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

Expert Group

Risk Assessment and Disposal of

Live Recombinant Veterinary Vaccines

Purpose

This paper briefs members on the current status of live recombinant veterinary vaccines and their possible adverse effect on the local biodiversity, and invite members' view on the recommendation for the disposal of the live recombinant veterinary vaccines.

Background

2. Live recombinant veterinary vaccines 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. The vaccines are attenuated and genetically defined live vaccines, which have definite, non-reverting mutations or deletions, for veterinary uses.

3. The attenuation was achieved through expressing the antigen-encoding genes isolated from pathogenic strains in non-virulent strains. It can also be achieved through deleting the disease-causing genes in the pathogenic strains, rendering them non-virulent.

4. Live recombinant veterinary vaccines are GMOs subject to the regulation under the Genetically Modified Organisms (Control of Release) Ordinance Cap.607 (the Ordinance). Currently, these vaccines have not been registered in Hong Kong as pharmaceutical products under the Pharmacy and Poisons Ordinance Cap.138. 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.

5. Vaccination with live microorganisms may lead to the shedding or spreading of the administrated microorganisms into the environment. The shed microorganisms may grow or reproduce and bring about adverse impact on the environment. Therefore, administration or import with the purpose of administration of live recombinant veterinary vaccines would in effect be considered as release of GMOs into the environment which requires prior approval from the Director of Agriculture, Fisheries and Conservation under the Ordinance.

Risk Assessment

6. In view of the rapid development in the production of live recombinant veterinary vaccines and the potential application of such vaccines in Hong Kong, a risk assessment was undertaken to assess the possible adverse biosafety effect of the commercially available strains of live recombinant veterinary vaccines on the local environment.

7. There are a number of potential adverse biosafety effects that could be resulted from the administration of the live recombinant veterinary vaccines, 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.

8. Based on the risk assessment, it is concluded that the risk to the biological diversity of the local environment posed by the live recombinant veterinary vaccines is acceptable. The detailed risk assessment report is attached at Annex.

Disposal of Live Recombinant Veterinary Vaccines

9. Under the Ordinance, a person must not knowingly release any unapproved GMO. Anyone who has control of a GMO knows that the GMO is unapproved and has been released into the environment shall also inform the Director of the release. Contravention of the above requirements may be liable to prosecution under the Ordinance.

10. At present, live recombinant veterinary vaccines are not approved or

exempted GMOs. Therefore, the above restrictions apply to these kinds of vaccines. However, the risk assessment undertaken indicates that the risk posed by the vaccines to the biological diversity of the local environment is acceptable. On the other hand, it is necessary to cater for the need of application of veterinary vaccines in emergency situations. It should be noted that the Pharmacy and Poisons Regulations (Cap.138A) exempts the registration of veterinary vaccines to be used for the purpose of treatment by a registered veterinary surgeon of a particular animal.

11. Section 46 of the Ordinance empowers the Secretary for the Environment to exempt any GMO from the application of section 5 (Restrictions on release into environment and maintenance of lives of GMOs), 7 (Restrictions on import of GMOs intended for release into environment) or 23 (Restrictions on export of GMOs intended for release into environment) if the Secretary is satisfied that the possible adverse biosafety effect that may result from the exemption is acceptable or manageable. The Ordinance also provides that an exemption may take effect generally or for any purposes or by reference to any circumstances, and either conditionally or unconditionally.

12. Considering the above, it is recommended to grant exemption to live recombinant veterinary vaccines from the application of section 5 and 7 of the Ordinance, provided that the live recombinant veterinary vaccines are registered with the Pharmacy and Poisons Board, or imported/administrated for the purpose of treatment by a registered veterinary surgeon of a particular animal.

Advice Sought

13. Members are invited to note the detail risk assessment report of live recombinant veterinary vaccines at Annex and comment on the proposed exemption of live recombinant veterinary vaccines under the Ordinance.

Agriculture, Fisheries and Conservation Department
June 2011

Annex

Risk Assessment Report

Live Recombinant Veterinary Vaccines

Purpose

Genetically modified or live recombinant veterinary vaccines 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 live recombinant veterinary vaccines 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.

Identities of the GMOs

Based on the information from the Biosafety Clearing-House, there are 16 strains of commercially available live recombinant veterinary vaccines. The target animals and diseases of the vaccines are listed in Table 1 below:

Table 1: The commercially available live recombinant vaccines for veterinary uses

#	Commercial Name	Target Animal	Target Disease
1	Proteqflu / Proteqflu-TE	Horse	Equine Influenza / + Tetanus
2	Recombitek Equine Influenza	Horse	Equine Influenza
3	Recombitek Equine WNV	Horse	West Nile Virus
4	Recombitek Canine Distemper	Dog	Distemper

5	Purevax Recombinant Leukaemia Vaccine	Cat	Feline Leukaemia
6	Purevax Feline Rabies	Cat	Feline Rabies
7	Equilis StrepE	Horse	*Streptococcus equi* Infection (Strangles)
8	PreveNile	Horse	West Nile Virus
9	AviPro® Megan® Vac 1	Chicken	*Salmonella* Infection
10	Poulvac ST	Chicken	*Salmonella* Infection
11	Vaxxitek HVT+IBD	Chicken	Infectious Bursal Disease
12	Innovax-ILT	Chicken	Marek's Disease
13	Innovax-ND-SB	Chicken	Marek's Disease, Newcastle Disease
14	Nobi-Porvac Aujeszky	Pig	Aujeszky Disease
15	Suvaxyn Aujeszky	Pig	Aujeszky Disease
16	Poulvac *E. coli*	Chicken	Pathogenic *E. coli* Infection

Besides, various similar strains of live recombinant veterinary vaccines have been developed and approved for use in China.

Recipient Organisms

Canarypox Virus - (for Vaccines # 1 - 6)

Canarypox virus belongs to the avipoxvirus family. It is a large, enveloped, double stranded DNA virus of which canary is the natural host (2). The ALVAC strain used in the preparation of the vaccines listed above was developed from an infectious strain attenuated by 200 serial passages on chick embryo fibroblasts and four successive plaque purifications. 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 (3).

Streptococcus equi - (for Equilis StrepE)

It is the bacterial pathogen that causes the strangle disease in equine species (horses, donkeys, mules and zebras). It normally does not impose any danger to humans or other domestic species (4).

Human Yellow Fever (YF) Virus - (for PreveNile)

It is an arthropod borne virus of the Flaviviridae family and the disease it caused can be fatal to human. Like other members of the Flaviviridae family, such as dengue and Japanese encephalitis viruses, this virus is transmitted by competent mosquito vectors (*Aedes* spp.) and is a risk in tropical parts of Africa and South America (5). The virus strain 17D used as the parental organism is an attenuated vaccine strain that has been widely used around the world, including Canada. This virus strain has no replication activity in live mosquito cells (6).

Salmonella typhimurium - (for AviPro® Megan® Vac 1 and Poulvac ST)

It is a bacterial pathogen that can infect a variety of domestic animals including chickens, horses, cattle, pigs, dogs and cats. It is also a leading cause of human gastroenteritis. The parental strain χ3761 *S. typhimurium* UK-1 isolated from a moribund horse in USA is virulent to young chicks and have an LD50 value of $3x10^3$ colony forming units (CFU) for one-day-old chicks (7, 8).

Turkey Herpesvirus - (for Vaxxitek HVT+IBD, Innovax-ILT and Innovax-ND-SB)

Turkey Herpesvirus (HVT) (FC-126 strain) is an enveloped double-stranded DNA virus that was originally isolated from domestic turkeys in the late1960s. It is a non-pathogenic 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) (9, 10).

Aujeszky's Disease Virus - (for Nobi-Porvac Aujeszky and Suvaxyn Aujeszky)

Pseudorabies (Aujeszky's Disease) Virus is an alphaherpesvirus that naturally infects pigs and causes the Aujeszky's Disease. The virus can also infect nearly all domesticated (including cattle, sheep, goats, cats and dogs) and wild mammals. However, it does not infect humans (11).

Escherichia coli - (for Poulvac *E. coli*)

Although non-pathogenic *E. coli* are normally found in the intestines of poultry, certain strains of *E. coli* will generate extra-intestinal infections, or colibacillosis, in

chickens. Colibacillosis is frequently associated with poor animal husbandry, and is a common secondary infection following bacterial or viral infection. The parental *E. coli* strain was isolated from a clinical case of avian colibacillosis. This strain is pathogenic to chickens, as it was shown to be invasive and capable of persisting for at least five weeks in specific pathogen-free (SPF) chicks (48).

Donor Organisms

Equine Influenza Virus - (for Proteqflu/Proteqflu-TE and Recombitek Equine Influenza)

The disease Equine Influenza caused by this virus is endemic to members of the Equidae family (i.e. horses, donkeys, mules and zebras) (12).

West Nile Virus - (for Recombitek Equine WNV and PreveNile)

It can affect many different species of animals. Humans are also susceptible to this virus. It is transmitted by bites of infected mosquitoes (13).

Canine Distemper Virus - (for Recombitek Canine Distemper)

It is a virus that infects dogs and other mammals, including ferrets and raccoons. But humans are not affected by this virus (14).

Feline Leukaemia Virus - (for Purevax Recombinant Leukaemia Vaccine)

It is a retrovirus that prominently infects cats. It is a common cause of cancers and various blood disorders in cats. There is no evidence showing that the virus can be transmitted from cats to humans (15).

Feline Rabies Virus - (for Purevax Feline Rabies)

It is transmitted through the saliva of infected animals. Humans can be infected by this virus (16).

Infectious Bursal Disease Virus - (for Vaxxitek HVT+IBD)

It principally affects chickens. But turkeys, ducks, and some other species of domestic

and wild birds can also be infected by this virus (17).

Laryngotracheitis Virus - (for Innovax-ILT)

Infectious Laryngotracheitis Virus causes respiratory diseases in chickens. It can also infect turkeys, pheasants, peafowl and perhaps other avian species (18).

Newcastle Disease Virus - (for Innovax-ND-SB)

It causes a contagious and fatal disease that affects most species of birds (19).

Chicken Herpesvirus - (for Innovax-ND-SB)

Chicken Herpesvirus (Marek's Disease Virus) is mainly found on chickens, but can also affect pheasants, quails, game fowls and turkeys (20).

Vectors

ALVAC® Vector for Vaccines # 1 - 6

It is derived from the non-disease causing Canarypox Virus and is considered to be the 'backbone' of ALVAC® vaccines. It is genetically stable and allows for the insertion of large amounts of foreign DNA. It was demonstrated that the ALVAC® vector is only capable of replication within certain avian species and not at all in mammals. Studies showed that the abortive step to viral replication occurs early in the life cycle of the virus with only early proteins being synthesized following infection of a cell (21).

Vectors for PreveNile

The vaccine was constructed by a two-plasmid system. Plasmid YF5'3'IV WN prME encodes the 5' UTR of Yellow Fever capsid, West Nile Virus prM gene, 5' end of West Nile Virus E gene and the 3' end of Yellow Fever NS5 and UTR. Plasmid YFM5.2 WN encodes the second half of West Nile Virus E gene and the non-structural genes of Yellow Fever NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5. West Nile prME gene fragments were amplified by RT-PCR and sub-cloned into the two-plasmid system by overlap-extension PCR. Silent Eag I and Bsp EI sites were introduced for *in vitro*

ligation steps necessary to create a full-length cDNA before *in vitro* transcription. Naked RNA initiates productive infection after transfection of a Vero cell line (22).

Vectors for AviPro® Megan® Vac 1

No vector was constructed specifically for this GMO. Bacteriophage P22HT *int* was used for transduction with standard methods and media. A library of *S. typhimurium* mutants was generated by random insertion of DNA transduced by the phage. Fusaric acid selection was then used for the desired deletion mutations (7).

Vector for Equilis StrepE

The vaccine strain was constructed by the electroporation of gene knock-out constructs and gene deletion (±1kb) constructs. Thus, the vaccine strain has no vector-derived antibiotic resistance markers or other foreign DNA (23).

Vector for Vaxxitek HVT+IBD

The vaccine was generated by inserting an IBDV VP2 gene expression cassette, which consists of a mammalian virus promoter/enhancer, DNA sequence encoding the VP2 capsid protein of IBDV, and a mammalian virus polyadenylation signal, into the HVT genome (10).

Vectors for Poulvac ST, Innovax-ILT & Innovax-ND-SB

Information is not available.

Vector for Poulvac *E. coli*

The mutant *aroA* gene was sub-cloned into a mobilized suicide vector (SacB, pKNG101), which was transferred to the parental pathogenic *E. coli* strain via conjugation (48, 49).

Insert and Modification

Proteqflu / Proteqflu-TE

The vaccines contain two vaccine strains vCP2242 and vCP1533. The

Haemagglutinin (HA) genes from Equine Influenza Virus A/equi-2/Ohio/03 (for vCP2242) and Equine Influenza Virus A/equi-2/Newmarket/2/93 (for vCP1533) were cloned into the ALVAC® vector and subsequently introduced into ALVAC, the non-disease causing strain of Canarypox Virus (24).

Haemagglutinin is a kind of antigenic glycoproteins found on the surface of influenza virus. Glycoproteins are important integral membrane proteins that play an important role in cell to cell interactions. Haemagglutinin is required by the virus to attach to host cells. The host's immune response is usually induced by such kinds of surface antigens (25).

Recombitek Equine Influenza

The haemagglutinin coding sequence from Equine Influenza Virus (EIV), either strain Kentucky/94 or Newmarket/2/93, was inserted into ALVAC (26).

Recombitek Equine WNV

Gene sequence from West Nile Virus was selected from those likely to stimulate protective immunity in horses and cloned into the ALVAC® vector and subsequently introduced into ALVAC (27).

Recombitek Canine Distemper

The haemagglutinin (HA) and fusion (F) protein genes from Canine Distemper Virus (CDV), flanked by the Vaccinia Virus H6 promoter, are introduced into ALVAC (28).

Fusion protein is a kind of glycoproteins found in the envelope of the virus. It mediates the fusion of the viral membrane with the host cell membrane (29).

Purevax Recombinant Leukaemia Vaccine

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 (30).

Env protein encoded by this gene is part of the viral envelope covering the viral core.

It is responsible for receptor binding and fusion with the host membrane. It can elicit host's antibody responses (29). The gene *gag* encodes for the structural protein which forms the skeleton of the viral core (31). The *pol* gene encodes for the RNA-dependent DNA polymerase. It is the enzyme produced by the RNA virus and is responsible for converting the viral RNA genome into DNA, which can be integrated into the host genome or used for protein production.

Vaccinia Virus H6 promoter is a DNA sequence essential for the transcription of the recombinant protein. It is obtained from the double stranded DNA virus Vaccinia Virus (30).

Purevax Feline Rabies

The rabies glycoprotein gene, flanked by the Vaccinia Virus H6 promoter, was introduced into ALVAC (32).

The glycoprotein protein is an accessory protein that promotes cell fusion during infection (33).

Equilis StrepE

The *aroA* gene was deleted from the disease-causing bacteria *S. equi*. The gene encodes for the 5-enolpyruvylshikimate 3-phosphate synthase involved in the biosynthesis of aromatic amino acids, which is required for the bacteria to grow (34).

PreveNile

The premembrane (prM) and envelope (Env) genes of the West Nile Virus were introduced into a live attenuated Human Yellow Fever Virus (6).

The premembrane is important for the correct conformational folding of the envelope (29).

AviPro® Megan® Vac 1

The *cya* and *crp* genes were deleted from the genome of χ3761 *S. typhimurium* UK-1 (7).

The *cya* gene encodes for adenylate cyclase and *crp* encodes for the cyclic adenosine monophosphate (cAMP) receptor protein. Both are key enzymes involved in the cAMP biosynthesis. cAMP is an important second messenger for intracellular signal transduction and is essential for the proper cell functioning (16).

Poulvac ST

The *aroA* gene from *S. typhimurium* LT2 strain was mutated and transduced into a wild type parental strain using bacteriophage (35).

Vaxxitek HVT+IBD

Turkey Herpesvirus (HVT) was modified by expressing the VP2 protein from Infectious Bursal Disease Virus (IBDV) (10).

VP2 protein is the major core proteins of IBDV and is identified as the major antigen to elicit hosts' neutralizing antibodies (36).

Innovax-ILT

Genes (unknown) from Laryngotracheitis Virus were introduced into Turkey Herpesvirus (37).

Innovax-ND-SB

An unknown gene from Newcastle Disease Virus and the SB-1 strain of Chicken Herpesvirus serotype 2 were introduced into Turkey Herpesvirus (38)

Nobi-Porvac Aujeszky

The glycoprotein gI gene and thymidine kinase gene are deleted from Aujeszky's Disease Virus (39).

Thymidine kinase is the enzyme required in the production of the DNA building block thymidine triphosphate (TTP). It is thus essential for the virus reproduction (40).

Suvaxyn Aujeszky

The glycoprotein E gene and thymidine kinase gene are deleted from Aujeszky's Disease Virus (41).

Poulvac *E. coli*

The mutant *aroA* gene was created by separately polymerase chain reaction (PCR) amplifying the 5' and 3' portions of the wild type gene (omitting a 100 base pair (bp) region in the centre of the gene) and ligating these two PCR products together into a shuttle plasmid. The resultant *aroA* gene was thereby inactivated by the deletion of the internal 100 bp region, and also by the incorporation of two stop codons via the internal PCR primers (48).

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

Vaccines # 1 - 6

The host ranges of the GMOs are expected to be the same as the parental organisms. The genetic modifications would not enhance the virulence or the ability to survive in target or non-target species.

PreveNile

The host range of the recombinant vaccine organism appears to be similar to the backbone vaccine strain, which is also not transmissible by mosquitoes (6).

AviPro® Megan® Vac 1

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 utilize 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 (LD50 value of $3x10^3$ CFU), the Δ*cya* Δ*crp S. typhimurium* strain has

lost the ability to cause disease, and has an LD50 value estimated to be greater than $4x10^9$ CFU in one-day-old chicks (7, 8).

Poulvac ST

The parent strain infects many species and the mutant vaccine strain can theoretically do the same. However, the latter cannot persist in vertebrates due to its requirement for para-aminobenzoic acid (PABA) (35).

Vaxxitek HVT+IBD

The recombinant HVT + IBD virus differs genetically from the parental HVT by the integration of the expression cassette described in section 4.2. The foreign DNA is inserted into what is thought to be a non-coding region of the HVT genome, and hence presumably does not disrupt any endogenous genes. The genetically modified organism does not contain any selectable markers such as antibiotic resistance genes. DNA sequencing of the recombinant HVT + IBD virus by the manufacturer determined that the recombination event between the donor plasmid and wild type parental HVT was perfectly homologous (10).

Innovax-ILT

The host range, tissue tropism, and shed / spread capabilities of the recombinant organism are expected to be the same as the parental HVT vaccine strain (37).

Innovax-ND-SB

The host range, tissue tropism, and shed/spread capabilities of the recombinant organism are expected to be similar to the parental HVT vaccine strain (38)

Nobi-Porvac Aujeszky

The evidence from testing indicated in particular that the genetic modification of the virus, was not expected to result in any post-release shift in biological interactions or host range or in any known or predictable effects on non-target organisms in the environment or other potentially significant interaction with the environment or in any increase in pathogenicity as compared to the parental virus strain (39).

Suvaxyn Aujeszky

The absence of the thymidine kinase gene in the Aujeszky's Disease virus increases

the GMO's 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 (41).

Poulvac *E. coli*

Due to the mutation of the *aroA* gene, the modified *E. coli* in the vaccine shows an impaired ability to persist in chickens and is consequently non-pathogenic. The *aroA*-mutant vaccine strain retains the expression of the surface appendages that have been shown to be important in the pathogenesis of avian colibacillosis, such as type 1 fimbriae and flagellae, which might aid in the generation of a robust and specific immune response (48).

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).

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.

Likely Potential Receiving Environment

Vaccines Using Canarypox Virus ALVAC as the Vector - (Vaccines # 1 - 6)

The parental poxvirus can survive for extended period of time under normal environmental conditions, in particular when it is associated with dried scab material or protected from direct sunlight. The recombinant virus is expected to have same survival characteristics as the parental poxvirus (30).

Vaccine Using *Streptococcus equi* as the Vector - (Equilis StrepE)

When exposed to natural environment (e.g. under direct exposure to sunlight), the parental strain can survive for a day or two. The survival of the bacteria may last for a

little bit longer if protected from sunlight or kept at low temperature, but it is probably still fairly short. The gene deletion is expected to weaken the capability of the recombinant bacteria to persist in the environment (42).

Vaccine Using Human Yellow Fever Virus as the Vector - (PreveNile)

The recombinant virus can stay alive for prolonged periods at -70^{o}C and 4^{o}C, but the viability is greatly reduced at room temperature or above (6).

Vaccines Using Turkey Herpesvirus as the Vector - (Vaxxitek HVT+IBD, Innovax-ILT and Innovax-ND-SB)

Herpesviruses are destructible by UV light from the sun. But HVT in dried feathers and poultry dust can remain infectious for up to a year. It is expected that the recombinant virus should have similar survival characteristics with the parental virus (10).

AviPro® Megan® Vac 1

The GM bacterium reportedly grows slower than the wild type parental organism, and has lost the ability to metabolize alternative carbohydrate sources. These defects and others induced by the gene deletions should hinder the persistence of the vaccine organism in the environment. It was described by the manufacturer as being sensitive to the antibiotics amikacin, ampicillin, carbenicillin, cefoxitin, cephalotin, chloramphenicol, gentamicin, tetracycline, tobramycin, trimethoprim sulfate, kanamycin, neomycin and streptomycin. The Δ*cya* Δ*crp S. typhimurium* should retain sensitivity to these same antibiotics (7).

Poulvac ST

Since the gene deletion renders it auxotrophic, the vaccine strain cannot survive in vertebrates or in the environment (35).

Vaccines Using Aujeszky's Disease Virus as the Vector

The parental virus can survive for extended periods under winter conditions below 4^{o}C. But it can be rapidly inactivated at 37^{o}C in sunlight and in dry conditions. It can remain infectious at pH5-9, though extreme acidity and alkalinity have deleterious effects on the virus. Flavoured environments, such as contaminated straw and feeding

troughs, can sustain the virus for 10-30 days at 24°C and for up to 46 days at -20°C. The persisting properties of the recombinant virus should be similar to that of the parental strain (43).

Poulvac *E. coli*

In back passage studies, the *aroA* deleted vaccine was unable to survive three back passages due to the inability to generate p-aminobenzoate (PABA) for continued survival and generate a self-sustaining population in the environment, thus eliminating the risk to other vertebrates that may have come in contact with the vaccine (50).

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

Evaluation of the Likelihood of the Adverse Effect Being Realized

Vaccines Using Canarypox Virus ALVAC as the Vector - (Vaccines # 1 - 6)

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 (24). These ALVAC-based 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.

Altered pathogenicity or host range

The safety of several recombinant ALVAC vaccines (Canine Distemper, Feline Rabies, Feline Leukaemia, Equine Influenza and Equine WNV) has been tested in canary birds in comparison with the original ALVAC vaccine. Similar tests were conducted on chicken 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 chicken and mice (3).

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 (44, 45). It was reported that all the genetically modified viruses tested to date were as safe as the parental strain and no change of host specificity was observed.

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 can 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 minimizes the risk of reverting to virulence by recombination (46). Thus, it is unlikely for the horizontal gene transfer and recombination to take place.

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). And the recombinant virus is considered to be genetically and phenotypically stable as no alternation was detected in an experiment with 20 cell culture passages (30). Thus the virulence reversion is unlikely to happen.

Possibility to spread to the environment

No virus shedding was detected from samples of saliva, urine and faeces collected from administered kitten (30). Thus, the likelihood of spreading to non-target animals is very low.

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.

Vaccines Using *Streptococcus equi* as the Vector - (Equilis StrepE)

Establishing an undesirable self-sustaining population

The attenuation of the recombinant bacteria is achieved by deleting the *aroA* gene which is essential for cell replication. It is thus expected that the recombinant bacteria could not form an undesirable self-sustaining population (47).

Altered pathogenicity or host range

The recombinant vaccine is regarded as non-pathogenic. Based on the nature of the genetic modification, the recombinant bacteria is unlikely to have its host range altered (47).

Horizontal gene transfer and recombination with other bacteria

It is possible that the GM bacteria could recombine with wild type *S. equi* or other homologous species, but it would only lead to the wild type species that is already present in the field (34).

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 deleted *aroA* gene 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 (34). 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.

Possibility to spread to the environment

It was demonstrated that the vaccine strain does not spread from vaccinated horses to in-contact horses (34). And because the recombinant bacteria do not infect animals other than equine species, the risk for the vaccine strain to spread to the environment is negligible.

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.

Vaccines Using Human Yellow Fever Virus as the Vector - (PreveNile)

Establishing an undesirable self-sustaining population

According to studies, the replication of the recombinant virus is very limited in the administered horses. The virus will not be transmitted to animals other than the intended targets, as no virus shedding or spreading was detected after vaccination. The recombinant virus is also not transmissible by mosquitoes (6). Thus, the virus is unlikely to establish an undesirable self-sustaining population.

Altered pathogenicity or host range

There have been *in vivo* and *in vitro* studies showing that the virulence properties and host range specificity of the recombinant virus are similar to those of the backbone Yellow Fever Virus strain (6).

Horizontal gene transfer and recombination with other virus

Intertypic recombination of Dengue Virus, which belongs to the same *Flaviviridae* family as the parental Yellow Fever Virus, was observed infrequently during extended periods of high viremia in humans. Interspecific recombination has not been reported in the literature. Since the virus has very limited replication in the horses and the viremia following West Nile Vrius infection is typically of low titre and short duration, recombination between West Nile Virus and the recombinant vaccine is unlikely to take place (6).

Reversion to virulence

Back passage attempts have demonstrated the genetic and phenotypic stability of the recombinant virus (6). The recombinant virus is unlikely to revert to virulence.

Possibility to spread to the environment

The recombinant virus has no shedding in horses and thus is not likely to spread to the environment (6).

Effects on local host species

Human is susceptible to the wild type Yellow Fever Virus. This recombinant virus is regarded to be as safe to human health as the backbone Yellow Fever vaccine strain, which has been widely used in the world since 1937 (6).

Vaccines Using Turkey Herpesvirus as the Vector - (Vaxxitek HVT+IBD, Innovax-ILT and Innovax-ND-SB)

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 chicken, given that

the virus 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. However, the recombinant virus, like the parental virus, can spread from vaccinated chickens to in-contact turkeys (10).

Altered pathogenicity or host range

Since only a gene with known function is added and the insertion site is at a non-coding region of the HVT genome, it is expected that the genetic modification would not alter the recombinant vaccine's pathogenicity or host range (10).

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. 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 (10). As the parental virus could latently infect avian cells and integrate into host chromosome for prolonged periods, recombination with other viruses can theoretically take place.

Reversion to virulence

A back passage study with nine passages in chickens was performed by the manufacturer and showed no increase in morbidity or mortality. Thus, the recombinant virus is considered to be phenotypically and genetically stable. Reversion to virulence due to recombination has not been observed on the parental HVT (10).

Possibility to spread to the environment

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.

Effects on local host species

The virus is shed in feather dander and was demonstrated capable of spreading from

vaccinated chickens to in-contact turkeys. Since quails are also susceptible to HVT, the recombinant virus may spread to these birds. There are three quail species: *Coturnix japonica* (Japanese Quail), *Turnix suscitator* (Barred Button-quail) and *Turnix tanki* (Yellow-legged Button-quail) found in Hong Kong, and theoretically they are susceptible to the recombinant virus. However, as the vaccine is non-pathogenic, the risk to the wild quail species in Hong Kong is considerable very low.

Vaccines Using *Salmonella typhimurium* as the Vector - (AviPro® Megan® Vac 1 and Poulvac ST)

Establishing an undesirable self-sustaining population

Deletion of the *aroA*, *cya* and *crp* genes impairs the essential cellular functions of the recombinant bacteria. As a result, the bacteria grow more slowly and are incapable to invade and infect hosts. Therefore, it is unlikely for the recombinant bacteria to establish an undesirable self-sustaining population (7).

Altered pathogenicity or host range

The pathogenicity of the recombinant bacteria is diminished. Its host range should not be different from that of the parental bacteria.

Horizontal gene transfer and recombination with other bacteria

The parental bacteria are known to acquire genes through horizontal gene transfer. The recombinant bacteria should have similar tendency in carrying out recombination.

Reversion to virulence

In the experiments with successive passages in chicks conducted by the manufacturer, no gross genetic changes were detected in the DNA surrounding the deletions. It thus confirmed the genetic and phenotypic stability of the two recombinant bacteria. Reversion to virulence of the double mutant (AviPro® Megan® Vac 1, Δ*cya* and Δ*crp*) by recombination is considered not feasible. 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 (7).

On the other hand, it is rare but still possible for the *aroA* mutant (Poulvac ST) to acquire functional *aroA* allele from wild type species by recombination and regain virulence. But it only leads to the wild type species that is already present in the field.

Possibility to spread to the environment

The recombinant bacteria were continuously shed by administered birds for up to 13 weeks post-inoculation. They also appear capable of spreading to in-contact birds (7).

Effects on local host species

A variety of species found in Hong Kong, such as horses, cattle, pigs, dogs and cats, are vulnerable to *S. typhimurium* infection. The wild relatives, such as *Prionailurus bengalensis* (Leopard Cat) might as well be susceptible to the 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 vaccines are non-pathogenic, the risk to the wild mammal species in Hong Kong is very low.

Vaccines Using Aujeszky's Disease Virus as the Vector

Establishing an undesirable self-sustaining population

The deletion of the essential genes, particularly the thymidine kinase that is required for DNA synthesis, would diminish the recombinant virus' ability to grow and infect the hosts. It is thus expected that the recombinant virus cannot establish a self-sustaining population (41, 43).

Altered pathogenicity or host range

The pathogenicity of the recombinant viruses is diminished. Their host range should not be different from that of the parental virus strains.

Horizontal gene transfer and recombination with other virus

It was justified that the intertypic recombination of the vaccine virus with other Aujeszky's Disease Virus is rare and the risk is considered to be acceptable (43).

Reverting to virulence

Experiments showed that the recombinant viruses did not revert to virulence and could not be recovered after 3 or 4 passages (43).

Possibility to spread to the environment

It was reported that the recombinant viruses did not spread from vaccinated pigs to in-contact susceptible pigs (43).

Effects on local host species

Though the parental virus can infect nearly all domesticated and wild mammals including cattle, sheep, goats, cats and dogs (11), the recombinant viruses 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.

Vaccines Using *E. coli* as the Vector - (Poulvac *E. coli*)

Establishing an undesirable self-sustaining population

The *aroA* defect means that the vaccine *E. coli* strain cannot survive without supplemental aromatic amino acids or PABA, which are not readily available in the environment (48).

Altered pathogenicity or host range

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

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. Further, the chances of horizontal gene transfer taking place after vaccination is significantly restricted, due to the inability of the vaccine organism to persist in an environment devoid of its requisite aromatic nutrients (48).

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 (48).

Possibility to spread to the environment

In a shed/spread study conducted by the manufacturer, swabs were taken from the feed bins, water bowls, floor, and top-front part of the cages following the vaccination of fifty chicks by coarse spray to monitor environment contamination by the vaccine organism. The vaccine organism was recovered from the environment at 3 and 7 days post-vaccination, but not after 10, 14, or 24 days, confirming the limited persistence of the *aroA* mutant *E. coli*.

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.

Evaluation of the Consequences should the Adverse Effect be Realized

- Establishing an undesirable self-sustaining population

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

- Altered pathogenicity or host range

  Since the genes modified are not relevant to the pathogenicity, it is speculated that the genetic modification would not enhance the pathogenicity. Even if pathogenicity is changed, it shall not result in significant adverse effect on Hong Kong's biodiversity. On the other hand, 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.

- 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.

- 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.

- Possibility to spread to the environment

  As the target animals are all domesticated, spreading to the environment would be limited. 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.

- Effects on local host species

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

Estimation of the Overall Risk Posed by the GMO

Vaccines Using Canarypox Virus ALVAC as the Vector - Vaccines # 1 - 6

Based on the above risk assessment, it is concluded that the potential risk of ALVAC-based recombination vaccines to biodiversity is low and acceptable.

Vaccines Using *Streptococcus equi* as the Vector - (Equilis StrepE)

Based on the above risk assessment, it is concluded that the potential risk of *S. equi*-based recombination vaccine 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.

Vaccines Using Human Yellow Fever Virus as the Vector - (PreveNile)

Based on the above risk assessment, it is concluded that the potential risk of Human Yellow Fever Virus-based recombination vaccine to biodiversity is low and manageable.

Vaccines Using Turkey Herpesvirus as the Vector - (Vaxxitek HVT+IBD, Innovax-ILT and Innovax-ND-SB)

The viruses could be shed by vaccinated chicks and persist in the environment in dust for prolonged periods. However, the pathogenicity to wild bird species in Hong Kong is considered acceptable. The overall risks are thus considered low and manageable.

Vaccines Using *Salmonella typhimurium* as the Vector - (AviPro® Megan® Vac 1 and Poulvac ST)

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

Vaccines Using Aujeszky's Disease Virus as the Vector - (Suvaxyn Aujeszky and Nobi-Porvac Aujeszky)

Based on the properties of the vaccines, it is considered that the potential risk of Aujeszky's Disease Virus-based recombination vaccines to biodiversity is low and acceptable.

Vaccine Using *E. coli* as the Vector - (Poulvac *E. coli*)

In view of the fact that the vaccine strain was rendered non-pathogenic, it is considered the potential risk of the recombination vaccine to biodiversity is low.

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Agriculture, Fisheries and Conservation Department

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