Review

Probability to produce animal vaccines in insect baculovirus expression system

Bhaskar Roy¹, Shuang Liang¹, Peng Zhang¹, mei-xian Wang¹, Fang Zhou¹, Wan Chi Cheong David² and Yun-gen Miao¹*

¹Key Laboratory of Animal Virology of Ministry of Agriculture, College of Animal Sciences, Zhejiang University, Hangzhou 310029, P. R. China.
²Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong SAR, P. R. China.

Accepted 20 May, 2011

The insect baculovirus expression system is a valuable tool for the production of vaccine. Many subunit vaccines have been expressed in this system. The first vaccine produced in insect cells for animal use is now in the market. In this study, we reviewed recent progress of animal's vaccine production for different expression levels and baculovirus genome stability, characteristic features of baculovirus expression vector system (BVES), virus link particles, baculovirus expression in mammalian cell and methodology of produce subunit vaccines. This review showed that BVES is a fantastic tool for vaccine development and it has wonderful feature for future animal vaccine development.

Key words: Baculovirus expression system, vaccine, subunit vaccine, DNA vaccine.

INTRODUCTION

Vaccine development has played a pivotal role in the history of medical biology for the treatment of diseases like polio, smallpox, diphtheria etc. This has resulted in increasing the life expectancy of people in the developing world. Some of the infectious diseases like smallpox, bird flu, plague etc, are a natural outcome of human beings coming in contact with infected animals. So, in order to prevent outbreak of these diseases, it is essential to control them at the source level. Vaccinating the animals against the causal agents can be an important step in this regard. The best example in this regard is the successful development of vaccines against smallpox. Scientists are now in the process of creating new vaccines with greater potential for protecting humans and animals (Lombard et al., 2007). Vaccines should be designed to prevent infection rather than to prevent clinical signs of disease and produce sterile immunity. Available technologies allow us to design vaccines, together with their companion diagnostic tests, which make it possible to distinguish between vaccinated and infected (Pastoret et al., 2007).

Several effective vaccines are already available for viral infection in the market. Some animal viral infections and diseases which are likely to be destroyable in the near future, such as African swine fever, the wild boar (Sus scrofa) for classical swine fever or the African buffalo (Syncerus caffer) for foot and mouth disease may be suffering from lack of suitable vaccine (Pastoret et al., 2007). The use of vaccines in animal production systems is also often more environmentally safe and friendly because it reduces the use of chemicals. An example is the anti-tick vaccine which is based on a cryptic intestinal antigen of the parasite (Lombard et al., 2007).

The baculovirus expression vector system (BEVS) has been extensively used since the past 23 years to express a large variety of proteins. Several researchers have proved that BEVS has the ability to produce significant amounts of the desired protein in a cellular environment, thereby enabling the correct folding, targeting and post-
translational modification of the expressed protein (Martijan et al., 2007). Baculovirus’s capability to transduce a wide variety of mammalian cells leads to the emergence of baculovirus as a novel vector for in vivo and in vitro gene delivery (Gould, 2004). The first vaccines produced in insect cells for animal use are now available commercially (Van, 2006).

This review will focus on past progress of vaccine development and possibility to produce vaccine in BEVS as a recent advance technology.

SUBUNIT VACCINE

Subunit vaccines are cell free vaccines, made from purified antigenic components of pathogenic microorganisms, thus, carrying less risk of adverse reactions than whole-cell preparations. However, some factors that must be considered in the expression of a subunit vaccine are its level of expression, immunogenicity and protective efficacy, purification and yield, fidelity to natural product, cost and safety. Examples of subunit vaccines include the vaccines used to protect against pneumonia caused by *Streptococcus pneumoniae* and against a type of meningitis (Nene et al., 1995).

Molecular biology and genetic engineering has good impact on vaccine development by providing the tools and techniques to produce a single protein in a nucleus system. For example, *Spodoptera frugiperda* SF21AE cells infected with recombinant virus expressed p67 as a 100-kDa molecule (Nene et al., 1995). Also, *Theileria parva* p67 subunit vaccines were produced in BVES which was against East Coast fever in cattle (Kaba et al., 2005).

Recombinant subunit vaccines

Recombinant subunit vaccines eliminates the risks associated with handling a pathogenic organism and the risks associated with live or killed products reverting to a pathogenic state due to incomplete inactivation (Greensfelder, 2000).

Conventional vaccinology

The conventional strategy of vaccine development requires the identity of the pathogenic microorganism and its dissection using biochemical, immunological and microbiological methods in order to identify the components important for immunity (Rappuoli, 2001). This method is applicable for pathogens that can be cultured under *in vitro* condition. An exception to this has been the hepatitis B vaccine where the pathogen, although, unable to grow *in vitro*, could be recovered in large quantities from the plasma of infected people (Buynak et al., 1976).

Reverse Vaccinology

This technology takes advantage of the genome sequence of the pathogen. The method was first used to identify antigen for a vaccine development against *Neisseria meningitidis* serogroup B (MenB) (Tettelin et al., 2000).

BEVS FOR VACCINE PRODUCTION

Insect BEVS has been used as an effective tool to make recombinant protein(s). There are many transfer vectors for inserting foreign genes into the viral genome (Jones and Morikawa, 1996).

The high levels of expression and proper post-translational modification were the major advantages in this system. The researchers have reported that baculovirus have capability to transduce a wide variety of mammalian cells both *in vivo* and *in vitro*. Also, modified AcNPVs (*Autographa californica* nuclear polyhedrosis virus) can express exogenous genes in mammalian cells (Carbonell et al., 1985; Volkman and Goldsmith, 1983; Boyce and Bucher, 1996).

Baculovirus has recently also been exploited as a vaccine expression/delivery vehicle (Yu-Chen et al., 2008; Vlak and Keus, 1990; Hou et al., 2003; Li et al., 2003). The recombinant baculovirus was used as expression vector for developing a prophylactic vaccine against severe acute respiratory syndrome-corona virus (SARS-CoV) (Bai et al., 2008). The baculovirus AcNPV is widely used as a vector for expression of foreign genes in insect cells (Hofmann et al., 1995). Monique M. Van Oers reported the successful expression of a wide variety of viral (glyco) proteins in baculovirus system for vaccine purposes (Van, 2006). Also, the successful expression of glycoprotein E1 of hog cholera virus in insect cells has been utilized to protect swines from hog cholera (Hulst et al., 1993). Besides, insect cells are excellent candidates for development of new safe and effective HCV subunit vaccine.

The literatures have shown that recombinant hemagglutinin subunit vaccines produced through BEVS is a more convenient effective and cheaper method for broadly targeted protection against avian influenza infections (Bethanie, 1997).

Characteristics

Baculovirus have lot of advantages for research purpose with regards to vaccine development. This includes its high expression levels, limitless size of expressed protein, efficient cleavage of signal peptides, processing of the protein and post-translational modifications, simultaneous expression of multiple genes and short time for protein expression (Patterson et al., 1995; Luckow
Table 1. Characteristics of the major protein expression systems.

| Characteristic     | Bacterial | Yeast | Mammalian | Insect |
|-------------------|-----------|-------|-----------|--------|
| Proteolytic cleavage | May be   | May be | Yes       | Yes    |
| Glycosylation     | No        | Yes   | Yes       | Yes    |
| Secretion        | May be   | Yes   | Yes       | Yes    |
| Folding          | May be   | May be | Yes       | Yes    |
| Phosphorylation  | No        | Yes   | Yes       | Yes    |
| Acylation        | No        | Yes   | Yes       | Yes    |
| Amidation        | No        | No    | Yes       | Yes    |
| Yield (dry weight) (%) | 1-5      | 1     | <1        | 30     |

Adapted from Vlak and Keus (1990).

and Summers, 1988; Beljelarskaya, 2002) (Table 1). BEVS technology combines the protein authenticity of higher eukaryotic systems with the efficiency of microbial systems, which is a highly efficient expression vector in insect and mammalian cells (Yu-chen, 2005) (Table 1). The baculovirus production process can be easily defined as a batch process with higher tolerance to osmolality and by-product concentration (Ikonomou et al., 2003).

**Baculovirus vectors**

Baculovirus is used as a viral vector for gene therapy because the production of many viral vectors requires either plasmid transfection into producer cell lines or helper virus infection (Yu-chen, 2005). The diversity of AcNPV-based transfer vectors, combined with available S. frugiperda Sf9 and Sf21 cell lines, establish baculovirus expression as a preferred system for functional eukaryotic gene expression and the large-scale production of recombinant proteins.

**Adaptations for secreted proteins**

Tan et al. has reported that the respiratory syncytial virus (RSV) fusion (F) protein was expressed in insect cells as recombinant glutathione-S-transferase (GST)-tagged proteins (Tan et al., 2003; Knops et al., 1991). Several researchers have reported that IL-18 expressed in BEVS is an effective vaccine adjuvant in mice and non-human primates (Eberl et al., 2000; Kim et al., 1999).

**Baculovirus vectors with mammalian cell**

Since 1983, baculoviruses have been used to express recombinant genes under strong insect-virus promoters in insect cells. In 1995, it was discovered that recombinant baculoviruses are able to deliver genes into mammalian cells.

The baculovirus vectors as mammalian cell gene delivery vectors are constantly increasing and the system improvement is going on. We may be concerned that baculoviruses are applied in cell-based assays for drug screening which requires long period of time to generate stable cell lines. Also, baculovirus vector can transfer very large DNA in to mammalian cells and vectors for toxic gene products can be generated.

The application of modified baculoviruses for in vivo gene delivery has also been demonstrated. The baculoviruses have the own property of replicating in insect cells while being incapable of initiating a replication cycle and producing infectious virus in mammalian cells. Baculoviruses are valuable tools for launching viral infection in cases where there is no appropriate cell culture system available (Kost and Condreay, 2002). Mammalian cells incubated with the culture supernatant of infected Sf9 (S. frugiperta) cells could serve as a very convenient way for rapid and efficient expression of foreign genes in mammalian cells, but it might be more suitable for primate adherent culture cells (Cheng et al., 2004).

**VIRAL SUBUNITS EXPRESSION IN THE BACULOVIRUS SYSTEM**

The nucleocapsid (N) gene of turkey coronavirus (TCV) was expressed in baculovirus expression system. The experiment had showed that baculovirus-expressed TCV, N protein is a suitable source of antigen for ELISA-based detection of TCV-specific antibodies in turkeys (Breslin et al., 2001).

In this case, HIV-1 glycoprotein 120 binding to recombinant N-methyl-D-aspartate (NMDA) receptor subunits is expressed in a baculovirus system. The result showed that HIV-1 gp120 could directly bind to the NMDA receptor (Xin et al., 1999). Also, virus-specified tubules of epizootic haemorrhagic disease virus which are using a baculovirus expression system were studied (Nel and Huismans, 1991).

**Viral envelope proteins**

The baculovirus-insect cell expression system is an
advance system for the production of viral antigens with vaccine potential for humans and animals. The wide variety of viral (glyco) proteins has been successfully expressed in this system for vaccine purposes (Van Oers and Vlak, 2007).

Recombinant dengue-2 virus envelope protein has been produced in baculovirus infected insect cells (Delenda et al., 1994; Zhang et al., 1988). Many scientists have shown that expressing vesicular stomatitis virus glycol-protein (VSV-G) in the viral envelope was generated by inserting the VSV-G coding sequence downstream of the polyhedrin promoter (Facciabene et al., 1988; Wilson et al., 2008).

Virus-like particles

Norwalk virus (NV) is a major cause of epidemic gastroenteritis. The NV capsid is composed of a single protein that forms recombinant (rNV) virus-like particles (VLPs) (Ball et al., 1996). Recombinant Norwalk virus-like particles (rNV VLPs), produced in insect cells were evaluated as a source of oral immunogen in CD1 and BALB/c mice by monitoring rNV-specific serum (Ball et al., 1998).

The ability to make a large variety of virus-like particles (VLPs) has been successfully achieved in the BEVS/insect cell system (Aucoin et al., 2007). Examples include the adeno-associated viral (AAV) particles (Sollerbrant et al., 2001), Nudaurelia capensis omega virus (NomegaV) (Maree et al., 2006) etc. Influenza virus-like particle (VLP) is being tested as a new generation of non-egg or non-mammalian cell culture-based candidate vaccine against influenza infection (Bright et al., 2007).

Inclusion of recombinant cytokines in the vaccine

Animal protection studies suggest that synergistic combinations of cytokines may be essential to protect the body from a viral challenge. In this regard, cytokines used in heterologous prime-boost strategies with viral vector vaccines or recombinant proteins, might afford the most potent vaccine approaches (Ahlers et al., 2003). For example, GM-CSF (granulocyte-macrophage colony-stimulating factor) DNA induces specific patterns of cytokines which could provide the basis for development of new strategies to develop cancer vaccines, including the use of cytokine genes as adjuvant (Perales et al., 2002; Kämpgen et al., 1994).

Baculoviruses as DNA vaccines

Baculoviral vectors with mammalian promoters driving the expression of viral genes have been used in a relatively less number of vaccine trials. Intramuscular injection with baculovirus (BV) expressing the E2 glycoprotein of hepatitis C virus controlled by the CMV promoter and an enhancer provided specific humoral and cellular responses (Facciabene et al., 2004).

Baculovirus vector with the influenza hemagglutinin (H1) controlled by chicken beta actin promotor gave a similar level of protection as a wild type baculovirus against a lethal influenza challenge in intranasally immunized mice (Abe et al., 2003). A complement regulatory protein in the BV envelope has been shown to protect the baculovirus gene therapy vector against complement mediated inactivation (Hüser et al., 2001). This type of strategy can be applied for vaccine production.

It has been reported that the baculovirus insect cell production technology could be used for rapid vaccine production and this technique is especially suitable for influenza vaccines (Cox, 2008; Safdar and Cox, 2007).

Combinations of vaccine strategies

The efficiency of combining different vaccine strategies is currently being explored to improve on the adjuvant therapy of melanoma (Kirkwood et al., 2006; Gogas et al., 2006). Recently, it has been found that in tuberculosis and HIV infection, the accent is currently being placed on the use of antimalarial combinations in order to overcome the problem of multidrug resistance which are particularly good candidates for combination therapy (Olliaro and Taylor, 2004).

It has been recently studied that in cancer treatment, there is the combination of vaccination with other therapies (Andersen et al., 2008; Colombo and Piconese, 2007). Vaccine development for humans against diseases is basically carried out using cattles as a model. It has been reported that prime boost vaccination strategies using (bovis bacillus Calmette-Guérin) combinations of BCG and DNA vaccines could provide better protection than either vaccine alone (Dle et al., 2005).

Viral marker vaccines and differential diagnosis technology

Improving the vaccine purity, potency, safety and efficacy is of paramount importance before the actual field trials. This technology makes it possible to retain the advantages, while overcoming the safety hazards and logistical disadvantages, inherent in many modified live vaccines (MLV) (Roth and Henderson, 2001; Henderson, 2005).

Subunit vaccines

These are used for marker vaccines development. Peptide Peptide vaccines are one form of subunit vaccine that is receiving additional interest (Frenchick et al., 1992).
Deletion mutants

Organisms with their virulence gene deleted can be used as live attenuated vaccines. Such genetically engineered vaccines are less likely to be a safety risk. Besides, gene deleted vaccine organism may also serve as an excellent vector for genes of other pathogens (Kimman, 1992).

Live vectored vaccines

Live recombinant vector strains express one or more protective genes from another microorganism. The advantage is that they may be effective in overcoming the interference with the maternal antibody immune response (Monteil et al., 2000). The use of viral vectors for development of veterinary vaccines has been reviewed (Sheppard, 1999; Wigdorovitz et al., 1996).

DNA vaccines

It consists of bacterial plasmids which are purified and injected into host animal cells that express the targeted antigen. Immune responses induced by DNA vaccines can be more variable than those by more conventional technologies; these must be assessed and balanced with the advantages of a particular DNA vaccine (Roth and Henderson, 2001).

Plant-based vaccines

Plants, plant cell cultures and plant viruses have been developed to produce antigens or antibodies for vaccines, therapeutics and diagnostic tests (Wigdorovitz et al., 1996).

Technologies for development of diagnostic test

Conventional technologies

Conventional diagnostic assays have relied on detection of the agent using a variety of techniques such as virus neutralization, hemagglutination inhibition etc. (Roth and Henderson, 2001).

Western blot technologies

There has been an increasing use of Western blot assays for highly specific confirmatory tests to more accurately identify cross-reactions, resulting in finding unexpected positive reactions from animals that come from populations with a low prevalence of the disease (Roth and Henderson, 2001).

Nucleic acid technologies

The development and optimization of nucleic acid amplification and detection techniques have resulted in the ability to quickly detect and identify a number of agents directly from host animal samples without waiting for culture results (Roth and Henderson, 2001). This technique has been used to detect a number of agents of veterinary interest including West Nile virus (Wigdorovitz et al., 1996).

Biosensor technologies

Biosensors involve the use of a receptor (usually an antibody) for the target pathogen or a disease-specific antibody and a transducer which converts a biological interaction into a measurable signal (David et al., 2008). Thus, in future, a number of technological developments will hold tremendous potential for creative design of marker vaccines and their companion diagnostic tests.

Baculovirus-produced vaccines against protozoan

Plasmodium

Immunogenicity testing of Plasmodium falciparum antigens, considered as a suitable candidate for development of malaria vaccines, was undertaken in rabbits. Also, there is a report on the comparative testing of six P. falciparum merozoite stage antigen-based malaria vaccine. Baculovirus MSP-1<sub>19</sub> immunizations produced the highest parasite-specific antibody titers in IFA (immuno-fluorescence assays). Antibodies induced by baculovirus MSP-1<sub>19</sub> gave the highest levels of growth inhibition in HB3 and 3D7 parasite cultures, followed by AMA-1+MSP-1<sub>19</sub> and the AMA-1/MSP-1<sub>19</sub> fusion. Comparative analysis of immunogenicity of vaccine antigens can be used to prioritize candidates before moving to expensive GMP production and clinical testing.

An experiment conducted using Baculovirus-Expressed Constructs on rabbit revealed that on expression; they induce Immunoglobulin G (I<sub>G</sub>) that recognizes VAR2CS A antigen on P. falciparum- infected erythrocytes. The end result thus, indicated that most domains of native VAR2CSA on the surface of intact infected erythrocytes are accessible to IgG and thus constitute a potential source of vaccine (Lea et al., 2006).

Babesia

Babesia rodhaini antigen p26 was expressed in Escherichia coli and when it was introduced into infected insect cells along with a recombinant baculovirus, good effective results were found (Igarashi et al., 2000).

CONCLUSION AND RECOMMENDATION

The past over view shows that animal vaccine development
has advanced with the availability of new knowledge and methodology in molecular biology and biotechnology.

Glycoproteins which are suitably expressed in the BEVS are good candidate sources for vaccine development. The combination of subunit vaccines and marker tests, both based on antigens expressed in insect cells, provides a powerful tool to combat disease and to monitor infectious agents. Apart from that it can be said that animal vaccine might be produced in baculovirus insect cell expression system. And our hypothesis is that baculovirus insect cell expression system tool will be a big advantage of vaccine development.

ACKNOWLEDGEMENTS

The work was supported by the National Basic Research Program of China under grand No. 2012CB114601 and the National Natural Science Foundation of China (No.30972141/ C120110), the Key project of Zhejiang Governmen (No.2011C14006) and Chinese Universities Scientific Fund.

REFERENCES

Abe T, Takahashi H, Hamazaki H, Miyano-Kurosaki N, Matsuura Y, Takaku H (2003). Baculovirus induces an innate immune response and confers protection from lethal influenza virus infection in mice. J. Immunol., 171(3): 1133-1139.

Ahlers JD, Belyakov IM, Berzofsky JA (2003). Cytokine, chemokine, and costimulatory molecule modulation to enhance efficacy of HIV vaccines. Curr Mol Med. 3: 285-301.

Andersen MH, Serensen RB, Schrarna D, Svane IM, Becker JC, Thor SP (2008). Cancer treatment: the combination of vaccination with other therapies. Cancer Immunol. Immunother., 57: 1735-1743.

Aucoin MG, Jacob D, Chahal PS, Meghrous J, Bernier A, Kamen AA (2007). Virus-like particle and viral vector production using the baculovirus expression vector system/insect cell system: adeno-associated virus-based products. Methods Mol Biol. 388: 281-296.

Ball JM, Estes MK, Hardy ME, Conner ME, Opekun AR, Graham DY (1996). Recombinant Norwalk virus-like particles as an oral vaccine. Arch. Virol. Suppl., 12: 243-249.

Ball JM, Hardy ME, Almar RL, Conner ME, Estes MK (1998). Oral immunization with recombinant Norwalk virus-like particles induces a systemic and mucosal immune response in mice. J. Virol., 72: 1345-1353.

Bai B, Lu X, Meng J, Hu Q, Mao P, Lu B, Chen Z, Yuan Z, Wang H (2008). Vaccination of mice with recombinant baculovirus expressing spike or nucleocapsid protein of SARS-like coronavirus generates humoral and cellular immune responses , Mol. Immunol. 45: 868-875.

Beljelarskaya SN (2002). A baculovirus expression system for insect cells. Mol. Biol., 36: 281-292.

Bethanion E (1997). Recombinant Hemagglutinin Subunit Vaccine Produced in a Baculovirus Expression Vector System. Avian Diseases, 47: 253-262.

Boyce FM, Bucher NL (1996). Baculovirus-mediated gene transfer into mammalian cells. Proc. Natl. Acad. Sci. U.S.A. 93: 2348-2352

Breslin JJ, Smith LG, Guy JS (2001). Baculovirus expression of turkey coronavirus nucleocapsid protein. Avian Dis. 45(1):136-43.

Bright RA, Carter DM, Daniluk S, Toapanta FR, Ahmad A, Gavrilov V, Massare M, Pushko P, Myle N, Rowe T, Smith G, Ross TM (2007). Influenza virus-like particles elicit broader immune responses than whole virion inactivated influenza virus or recombinant hemagglutinin. Vaccine 25: 3871-3878

Buynak EB, Roehm RR, Tytell AA, Bertland AU II, Lampson GP,Hillerman MR (1976). Vaccine against human hepatitis B. JAMA 235: 2832-2834.

Carbonell LF, Klowden MJ, Miller LK (1985). Baculovirus-mediated expression of bacterial genes in dipteran and mammalian cells. J. Virol.56: 153-160.

Cheng T, Xu CY, Wang YB, Chen M, Wu T, Zhang J, Xia NS (2004). A rapid and efficient method to express target genes in mammalian cells by baculovirus. World J. Gastroenterol. 10: 1612-1618.

Colombo MP, Picone S (2007). Regulatory-T-cell inhibition versus depletion: the right choice in cancer immunotherapy. Nat. Rev. Cancer, 7: 880-887.

Cox MM (2008). Progress on baculovirus-derived influenza vaccines. Curr. Opin. Mol. Ther., 10: 56-61.

David EA, David RC, Edmond JR, Alson MC, Mercy PKS, William DM, Anthony AH, Shirley L, Alan WT (2008). Comparative testing of six Plasmodium falciparum merozoite stage antigen-based malaria vaccine candidates. Cln. Vaccine Immunol. 15(9): 1345-1355.

Delenda C, Frenkel MP, Deubel V (1994). Protective efficacy in mice of a secreted form of recombinant dengue-2 virus envelope protein produced in baculovirus infected insect cells. Arch Virol. 139(1-2):197-207.

Die BM, Skinner MA, Wedlock DN, De Lisle GW, Vordermeier HM, Glyn HR (2005). Cattle as a model for development of vaccines against human tuberculo, Bud Tuberculosis (Edinb), 85: 19-24.

Eberhard PE, E.Coulston PS, Okamura H, Wilson RA, Mountford AP (2000). IL-18 potentiates the adjuvant properties of IL-12 in the induction of a strong Th1 type emmuni respose against a recombinant antigen.Vaccine.18: 2002-2008.

Facciaciabene A, Aurisicchio L, La Monica N (1988). Baculovirus vectors elicit antigen-specific immune responses in mice. J. Virol., 78: 8663-8672.

Facciaciabene A, Aurisicchio L, La Monica N (2004). Baculovirus vectors elicit antigen-specific immune responses in mice. J. Virol., 78(16): 8663-8672.

Frenchick PJ, Sabara MJ, Ready KFM, Babik LA (1992). Biochemical and immunological characterization of a novel peptide carrier system using rotavirus VP6 particles. Vaccine 10: 783-791.

Gogas H, Ioannovich J, Dafni U (2006). Prognostic significance of autoimmunity during treatment of melanoma with interferon. N. Engl. J. Med., 354: 709-718.Gould AR (2004). Virus evolution: disease emergence and spread. Aust. J. experim. Agric., 44: 1085-1094Lombard M, Pastorot PP, Moulin AM (2007). A brief history of vaccines and vaccination. Rev. Sci. Tech. Off. int. Epiz., 26: 29-48.

Greensfelder L (2000). Infectious diseases. Polio outbreak raises questions about vaccine. Sci., 290: 1867-1869.

Henderson, LM (2005). Overview of marker vaccine and differential diagnostic test technology. Bio., 33: 203-209.

Hofmann C, Sandig V, Jennings G, Rudolph M, Schlag P, Strauss M (1994). Protective efficacy in mice of recombinant surface antigens p26 with Freund's adjuvants against Babesia rodhaini infection. J. Vet. Med. Sci., 62(7): 717-723.

Hikonomou L, Schneider YJ, Agathos SN (2003). Insect cell culture for diagnostic test technology. Bio., 33: 203-209.

Ikonomou L, Schneider YJ, Agathos SN (2003). Insect cell culture for diagnostic test technology. Bio., 33: 203-209.

Jones I, Morikawa Y (1996). Baculovirus vectors for expression in insect cells. J. Virol., 70: 5435-5442.

Kaba SA, Musoke AJ, Schaap D, Schetters T, Rowlands J, Vermeulen
