but in terms of identification of potential risks, the Norwegian and European environments were emphasized. Domesticated animals is the focus veterinary animals in this report, thus, GM-VVs only used in vaccination of domesticated animals are covered. Wildlife and humans are, however, potential non-targets that are at risk of exposure to GM-VVs when applied to livestock, they are therefore briefly highlighted where necessary.

3 Overview of GM-virus based veterinary vaccine vectors

3.1 Viruses/vectors relevant to veterinary GM-vaccines

Table 1 summarizes the common virus vectors that are in use in veterinary GM vaccines. The table focuses on vectors that have been used in licensed vaccines or as a minimum applied in field trials. This means that the vectored vaccines have at least been tested in the target animal species. Therefore, vectors that have only been tested in cell lines or experimental animals that are not the target animal species were not included.

Table 1: Common vectors employed in veterinary GM virus-derived vaccines and diseases against which the vaccines are used

Virus Vector	Target Disease (Animal)	Reference
Herpes virus vectors		
-Herpes virus of Turkey vector	Bursal disease, Marek's disease, Newcastle disease, Laryngotracheitis virus, Avian influenza (Chicken)	(Esaki, 2013); (Esaki et al., 2013); (Soejoedono et al., 2012); (Tsukamoto et al., 2000)
-Cyprinid Herpes virus vector	Cyprinid Herpesvirus Type 3 disease of fish (Common and Koi Carp)	(Michel et al., 2010);(Agriculture)
-Feline Herpes virus vector	Feline immunodeficiency virus (Cat)	(Wardley et al., 1992)
-Bovine Herpes virus vector	Caprine herpesvirus type 1 induced genital disease (Goat); Bovine Diarrhea (Cattle)	(Donofrio et al., 2013); (Kweon et al., 1999)
Adenovirus vectors		
-Turkey adenovirus vector	Avian influenza (Chicken)	(van Ginkel et al., 2009)
-Porcine adenovirus vector	Pneumoniae (Hyopneumoniae –caused by Mycoplasma); Classical Swine Fever (Pig)	(Okamba et al., 2010); (Hammond et al., 2003)
-Canine adenovirus vector	Canine distemper (Dog)	(Fischer et al., 2002)
-Human Adenovirus serotype 5 (HAd5)	Rabies (Dog, Fox); Porcine respiratory and reproductive syndrome, Porcine foot and mouth disease, Swine influenza (Pig); Rinderpest (Cattle)	(Ferreira et al., 2005);(Gagnon et al., 2003);
Poxvirus vectors		

Virus Vector	Target Disease (Animal)	Reference
-Vaccinia virus vector	Rabies (Dog, Cat)	(Hermann et al., 2011); (Blancou et al., 1989)
-Modified Vaccinia virus Ankara (MVA) vector	Canine Leishmaniasis (Dog)	(Carson et al., 2009; Carson et al., 2010)
-NYVAC vaccine vector	Distemper (Ferret, Dog); Equine Rhinopneumonitis (Horse); Pseudorabies (Pig)	(Paillot et al., 2006), (Brockmeier and Mengeling, 1996)
-Fowlpox virus vector	Avian Encephalomyelitis, Infectious Laryngotracheits, Fowlpox, Infectious Bursal Disease (Chicken); Newcastle disease, Mycoplasma gallisepticum (Chicken, Turkey); Canine Distemper (Ferret)	(Butter et al., 2013);(Bublot et al., 2010);(Jones et al., 1997); (Boursnell et al., 1990)
-Canarypox virus vector	Feline leukemia, Feline rabies (Cat); Equine influenza, Equine Encephalomyelitis/West Nile disease, African Horse sickness (Horse); Distemper (Ferret, Dog); Parvovirosis, Coronavirus, Adenovirus types 1 and 2, Parainfluenza, (Dog); Bluetongue (Sheep); Rabbit hemorrhagic fever (Rabbit); West Nile Disease (Cat, Dog)	(Guthrie et al., 2009); (Welter et al., 2000);{(Boone et al., 2007);(Karaca et al., 2005);(Jas et al., 2012)
-Raccoonpox virus vector	Rabies (Dog,Cat); Plagues (Dog)	(Tripp et al., 2015); (Osorio et al., 2003)
-Capripoxvirus vector	Bluetongue disease, Sheep pox & Goat pox (Sheep, Goat); Lumpy Skin (Cattle)	(Perrin et al., 2007); (Davies and Mbugwa, 1985);(Kitching, 2003);(Hunter and Wallace, 2001)
-Swinepox virus vector	Swinepox, Swine Influenza (Pigs)	(Xu et al., 2012)
-Myxoma virus vector	Rabbit hemorrhagic disease (Rabbit); Bluetongue disease (Sheep)	(Top et al., 2012)
Other virus vectors		
-Bovine diarrhea virus vector	Classical swine fever (Pig)	(Leifera, 2009)
-Baculovirus vector	Porcine Circovirus Type 2 (Pig); Infectious Bronchitis (Chicken); Avian influenza (Ferret)	(Zhang et al., 2014); (Tretyakova et al., 2013)
-Vesicular stomatitis virus vector	Avian influenza (Chicken)	(Halbherr et al., 2013)
-Rous Sarcoma virus vector	Avian influenza (Chicken)	(Hunt et al., 1988)
-Newcastle disease virus vector	Avian influenza (Chicken, Turkey); Avian influenza (Duck); Avian influenza, Newcastle disease (Chicken)	(Schroer et al., 2011); (Ferreira et al., 2014); (Ferreira et al., 2012)
-Duck entiritis virus vector	Avian influenza (Duck)	(Liu et al., 2013)
-Avian paramyxovirus vector	Newcastle disease (Chicken)	(Kumar et al., 2011)

Virus Vector	Target Disease (Animal)	Reference
-Pseudorabies virus vector	Influenza, Classical swine fever (Pig)	(Klingbeil et al., 2014);(Sun et al., 2013)

The development and use of live virus vectors in veterinary medicine is generally based on the same principle as in human medicine. Consequently, some of the vectors applied in human medicine, including vectors derived from Vaccinia virus, Modified Vaccinia virus Ankara (MVA), Herpes viruses (excluding Herpes Simplex virus that infects human), Adenoviruses, Canarypox virus and Baculovirus find application in veterinary medicine. Differences however exist in the types of modifications, including antigenic transgenes, used in a particular veterinary vaccine construct. Table 1 shows, in order of frequency of use, that Poxviruses, Herpes viruses and Adenoviruses are the most commonly applied vectors in veterinary GM vaccines.

3.1.1 Poxvirus

Poxviruses are used as live virus vectors to vaccinate different domesticated animal species (e.g. dog, cat, cattle, chicken; Table 1) and wildlife against different diseases. An example is the rabies vaccine (RABORAL Vaccinia rabies glycoprotein;V-RG), which uses the Copenhagen strain of the Vaccinia virus as a vector in which the thymidine kinase gene (that is essential for DNA synthesis) was replaced by the glycoprotein gene from the Evelyn-Rokitnicki-Abelseth rabies virus strain (Hermann et al., 2011). Vaccinia virus is the parental virus for Modified Vaccinia virus Ankara (MVA) and Vaccinia virus vaccine vector (NYVAC), both of which as vectors provide excellent platforms for GM-VV against several heterologous diseases of livestock. MVA and NYVAC were respectively derived from the Ankara and Copenhagen strains of Vaccinia. Both MVA and NYVAC are replication defective and thus attenuated and safer compared to the replication competent parental Vaccinia. MVA was derived by serial passages in an unnatural host (chick embryo fibroblast) where several point and deletion mutations resulted in its host range restriction and defective replication characteristics in most mammalian cells (Meyer et al., 1991). NYVAC was attenuated by precise deletion of 18 open reading frames from the genome of the Copenhagen vaccine strain, leading to its attenuation phenotype, including host range restriction and defective replication in mammalian cells (Tartaglia et al., 1992). Unlike NYVAC, the mutations resulting in MVA's attenuation was not precise, as such the molecular basis for the attenuation phenotype is not completely characterised. Nonetheless, MVA together with NYVAC serve as vector platforms for the construction of GM vaccines for different diseases of both livestock and human.

Canarypox virus (ALVAC) and fowlpox virus (FWPV) are other examples of poxviruses commonly employed as veterinary vaccine vectors. They belong to the Avipoxvirus genus; Avipoxviruses cause diseases in domesticated pet and wild birds of many species. ALVAC and FWPV serve as the most important vaccine vectors for immunization against several types of avian diseases. Both ALVAC and FWPV can also infect mammalian cells, but replication in these cells is abortive. This is a relevant characteristic of the two viruses as vector platforms for diseases that plague mammalian livestock in that it reduces the chance that human and mammalian livestock can inadvertently be infected. Canarypox in particular finds wide application in vaccines used to vaccinate feline, canine, equine and sheep (Table 1). Although defective replication in mammalian cells confers a major safety advantage to ALVAC and FWPV vaccine vectors, the molecular basis for the restricted replication is yet not fully understood (Weli and Tryland, 2011). Other examples of poxviruses that have found application in virus-based GM veterinary vaccines include Raccoonpox virus (Genus: orthopoxvirus; Sub-family: Chordopoxvirinae) that has raccoon as natural host; Capripoxvirus (Sub-family: Chordopoxvirinae); with sheep, goat and cattle as natural hosts; and Swinepox virus (Genus: Suispox, Sub-family: Chordopoxvirinae) that infects pigs.

3.1.2 Adenovirus

Animal adenoviruses are typically species specific, and as vaccine vectors, present negligible risks to other animals or human. Two examples of animal adenovirus vectors are Turkey adenovirus and Porcine adenovirus vectors used for vaccination against Avian influenza, Canine distemper and *Mycoplasma hyopneumoniae* in chicken, cats and pigs (Fischer et al., 2002; Okamba et al., 2010; van Ginkel et al., 2009). Human adenoviruses have also served as vectors for veterinary GM vaccines. The human adenovirus (HAd) mainly used in this respect is the serotype 5 (HAd5). The HAd5 has been used in the development of vaccines against several types of animal diseases including rabies, porcine respiratory and reproductive syndrome, foot and mouth disease of pig, swine influenza and rinderpest (Ferreira et al., 2005; Gagnon et al., 2003).

3.1.3 Herpesvirus

Similar to adenoviruses, herpes viruses are species specific. An example is the Cyprinid Herpes virus 3 (CyHV-3) that causes an emerging and mortal disease in the common and Koi Carp fish (*Cyprinus carpio*) (Waltzek et al., 2005). *Cyprinus carpio* is the only fish species in which the virus causes disease (Michel et al., 2010), thus, the vaccine vector presents negligible risk to other non-target fish species. CyHV-3 has been successfully developed as a vaccine vector for the vaccination of common and Koi Carp fish against CyHV-3 disease. Some other examples of herpes viruses that have served as platforms for species specific

GM vaccines include herpes virus of turkey, feline herpesvirus and bovine herpesvirus (Table 1). Unlike the human adenovirus, the human herpes viruses (herpes simplex virus 1 & 2) have not found applications in veterinary GM vaccines.

3.2 Common modifications in GM virus vectored vaccines:

Recombinant gene technology has been used to modify viruses into GM vaccine vectors by deletion/truncation of parts or whole genes, or by insertion mutation, i.e. where an immunogenic gene foreign to the virus (transgene) is inserted into the virus vector. In both strategies, the viruses must be attenuated by the precise removal of undesirable genes - genes responsible for virulence and replication are common targets for removal. Other genetic manipulations include placement of the transgene(s) under the control of strong promoters, e.g., the cauliflower mosaic virus, the vaccinia virus or a synthetic promoter. This ensures high expression of the transgene(s). In the insertion mutation strategy, it is common practice to replace the undesirable genes with the transgene(s) to ensure that the vector does not revert to the wild-type genotype through recombination. Also, non-essential genes can often be replaced by markers for monitoring of unintended vaccine virus spread – presence of these markers from a field isolate will indicate that the isolate is a virus vector used in vaccination. For replication defective vaccine vectors, double mutations in replication essential genes are additionally employed to avoid reversion to replication competent strains.

Insertion mutation strategies (i.e. transgenesis) used in veterinary vaccines are numerous. The Canarypox virus is the most common vaccine vector employed in the delivery and expression of immunogenic transgenes for vaccination of several animals against heterologous diseases. An example, ALVAC expressing the canine distemper virus (CDV) hemagglutinin (H) and fusion (F) proteins, which induces neutralizing antibodies and protection of ferrets against canine distemper (Welter et al., 2000). Other ALVAC examples include protective immunization of horses with ALVAC co-expressing synthetic genes encoding the outer capsid proteins, VP2 and VP5 of African horse sickness virus (Guthrie et al., 2009) and bluetongue disease virus (Boone et al., 2007) used in the vaccination of horse and sheep respectively. An ALVAC vaccine, ALVAC-FL, which carries the feline leukemia virus subgroup A *env* and *gag* genes is also being used to protect cats against leukemia (Tartaglia et al., 1993) (Table 1). Other examples of ALVAC vaccines used in veterinary medicine can be found in the review by Weli and Tryland (2011).

Insertion of genes from different avian disease-causing viruses into the genome of FWPV and herpes virus of turkey (HVT) is also a successful strategy in veterinary GM virus vector construction mostly for poultry vaccination (Table 1), although FWPV has also found application in the vaccination of ferrets against canine

distemper (Jones et al., 1997). Examples include the modification of HVT to express the antigenic protein, VP2, of infection bursal disease (IBD) virus (Tsukamoto et al., 2000), glycoprotein B of infectious laryngotracheitis virus (Esaki, 2013), fusion protein of Newcastle disease (Esaki et al., 2013), hemagglutinin protein of avian influenza (Soejoedono et al., 2012).

Remarkable examples of the use of adenoviruses as GM vaccine vectors exist. Animal adenoviruses are specie specific, and several vaccine vectors derived from different animal adenoviruses are at advanced field trials (Ferreira et al., 2005). With regard to non-target effect, adenovirus-derived vaccine vectors will pose negligible risks to humans or other animal species (Tuboly and Nagy, 2001). Several vaccine vectors derived from human adenovirus serotype 5 (HAd5) have also been tried successfully as veterinary vaccines against canine, bovine and swine diseases. Although HAd5 is one of the most efficient vector systems for the delivery of vaccine antigens - being able to induce both humoral and mucosal immunity in vaccinated animals, their application is still limited due to the potential risks this may pose to humans (Ferreira et al., 2005).

Viruses have also been genetically modified to serve as vaccines or vaccine vectors by the precise removal (deletion or truncation) of undesirable genes from the viral genome using recombinant technology. These gene-deleted viruses are used for homologous vaccination, i.e. to achieve protective immunity against the parental virus. For example, deletion of either the phosphoprotein (P) gene or the matrix (M) gene of rabies virus, which are required by the virus for effective replication in the host, renders the virus replication deficient and unable to spread into the central nervous system of the host animal. This strategy has been successfully used in vaccination of animals against rabies (Cenna et al., 2009). Removal of the thymidine kinase (TK) gene, which in infectious laryngotracheitis virus (ILTV) is essential for virulence (but not replication), causes attenuation without impairing replication and immunogenicity of the virus (Han et al., 2002). The recombinant ILT vaccine is popularly used in the vaccination of chicken against infectious laryngotracheitis. Recombinant GM virus vaccine vectors such as ILTV and the P/M-deleted rabies virus that are based on deletion mutants and which do not contain foreign genome, are considered GMOs because the modifications were achieved through techniques of recombinant technology.

NYVAC and MVA are also deletion mutants. In NYVAC, the K1L, C7L (host range genes), N1L and C3L (virulence genes) were precisely deleted resulting in replication deficiency in most mammalian and avian cells as well as virulence attenuation in these hosts (Tartaglia et al., 1992). Similarly, deletions and multiple truncations in genes responsible for replication and virulence in most mammalian cells resulted in defective replication and attenuation of MVA (Meyer et al., 1991). The host range restriction, virulence attenuation and history of safe use (the parental Vaccinia virus and MVA were used in the eradication of Smallpox) are

some advantages of MVA and NYVAC as platforms for the construction of vaccine vectors for the vaccination of a wide range of animals against several heterologous diseases (Quinan et al., 2014). However, the lack of clarification on the precise factors behind MVA attenuation is a major drawback in terms of its safety as a vaccine vector.

4 Potential areas of use of GM –VVs in Norway and possible routes of environmental exposure

4.1 General description

The motivation for GM-VVs for farm and sport animals is generally based on animal health requirements, but public health consideration has also driven the use of GM-VV in the vaccination of reservoirs of diseases, e.g. the famous RABORAL Vaccinia rabies glycoprotein; V-RG, used in the vaccination of wild foxes in Europe aimed at rabies control. On the other hand, GM-VV vaccination of companion animals such as cat and dog is driven by both compassion, commercial and public health interest. Diseases for which GM-VVs are used are those for which there exist no effective conventional vaccines. Common diseases against which GM-VVs are commonly applied are listed in Table 1. The most successful intervention is diseases of poultry origin, where 33% are related to commercial important poultry diseases (Butter et al., 2013; Cliquet et al., 2013; Esaki et al., 2013; Gutierrez et al., 2013; Gutierrez et al., 2014; Hackl et al., 2015; Halbherr et al., 2013; Knipe and Howley, 2013; Singleton et al., 2013; Thomas and Versteeg, 2013; Tretyakova et al., 2013; WHO, 2013) are managed by GM-VVs.

In Norway, the domesticated animals of commercial importance include fish, poultry, cattle, sheep, goats, pigs and reindeers. Sports and companion animals, e.g. horses, cats and dogs are also important in Norway due to the significant commercial activities related to their health and care. This section will evaluate possible routes of environmental exposure should GM-VVs be applied in the vaccination of these animals in Norway.

The degree to which a GMO is spread from domesticated animals to the environment is heavily influenced by the way the animals are kept and managed.

Important points to consider are:

- degree and occurrence of direct contact between the actual species and other domesticated and wild species, both in the premises the animals are kept, where the animals are kept within fences and where they roam freely.

- water flow freely through open sea-pens, facilitating transport of microorganisms and parasites to and from the fish inside the pens.

- contact between herds of the same species, both at farm and on pastures

- exposure to ectoparasites as crustaceans (on fish), insects and mites (among them ticks) that may transmit infectious organisms

- to which degree the animals are transported, for example between juvenile and adult production sites (fish), farms and pastures (ruminants), between racetracks (horses), with their owners (pets) and from the farm to the slaughtering facility. Transport of animals reduces the control of spread of disease and may cause spillover of an infectious organism to hosts that not are taken into account.

- treatment and spread of manure, bedding and spillage and how this get in contact with wildlife

- run-off from pastures and areas where manure, bedding and spillage are spread to lakes, streams, seas and the sea floor.

- disposal and treatment of carcasses and other by-products and contact with these and scavengers and carnivores

- usage of untreated products and by-products from the animals

4.2 Domesticated animal production

GM vaccines are currently not authorised for use in Norway, but domesticated animal production as well as health care and sustenance of companion and sports animals, are challenged by a variety of infectious diseases. These diseases cause animal suffering as well as economic loss. This section highlights these challenges, and describes the management of various diseases that plague domesticated animal production, including companion and sports animals. Table 2 shows the total dose of conventional vaccines used in domesticated animal production for the year 2015. The huge total dose of the various vaccines used in 2015 alone indicates that there exists a significant burden on the management and control of the diseases using conventional vaccines. Information on the effectiveness of the conventional vaccines in the diseases management in Norway was not available at the time of writing this report. However, increased demand is expected for authorization of GM vaccines for domesticated animal vaccination. Especially GM

vaccines for diseases of poultry and fish, given that the demand for poultry and fish vaccines in Norway is enormous (Table 2).

Table 2: Total number of vaccine doses sold in Norway for veterinary use in 2015 (source Reseptregisteret, FHI)

Species	Vaccine doses in 2015
Cat	111143
Cattle	178545
Dog	342181
Fish	338786500
Horse	48032
Mink	3978152
Poultry	20256050
Sheep	662590
Pig	746235
Total	**365109428**

4.2.1 Fish

Norwegian aquaculture is dominated by Atlantic salmon farming and about 1000 farms for large fish production and 280 for smolt (1/4 are with recirculated water, ¾ with flow-through) were in operation in 2015 (Veterinærinstituttet, 2015). The production cycle for a marketable salmon takes about 2½ years and proceeds through an initial phase (12 months) in freshwater (from hatch to about 100 g size) followed by approximately 18 months in seawater where the fish grows to a size of 3-6 kg. Cage culture in places with sufficient ocean currents is the dominant form as this ensures adequate supply of clean water and removal of metabolic waste from the farm. However, this means that the farmed animals are in open contact with their environment, representing a risk of disease transmission both ways. In addition to salmon, Norwegian fish farms also produce rainbow trout, cod, halibut, arctic char and turbot, albeit at much smaller volumes. General environmental risk assessment of using GM vaccines in these species will be similar so we focus this discussion on Atlantic salmon (*Salmo salar*). Smolts are produced at about 280 sites spread mainly along the west coast and up to northern Norway. Before smoltification and transfer to their sea cages, all animals (about 300 million fish) are mandatorily vaccinated by intraperitioneal injection of a combination vaccine containing 3 inactivated bacterial antigens (*Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio salmonicida*). In addition, dependent on risk and location, farmers can choose to vaccinate against bacterial disease caused by *Moritella* or *Yersinia* or viral disease caused by salmon pancreas disease virus (SPDV), infectious pancreatic disease virus (IPNV) or infectious salmon anemia virus (ISAV) (Veterinærinstituttet,

2015). In most cases these are combined into the mandatory vaccines resulting in up to 6-7 antigens in one vaccine. In general, the bacterial vaccines give good protection and have contributed to the strong reduction in the use of antibiotics the last 20 years. The effect of vaccination against viral infections in salmon is less well documented. Although the frequency of infections caused by IPNV have declined the last years this can partly be explained by successful breeding programs selecting for resistance (Moen et al., 2015). In addition to these microbial infections, important infections are caused by two parasitic copepods: the salmon sea lice (*Lepeophtheirus salmonis*) and (*Caligus elongatus*). The former is a main cause of production loss in salmon farming today.

Infectious disease in fish farming can spread through seawater, via wild fish and via transport of smolt and large fish in well boats with flow through systems. There are also reports of vertical transmission from breeding stocks (Nylund et al., 2007). Many hygienic measures have been implemented to control spread of disease; health controls in smolt producing farms, increased use of water recirculation in smolt production, disinfection of effluents from slaughtering, spatial and temporal separation of different year classes, isolation and fallowing of infected sites. These measures have reduced the incidence of some viruses (notably ISAV), but infectious disease still remains a serious problem in salmon farming (Woo and Gregory, 2014).

4.2.2 Cattle

In 2015 there were 228 399 dairy cows and 71 363 suckler cows spread on 8 889 and 4 851 herds, respectively (provisional numbers from (Statistics Norway, 2016). Average herd size is consequently 25 per dairy and 14.7 per beef herd. Calves and bulls are not included in these numbers. Most dairy farms are located in the counties of Oppland, Rogaland, Sogn og Fjordane, Møre og Romsdal and Nord- and Sør-Trøndelag, while farms with beef production are most common in Rogaland, Oppland, Nord-Trøndelag, Hedmark, Hordaland and Sør-Trøndelag. Number of dairy cows has declined with 13.9 percent from 2006 to 2015, while there has been an increase on 29.8 percent in number of suckler cows in the same period. Both dairy and beef cattle are housed in barns much of the year. The level of containment is however low, and insect vectors, small birds and mammals will most often have access to the stables. According to Norwegian legislation (Forskrift om hold av storfe, 2004), cattle shall be given access to free motion at least 8 weeks of the year. The animals will most often be given access to fenced pastures near the farm, but in some areas, use of forest or mountain pastures where the cattle roam freely is common. To relieve the workload on the farms, many dairy cattle graze on "common pastures" during the summer months. Here herds from different farms are kept and managed together.

Cattle, which are traded, are transported between farms. It is common however, that these are followed by health certificates that testifies that the animals are free of clinical disease, and trade between the different regions (North, Middle, West and East) of Norway is not recommended.

There are relatively few slaughterhouses in Norway. Consequently, cattle may be transported over long distances before slaughter. By-products from the slaughter process not intended for human consumption are in some abattoirs utilized in production of pet or fur animal food. Other by-products are transported to approved facilities for destruction.

Manure, bedding and spillage from housed cattle is kept in manure pits or yards until spring and used as fertilizer and soil conditioner on acres and fields. Cattle carcasses from animals that have died on the farm or on pasture are according to the legislation (Forskrift om animalske biprodukter som ikke er beregnet på konsum, 2007) treated as animal by-products and has to be transported to an approved facility for destruction.

There are few contagious diseases that are prevalent among cattle in Norway (Årsmelding Helsetjenesten for storfe, 2014). However, sporadic outbreaks of winter dysentery (bovine coronavirus) and bovine respiratory syncytial virus have been observed. There are also sporadic outbreaks of bovine ringworm, occasional cases of *Salmonella* enteritis and rare cases of paratuberculosis (*Mycobacterium avium* var. *paratuberculosis*) (Årsmelding Helsetjenesten for storfe, 2014). Calves often suffer from opportunistic respiratory infections where several viruses (BRSV, parainfluenza virus type 3; PIV-3) and *Mannheimia haemolytica* play roles.

It is not recommended to routinely vaccinate cattle against any diseases (Felleskatalogen over preparater i veterinærmedisinen, 2014-2015). There are, however, several registered vaccines available:

- Combined vaccines against PIV-3, BRSV and *M. haemolytica*

- Bovine ringworm (*Trichophyton verrucosum*)

- Clostridial diseases (*Clostridium perfringens* type A toxoid, *Clostridium perfringens* type B, *Cl. perfringens* type C toxoid, *Cl. perfringens* type D toxoid, *Cl. chauvoei*, *Cl. haemolyticum*, *Cl. novyi*, *Cl. septicum* toxoid, *Cl. tetani*. *Cl. sordellii*)

- Neonatal diarrhoea (bovint rotavirus, bovint coronavirus and *E. coli* K99/F41) – passive immunisation of newborn calves through vaccination of mother cow

In addition to these vaccines against diseases that are found in Norway, introduction of highly contagious diseases may be met by prophylactic vaccination to prevent further spread of disease. Such diseases may

be foot and mouth disease, bluetongue, Schmallenberg, Contagious bovine pleuropneumonia (caused by *Mycoplasma mycoides* subsp. *mycoides*) and bovine virus diarrhoea (BVD).

4.2.3 Goats & sheep

In 2015 there were 1.056.525 sheep (kept over the winter) and 31.724 dairy goats in Norway. Average herd size was 74 sheep and 108 dairy goats. Each ewe gives on average birth to two lambs (Årsmelding. Sauekontrollen, 2014). Consequently, the number of sheep released on pasture in spring 2016 will be around 3 millions. 20% of the sheep are found in Rogaland, while 10% are found in both Hordaland and Oppland and 9% in Sogn & Fjordane and Nordland. The number of sheep has shown a slight increase recently, but the number has although decreased with about 2% the last decade.

Dairy goats are found in Troms (22%), Sogn & Fjordane (16%), Møre & Romsdal (13%), Oppland (11%) and Nordland (10%). Also the number of goats increased from 2014 to 2015, but has decreased with 30% the last decade.

Sheep and goats are housed in barns on the farm during the winter, but as for cows, the level of containment is minimal.

While some farmers keep their sheep on fenced infield pastures around the farm also in the summer, the majority of sheep are kept at the farm pastures only until the snow has melted in the hills or mountains they use as grazing pastures. Here they are released and roam over large areas, most often without being herded. Farmers frequently use licking stones as a mean to keep the animals within an area. These also cause wild ruminants to congregate, increasing the degree of contact between them and sheep. Loss of lambs on summer pastures is a major problem for the sheep industry, and around 10% of lambs released on summer pasture are lost (Årsmelding. Sauekontrollen, 2014). The losses may be very high (above 25%) in some farms, but this varies a lot between farms and regions. A substantial proportion of the loss is caused by predation by carnivores. The carcasses of sheep that die of other causes on hill/mountain pastures will be eaten by scavengers.

Goats are also released in semi-natural hill or mountain pastures, but as these animals are milked in the morning and evening, they do not roam that far from the summer farm.

Treatment of manure, bedding and spillage and carcasses from animals that die on the farm is as for cattle. On hill- and mountain pastures, manure will naturally be spread all over the used area.

There are few important contagious diseases among sheep and goat in Norway (Veterinærinstituttets faglige aktivitetsrapport, 2014). Maedi-visna in sheep and caprin arthritis-encephalitis (CAE), both caused by related lentiviruses, seem to be under control. There has not been outbreaks of maedi-visna since 2005, and the occurrence of CAE is declining, though one goat and four sheep herds were positive in 2014. The prevalence of paratuberculosis (see above) in goats and caseous lymphadenitis (pseudotuberculosis) caused by *Corynebacterium pseudotuberculosis/ovis* is very low, due to a long-lasting and intensive eradication program ("Friskere geiter"). While enzootic abortion caused by *Chlamydophila abortus* seem to be absent from Norway, *Toxoplasma gondii* may occasionally cause abortion in some herds. Intestinal parasite infection, i.e. coccidia and nematodes (*Nematodirus, Ostertagia, Haemonchus* etc.), constitute major problems for both lambs and adults. Tick-borne fever caused by the bacterium *Anaplasma phagocytophilum* is also a major cause of loss at pasture in coastal districts.

It is common to vaccinate both ewes and lambs with combined vaccines against clostridial diseases and infections with Pasteurellaceae, i.e. *Mannheimia haemolytica* and *Bibersteinia trehalosi* (see above for further description). Ewes are vaccinated well before lambing, to provide maximum antibody transfer to the lambs through the colostrum. Lambs are often vaccinated when they come home from mountain pastures. Vaccination against clostridial diseases is also common in goat herds that have experienced problems. In herds with paratuberculosis, the authorities may instruct the farmer to vaccinate against this disease. Autovaccination has sometimes been used during severe outbreaks of orf (*Echtyma contagiosa*).

In herds experiencing problems, vaccination against *Toxoplasma gondii* may be used to prevent abortion.

A vaccine against paratuberculosis (*Mycobacterium avium* var. *paratuberculosis*) has also been imported to Norway.

Introduction of highly contagious diseases may be met by prophylactic vaccination to prevent further spread of disease. Such diseases may be foot and mouth disease, bluetongue, Schmallenberg, brucellosis, lumpy skin disease (*Capripoxvirus*) and other diseases (see attachment to (Forskrift om varsel og melding om sjukdom hos dyr, 2014)).

Cattle, sheep and goats may share some infectious organisms and parasites with wild cervids (and muskoxen). The population density of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and moose (*Alces alces*) is very high in certain areas. The wild reindeer (*Rangifer tarandus*) populations are not found in such densities, but as this species is a very social herd animal, the contact rate between them and domesticated animals may be very high, when this animal species is established within an area.

4.2.4 Poultry

The number of poultry in Norway has increased the last decades, probably due to increased demand for low-fat meat and protein-rich food. Between 2004 and 2014, there was a 100% increase in broiler meat production volume and a 55% increase in turkey meat production (Husdyrkonsesjon, 2016). However, from 2014 to 2015 the poultry meat production diminished by 13%, probably due to decreased demands associated with much public attention on the issue of vancomycin-resistant microbes in poultry meat.

In 2015, there were over 4.44 million egg-producing hens in the country, in 2.061 farms. Approximately 65.000 tons of eggs were produced. The number of egg-producing farms has increased slightly (2%) lately, but in a ten-year perspective, the number of farms has decreased by 18.5%. In contrast to this, the production has increased by 30% and the number of animals by 24% since 2005. Over a quarter of the egg-hens are concentrated in Rogaland, with the next quarter equally divided between Hedmark and Nord-Trøndelag and just below 10% in Østfold.

In 2014, 73 millions broilers were slaughtered for consumption. This decreased by 14% in 2015, to 63 millions. All broilers are kept on as few as about 677 farms, mostly located in Nord-Trøndelag, Hedmark, Rogaland, Østfold and Sør-Trøndelag.

There are only 61 farms with turkey production in Norway. Most of them are located in Østfold. Production of ducks, geese, guinea fowls and quail is very limited. There was a boom of ostrich production in the nineties, but it seems not many active farms are left.

Both laying hens, broiler hens, chickens and turkeys are mostly housed in purpose-designed modern buildings under strict hygiene and limited admittance for people not involved in the production. Direct contact with wild birds and mammals is generally avoided, but in ecological egg production, the birds will have access to outdoor areas and hence get in contact with sparrows and other Passeriformes, wild gallinaceous birds and waterfowl as gulls and ducks. Chickens in ordinary commercial production are slaughtered at the age of 28 days – at approximately 1.2 kg. In ecological production, the chickens live considerably longer – approximately 80 days. Turkeys are slaughtered at 12 (hens) and 18 (cocks) weeks of age. Thorough cleaning and time lags between "batches" of chickens and turkeys prevent transmission of disease over time within a house.

Backyard poultry, are kept in small herds as a leisure activity. These herds are often a mixture of animals of different breeds and species. The housing of these animals may be provisional and they may be in regular contact with other bird species and mammals.

Infectious diseases are not prevalent in modern commercial Norwegian poultry production. Necrotizing enteritis (*Clostridium perfringens*) is occasionally seen in commercial broiler chickens and turkeys, but is not very prevalent.

There are, however, many contagious diseases of concern, which may be introduced into commercial poultry production – often via backyard herds. Some of these are avian encephalomyelitis (picornavirus), bluewing disease/infectious anaemia (Circovirus), infectious bursitis/Gumboro disease (Birnavirus), infectious laryngotracheitis (Alfa-Herpesvirus 1), infectious bronchitis (Coronavirus), avian rhinotracheitis, egg drop syndrome (Adenovirus), avian reovirus, fowlpox, fowl cholera (*Pasteurella multocida*), *Erysipelothrix rhusiopathiae* and others.

Vaccines against the following diseases has been used in recent years:

- Avian encephalomyelitis (AEV)
- Bluewing disease/infectious anaemia (CAV)
- Gumboro /infectious bursitis (IBDV)
- Marek's disease (Avian herpesvirus 2)
- Coccidiosis (*Eimeria* spp.)
- Pasteurellosis (*Pasteurella multocida*)
- Erysipelas (*Erysipelothrix rhusiopathiae*)

4.2.5 Cats & dogs

The exact number of dogs and cats in Norway is unknown, as there are no public statistics. According to the Norwegian breeding association there are approximately 400.000 dogs in Norway (Norsk Kennel Klub, 2010). Other sources state that there are 500.000 dogs in the country, and 40% of all households have one or more dogs (Veterinærinstituttet). The number of cats is even higher than the number of dogs. Wikipedia states that there are 750.000 in Norway.

Dogs are used in a wide variety of settings, from the farmer's shepherd dog, various hunting dogs, sledge dogs, save- and rescue dogs, military dogs, police dogs, the ordinary family pet to the chic and urban miniature dog carried around in a purse. In general, dogs travel together with their owners and are hence transported over long distances, also to and from foreign countries. Consequently, dogs are exposed to a wide variety of environments.

Cats were traditionally kept at farms and houses to keep the number of rodents as low as possible. In this function, they were allowed to roam freely around the farm and its surroundings. This is also common on farms today. In addition, people in rural areas keep cats in this manner even if they do not live on a farm. However, there are also cats that most of the time are kept indoor and at least under control by their owners.

Dogs are closely related to both the red fox (*Vulpes vulpes*), the arctic fox (*Vulpes lagopus*) and the grey wolf (*Canis lupus*), and share many pathogens with these species. Especially the prevalent red fox pose an important reservoir for infectious diseases for domesticated dogs, the arctic fox and wolves. The raccoon dog (*Nyctereutes procyonoides*) which has become prevalent in Finland, and is regarded an invasive species to Norway, also shares pathogens with the other Canidae and may become an important reservoir for infectious diseases in the future.

The domesticated cat do similarly share many pathogens with the lynx (*Lynx lynx*), and there are some pathogens that are shared between the two groups of carnivores.

In addition, some of the pathogens that infect Canidae, and Felidae, may also cause disease in Mustelidae.

A large proportion of the dog population is vaccinated against canine distemper virus, canine adenovirus type 1 (infectious hepatitis), canine adenovirus type 2, canine parvovirus and canine parainfluenzavirus (kennel cough). In addition, vaccines against *Leishmania infantum*, rabies virus, *Leptospira* spp. (*L. interrogans* serogroup *canicola, icterohaemorrhagica* and *australis* and *L. kirschneri* serogroup *Grippotyphosa*), canine herpesvirus, *Bordetella bronchisepta* and *Borrelia burgdorferi* sensu lato are available. Vaccination against rabies is mandatory for dogs travelling back from many countries.

Many cats are vaccinated against Feline Panleukopenia virus, Feline Rhinotracheitis virus (Herpesvirus), Feline Calicivirus, Feline Leukemiavirus and *Chlamydia psittaci.* As with dogs, vaccination against rabies is mandatory for cats travelling back to Norway from countries that are not regarded as free from this disease.

A recombinant vaccine strain of Canarypox virus expressing feline interleukin 2 is also marketed in Norway, intended for use as local treatment against fibrosarcoma in cats (Felleskatalogen over preparater i veterinærmedisinen, 2014-2015).

4.2.6 Horses

The number of horses in Norway has grown considerably in recent years and was in a survey performed in 2012 estimated to around 125.000 animals owned by about 50.000 owners (Vik and Farstad, 2012). Most of the horses were kept for leisure activities (72%), but a considerable proportion used their horses in harness racing (32%) or other types of competition (23%) – like horse racing, showjumping, dressage or eventing. 27% of the horses were kept by farmers, of which 50% were engaged in other animal production, while 10% of the horse owners earned their living in the horse industry.

Most horses are kept together with other horses. According to Vik and Farstad (2012) the mean "herd size" was around 4. Horses used for competition are frequently transported over long distances.

According to the legislation (Forskrift om velferd for hest, 2005), horses shall be given exercise or allowed free movement for at least two hours each day. Many horses are given access to pasture – where they may get in contact with wildlife. Many stables are also accessible for small mammals and birds.

There are no wild equids in Norway, and there seem to be few infectious agents that are transmitted between horses and wildlife.

Horses in Norway are vaccinated against:

- horse influenza
- tetanus (*Clostridium tetanus* toxoid)
- botulism (*Clostridium botulinum* toxoid)

4.2.7 Pigs

The numbers of breeding and slaughter pigs in Norway have been relatively stable during the last decade. The number of breeding pigs has shown a decrease of 5.6 % since 2007, while the number of pigs sold as feeder pigs or sent to slaughter in the same period increased by 9.4%. In the annual survey in 2015, there were 92.600 breeding pigs, while 1.565.800 pigs were sold as feeder pigs or slaughtered in the run of that year (Statistics Norway, 2016). However, there has been a major decline of 36% in number of farms with pig production to 1121 farms with breeding pigs and 1170 farms producing slaughter pigs, meaning that the average herd size has increased correspondingly. In 2015, 64% of the breeding sows were found in herds with more than 100 adult pigs (mean herd size 118 sows). In 2014, an average sow produced 24,3 piglets in 2,16 litters (Kjøttets tilstand, 2015).

A major part of the pig production takes place in Rogaland county (28,6%), but this is also an important industry in the counties Nord-Trøndelag and Hedmark.

Pig production is a highly professionalized activity and the farms follow strict hygiene routines to avoid transmission of disease. There is therefore limited direct contact between pigs, wildlife and the rest of the environment. The exception is the organic production, where access to outdoor areas is mandatory, but only 0,4% of the breeding sows and 0,3% of the feeder pigs are found on organic farms (Kjøttets tilstand,

2015). The minor extent of organic pig farming in Norway stands in contrast to the situation in Sweden and Denmark, where this production contributes to a larger proportion of the total volume.

The wild boar (*Sus scrofa scrofa*), to which the domesticated pig is regarded as a subspecies (*Sus scrofa domesticus*) by many, has just recently started to invade Norway from Sweden and is currently regarded and managed as an alien invasive species. Breeding family groups have established along the southernmost part of the Norwegian-Swedish border (Artsdatabankens faktaark nr. 239, 2012). As the wild boar is prevalent in Sweden, we may expect that this species will spread further into Norway and establish in larger areas.

The health of Norwegian pigs is generally very good, and there are few outbreaks of contagious diseases. In 2015 there were, apart from two cases of necrotizing enteritis – caused by *Clostridium perfringens* type C, no cases of A- or B-listed diseases among swine in Norway (Årsrapport Helsetjenesten for svin, 2015). Enzootic pneumonia, caused by *Mycoplasma hyopneumonia*, which previously was prevalent, was not observed in any of 398 tested herds, and there was only one case of swine dysentery (caused by *Brachyspira hyodysenterica*). Atrophic rhinitis, caused by toxigenic strains of *Pasteurella multocida*, possibly in interaction with *Bordetella bronchisepta* was, however, seen in one outbreak, and there was another outbreak with pleuropneumonia, caused by *Actinobacillus pleuropneumoniae,* in 28 herds.

The most common health problem in pigs is joint infection/inflammation, especially in piglets. Gastritis/enteritis/colitis is also a group of diseases of major importance.

Most herds use vaccination against neonatal diaorrhea caused by specific strains of *Escherichia coli*, erysipelas caused by *Erysipelothrix rhusiopathiae* and porcine parvovirus.

When required, they may also be vaccinated against:

- oedema disease caused by Shiga-toxin producing *E. coli*
- necrotizing enteritis
- atrophic rhinitis
- tetanus
- intestinal adenomatosis caused by *Lawsonia intracellularis*
- enzootic pneumonia
- pleuropneumonia
- Glässer's disease caused by *Haemophilus suis*
- porcine circovirus
- porcine parvovirus
- porcine influenza virus A

In addition to vaccination against disease, vaccines containing a synthetic gonadotropin releasing hormone (GnRH) analogue, meant to prevent development of boar taint trough androstenone and skatole in the meat of male, uncastrated pigs are also available.

*Due to time constraint, detailed description of farm management of reindeer with respect to spread of GMVVs was not provided.

5 Potential environmental risks associated with GM veterinary vectors

In the EU, a standard ERA of veterinary GM medicinal products for applicants addresses the following main areas, risk to humans, risk to the environment and subsequently assessment of the overall risk (EC, 2006; EMEA, 2004). Notably, for the purpose of this report the risk to humans will not be discussed in detail since this lies outside our mandate. Additionally, for coherence in the event of an actual hearing process, the latter is in principle, assigned to the Norwegian Medicines Agency.

Regarding assessment of risk to the environment, the key points iterated below are considered.

- Hazard identification: This includes hazardous characteristics of the GMO that could lead to harm to the environment concerning its capacity to transmit to non-target species, shedding of live product organisms, capacity to survive, establish and disseminate, potential for gene transfer, products of expression of inserted sequences, phenotypic and genotypic stability, pathogenicity to other organisms and potential for other effects.
- Assessment of likelihood: Encompasses the probability and frequency of hazard(s) identified
- Assessment of the level of risk: Involves the combined effects of the above components of hazard and its subsequent likelihood of occurrence. Here, risk matrix (Table 3) can be employed to illustrate the estimation.
- Assessment of the consequence: Each potential consequence is assigned a relative weighting on the standards of high, moderate, low or negligible. The risk matrix is again useful at this stage (Table 4).
- Assessment of the overall risk to the environment: A weight of evidence approach is usually employed as estimates are often qualitative.
- Risk management strategy

The environment is here defined as the surrounding ecosystem including animals, plants and microorganisms. The Department for Environment, Food and Rural Affairs (Defra), responsible for environmental protection, food production and standards, agriculture, fisheries and rural communities in the United Kingdom of Great Britain and Northern Ireland, has in 2008 published a "desk study" to review environmental risks from marketing GM veterinary and human medicines (Department for Environment Food and Rural Affairs, 2008). We lean our consideration on this report and update it to current knowledge.

5.1 Hazard identification

As stated in previous sections, a proper ERA of GM-VVs requires knowledge of the characteristics of the specific GM virus, its modifications and application. The first part of an ERA is the identification of properties of the GMO, on a case-by-case basis, that can constitute hazards to the environment. Here, we attempt to outline some common risk factors for the environment, bearing in mind that only the surrounding environment (and not the vaccinated animal) is considered.

5.1.1 Survival and spread of GM-VV

The survival and subsequent spread of the GM-VV is a key concern with regard to environmental risks. The environment receives, maintains or protects and transports viruses to susceptible hosts. For viruses in general, spread is dependent on the virus being able to replicate, and then again released from the infected animal. Survival is determined by a virus' resistance to environmental factors such as ultraviolet light and exsiccation, as well as its ability to infect a suitable host. Viruses that are both replication-incompetent and with a limited ability to survive in the environment pose a lower risk to the environment. They will not be spread from the recipient animal and the transgene(s) will be contained biologically within the animal.

5.1.1.1 Replication-incompetent GM-VV

With replication-incompetent GM-VV, virus-survival is mainly of importance if stocks of GM-VV are spilled or intentionally released to the environment.

5.1.1.2 Replication defective GM-VV

Replication defective GM-VV can only infect a limited number of cells (Awasthi et al., 2015; Moussa et al., 2015). Spread of these vectors from the vaccinated animal into the environment (shedding) is in most cases likely to be minimal or occurring at low levels and for a short period of time after administration only (Van den Akker, 2008).

5.1.1.3 Replication competent GM-VV

Replication competent GM-VV are able to replicate within the recipient's cells post-administration. Consequently, the recipient animal is able to spread GM-VV into the environment or directly to other organisms. The spread of replication competent GM viruses to other organisms may occur through shedding, contact transmission, (the transfer during physical contact, or contact of contaminated materials, between people or animals), or arthropod vector transmission (Department for Environment Food and Rural Affairs,

2008). An environmental risk assessment of any replication competent GM-VV remains to be a case-by-case examination, as for some replication competent GM-VVs the likelihood of shedding will be negligible under the proposed conditions of use, and therefore subsequent environmental exposure will be negligibility (Van den Akker, 2008).

The potential risk posed by the release of replication competent GM-VV through shedding or other process may be reduced if the GM-VV is administered in a situation in which it can be physically contained.

5.1.2 Shedding of progeny GM-VV

Shedding, the expelling of viral particles from the body has been reported for several GM-VV (de Wit et al., 2015; Decaro et al., 2014; Hammond et al., 2001). For example, canine parvovirus (CPV) modified live virus vaccines are able to infect vaccinated dogs replicating in the bloodstream and enteric mucosa, and shedding could be detected up to 19 days after inoculation (Decaro et al., 2014). Other studies, based on replication-competent Ad5 (rcAd5) and Ad26 (rcAd26) based adenovirus vectors, could demonstrate shedding up to 35 days post-inoculation (Abbink et al., 2012). In July 2009, the European Medicines Agency released a guideline to address virus and vector shedding (EMEA/CHMP/ICH/449035/2009). However, the scope of this EMA guideline excludes shedding as it relates to environmental concerns.

The potential risk posed by shedding GM-VV should be evaluated for each GM-VV separately.

5.2 Assessment of likelihood

5.2.1 Dissemination as a consequence of mechanism of delivery

The vaccination of wild animals for the control of diseases such as rabies through the distribution of doped bait may result in the dissemination of the GM-VV if the bait is not eaten by the target animal.

Even though this practice does not apply to Norway, rabies vaccination of wild animals is a relevant example for this report and allows us to elucidate eventual risks associated with a widely used mechanism of delivery. Currently, four vaccine strains are authorized in the European market (EFSA, 2015):

- The SAD Bern vaccine was adapted from the ERA strain after various *in vitro* passages in baby hamster kidney cells. This strain, which is considered to be the ancestor strain of all available vaccines, was provided to other European laboratories in the 1980s for further vaccine development.
- The SAD B19 vaccine was developed from SAD in vitro selections using cloned baby hamster kidney (BHK21) cells.

- The SAG2 vaccine (Street Alabama Gif) was selected from the SAD Bern strain after two successive in vitro mutations of the Arginine 333 codon by using specific anti-rabies glycoprotein-neutralising monoclonal antibodies.
- The V-RG vaccine (Vaccinia Recombinant Glycoprotein) is a vaccinia virus (Copenhagen strain) recombinant coding for the rabies glycoprotein gene from the ERA strain.

Those live replication competent virus vaccines, both genetically modified and cell culture adapted, are distributed by baits directly into the environment, and the baits stay intact, depending on environmental conditions, for approximately one week or more. Both the WHO and The European Pharmacopoeia suggest to perform molecular characterization of rabies isolates from target and non-target animals sampled in vaccinated areas as part of any vaccination programme, in order to distinguish field rabies virus from vaccine-associated cases (EU, 2005; WHO, 2013)

Some modified-live rabies virus oral vaccines may have residual pathogenicity, depending on the level of attenuation of the viral strain (WHO, 2013), as the successive selections from the original strain may produce hazardous and uncontrolled results, and variants may remain pathogenic both in target and non-target species. Recent studies on the SAD Bern and SAD B19 GM-VVs show that they are relatively genetically stable in the environment (Cliquet et al., 2013; Orlowska and Zmudzinski, 2015). Though genetically stable there have been six vaccine-induced rabies cases reported in red foxes in vaccinated areas in Germany caused by SAD B19 (Muller et al., 2009). A Slovenian study demonstrated the presence of SAD B19 vaccine in the brain tissue and the salivary glands of a naturally infected red fox (Hostnik et al., 2014).

Another general environmental issue relevant for the administration of veterinary medicines and vaccines by baits, is the use of the antibiotic tetracycline as biomarker. Tetracycline is incorporated into bones and teeth and can be detected by fluorescence microscopy several weeks post-consumption (Johnston et al., 2005). Little is known about antimicrobial resistance induced by tetracycline used as biomarkers in the baits that are spread in the environment. Some studies suggest associations between extensive use of anti-microbial drugs and anti-microbial drug resistant microorganisms found in different wild animals or ecosystems. (Benedict et al., 2015; Guerrero-Ramos et al., 2016; Kashoma et al., 2015; Traversa et al., 2015). The use of tetracyclines as biomarkers in baits could potentially give rise to safety issues related to ecotoxicity and antimicrobial resistance, although no risk assessment has ever been performed on these aspects (EFSA, 2015). In this opinion, the VKM panel on Microbial Ecology considers baiting to be unlikely as a primary factor associated with the occurrence of resistance.

5.2.2 Arthropod transmission of GM-VV

Numerous diseases are transmitted by arthropod vectors and it is to assume that arthropod vectors can also transmit GM-VV. To the authors' knowledge there is no study published focusing on the arthropod transmission of GM-VV.

5.2.3 Occupational and non-occupational exposure to GM-VV

Needle stick injuries are an inherent risk of handling needles, and are of concern because of the potential exposure to infectious agents and syringe contents. Accidental needle stick injuries and conjunctival or open wound exposures of humans with GM-VV may be associated with both local and systemic adverse events. Agriculture workers and especially veterinarians are at highest risk of exposure to veterinary vaccines (Berkelman, 2003; Buswell et al., 2016; Muroga et al., 2015; Riley et al., 2016; Thompson and McNicholl, 2010). A recent surveillance study about biological hazards reported by veterinarians working in western Canada by Epp and Waldner (Epp and Waldner, 2012) revealed that between 2007 and 2012 26% (214/810) of veterinarians reported accidental exposure to vaccines (mainly West Nile virus, *Giardia*, *Leptospira spp.*) due to needle sticks.

Aquaculture workers may also be at risk of accidental self-injection with a fish vaccine. In a Norwegian study published in 1991 Professional vaccinators reported from one to more than 50 stabs or self-injections during the vaccination season (Leira and Baalsrud, 1997). In addition to needle stick injuries, conjunctival spray exposure and spray exposure of open wounds have been reported for *Brucella abortus* strain RB51 vaccine, an attenuated live bacterial vaccine (Ashford et al., 2004).

Non-occupational accidental exposure to oral rabies vaccine have been reported inter alia from a multistate oral rabies vaccination (ORV) program for wildlife in the USA. The program uses baits containing liquid vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine. In August 2009, during the autumn baiting campaign in western Pennsylvania, a 35 year old, immunocompromised woman handled a ruptured bait, which had leaked liquid rabies vaccine onto a patch of abraded skin on her right hand. The patient subsequently developed vaccinia virus infection (Centers for Disease and Prevention, 2009).

5.3 Assessment of the level of risk

This is case-specific and is determined by the combined effect of the magnitude of the hazard, should it occur and the likelihood of occurrence. As stated earlier, the risk matrix is a beneficial tool to illustrate how risk can be estimated, but is not definitive in itself. Weight of evidence on existing knowledge and history of safe use of the GMVV in question may produce different outcomes.

Table 3: Risk matrix to illustrate risk estimation, adapted from guideline for applicants (EC, 2006).

Magnitude of hazard		Likelihood of hazard			
		High	**Moderate**	**Low**	**Negligible**
	Severe	High	High	Moderate	Negligible
	Moderate	High	High	Moderate/Low	Negligible
	Low	Moderate/Low	Low	Low	Negligible
	Negligible	Negligible	Negligible	Negligible	Negligible

5.4 Assessment of the consequence

Similar to estimation of risk, evaluation of the consequence in the event of an adverse effect can be estimated by employing the risk matrix.

Table 4: Estimation of consequence (EC, 2006).

Consequences of hazard		Likelihood of hazard			
		High	**Moderate**	**Low**	**Negligible**
	Severe	High	High	Moderate	Negligible
	Moderate	High	High	Moderate/Low	Negligible
	Low	Moderate/Low	Low	Low	Negligible
	Negligible	Negligible	Negligible	Negligible	Negligible

5.5 Assessment of the overall risk to the environment

This section entails a concise summary of information gathered from the analyses and estimations in the previous assessments. Qualitative expressions such as high, medium, low or negligible are required.

5.6 Risk management strategy

In the final step of the ERA of GMVV, when the overall risk to the environment has been determined, it is necessary to evaluate whether risk management strategies need to be implemented to minimize the occurrence of potential hazards. A set of relevant protective measures has to be proposed in cases where the overall risk to the environment is not negligible. However, the basic approach to minimizing risk is best addressed during product design and development.

6 Uncertainties

EFSA recommends that assessments identify areas of uncertainties and state clearly their subsequent impact on the overall assessment outcome for the purpose of clarity and transparency in risk assessment processes. Additionally, this is critical in the subsequent selection of risk management options (EFSA draft opinion).

There are some concerns with regards to lack of clarification on factors underlying the attenuation of particular vaccine vectors. As stated in section 3, it appears that for some viral vectors, the molecular basis for their attenuation are not well characterized. Inaccessibility to data or the lack thereof on the vaccination status of domesticated animals, especially of companion animals in Norway is another drawback (see section 7). In addition, there is lack of proper documentation on the impact of vaccination against viral infections in Norwegian aquaculture, i.e. in salmon (see section 4.2.1).

Considering the replicative capability of viruses in all life forms (plants, animals and microorganisms), it is apparent that the sources of uncertainties that can be addressed may be numerous and their magnitude rather high. On the other hand, it can be argued that the ubiquitous nature of viruses in nearly all ecosystems implies that the use of viruses as vaccine platforms poses no immediate or discernible adverse effect to the environment.

Notably, the interactions between even well described microorganisms and the biotic or abiotic environment they are released into are complex. The fact that viruses are important means of horizontal gene transfer regarding genetic diversity in evolution cannot be disputed. Taking also into consideration the inherent lability of biological systems, there will always be considerable uncertainties regarding the impact of GM viral vectors in veterinary vaccines on our environment.

There may be unanswered questions in the use of recombinant viruses regarding the risk of reversion to virulence, shedding and subsequent release to the environment. However, decisions will have to be made ahead of conclusive scientific evidence. A case-by-case approach is therefore emphasized. Consequently, risk assessments may inevitably involve preliminary assumptions according to expert judgement. The resulting ambiguity of this mixture of scientific knowledge and non-objective assumptions may be acceptable to the public if the processes and decisions are transparent and the uncertainty is well communicated (Van Der Sluijs, 2005).

7 Conclusions (with answers to the terms of reference)

1. **Provide an overview and short description of genetically modified virus vectors used as vaccines in veterinarian medicines**

The DNA virus genera of poxvirus, herpesvirus and adenovirus are the most commonly employed in GM vaccine vectors of livestock vaccination. Most virus vector strains are specific to the animal species, but some have been used across species. For example, Canarypox virus and human adenovirus serotype 5 (HAd5) vectors derived from the genera of pox- and adenoviruses respectively. Canarypox virus has been used to vaccinate cat, horse, ferret, dog, sheep and rabbit; HAd5 has been used in field trial for vaccination of dog, fox, pig and cattle. Target veterinary diseases for vaccination are those for which there currently exist no efficient therapeutic and prophylactic measures. The disease situation is the main driver determining the choice of which GM-VVs are produced; the most successful application of GM-VVs in the vaccination of domesticated animals being in the control of avian diseases.

2. **Summarize available information of relevance for environmental risk assessment of genetically modified virus vaccines used as veterinarian medicines**

The hazards associated with the use of GM-VV and the potential risk they pose to the environment are identified case-by-case, and requires knowledge of the characteristics of the specific GM virus, its modification and intended use. This Report did not focus on the actual vaccines, but on the vectors from which the vaccines are derived. Thus, type of modifications, such as properties of transgenes (whether hazardous or non-hazardous) and how they affect the virus vectors, could not be discussed in the Report.

Hazards and potential risk to the environment are linked to shedding, survival and potential dissemination of the GM-VV. Replication defective GM-VV portend less risk to the environment in comparison to replication competent GM-VV as shedding and eventual spreading of the former from vaccinated animal into the environment, in most cases, are likely to be minimal or occur at low levels and for a short period of time after administration. Even for replication competent GM-VV, the risk of spread of shed GM-virus particles can be reduced or mitigated if the GM-VV is applied in a physically contained environment. Dissemination can also be increased by mechanism of GM-VV administration, e.g. in the vaccination of wild foxes against rabies using doped bait. Insects may also be vehicles of GM-VV dissemination.

3. **Identify environmental risk factors and possible knowledge gaps of special relevance for the assessment of use of genetically modified virus vaccines under Norwegian conditions.**

The Norwegian physical environment and 'veterinary' environments are unique especially in relation to climate, diversity of both macro- and microorganisms, farm and animal handling practices, (e.g. animal population, confinement, distances of animal transportation, manure and carcass disposal, and government regulations). These are factors that are relevant for ERA of GM-VV within the Norwegian context. Therefore, should a GM-VV be applied in Norway for domesticated animal vaccination, the potential risk to the environment, compared to other geographic regions, is likely to be different, although bio-distribution in the animal and potential shedding to the environment will be similar.

The shed vaccine virus particles may preserve better in the Norwegian cold environment compared to warmer climates, but dissemination and spread between regions in Norway may be difficult due to the stringent regulated farm practices, e.g. the hygienic practices used to control viral diseases in salmon farming, the highly regulated treatment and disposal of carcasses and manure, the restriction in distances of animal transportation. In addition, dissemination by insects, for arthropod borne vectors, will be dependent on presence and survival of such insects in the Norwegian environment –this is limited, although the impact of climate change has been cited as a potential game changer to this.

There is little to no experience of the use of GM-VVs in domesticated animal vaccination in Norway, thus, no post-field or post-market monitoring information of such products in the country could be derived. This constituted a major source of knowledge gap in this study. Other sources of knowledge gaps (discussed in sections 6 and 8) include lack of (or inaccessible) data on the vaccination status of domesticated animals, especially of companion animals in Norway –such data would have provided information on the size and need of vaccines in Norway. Of the more than 360 million veterinary vaccines doses sold in Norway in 2015, 92 % is for fish. More data on their use would also give an indication of dose and potential spread, should these conventional vaccines be replaced by GM-VVs in the future.

8 Data gaps

As stated in section 7, experience of the use of GM-VVs in domesticated animal vaccination in Norway is limited. Consequently, no post-field or post-market monitoring information of such products are available.

Specific knowledge / data gaps identified are listed below:

- GM virus shedding: field trial studies on shedding of GM-VV is rarely reported in the published literature, and this can hamper evaluation of the extent to which GM-viruses can be released into the environment;
- Lack of post-release information, e.g. post approval/post marketing data on ERA of approved, previously approved, rejected or withdrawn GM-VVs due to confidential business information;
- The Report focused on vectors not on the actual vaccines. Therefore, critical information on the characteristics of the actual vaccines, e.g. properties of transgenes and other inserts, which can be relevant for ERA of GM-VV could not be provided;
- Baseline information on naturally circulating relatives of vaccine-relevant viruses in the Norwegian environment is lacking. This information may be beneficial in ERA in order to assess the potential of transfer of genetic materials (through recombination) between GM-VV and related viruses that are circulating in the environment;
- Climate change may impact the ERA of GM-VV in Norway, because it may affect survivability of GM-virus particles in the environment, diversity of insects that can assist in the transmission and spread of GM-viruses, etc. The impact of climate change on factors that are relevant to ERA is currently unknown;
- Arthropod transmission of GM-VV.

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