For Discussion on **Discussion Paper GMO 03/2015**
8 May 2015

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 to monitor 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. Subsequently, 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 has conducted a review of the exemption of LRVVs and the results of the review are presented in the ensuing paragraphs.

Live Recombinant Veterinary Vaccines

6. Live recombinant veterinary vaccines (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. 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 49 veterinary vaccines that are currently registered in Hong Kong[2], none of them is genetically modified or LRVV. However, according to Section 36(1A) of the Pharmacy and Poisons Regulations Cap. 138A, such vaccines could be imported or administered without registration for the purpose of treatment by a registered veterinary surgeon of a particular animal.

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

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[1] Genetically Modified Organisms (Control of Release) (Exemption) Notice. http://www.legislation.gov.hk/blis_pdf.nsf/6799165D2FEE3FA94825755E0033E532/4672C6F893E588D6482579EC0053FFAE/$FILE/CAP_607B_e_b5.pdf

[2] Drug Office, 2015. Registered Pharmaceutical Products. Published online and continuously updated: http://www.drugoffice.gov.hk/eps/do/en/consumer/reg_pharm_products/index.html (retrieved on 17 April, 2015)

Ordinance).

Risk Assessment of Live Recombinant Veterinary Vaccines

9. A risk assessment of LRVVs was undertaken in March 2015 to assess the possible adverse effects of LRVVs on the biological diversity in the local environment. The detailed risk assessment report is attached at Annex.

10. The current review has covered the risk assessment of 28 LRVVs. Although this collection may not be an exhaustive list of such products available in the international market, they represent the full range of available LRVVs for a comprehensive assessment.

11. There are a number of potential adverse biosafety effects that 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.

12. 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 likelihoods of recombination and horizontal gene transfer are considered to be low and 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.

Advice Sought

13. 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, subject to further review in three years' time.

14. Members are invited to note and provide their views on the review on the exemption of LRVVs.

Agriculture, Fisheries and Conservation Department
April 2015

Annex

Risk Assessment Report

2015

Live Recombinant Veterinary Vaccines

1. Introduction

Genetically modified or live recombinant veterinary vaccines (LRVVs) are vaccines where a live microorganism (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.

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

Based on the information from various sources, there are 28 commercially available LRVVs (2, 3, 4, 5). 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. The 28 LRVVs are listed in the following tables with their commercial names (GMO name, if available), target animals and diseases (Table 1) and their parental organisms, donor organisms, and vectors (Table 2).

Table 1. The commercially available LRVVs.

#	Commercial Name (GMO Name)	Target Animal	Target Disease
1	Purevax ® FeLV, Purevax® RCPCh FeLV, and Purevax® RCP FeLV (vCP97) References: 6, 7, 8	cat	Feline Leukemia
2	Oncept IL-2 (vCP1338) References: 9, 10	cat	Fibrosarcoma
3	Purevax® Feline Rabies, Purevax® Feline 3/Rabies, Purevax® Feline 4/Rabies, and Purevax® Feline Rabies 3 YR (vCP65) References: 6, 11, 12	cat	Rabies
4	Bovela References: 13, 14	cattle	Bovine Viral Diarrhoea
5	Hiprabovis IBR Marker Live (IBRV strain CEDDEL) References: 15	cattle	Infectious Bovine Rhinotracheitis
6	VECTORMUNE® HVT AIV Reference: 16	chicken	Avian Influenza and Marek's Disease
7	禽流感、新城疫重組二聯活疫苗（rLH5-6株）[Avian Influenza and Newcastle Disease Recombinant Vaccine, Live] (Strain rLH5-6) References: 17, 18, 19	chicken	Avian influenza and Newcastle diseases
8	Poulvac® *E. coli* (ATTC no. PTC 5094) References: 20, 21, 22, 23	chicken	Pathogenic *Escherichia coli* Infection
9	VECTORMUNE® FP MG References: 24, 25	chicken	Chronic Respiratory Disease and Fowlpox
10	VECTORMUNE® FP-LT and VECTORMUNE® FP-LT + AE References: 26, 27, 28	chicken	Laryngotracheitis and Fowlpox

11	Vaxxitek HVT+IBD (vHVT013-69) References: 29, 30, 31	chicken	Infectious Bursal Disease and Marek's Disease
12	VECTORMUNE® HVT-IBD References: 32, 33	chicken	Infectious Bursal Disease and Marek's Disease
13	Innovax-ILT and Innovax-ILT-SB (strain HVT/ILT-138) References: 34, 35, 36	chicken	Laryngotracheitis and Marek's Disease
14	Innovax-ND and Innovax-ND –SB (strain HVT/NDV-F) Reference: 37, 38, 39	chicken	Newcastle Disease and Marek's Disease
15	AviPro® Megan® Vac 1 and AviPro® Megan® Egg References: 40, 41, 42, 43	chicken	*Salmonella* Infection
16	Poulvac® ST (strain STM-1, Australian Government Analytical Laboratories Accession number N93/43266) References: 44, 45, 46	chicken	*Salmonella* Infection
17	Recombitek® Canine Distemper (C3, C4, C4/CV, C6, C6/CV) and Purevax® Ferret Distemper References: 47, 48	dog and ferret	Distemper
18	Proteqflu, Proteqflu-TE, Recombitek® Equine Influenza (vCP1529, vCP1533, vCP2422) References: 49, 50, 51, 52, 53, 54	horse	Equine Influenza
19	Equilis StrepE (strain TW928, Centraalbureau voor Schimmelcultures at Baam CBS 813.95) Reference: 55, 56	horse	*Streptococcus equi* Infection
20	Recombitek Equine Western Nile, ~ EW, ~EWT, Proteq West Nile, and Proteq rWNV-EWT (vCP2017) Reference: 57, 58, 59	horse	West Nile Virus

21	Porcilis® Begonia, Porcilis® AD Begonia or Nobi-Porvac Aujeszky References: 60, 61, 62, 63	pig	Pseudorabies
22	PRV/Marker Gold® (S-PRV-155 Iowa, ATCC Accession No. VR 2311) Reference: 64, 65	pig	Pseudorabies
23	Suvaxyn Aujeszky 783 + O/W References: 66, 67, 68	pig	Pseudorabies
24	撲偽優 [Swine Pseudorabies Vaccine, Live] (Strain SA215) References: 69, 70, 71	pig	Pseudorabies
25	中牧偽寧 and 科衛寧 [Swine Pseudorabies Vaccine, Live] (Strain HB-98) References: 72, 73, 74	pig	Pseudorabies
26	仔豬大腸桿菌病 K88、LTB 雙價基因工程活疫苗 [*Escherichia coli* Diarrhea (K88、LTB) Gene Modified Vaccine for Newborn Piglets, Live] (MM-3 = strain C600(pMM085)) References: 75, 76, 77	pig	Pathogenic *Escherichia coli* Infection
27	Raboral V-RG References: 78, 79	raccoon & coyotes	Rabies
28	ONRAB (AdRGI.3) References: 80, 81, 82, 83	raccoon & skunks	Rabies

Table 2. The parental organism, donor organism and vector(s) of the commercially available LRVVs.

Notes:

[1]: See column 1 and 2 of Table 1 for the commercial names of the vaccines.

[2]: The methodology of the transformation or information about the vector is not available.

Vaccines #[1]	Parental Organism	Donor Organism	Vector
1	Canarypox Virus strain ALVAC (ATCC no. VR-2547)	Feline Leukemia Virus subgroup A strain Glasgow-1	pBlueScript© SK+
2	Canarypox Virus strain ALVAC (ATCC no. VR-2547)	cat	pUC8
3	Canarypox Virus strain ALVAC (ATCC no. VR-2547)	Rabies Virus strain ERA	pUC9
4	Bovine Viral Diarrhoea Virus type 1 (strain KE-9) and type 2 (strain NY-93)	Nil	Unknown[2]
5	Bovine Herpes Virus type , strain FM)	Nil	Unknown
6	Turkey Herpesvirus serotype 3	Avian Influenza H5N1 (A/swan/Hungary/4999/2006)	Unknown
7	Newcastle Disease Virus	Avian Influenza H5N1 (A/duck/Guangdong/S1322/2006)	Unknown
8	*E coli*, type O78, strain EC34195	Nil	pKNG101
9	Fowlpox Virus (FP strain)	*Mycoplasma gallisepticum* (strains S6 & R) and Marek Disease Virus (serotype 1 GA)	pUC18
10	Fowlpox Virus	Infectious Laryngotracheitis Virus (strain LT	pUC18

	(Cutter strain)	632 and NS175)	
11	Turkey Herpesvirus serotype 3 strain FC-126	Infectious Bursal Disease Virus, strain Faragher 52/70	Unknown
12	Turkey Herpesvirus serotype 3, strain FC-126	Infectious Bursal Disease Virus, strain Delaware variant "E USA"	pUC18
13	Turkey Herpesvirus serotype 3, strain FC-126	Infectious Laryngotracheitis Virus	pNEB193
14	Turkey Herpesvirus serotype 2, strain PB1	Newcastle Disease virus, strain "clone 30"	pGEM-3Z
15	χ 3761 *Salmonella typhimurium* UK-1	Nil	Enterobacteria phage P22HT int
16	*Salmonella typhimurium*, strain 82/6915	*Salmonella typhimurium* LT2 strain1545	Enterobacteria phage P22
17	Canarypox Virus strain ALVAC (ATCC no. VR-2547)	Canine Distemper Virus	Unknown
18	Canarypox Virus strain ALVAC (ATCC no. VR-2547)	Equine Influenza H3N8 (vCP1529 - A/Eq/Kentucky/94 vCP1533 - A/equi-2/Newmarket/2/93 vCP2422 - A/equine-2/Ohio/03)	pUC8
19	*Streptococcus equi* strain TW	Nil	Unknown
20	Canarypox Virus strain ALVAC (ATCC no. VR-2547)	Western Nile Virus (strain NY99)	pBlueScript© II SK+
21	Pseudorabies virus (strain Begonia)	Nil	pBR322
22	Pseudorabies virus (Shope strain from	Nil	pSP19, pSP65

	USDA)		
23	Pseudorabies virus (strain NIA-3)	Nil	pBR322
24	Pseudorabies virus (strain PRV Fa)	Nil	pBR322, pCMV-*β*
25	Pseudorabies virus (strain PRV Ea)	Nil	pBR322, pUC18
26	*Escherichia coli* (strain C600)	Nil	pBR322, pGA22
27	Vaccinia Virus strain Copenhagen (tk- phenotype)	Rabies Virus strain ERA	pBR322
28	Human Adenovirus type 5 (HAd5)	Rabies Virus strain ERA	pBR322

3. Recipient/parental Organisms

3.1 Canarypox Virus - (for vaccines # 1 - 3, 17, 18, 20)

Canarypox virus belongs to the avipoxvirus family. It is a large, enveloped, double stranded DNA virus of which canary is the natural host (80). The ALVAC strain (ATCC, accession number VR-2547) used in the preparation of the vaccines listed above is a purified attenuated canarypox strain, KANAPOX, originated from the field (strain Rentschler) after 200 serial passages on chick embryo fibroblasts. Because it is non-replicative in mammals, and genetically and physically stable, the ALVAC strain is considered as a ubiquitous vaccine vector with high biosafety (81).

3.2 Turkey Herpesvirus - (for vaccines # 6, 11 - 14)

Turkey Herpesvirus (HVT or MDV-3) is an enveloped double-stranded DNA virus that was originally isolated from domestic turkeys in the late 1960s. It is an alphaherpesvirus and has been widely used as a vaccine against Marek's Disease since the early 1970s, due to its antigenic relationship to Marek's Disease Virus (MDV) (86, 87). Its natural host is turkey and it is easily transmissible among turkeys, although it causes no apparent disease. Chicken will also be infected, but it is non-pathogenic in chicken and it is not readily transmissible among chicken (88, 89).

3.3 Pseudorabies Virus - (for vaccines # 21 - 25)

Pseudorabies (also called Aujeszky's Disease) Virus is an alphaherpesvirus that infects central nervous system and other organs, such as the respiratory tract, in a variety of mammals except humans and the tailless apes. The virus can infect nearly all domesticated (including cattle, sheep, goats, cats and dogs) and wild mammals (88, 89, 90). The above vaccines are all weakened strains of various pathogenic strains of the virus attenuated by genetic engineering method.

3.4 *Escherichia coli* - (for vaccines # 8 and 26)

Although non-pathogenic *E. coli* are normally found in the intestines of animals, certain strains of *E. coli* will generate extra-intestinal infections, or colibacillosis, in chickens and pigs. Colibacillosis is frequently associated with poor animal husbandry, and is a common secondary infection following bacterial or viral infection (88, 89, 90). The parental *E. coli* strain of the GMO in vaccine #8 was a pathogenic isolate from

clinical cases of colibacillosis (20). The parental strain of the GMO in vaccine #26 is *E. coli* strain C600, which is a strain of non-pathogenic bacteria widely used in microbiology as a model organism. C600 was derived from strain *E. coli* K-12, which is a debilitated strain normally not colonizing the human intestine and survive poorly in the environment. It has a history of safe commercial use, and is not known to have adverse effects on microorganisms or plants (107).

3.5 Fowlpox Virus - (for vaccines # 9 and 10)

Fowlpox is a slow-spreading viral infection of chickens and turkeys, caused by the fowlpox virus (FPV), DNA virus of the genus *Avipoxvirus* of the family Poxviridae. It is characterised by proliferative lesions in the skin that progress to thick scabs and by lesions in the upper gastrointestinal and respiratory tracts. Virulent strains may cause lesions in the internal organs. Fowlpox is seen worldwide (88, 89). FPV causes a non-productive infection in mammalian host, and it has been used to develop recombinant vector vaccines since 1980s, for use not only in poultry, but also in mammals including humans (91). The strains of FPV (Cutter and FP strain) have used been used as a vaccine for the active immunization against fowlpox in chicken (e.g. CEVAC® FP L). They have been attenuated through successive passages in culture.

3.6 *Salmonella typhimurium* - (for vaccines # 15 and 16)

Salmonella typhimurium is a bacterial pathogen that can infect a variety of domestic animals including chickens, horses, cattle, pigs, dogs and cats (89, 90). It is also a leading cause of human gastroenteritis (92). The above two vaccines are weakened forms of virulent parental strains through genetic engineering.

3.7 Bovine Herpes Virus type 1 - (for vaccines #5)

Infectious Bovine Rhinotracheitis (IBR), caused by bovine herpesvirus 1 (BoHV-1), is a disease of domestic and wild cattle. The disease is characterised by clinical signs of the upper respiratory, but it can also affect the genital tract. Although the mortality is low, secondary infection could lead to more severe disease (89). The parental organism of the vaccine is a virulent strain isolated from an outbreak of IBR. It was subsequently weakened by genetic engineering (15).

3.8 Bovine Viral Diarrhoea Virus type 1 (strain KE-9) and type 2 (strain NY-93) - (for vaccines #4)

Bovine Viral Diarrhea is caused by the Bovineviral diarrhea virus (BVDV) which is a pestivirus closely related to classical swine fever. Although cattle are the primary host for BVDV, several reports suggest most even-toed ungulates are also susceptible. The clinical presentation can range from inapparent or subclinical infection to acute and severe enteric disease to the highly fatal mucosal disease complex (88, 89). The above vaccine comes from two pathogenic parental BVDV strains (type 1 (strain KE-9) and type 2 (strain NY-93)) (13, 14).

3.9 Human Adenovirus type 5 - (for vaccines #28)

Human adenoviruses are members of the family Adenoviridae and genus *Mastadenovirus*. In human, they cause generally mild respiratory tract infections which are self-limiting or even asymptomatic infection. General infections are commonly observed in young children. The serotype 5 is the most common among the 51 serotypes known (92). Human adenoviruses generally affect only human and do not replicate in most animal (93). They have been widely used as a vector for developing recombinant vaccine due to its well characterised molecular structure, genomic stability, and ability to grow to high titers in a wide spectrum of cells (94, 95).

3.10 Newcastle Disease Virus - (for vaccines # 7)

Newcastle disease is a highly contagious avian viral disease present in many parts of the world. It is an infection of domestic poultry and other bird species with virulent Newcastle disease virus (NDV), an RNA virus which is also called avian paramyxovirus serotype 1. It is a worldwide problem that presents primarily as an acute respiratory disease. Clinical manifestations vary from high morbidity and mortality to asymptomatic infections (88, 89). The parental organism used for the above vaccine is the attenuated “La Sota” strain which has been used as a live vaccine for this disease.

3.11 *Streptococcus equi* - (for vaccines # 19)

Strangles is an infectious, contagious disease affecting the upper respiratory tract of Equidae. The causative organism, *Streptococcus equi equi*, is highly host-adapted and produces clinical disease only in horses, donkeys, and mules. It normally does not impose any danger to humans or other domestic species (88, 89). The above vaccine is originated from a parental strain (strain TW) which is a pathogenic isolate obtained in 1990 (53, 54).

3.12 Vaccinia Virus - (for vaccines # 27)

Vaccinia virus is closely related to cowpox and smallpox, and was used as a vaccine against smallpox. However, its origin and original host range was not defined. Vaccinia-related viruses continue to cause occasional outbreaks of minor infections in dairy cattle in South America and buffalo in the Indian subcontinent. These viruses often spread to people in contact with cattle (88). Nowadays, vaccinia virus is being used in genetic study and development of recombinant vaccine. The parental strain of the above vaccine is itself genetically modified by the insertional inactivation of the thymidine kinase gene (*tk*-) of the Copenhagen strain. It has been shown to be less pathogenic in tested animals because of its inability to synthesis thymidine kinase and hence thymidine triphosphate, an essential metabolite for DNA synthesis (78, 79).

4. Donor Organisms

4.1 Avian Influenza Virus (H5 subtype) - (for vaccines #6, 7)

Avian influenza (AI) viruses infect domestic poultry as well as pet, zoo, and wild birds. They are divided into 16 hemagglutinin (H1-16) and 9 neuraminidase (N1-9) subtypes. Most AI viruses (H1-16 subtypes) are of low pathogenicity, but some of the H5 and H7 AI viruses are highly pathogenic for chickens, turkeys, and related gallinaceous domestic poultry (88). The donor organisms of the above two vaccines belong to the H5N1 subtype.

4.2 Canine Distemper Virus - (for vaccine # 17)

Canine distemper is a highly contagious, systemic, viral disease of dogs seen worldwide, and it is considered to be an important threat to a whole range of wildlife

(e.g. fox, ferret, raccoon, civet, tiger, red panda, bear, primates, elephant, etc.). Canine distemper is caused by the canine distemper virus (CDV), a paramyxovirus closely related to the viruses of measles and rinderpest, but it does not cause disease in human (88, 96, 97).

4.3 Equine Influenza Virus - (for vaccine #18)

Equine influenza is an acute respiratory infection of the Equidae family (i.e. horses, donkeys, mules and zebras) caused by two distinct subtypes (H7N7 and H3N8) of influenza A virus within the genus *Influenzavirus A* of the family Orthomyxoviridae (89). The donor organisms of the above two vaccines belong to the H3N8 subtype.

4.4 *Escherichia coli* - (for vaccines #26)

Please refer to section 3.4 for information of *Escherichia coli*. The donor organisms of the GMO in this vaccine are *E. coli* 79-1454 (08:K88acK31:H-) isolated from Shanghai (for the *K88* gene) (76) and pCG86 (for the *LT* gene) which is a plasmid isolated from wild-type *E. coli* from a piglet (108).

4.5 Feline Leukaemia Virus - (for vaccine # 1)

Feline leukemia is a contagious, viral disease prominently infecting cats caused by Feline Leukemia Virus (FeLV) which is a retrovirus in the family Oncovirinae. In addition to causing leukemia, it has been associated with various other types of cancer, anemia, and immune suppression leading to increased susceptibility to various infectious diseases. There is no evidence showing that the virus can be transmitted from cats to humans (88, 97).

4.6 Infectious Bursal Disease Virus - (for vaccines # 11, 12)

Infectious bursal disease (IBD) is caused by a virus that is a member of the genus *Avibirnavirus* of the family Birnaviridae. Although turkeys, ducks, guinea fowl and ostriches may be infected, clinical disease occurs solely in chickens. The IBD virus (IBDV) causes lymphoid depletion of the bursa and immune-depression which weaken the bird and lead to secondary infections (89). The donor organisms of the two vaccines above belong to the pathogenic serotype 1.

4.7 Laryngotracheitis Virus - (for vaccines # 10, 13)

Infectious Laryngotracheitis (ILT) is an acute, highly contagious, herpesvirus infection of chickens characterised by severe dyspnea, coughing, and rales. The disease is caused by Gallid herpesvirus I, commonly known as infectious laryngotracheitis virus (ILTV). It can also affect pheasants, partridges and peafowl, but there is no known risk of human infection with ILTV (88, 89).

4.8 *Mycoplasma gallisepticum* and Marek's Disease - (for vaccine # 9)

Mycoplasma gallisepticum is commonly involved in the polymicrobial "chronic respiratory disease" of chickens and "infectious sinusitis" in turkeys. These diseases affect chickens and turkeys worldwide, causing the most significant economic losses in large commercial operations (88, 89).

Marek's disease is one of the most ubiquitous avian infections. It is identified in chicken flocks worldwide, but it can also affect quail naturally. Marek's disease virus (MDV) is a member of the genus *Mardivirus* within the subfamily Alphaherpesvirinae. The genus *Mardivirus* also includes the avirulent strains MDV-2 and MDV-3 (or HVT) which has been mentioned in the section of parental organism above (88, 89).

4.9 Newcastle Disease Virus - (for #14)

Newcastle disease is a highly contagious avian viral disease present in many parts of the world. It is an infection of domestic poultry and other bird species with virulent Newcastle disease virus (NDV), an RNA virus. It is a worldwide problem that presents primarily as an acute respiratory disease. Clinical manifestations vary from high morbidity and mortality to asymptomatic infections (88, 89).

4.10 Rabies Virus - (for vaccines #3, 27, 28)

Rabies is an acute, progressive viral encephalomyelitis that principally affects carnivores and bats, although any mammal can be affected. It is transmitted through the saliva of infected animals. Humans can be infected by this virus. The disease is fatal once clinical signs appear (88). The donor organism of the above three vaccines is an attenuated rabies strain (ERA strain) which had been used for

vaccination of domestic cats and wildlife (78).

4.11 *Salmonella typhimurium* LT2 strain 1545 - (for #16)

Salmonella typhimurium is a bacterial pathogen that can infect a variety of domestic animals including chickens, horses, cattle, pigs, dogs and cats (89, 91). It is also a leading cause of human gastroenteritis (92). The strain used as donor organism for the above vaccine is an attenuated strain with gene deletion (44, 46).

4.12 West Nile Virus - (for #20)

West Nile fever is a mosquito-borne viral disease that can affect birds, humans and horses causing inapparent infection, mild febrile illness, meningitis, encephalitis, or death. West Nile virus (WNV) is a member of the genus *Flavivirus* in the family Flaviviridae (88, 89).

4.13 Vaccines #4, 5, 8, 15, 20, 21-25 are attenuated strains after targeted gene deletion, and hence they do not have donor organism.

5. Vectors

5.1 Bacteriophage p22 – for vaccines #15, 16

The Enterobacteria phage P22 is a bacteriophage, i.e. a kind of virus that infects bacteria, related to bacteriophage λ which infects *Salmonella typhimurium*. Like many phage viruses, it has been used in molecular biology to induce mutations in cultured bacteria and to introduce foreign genetic material. P22 has been used in generalised transduction and is an important tool for *Salmonella* genetics (98, 99).

5.2 pBlueScript© SK+ and pBlueScript© II SK+ - for vaccines #1 and 20

These two plasmids are commercially available phagemids containing several useful sequences for use in cloning with bacteriophage. The sequences include an antibiotic resistance sequence to ampicillin and an *E. coli* and f1 helper phage origin of replication. They also contain the *lacZ* gene for production of *β*–galactosidase which can change the colourless chemical “X-gal” into galactose and a blue-coloured

dyne or vice versa. This colour change allows selection of transformed cell cultures (100).

5.3 pBR322 – for vaccines #21, 23-28

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 (100).

5.4 pCMV-β - for vaccine #24

This plasmid is a commercially available mammalian reporter vector designed to express high level of β-galactosidase in mammalian cells. The *lacZ* gene may be replaced by other t-DNA for transforming mammalian cells. It also contains ampicillin resistant gene (100).

5.5 pGA22 – for vaccine #26

This plasmid carries several antibiotic resistance genes (ampicillin, tetracycline, chloramphenicol and kanamycin). It has very low copy number (less than 10 as compared to 15-20 for pBR322) (101).

5.6 pGEM-3Z – for vaccine #14

This plasmid carries the *lacZ* alpha-peptide and multiple cloning region arrangement from pUC18. In addition, the vector contains both the SP6 and T7 RNA polymerase promoters flanking the multiple cloning region (100, 105).

5.7 pKNG101 – for vaccine #8

This vector contains a conditional origin of replication, a gene (*SmR*) for antibiotic (streptomycin) resistance, and a gene (*sacB*) which codes for an enzyme that produces substance harmful to the cells in the presence of high level of sucrose. The presence of such "counterselectable" marker will facilitate targeted gene deletion by selection against transformed cells which have the vector plasmid integrated with the genome. Moreover, the conditional origin of replication of the plasmid will limit its replication during cell division, and thus it will become diluted away and/or degraded after a few generations (102).

5.8 pNEB193 – for vaccine #13

This plasmid is a pUC19 derivative that carries unique *AscI*, *PmeI*, and *PacI* sites. The only differences between pUC19 and pNEB193 are in the polylinker region. pNEB193 is isolated from *E. coli* TB1(hsd M+) by a standard plasmid purification procedure (100, 104).

5.9 pSP19 and pSP65 - for #22

The two plasmids contain genes for ampicillin and tetracycline resistance (100).

5.10 pUC vectors (including pUC8, pUC9, pUC18, pUC19) – for vaccines # 2, 3, 9, 10, 12, 18, 25

The pUC vectors are also widely used cloning vectors. They generally contain a gene for ampicillin resistance (*AmpR*) and *β*–galactosidase (*lacZ*) to facilitate selection of transformed cell cultures. The pUC vectors are developed from pBR322, but they have much higher copy number (~500-700 as compare to 15-20 in pBR322) (100).

6. Insert and Modification

6.1 Purevax ® FeLV (vCP97) - #1

The *env* and *gag* genes and part of the *pol* gene obtained from the Glasgow strain of Feline Leukaemia Virus, flanked by the vaccinia virus H6 promoter, are introduced into ALVAC vector plasmid modified from pBluescript-SK. This vector plasmid was used to insert the transgene cassette into the C3 locus of the ALVAC virus and generated the vCP97 (6, 7, 8).

6.2 Oncept IL-2 (vCP1338) - #2

Using the published feline interleukin-2 sequence, PCR primers were designed to amplify the IL-2 coding region. The gene was cloned together with vaccinia virus H6 promoter into an ALVAC vector plasmid and flanked by the left and right arms of the C5 insertion locus. The vector plasmid was modified from pUC8. This vector plasmid was used to insert the transgene cassette into the C5 locus of the ALVAC

virus and generated the vCP1338 (9).

6.3 Purevax Feline Rabies (vCP65) - #3

The gene codes for glycoprotein of the rabies strain ERA together with the vaccinia virus H6 promoter at the upstream position was inserted into an ALVAC vector plasmid which originate from a pUC9. This vector plasmid was used to insert the transgene cassette into the C5 open reading frame of the ALVAC virus and generated the vCP65 (6, 11).

6.4 Bovela - # 4

The vaccine contains two GMO strains originated from two strains of bovine viral diarrhoea virus (strains KE-9 and NY-93). Both GMO strains contain two identical deletions: one in the N^{pro} gene prohibiting the N-terminal protease N^{pro} from being expressed and the other is in the E^{rns} gene resulting in abrogation of the ribonuclease function (13, 14).

6.5 Hiprabovis IBR Marker Live - #5

The parental organism (bovine herpes virus type 1) was attenuated by the construction of a double deletion corresponding to the genes of the glycoprotein *E* (*gE*) and the enzyme thymidine kinase (*tk*), resulting in the GMO (strain CEDDEL). Both deletions are known to reduce virulence in the vaccine strain. Moreover, the *gE* deletion permits the use of this strain as marker vaccine in IBR eradication programmes (15).

6.6 Vectormune HVT AIV – #6

A serotype 3 turkey herpesvirus was modified to express an avian influenza H5 type key protective antigen (16).

6.7 禽流感、新城疫重組二聯活疫苗（rLH5-6 株）(Avian Influenza and Newcastle Disease Recombinant Vaccine, Live, Strain rLH5-6) – #7

An attenuated strain of the Newcastle disease (strain La Sota) was modified to express the HA gene of H5N1 avian influenza. A *PmeI* site was introduced in the P-M intergenic region at nucleotide position 3165 of the NDV genome. The HA gene of H5N1 avian influenza was then inserted into the *PmeI* site (17).

6.8 Poulvac *E. coli (ATTC no. PTC 5094) – #8*

A 100 base pairs (bp) region deletion and two stop-codons insertion were made at the centre of the essential *aroA* gene of the parental *E. coli* strain. The non-functional mutant *aroA* gene resulted in an auxotrophic mutant which cannot produce several of its own amino acids (20). The deletion was achieved with the help of a suicide plasmid which harbored the mutant gene and another *E. coli* (*E. coli* K12 S17 λ pir) which helps to introduce the plasmid into the parental *E. coli* by conjugation.

6.9 Vectormune FP MG - #9

The *40K* and *mcg3* genes of *Mycoplasma gallisepticum* (strains S6 and R) were inserted within a 3.0-kb Hpal-Spel of the FPV parental strain. The inserted fragment also contained a synthetic *Ps* promoter and one signal sequence derived from gene *gB* of the Marek Disease Virus (MDV), serotype 1 GA. Promoter *Ps* emulates the consensus early/late promoter of poxvirus. The MDV *gB* sequence was added to the amine terminal of genes MG 40K and mcg3 to facilitate the recognition of the polypeptide and stimulate immunological responses (25).

6.10 Vectormune FP LT - #10

Two genes, *UL-32* and *gB*, from ILTV (field strain NS175 and 632, respectively) were introduced into the parental FPV, in addition to a marker gene *lacZ*, from *E. coli*, using two synthetic promoters and a region where an homologous recombination may occur with the FPV genome. Genes *gB* and *UL32* code for the LTV protective antigens, while gene *lacZ* operates as a reporter gene (28).

6.11 Vaxxitek HVT+IBD (vHVT013-69) - #11

Double stranded DNA encoding the VP2 structural protein was obtained using the IBDV Faragher 52/70 IBDV strain. This fragment was cloned into an expression cassette with a stop codon introduced at the end of the VP2 open reading frame. The expression cassette also includes a mammalian virus promoter and a mammalian virus polyadenylation signal corresponds to DNA sequences available on commercially available plasmids. The expression cassette was then inserted into the parental turkey herpesvirus (HVT) (30).

6.12 Vectormune HVT-IBT - #12

VP2 encoding sequence of the IBDV serotype 1 strain Delaware Variant E is cloned into a vector between a chicken β-actin promoter and signal sequences SV40 and UL46. This vector plasmid with the VP2 gene was then used to transform the HVT (parental organism) to give the GMO (33).

6.13 Innovax-ILT - #13

Genes *gD* and *gI* of a ILTV with their respective endogenous ILTV promoters and single shared endogenous polyadenylation signal was inserted into HVT (strain FC-126) (36).

6.14 Innovax-ND-SB - #14

The protein F coding sequence was obtained from NDV, clone 30, an attenuated strain. The F-protein cDNA was cloned into a vector with the Rous sarcoma virus LTR promoter, resulting in the vector plasmid for transforming the HVT parental organism. The resultant GMO named HVT/NDV-F can express the NDV F-protein and induce immunity against ND in chicken (37, 39).

6.15 AviPro® Megan® Vac 1 and AviPro® Megan® Egg - # 15

The *cya* and *crp* genes were deleted from the genome of χ3761 *S. typhimurium* UK-1. These two genes encode for key enzymes involved in the cAMP biosynthesis, and their deletions have resulted in attenuation of the bacteria (40, 41).

6.16 Poulvac ST (strain STM-1, Australian Government Analytical Laboratories Accession number N93/43266) - #16

The STM-1 mutant was generated by phage transduction using P22 transduction of *aroA Tn:10* from strain 1545 to the wild-type *S. typhimurium* isolated from the chicken flock. Transposon *Tn:10* insertion mutants were selected and then a transposon deleted, *aroA* deleted mutant was isolated. The insertion site of the transposon Tn10 is in the *aroA-serC* operon which codes for an essential enzyme. The insertion resulted in attenuation of the bacteria (2, 44).

6.17 Recombitek Canine Distemper - #17

The haemagglutinin (HA) and fusion (F) protein genes from CDV of the Onderstepoort strain, are introduced into ALVAC. Vaccinia virus H6 promoter was cloned upstream of HA and F genes to direct the transcription of recombinant proteins which stimulate the immunological response in the target animal (47).

6.18 Proteqflu / Proteqflu-TE / Recombitek-Equine Influenza (vCP1533, vCP1529 and vCP2242) - #18

The vaccine contains combination of two of the three GMOs, depending on where they are being marketed. The haemagglutinin (*HA*) genes from Equine Influenza Virus A/equi-2/Ohio/03 (for vCP2242), A/equi-2/Newmarket/2/93 (for vCP1533) and A/equi-2/Kentucky/94 (vCP1529) were cloned into the ALVAC® vector and subsequently introduced into ALVAC, the non-disease causing strain of canarypox virus (49-54).

6.19 Equilis StrepE (TW928, Centraalbureau voor Schimmelcultures at Baam CBS 813.95) - #19

The details of the modification process were not available. The wild-type gene of strain TW, i.e. the parental strain, was replaced by selected gene interrupted by an antibiotic resistance gene. The mutated gene was used to replace the wild-type gene of the parental strain. The mutant strain selected for this vaccine has a 1kb deletion (55, 56).

6.20 Recombitek Equine WNV (vCP2017) - #20

Gene sequence from West Nile Virus of the strain NY99 was cloned into the ALVAC® vector and subsequently introduced into ALVAC (59).

6.21 Porcilis Begonia - #21

Two genes, *gI* and *tk* (thymidine kinase) gene, of the PRV, strain Begonia (60, 62), were inactivated to reduce its virulence and viability. This is achieved by insertion of TAG translational stop codons at the genes resulting in premature translational termination (63, 64). Thymidine kinase is the enzyme required in the production of the DNA building block thymidine triphosphate, whereas GI probably contributes to

virulence by facilitating the spread of the virus through the central nervous system (64).

6.22 PRV/Marker Gold (S-PRV-155 Iowa, ATCC Accession No. VR 2311) - #22

The GM live vaccine was developed by site directed mutagenesis of the pseudorabies virulent strain (Shope strain from USDA) in three steps. The parental strain was first transformed by homologous transformation using a vector plasmid with deletions in the TK gene, resulting in the mutant strain S-PRV-002 (ATCC No. VR 2107) (65). This mutant strain was then transformed using a vector plasmid with deletions in the gI gene, and then transformed using another vector plasmid with deletions in the gpX gene. In each of the two deletion events, the lacZ (β–galactosidase) reporter genes were removed subsequently. The resulting GMO designated as S-PRV-155 is a pseudorabies virus that has a deletion in the *TK* gene in the long unique region, a deletion in the repeat region, a 1460 base pair deletion in the *gI* coding region, and a 1414 base pair deletion in the *gpX* coding region (64).

6.23 Suvaxyn Aujeszky (strain NIA3-783) - #23

The GM live vaccine was developed by site directed mutagenesis of the pseudorabies virulent strain NIA-3 in two steps. The first step was to delete the thymidine kinase gene (*tk*) which increased its safety, and the second step was to delete the glycoprotein E gene (*gE*) (66).

6.24 撲偽優 Swine Pseudorabies Vaccine, Live (Strain SA215) - #24

The PRV, strain FA, was transformed by two steps of homologus recombination. The parental strain was first transformed by a plasmid with part of the *TK* gene deleted, resulting in a *TK*- mutant PRV virus. This mutant was than transformed by another plasmid with *LacZ* gene and deletion at the *gI* and *gE*, resulting in the *TK-/gE-/gI-/LacZ*+ mutant, i.e. the strain SA215 (69).

6.25 中牧偽甯 and 科衛寧 (Swine Pseudorabies Vaccine, Live) (Strain HB-98) - #25

The PRV, strain Ea, was transformed by homologus recombination of the parental strain by a plasmid vector with part of the *TK* and *gG* genes deleted. The

transformation cassette also contains a *LacZ* gene which operates as a reporter gene (72).

6.26 仔豬大腸桿菌病 K88、LTB 雙價基因工程活疫苗 [*E. coli* Diarrhea (K88、LTB) GM Vaccine for Newborn Piglets, Live] (MM-3) - #26

The gene coding for the heat-labile enterotoxin LT in *E. coli* was altered by a single base pair inserting which resulted in a stop codon. The genes of colonization factor K88, avirulent mutated gene of heat-labile enterotoxin *LT A-B+* of enterotoxigenic *E. coli* (ETEG), and a chloramphenicol resistance maker gene were ligated into a plasmid forming the vector pMM085. This vector plasmid was used to transform the *E. coli* strain C600, resulted in a GMO named strain C600(pMM085) or MM-3 (75, 76, 77).

6.27 Raboral V-RG - #27

The glycoprotein G from the rabies virus (ERA strain) was cloned. The cloned gene was altered by site-directed mutagenesis and removal of the poly(dG) tail. This mutated gene was then inserted into the thymidine kinase locus of the vaccinia virus strain Copenhagen (*tk*- phenotype) (2, 78).

6.28 ONRAB (AdRGI.3) - #28

This vaccine construct is a human adenovirus-vectored recombinant vaccine containing the glygoprotein G gene sequence from the ERA strain of wildlife rabies vaccine virus, inserted between two *Bgrll* sites in the E-3 region of the human adenovirus type 5 (80).

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

7.1 Vaccine using bovine herpes virus type 1 as parental organisms (vaccine #5)

The GMO is defective in the expression of two proteins, glycoprotein *E* (*gE*) and the enzyme thymidine kinase (*tk*), which are present in all the wild-type IBRV strains, and

no foreign genetic material was introduced in the transformation. As such, the GMO is unlikely to have a wider host range than the parental organism. Instead, the GMO was found to be less virulent and less transmissible among its hosts as compared to its parental organism (15).

7.2 Vaccine using bovine viral diarrhoea virus (type 1 and 2) as parental organism (#4)

The vaccine virus strains were generated by two targeted deletions identical for BVDV-1 and BVDV-2 without any insertion of foreign DNA sequences. The GMO has been shown to be less virulent and less transmissible than the parental organisms. Following vaccination of target and non-target species, no evidence of transmission was observed (14).

7.3 Vaccines using canarypox virus ALVAC as parental organism (# 1 – 3, 17, 18, 20)

The host ranges of the GMOs are expected to be the same as the parental organisms (7, 11, 47, 53, 57). The genetic modifications do not enhance the virulence or the ability of the virus to survive in target or non-target species.

7.4 Vaccines using *Escherichia coli* as parental organisms (#8, 26)

Vaccine #8 – Due to the mutation of the *aroA* gene, the GM *E. coli* in the vaccine shows an impaired ability to persist in chickens and is consequently non-pathogenic. The GM vaccine strain retains the expression of the surface appendages that have been shown to be important in the generation of specific immune response (21).

Vaccine #26 – The GM *E. coli* differs genetically from the parental strain in the presence of the plasmid pMM085 with the inactivated LT A-B+ gene, the K88 antigen gene and the chloramphenicol resistance gene. It was shown to be non-pathogenic like its parental strain (strain C600) and yet offered high degree of protection to piglets against colibacillosis (75).

7.5 Vaccines using fowlpox virus as parental organisms (#9, 10)

Vaccine #9 – The GMO differs from the parental strain in the expression of key protective antigens of *Mycoplasma gallisepticum*. It was shown to be similar to the

parental fowlpox vaccine regarding their tissue tropism, transmissibility among the chicken and turkeys, and cytopathic effects on cell culture of mammal lineage (25).

Vaccine #10 – The GMO differs from the parental strain in the expression of genes *UL32* and *gB* of ILTV. Several studies were carried out to assess whether the behavior of the GM vaccine varies from that of the parental vaccine. All parameters assessed showed that the biological features of the GM vaccines are not different from those of the parental virus, regarding both *in vivo* replication and tissue tropism (28).

7.6 Vaccine using human adenovirus type 5 as parental organism (#29)

The GM live vaccine is expected to have the same narrow host range as the parental wild-type virus. It has been tested on a range of mammal species without causing any adverse reactions. Studies have indicated that the GM live vaccine is not likely to be any more pathogenic than the parental wild-type virus (83).

7.7 Vaccine using Newcastle disease virus as parental organism (#7)

The GM live vaccine was found to show lower level of virulence but less viability in host organism (chickens) as compared to the NDV without the insertion of the H5 AIV HA gene. The GM live vaccine expresses the HA genes of H5N1 avian influenza virus as well as the antigen of NDV (17).

7.8 Vaccines using pseudorabies virus as parental organisms (#21-25)

Vaccine #21 – The evidence from testing indicated that the genetic modification of the GMO was not expected to result in any potentially significant post-release shift in biological interactions, host range, effects on non-target organisms in the environment, other interaction with the environment, or increase in pathogenicity as compared to the parental virus strain (60)

Vaccine #22 – The GM live vaccine was modified by deletion in three genes (*TK*, *gI* and *gpX*) without insertion of foreign gene. As such, the GMO is unlikely to have a wider host range than the parental organism. The deletion in *TK* gene should inactivate the thymidine kinase and render the virus non-pathogenic as compared to the parental organism (64).

Vaccine #23 – The absence of the thymidine kinase gene in the GM live vaccine increases its safety by reducing its ability to grow. The absence of the glycoprotein

E gene in the virus reduces significantly the multiplication of the vaccine virus in the central nervous system of pigs (66, 67). The GM live vaccine has been proven to be less virulent and it does not spread to other susceptible pigs in contact with the vaccinated pigs (68).

Vaccine #24 – The GM live vaccine has been attenuated by targeted gene deletion. As compared to the virulent parental pseudorabies virus strain Fa, the GMO was not transmissible among pigs and did not cause significant pathogenic effect in pigs. It was also found to be safe to other tested animals (71).

Vaccine #25 – The GM live vaccine has been attenuated by targeted gene deletion. As compared to the virulent parental pseudorabies virus strain Ea, the GMO showed lower virulence to porcine cell culture and did not cause significant pathogenic effect in pigs. It was also found to be safe to other tested animals (72, 73, 74).

7.9 Vaccines using *Salmonella typhimurium* as parental organisms (#15, 16)

Vaccine #15 - No differences were observed in the lipopolysaccharide profile of the vaccine organism compared to the parental *S. typhimurium* strain, and both bacteria express the O-antigen. The modified vaccine organism retains the naturally occurring ~91 kb plasmid present in the parental bacterium. Both bacteria can ferment glucose and mannose. However, in contrast to wild type *S. typhimurium*, the vaccine organism is unable to utilise maltose, mannitol, sorbitol, sucrose, melibiose, rhamnose or xylose as the sole carbon source, due to the loss-of-function mutations in *cya* and *crp*. The growth rate of the mutant strain on Luria-Bertani broth is slightly reduced compared to the parental organism. Whereas the parental *S. typhimurium* strain is virulent to young chicks, the Δ*cya* Δ*crp S. typhimurium* strain has lost the ability to cause disease in chicks (40, 41, 42).

Vaccine #16 - Compared to its parental strain, the GMO has been mutated by deletion in two genes, *aroA* and *serC*. In contrast to its virulent parental strain, the GM live vaccine cannot persist in vertebrates due to its requirement for para-aminobenzoic acid (PABA), a nutrient not available in vertebrate host animals. It was found to be non-virulent to chicken as well as other tested animals. Studies also found that the vaccinated animals did not shed the GMO to its surrounding environment or spread the GMO to other birds that are kept together (45, 46).

7.10 Vaccine using *Streptococcus equi* as parental organism (#19)

The GM strain selected for this vaccine has a 1kb deletion in its genome. Compared to its parental strain, the GM live vaccine was found to be much less virulent to the tested animals, and was non-pathogenic to its host (55, 56).

7.11 Vaccines using turkey herpesvirus as parental organisms (#6, 11-14)

Vaccine #6 – The GM live vaccine has been modified to express the protective antigen of H5 influenza. This genetic modification would not enhance the virulence or the ability of the virus to survive in target or non-target species. The host range of the GMO is expected to be the same as the parental organisms.

Vaccine #11 – The recombinant HVT + IBD virus differs genetically from the parental HVT by the integration of the expression cassette which contains the gene encoding the VP2 structural protein of IBDV and a stop codon. The foreign DNA has been inserted into what a non-coding region of the HVT genome. The genetically modified organism does not contain any selectable markers such as antibiotic resistance genes. The specificity of the host range of the GM live vaccine did not show changes in relation to wild-type HVT that is restricted to the avian species (29, 30, 31).

Vaccine #12 – The GMO differs genetically from the parental HVT by the integration of the expression cassette containing the gene E VP2 of the donor IBDV strain. The foreign DNA is inserted into what is thought to be a non-coding region of the HVT genome. The genetically modified organism does not contain any selectable markers such as antibiotic resistance genes. Both the parental strain and the GMO were found to be non-pathogenic to the host. Both of them were not transmitted from infected birds to the un-infected birds during the study period (32, 33).

Vaccine #13 – The GMO differs from the parental HVT strain in the insertion of two genes, *gD* and *gI*, of a ILTV with their respective endogenous ILTV promoters and single shared endogenous polyadenylation signal (36). The host range, tissue tropism, and shed / spread capabilities of the GMO are expected to be the same as the parental HVT vaccine strain (34).

Vaccine #14 – The only difference between the GMO and the original HVT strain is the insertion of fragment of NDV containing gene F and the respective promoter region. The host range, tissue tropism, and shedding/spreading capabilities of the

recombinant organism are expected to be similar to the parental HVT vaccine strain (38, 39).

7.12 Vaccine using vaccinia virus as parental organism (#27)

The GMO differs genetically with the parental strain (strain Copenhagen tk- phenotype) in the insertion of the glycoprotein G gene of the donor strain into the thymidine kinase (*tk*) locus. Both the parental strain and the GMO are non-pathogenic to foxes, and the GMO showed more mild cutaneous reaction in fox than the parental strain.

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 1.

10. Likely Potential Receiving Environment

10.1 Vaccine using Bovine Herpes Virus type 1 as parental organisms (vaccine #5)

The GM live vaccine is used to vaccinate cattle against the wild-type BoHV-1. It is likely to be used in modern conventional cattle farm. The strain of GMO in this live vaccine is a gene deleted mutant, and hence it is unlikely to have a wider host range than the parental strain which affects cattle as well as other even-toed ungulates. A study has been carried out to establish the dissemination capacity of the GMO in vaccinated animals. The results obtained demonstrated that the GMO was not detected in organs, body fluids or secretions of inoculated animals, and therefore, its

transmission capacity to non-vaccinated animals can be considered as zero (15).

10.2 Vaccine using Bovine Viral Diarrhoea Virus (type 1 and 2) as parental organism (vaccine #4)

The GM live vaccine is used to vaccinate cattle against the wild-type BVDV. It is likely to be used in modern conventional cattle farm. The two strains of GMOs in this live vaccine are gene deleted mutants, and hence they are unlikely to have a wider host range than the parental strains which may affect most even-toed ungulates. The live vaccine has been shown to induce immunological response in sheep through vaccination but not in pigs. Although GM virus was detected in the milk but not the urine produced by the vaccinated cattle, studies have shown that the GMOs were not transmitted to control contact sheep, pigs or calves (14).

10.3 Vaccines Using Canarypox Virus ALVAC as the parental organism - (vaccines # 1 – 3, 17, 18, 20)

The GM live vaccines are used to vaccinate pet or sport mammals, e.g. cats (#1, 2, 3), dogs (#17) and horse (#18, 20), or fur animals, such as ferret (#17). The original host of the parental organism is a bird, i.e. canary, and the parental strain used has already been highly attenuated. The ALVAC strain does not replicate in mammal cell cultures, and the GMOs could not be isolated from the blood samples, *or* faecal or pharyngeal swabs of the vaccinated animals (7, 10, 11, 48, 53, 58).

10.4 Vaccines using *E. coli* as parental organisms (vaccines #8, 26)

Vaccine #8 – The GM live vaccine is intended for mass administration to chickens one day of age or older by spray as an aid in the prevention of disease caused by *Escherichia coli*. It is most likely to be used in commercial poultry farms. In back passage studies, the *aroA* deleted GM live vaccine was unable to survive three back passages. The GM live vaccine could be not detected in the environment 10 days after vaccination by coarse spraying. The GMO could not be recovered from non-vaccinated birds housed in close proximity to vaccinated chicks. Due to the inability to generate p-aminobenzoate (PABA) for continued survival, the GMO cannot establish self-sustaining population in the environment (21, 22, 23).

Vaccine #26 – The GM live vaccine is intended for mass administration to pigs in

farm to prevent infection caused by enterotoxgenic *E. coli* (ETEC). It had been shown to be non-pathogenic to pigs and could be used to protect pigs from ETEC (75). The GM live vaccine is also known to lose the immunity-conferring plasmid *in vivo* readily after the vaccination (106).

10.5 Vaccines using fowlpox virus as parental organisms (vaccines #9, 10)

Vaccine #9 – The GM live vaccine is intended for mass administration to chickens for prevention of disease caused by fowlpox (FP) and *Mycoplasma gallisepticum* (MG). It is likely to be used in commercial poultry farm. Both the parental strain and the GM live vaccine has been shown to be non-pathogenic to chickens and failed to cause any syndrome of both FP and MG. Study had also shown that both the GMO and the parental strain did not persist in the chickens ten days after the vaccination, and they were not transmissible. Similar results were obtained when they were tested on other bird species (25).

Vaccine #10 – The GM live vaccine is intended for mass administration to chickens for prevention of laryngotracheitis and fowlpox. It is likely to be used in commercial poultry farm. The GMO has been found to be safe in the target species (chickens) and does not spread after vaccination to other chickens or birds. It has a narrow host range and its capacity to disseminate in the environment is extremely limited (29, 30).

10.6 Vaccine using human adenovirous type 5 as parental organism (vaccine #28)

The GM live vaccine is intended to vaccinate wild animals, especially skunk and fox, against the arctic strain of rabies. It is applied in the form of baits preferred by wild animals and these baits are distributed in their habitats. The GM live vaccine was found to be safe in experimental studies in skunks (intended target species) as well as in several non-target species. The limited host range of human adenovirus reduces the risk of spread in target and non-target wild or domestic animals (83).

10.7 Vaccine using Newcastle disease virus as parental organism (vaccine #7)

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

farm. The GMO is expected to have the same host range as its parental strain.

10.8 Vaccines using pseudorabies virus as parental organisms (vaccines #21-25)

The GM live vaccines are intended for mass administration to pigs in farms for prevention of the pseudorabies disease. They are generated by selected gene deletion of parental pathogenic strains resulting in immunogenic GM strains with reduced viability and virulence that are safe to use as vaccines. All of the above GM live vaccines have deletion in at least part of the thymidine kinase gene which reduces their viability in the host. Deletions at the glycoprotein genes should also reduce their ability to infect the host or spread within the host. As such they are unlikely to have wider host range than their parental strains.

10.9 Vaccines using *Salmonella typhimurium* as parental organisms (vaccines #15, 16)

The GM live vaccines are intended for mass administration to chickens for prevention of disease caused by *S. typhimurium, S. enteritidis* and *S. heidelberg*. They are likely to be used in commercial poultry farm.

Vaccine #15 –The GM bacterium reportedly grows slower than the wild type parental organism, and has lost the ability to metabolise alternative carbohydrate sources. These defects and others induced by the gene deletions should hinder the persistence of the vaccine organism in the environment. (42).

Vaccine #16 – As compared to the parental strains, the gene deletion in the GMO has rendered them auxotrophic, and hence they cannot survive in vertebrate hosts or in the environment (45). The isolation of the GMO by environmental scanning of a test aviary and commercial poultry farms showed that the vaccine strain does not persist in the environment or in the birds for more than 21 days after vaccination. There was no recovery of the micro-organism of any of the uninoculated birds kept in touch with any of the inoculated recta. This indicates that the GM live vaccine does not spread to nearby birds, and cannot be isolated after 21 days of inoculation in birds held on the first day of life (46).

10.10 Vaccine using *Streptococcus equi* as parental organism (vaccine #19)

The GM live vaccine is intended for use in horses for prevention of the strangles

disease caused by *Streptococcus equi*. It is likely to be used for horses kept as pet or for sport purposes. It is resulted from gene deletion that is expected to weaken its capability to persist in the environment. It was found that the GMO cannot compete with other microorganisms in its natural environment and that it is only able to survive in the natural environment when the growth condition is limited for bacteria, including the GMO itself. Safety test of the GMO has also shown that it does not spread to other horses through the natural route (56).

10.11 Vaccines using turkey herpesvirus as parental organisms (vaccines #6, 11-14)

The GM live vaccines are intended for mass administration to chickens for prevention of diseases. They are likely to be used in commercial poultry farm. 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 transmittable to turkey (the natural host of HVT) in contact with the vaccinated chicken, no transmission of the recombinant organism was seen to occur from vaccinated chickens to other in-contact, unvaccinated chickens or ducks (30, 33, 34, 38).

10.12 Vaccine using vaccinia virus as parental organism (vaccine #27)

The GM live vaccine is intended to vaccinate wild raccoons and coyotes against rabies. It is applied in the form of baits preferred by wild animals and these baits are distributed in their habitats. Since the parental organism is a *tk*- strain, the GMO should have low viability in natural environment like its parental strain. Studies have shown that the GMO was only found in tonsils and buccal mucosa of the tested foxes shortly after the vaccination but not in other organs or the saliva or faeces. Transmission from the vaccinated animals was considered to be rare (79).

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 live recombinant veterinary vaccines 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

12.1 Vaccine using Bovine Herpes Virus type 1 as parental organism (#5)

12.1.1 Establishing an undesirable self-sustaining population

The self-sustaining of the recombinant virus, which means the indefinite survival of the population by replicating and spreading from host to host, was determined to be highly improbable. The deletions in *tk* and *gE* genes of the parental strain would greatly reduce its viability and virulence (15).

12.1.2 Altered pathogenicity or host range

The GMO is not expected to spread better nor has wider host range in cattle populations than wild-type strains of the virus. Instead, the GMO was found to be non-pathogenic because of the deletions in two essential genes (15).

12.1.3 Horizontal gene transfer and recombination with other bacteria

It is considered that the only potential genetic transmission which could be foreseen is genetic exchange by homologous recombination between the recombinant virus and

virulent strains of IBRV. For this to take place, both viruses would have to co-infect the same cell, in the same animal, which limits the chance of such an event to occur. Should such a recombination occur between the GMO and a wild-type IBRV strain, the deleted sequences of the *gE* and *tk* enzyme could only be replaced by the corresponding gene sequences donated by the virulent strain. The expected result of such a recombination would be the creation of a recombinant IBRV strain with the same complement of genes as the wild-type virus. (15).

12.1.4 Reversion to virulence

The GMO did not show any reversion to virulence upon different passages in calves and in cell cultures (15).

12.1.5 Possibility to spread to the environment

Laboratory and field studies indicated that horizontal transmission is practically non-existent. Cattle inoculated by intramuscular route do not excrete the GMO, so the possibility of excessive excretion of the virus is highly unlikely. The vaccinated animal acts almost as a dead end host, therefore the virus would be eliminated from the population (15).

12.1.6 Effects on local host species

The parental organism (BoHV-1) affects primarily domestic and wild cattle (87). Locally the feral cattle may be affected by the virus. However, the safety studies demonstrated that the GMO is not shed by the vaccinated target animals into the environment, hence the risk to local animals is very low (15).

12.2 Vaccine using Bovine Viral Diarrhoea Virus (type 1 and 2) as parental organism (#4)

12.2.1 Establishing an undesirable self-sustaining population

The self-sustaining of the recombinant virus was determined to be highly improbable. Both GMO strains in this live vaccine contain two identical deletions. The deletion in N^{pro} gene should result in reduced growth rates and attenuation in the natural host. The deletion in the E^{rns} gene should result in replicons that are unable to produce infectious virus particles. As such, the virulence and transmissibility of the GMO is

reduced as compared to the parental strains. Data indicated that the shedding capacity of the GMOs is low and they could not be recovered after the 2nd back-passage (14).

12.2.2 Altered pathogenicity or host range

The two GMO strains had been modified by targeted deletions without any insertion of foreign DNA sequences. As such the GMOs are unlikely to have wider host range than the parental strains. Instead, they have been shown to be non-pathogenic and far less transmissible than the parental strains (14).

12.2.3 Horizontal gene transfer and recombination with other viruses

The viral RNA genome is the only available genetic information and RNA cannot recombine with DNA. Therefore, the potential hazard of "gene transfer" from the viral RNA genome to the genomes of animals or environmental bacteria is negligible. Recombination with other viruses would take place during a double infection of cells with wild viruses which is very unlikely owing to super-infection exclusion mechanism and the low replication rate of the vaccine strain (14).

12.2.4 Reversion to virulence

The vaccine strains were attenuated by double individual genomic deletions: N^{pro} codons 5 to 168 and E^{rns} codon 349. Therefore the reversion by mutation is very unlikely. The reversion of virulence by recombination requires a double infection of cells with wild viruses which is very unlikely owing to super-infection exclusion mechanism and the low replication rate of the vaccine strain. Moreover, animal studies showed that the GMOs could not be recovered after the 2nd back-passage, which is a pre-requisite for reversion to virulence (14).

12.2.5 Possibility to spread to the environment

The excretion of BVDV via nasal secretions, blood, urine and faeces were assessed to be a hazard with a low risk, especially since the GMOs could not be detected in nasal secretions which are known to be the most important source of spread of BVDV into the environment. Transmission of vaccine virus strains could not be shown between target and non-target animals. Following vaccination of cattle, pigs and sheep, no

evidence of transmission in those species could be observed (14).

12.2.6 Effects on local host species

BVDV is restricted under natural infection conditions to members of the order Artiodactyla. Locally, the feral cattle and the red muntjac (*Muntiacus muntjak*) may be affected by the GMOs. However, since the GMOs in this vaccine are non-pathogenic and not transmissible, the risk to local animals is very low.

12.3 Vaccines Using Canarypox Virus ALVAC as the parental organism - (vaccines #1 – 3, 17, 18, 20)

12.3.1 Establishing an undesirable self-sustaining population

The self-sustaining of the GMOs was determined to be highly improbable. The ALVAC strain used in the preparation of the vaccines listed above is a purified canarypox clone isolated from the attenuated viral strain, KANAPOX, developed from an infectious strain attenuated by 200 serial passages on chick embryo fibroblasts. These ALVAC-based GM live vaccines were designed to be used in mammals. As no virus shedding or spreading was detected after vaccination, non-target animals will not be infected. As a result, the virus is unlikely to replicate and establish an undesirable population in the non-target animals of the local environment (85).

12.3.2 Altered pathogenicity or host range

The safety of several recombinant ALVAC vaccines has been tested in canary birds in comparison with the original ALVAC vaccine. Similar tests were conducted on chickens and mice. Like the parental vaccine, the recombinant viruses cause mild local lesions at the inoculation sites of the infected canary birds, which were recovered soon afterwards. No lesion was observed on inoculated chickens and mice (85). The safety of various ALVAC vaccines was also assessed under laboratory and field conditions in a variety of species including mice, horses, humans, dogs and chickens. Safety of ALVAC vaccines has been confirmed in animals of a variety of ages with varying immune statuses and using various routes and doses of administration. It was reported that all the GM viruses tested to date were as safe as the parental strain and no change of host specificity was observed (85).

12.3.3 Horizontal gene transfer and recombination with other virus

Molecular interaction between poxvirus within co-infected cells could result in recombination. Since mammals are not infected by avipoxvirus, *in vivo* recombination between the recombinant virus and its wild-type relatives could hardly happen. Although cats can be infected by cowpox virus which could recombine with avipoxvirus, the chance for the recombinant virus and cowpox virus to exist in the same cell is extremely low. The wide genetic distance between avipoxvirus and cowpox virus further minimises the risk of reverting to virulence by recombination. Thus, it is unlikely for the horizontal gene transfer and recombination to take place (85).

12.3.4 Reversion to virulence

Virulence reversion could occur when the non-pathogenic virus recombines with its virulent relatives or spontaneously mutated during consecutive passages in different cells. In this case, recombination could hardly take place because the virulent strains could only be found in avian species but not the inoculation targets (i.e. mammals). The recombinant virus is also considered to be genetically and phenotypically stable as no alternation was detected in an experiment with 20 cell culture passages. Thus, the virulence reversion is unlikely to happen (85).

12.3.5 Possibility to spread to the environment

No virus shedding was detected from samples of saliva, urine and faeces collected from the tested animals. Thus, the likelihood of spreading to non-target animals is very low (85).

12.3.6 Effects on local host species

Canaries are not native species in Hong Kong. They are imported as pets and kept in cages. Because the recombinant viruses have the same mild pathogenicity and host range as the non-recombinant ALVAC virus, they should not impose any threats to the wild bird species in Hong Kong.

12.4 Vaccines using *E. coli* as parental organism (vaccines #8, 26)

12.4.1 #8 - Poulvac® *E. coli*

12.4.1.1 Establishing an undesirable self-sustaining population

The *aroA* defect means that the GM *E. coli* vaccine strain cannot survive without supplemental aromatic amino acids or PABA, which are not readily available in the environment. The GMO was found to be unable to multiply in the environment. When it was tested under conditions mimicking a commercial poultry farm, the GMO could not persist for more than 24 hours. In a shed/spread study the GMO was not recovered 10 days after the vaccination by coarse spray, confirming the limited persistence of the GMO (21, 22, 23).

12.4.1.2 Altered pathogenicity or host range

Based on the nature of the genetic modification, the recombinant bacteria are unlikely to have its host range altered.

12.4.1.3 Horizontal gene transfer and recombination with other bacteria

E. coli are known to acquire foreign DNA through horizontal gene transfer, via conjugation (typically only plasmid DNA transferred), by transduction (involving bacteriophage), or by free DNA uptake (transformation). However, such a horizontal gene transfer event would not increase the bacteria's pathogenicity beyond that of the wild-type parental *E. coli*, which may already be present at the farm, or not unlike other pathogenic *E. coli* in the poultry flock being vaccinated. Furthermore, the chances of horizontal gene transfer taking place after vaccination is significantly restricted, due to the inability of the GMO to persist in an environment devoid of its requisite aromatic nutrients (21, 22, 23).

12.4.1.4 Reversion to virulence

The manufacturer estimates that the reversion rate of the vaccine strain back to the parental strain is less than 10^{-11} for the *aroA* mutation. Deletion of a large part of a gene provides confidence that random spontaneous mutations will be unable to repair the loss of function, especially compared to a system of inactivation dependent on the modification of only a few nucleotides. The genetic stability of the master seed under normal culture conditions has been demonstrated up to n+5 passages, which is the upper limit of fermentation specified for the production of the vaccine (21, 22,

23).

12.4.1.5 Possibility to spread to the environment

In a shed/spread study, the GMO was not recovered 10 days after the vaccination by coarse spray, confirming the limited persistence of the GMO. Moreover, several studies have shown that transmission was not observed for non-vaccinated birds after they had been kept with vaccinated birds for prolonged period (21, 22, 23).

12.4.1.6 Effects on local host species

A lot of species in Hong Kong are susceptible to *E. coli* infection. However, since the vaccine is non-pathogenic, the risk to the wild species in Hong Kong is very low.

12.4.2 #26 -仔豬大腸桿菌病 K88、LTB 雙價基因工程活疫苗 (MM-3 = strain C600(pMM085))

12.4.2.1 Establishing an undesirable self-sustaining population

The parental strain of the GMO is *E. coli* strain C600, which is a laboratory strain derived from strain *E. coli* K-12, a debilitated strain normally not colonizing the human intestine (107). C600 cannot survive without supplemental thiamine, threonine and leucine. The GMO is therefore unlikely to form a self-sustaining population. Moreover, the GMO is also known to readily lose its immunogenic insertion or plasmid readily in the absence of antibiotic pressure (106).

12.4.2.2 Altered pathogenicity or host range

Based on the nature of the genetic modification, the recombinant bacteria are unlikely to have its host range altered.

12.4.2.3 Horizontal gene transfer and recombination with other bacteria

E. coli are known to acquire foreign DNA through horizontal gene transfer, via conjugation (typically only plasmid DNA transferred), by transduction (involving bacteriophage), or by free DNA uptake (transformation). However, the chance of horizontal gene transfer taking place after vaccination is significantly restricted, due to the inability of the GMO to persist in an environment without antibiotic pressure.

12.4.2.4 Reversion to virulence

Loss of the inserted gene or plasmid will regenerate the original parental strain which is non-virulent (106).

12.4.2.5 Possibility to spread to the environment

The parental strain does not survive without supplemental thiamine, threonine and leucine. The GMO also readily loses its gene insertion or plasmid in the absence of antibiotic pressure.

12.4.2.6 Effects on local host species

A lot of species in Hong Kong are susceptible to *E. coli* infection. However, since the vaccine is non-pathogenic, the effect on the wild species in Hong Kong should be negligible.

12.5 Vaccines using fowlpox virus as parental organism (vaccines #9, 10)

12.5.1 Establishing an undesirable self-sustaining population

The dissemination of wild-type fowlpox has been known to be slow. The parental strains of the two GM live vaccines are attenuated strains of fowlpox. The GMOs were not isolated from the vaccinated chickens 10 days after the vaccination. Both the GMOs and their parental strains were not shown to be transmissible from the vaccinated birds. The vaccinated birds therefore act as a dead end host, and hence the GMOs will not be able to establish a self-sustainable population (25, 27, 28).

12.5.2 Altered pathogenicity or host range

The GMOs has been shown to be able to colonize turkey and chicken which is the same as the host range of their parental strains. Inoculations of other bird species (quail, fowl, and pigeon) or mammal lineage cell culture give similar negative results for both the parental strains and the GMOs (25, 27, 28).

12.5.3 Horizontal gene transfer and recombination with other bacteria

Genetic recombination could be remotely possible with other FPV viruses. The consequence may be the emergence of other FPV expressing the *gB* and *UL-32* genes of LTV or part of these genes and at the same time GMO losing some of the inserted

sequences. However, the potential impacts for these events to occur would be no greater than for the parent strain FPV recombining with other FPV (25, 27, 28).

12.5.4 Reversion to virulence

Safety studies associated to vaccine genetic stability and purity were also conducted. Lack of virulence reversion demonstrated that the GMO is genetically and phenotypically stable after five successive retro-passages in chicken. No adverse reactions or clinical signs of FP and MG were recorded during each passage or for twenty-one days at the group of the fifth passage (25, 27, 28).

12.5.5 Possibility to spread to the environment

The FP viruses are not shed from chicken vaccinated with the GMOs or their parental strains. Hence the GMO is unlikely to be spread to the environment (25, 27, 28).

12.5.6 Effects on local host species

The GMOs and their parental strains have narrow host range. Moreover, both the parental strains and the GMOs are not transmissible by natural means. The effect of these two GMOs on the wild species in Hong Kong should be negligible.

12.6 Vaccine using human adenovirus type 5 as parental organism (vaccine #28)

12.6.1 Establishing an undesirable self-sustaining population

The parental organism and the GMO have a narrow host range and have no pathogenic effect on most animal species. The live vaccine is presented as baits enclosed in package that can only be opened with bites by the wild animals, hence the chance that human getting in contact with the vaccine is low. The widespread immunity in humans over the age of five for related human type 5 adenoviruses would make person-to-person spread of infection unlikely (83).

12.6.2 Altered pathogenicity or host range

All of the studies in target and non-target species indicate that the host range and tropism of the rabies vaccine, live adenovirus vector, were not altered from the parent human adenovirus type 5 strain (83).

12.6.3 Horizontal gene transfer and recombination with other bacteria

The GMO only persists in the vaccinated animal for less than 21 days after vaccination. Hence, the chance of recombination with co-infecting viral genetic materials is very low. In case recombination does occur with other adenovirus, it will result in another rabies glycoprotein expressing adenovirus and regenerate the parental strain which is non-virulent to most animal species (83).

12.6.4 Reversion to virulence

Back passage studies with cotton rats showed that the virus titers dropped to levels insufficient for continued passaging after the third passage. The GMO was also found to be genetically and phenotypically stable up to passage MSV+10, and is free of extraneous agents (83).

12.6.5 Possibility to spread to the environment

The potential for accidental exposure to human or the natural host of the parental virus is low, since the wildlife baits are typically distributed in rural areas away from houses. The widespread immunity in humans over the age of five for related human type 5 adenoviruses would make person-to-person spread of infection unlikely (83).

12.6.6 Effects on local host species

The GMO and its parental strain have a very narrow host range and are non-pathogenic to most animal species in Hong Kong. The effect of this GMO to the wild species in Hong Kong should be very limited.

12.7 Vaccine using Newcastle disease virus as parental organism (vaccine #7)

12.7.1 Establishing an undesirable self-sustaining population

The parental strain for the GMO is the strain LaSota which is an attenuated NDV strain used widely to vaccinate chicken to prevent Newcastle disease. It was shown that recombinant vaccine with the insertion of the *H5 AIV HA* gene was less virulent and grew slower than the recombinant LaSota virus without the insertion (17). As such, the GMO is unlikely to establish self-sustaining population.

12.7.2 Altered pathogenicity or host range

The GM live vaccine with the gene insertion was shown to be less virulent and grow slower than the recombinant LaSota virus without the insertion. The insertion occurred at the intergenic region between the genes coding for the phosphoprotein *P* and the matrix protein *M*, and hence it should not alter the protein originally encoded by these viral genes (17).

12.7.3 Horizontal gene transfer and recombination with other bacteria

Genetic recombination could be remotely possible with other NDV viruses. The consequence may be the emergence of other NDV expressing the *H5 AIV HA* or part of these genes and at the same time GMO losing some or all of the inserted sequences. However, the potential impacts for these events to occur would be no greater than for the parent strain NDV recombining with other NDV (17).

12.7.4 Reversion to virulence

Genetic stability of the GM live vaccine was studied by passaging the GMO for 20 times in 10-day-old embryonated SPF chicken eggs. The GMO was found to be stable with respect to its DNA sequence, HA expression, virulence and viability levels after the passage study (17).

12.7.5 Possibility to spread to the environment

The GM live vaccine was detected from the lungs and the oropharyngeal swabs of the chickens, and hence it may be spread to other birds in contact with the vaccinated chickens (17).

12.7.6 Effects on local host species

The possibility of spreading of the GM live vaccine from the vaccinated chickens cannot be ruled out. However, the GMO is less virulent than its attenuated parental strain, thus its effect on local wild birds should be very limited.

12.8 Vaccines using pseudorabies virus as parental organisms (vaccines #21-25)

12.8.1 Establishing an undesirable self-sustaining population

All of the GMOs in the above vaccines have been attenuated by selective gene

deletion. The deletion in the gene encoding the thymidine kinase (*tk*) which is for the production of metabolite required for DNA synthesis, would diminish the ability of the GMOs to grow. The other genes (*gE, gG, gI, gpX*, etc.), on the other hand, codes for glycoproteins which account for the infection of cells, and thus their deletions will reduce the ability of the GMOs to spread and infect. It is thus expected that the GMOs are much weakened and cannot establish a self-sustaining population (61, 64, 68, 71, 72).

12.8.2 Altered pathogenicity or host range

The pathogenicity of the GMOs are diminished because the deletions in glycoprotein genes (*gE, gI, gG,* and *gpX*) limit their ability to infect cells (61, 64, 68, 71, 72). Their host range should not be different from that of the parental virus strains.

12.8.3 Horizontal gene transfer and recombination with other virus

It was justified that the intertypic recombination of the vaccine virus with other pseudorabies virus is rare and the risk is considered to be acceptable (68).

12.8.4 Reverting to virulence

Experiments showed that the vaccine #23 did not revert to virulence and could not be recovered after three or four passages (68). The above GMOs possess deletions in two or more genes, thus the possibilities for them to revert to virulence by recombination with wild-type pseudorabies viruses are very low.

12.8.5 Possibility to spread to the environment

It was reported that vaccine #23 did not spread from vaccinated pigs to in-contact susceptible pigs (68). The deletions in genes should render the GMOs much reduced in viability and transmissibility.

12.8.6 Effects on local host species

Though the parental viruses can infect nearly all domesticated and wild mammals including cattle, sheep, goats, cats and dogs, the GMOs are attenuated and have very limited replication in the hosts and thus it is unlikely that they would have any significant effects on local non-target species.

12.9 Vaccines using *Salmonella typhimurium* as parental organisms (#15, 16)

12.9.1 Establishing an undesirable self-sustaining population

Mutations of the *aroA* (vaccine #15), and the *cya* and *crp* genes (vaccine #16) impairs the essential cellular functions of the GMOs. As a result, the GMOs grow more slowly, and thus it is unlikely for the GMOs to establish undesirable self-sustaining populations (42, 45).

12.9.2 Altered pathogenicity or host range

The pathogenicity of the both GMOs in vaccine #15 and #16 are diminished and both were found to be non-pathogenic to chickens as well as other tested birds and animals. The host range of the GMO in vaccine #15 should not be different from that of the parental strain. Studies performed by the manufacturer suggest that the GMO retains this tissue tropism, although it might have a diminished capacity to colonize the internal organs (42). On the other hand, vaccine #16 cannot persist in vertebrate due to its requirement for para-aminobenzoic acid (PABA) which is absent in vertebrate cells (45).

12.9.3 Horizontal gene transfer and recombination with other bacteria

The parental strains of the two GMOs are known to acquire genes through horizontal gene transfer. The GMOs should have similar tendency in carrying out recombination. However, the chance that the normal functions of the two mutated genes in vaccine #15 are restored by recombination is considered to be very low since the locations of the two genes on the chromosome are separated at great distance. One single recombination event cannot cover the entire section and double recombination in the same cell is extremely rare (42). On the other hand, it is rare but still possible for the *aroA* mutant (#16) to acquire functional *aroA* allele from wild type species by recombination and regain virulence. But it only leads to the wild type strains that are already present in the field. The probability of such reversion was estimated to be lower than 10^{-18} (45).

12.9.4 Reversion to virulence

In the experiments with successive passages in chicks conducted by the manufacturers,

no vaccine organisms were recovered after the third passage for the GMO of vaccine #15. No gross genetic changes were detected in the DNA surrounding the deletions after the last back passage (42). For vaccine #16, the GMO demonstrated no reversion to virulence through five back passages in chickens (45).

12.9.5 Possibility to spread to the environment

The GMO in vaccine #15 was continuously shed by administered birds for up to 13 weeks post-inoculation. They also appear capable of spreading to in-contact birds (42). On the other hand, the GMO in vaccine #16 did not spread from vaccinates to contacts. Studies demonstrated that the vaccine is not shed beyond 21 days after vaccination. Since the gene deletion renders it auxotrophic, the vaccine strain cannot survive in vertebrates or in the environment (45).

12.9.6 Effects on local host species

A variety of species found in Hong Kong, such as horses, cattle, pigs, dogs and cats, as well as other native mammals and birds are vulnerable to *S. typhimurium* infection. Humans are also potential host to this bacterial pathogen and thus can become carriers to spread the bacteria to local host species. However, since the GMOs are non-pathogenic and grow poorly in the natural environment, the risk to the wild mammal or bird species in Hong Kong is considered negligible.

12.10 Vaccines Using *Streptococcus equi* as the Vector - (#19)

12.10.1 Establishing an undesirable self-sustaining population

The GMO of the vaccine has been attenuated by gene deletion. The survival of the GMO in the vaccine and the wild type strains of *S. equi* in the environment were investigated. It was shown that the GMO cannot compete with other bacteria in its natural environment and that the GMO is only able to survive in its natural environmental when the conditions for bacterial growth, including the GMO itself, are limited (56).

12.10.2 Altered pathogenicity or host range

The recombinant vaccine has been shown to be non-pathogenic in horse. Based on the nature of the genetic modification, the GMO is unlikely to have its host range

altered (56).

12.10.3 Horizontal gene transfer and recombination with other bacteria

The strain was shown to be genetically stable. The possibility of recombination with wild-type organism was shown to be remote. *Streptococcus equi* has been shown to be genetically homogenous, indicating no or very limited horizontal gene transfer or influx of heterologous DNA (56).

12.10.4 Reversion to virulence

Experiments with five and six passages in horses were performed to test the virulence reversion. In the study using five passages, the gene deletion was not re-acquired as confirmed by PCR result. In the horses through which the sixth passage was made, the recombinant vaccine was completely eradicated two weeks after inoculation (56). Thus, the virus should be regarded as genetically and phenotypically stable. Recombination could take place only if the horse is already contracted with the wild-type bacteria species, leading only to the normal wild-type *S. equi* already present in the field.

12.10.5 Possibility to spread to the environment

It was demonstrated that the vaccine strain does not spread from vaccinated horses to in-contact horses (56). As the recombinant bacteria do not infect animals other than equine species, the risk for the vaccine strain to spread to the environment is negligible.

12.10.6 Effects on local host species

Hong Kong has no native equine species. Horses that are to be imported are required to go through a series of inspection and quarantine measures to ensure their healthiness. Thus, no local host species would be affected.

12.11 Vaccines using turkey herpesvirus as parental organisms (#6, 11-14)

12.11.1 Establishing an undesirable self-sustaining population

The parental virus can replicate in cells of avian origin (particularly chicken, turkey, duck and quail). The shedding of the virus can be detected in feather dander.

However, it is not reported that the Turkey Herpesvirus can spread between chickens, given that the parental strain has been used as vaccine against Infectious Bursal Disease since 1970s. Data submitted by the manufacturer showed that the recombinant virus will not spread from vaccinated chickens to in-contact chickens (30, 32, 34, 38). However, the recombinant virus, like the parental virus, can spread from vaccinated chickens to in-contact turkeys (30, 32, 34, 38).

12.11.2 Altered pathogenicity or host range

The GMO in the vaccine, like the parental HVT strain, has been shown to be non-pathogenic to all tested animals. The parental strains and the GMOs were also found to have similar host range, tissue tropism, and shed/spread capabilities (30, 32, 34, 39). This is supposed to apply also to the other GMOs with the same parental organism (34).

12.11.3 Horizontal gene transfer and recombination with other virus

In experiments performed by the manufacturer, the recombinant virus was inoculated to chickens together with either a serotype 1 or serotype 2 MD virus. The infected chickens were not sick or killed by the infection. Moreover, analysis of the DNA recovered from infected lymphocytes showed no evidence of virus recombination (30). In addition, the parental HVT has been widely used as a component of bivalent vaccines with other MD viruses with high safety over the past 30 years. These two events indicate that either there is no recombination or the recombination does not result in producing pathogenic virus (30, 32). It must be noted that an MDV-2 strain and an HVT strain have been combined in existing multivalent vaccines which have been used for years with no indication of acquiring virulence (34, 38).

As the parental virus could latently infect avian cells and integrate into host chromosome for prolonged periods, the introduction of the foreign expression cassette contained within the genetically modified virus into the host genome can theoretically take place. However, there was nothing in the manufacturer's safety studies to suggest that this conjectured recombination event is occurring and causing adverse health effects (32).

12.11.4 Reversion to virulence

The parental organism is not known to be pathogenic to any tested animal species. Back passage studies in chickens were performed by the manufacturer and showed no increase in morbidity or mortality. Reversion to virulence due to recombination has not been observed on the parental HVT (30, 32, 34, 38).

12.11.5 Possibility to spread to the environment

The GMO in the vaccine were found feather follicles, and hence it may be shed in feather dander like the parental organism (30, 32, 34, 38). If the poultry dust from farms rearing vaccinated chickens is not properly disposed, the recombinant virus could spread to quails or other birds in Hong Kong.

12.11.6 Effects on local host species

The GMOs and their parental strains are shed in feather dander and were demonstrated to be capable of spreading from vaccinated chickens to in-contact turkeys. Since quails and ducks are also susceptible to HVT, the recombinant virus may spread to these birds. Theoretically the native quails and migratory ducks may be susceptible to the recombinant virus. However, as the vaccine is non-pathogenic to all bird species, and the dissemination of the parental strains and the GMOs are limited to in-contact birds, the risk to the wild bird species in Hong Kong are considerable very low.

12.12 Vaccine using vaccinia virus as parental organism (#27)

12.12.1 Establishing an undesirable self-sustaining population

The parental vaccinia virus has a board host range and can affect a number of mammal and bird species. However, the parental strain being used is an attenuated phenotype with inactivated thymidine kinase gene which compromises the growth of the parental strains and the GMO. The GMO has been shown to be non-pathogenic and transmitted poorly. Therefore, it is unlikely to establish an undesirable self-sustaining population (79).

12.12.2 Altered pathogenicity or host range

The parental vaccinia virus is considered a “laboratory virus” with no natural host, but

it can infect an extremely board range of mammals and birds. The GMO is supposed to retain the host range of the parental virus. The GMO was found to be non-pathogenic to all tested animals. Inoculation of the GMO caused only mild localised inflammation or typical pox lesion at the inoculation site. As compared to the parental strains, the intensity of the cutaneous reaction was less pronounced with the GMO (79).

12.12.3 Horizontal gene transfer and recombination with other bacteria

Recombination of the GMO with another (ortho-)poxvirus might take place on rare occasions in case if there is simultaneous infection of the same host cell. The likelihood of a recombination generating a more virulent progeny virus is considered even lower. As compared to the parental virus, the insertion of rabies G-glycoprotein gene should not cause an alleviated risk of reversion of virulence (79).

12.12.4 Reversion to virulence

The genetic stability of the GMO was checked throughout seven passages in mice and no evidence was found for genomic breakdown or for change in biological properties of the GMO. In another study, the GMO was not isolated after the third and fourth passage in through mice brains (79).

12.12.5 Possibility to spread to the environment

Transmission of the GMO was found to be rare, although there had been cases of transmission between close-contact individuals in raccoons, such as between pair-bonded individuals and from mother to offspring (79).

12.12.6 Effects on local host species

The parental strain of the GMO has a wide host range and hence the GMO may infect a lot of local mammals and birds. However, the transmission of the GMO is rare and it is also non-pathogenic to most tested animals. The effects of the GMO on the local host species may thus be negligible.

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 the target animals are all domesticated, spreading to the environment would be limited. The GMO assessed generally are 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 Vaccine using Bovine Herpes Virus type 1 as parental organisms - (vaccine #5)

The GMO of this LRVV is not transmissible and has been much attenuated. The overall risks of the LRVV to local biodiversity are thus considered low and acceptable.

14.2 Vaccine using Bovine Viral Diarrhoea Virus (type 1 and 2) as parental organism - (vaccine #4)

The GMOs in this LRVV are much attenuated and not transmissible. The overall risks of the LRVV to local biodiversity are thus considered low and acceptable.

14.3 Vaccines Using Canarypox Virus ALVAC as parental organism – (vaccines # 1 - 3, 17, 18, 20)

The ALVAC virus does not replicate nor spread from the administered mammals. The GMOs were also shown to cause only mild lesions in canary bird which is the natural host of the parental virus. Based on the above risk assessment, it is concluded that the potential risk of ALVAC-based recombination vaccines to biodiversity is low and acceptable.

14.4 Vaccines using *E. coli* as parental organisms - (vaccine #8, 26)

14.4.1 Vaccine #8

In view of the fact that the GMO was rendered non-pathogenic, it is considered the potential risk of the LRVV to biodiversity is low and acceptable.

14.4.2 Vaccine #26

The GMO is non-pathogenic and is modified from a non-virulent laboratory strain. It is considered the potential risk of the LRVV to biodiversity is low and acceptable.

14.5 Vaccines using fowlpox virus as parental organisms – (vaccine #9, 10)

The GMOs and their parental strains have narrow host range. The viruses are not

shed from chicken vaccinated with the LRVVs. Naturally the parental strains are non-pathogenic and also spread very slowly. The overall risks of these LRVVs to local biodiversity are thus considered low and acceptable.

14.6 Vaccine using human adenovirous type 5 as parental organism - (vaccine #28)

The parental organism and the GMO have a narrow host range and have no pathogenic effect on most animal species. The risk of this LRVV to the local biological diversity is considered low and acceptable.

14.7 Vaccine using Newcastle disease virus as parental organism – (vaccine #7)

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

14.8 Vaccines using pseudorabies virus as parental organisms - (vaccine #21-25)

The five LRVVs contained genetically attenuated strains with poor survival in the environment. It is considered that the potential risk of pseudorabies-based recombination vaccines to biodiversity is low and acceptable.

14.9 Vaccines using *Salmonella typhimurium* as parental organisms - (vaccine #15, 16)

The only biosafety risk that theoretically may be expected is the recombination of the GMO in the LRVVs with wild type *S. typhimurium*, which would result only in the presence of the normal wild type bacteria that is already present. Thus the risk of the LRVVs to the local biological diversity is considered low and acceptable.

14.10 Vaccines Using *Streptococcus equi* as parental organism - (vaccine #19)

Based on the above risk assessment, it is concluded that the potential risk of GMO in the *S. equi*-based LRVV to biodiversity is low and acceptable. The current inspection and quarantine measures imposed on imported horses should be adequate

for preventing the virulent strain of the bacteria from entering into Hong Kong. It thus reduces the risk of reversion to virulence by recombination.

14.11 Vaccines Using Turkey Herpesvirus as the Vector - (vaccine #6, 11-14)

The GMOs in the LRVVs and the parental viruses could be shed by vaccinated chicks and persist in the environment in dust for prolonged periods. 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.

14.12 Vaccine using vaccinia virus as parental organism – (vaccine #27)

The parental strain being used is an attenuated phenotype with inactivated thymidine kinase gene which compromises the growth of the parental strains and the GMO. The GMO has been shown to be non-pathogenic and transmitted poorly. The overall risks of this LRVV to local biodiversity are thus considered low and acceptable.

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