For Discussion on **Discussion Paper GMO 02/2018**
19 April 2018

Genetically Modified Organisms (Control of Release) Ordinance Cap. 607

Expert Group

Review of the Exemption of

Live Recombinant Veterinary Vaccines

Purpose

This paper briefs members on a review of the exemption of live recombinant veterinary vaccines in Hong Kong.

Background

2. The Genetically Modified Organisms (Control of Release) Expert Group (the Expert Group) in its first meeting held on 5 July 2011 discussed the risk assessment and disposal of live recombinant veterinary vaccines (LRVVs) in Hong Kong. The risk assessment undertaken in 2011 for LRVVs indicated that they were highly unlikely to pose any risk to the biodiversity of the local environment and the possible biosafety effect of LRVVs was deemed acceptable. On the other hand, it was considered necessary to cater for the need of application of veterinary vaccines in emergency situations such as an outbreak of a pandemic disease. If not exempted, the application of such vaccines in case of emergency could be hindered by the lengthy approval process as stipulated in the Genetically Modified Organisms (Control of Release) Ordinance, Cap. 607 (the Ordinance). In this connection, the Expert Group recommended that LRVVs should be exempted from the application of section 5 (restrictions on release into environment and maintenance of lives of genetically modified organisms (GMOs)) and section 7 (restrictions on import of GMOs intended for release into environment) of the Ordinance.

3. The Expert Group also advised that the Agriculture, Fisheries and Conservation Department (AFCD) should continue monitoring the latest progress and development of LRVVs and carry out a review of the exemption of LRVVs in a three years' time for reporting to the Expert Group.

4. The Genetically Modified Organisms (Control of Release) (Exemption) Notice took effect on 23 June 2012 to exempt all varieties of LRVVs from the application of sections 5 and 7 of the Ordinance[1].

5. AFCD conducted a review of the exemption of LRVVs and consulted the Expert Group on 8 May 2015. The second round of review has been completed by AFCD, and the results of the review are presented in the ensuing paragraphs.

Live Recombinant Veterinary Vaccines

6. LRVVs are vaccines where a live microorganism (bacteria or virus) has been modified to express its entire genome or a portion of foreign RNA or DNA sequences or proteins and where the replicative competent vector acts as a carrier and may itself act as a protective immunogen for veterinary uses. Therefore, LRVVs are GMOs which are attenuated with definite, non-reverting mutations or deletions. The live vaccine may be a non-virulent strain expressing the antigen-encoding genes isolated from pathogenic strain(s), or it may be a pathogenic strain turned into non-virulent by selective modification or deletion of gene(s) contributing to its virulence.

7. Veterinary vaccines are pharmaceutical products that are required to be registered under the Pharmacy and Poisons Regulations Cap. 138A in order for them to be sold, offered for sale, distributed or possessed for the purposes of sales, distribution or other use in Hong Kong. Among the 45 veterinary vaccines that are currently registered in Hong Kong[2], there are 28 live vaccines, of which none of them is LRVV. However, according to Section 36(1A) of the Pharmacy and Poisons Regulations Cap. 138A, unregistered pharmaceutical products including vaccines could be imported or administered for the purpose of treatment by a registered veterinary surgeon of a particular animal. According to the importation record kept by the Department of Health in the last three years, three LRVVs listed in the 2015 review assessment had been imported to Hong Kong.

8. Vaccination with live microorganisms may lead to the shedding or spreading of the administrated microorganisms into the environment. The shed microorganisms may grow or reproduce and bring about adverse impact on the environment. Therefore, the

[1] Genetically Modified Organisms (Control of Release) (Exemption) Notice. https://www.elegislation.gov.hk/hk/cap607B!en@2012-08-02T00:00:00

[2] Drug Office 2018. Registered Pharmaceutical Products. Published online and continuously updated: http://www.drugoffice.gov.hk/eps/do/en/consumer/reg_pharm_products/index.html (retrieved on 29 March 2018)

administration or import with the purpose of administration of LRVVs would in effect be considered as release of GMOs into the environment and would be subject to the regulation under the Ordinance.

Risk Assessment of Live Recombinant Veterinary Vaccines

9. In general, regulation of LRVV is being conducted by relevant authorities through registration, risk assessments or relevant safety studies[3,4,5].

10. Risk assessment of LRVVs was previously undertaken in March 2015 to assess the possible adverse effects of LRVVs on the biodiversity in the local environment. A review was conducted in March 2018 to update the list of LRVVs. The updated risk assessment report is attached at Annex.

11. The current review has covered the risk assessment of 32 LRVVs, focusing on the 9 LRVVs which have recently become commercially available and thus were not included in the previous risk assessment. Although this may not be an exhaustive list of such products available in the international market, the assessment could be deemed comprehensive by including the major categories of LRVVs.

12. A number of potential adverse biosafety effects could be resulted from the administration of the LRVVs, including establishment of an undesirable self-sustaining population, altered pathogenicity or host range, horizontal gene transfer and recombination with other virus/bacteria, reversion to virulence, possibility to spread to the environment and effects on local host species.

13. All of the LRVVs assessed are non-pathogenic and attenuated with no or very limited transmission capabilities. The assessed LRVVs also showed high genetic stability. The likelihood of recombination and horizontal gene transfer is considered to be low and that of the generation of virulent strains is even lower. Based on the review, it is concluded that the potential risk to the biodiversity of the local environment posed by the LRVVs is very low and the possible biosafety effect of LRVVs is deemed acceptable.

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[3] European Medicines Agency 2005. Guideline on live recombinant vector vaccines for veterinary use. EMEA/CVMP/004/01-FINAL. Accessed on 2 November 2017.

[4] Malaysian Veterinary Council 2015. List of approved veterinary vaccines in Malaysia. Accessed on 2 November 2017.

[5] United States Department of Agriculture 2017. Licensed Veterinary Biological Product Information. Accessed on 2 November 2017.

Advice Sought

14. In the light of the findings of the latest review, it is proposed that the current control and exemption under the Ordinance shall be maintained. Members are invited to note and provide their views on the review on the exemption of LRVVs.

Agriculture, Fisheries and Conservation Department
April 2018

Annex

Risk Assessment Report

2018

Live Recombinant Veterinary Vaccines

1. Introduction

1.1 Genetically modified or live recombinant veterinary vaccines (LRVVs) are vaccines where a live microorganism, e.g. bacteria or virus, has been modified to express entire genomes or a portion of foreign RNA or DNA sequences or proteins and where the replicative competent vector acts as a carrier and may itself act as a protective immunogen. The vaccines are attenuated and genetically defined live vaccines, which have definite, non-reverting mutations or deletions, for veterinary uses (1). In view of the rapid development in the production of LRVVs and the potential application of such vaccines in Hong Kong, a risk assessment is undertaken to assess the possible adverse biosafety effect of the live recombinant veterinary vaccines on the local environment.

1.2 This risk assessment report was prepared in accordance with Schedule 3 of the Genetically Modified Organisms (Control of Release) Ordinance Cap. 607 with respect to the requirements on risk assessment on possible adverse biosafety effects of GMOs on the local environment.

2. Identities of the GMOs

2.1 Based on the information from various sources, there are 32 commercially available LRVVs (2, 3, 4). Although this may not be an exhaustive list of such products, they include the major categories and provide a basis for a comprehensive assessment of LRVVs available. Within the 32 LRVVs, 23 listed and assessed previously in the Discussion Paper GMO/03/2015 are still currently commercially available (Table 1). Amongst them, three unregistered LRVVs were imported to Hong Kong by the registered veterinary surgeon for treatment of particular animals. The following risk assessment would focus on the LRVVs that have not been assessed previously or are newly commercialised (Table 2).

Table 1. The commercially available LRVVs listed and assessed previously (see GMO/03/2015 for more details).

#	Commercial Name (GMO Name)	Target Animal	Target Disease	Parental Organism	Donor Organism	Vector
1	Purevax ® FeLV**, ~ RCPCh FeLV, and ~ RCP FeLV (vCP97)	Cat	Feline Leukemia	Canarypox Virus strain ALVAC (ATCC no. VR-2547)	Feline Leukemia Virus subgroup A (strain Glasgow-1)	pBlueScript© SK+
2	Oncept IL-2 (vCP1338)	Cat	Fibrosarcoma	Canarypox Virus strain ALVAC (ATCC no. VR-2585)*	Canarypox Virus (strain NYVAC)*	pUC8
3	Purevax® Feline Rabies, ~ Feline 3/Rabies, ~ Feline 4/Rabies, and ~ Feline Rabies 3 YR (vCP65)	Cat	Rabies	Canarypox Virus strain ALVAC (ATCC no. VR-2547)	Rabies Virus (strain ERA)	pUC9
4	Bovela	Cattle	Bovine Viral Diarrhoea	Bovine Viral Diarrhoea Virus type 1 (strain KE-9) and type 2 (strain NY-93)	Nil	pXIKE-B-NdN and pKANE99C*
5	Hiprabovis IBR Marker Live (IBRV strain CEDDEL)	Cattle	Infectious Bovine Rhinotracheitis	Bovine Herpes Virus type 1 (strain FM)	Nil	Unknown
6	Vectormune® HVT AI	Chicken	Avian Influenza and Marek's Disease	Turkey Herpesvirus serotype 3	Avian Influenza H5N1 (*Ck/Qal-Egypt/1/08*)*	Unknown
8	Poulvac® *E. coli*	Chicken	Pathogenic *Escherichia*	*E coli*, type O78	Nil	pKNG101

			coli Infection	(strain EC34195)		
9	Vectormune ® FP MG	Chicken	Chronic Respiratory Disease and Fowlpox	Fowlpox Virus (strain FP)	*Mycoplasma gallisepticum* (strains S6 & R) and Marek Disease Virus (serotype 1 GA)	pUC18
10	Vectormune ® FP-LT and Vectormune ® FP-LT + AE	Chicken	Laryngotracheitis and Fowlpox	Fowlpox Virus (strain Cutter)	Infectious Laryngotracheitis Virus (strain LT 632 and NS175)	pUC18
11	Vaxxitek HVT+IBD (vHVT013-69)	Chicken	Infectious Bursal Disease and Marek's Disease	Turkey Herpesvirus serotype 3 (strain FC-126)	Infectious Bursal Disease Virus (strain F52/70)	Unknown
12	Vectormune ® HVT-IBD	Chicken	Infectious Bursal Disease and Marek's Disease	Turkey Herpesvirus serotype 3 (strain FC-126)	Infectious Bursal Disease Virus (strain Delaware variant "E USA")	pUC18
13	Innovax® ILT and ~ ILT-SB (HVT/ILT-138)	Chicken	Laryngotracheitis and Marek's Disease	Turkey Herpesvirus serotype 3 (strain FC-126)	Infectious Laryngotracheitis Virus	pNEB193
14	Innovax® ND** and ~ ND –SB (HVT/NDV-F)	Chicken	Newcastle Disease and Marek's Disease	Turkey Herpesvirus serotype 2 (strain SB-1)	Newcastle Disease Virus, (strain "clone 30")	pGEM-3Z

15	AviPro® Megan® Egg	Chicken	*Salmonella* Infection	*Salmonella typhimurium* (UK-1 strain χ 3985)*	Nil	Enterobacteria phage P22HT int
16	Poulvac® ST (STM-1)	Chicken	*Salmonella* Infection	*Salmonella typhimurium*, (strain 82/6915)	*Salmonella typhimurium* LT2 strain1545	Enterobacteria phage P22
17	Recombitek® Canine Distemper (C3, C4, C4/CV, C6, C6/CV) and Purevax® Ferret Distemper**	Dog and ferret	Distemper	Canarypox Virus (strain ALVAC)	Canine Distemper Virus	Unknown
18	Proteqflu, Proteqflu-TE, Recombitek® Equine Influenza (vCP1529, vCP1533, vCP2422)	Horse	Equine Influenza	Canarypox Virus (strain ALVAC)	Equine Influenza H3N8 (A/Eq/Kentucky/94, A/equi-2/Newmarket/2/93**,** A/equine-2/Ohio/03)	pUC8
19	Equilis StrepE (strain TW928)	Horse	*Streptococcus equi* Infection	*Streptococcus equi* (strain TW)	Nil	pTYB4*
20	Recombitek® Equine Western Nile, ~ EW, ~EWT, Proteq West Nile, and Proteq rWNV-EWT (vCP2017)	Horse	West Nile Virus	Canarypox Virus (strain ALVAC)	Western Nile Virus (strain NY99)	pBlueScript© II SK+
22	PRV/Marker Gold® (S-PRV-155)	Pig	Pseudorabies	Pseudorabies Virus (strain ISU S62/26*)	Nil	pSP19, pSP65
23	Suvaxyn Aujeszky 783 + O/W	Pig	Pseudorabies	Pseudorabies Virus (strain NIA-3)	Nil	pBR322
27	Raboral V-RG®	Dog, cat, cattle,	Rabies	Vaccinia Virus	Rabies Virus	pBR322

		raccoon & coyotes		(strain Copenhagen tk-phenotype)S	(strain ERA)	
28	ONRAB (AdRGI.3)	Raccoon & skunks	Rabies	Human Adenovirus type 5 (HAd5)	Rabies Virus (strain ERA)	pBR322

*updated information

**imported to Hong Kong during 2015-2018 (as of 29 March 2018)

Table 2. The commercially available LRVVs not assessed previously or newly commercialised.

#	Commercial Name (GMO Name)	Target Animal	Target Disease	Parental Organism	Donor Organism	Vector
1	Vectormune® HVT LT References: 5, 6, 7, 8	Chicken	Laryngotracheitis and Marek's Disease	Marek's Disease Virus serotype 3, HVT (strain FC-126)	Laryngotracheitis Virus (gD and gI genes)	pWE15 or pSP64
2	Vectormune® HVT IBD & Rispens References: 7, 9, 10	Chicken	Infectious Bursal Disease and Marek's Disease	HVT	Infectious Bursal Disease Virus (VP2 gene of strain F52/70)	pUC18
3	Vectormune® HVT NDV, ~ ND References: 7, 11, 12	Chicken	Newcastle Disease and Marek's Disease	HVT (strain FC-126)	Newcastle Disease Virus (F gene of strain Clone 30)	pBR322 or pUC series
4	Innovax-ND-IBD (HVP360) References: 11, 13	Chicken	Newcastle Disease, Infectious Bursal Disease and Marek's Disease	HVT (HVP360)	Newcastle Disease Virus (F gene of strain Clone 30), Infectious Bursal Disease Virus (VP2 gene of strain F52/70)	pBR322
5	Recombinant Newcastle Disease LaSota Vaccine References: 14, 15, 16	Chicken	Newcastle Disease and Infectious Bursal Disease	Newcastle Disease Virus, Paramyxovirus type 1 (strain LaSota)	Infectious Bursal Disease Virus (VP2 gene of strain F52/70)	pBR322
6	無致病性的重組減毒狂犬病毒疫苗 [Non-pathogenic recombinant attenuated rabies virus vaccination] References: 17	Dog	Rabies	Rabies Virus (strain CTN-1)	G gene	unknown

7	*Rhodococcus equi* Vaccine (*R. equi* RG2837) References: 18, 19	Horse	Pneumonia	*Rhodococcus equi*, RHOE1 (strain 103S)	Nil	pSelAct
8	Suvaxyn CSF Marker References: 20, 21	Pig	Classical Swine Fever	Bovine Viral Diarrhoea Virus	Classical Swine Fever Virus (Strain Alfort 187)	pGEM-T Easy
9	豬偽狂犬病病毒疫苗 [Porcine pseudorabies virus vaccine] References: 22, 23	Pig	Pseudorabies	Pseudorabies Virus (strain HN1201)	gD gene	pFastBac/HBM-TOPO

3. Recipient/parental Organisms

3.1 Marek's Disease Virus - (for vaccines # 1 - 4)

Marek's Disease Virus (MDV) is a double-stranded DNA virus with no RNA intermediate, belonging to the genus *Mardivirus* that includes three species or serotypes designated as *Gallid herpesvirus* 2 (Rispens) (serotype 1), *Gallid herpesvirus* 3 (SB-1) (serotype 2) and *Meleagrid herpesvirus* 1 or *herpesvirus* of turkeys (HVT or MDV-3) (serotype 3). Rispens includes all the virulent and pathogenic strains and some attenuated vaccine strains (e.g. CVI-988). SB-1 includes the naturally avirulent strains (e.g. SB-1, 301B/1) of which some are also used as vaccines, particularly with HVT for protection against the very virulent strains. HVT, or Turkey Herpesvirus, also contains naturally avirulent strains (e.g. FC-126, HVP360) and is used as a recombinant viral vaccine vector and vaccine against Marek's Disease (13, 24). The vaccine strains derived from turkey should lose their pathogenic properties but retain the immunogenicity (24). The viral vectors do not revert to virulence and are species-specific, such that they are not likely to be transmitted to or replicate in other hosts (7).

3.2 Newcastle Disease Virus - (for vaccine # 5)

Newcastle disease is a highly contagious avian viral disease and is primarily an acute respiratory disease, present in many parts of the world. It is caused by an infection of domestic poultry and other bird species with virulent Newcastle disease virus (NDV), a RNA virus which is also called avian Paramyxovirus serotype 1 (APMV-1). APMV belongs to the genus *Avulavirus* that includes ten species or serotypes designated as APMV-1 to APMV-10 (25). Clinical manifestations vary from high morbidity and mortality to asymptomatic infections. The parental organisms used as live vaccines for this disease, e.g. LaSota and B1 strains, are widely administrated to poultry by mass application in drinking water or by spray (26). Both virulent and weakly virulent NDVs are transmissible to humans, usually causing transitory infections e.g. acute conjunctivitis after direct introduction to the eye (25, 26).

3.3 Rabies Virus - (for vaccine # 6)

Rabies Virus is a RNA virus which is neurotropic and transmissible to all mammals including humans. It belongs to the genus *Lyssavirus* that includes eleven species or serotypes, of which the classical rabies virus (RABV) is used to produce rabies vaccines

(27). The non-pathogenic oral rabies vaccines are developed such that the parental organism, CTN-1 strain, is naturally passed on in rabies virus with the technology of reverse molecular genetics, leading to reduced pathogenicity (17).

3.4 *Rhodococcus equi* - (for vaccine # 7)

Rhodococcus equi is a coccobacillus bacterium which is considered as one of the most significant pathogens causing fatal pneumonia in foals (28). *R. equi* RG2837 is the parental organism of the live recombinant *R. equi* vaccine. It differs from its wild-type strain *R. equi* RE1 by the absence of most of the coding sequences of chromosomal genes ipdA, ipdB, ipdA2 and ipdB2 which are involved in the cholesterol catabolism. Although *R. equi* RG2837 possesses the virulent VapA plasmid which triggers the clinical signs of pneumonia, four introduced deletions in the bacterial chromosome prevent its survival and proliferation of macrophages (29).

3.5 Bovine Viral Diarrhoea Virus - (for vaccine # 8)

Bovine Viral Diarrhea Virus (BVDV) is in the same genus, *Pestivirus*, as Classical Swine Fever Virus (CSFV), and thus are antigenically and structurally similar to each other such that the clinical presentations are indistinguishable (30, 31). BVDV is a RNA virus that includes two genotypes, types 1 and 2 (30). As the parental organism, the modified BVDV would produce the envelope protein gene (E2) which is a part of the outer coat of CSFV, triggering the immune system to produce antibodies against the Classical Swine Fever (30).

3.6 Pseudorabies Virus - (for vaccine # 9)

Pseudorabies (or Aujeszky's Disease) is caused by Pseudorabies Virus (PRV), *Suid herpesvirus 1* (SHV-1), which is a DNA virus belonging to the genus Varicellovirus. SHV-1 infects central nervous system and other organs, such as the respiratory tract, in a variety of mammals excluding humans and the tailless apes. The virus can infect nearly all domesticated mammals (including cattle, sheep, cats and dogs) and wild mammals. The conventional vaccines are usually made with a virus that lacks a specific glycoprotion e.g. gE, gG or gC (32). The parental organism, HN1201 strain, would express the PRV gD recombinant protein gene in the vaccine, which thus induces neutralizing antibody generation and prevents infections of virulent strains of PRV (22, 23).

4. Donor Organisms

4.1 Laryngotracheitis Virus - (for vaccine # 1)

Infectious Laryngotracheitis (ILT) is an acute, highly contagious respiratory disease of chickens, caused by an alphaherpesvirus, *Gallid herpesvirus 1*, which is also commonly known as Infectious Laryngotracheitis Virus (ILTV). ILTV is a DNA virus that can affect pheasants, partridges and peafowl with high morbidity and moderate mortality, yet there is no known risk of human infection (33). The donor organisms are ILTV gD and gI protein genes which naturally overlap and need to be co-expressed for proper immunisation of ILTV (6).

4.2 Infectious Bursal Disease Virus - (for vaccines # 2, 4, 5)

The Infectious Bursal Disease Virus (IBDV) is a double-stranded RNA virus in the genus *Avibirnavirus* that include two serotypes, causing Infectious Bursal Disease (IBD), also known as Gumboro Disease. The disease would lead to lymphoid depletion of the bursa and immune-depression and cause secondary infections. Although turkeys, ducks, guinea fowls and ostriches may be infected, clinical disease occurs solely in chickens (34). The donor organism of the three vaccines above is strain F52/70 which belongs to the pathogenic serotype 1. Chickens vaccinated with such vaccines would develop anti-VP2 antibodies specifically, where VP2 is the capsid antigen of IBDV (11, 13, 16).

4.3 Newcastle Disease Virus - (for vaccines # 3, 4)

The donor organism of the above vaccines is strain Clone 30 of which the F protein gene, i.e. fusion protein, can be the basis of an effective immune response against NDV (11, 13).

4.4 Classical Swine Fever Virus - (for vaccine # 8)

Classical Swine Fever Virus (CSFV) is highly contagious among pigs, belonging to genus *Pestivirus* with only one serotype. The disease which can be fatal causes fever, skin lesions and convulsions. The donor organism, Alfort 187 strain (genotype 1.1), is commonly used among the genotypes or field virus isolates of CSFV. It is used to replace the E2 gene of BVDV as in the vaccine (21, 32).

4.5 Pseudorabies Virus - (for vaccine # 9)

The donor organism, PRV gD protein gene in the porcine kidney cell line PK-15, would be seeded in and expressed by the strain HN1201 of SHV-1 in the vaccine to induce neutralizing antibody generation for protection from pseudorabies (22, 23).

5. Vectors

5.1 pWE15, pSP64 – (for vaccine # 1)

Both pWE15 and pSP64 are cosmids, i.e. plasmids that contain phage sequences that allow the vector to be packaged and transmitted to bacteria like a phage vector. pWE15 is developed from pBR322 (see Section 5.3) while pSP64 is non-viral and contains genes for ampicillin resistance (*AmpR*) (35, 36).

5.2 pUC vectors (including pUC8, pUC9, pUC18, pUC19) – (for vaccines # 2, 3)

The pUC vectors are widely used cloning vectors. They generally contain a gene for *AmpR* and *β*–galactosidase (*lacZ*) to facilitate selection of transformed cell cultures. The pUC vectors are also developed from pBR322 (see Section 5.3) (36).

5.3 pBR322 – (for vaccines # 3 – 5)

It is one of the most widely used cloning vectors for genetic engineering. Its natural host is *E. coli*. It contains genes for ampicillin and tetracycline resistance (38).

5.4 pSelAct – (for vaccine #7)

This plasmid is a commercially available mammalian vector designed to express a gene of interest in mammalian cells, containing two transcription units such that the first drives the expression of the gene of interest and the second drives the expression of the resistance gene (39).

5.5 pGEM-T Easy - (for vaccine # 8)

The multiple cloning region of the vector is flanked by recognition sites for the restriction enzymes EcoRI, BstZI and NotI to allow the release of insert using either of these enzymes (40, 41).

5.6 pFastBac/HBM-TOPO - (for vaccine # 9)

The pFastBac™ HBM TOPO® vector contains the honeybee melittin (HBM) secretion signal and a His-Tag with a Tobacco Etch Virus (TEV) cleavage signal so that it enables secreted protein expression and easy purification of Histidine fusion proteins respectively (42).

6. Insert and Modification

6.1 Vectormune® HVT LT - # 1

The vaccine consists of genetically engineered strain FC-126 of HVT inserted with gD and gl genes of ILTV with the plasmid pWE15 or pSP64, as an aid in the prevention of laryngotracheitis and Marek's disease (6, 7).

6.2 Vectormune® HVT IBD & Rispens - # 2

The vaccine also contains HVT inserted with the capsid protein VP2 gene of IBDV strain F52/70 and mixed with conventional MDV serotype 1 Rispens strain, for prevention of both standard and variant types of Infectious Bursal Disease as well as very virulent Marek's Disease (7, 9, 10).

6.3 Vectormune® HVT NDV, ~ ND - # 3

Likewise, the vaccine contains strain FC-126 of HVT inserted with F gene of NDV strain Clone 30 with the plasmid pBR322 or pUC series, to trigger protection against Newcastle Disease (11, 12).

6.4 Innovax-ND-IBD - # 4

The vaccine consists of strain HVP360 (which is derived from strain FC-126 of HVT) inserted with F gene of NDV strain Clone 30 and VP2 gene of IBDV strain F52/70 with the plasmid pBR322, to prevent both Newcastle Disease and Infectious Bursal Disease (13, 14).

6.5 Recombinant Newcastle Disease LaSota Vaccine - # 5

The strain LaSota of Paramyxovirus type 1 is inserted with VP2 gene of IBDV strain F52/70 with the plasmid pBR322 to form low virulent vaccine strain rLasota-VP2, preventing Infectious Bursal Disease (15, 16). Preferably, the attenuated vaccine strain

LaSota is AV1615 (15).

6.6 Non-pathogenic recombinant attenuated rabies virus vaccination - # 6

The technology of reverse molecular genetics is used for making the attenuation of rabies virus strain more target-specific and stable for the insertion of external genes. In this vaccine, the various fixed virus strains CTN-1 are removed and G protein genes that carry street rabies virus are inserted at position 164 (Asn-Ser) and 333 (Arg-Glu) (16).

6.7 *Rhodococcus equi* Vaccine - # 7

The vaccine includes *Rhodococcus equi* RG2837 which is a double-deletion mutant of the *R. equi* wild-type RE1. RG2937 has the virulence plasmid VapA that the original strain possesses but four introduced deletions in the bacterial chromosome prevent survival and proliferation of R. equi RG2837 in macrophages. As this is a requirement for manifestation of an infection, the deletion mutant is suitable to be used as a live-attenuated vaccine (18, 19).

6.8 Suvaxyn CSF Marker - # 8

The E2 gene encoding region of BVDV is deleted followed by the insertion of the chimera CP7_E2alf on the CSFV strain Alfort 187 with CP 7, which is an infectious cDNA clone of BVDV, to the respective sequence with the plasmid pGEM-T Easy, inducing immunity against CSFV (20, 21).

6.9 Porcine pseudorabies virus vaccine - # 9

The vaccine consists of strain HN1201 of PRV inserted with gD gene with the plasmid pFastBac/HBM-TOPO, to produce neutralizing antibodies which can resist PRV virulent attacks (22, 23).

7. Differences between the Biological Characteristics of the GMO and those of the Recipient or Parental Organism

7.1 Vaccines using Marek's Disease Virus as parental organisms - # 1 - 4

The GM vaccines differ genetically from the parental HVT by the integration of the

expression cassette which contains i) the insertion of two genes, *gD* and *gI*, of a ILTV with their respective endogenous ILTV promoters and single shared endogenous polyadenylation signal, ii) the gene encoding the VP2 structural protein of IBDV and a stop codon, or iii) the insertion of fragment of NDV containing F gene and the respective promoter region. The foreign DNA has been inserted into what a non-coding region of the HVT genome. Both the parental strain and the GMO were found to be non-pathogenic to the host and were not transmitted from infected birds to the un-infected birds. The host range, tissue tropism, and shedding or spreading capabilities of the recombinant organism are expected to be similar to the parental HVT vaccine strain (6, 7, 8).

7.2 Vaccine using Newcastle Disease Virus as parental organism - # 5

The GM vaccine differs genetically from the parental APMV-1 by the insertion of the gene encoding the VP2 structural protein of IBDV with reduced pathogenicity. The GMO remains eligible virus proliferation and features similar to wild virus (15, 16).

7.3 Vaccine using Rabies Virus as parental organism - # 6

The GM attenuated rabies vaccine is non-pathogenic and more stable in allowing the removal of various fixed virus strains CTN-1 and insertion of external G protein genes at Glu333 and Ser164 compared to the parental Rabies Virus. The virulence-related genetic positions in the virus would neither undergo back mutation nor changes (17).

7.4 Vaccine using *Rhodococcus equi* as parental organism - # 7

The GM *R. equi* RG2837 differs from its wild-type strain RE1 by the loss of most of the coding sequences of cholesterol catabolic genes ipdA, ipdB, ipdA2 and ipdB2, resulting in the attenuation i.e. reduced ability to establish in human macrophages. In comparison with the parent strain RE1, the GM vaccine strain demonstrates a lower pathogenic potential for humans. No difference in the survival time of the GM and wild-type strains is detected (18, 19).

7.5 Vaccine using Bovine Viral Diarrhoea Virus as parental organism - # 8

The GM vaccine differs genetically from the parental BVDV by the replacement of the E2 gene of BVDV with the corresponding gene of CSFV, while the re-isolation of the vaccine

virus is rare due to the limited replication in the target host. No indications exist for recombination events or an increase in mutational events compared to its parental virus (20, 21).

7.6 Vaccines using Pseudorabies Virus as parental organisms - #9

The GM vaccine would express gD recombinant protein gene to resist PRV virulent attacks compared to the parental PRV (22, 23).

8. Detection and Identification of the GMO

As the DNA sequences involved in the genetic modifications are readily accessible in the literature, the GMOs can be detected and identified with high sensitivity by Polymerase Chain Reaction (PCR).

9. Intended Use of the GMO

The GMOs are used as main active components of the veterinary vaccines for vaccination against diseases listed in Table 2.

10. Likely Potential Receiving Environment

10.1 Vaccine using Marek's Disease Virus as parental organism - # 1 - 4

The GM vaccines are intended for mass administration to chickens for prevention of diseases and so they are likely to be used in commercial poultry farms. The genetic modifications are unlikely to change the host range, mode of transmission or their non-pathogenic nature. Although the GMOs were found to be transmitted to turkey (the natural host of HVT) in contact with the vaccinated chicken, no transmission of the GMO was recorded to occur from vaccinated chickens to other in-contact, unvaccinated chickens or ducks (9, 24).

10.2 Vaccines using Newcastle Disease Virus as parental organisms - # 5

The GM live vaccine is intended for mass administration to chickens for prevention of avian influenza and Newcastle disease. It is likely to be used in commercial poultry farms. The GMO is expected to have the same host range as its parental strain (25, 26).

10.3 Vaccine using Rabies Virus as parental organism - # 6

The oral GM vaccine is intended for domestic dogs, wild animals, stray animals and large groups of farm animals to provoke general immune response to rabies virus (17).

10.4 Vaccine using *Rhodococcus equi* as parental organism - # 7

The GM vaccine is intended for prevention of horse pneumonia of horse and so it is likely to be used in horse farms. Immuno-compromised humans are sensitive to a range of naturally occurring *R. equi* strains, however, it is very unlikely that immuno-compromised humans being infected with GMO (18).

10.5 Vaccine using Bovine Viral Diarrhoea Virus as parental organism - # 8

The GM vaccine is used to vaccinate both wild boars and domestic pigs against the wild-type BVDV (20, 21). It is likely to be used in modern conventional pig farms.

10.6 Vaccines using Pseudorabies Virus as parental organisms - # 9

The GM vaccine is likely to be used in commercial pig farms, yet it can also be potentially used to vaccinate a variety of mammals e.g. cattle, sheep and other livestock, except humans and tailless apes, against the infection of pseudorabies virus (23, 32).

11. Identification of any Novel Genotypic and Phenotypic Characteristics Associated with the GMO that may have an Adverse Effect on Biological Diversity in the Likely Potential Receiving Environment

The potential adverse effects associated with the novel genotypic characteristics of LRVVs may include:

1. Establishing an undesirable self-sustaining population

2. Altered pathogenicity or host range

3. Horizontal gene transfer and recombination with other virus / bacteria

4. Reversion to virulence

5. Possibility to spread to the environment

6. Effects on local host species

12. Evaluation of the Likelihood of the Adverse Effect Being Realised

GM vaccines / Adverse effects	12.1 With GM Marek's Disease Virus (#1–4)	12.2 With GM Newcastle Disease Virus (#5)	12.3 With GM Rabies Virus (#6)	12.4 With GM *Rhodococcus equi* (#7)	12.5 With GM Bovine Viral Disease Virus (#8)	12.6 With GM Pseudorabies Virus (#9)
Establishing an undesirable self-sustaining population	Low survivability in the environment (5, 9)	It is expected that the GM virus is much weakened and cannot establish a self-sustaining population	N/A	The genetic deletion does not lead to the generation of a bacterium with persistent or invasive characteristics (18)	The cytopathogenic biotype is proposed to lead to self-limiting infections (20)	No information was found on the survival of variant PRV (43)
Altered pathogenicity or host range	No particular risk under normal conditions (5, 9)	Only naturally occurring avirulent strains are used (25)	The oral rabies vaccine is non-pathogenic (17)	No reason to assume that the genetic modification has led to an amplification of the pathogenicity or a change in the host spectrum (18)	No serious adverse reactions are identified in the non-target species i.e. calves, young goats, lambs, and rabbits (20)	Their host range should not be different from that of the parental virus strains
Horizontal gene transfer and recombination with	No particular risk under normal conditions (5, 9)	Poison recombination occurs between strains is unlikely	N/A	N/A	The risk of transmissibility is not further limited but no	Consumption of uncooked pork or offal has been

other virus / bacteria		(15)			horizontal transmission has been confirmed (20)	linked to PRV transmission in dogs and cats (43)
Reversion to virulence	No particular risk under normal conditions (5, 9)	Reversion of virulence is unlikely (15)	The GM virus would neither undergo back mutation nor changes (17).	Extremely low likelihood of a repair of the gene deletion by recombination (18)	The risk of reversion is not further limited (20)	N/A
Possibility to spread to the environment	Low survivability in the environment and prevention of entry to sewers and public waters (5, 9)	N/A	N/A	The possible discharge of small amounts of the vaccine strain, e.g. by birds or small mammals, does not represent a risk to humans, animals or the environment (18)	The risk to the environment is indicated to be minimal when used as recommended exclusively in restricted control zones (20)	The virus can spread through the air for short distances (44)
Effects on local host species	No particular risk under normal conditions (5, 9)	The risk to local wild bird is very low	The risk to wild and domestic animals is low	The risk to local animals is very low	The risk to feral cattle and the red muntjac is very low	Wild boars seldom exhibit signs of infection (43) so the risk to local wild boars is low

13. Evaluation of the Consequences should the Adverse Effect be Realised

13.1 Establishing an undesirable self-sustaining population

As the vaccines are not pathogenic and some of them are not viable in wild environment, establishment of an undesirable self-sustaining population should not result in significant adverse effect on Hong Kong's biodiversity.

13.2 Altered pathogenicity or host range

If the host range is changed, there might be adverse effects on Hong Kong's biodiversity as the pathogenicity on different hosts may be different. Since the genes modified are not relevant to the host range, it is expected that the genetic modification would not change the host range. Even if pathogenicity is changed after the modification, the resulting GMOs all have reduced pathogenicity. Hence, they shall not result in significant adverse effect on Hong Kong's biodiversity.

13.3 Horizontal gene transfer and recombination with other viruses / bacteria

If horizontal gene transfer and recombination with other viruses / bacteria occur, the chance to produce viral or bacterial strains with severe pathogenicity shall not be different from those happening among non-GM strains.

13.4 Reversion to virulence

As the diseases are already present in nature, the reversion to virulence shall not result in significant adverse effect on Hong Kong's biodiversity.

13.5 Possibility to spread to the environment

As most target animals are domesticated, spreading to the environment would be limited. The GMO assessed generally non-transmissible are less transmissible than the parental strains. And as the diseases are already present in nature, the spreading of the non-pathogenic vaccine strains to the environment shall not result in any significant adverse effect on Hong Kong's biodiversity.

13.6 Effects on local host species

As the vaccines are not pathogenic, adverse effect on local host species is not

anticipated.

14. Estimation of the Overall Risk Posed by the GMO

14.1 Vaccines using Marek's Disease Virus as parental organism - # 1 - 4

The GMOs in the LRVVs and the parental viruses could be shed by vaccinated chicks and persist in the environment. However, the GMOs or their parental strains are non-pathogenic to wild bird species. The overall risks of these LRVVs to local biodiversity are thus considered low and acceptable.

14.2 Vaccine using Newcastle Disease Virus as parental organisms - # 5

The parental strain for the GMO is an attenuated strain. Therefore, the risk of this LRVV to the local biological diversity is considered low and acceptable.

14.3 Vaccine using Rabies Virus as parental organism - # 6

Since the parental strain for the GMO is a non-pathogenic strain, the risk of this LRVV to the local biological diversity is considered low and acceptable.

14.4 Vaccine using *Rhodococcus equi* as parental organism - # 7

The parental strain for the GMO is an attenuated strain. Therefore, the risk of this LRVV to local biodiversity is thus considered low and acceptable.

14.5 Vaccine using Bovine Viral Diarrhoea Virus as parental organism - # 8

The GMO in this LRVV is much attenuated and not transmissible. The risk of the LRVV to local biodiversity is thus considered low and acceptable.

14.6 Vaccines using Pseudorabies Virus as parental organisms - # 9

Although the virus could spread through air for short distances and no information was found on its survival, the GMO is expressed to induce neutralizing antibodies against the disease. It is considered that the potential risk of pseudorabies-based recombination vaccines to biodiversity is low and acceptable.

Agriculture, Fisheries and Conservation Department

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