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(54) **Title:** VACCINE ACCELERATOR FACTOR (VAF) FOR IMPROVEMENT OF VACCINATIONS IN POULTRY

(57) **Abstract:** The present invention provides a vaccine accelerator factor (VAF) which is an *in ovo* nucleotide immuno-stimulant. The VAF contains one or more DNA constructs, each having a DNA molecule and a vector. Each of the DNA molecule contains one or more genes or gene fragments, each encoding an antigenic peptide of an avian virus. The VAF is preferably administered to the amniotic fluid of an egg after being fertilized for about 17-19 days. The VAF can be co-administered with a viral vaccine containing one or more attenuated or inactive avian viruses. Alternatively, the VAF can be administered prior to the administration of the viral vaccine, which is administered at hatch or post-hatch. The VAF stimulates and accelerate a protective immune response of a viral vaccine.

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VACCINE ACCELERATOR FACTOR (VAF) FOR IMPROVEMENT OF VACCINATIONS IN POULTRY

FIELD OF THE INVENTION:

The present invention relates to a vaccine accelerator factor (VAF) which is an *in ovo* nucleotides-immuno-stimulant containing one or more DNA constructs, each having a DNA molecule and a vector. Each of the DNA molecule contains one or more genes or gene fragments, each encoding an antigenic peptide of an avian virus. The VAF is preferably administered to the amniotic fluid of an egg, which has been fertilized for about 17-19 days. The VAF can be co-administered with a viral vaccine containing one or more attenuated or inactive avian viruses or a recombinant DNA vaccine. Alternatively, the VAF can be injected into egg prior to the administration of the viral vaccine. In that case, the viral vaccine is preferably administered at hatch or post-hatch. The VAF stimulates and accelerate a protective immune response of a viral vaccine against the avian virus of which the DNA molecule of the VAF contains a gene or a fragment thereof.

BACKGROUND OF THE INVENTION:

Routine vaccinations used in veterinary practice have had a highly beneficial impact on the health and welfare of livestock and companion animals. Poultry vaccines can be administered via different routes and by various methods. For example, a post-hatch spray vaccination method has been widely used. This method can mass-immunize day old chicks through aerosol spray. Also, live or attenuated vaccines can be administered to poultry through traditional method, i.e., by subcutaneous injection to chicks, rearing stock and breeders. Furthermore, poultry vaccines can be delivered via eye drops and/or intranasal routes during brooding of chicks. Finally, and

most prevalently, vaccines can be administered to poultry via drinking water. This vaccination method has the advantage of low cost, but its effectiveness, particularly against some infections, is limited due to less control of vaccination.

Most recently, some poultry vaccines have been administered to eggs through an *in ovo* injection method. A patented EMBREX INOVOJECT® system has been used to facilitate this kind of injection. The first *in-ovo* vaccination machine for use on chicken hatching eggs was developed by Embrex, Inc., of Raleigh, N.C. in the late 1980s. (See U.S. Pat. Nos. 5,056,464 and 5,699,751).

This *in-ovo* machine is currently used in about 80% of the U.S. broiler hatcheries, primarily for administering Marek's disease (MD) vaccines. The popularity of this machine, which has proven to be safe and effective in vaccination of chicks against MD, is also being used increasingly to administer infectious bursal disease (IBD) and Newcastle disease (ND) vaccines.

In ovo vaccination of virus-containing vaccines was extensively described by Sharma et al. (U.S. Pat. No. 4,458,630). In particular, it teaches that live Marek's disease virus can be injected into amniotic fluid within the egg, whereafter the embryo is infected and the vaccine virus replicates to a high titer which induces the formation of protective antibodies in the treated embryo. (See Sharma (1985), Avian Diseases 29, 1155, 1167-68).

It is well-known in the worldwide poultry business that certain viral diseases, such as Marek's disease virus (MDV), infectious bursal disease virus (IBDV), Newcastle disease virus (NDV), infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILTV), avian encephalomyelitis (AEV), chick anemia virus (CAV), Fowlpox virus (FPV), avian influenza virus (AIV), reovirus, avian leukosis

virus (ALV), reticuloendotheliosis virus (REV), avian adenovirus and hemorrhagic enteritis virus (HEV), may cause major outbreak and result in significant economic losses in the commercial poultry industry. Among them, MDV, IBDV, NDV and IBV, are particularly important due to their virulent nature.

Marek's Disease (MD) is a malignant, lymphoproliferative disorder disease that occurs naturally in chickens. The disease is caused by a herpesvirus: Marek's Disease Virus (MDV). MD is ubiquitous, occurring in poultry-producing countries throughout the world. Chickens raised under intensive production systems will inevitably suffer losses from MD. The symptoms of MD appear widely in the nerves, genital organs, internal organs, eyes and skin of the infected birds, causing motor trouble (due to paralysis when the nerves have been affected), functional trouble of the internal organs (due to tumors), and chronic undernourishment (if the internal organs are attacked by the virus). MD affects chickens from about 6 weeks of age, occurring most frequently between ages of 12 and 24 weeks.

At of this time, there are no methods of treating MD. The control of the disease is based primarily on management methods such as insolating growing chickens from sources of infection, the use of genetically resistant stock, and vaccination. However, management procedures are normally not cost-effective and the progress has been disappointing with respect to the selection of poultry stock with increased genetically controlled resistance. Nowadays, control of MD is almost entirely based on vaccination.

Infectious bursal disease virus (IBDV) is responsible for a highly contagious immunosuppressive disease in young chickens which causes significant losses to the poultry industry worldwide (See Kibenge (1988), J. Gen. Virol., 69:1757-1775).

Infection of susceptible chickens with virulent IBDV strains can lead to a highly contagious immunosuppressive condition known as infectious bursal disease (IBD). Damage caused to the lymphoid follicles of the bursa of Fabricius and spleen can exacerbate infections caused by other agents and reduce a chicken's ability to respond to vaccination as well (See Cosgrove (1962), Avian Dis., 6:385-3894).

IBDV is a member of the Birnaviridae family and its genome consists of two segments of double-stranded RNA (See Dobos et al (1979), J. Virol., 32:593-605). The smaller segment B (about 2800 bp) encodes VP1, the dsRNA polymerase. The larger genomic segment A (about 3000 bp) encodes a 110 kDa precursor polypeptide in a single open reading frame (ORF) that is processed into mature VP2, VP3 and VP4 (See Azad et al (1985), Virology, 143:35-44). From a small ORF partly overlapping with the polypeptide ORF, segment A can also encode VP5, a 17-kDa protein of unknown function (See Kibenge et al (1991), J. Gen. Virol. 71:569-577).

While VP2 and VP3 are the major structural proteins of the virion, VP2 is the major host-protective immunogen and causes induction of neutralizing antibodies (See Becht et al. (1988), J. Gen. Virol., 69:631-640; Fahey et al. (1989), J. Gen. Virol., 70:1473-1481). VP3 is considered to be a group-specific antigen because it is recognized by monoclonal antibodies (Mabs) directed against VP3 from strains of both serotype 1 and 2 (See Becht et al (1988), J. Gen. Virol., 69:631-640). VP4 is a virus-coded protease and is involved in the processing of the precursor protein (See Jagadish et al. (1988), J. Virol., 62:1084-1087).

In the past, control of IBDV infection in young chickens has been achieved by live vaccination with avirulent strains, or principally by the transfer of maternal antibody induced by the administration of live and killed IBDV vaccines to breeder

hens. Unfortunately, in recent years, virulent variant strains of IBDV have been isolated from vaccinated flocks in the United States (See e.g., Snyder et al. (1988), Avian Dis., 32:535-539; Van der Marel et al. (1990), Dtsch. Tierarztl. Wschr., 97:81-83), which drastically undermine the effectiveness of using live vaccination for IBDV.

Efforts to develop a recombinant vaccine for IBDV have also been made, and the genome of IBDV has been cloned (See Azad et al (1985) "Virology", 143:35-44). The VP2 gene of IBDV has been cloned and expressed in yeast (See Macreadie et al. (1990), Vaccine, 8:549-552), as well as in recombinant fowlpox virus (See Bayliss et al (1991), Arch. Virol., 120:193-205). When chickens were immunized with the VP2 antigen expressed from yeast, antisera afforded passive protection in chickens against IBDV infection. When used in active immunization studies, the fowlpox virus-vectored VP2 antigen afforded protection against mortality, but not against damage to the bursa of Fabricius.

Newcastle disease virus (NDV) is an enveloped virus containing a linear, single-strand, nonsegmented, negative sense RNA genome. Typically, virus families containing enveloped single-stranded RNA of the negative-sense genome are classified into groups having non-segmented genomes (e.g., Paramyxoviridae and Rhabdoviridae) or those having segmented genomes (e.g., Orthomyxoviridae, Bunyaviridae and Arenaviridae). NDV, together with parainfluenza virus, Sendai virus, simian virus 5, and mumps virus, belongs to the Paramyxoviridae family.

The structural elements of the NDV include the virus envelope which is a lipid bilayer derived from the cell plasma membrane. The glycoprotein, hemagglutinin-neuraminidase (HN) protrude from the envelope allowing the virus to

contain both hemagglutinin and neuraminidase activities. The fusion glycoprotein (F), which also interacts with the viral membrane, is first produced as an inactive precursor, then cleaved post-translationally to produce two disulfide linked polypeptides. The active F protein is involved in penetration of NDV into host cells by facilitating fusion of the viral envelope with the host cell plasma membrane. The matrix protein (M), is involved with viral assembly, and interacts with both the viral membrane as well as the nucleocapsid proteins.

The main protein subunit of the NDV nucleocapsid is the nucleocapsid protein (NP) which confers helical symmetry on the capsid. In association with the nucleocapsid are the P and L proteins. The phosphoprotein (P), which is subject to phosphorylation, is thought to play a regulatory role in transcription, and may also be involved in methylation, phosphorylation and polyadenylation. The L gene, which encodes an RNA-dependent RNA polymerase, is required for viral RNA synthesis together with the P protein. The L protein, which takes up nearly half of the coding capacity of the viral genome is the largest of the viral proteins, and plays an important role in both transcription and replication.

The replication of all negative-strand RNA viruses, including NDV, is complicated by the absence of cellular machinery required to replicate RNA. Additionally, the negative-strand genome can not be translated directly into protein, but must first be transcribed into a positive-strand (mRNA) copy. Therefore, upon entry into a host cell, the virus can not synthesize the required RNA-dependent RNA polymerase. The L, P and NP proteins must enter the cell along with the genome on infection. Both the NDV negative strand genomes (vRNAs) and antigenomes (cRNAs) are encapsidated by nucleocapsid proteins; the only unencapsidated RNA species are

virus mRNAs. The cytoplasm is the site of NDV viral RNA replication, just as it is the site for transcription. Assembly of the viral components appears to take place at the host cell plasma membrane and mature virus is released by budding.

In U.S. Pat. No. 5,427,791, Ahmad et al. describe the embryonal vaccination against NDV, which requires the modification of the viruses through the use of ethyl methane sulfonate (EMS). However, EMS is a mutagen so that the vaccine prepared by the use of EMS is suspected to act as a mutagen as well, which is undesirable for regular administration of the vaccine. Nevertheless, without the modification with EMS, the NDV vaccine cannot be applied for *in ovo* vaccination as almost all of the embryos will die upon injection of the eggs with the unmodified virus.

Infectious bronchitis virus (IBV), the prototype of the family Coronaviridae, is the etiological agent of infectious bronchitis (IB). The virus has a single-stranded RNA genome, approximately 20 kb in length, of positive polarity, and is usually about 80-100 nm in size, being round with projecting 20 nm spikes. IBV is the causative agent of an acute, highly contagious disease in chickens of all ages, affecting the respiratory, reproductive and renal systems.

IBV contains three structural proteins: the spike (S) glycoprotein, the membrane glycoprotein, and the nucleocapsid protein. The spike glycoprotein is so called because it is present in the teardrop-shaped surface projections or spikes protruding from the lipid membrane of the virus. The spike protein is believed likely to be responsible for immunogenicity of the virus, partly by analogy with the spike proteins of other corona-viruses and partly by in vitro neutralization experiments (See, e.g., D. Cavanagh et al. (1984), Avian Pathology, 13, 573-583). There are two spike glycoproteins, which are S1 (90,000 daltons) and S2 (84,000 daltons). The polypeptide

components of the glycopolypeptides S1 and S2 have been estimated after enzymatic removal of oligosaccharides to have a combined molecular weight of approximately 125,000 daltons. It appears that the spike protein is attached to the viral membrane by the S2 polypeptide.

IBV has been wide-spread in countries where an intensive poultry industry has been developed. Young chickens up to 4 weeks of age are most susceptible to IBV, infection leading to high rates of morbidity and to mortality resulting from secondary bacterial infection. Infection also results in a drop in egg production, or failure to lay at full potential, together with an increase in the number of down-graded eggs with thin, misshapen, rough and soft-shells produced, which can have a serious economic effect.

Administering live vaccines to a developing chick in the egg (*in-ovo*) has proven to be a fast (40,000 eggs per hour), effective (100% of the eggs receive the vaccine), and labor saving ($100,000 per year per hatchery) method to vaccinate baby chicks against certain diseases before they hatch.

Recently, Embrex, Inc. has developed a live viral vaccine called VNF® (Viral Neutralizing Factor). The VNF contains an antibody (immunoglobulin) specific for the virus used in the vaccine. This specific antibody is mixed in an appropriate ratio with the vaccine virus to form a virus-antibody complex (immune complex) vaccine. The amount of the antibody in a complex vaccine is so small that it does not provide passive immunity or neutrialize the vaccine virus. On the other hand, the amount of antibody added to the vaccine virus is enough to delay by several days the normal course of vaccine virus replication.

This delayed vaccine virus replication allows for the safe *in ovo* administration of moderately attenuated vaccine viruses in young animals. Moderately attenuated

vaccine viruses are better at overcoming maternal immunity and at stimulating strong protective immune responses than highly attenuated vaccine viruses. Therefore, the virus-antibody complex vaccine technology allows for the safe hatchery use of vaccine viruses that are not eliminated by maternal antibody. Vaccination in the hatchery (either *in ovo* or at hatch) is better controlled and more uniform than field vaccination. Thus, the virus-antibody complex vaccine technology improves vaccine efficacy as well as safety.

In the invention to be presented in the following sections, immuno-stimulatory nucleotides, particularly a DNA sequence, specific for avian viral vaccines (collectively "vaccine accelerator factor" and abbreviated as "VAF") will be introduced. The VAF can be either co-administered with or separately administered from the commercially available vaccines. The VAF can be either a single or multiple DNA constructs, each containing a single DNA molecule which is a viral gene or a fragment thereof. Additionally, the VAF can be a multivalent DNA construct, which contains two or more viral genes or fragments thereof linking together in one DNA construct. The viral genes or fragments used in preparation of the VAFs are those that encode viral peptides which are antigenic to and can induce both the humoral and the cellular immune system in a host. The VAFs are preferably applied *in ovo* in an appropriate quantity.

Although the VAFs share the same advantages as the VNF, i.e., to improve vaccine efficacy as well as safety, the VAFs differ from the antibody used in Embrex VNF. Most importantly, the VAF stimulates immunological responses, such as antibody induction, T-cell activation with cytokine secretion, and the production of cytotoxic T lymphocytes, while the antibody in VNF acts to delay the normal course of

vaccines much faster and enables the live vaccines to generate much higher titres of antibody than without the VAF. Also, the VAF's effect on stimulating the immune response is not influenced by maternal immunity. Thus, the VAF, in sum, improves pre-hatch immune response, which in turn improves life long poultry health and reduces vaccination costs.

Commercial poultry are an extremely important source for food. Through the combined use of VAF and appropriate vaccination program, the potential losses caused by infections of commercial flocks can be minimized.

SUMMARY OF THE INVENTION:

The present invention provides a vaccine accelerator factor (VAF), which is a nucleotides- or DNA-containing *in ovo* immuno-stimulant. The VAF contains one or more DNA constructs. Each of the DNA constructs comprises a DNA molecule and a vector. The DNA molecule comprises at least one gene or a fragment of the gene encoding an antigenic peptide of an avian virus. The VAF stimulates and accelerates a protective immune response of a viral vaccine against the avian virus of which the DNA molecule contains a gene or a fragment thereof.

There are four major kinds of VAFs. The first kind of VAF contains one DNA construct. The DNA molecule of the DNA construct contains one gene or a fragment of this gene encoding an antigenic peptide of an avian virus. This kind of VAF stimulates and accelerate a viral vaccine against the same avian virus. The second kind of VAF also contains one DNA construct. But the DNA molecule of the DNA construct in the second kind of VAF contains more than one viral gene or a fragment thereof. In other words, the DNA construct is a "multivalent" DNA VAF where two or more genes or

fragments thereof, each from a different avian virus, are linked together with a vector. The multivalent DNA VAF is capable of stimulating and accelerating two or more viral vaccines or a viral vaccine containing two or more viral antigens against the same avian viruses represented in the VAF. The third kind of VAF contains more than one DNA construct. Each of the DNA molecule of the DNA construct in this VAF contains only one gene or a fragment of the gene. In other words, this kind of VAF is a "multiple" DNA VAF. A "multiple" DNA VAF is also capable of stimulating and accelerating two or more viral vaccines or a viral vaccine containing two or more viral antigens against the same avian viruses represented in the VAF. Finally, the fourth kind of VAF contains more than one DNA construct. Each of the DNA molecule of the DNA construct either contains one gene or a fragment thereof or more than one gene or a fragment thereof. In other words, this kind of VAF is a "multiple multivalent" DNA VAF. This multiple multivalent DNA VAF is capable of stimulating and accelerating no less than three viral vaccines or a viral vaccine containing no less than three viral antigens against the same avian viruses as represented in the VAF.

The viral vaccines used in combination with the VAF include, but are not limited to, any commercially available viral vaccines, such as vaccines containing live-virus, inactivated virus, or recombinant DNA viral vaccines. The viral vaccines are administered to the fowl either together with or subsequent to the *in ovo* injection of the VAF. In a preferred enbodiment, a live vaccine at 1/5 volume of the full dose recommended by the manufacturer of the viral vaccine is injected into the fowl post hatch after the fowl eggs receive an *in ovo* injection of VAF.

The vector of the DNA construct can be a plasmid or a viral carrier. The preferred vector is a plasmid. Examples of the plasmid include, but are not limited to,

pcDNA3, pVAX1, pSectag, pTracer, pDisplay, pUC system plasmid (such as pUC7, pUC8, pUC18), and pGEM system plasmid. Alternatively, any plasmid which contains a promoter such as CMV promoter, SV40 promoter, RSV promoter, and β-actin promoter, can also be used for preparing the DNA construct. The most favorable plasmid is pcDNA3. The preferred viral carrier is one of the following viruses: a bacteriophage, SV40, an adenovirus, a polyoma virus, a baculovirus, a herpes virus, a vaccinia virus, or a pox virus.

Examples of the avian virus include, but are not limited to Marek's disease virus (MDV), infectious vursal disease virus (IBDV), Newcastle disease virus (NDV), infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILTV), avian encephalomyelitis (AEV), avian leukosis virus (ALV), fowlpox virus (FPV), avian paramyxovirus (APV), duck hepatitis virus (DHV), and hemorrhagic enteritis virus (HEV).

The genes contained in the DNA molecules that are particularly suitable for stimulating and accelerating a protective immune response against avian viral diseases include, but are not limited to, the entire of gB gene of Merk's Disease virus (MDV) having the DNA sequence of SEQ ID NO:1 or a fragment thereof; the entire VP2 gene of infectious bursal disease virus (IBDV) having the DNA sequence of SEQ ID NO:2 or a fragment thereof; the entire HN gene of Newcastle disease virus (NDV) having the DNA sequence (which is from bases 6321 to 8319) of SEQ ID NO:3 or a fragment thereof (i.e., SEQ ID NO:3 is the entire genome of the NDV); the entire S1 gene of infectious bronchitis virus (IBV) having the DNA sequence of SEQ ID NO:4 or a fragment thereof; the entire glycoprotein G gene of infectious laryngotracheitis virus (ILTV) having the DNA sequence of SEQ ID NO:5 or a fragment thereof; the entire

VP1, VP0, or VP3 gene of avian encephalomyelitis virus (AEV) or a fragment therof (the VP1 gene has the DNA sequence of SEQ ID NO:6; the VP0 gene has the DNA sequence of SEQ ID NO:7; and the VP3 gene has the DNA sequence of SEQ ID NO:8); the entire paraglycoprotein G gene of avian parainfluenza virus (APV) having the DNA sequence of SEQ ID NO:9 or a fragment thereof; the entire type A penton base gene of hemorrhagic enteritis virus (HEV) having the DNA sequence of SEQ ID NO:10 or a fragment thereof; and the entire envelope antigen gene of fowlpox virus (FPV) having the DNA sequence of SEQ ID NO:11 or a fragment thereof.

The VAF is preferred to be injected into the egg, particularly in the amniotic fluid of the egg, of a fowl. The egg is preferred to be fertilized for about 17-19 days. The preferred fowl includes chicken, turkey, duck, and goose.

The present invention also provides a method for vaccinating fowl egg, which includes injecting into a fowl egg the VAF as shown above. The VAF is prepared by ligating a DNA molecule to a plasmid or virus carrier to form a DNA construct. For the VAF which contains more than one DNA construct, two or more of the DNA constructs are mixed together. The insertion of the DNA molecule into the vector can be achieved by conventional method, i.e., by ligation the DNA molecule with an enzyme such as T4 DNA ligase when both the genes and the desired vector have been cut with the same restriction enzyme(s) as complementary DNA termini are thereby produced. For pcDNA3, the preferred restriction enzymes are BamH1 and EcoR1.

DETAILED DESCRIPTION OF THE INVENTION:

Traditional avian vaccines comprise chemically inactivated virus vaccines or modified live-virus vaccines. Inactivated vaccines require additional immunizations, which are not only expensive to produce but also laborious to administer. Further,

some infectious virus particles may survive the inactivation process and may cause disease after administration to the animal.

In general, attenuated live virus vaccines are preferred over inactivated vaccines because they evoke an immune response often based on both humoral and cellular reactions. Such vaccines are normally based on serial passage of virulent strains in tissue culture. However, the attenuation process induces mutations of the viral genome, resulting in a population of virus particles heterogeneous with regard to virulence and immunizing properties. In addition, it is well known that the traditional attenuated live virus vaccines can revert to virulence resulting in disease outbreaks in inoculated animals and the possible spread of the pathogen to other animals.

A comprehensive post-hatch vaccination program involves administrations of different vaccines at various times. The vaccination program starts from 1 day of age and lasts up to 65 weeks of age. Many of the vaccines require repeated administrations, i.e., using vaccines of different immunogenic activities or using different route of administration at various times.

However, as vaccine viruses become more and more attenuated and safer for use in eggs as well as in young animals, they also lose the ability to stimulate a quick and strong protective immune response. This problem is compounded in young animals when maternal immunity is present because maternal antibody often interferes with the replication of more highly attenuated vaccine viruses. Thus, the more attenuated vaccine viruses usually do not stimulate protective immunity in individuals with maternal immunity.

In the poultry industry where high levels of maternal immunity is common, maternal antibody interference has usually been addressed by giving several

vaccinations during the first few weeks of life. Normally, a highly attenuated vaccine virus is used at hatch and a moderately attenuated virus is used during the second or third week of life. In this manner it is hoped that at least one of the vaccinations is timed correctly so that the individual is vaccinated prior to exposure to the pathogenic agent. This approach has served the poultry industry relatively well for many years, but it is generally accepted that a certain percentage of individuals in a flock are not going to be properly vaccinated. The percentage of improperly vaccinated individuals increases if the secondary vaccinations are not timed correctly for that particular flock's spread of maternal antibody or if the secondary vaccinations are not adminstered properly and uniformly.

Thus, the employment of the VAF to stimulate and accelerate the immune response of a vaccine would be advantageous for the poultry industry. Particularly, the VAF is injected into an egg of a fowl at 17-19 days of fertilization. Because the VAF contains at least a gene or a fragment of the gene capable of expressing an antigenic peptide of an avian virus *in ovo*, the administration of the VAF to an egg induces an initial immune response against the avian virus.

Also, the administration of the VAF, together with or prior to the administration of the avian viral vaccine(s), substantially improves the efficacy of the immune response generated by the viral vaccine(s). Especially, the dosage of the vaccine used in combination with the use of VAF for achieving the same immune response is far less than that without the administration of the VAF (as low as 20% of the normal dosage). Also, the time period for achieving the same immune response is much shorter when the VAF is used.

Furthermore, it is well known that some of the commercially available viral vaccines, such as the vaccines for infectious bronchitis virus (IB) and Newcastle disease virus (ND), are not suitable for *in ovo* injection. That is because the vaccine for IB is known to cause embryonic damage, and the vaccine for ND can only be properly stimulated through local muscular injection. The introduction of the VAF to the immunization of these diseases will improve the overall prevention of the diseases.

For the purpose of preparing the VAF, the DNA sequence of a gene need not contain the full length of DNA encoding the polypeptides. In most cases, a fragment of the gene which encodes an epitope region should be sufficient enough for immunization. The DNA sequence of an epitope region can be found by sequencing the corresponding part of other viral strains and comparing them. The major antigenic determinants are likely to be those showing the greatest heterology. Also, these regions are likely to lie accessibly in the conformational structure of the proteins. One or more such fragments of genes encoding the antigenic determinants can be prepared by chemical synthesis or by recombinant DNA technology. These fragments of genes, if desired, can be linked together or linked to other DNA molecules.

Also, the viral genes need not be in DNA. In fact, some of the frequently found avian viral diseases are caused by double- or single-stranded RNA viruses. For example, Marek's disease virus is a double-stranded RNA virus, while infectious bursal disease virus (IBDV), Newcastle disease virus (NDV) and infectious bronchitis virus (IB) are single-stranded RNA viruses. The RNA viral sequences, however, can be reverse-transcribed into DNA using RT-Polymerase chain reaction (RT-PCR) technology and then incorporated into a vector by the conventional recombinant DNA technology.

In addition, because of the degeneracy of the genetic code it is possible to have numerous RNA and DNA sequences that encode a specified amino acid sequence. Thus, all RNA and DNA sequences which result in the expression of a polypeptide having the antibody binding characteristics are encompassed by this invention.

To construct a recombinant VAF, either univalent or multivalent, the DNA sequence of the viral gene can be ligated to other DNA molecules with which it is not associated or linked in nature. Optionally, the DNA sequence of a viral gene can be ligated to another DNA molecule, i.e., a vector, which contains portions of its DNA encoding fusion protein sequences such as β-galactosidase, resulting in a so-called recombinant nucleic acid molecule or DNA construct, which can be used for transformation of a suitable host. Such vector is preferably derived from, e.g., plasmids, or nucleic acid sequences present in bacteriophages, cosmids or viruses.

Specific vectors which can be used to clone nucleic acid sequences according to the invention are known in the art and include either a plasmid or a virus carrier. Examples of the plasmid include, but are not limited to, pBR322, pcDNA3, pVAX1, pSectag, pTracer, pDisplay, pUC system plasmids (e.g., pUC7, pUC8, pUC18), pGEM system plasmids, Bluescript plasmids or any other plasmids where CMV promoter, SV40 promoter, RSV promoter, or β-actin promoter is included. The preferred plasmid is pcDNA3. Examples of the virus carrier include, but are not limited to, bacteriophages (e.g., λ and the M13-derived phages), SV40, adenovirus, polyoma, baculoviruses, herpes viruses (HVT), vaccinia virus, or pox viruses (e.g., fowl pox virus).

The methods to be used for the construction of a recombinant nucleic acid molecule are known to those of ordinary skill in the art. For example, the insertion of the nucleic acid sequence into a cloning vector can easily be achieved by ligation with an enzyme such as T4 DNA ligase when both the genes and the desired cloning vehicle have been cut with the same restriction enzyme(s) so that complementary DNA termini are thereby produced.

Alternatively, it may be necessary to modify the restriction sites so as to produce blunt ends either by digesting the single-stranded DNA or by filling in the recessive termini with an appropriate DNA polymerase. Subsequently, blunt end ligation with an enzyme such as T4 DNA ligase may be carried out. If desired, any restriction site may be produced by ligating linkers onto the DNA termini. Such linkers may comprise specific oligonucleotide sequences that encode restriction site sequences. The restriction enzyme cleaved vector and nucleic acid sequence may also be modified by homopolymeric tailing.

The present invention provides four kinds of VAFs. The first kind is a VAF containing one DNA construct (i.e., univalent VAF), which comprises a DNA molecule and a vector. The DNA molecule contains one DNA sequence (which can be a gene or a fragment of a gene) encoding one antigenic peptide which provide immuno-protection against one avian virus. The second kind of VAF comprises two or more of the kind of the univalent VAF, each carrying a different DNA sequence against a different avaian virus.

The third kind of VAF contains one DNA construct in which the DNA molecule contains two or more genes or gene fragments linked together, each from a different avian virus (i.e., a multivalent VAF or multivalent recombinant VAF). These genes or

gene fragments are carried by a useful vector, which can be either a plasmid or a virus carrier. The multivalent recombinant VAF encodes two or more antigenic polypeptides which afford protection against at least two viral diseases including, but not limited to, MD, IBD, ND or IB. The viral genes or gene fragments are operatively attached to the vector in reading frame so that they can be expressed in a host. The different structural DNA sequences carried by the vector may be separated by termination and start sequences so that the proteins can be expressed separately or they may be part of a single reading frame and therefore be produced as a fusion protein by methods known in the art. The fourth kind of VAF is a mixture of the univalent and multivalent DNA VAF.

The viral genes or gene fragments are preferably derived from Marek's disease virus (MDV), infectious bursal disease virus (IBDV), Newcastle disease virus (NDV), infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILTV), avian encephalomyelitis (AEV), Fowlpox virus (FPV), avian influenza virus (AIV), avian leukosis virus (ALV), duck hepatitis virus B genome, and hemorrhagic enteritis virus (HEV), inserted into a commercially available plasmid.

The preferred DNA sequences include, but are not limited to, the entire of gB gene of Merk's Disease virus (MDV) having the DNA sequence of SEQ ID NO:1 or a fragment thereof; the entire VP2 gene of infectious bursal disease virus (IBDV) having the DNA sequence of SEQ ID NO:2 or a fragment thereof; the entire HN gene of Newcastle disease virus (NDV) having the DNA sequence of SEQ ID NO:3 or a fragment thereof; the entire S1 gene of infectious bronchitis virus (IBV) having the DNA sequence of SEQ ID NO:4 or a fragment thereof.

The DNA sequence encoding the gB polypeptide of MDV has the nucleic acid sequence as SEQ ID NO:1. The DNA sequence contains 3650 bp of linear DNA.

The DNA sequence encoding the VP2 polypeptide of IBDV has the nucleic acid sequence as SEQ ID NO:2. The DNA sequence contains 3004 bp of linear DNA molecule which is reversely transcribed from IBDV's RNA template.

The DNA sequence of the entire genome of NDV contains 15186 bps of DNA, wherein (1) base No. 56 to 1792 encodes NP polypeptide, which is nucleocapsid protein; (2) base No. 1804-3244 encodes P polypeptide, which is a phosphoprotein; (3) base No. 3256-4487 encodes M polypeptide, which is a matrix protein; (4) base No. 4498-6279 encodes F polypeptide, which is a fusion protein; (5) base 6321-8319 encodes HN polypeptide, which is a hemagglutinin-neuraminidase; (6) base No. 8370-15073 encodes L polypeptide, which is a large polymerase protein. The NDV genome has the DNA sequence as SEQ ID NO:3.

The DNA sequence of the S1 polypeptide contains 1611 bp of linear DNA sequence as shown in SEQ ID NO:4, which is reversely transcribed from IBV's RNA templates.

The following examples are illustrative, but not limiting the scope of the present invention. Reasonable variations, such as those occur to reasonable artisan, can be made herein without departing from the scope of the present invention.

I. Materials and Methods

(A) Virus and vaccines

Avian infectious bronchitis virus (IBV), infectious bursal disease (IBD) and Newcastle disease (ND) vaccines were purchased from Intervet Inc.

(B) Viral RNA isolation and RT-PCR

Two hundred microliter recovered attenuated vaccines (Intervet Inc.) were resolved in iced cold GTC buffer (4 M guanidium isothiocyanate, 25mM sodium citrate, pH 7.0, 0.5% Sarkosyl, 0.1 M - mercaptoethanol) and sodium acetate (pH 4). An equal volume of phenol-chloroform (1:1) was added and placed on ice for 15 minutes after vortexing. The aqueous phase was collected after centrifuge and the RNA was precipitated with an equal volume of isopropanol. RNA was pelleted by centrifugation at 12000 rpm for 20 min at 4°C and then suspended in diethylpyrocarbonate (DEPC) treated deionized distill water and stored at -70°C.

(C) Oligonucleotides

Oligonucleotide primers for RT-PCR amplification were purchased from Promega, and were designed according to the genome of the Avian infectious bronchitis virus (Beaudette CK strain), Newcastle disease virus (Lasota strain) and Infectious bursa disease virus respectively. The sequences of the primers used for PCR were :

IBS1F' 5' CGGGATCCGCCGCCGCCATGTTGGTAACACCTCTT 3'; (SEQ ID NO: 12)

IBS1R' 5' CGGAATTCTTAACGTCTAAAACGACGTGT 3'; (SEQ ID NO:13)

NDF F' 5' CGGGATCCGCCGCCGCCATGGGCTCCAGACCTTCTACC 3'; (SEQ ID NO:14)

NDF R' 5' CCGCTCGAGTTACATTTTTGTAGTGGCTCTCATT 3'; (SEQ ID NO:15)

NDHN F' 5' CGGGATCCGCCGCCGCCATGGACCGCGCCGTTAGGCAAG 3'; (SEQ ID NO:16)

NDHN R' 5' GCTCTAGATTACTCAACTAGCCAGACCTG 3'; (SEQ ID NO:17)

IBDVP2F' 5' CGGGATCCGCCGCCGCCATGACAAACCTGCAAGAT 3'; (SEQ ID NO:18)

IBDVP2R' 5' CGGAATTCTTACCTTATGGCCCGGATTAT 3'. (SEQ ID NO:19)

(D) Reverse transcription polymerase reaction (RT-PCR)

Reverse transcription of IBV, NDV and IBDV RNA were carried out at 42°C for 30 min in 2.5 x *Taq* buffer (200 mM NaCl, 15 mM Tris-HCl, pH7.4, 15 mM $MgCl_2$, 15 mM β -mercaptoethanol, and 0.25 mM each of dATP, dCTP, dGTP, and dTTP). In addition to the *Taq* buffer, the reaction mixture (40 μl) also contained viral RNA, 2.4 U of avian myeloblastosis virus (AMV) reverse transcriptase (Promega), 16 U of RNasin (Promega), and 0.01 nmol reverse primer (*IBDVP2R, NDF F, NDHN F or IBS1R*). The final volume of the reaction mixture was 40 μl. After reverse transcription, the following reagents were added to the reverse transcription mixture: 0.02 nmol of each nucleotide triphosphate (dATP, dCTP, dGTP, dTTP), 0.01 nmol of forward primer (*IBDVP2F, NDF R, NDHN R or IBS1F*) and 1.5 U of *Taq* DNA polymerase (Strategene). Water was then added to a final volume of 100 μl. The reaction was carried out for 32 cycles in a Thermal Cycler (Perkin Elmer-Cetus). Each PCR cycle

consisted of 1 min of denaturation at 94°C, 1 min of annealing at 57°C, and 2 min of DNA chain elongation at 72°C.

(E) Preparation of DNA constructs

The plasmids pCMV-VP2, pCMV-S1, pCMV-NDF and pCMV-NDHN were constructed with the VP2, S1, NDF and NDHN genes from IBD vaccine, IBV vaccine and NDV vaccine respectively, placed downstream of the commercial plasmid pcDNA3. (Invitrogen, U.S.A.). All of the genes were inserted into the pcDNA3 vector using restriction enzymes *Bam*HI , *Eco*RI, *Xba*I and *Xho*I (underlined characters in the sequence of the primers). Sequences of the all genes in the pcDNA3 vector were verified by sequencing in both directions.

(F) Preparation of DNA and DNA delivery

The quantity of plasmid DNA that had been purified by affinity chromatography (Qiagen. Inc.) was determined by spectrophotometric measurements at 260 and 280 nm. The DNA in aliquots to 100 μg was suspended in 100 μl of PBS (0.14M NaCl, 10mM sodium phosphate, pH 7.4). For DNA delivery, 1cc syringe with a 20 gauge 1 and 1/2 inch needle were used. For the *in-ovo* groups, the embryos (18-day-old fertilized and developing eggs from the setting trays) were injected with 0.1 milliliters of VAF (100 μg) into the large end of each egg through the air cell with a needle. The eggs were then transferred into the hatchery where they remained until they hatched at about 21 days of age. For the IM (Intramuscular), all of the vaccines (1/5 dose of live vaccines) were injected into the chicken's thoracic muscle at 10 days post hatchery.

II. *Experimental design*

Specific Pathogen Free (SPF) fertilized eggs (n=60) were randomized into 12 groups. All groups (five eggs each group), all eggs were given 100 μl in volume each. 100 μg pCMV-NDF+ 100 μg pCMV-NDHN mixture was injected in each egg of group A, 100 μg pCMV-S1 was injected in each egg of group B, 100 μg pCMV-VP2 was injected in each egg of group C, 100 μg pCMV-NDF + 100 μg pCMV-NDHN+100 μg pCMV-S1(ND+IB) was injected in each egg of group D, 100 μg pCMV-NDF+ 100 μg pCMV-NDHN+100 μg pCMV-VP2 (ND+IBD) was injected in each egg of group E, 100μg pCMV-VP2+100 μg pCMV-VP2 mixture (IB+IBD) was injected in each egg of group F, 100 μg pCMV-NDF+ 100 μg pCMV-NDHN+100 μg pCMV-S1+100 μg pCMV-VP2 mixture (ND+IB+IBD) was injected in each egg of group G, one dose of commercialized in-ovo IBD vaccine (Embrex, Inc) was injected in each egg of group H as positive control, 100 ul PBS was injected in each egg of group I, J, K and L. All chickens in this experiment were given 100 μl in volume (1/5 dose of live vaccines), injected into the chicken's thoracic muscle each at 10 days post hatchery. Chickens in group A and I were injected with NDV vaccine, group B and J were injected with IBV vaccine, group C and K were injected with IBDV vaccine, group D were injected with the mixture of NDV+IB vaccines, group E were injected with the mixture of NDV+IBD vaccines, group F were injected with the mixture of IB+IBD vaccines and group G and L were injected with the mixture of NDV, IB and IBD vaccines.

III. *Manual In ovo Injection Procedure:*

The *in ovo* injection could either be performed by automatic *in ovo* vaccination system (such as Embrex Inovoject® system) or by manual procedure. A manual *in ovo* procedure was described as follows:

1. The eggs were candled to remove infertile, contaminated, and/or upside down eggs. Also, dirty eggs were moved to avoid bacterial contamination during the injection.

2. The top of the air cell of each egg was sanitized by a diluted bleach solution (prepared by mixing a 10% solution of household bleach in distilled water to reach a final concentration of 0.5% hypochlorite). Preferably, the diluted bleach solution was prepared fresh and mixed just before use. A cotton swab soaked in the diluted bleach was used to wipe the surface on top (air cell) of the egg. Alternatively, iodine could be used to replace the diluted bleach, although the diluted bleach was more effective. If clean eggs were used, there was no need to clean the eggs with the diluted bleach solution.

3. Once the bleach solution has dried on the eggs surface (about 5 minutes), the eggs were sprayed with 70% isopropyl alcohol. After the 70% isopropyl alcohol was dried, a hole was punched in the air cell end on top of the egg by inserting an 18 gauge 1 and 1/2 inch needle through a rubber stopper so that 1/4 to 1/2 inch of the needle tip emerges from the stopper. This hole would be used to insert the needle when injecting the vaccine. When vaccine or VAF was inserted into the egg, the needle and syringe must be straight up and down (not at an angle). The embryo was not hurt by this way so that the fowl could hatch normally.

4. The VAF was preferably injected into the egg by a 1cc syringe with a 20 gauge 1 inch needle. For multiple or multiple multivalent VAFs, it was important that the DNA constructs were mixed under sterile conditions. The sterility of the VAF could be confirmed by sampling it before and after the injection by putting 1/2 mL of

agar to determine the presence of bacteria.

6. For *in ovo* live vaccine injection, normally, 100 μL of the commercially available vaccine was injected into an egg (which constituted 100% full volume). If the live vaccine was used in connection with the VAF, less volume (at low as 20 μL) of the vaccine could be used to achieve the same titer in the young animals.

IV. *Serology detection*

All of the serum samples from a fowl were collected at 10 days (injected with low dose live vaccines at the same time), 17days, 24 days and 31 days post hatchery. The antibody titers were detected by ELISA using IB, IBD and NDV antibody test kits which purchased from IDEXX Laboratories, Inc. All of the samples were detected duplicated. Dilute test samples five hundred fold (1:500) with sample diluents prior to being assayed. The test procedure was applied according to the kit's manual. For the assay to be valid, measure and record absorbance values at 650 nm, A (650). The relative level of antibody in the unknown was determined by calculating the sample to positive (S/P) ratio. Endpoint titers were calculated using the formula: $Log_{10}Titer = 1.09(Log_{10}S/P)+ 3.36$

IV. Results

As shown in Table 1, the results demonstrated that, for the detection of anti-IBD antibodies, the IBDV recombinant antigen VP2 DNA construct (i.e., a single, monovalent DNA contruct-containing VAF) could be expressed and played the role of primary stimulation of IBVD immunization. The titers increased rapidly after a low dose vaccine booster. The titers of group C, E, F and G at 17 days post hatchery (i.e., 7 days post IM injection) were significantly higher than those of group K and L. Most importantly, the expression of IBDV antigen was not interfered by other monovalent VAFs (NDV and IBV). The same results were also applied to IB and NDV VAFs. The titers of group B, D, F and G were higher than those of group J and L at 17 days post hatchery (Table 2) and the titers of group A, D, E and G were higher than those of group I and L at 17 days post hatchery (Table 3). The only unpredicted result was the anti-NDV titer could not be highly induced by the triple valent VAF (Table 3, group G), but anti-IBD and anti-IB could (Tables 1 and 2, group G).

Table 1. Serum Samples Detected by IDEXX IBD Antibody Test Kit (Ab Titers Correspond to the Average Titers ±SD)

Immunization and Sample Collection Schedule (days)				
Animal Group	10 Days PH*	17 Days PH	24 Days PH	31 Days PH
C(IBD)	- **	4535±1267	16623±3105	21254±3852
E(IBD+ND)	-	1685±655	17339±2185	19041±2967
F(IBD+IB)	-	8252±2205	10057±1295	17561±2006
G(IBD+IB+ND)	-	9111±1701	13127±1763	16694±2134
H(IBD positive)	6553±851	13025±2131	18015±1592	18853±2614
K(PBS/IBD)	-	-	1853±302	17002±2965
L(PBS/IBD+IB+ND)	-	-	6923±1168	18063±2531

*P H: post hatchery

** - : average titers less than 396 (be considered negative by IDEXX kit)

Table 2. Serum Samples Detected by IDEXX IB Antibody test kit (Ab Titers Correspond to the Average Titers ±SD

Immunization and Sample Collection Schedule (days)				
Animal Group	10 Days PH*	17 Days PH	24 Days PH	31 Days PH
B(IB)	- **	441±117	2426±264	3214±877
D(IB+ND)	-	586±182	805±221	1988±501
F(IB+IBD)	-	509±89	685±186	1192±237
G(IBD+IB+ND)	-	499±81	688±78	2551±531
J(PBS/IB)	-	-	485±76	1662±441
L(PBS/IBD+IB+ND)	-	-	819±202	1332±488

*P H: post hatchery

** - : average titers less than 396 (be considered negative by IDEXX kit)

Table 3. Serum Samples Detected by IDEXX ND Antibody Test Kit (Ab Titers Correspond to the Average Titers ±SD)

Immunization and Sample Collection Schedule (days)				
Animal Group	10 Days PH*	17 Days PH	24 Days PH	31 Days PH
A(ND)	-	466±101	2394±456	8103±2198
D(ND+IB)	-	706±140	1778±378	6811±2206
E(ND+IBD)	-	517±104	3021±411	5991±1695
G(IBD+IB+ND)	-	-	-	783±201
I(PBS/ND)	-	-	1853±324	3912±304
L(PBS/IBD+IB+ND)	-	-	4027±662	5807±1996

*P H: post hatchery

** - : average titers less than 396 (be considered negative by IDEXX kit)

While the invention has been described by way of examples and in terms of the preferred embodiments, it is to be understood that the invention is not limited to the disclosed embodiments. On the contrary, it is intended to cover various modifications as would be apparent to those skilled in the art. Therefore, the scope of the appended claims should be accorded the broadest interpretation so as to encompass all such modifications.

WE CLAIM:

1. A vaccine accelerator factor (VAF) comprising:

one or more DNA constructs; each of said DNA constructs comprising a DNA molecule and a vector; said DNA molecule comprising at least one gene or a fragment of said gene encoding an antigenic peptide of an avian virus; wherein said VAF stimulates and accelerates a protective immune response of a viral vaccine against said avian virus of which said DNA molecule contains a gene or a fragment thereof.

2. The VAF according to claim 1, wherein said VAF comprises one DNA construct; wherein said DNA molecule of said DNA construct comprises one gene or a fragment thereof.

3. The VAF according to claim 1, wherein said VAF comprises one DNA construct; wherein said DNA molecule of said DNA construct comprises more than one gene or fragments thereof.

4. The VAF according to claim 1, wherein said VAF comprises more than one DNA construct; wherein said DNA molecule of said DNA construct comprises one gene or a fragment thereof.

5. The VAF according to claim 1, wherein said VAF comprises more than one DNA construct; wherein said DNA molecule of said DNA construct comprises either one gene or fragments thereof or two or more genes or fragments thereof.

6. The VAF according to claim 1, wherein said avian virus is one selected from the group consisting of Marek's disease virus (MDV), infectious vursal disease virus (IBDV), Newcastle disease virus (NDV), infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILTV), avian encephalomyelitis (AEV), avian leukosis virus (ALV), fowlpox virus (FPV), avian parainfluenza virus (APV), duck hepatitis virus (DHV), and hemorrhagic enteritis virus (HEV).

7. The VAF according to claim 1, wherein said vector is a plasmid or a viral carrier.

8. The VAF according to claim 7, wherein said plasmid is one selected from the group consisting of pcDNA3, pVAX1, pSectag, pTracer, pDisplay, pUC system plasmid, and pGEM system plasmid.

9. The VAF according to claim 8, wherein said plasmid comprises a promoter which is selected from the group consisting of a CMV promoter, SV40 promoter, RSV promoter, and β-actin promoter.

10. The VAF according to claim 7, wherein said viral carrier is one selected from the group consisting of a bacteriophage, SV40, an adenovirus, a polyoma virus, a baculovirus, a herpes virus, a vaccinia virus, and a pox virus.

11. The VAF according to claim 1, wherein said gene of said DNA molecule is an entire of gB gene of Marek's Disease virus (MDV) having the DNA sequence of SEQ ID NO:1.

12. The VAF according to claim 1, wherein said gene of said DNA molecule is an entire VP2 gene of infectious bursal disease virus (IBDV) having the DNA sequence of SEQ ID NO:2.

13. The VAF according to claim 1, wherein said gene of said DNA molecule is an entire HN gene of Newcastle disease virus (NDV) having the DNA sequence of bases 6321 to 8319 in SEQ ID NO:3.

14. The VAF to claim 2, wherein said gene of said DNA molecule is an entire S1 gene of infectious bronchitis virus (IBV) having the DNA sequence of SEQ ID NO:4.

15. The VAF according to claim 1, wherein said gene of said DNA molecule comprises an entire glycoprotein G gene of infectious laryngotracheitis virus (ILTV) having the DNA sequence of SEQ ID NO:5.

16. The VAF according to claim 1, wherein said gene of said DNA molecule comprises an entire VP1, VP0, or VP3 gene of avian encephalomyelitis virus (AEV); wherein said VP1 gene

has the DNA sequence of SEQ ID NO:6, said VP0 gene has the DNA sequence of SEQ ID NO:7, and said VP3 gene has the DNA sequence of SEQ ID NO:8.

17. The VAF according to claim 1, wherein said gene of said DNA molecule is an entire paraglycoprotein G gene of avian parainfluenza virus (APV) having the DNA sequence of SEQ ID NO:9.

18. The VAF according to claim 1, wherein said gene of said DNA molecule is an entire type A penton base gene of hemorrhagic enteritis virus (HEV) having the DNA sequence of SEQ ID NO:10.

19. The VAF according to claim 1, wherein said gene of said DNA molecule is an entire envelope antigen gene of fowlpox virus (FPV) having the DNA sequence of SEQ ID NO:11.

20. The VAF according to claim 1, wherein said VAF is injected into an egg of a fowl.

21. The VAF according to claim 20, wherein said VAF is injected into an amniotic fluid of said egg.

22. The VAF according to claim 20, wherein said VAF is co-administered with a viral vaccine to said egg.

23. The VAF according to claim 20, wherein said VAF is administered to said egg prior to administration of said viral vaccine.

24. The VAF according to claim 16, wherein said egg is fertilized for about 17-19 days.

25. The VAF according to claim 1, wherein said viral vaccine is one selected from the group consisting of an attenuated live virus vaccine, an inactivated virus vaccine, or a recombinant DNA vaccine of said avian virus.

26. The VAF according to claim 23, wherein said viral vaccine is administered to said fowl at hatch or post hatch.

27. The VAF according to claim 26, wherein about 1/5 by volume of a manufacturing recommended amount of said viral vaccine is administered to said fowl.

28. The VAF according to claim 20, wherein said fowl is one selected from the group consisting of chicken, turkey, duck, and goose.

29. A method for vaccinating a fowl against viral infection comprising:

co-administering an effective amount of said VAF according to claim 1 with said viral vaccine by injecting said VAF and said viral vaccine into an amniotic fluid of an egg.

30. The method according to claim 29, wherein said egg is fertilized for about 17-19 days.

31. A method for vaccinating a fowl against viral infection comprising:

administering an effective amount of said VAF according to claim 1 to an amniotic fluid of an egg at about 17-19 days of fertilization; and

adminstering said viral vaccine at hatch of said egg.

SEQUENCE LISTING

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<120> VACCINE ACCELERATOR FACTOR (VAF) FOR IMPROVEMENT OF VACCINATIONS IN POULTRY

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<210> 4

<211> 1611

<212> DNA

<213> infectious bronchitis virus (IBV)

<400> 4

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<210> 5

<211> 960

<212> DNA

<213> infectious laryngotracheitis virus (ILTV)

<400> 5

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<210> 6

<211> 810

<212> DNA

<213> avian encephalomyelitis virus (AEV)

<400> 6

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gcggtgccag tggtcagctc attcactgac acatctagca aaacagtgat gaatacatat 780

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<210> 7

<211> 726

<212> DNA

<213> avian encephalomyelitis virus (AEV)

<400> 7

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gcacag 726

<210> 8

<211> 735

<212> DNA

<213> avian encephalomyelitis virus (AEV)

<400> 8

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<210> 9

<211> 1500

<212> DNA

<213> avian parainfluenza virus (APV)

<400> 9

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<210> 10

<211> 1440

<212> DNA

<213> hemorrhagic enteritis virus (HEV)

<400> 10

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<210> 11

<211> 2995

<212> DNA

<213> fowlpox virus (FPV)

<400> 11

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