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Review Article

VETERINARY VACCINES: PAST, PRESENT AND FUTURE – A REVIEW

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Abstract: The use of vaccines in veterinary field has progressed from an experimental adventure to a routine and relatively safe practice. Veterinary vaccines have a major role in protecting animal health and human health, reducing animal suffering and greatly reducing the need for antibiotics to treat food and companion animal. The continued interaction between animals and human researchers and health professionals will be of major importance for adapting new technologies, providing animal models of disease, and confronting new and emerging infectious disease. This review addresses the history, current veterinary vaccine practices and potential future improvements of vaccine use in veterinary field.
Keywords: Vaccine, Public health, Infectious disease, Vaccine technology.

Introduction

The term "vaccine" (from the Latin term "vacca," meaning cow) was first coined by Edward Jenner to describe the inoculation of humans with the cowpox virus to confer protection against the related human smallpox virus and illustrates the close relationship between human and animal infectious disease sciences (Meeusen *et al*., 2007). According to OIE (2015) vaccines include all products designed to stimulate active immunity of animals against disease, without regard to the type of microorganism or microbial component or toxin from which they may be derived or that they contain. So vaccines are biological preparations made from killed or attenuated pathogens that upon administration should elicit specific and adaptive immunity to the target pathogen.

An absolute requirement for veterinary vaccines is safety and sustained efficacy. A Special care must be taken during production of vaccine for food animals, as compared to pet or companion animals. Vaccination constitutes the highly cost-effective measure to prevent or reduce clinical signs after infection and to eradicate infectious diseases, compared to the cost

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of chemotherapies and prophylaxis against many infectious diseases. The associated evolution of new technology in the field of molecular biology and immunology has furthermore had a large impact on the development of new vaccine strategies and the quality of the products that are produced. It has enabled the design of vaccines targeted for the control and eradication of specific pathogens within the framework of regional, national and international requirements. To meet the growing demand for livestock products with its quality, improve animal health in, particularly as it relates to infectious disease control, limits on residues in commodities, and animal welfare in overall world.

History for vaccine development

In terms of its practices and concerns, human vaccinology with its primary focus on the individual, seems far removed from veterinary medicine, with its concern for the health of the herd (Lombard *et al*., 2007). In some cases the human vaccine was developed first, while in other cases it was the animal vaccine, yet the history of vaccines clearly demonstrates the importance of these 'two medicines' working together. A veterinary vaccine is that a veterinarian applies to companion or wild animals or herds of livestock. Yet the usefulness of veterinary vaccines extends beyond these limits since many of them also protect humans from anthropozoonoses, diseases transmitted from animals to humans, or vice versa.

Earliest description of vaccines at 10thcentury: In the China, their people have been inoculated against smallpox, probably by having powder from pulverized smallpox scabs blown into the nostril. Inoculation may also have been practiced by scratching matter from a smallpox sore into the skin. This was the first documented account of variolation was in the 10th century. This Chinese "injected" the infection in a completely unique way, called "nasal insufflation", but was not carried out in other nations.

Edward Jenner's Era (1749-1823): Edward Jenner, "the father of immunology" was the pioneer of world's first smallpox vaccine.His work is said to have "saved more lives than the work of any other human".Noting the common observation that milkmaids were generally immune to smallpox, Jenner postulated that the pus in the blisters that milkmaids received from cowpox (a disease similar to smallpox, but much less virulent) protected them from smallpox.Jenner tested this hypothesis by inoculating James Phipps, an eight-year-old boy who was the son of Jenner's gardener. The scraped pus from cowpox blisters on the hands of Sarah Nelmes, a milkmaid who had caught cowpox from a cow called Blossom.

Louis Pasteur (1880-1885): Pasteur's important discovery in the study of vaccination came in 1879 concerned with a disease called chicken cholera. Pasteur stated in his statement that

chance only favours the prepared mind, and it was chance observation through which he discovered that cultures of chicken cholera organism lost their pathogenicity and retained "attenuated" pathogenic characteristics over the course of many generations. He inoculated chickens with the attenuated form and demonstrated that the chickens were resistant to the fully virulent strain. Pasteur began investigating anthrax in 1879. During that time an anthrax epidemic in France and in some other parts of Europe had killed a large number of sheep, and the disease was also attacking humans as well. Pasteur was applied the same principle of vaccination to anthrax and prepared attenuated cultures of the bacillus after determining the conditions that led to the organism's loss of virulence.

Also Pasteur developed another vaccine against rabies. Rabies was a dreaded and horrible disease that had fascinated popular imagination for centuries because of its mysterious origin and the fear it generated.Pasteur suspected that the agent that caused rabies was a microbe (the agent was later discovered to be a virus). It was too small to be seen under Pasteur's microscope. Pasteur chose to conduct his experiments using rabbits and transmitted the infectious agent from animal to animal by intracerebral inoculations until he obtained a stable preparation.Thus, rather unknowingly, he had produced, instead of attenuated live microorganisms, a neutralized agent and opened the way for the development of a second class of vaccines, known as inactivated vaccines.

Yellow fever vaccine: In 1937, Max Theiler, working at the Rockefeller Foundation, developed a safe and highly efficacious vaccine for yellow fever that gives a lifelong immunity from the virus. For his work on the yellow fever vaccine, he received Nobel Prize in the year 1951. The vaccine consists of a live, but attenuated strain of the yellow fever virus called 17D. The 17D vaccine has been used commercially since the 1950s. This vaccine is very safe, with few adverse reactions having been reported and millions of doses administered and highly effective with over 90% of vaccines developing a measurable immune response after the first dose.

Tuberculosis vaccine: The history of Bacille Calmette-Guerin (BCG) is tied to that of smallpox. Jean Antoine Villemin first recognized bovine tuberculosis in 1854 and transmitted it while Robert Koch first distinguished *Mycobacterium bovis* from *Mycobacterium tuberculosis*. Albert Calmette, a French physician and bacteriologist, and his co-worker Camille Guerin, a veterinarian, were working at the Institute Pasteur de Lille (Lille, France) in 1908. Their work included sub-culturing virulent strains of the tubercle bacillus and testing different culture media. They noted a glycerin-bile-potato mixture grew bacilli that seemed

less virulent, and changed the course if repeated sub-culturing would produce a strain that was attenuated enough to be considered for use as a vaccine. BCG strain was isolated after 239 times sub-culturing during 13 years from virulent strain on glycerine potato medium.

Polio vaccine: Two polio vaccines are used throughout the world to provide immunity to the virus that causes poliomyelitis (or polio). The first was developed by Jonas Salk through the use of HeLacells and it consists of an injected dose of inactivated poliovirus. An oral vaccine was developed by Albert Sabin using attenuated or weakened poliovirus. Interruption of person to person transmission of the virus by vaccination has been crucial in global polio eradication. The two vaccines have eliminated polio from most countries in the world, and reduced the worldwide incidence from an estimated 350,000 cases in 1988 to just 223 cases in 2012 (CDCP, 2015).

Rinderpest Vaccine: In the past, classical rinderpest was an acute, viral disease of domestic cattle, yaks and wild African buffaloes and Asian water buffaloes. It was characterized by high morbidity and mortality rates. Sheep, goats, pigs and wild ungulates might also be affected. Walter Plowright developed the tissue culture rinderpest vaccine (TCRPV), also called the Plowright tissue culture vaccine (PTCV) in 1962. He harvested samples of the Kabete "O" RPV, the most virulent strain of RPV from the gums of infected animals and adapted it for culture in single layers of calf kidney cells. After ninety or more passages through the cells, the resulting virus had become completely nonpathogenic and could be used for inoculation.

Present day Vaccines

Live vaccines: A large number of live organisms are used as vaccines, because live vaccines have several advantages. Although live vaccines are produced in several ways, the most common method for creating vaccine strains is made through passing organisms in cell cultures, embryos, or suitable materials. For instance, a selected virus strain is serially passed in chicken embryos, resulting in better replication in chick cells but with a lost ability to replicate in animals cells of the target host. Also, the live vaccine viruses can be generated by inducing random mutations on viral genome and followed by selecting a non-virulent mutant incapable of causing clinical diseases.

The immunity produced by live vaccines is often solid and gives long term protection. Live organisms in the vaccine grow in the host and thus mimic the natural infection (Griffin *et al*., 2002). Nevertheless, these vaccines still have a residual virulence or a risk of reversion to a virulent phenotype. A single point mutation on certain gene may tend to induce attenuation of

virus but may lead to back mutation, resulting in the wild type virulent virus. Despite these drawbacks of live vaccines, live vaccines play an important role in preventing and eradicating diseases in animals industry. Interestingly, a potent adjuvant is not necessary for the formulation of live vaccines because live vaccines are capable of infecting target cells and provoking immune responses to injected organisms. One such example is a new live vaccine (Enterisol Ileitis) against porcine proliferative enteropathy caused by the intracellular bacterium *Lawsoniaintracellularis* (Kroll *et al.,* 2004).

Inactivated vaccines: Inactivated vaccines are safer than live vaccines because they cannot replicate at all in a vaccinated host, resulting in no risk of reversion to a virulent form capable of causing diseases. However, they generally provide a shorter length of protection than live vaccine and generally elicit weak immune responses, in particular cell-mediated immunity, as opposed to live viral vaccines. For this reason, inactivated vaccines are administered with potent adjuvant (Lee *et al*., 2012), and require boosters to elicit satisfactory and a long-term immunity. Vaccines of this type are generally created by inactivating propagated viruses by treatment with heat or chemicals such as formalin or binary ethylenemine (Gupta *et al.,*1987). These agents do not cause alteration to the protein structure and thus maintain antigenicity to a larger extent. This procedure can destroy the pathogen's ability to propagate in the vaccinated host, but keeps it intact so that the immune system can still recognize it.

For live Avian Influenza Virus vaccines, the possibility of re-assortment between live vaccine strain and field isolates and of back mutation from low-pathogenic to highly pathogenic viruses lead to serious concerns for vaccine safety. Thus, prior stimulation of the immune system using some immune-modulators followed by vaccination with inactivated vaccines may be needed to confer better protective immunity within a short period of time. A killed vaccine against periodontitis for dogs is available against the pathogens like Porphyromonasgulae, P. denticanis and P. salivosa with brabd name "Periovac" (Meeusen *et al.,* 2007).

Subunit vaccines: Subunit vaccines usually contain a part of the target pathogen so that the immune response would be against the component only (van Overbeke *et al*., 2001). Such vaccine can be prepared by isolating a particular immunogenic protein from the pathogen and presenting it as an antigen on its own. An antigen derived from bacterial surface components is cloned, expressed, purified, and its protective potential is assessed in an animal infection model. Over the past decade modern genetic techniques enabled easily identification of vaccine antigen in lieu of previous available biochemical or antigen data.

The genes are expressed using foreign protein expression systems, including *Escherichia coli*, yeast, insect or mammalian cells, and are then purified and injected into a host to elicit immunity. The resulting product is combined with proper adjuvant and used as the subunit recombination vaccine. Such vaccine has a benefit due to its inability to replicate in the host, and well tolerated due to the addition of a good purification step. An example for this vaccine against *Actinobacillus pleuropneumoniae* in pigs is available in the form of extracted ApxI, ApxII, ApxIII and outer membrane proteins (Chiers *et al.,* 1998).

Toxoid vaccines: Some bacterial diseases are not directly caused by bacteria themselves but by a toxin produced by the bacteria. Tetanus is caused by neurotoxin that is produced by *Clostridiumtetani*, rather than bacterial infection. Vaccines for this type of pathogen can be generated by inactivating the toxin responsible for causing clinical signs. Although inactivated toxin could be considered a killed vaccine, sometimes it should be in its own category to highlight that it contains an inactivated toxin but not bacteria.

The classical example of the use of this type of vaccine protection is that of clostridial toxoid vaccines against the economically important clostridial infections. The major toxins responsible for disease have been identified and the use of toxoid vaccines to control clostridial diseases is widely practiced. Bacterial toxins are inactivated to produce bacterial toxoids in such a way that toxicity is lost but antigenicity retained. The usual method of treatment is with formaldehyde (Petre *et al*., 1996). Toxoids are normally adsorbed on to an adjuvant, usually a mineral salt such as aluminium hydroxide, aluminium phosphate or potassium aluminium sulphate. By far the most potent adjuvants are mineral oils or their derivatives, which enhance immunogenicity by at least an order of magnitude over conventional adjuvants (Han *et al*., 2014).

Conjugated vaccine: Conjugated vaccines are somewhat similar to recombinant subunit vaccines, which are usually composed of two different components. They have been generated against pathogens whose polysaccharide capsule protects them from the phagocytosis (Pollard *et al*., 2009). Since the polysaccharide is poorly immunogenic, linking the polysaccharide to immunogenic protein enables the immune system to recognize them as if they were protein antigens. They are produced by chemically linking the polysaccharide to a carrier protein, which creates stronger, combined immune responses to the piece derived from bacteria as well as the carrier protein. Immunity to a piece of the bacteria can protect from future infection. Such types of vaccines are currently in use for *Streptococcus pneumonia* (Seong *et al*., 1999).

For examples, a conjugated vaccine composed of Vi capsular polysaccharide of *Salmonella Typi* conjugated with diphtheria toxoid (Micoli *et al*., 2010), was generated and inoculated into mice in order to validate its immunogenicity. Immunization of a single dose of the conjugate induced the high titers of anti-ViIgG, whereas inoculation of the large amount of unconjugated Vi polysaccharide alone showed the suppression of anti-Vi antibody.

Modern Vaccine Strategies

Many conventional vaccines currently employed in human or veterinary medicine contain live microorganisms orvirus that has been attenuated. However, conventional methods of attenuation rely on spontaneous, random mutations occurring during multiple passages and the basis for the attenuation is usually not known. An alternative approach is to use genetic engineering to define specific genes or regions of the genome which are responsible for virulence, then delete these in order to obtain a replicating, non-pathogenic virus for use as the immunogen. This approach has been particularly fruitful with bacteria and DNA viruses with large genomes, such as herpes viruses and poxviruses.

Recombinant vaccines: The recombinant proteins can be a component of safe and non-replicating subunit vaccines. When manipulating DNA that encodes such proteins, a large quantity of proteins can be expressed, purified, and then immunized into a target host in order to stimulate immune reaction against the pathogen.

When the carrier virus propagates or when the producer cells metabolize, the inserted gene is also expressed and released into cytoplasm. The end result of this approach is a recombinant vaccine; the immune system of vaccinated host will recognize the expressed protein and provide future protection against the target virus. For examples, the open reading frame 2 (ORF2) protein of porcine circovirus type 2 (PCV2), a major agent responsible for developing post-weaning multi-systemic wasting syndrome in pigs, was recently produced in baculovirus expression system, and the subunit vaccine containing ORF2 protein has been commercialized (Blanchard *et al.,*2003). Meanwhile, a subunit vaccine capable of preventing Newcastle disease virus (NDV) in poultry was successfully registered (Palya *et al*., 2012). Hemagglutinin-neuraminidase (HN) protein, a protective antigen, of NDV was produced in plant cells and demonstrated to protect a vaccinated chicken once challenged with wild type virus.

DNA vaccines: DNA, the essential part of the life is making way in to new vaccine technology. Plasmid vectors from the bacteria have revolutionized the world of vaccine

design by its new technology – DNA vaccines. Small portion of the nucleotides from the pathogen held under the control of promoter in a plasmid vector can be used as a vaccine.

DNA vaccine is a cloning of "Gene of Interest" in plasmid vector under strong promoter and introduction of such plasmids directly into host tissue can generate immune response against the gene of interest. In the host cells the gene of interest is transcribed, translated, processed and presented to immune system. To get the effective vaccine effect it is mandatory to find a good route of delivery like injecting them intramuscularly or by gene gun. Skin being the largest area in the body has been the hunting ground for scientists to deliver vaccines and drugs. The advantage of skin is that it is easily approachable; it contains Langerhan cells, antigen presenting cells and migrating lymphocyte cells, which make it a perfectroute to manipulate the immune system.

Marker vaccine (DIVA strategy): For several viral infections of livestock, effective conventional vaccines are available but cannot be used as they would interfere with disease surveillance based on serological testing and may result in the loss of a country's disease-free status. Vaccines are increasingly assessed for their ability to reduce virus transmission and thus the establishment of herd immunity (van Oirschot *et al*., 1996).

The ability to identify and selectively delete genes from apathogen has allowed the development of "marker vaccines" that, combined with suitable diagnostic assays, allow differentiating infected from vaccinated animals (DIVA) by differentiation of antibody responses induced by the vaccine (no antibodies generated to deleted genes) from those induced during infection with the wild-type virus. Such DIVA vaccines and their companion diagnostic tests are now available or in development for several diseases like classical swine fever and FMD. Infectious bovine rhino trachitis, caused by bovine herpes virus type 1 (BHV-1) infection of cattle, and pseudorabies (Aujeszky's disease) in pigs have been identified internationally as being candidates for eradication from national herds, and so there has been an impetus for the development of DIVA vaccines and diagnostics (Ferrari *et al*., 2000).

Chimeric vaccine: An interesting development in genetically engineered viral vaccines is the production of chimera viruses that combine aspects of two infective viral genomes. Chimeric vaccines are created by cloning pieces of one virus into another virus and deriving a "chimer."Live chimeric PCV1-PCV2 vaccine with the capsid gene of PCV2 cloned in the backbone of the nonpathogenic PCV1 has been developed and shown to be nonpathogenic. An inactivated version of the live vaccine, Suvaxyn PCV2 (Fenaux *et al*., 2004) has

previously been licensed and was commercially introduced to pig population in 2006. Both the inactivated and the live-attenuated PCV2 vaccines were demonstrated to be very effective and induced protective immunity in the singular PCV2-challenge model. It has been shown that the live chimeric PCV1-2 vaccine virus is genetically stable when it is serially passage in cell culture as well as in pigs. Another one is a live *Flavivirus* chimera vaccine against West Nile virus (WNV) in horses (Preve Nile) was registered in the United States in 2006 (Monath *et al.,*2001).

Veterinary Vaccine: Future

New technologies such as recombinant DNA technology have been used to improve traditional or develop new vaccines against diseases. After 16 years of research the world's first regulatory approval for a plant made vaccine for veterinary purposes occurred in early 2006 and marketed the possible transition of plant made vaccine research to plant made vaccine development. The licensure of the plant made Newcastle disease virus vaccine demonstrated that the technology has technical and industrial feasibility for application with animals.

Edible vaccine: Edible vaccines are a type of subunit vaccine where the reactor is a plant or plant cells. The vaccine may be delivered in plant tissues or through a purified or partially purified extract (Lal *et al*., 2007). Producing anedible vaccine begins by selecting a suitable protein fragments or antigen. The corresponding gene of interest is cloned into an expression cassatte that contains plant regulatory sequences capable of driving gene expression and showing the gene's end. This cassatte is then used in plant transformation. It is also possible to clone gene of interest in plants like tobacco, potato or corn. The genes of transmissible gastro enteritis and New Castle Disease (Hahn *et al*., 2007) coding for protective antigens were cloned in plants and these plants products were used for vaccination.

Reverse genetics based vaccine: Reverse genetics is an approach to discover the function of a gene by analyzing the phenotypic effects of specific engineered gene sequences. Reverse genetics seeks to find what phenotypes arise as a result of particular genetic sequences. Influenza viruses cause annual epidemics and viruses undergo continual antigenic variation, which requires the annual reformulation of trivalent influenza vaccines, making influenza unique among pathogens for which vaccines have been developed. This technique has long been used to generate strains for the preparation of either inactivated or live attenuated influenza vaccines (Fenaux *et al*., 2004).

Biofilm based and anti-ideotypic vaccine: Bacterial biofilm is a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface, which constitutes a protected mode of growth that allows survival in hostile environment. Bacteria biofilm on suitable substrate after inactivation can be used as a successful oral vaccine (Azad *et al*., 2000).

Anti-idiotypic vaccines comprise antibodies that have three-dimensional immunogenic regions, designated idiotopes that consist of protein sequences that bind to cell receptors. Idiotopes are aggregated into idiotypes specific of their target antigen. Anti-idiotypic vaccine based on structural variants found in immunoglobulins called idiotopes. Anti ideotypes resemble the native protein which can be used as vaccine candidate. They are mainly used (ex. Racotumomab) for high risk cancer patients (Ladjemi, 2012).

Conclusions

Vaccinology has become a recognized science that combines disciplines of immunology, microbiology, protein chemistry and molecular biology with practical considerations of production costs, regulatory affairs and commercial returns. Veterinary vaccines have already made enormous impacts not only on animal health, welfare, and production but also on human health. A continuous interchange between animal and human disease control agencies and scientists will be essential to be prepared for the ever-present threat of new, emerging diseases.

Present and future immunological products increase the ability to keep animals healthy rather than awaiting the onset of disease and its associated negative effects. Research and development form the basis for the generation of new and improved veterinary vaccines. Animal scientists can borrow heavily from medical research, particularly in the areas of welfare and geriatric medicine for companion animals, which are becoming increasingly lucrative markets for animal health companies. While at present, vaccines are not available for all infections, access to modern research into vaccines holds great promise and opportunity for the future, as new techniques are mastered. Practical aspects in vaccine development such as product stability and less dependence on cold-storage are to be addressed. The research inputs for veterinary vaccines are to be raised in terms of funding, awareness and social and political will.

References

[1] Azad, I.S., Shankar, K.M., Mohan, C.V. and Kalita, B. (2000). Uptake and processing of biofilm and free-cell vaccines of Aeromonashydrophila in Indian major carps and common carp following oral vaccination antigen localization by a monoclonal antibody.*Dis. Aquat. Org*., **43**: 103–108.

[2] Blanchard, P., Mahe, D., Cariolet, R., Keranflech, A., Baudouard, M.A., Cordioli, P., Albina, E. and Jestin, A. (2003). Protection of swine against postweaningmultisystemic wasting syndrome (PMWS) by porcine circovirus type 2 (PCV2) proteins. *Vaccine,* **21**: 4565–4575.

[3] CDCP (2015). Report by Centers for Disease Control and Prevention. Epidemiology & Prevention of Vaccine – preventable Disease. 13th Ed. Cited from www.post-polio.org. Page: 297-310.

[4] Chiers, K., van Overbeke, I., De Laender, P., Ducatelle, R., Carel, S. and Haesebrouck, F.(1998). Effects of endobronchial challenge with *Actinobacilluspleuropneumoniae* serotype 9 of pigs vaccinated with inactivated vaccines containing the Apx toxins. *Vet. Q.*, **20**:65-69.

[5] Fenaux, M., Opriessnig, T., Halbur, P.G., Elvinger, F. and Meng, X. J. (2004). A chimeric porcine circovirus (PCV) with the immunogenic capsid gene of the pathogenic PCV type 2 (PCV2) cloned into the genomic backbone of the nonpathogenic PCV1 induces protective immunity against PCV2 infection in pigs. *J. Virol.,***78**:6297–6303.

[6] Ferrari, M., A., Brack, M.G., Romanelli, T.C., Mettenleiter, A., Corradi, N., Dal Mas, M. N., Losio, R., Silini, C. and Pratelli, A. (2000). A study of the ability of a TK-negative and gI/gE-negative pseudorabies virus (PRV) mutant inoculated by different routes to protect pigs against PRV infection. *J. Vet. Med. B Infect. Dis. Vet. Public Health.,***47**: 753–762.

[7] Griffin, D., Ensley, S., Smith, D. and Dewell, G. (2002). Understanding vaccines. *NebGuide*, 1445-1448.

[8] Gupta, R.K., Sharma, S.B., Ahuja, S. and Saxena, S. N. (1987). The effects of different inactivating agents on the potency, toxicity and stability of pertussis vaccine. *J. Biol.Stand*., **15(1)**: 87-98.

[9] Hahn, B.S., Jeon, I.S., Jung, Y. J., Kim, J.B., Park, J.S., ha, S.H., Kim, K. H., Kim, H. M., Yang, J. and Kim, Y.H. (2007). Expression of hemagglutinin-neuraminidase protein of Newcastle disease virus in transgenic tobacco. *Plant Biotechnol. Rep*., **1**: 85-92.

[10] Han, K., Zheng, H., Huang, Z., Qiu, Q., Chen, B. and Xu, J. (2014). Vaccination coverage and its determinants among migrant children in Guangdong, China. *BMC Public Health*.,**14**: 203.

[11] Kroll, J.J., Roof, M.B. and McOrist, S. (2004). Evaluation of protective immunity in pigs following oral administration of an avirulent live vaccine of *Lawsoniaintracellularis*. *Am. J. Vet. Res*., **65:**559-565.

[12] Ladjemi, M.Z. (2012). Anti-idiotypic antibodies as cancer vaccines: achievements and future improvements. *Front. Oncol.,***2**: 158.

[13] Lal, P., Ramachandran, V.G., Goyal, R. and Sharma, R. (2007).Edible vaccines: current status and future. *Indian Journal of MedicalMicrobiology,* **25(2)**: 93-102.

[14] Lee, N.H., Lee, J.A., Park, S.Y., Song, C. S., Choi, I. S. and Lee J.B. (2012). A review of vaccine development and research for industry animals in Korea. *Clin. Exp. Vaccine Res*.,**1**: 18-34.

[15] Lombard, M., Pastoret, P. P. and Moulin, A. M. (2007). A brief history of vaccines and vaccination.*Rev. Sci.Tech. Off. Int. Epiz*., **26(1)**: 29-48.

[16] Meeusen, N.T., Walker, J., Peters, A., Pastoret, P. P. and Jungersen, G. (2007).Current Status of Veterinary Vaccines.*Clin. Microbiol. Rev*., **20(3):**489–510.

[17] Micoli, F., Rondini, S., Pisoni, I., Proietti, D., Berti, F., Costantino, P., Rappuoli, R., Szu, S., Saul, A. and Martin, L. B. (2010). Vi-CRM197 as a new conjugate vaccine against *Salmonella Typhi. Vaccine.*,**29(4)**: 712-720.

[18] Monath, T.P., Arroyo, J., Miller, C. and Guirakhoo, F. (2001). West Nile virus vaccine. *Curr.Drug Targets Infect. Disord*.,**1**: 37–50.

[19] Palya, V., Kiss, I., Tatar-Kis, T., Mato, T., Felfoldi, B. and Gardin, Y. (2012). Advancement in vaccination against disease: recombinant HVT NDV provides high clinical protection and reduces challenge virus shedding with the absence of vaccine reactions. *Avian dis.,* **56(2)**: 282-287.

[20] Petre, J., Pizza, M., Nencioni, L., Podda, A., De Magistris, M. T. and Rappuoli, R. (1996). The reaction of bacterial toxins with formaldehyde and its use for antigens stabilization. *Dev. Biol. Stand*., **87**: 125-134.

[21] Pollard, A.J., Kirsten, P. and Beverley, C.P. (2009). Maintaining protection against invasive bacteria with protein–polysaccharide conjugate vaccines.*Nature Reviews Immunology*, **9:** 213-220.

[22] Seong, S.Y., Cho, N.H., Kwon, I.C. and Jeong, S.Y. (1999). Protective immunity of microsphere based mucosal vaccines against lethal intranasal challenge with *Streptococcus pneumonia*e. *Infect. Immun*., **67**:3587-92.

[23] vanOirschot, J.T., Kaashoek, M.J. and Rijsewijk, F.A. (1996). Advances in the development and evaluation of bovine herpesvirus 1 vaccines. *Vet. Microbiol*.,**53**: 43–54.

[24] vanOverbeke, I., Chiers K., Ducatelle, R. and Haesenbrouck, F. (2001). Effect of endobronchial challenge with *Actinobacilluspleuropneumoniae* serotype 9 of pigsvaccinated with a vaccine containing Apx toxins ant transferrin-binding proteins. *J. Vet. Med. B Infect. Dis. Vet.Public.Health*.,**48**: 15–20.