POULTRY VIRUSES

FROM THREAT TO
THERAPY

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ABSTRACT

Infectious diseases are one of the major causes of economic losses in poultry industries. In many instances, there are no specific signs that are associated with a particular disease. Besides clinical signs and findings from post mortem examinations, diagnostic tools based on serological and molecular detections are used to confirm the causative agents. However, the identification of causative agents and the detection of specific antibody responses in relation to a clinical problem are complicated due to the concurrent infections and improper use of vaccines. Currently, the poultry industry is threatened by more virulent viruses of endemic diseases or by exotic and emerging diseases that can cause major economic losses to this sector. The emergence and re-emergence of avian influenza virus (AIV), particularly the highly pathogenic avian influenza (HPAI) H5N1, the presence of endemic low pathogenic avian influenza (LPAI) H9N2 in poultry flock and recently the detection of the novel H7H9 and H10N8 in certain regions in China posed threats to the poultry industry and public health sector. Infection with HPAI such as H5N1 is easy to diagnose. However, the real challenge is to confront H7N9, H9N2, H10N8 and other LPAI which act in concert with other factors such as management, environment, nutrition and concurrent infections which possess a continuous threat to the entire poultry production system. The repeated outbreaks of diseases caused by variant strains of infectious bronchitis virus (IBV), velogenic Newcastle disease viruses (NDV) and more virulent viruses of infectious bursal disease virus (IBDV), infectious laryngotracheitis virus (ILTV) and Marek's disease virus (MDV) in well managed poultry flocks have prompted the need to evaluate the underlying factors contributing to the failure of vaccinations

to provide protection against clinical infections and transmission of disease.

In the field, vaccination failure is a complex event involving various factors associated with vaccine strains and vaccination program, the virulence of field pathogens and the host immune competence. In many situations, immunosuppressive agents, primarily the MDV, IBDV and chicken anemia virus play an important role in increasing the susceptibility of chickens to opportunistic infections and/or suppressing effective vaccine induced responses. Vaccination is the most cost-effective method available in preventing economic losses and increasing the lifespan of animals. Undoubtedly, diagnostic tools, vaccines and vaccination equipment have improved over the years through the use of innovative technology. However, vaccine is not evolution-proof and it may enhance virus evolution especially in the absence of sterilizing immunity allowing wild type viruses to be transmitted through vaccinated chickens.

Despite advancements in genetic engineered tools, conventional laboratory diagnosis using serological tests and conventional vaccines are used extensively in health and disease management of poultry. Nearly all poultry vaccines are conventional vaccines which consist of live-attenuated and killed vaccines that have generally worked well. However, avian pathogens continue to change and develop ways to evade the immunity induced by the current vaccines. In addition, as the poultry industry become more intensive, accurate, economical and practical laboratory diagnostic tools are important for the effective control of disease outbreaks. The advancements in the use of molecular detection method using real-time PCR approach, highly automated instruments for antibody detection and development of rapid on site assays for

virus antigen detection may have significant impact in the field of disease prevention and control. In the area of vaccinology, most of the advances in the development of recombinant vaccines against poultry diseases are based on the development of recombinant viral vectored, DNA plasmid and reverse genetic vaccines. However, it is anticipated that more recombinant based vaccines will be used in the field in the near future. In addition, the advancement on "omic" technology are paving novel approaches for the development of new generation adjuvants and vaccines as well as breeding for disease resistance based on our improving knowledge of the chicken immunogenomic response to disease. However, the development and delivery of new or improved poultry diagnostics, vaccines and pharmaceutical which fulfill the industry, regulatory and public acceptance is a challenging process.

Although the majority of poultry viruses are pathogenic to chickens, some of them especially NDV has the potential to act as a live saver in humans due to its unique properties as viral vectored vaccine against other infectious diseases and selective oncolytic properties on human cancer cells. The continuous encouraging results of NDV oncolytic virotherapy in human clinical trials will facilitate the approval of NDV as an ancillary therapy of human cancer in the near future. Innovative research on the use of NDV as human vaccines and therapeutics should be explored via multi-disciplinary approach by various expert groups.

In conclusion, as the poultry industry is expanding and the globalization of poultry and poultry by products, much is needed to improve the control and prevention of diseases. The emerging and emerging of diseases especially transboundary diseases can impact socioeconomic of a country and global food security. The strengthening our scientific and technical capacity, especially

through innovative technology and strategy, will help to meet the current challenges and ever changing needs of the nation and the world.

INTRODUCTION

Livestock production is growing rapidly as a result of the increasing demand for animal products. FAO projections suggest that global meat production and consumption will rise from 233 million tonnes (2000) to 300 million tonnes (2020), and milk from 568 to 700 million tonnes over the same period. Egg production will also increase by 30 percent. This forecast shows a massive increase in animal protein demand, needed to satisfy the growth in the human population. Asia is experiencing the world's highest growth rates in production and consumption of livestock products (meat, milk and eggs). The Malaysian livestock industry is an important and integral component of the agricultural sector, providing gainful employment and producing useful animal protein food for the population. Poultry and pig farming represent by far the major proportion of the livestock industry in terms of output value. Malaysia is self-sufficient in poultry, pork and eggs, but imports about 80% of its beef requirement (http://www.mida.gov.my/env3/index.php?page=food-industries).

Although our poultry industry is self-sufficient, the industry is facing constant threats and challenges. Productivity is influenced primarily by the soaring prices of feed whilst infectious diseases caused by viruses and bacteria have significant economic impact to the entire value chain of poultry production. Even though viruses are smaller than bacteria, they are more difficult to control and are classified into different families based on their genomic nature (Figure 1). Viral diseases such as avian influenza (AI) and Newcastle disease (ND) which are also known as transboundary diseases are highly contagious or transmissible and have the potential for very rapid spread, irrespective of national borders, causing serious socio-economic and possibly public health consequences (Omar, 2013). In many cases, outbreaks of the diseases have been widespread

and often transnational and have severely challenged the ability of governments to control them. In the case of avian influenza virus, the impact of the virus to the public health sector cannot be taken lightly since the four pandemics that had occurred in the last 100 years were associated influenza virus of avian species crossing to humans. In addition, some viral infectious diseases such as infectious bursal disease (IBD) (Gumboro disease) and chicken infectious anemia (CIA) and Marek's disease (MD) which may induce immunosuppression are able to cause significant impact to the health status of the birds, preventing them from responding optimally to the vaccinations against different diseases.

Viruses, particularly the RNA viruses can evolve rapidly due to their short generation time and higher mutation rate compared to the DNA viruses. Some viruses such as AI virus can shuffle (re-assort) their genes with other influenza viruses when two or more strains are infecting the same cell whilst, most RNA viruses also evolve by recombination process by exchange of genetic materials with other related viruses. This has been demonstrated in existence of more than 100 different strains of avian infectious bronchitis virus (IBV), a virus classified under the family coronavirus (Zarirah *et al*., 2009). Likewise, studies have shown that the emergence of new coronavirus affecting humans such as severe acute respiratory syndrome coronavirus (SARS-CoV) and more recently in 2012, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Hofer, 2013). Meanwhile, other viruses including DNA virus change slower via mutation process in their genes which gradually accumulate over time. In the presence of selection pressure such as anti-viral drugs, vaccines and/or new susceptible hosts, the mutation rate of viruses may increase. These different processes can occur simultaneously producing a new strain or subtype of viruses that are far more superior that the predecessor in many ways i.e. highly

virulent, resist antiviral drug, vaccine-escape strains and increase in host range susceptibility (Lee *et al*., 2012).

Our research group is working through its researches and with industries to address the various challenges from several different poultry viral diseases namely the avian influenza virus (Orthomyxovirus), Newcastle disease virus (Paramyxovirus), infectious bronchitis virus (Coronavirus), infectious bursal disease virus (Birnavirus), chicken infectious anemia virus (Circovirus), fowl adenovirus (Adenovirus) and Marek's disease virus (Herpesvirus) (Figure 1). Effective disease control requires rapid and accurate detection tools coupled with effective vaccines to control the outbreak. Hence, our group has been interested to develop diagnostics, vaccines and other therapeutics against several economically important poultry diseases. Currently, most of the laboratory diagnosis of avian disease is via conventional method including serological based assays (Omar, 2005). Nevertheless, laboratory diagnosis based on molecular approach using PCR, real-time PCR and sequencing is getting more and more important (Figure 2). In the case of vaccines, the majority of the poultry vaccines are conventional live attenuated and killed vaccines that are formulated against several important bacterial and viral diseases. Numerous studies have been carried out on the development of new generation of vaccines using genetic engineered (reverse vaccinology) technology (Figure 5 and 6). Emphasis is also given to develop new adjuvants which are able to enhance vaccine induced immunity. However, only a handful of some of the developed new vaccines and adjuvants show huge potential for application in a commercial setting.

In order to increase the value proposition of our research and innovation, early buy in and active engagements are being carried out with industries in an effort to bring new diagnostics

and vaccines for animals since prudent measures for control and surveillance of poultry infectious diseases are needed to safeguard both animal and human safety and welfare. This is in line with the Department of Veterinary Service (DVS) decision in looking into ways of producing more poultry vaccines, in mitigating Malaysia's heavy reliance on imported vaccines (http://www.thepoultrysite.com/poultrynews/22972/malaysia-looking-to-produce-poultry-vaccines). Currently, our group has successfully commercialized 4 poultry vaccines, a few more vaccines and diagnostic kits are scheduled to be commercialized by next year through relevant industry partners.

In an effort to explore the use of poultry viruses in comparative medicine and biomedical sciences, our group is also working closely with various expert groups in exploring the possibilities of using poultry viruses namely NDV as oncolytic virotherapy against human cancer. The use of oncolytic virus has enable researchers to develop strategies for both selective destruction of cancer cells and activation of immune responses against residual cancer cells (Nakajima *et al.*, 2013). Numerous studies both in vitro and in vivo animal models have demonstrated the potentials of NDV as a safe and effective therapy against human cancer. Additionally, the potentials of NDV as an oncolytic therapy is currently been tested at phase II/III clinical trials in several countries (Lam *et al*., 2011). However, more work still need to be conducted especially to address the fundamental understanding of NDV selective lysis of cancer cells as well as the safety and ethical issues for public and regulatory acceptance of NDV as one approach that is available for treatment of cancers.

Family Name	Morphology	Enveloped (E) or Naked (N)	Approximate Size (nm)	Nucleic Acid
Poxviridae (poxviruses)		E	350 × 250	Linear ds DNA
Herpesviridae (herpesviruses)		E	200	Linear ds DNA
Adenoviridae (adenoviruses)		N	75	Linear ds DNA
Parvoviridae (parvoviruses)		N	20	Linear ss DNA
Papovaviridae (papovaviruses)		N	50	Circular ds DNA
Baculoviridae (baculoviruses)		E	300 × 40	Circular ds DNA
Picornaviridae (picornaviruses)		N	27	Plus-strand RNA
Togaviridae (togaviruses)		E	50	Plus-strand RNA
Retroviridae (retroviruses)		E	50	Plus-strand RNA
Orthomyxoviridae (orthomyxoviruses)		E	110	Segmented: 8 minus-strand RNA molecules
Paramyxoviridae (paramyxoviruses)		E	200	Minus-strand RNA
Rhabdoviridae (rhabdoviruses)		E	170 × 70	Minus-strand RNA
Reoviridae (reoviruses)		N	65	Segmented: 10–13 ds RNA molecules

Figure 1 Classification of viruses. Viruses are classified based on the presence of lipid envelope and their nucleic acid either DNA or RNA

WHY VIRAL DISEASES ARE DIFFICULT TO CONTROL?

Poultry diseases caused by pathogenic bacteria and viruses are causing constant threats to poultry industry not only in large commercial flocks, but also in backyard chickens throughout the world. However, viral diseases are the most common causes of losses compared to bacteria diseases due to several main reasons as follows;

- Viral diseases can reduce flock performance without showing obvious clinical diseases, hence, being subclinical they can spread easily.
- Viruses can act as the primary agent causing damage to primary tissue for other infectious agents to infect and cause more damaging diseases, hence, causing a complex disease syndrome that is difficult to detect and manage.
- Effects of viral diseases are more damaging since they usually can affect more than one body systems. Furthermore, some viral diseases are able to cause immunosuppression due to their ability to destroy immune cells such as lymphocytes and/or macrophages.
- The only means to control viral diseases is through vaccination which requires healthy host immune systems. Besides, antibiotic works only against bacterial infection.
- Many farms are practising multi-age rearing, viruses can build up on farms and this can put a huge toll on vaccine to work effectively.

- Vaccine is not evolution-proof. The use of vaccine may enhance virus evolution especially in the absence of sterilizing immunity allowing wild type viruses to be transmitted through vaccinated chickens.
- Many viruses are able to resist decontamination procedure hence, they persist in farm environment and infected chickens act as carriers intermittently shed the virus. More importantly, many of the routine diagnostic tests are unable to detect the virus shedding.

If you ask poultry farmers in many countries including Malaysia as to which poultry viral diseases that they are really concerned of, their most likely answers would be avian influenza (AI), Newcastle disease (ND), infectious bronchitis (IB), infectious bursal disease (IBD @ Gumboro), egg drop syndrome (EDS) and chicken infectious anemia CIA). Other viral diseases of concerned especially in layers and breeder are diseases associated with viruses that are able to cause tumor (oncogenic viruses) such as Marek's disease and avian leucosis. However, in the field situation, the scenario may be different. Viral challenges can be the triggers by a whole range of problems including non-infectious disorders but can have an even more devastating effect on overall flock performance, production and profits. These viral diseases have been considered to cause serious threat to the poultry industry either by causing direct economic losses due to high morbidity and mortality or indirect losses due to immunosuppression and/or restriction to export due to international trading policies. The viruses associated with these diseases in Malaysia has been isolated and characterized by several researchers. Most of the work focused on isolation and characterization of the viruses such as NDV (Salih *et al*., 2000b; Yusoff and Tan, 2001; Tan *et al*., 2004b; 2010; Berhanu *et al*.,

2010; Tan *et al*., 2010), variant IBV (Zarirah *et al*., 2009; Balkis *et al*., 2005), very virulent IBDV (Chong *et al*., 2001; Hoque *et al*., 2001; 2002; Phong *et al*., 2002b; 2003; Kong *et al*., 2004a, b; Tan *et al*., 2004a; Nurulfiza *et al*., 2006; 2011), CAV (Chowdhury *et al*., 2002; 2003; Hasmah *et al*., 2004; Hailemariam *et al*., 2008), ALV-J (Thapa *et al*., 2004), adenovirus (Alemnesh *et al*., 2012; Juliana *et al*., 2012) and several different subtypes of AIV (Balasubramaniam *et al*., 2011; 2012; 2013; Chaharaein *et al*., 2006; 2009) for the development of diagnostics and vaccines. The isolation and characterization of some of these viruses in other countries using expertise from UPM researchers have also been carried out in studies by Bahmaninejad *et al*. (2008), Hosseini *et al.* (2004), Kianizadeh *et al.* (2002) and Sharma *et al.* (2005) and Kammon *et al.* (2013).

Although various factors such as environment, nutrition and management play an important role in the full expression of diseases, the most significant underlying factor is usually the infectious agent, how different and virulent is the infecting viruses. Since most RNA viruses such as AIV, IBV, NDV undergo various processes leading to emergence of new genotypes or strains of viruses with different phenotypic characteristic. For instance, the emergence of HPAI H5N1 strain that is able to cause fatal infection in migratory birds and causing efficient transmission of the virus in experimental infection in mouse model as well as causing fatal infection in larger mammals such as tigers and cats (reviewed by To *et al*., 2012). Low pathogenic AIV such as H9N2 has been known for more than 20 years to cause mild infection in human and now there are evidences indicating the virus is drifting rapidly and becoming more virulent in chickens and humans (Shanmuganatham *et al*., 2013). More recently, we faced another concern of AIV jumping host causing infection in human, the emergence of novel low

pathogenic AIV H7N9 and H10N8 strains that cause mild infections in chickens but is able to infect and cause fatal human infections (Gao *et al*., 2013; Garcia-Sastre and Schmolke, 2014). Currently, H7N9 is endemic in certain part of China, and Malaysia reported the first H7N9 in human from a tourist from Guangdong Province, China who visited Sabah early this year (http://www.cidrap.umn.edu/news-perspective/2014/02/malaysia-reports-first-h7n9-case-outside-china). The current H7N9 infection in China is spreading to the neighbouring countries where the virus has been detected in live poultry markets and humans in northern part of Vietnam. The detection of new strain of H10N8 early this year in eastern China resemble of H7N9 virus, where the virus cause mild infection in poultry but fatal human infection. So far, there is no evidence of efficient human to human transmission in the spread of H7N9 and H10N8 viruses. However, recent study confirms that the HPAI H5N1, and the H7N9 and H10N8 viruses have internal genes from H9N2, indicating that the on-going circulating of H9N2 virus may play important role in the emergence of new strains of AIV with significant threat to human (Garcia-Sastre and Schmolke, 2014).

Even though LPAI viruses such as H5N2 and H9N2 are less virulent in chickens, they are able to trigger other infections primarily bacteria and mycoplasmas to take place and subsequently causing a more serious diseases. Meanwhile, there are a number of avian viruses that are able to exert direct or indirect insult to the chicken immune system causing immunosuppression (reviewed by Omar, 2005). This can pose a significant impact to the health status of the birds, preventing the bird from responding optimally to the vaccinations against different diseases. In the case of NDV, although the virus can be divided into at least 10 different genotypes, most of the poultry farms in this region including Malaysia are facing constant threats from the outbreaks of virulent genotype VII

NDV despite vaccination effort (reviewed by Miller *et al*., 2010). In fact, outbreaks of genotype VII NDV have been reported in various countries in Far East and South America. Furthermore, the emergence of variant strains of IBV, ie Qx-like and very virulent IBDV that are able to break immunity induced by vaccination with classical strains of the respective viruses are commonly reported in poultry flocks (reviewed by Jackwood, 2012; Ingrao *et al*., 2013). DNA viruses although to the less extend compared to RNA viruses also undergo genetic alteration especially in the presence of immune selection. Studies have also showed the continuous emergence of very virulent MDV that are able to break vaccine induce immunity (reviewed by Nair, 2005), whilst in the case of infectious laryngotracheitis (ILT), the emergence of pathogenic ILT virus (ILTV) strains from recombination of different live ILTV vaccine strains (Lee *et al*., 2012). All in all, viruses have developed various strategies to evade and invade the immune functions and co-exist within its host and induce an infectious disease in a host with depressed resistance.

CURRENT DIAGNOSTICS AND VACCINES FOR POULTRY

Laboratory Diagnostic Assays

Generally diagnostic assays for detecting infectious agents can be divided as serological or molecular based (Figure 2) where most viral infections can be diagnosed by the detection of (part of) the virus itself or the specific antibody responses (Table 1). The most commonly used laboratory diagnosis method is the virus isolation (VI) via embryonated chickens eggs or cell culture which require serology tests for confirmation. Serological tests that are commonly used are enzyme-linked immunosorbent assays (ELISA),

immunofluorescence assay (IFA), immunoperoxidase assay (IPA), agar gel diffusion precipitin test (AGPT) and virus neutralization test (VNT). In some viral agent such as NDV, AIV and IBV assays such as hemagglutination (HA) and hemagglutination inhibition (HI) are commonly used to diagnose a clinical problem (reviewed by Omar, 2005).

Among the available serology tests, the ELISA procedure is the most commonly used for the evaluation of antibodies in poultry flocks following immunization programs (Phong *et al*., 2002a; Wong *et al*., 2009; Omar, 2005). ELISA can also be used to detect infection by statistically analyzing the kinetics of the antibody titer from paired serum sets. In addition, the ELISA has been showed to correlate well with other serological test namely HI in the detection of NDV and IBV. Our group has developed ELISA against NDV and IBDV by using recombinant viral protein such as nucleoprotein (NP) and VP2 protein, respectively, expressed in *E. coli* (Rabu *et al*., 2002; Hoessini *et al*., 2007). Recently, we have also studied the potential of NS2 protein expressed in yeast expression system as diagnostic assay to detect AIV (Abubakar *et al*., 2011). The performances of these assays are currently under evaluation. One of the major requirements in laboratory diagnosis of a disease is the speed and accuracy of the test. However, the majority of on-site test kits that are currently available are very rapid but lack sensitivity (Figure 3). Nevertheless, the advancements in monoclonal antibody technology in detecting different subtypes and strains of AIV, IBDV and IBV, respectively (reviewed by Omar, 2005) probably may address some of these limitation. However, this assay is limited by the monoclonal antibodies used and additional monoclonal antibodies will be needed as new antigenic strains are identified. Virus neutralization test (VNT) has been extensively used for serological testing and is considered as gold standard assay for

variety of diseases. However, this method is more tedious and not suitable to be used as a routine procedure.

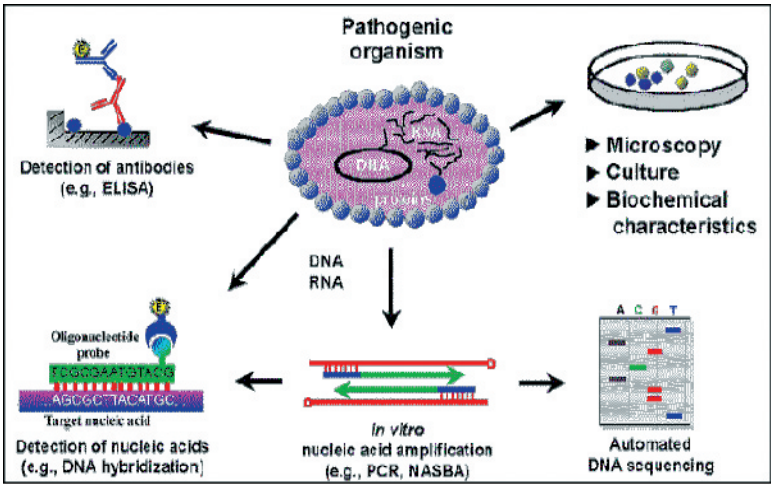


Figure 2 Different technologies in laboratory diagnosis. Conventional approach such as virus isolation and culture, serology based on ELISA and molecular based such as PCR, NASBA and sequencing.

Rapid progress has been made in the development of diagnostic method using molecular techniques (Figure 2 and 4). Polymerase chain reaction (PCR) based detection techniques have been developed to detect various poultry viruses based on the amplification of specific genes. The use of PCR technology followed by restriction fragment length polymorphism (RFLP) analysis or development of specific probes in a hybridization based detection technique has been shown to detect specific strains of a viral agent. However, their applications in diagnosing large number of clinical samples are problematic due to the insensitivity of the agarose gel detection method and the use of hazardous reagents such as ethidium

bromide and radioactive materials. Compared to the conventional PCR, nested or multiplex PCR, colorimetric PCR, real-time PCR have several advantages as laboratory diagnostic tools (Tan *et al*., 2004b; 2009; Phong *et al.*, 2003; Chaharaein *et al*., 2006; 2009; Hairul *et al*., 2008). However, besides conventional PCR, only nested, multiplex and real-time PCR are currently being used in the detection of poultry viruses.

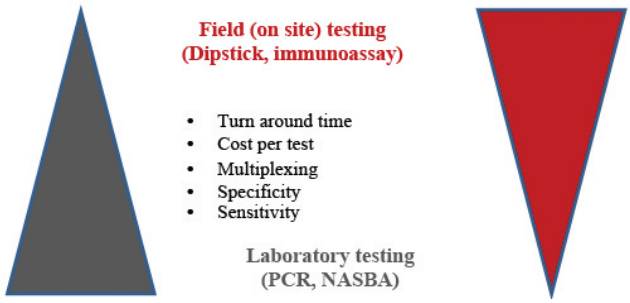


Figure 3 Comparison between field (on-site) and laboratory based tests

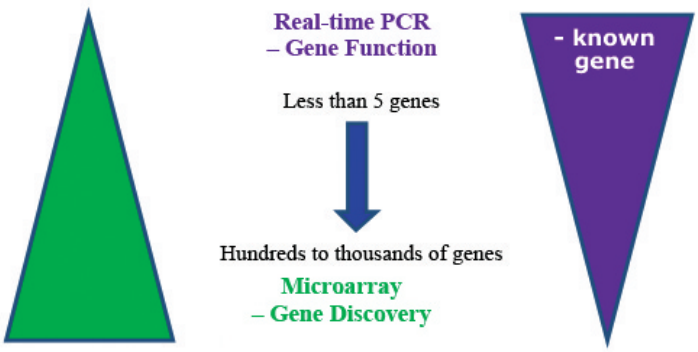


Figure 4 Comparison between gene function/expression and gene discovery

Avian Vaccinology

Poultry industry is growing rapidly in size worldwide. Under the intensive management condition, prevention of infection through effective vaccination is vital to minimize losses due to infectious diseases. Vaccines are available for variety of poultry diseases. Active and passive immunizations are practiced to control diseases in poultry. However, numerous factors can modulate the ability of the host to produce immunity. Vaccination should be carried out at a time when the host is immunologically competent. In most cases, this means that for an optimal response, vaccine should not be administrated until at least third week of age. In addition, both humoral and cell-mediated immunity (CMI) are age-dependent. Unfortunately, exposure to many infectious agents can occur before the optimum vaccination time. Thus, it is necessary to compromise this principle. In this case, depending on the type of the vaccines, immune responses may be obtained, but it is of a lower level and often requires subsequent vaccination to boost immunity and protection levels. Exceptions to this principle, for instance is MD vaccine which causes persistent life-long infections and thus provide a continuous antigenic stimulation (reviewed by Omar, 2006a). The use of veterinary vaccines has somewhat different requirements than the use of human vaccines. One of the limitations of veterinary vaccines is the cost especially poultry vaccines when they are required on a large scale of tens of billions of dose annually. Hence, most poultry vaccines consist of viruses of the same genus but different strains and/or totally of different families of viruses. The bivalent and trivalent MDV vaccines which constitute of the serotype 1, 2 and/or 3 MDV and polyvalent vaccines constitute of NDV, IBV and reovirus are commonly used in poultry industry (reviewed by Omar, 2006b). In addition, most of the vaccines are administered through mass vaccination methods

such as through drinking water and spraying. The technology of administrating vaccines in ovo has also been developed for some viruses namely MDV, NDV, IBDV and fowlpox virus. Another interesting development in poultry vaccines is the development of strong IBDV immunity in chicks with high maternal antibody after administration of IBDV and IBDV-specific antibodies, whereby this type of IBD vaccine is commercially available.

Avian Viral Immunology

Chicken immune system is very similar to mammals and shares common features in the classification of innate and acquired immunity and the presence of different population of cells such as B cells, T cells, NK cells, macrophages and inflammatory cells. The mechanisms associated with vaccine induced immunities against different pathogens have been reviewed by several groups (reviewed by Omar, 2005). Simply providing immune responses following vaccination may not be enough. The immune responses must be of the right type and against the right antigens. Neutralizing antibodies directed against conformationally-dependent epitopes of the fusion (F) and haemagglutinin-neuramidase (HN) of NDV, VP2 of IBDV, spike (S1) of IBV, haemagglutinin (HA) of AIV, VP1 and VP2 of CAV induced protection against lethal challenge with the respective viruses (reviewed by Omar, 2005). Meanwhile, it has been showed that CMI play important role in inducing protection against infections with ILT, MDV and IBV but not against NDV and IBDV (Omar and Schat, 1998; Omar, 2005). Protection against systemic infections of NDV and IBDV might be induced by injection of killed virus vaccine. However, this attempt might be useless in protecting against respiratory or digestive tract infections as local or mucosal immunity might be needed. However, studies have also indicated that oil adjuvant killed vaccines gave markedly

superior responses obtained following a primary inoculation with a live vaccine. The antibody responses following this procedure are exceptionally high, long lasting and are readily transferred to day-old chicks.

Besides immune cells, various immune mediators such as cytokine, interferon, interleukin, chemokine, nitric oxide and many others also play important roles in providing a full spectrum of protection against viral infections (reviewed by Kaiser *et al*., 2010). In addition, mediators from Th1 cells and macrophages such as interferon-gamma (IFN-γ), tumour necrosis factors and nitric oxide are important in certain virus infections especially that are highly cell-associated such as Marek's disease virus (reviewed by Schat, 2004).

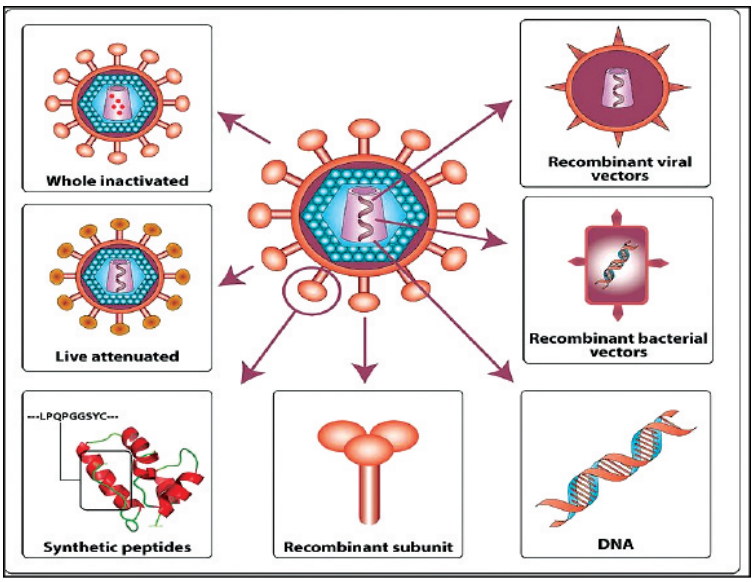


Figure 5 Different types of conventional and recombinant viral vaccine. Live and inactivated vaccines compared to recombinant vaccines based on viral vector, bacterial vector, peptide, subunit and DNA plasmid.

Table 1 Comparison of the properties of different diagnostic techniques

Tests	Specificity	Serotyping	Cost/test	Operator skill needed	Throughput	Speed	Sensitivity
Electron Microscope	Morphology only	Difficult, but possible[a]	medium to high	Yes	Low	< 1 days	Low
EIA/IFA/IPA	Any cultivable virus	Neutralization only	Expensive especially on negative[b]	No/Yes[c]	Low	2 days – 1 month	High if cells susceptible
Serology	Type/group	Only by neutralization	Medium to Inexpensive	No, if automated	High	Have to wait for antibody to be formed	High, if correct antibody is available
PCR	High	Yes, if reagent available	Expensive	Yes	Medium to high	< 10 hours	Very high
Sequencing	Very high	Yes, if specific marker available	Expensive	Yes	High	1 day	Very high

[a] *Possible using immune electron microscopy*

[b] *Reflects longer time in culture before discarding*

[c] *To do and read the test*

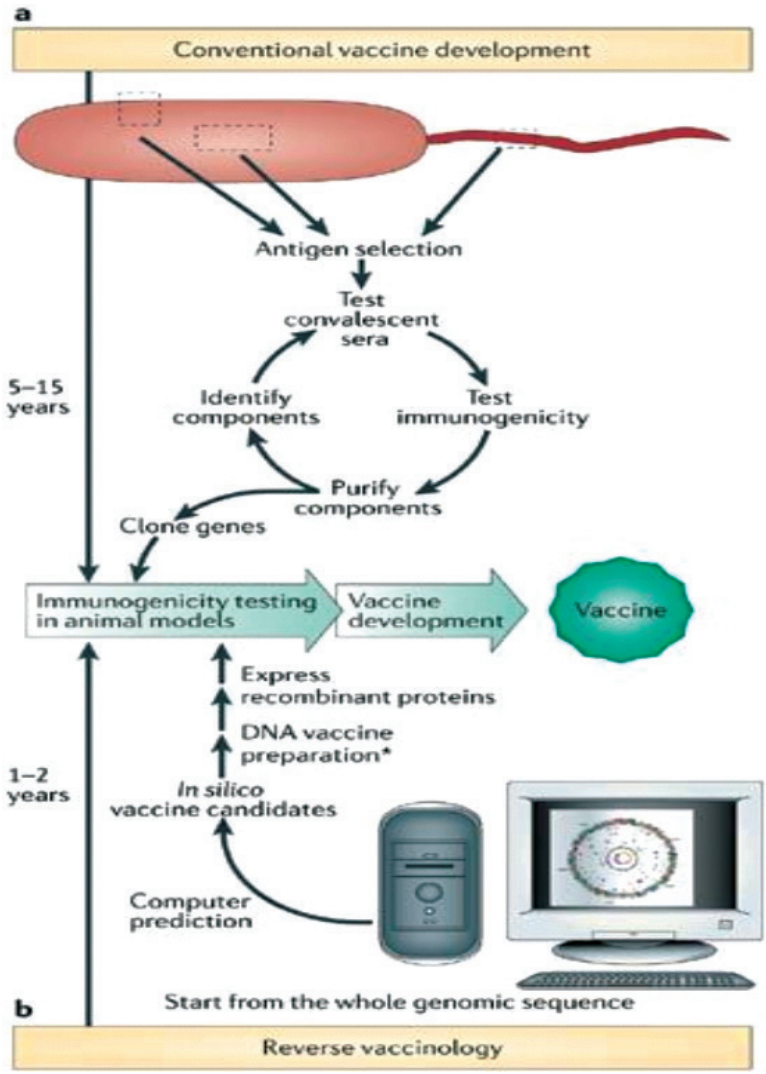


Figure 6 Comparison between reverse vaccinology over conventional vaccine development. Fast access to virtually every single antigen even from non-cultivable microorganisms can be approached from reverse vaccinology

ADVANCES IN POULTRY DIAGNOSTICS AND VACCINOLOGY

Real-time PCR based Diagnostics

In designing an effective disease control program one should consider the diagnostic methods used to diagnose diseases caused by infectious agents (Figure 4). In addition, the evolution of viruses and the emergence and reemergence of pathogenic and zoonotic viruses such as highly pathogenic AIV, zoonotic AIV strains, variant strains of IBV, very virulent strains of MDV and IBDV have placed considerable pressure on development of new diagnostics. Molecular diagnostic based on PCR based technologies especially real-time PCR has been proven as a valuable diagnostic for rapid and sensitive detections of evolving pathogens in the environments (reviewed by Suarez *et al*., 2007).

In general, real-time PCR can be divided into 2 types; first probe-based such as Taqman probes and molecular beacons both consist of an oligonucleotide specific to the gene of interest with a dye molecule on one end and quencher on the other (Espy *et al*., 2006). Secondly, real-time PCR which is based on dyes that bind with dsDNA such as SYBR Green which has been considered the simplest method for real-time PCR but often difficult to optimize due to high dependency of the primers to target sequences. Fluorescence probe based real-time PCR have been developed and are used in diagnostic laboratory, i.e., for the detection and differentiation of NDV pathotypes (Aldous *et al*., 2001), IBDV subtypes (Jackwood *et al*., 2003a), IBV strains (Jackwood *et al*., 2003b), CAV strains (Markowski-Grimsrud *et al*., 2002) and AIV subtypes (Spackman *et al*., 2002). Using similar technology, multiplex real-time PCR assays that are able to detect and differentiate viruses has also been developed. Recently, our group has also developed Taqman

real-time for the detection of different NDV genotypes as a tool to determine virus shedding from oral and cloacal of NDV vaccinated challenged chickens (Rasoli *et al*., 2014).

The use of SYBR Green I based real-time PCR has also been explored for the detection and quantitation of poultry virus. Detection and differentiation of different strains of viruses via Sybr Green I based real-time PCR can only be achieved based on meting curve analysis (reviewed by Espy *et al*., 2006). Hence, we have developed a triplex Sybr Green I based real-time PCR that able to detect and subtypes H9N2 virus based on detection of the NP, H9 and N2 genes (Ong *et al*., 2007). Subsequently, we have developed Sybr Green I based real-time PCR for the differentiation of lentogenic and virulent (mesogenic, velogenic) strains of NDV (Tan *et al*., 2009) and also very virulent IBDV from classical vaccine strain (Kong *et al*., 2009).

New and Emerging Diagnostics

Currently, there is as yet no formal mechanism for the approval of diagnostic tests, although OIE has established guidelines of diagnostic tests, including commercial kits, through its worldwide network of over reference laboratories in different countries (http://www.oie.int/our-scientific-expertise/reference-laboratories/introduction/). Under this guideline, four development requirements have been identified, validation, standard protocols, reference materials and proficiency test. In addition, the molecular diagnostics market is the fastest growing segment and is being driven by multiple growth factors such as the need for automated and easy-to-handle techniques such as on-site test. One of recent advances in new diagnostic tools is the advent of microarray technology. A typical microarray consist of DNA probe (PCR products or

oligonucleotides) which can be efficiently printed on glass slide containing up to 100,000 spots, thus allowing for simultaneous gene expression analysis of many genes. These immobile DNA probes can then be hybridized with a labeled cDNA target samples in order to elucidate the genes expressed in that samples and determine at what relative level they are being expressed. Microarrays have been adopted for multiplexed detection of various targets such as pathogens as well as to profile the expression of thousands of genes simultaneously particularly immune-mediated genes following pathogens infections (Clewley *et al*., 2004; Gheyas and Burt, 2013).

Microarray has been used for typing and subtyping of various avian influenza virus strains depending on the different viral genes (Mukherjee and Chakrabarti, 2012). However, in most studies, the sensitivity of microarray was found to be 10- to 100-fold lower than that of real-time RT-PCR with respect to HA subtyping and pathotyping. Nevertheless, the specificity of the array was excellent, as no pathogens relevant for differential diagnosis yielded a positive reaction. Recently, the applications of microarray as a tool for diagnosis and genome-wide transcriptional profiling of host responses to virus infection has been reviewed by various investigators (Mukherjee and Chakrabarti, 2012; Gheyas and Burt, 2013). Similar approach has been used to identify the differentially expressed mRNA in chicken cells infected with different pathogens such as IBDV, MDV and AIV. Hence, it is envisaged that future improvement in microarray technology may lead to the development of inexpensive and effective diagnostic for screening of animals. In addition, a simple and rapid on-site test for use in the field and laboratories to support clinical suspicion of disease is of importance in developing effective disease control strategy.

Advances in Poultry Immunology

Chickens have several lymphoid tissues and organs such as bursa of Fabricius, Haderian gland and cecal tonsils that are unique compared to mammals. In addition, chickens lack distinct aggregate of lymphoid cells, commonly known as lymph nodes in mammals (Oláh and Vervelde, 2008). However, the chicken immune functions are very similar to mammals and share common features in the classification of innate and acquired immunity and the presence of different population of cells such as B cells, T cells, NK cells, macrophages and inflammatory cells. However, instead of neutrophils, chickens have heterophils that have similar inflammatory functions (Oláh and Vervelde, 2008). Advances in the characterization of chicken genome has identified numerous genes that are orthologs to human and mouse models such as Toll-like receptors that are involved in innate immunity and various immune mediators namely interleukins, cytokines, chemokines, interferons and their receptors (reviewed by Kaiser, 2010). The identification of IL-4 and related cytokines such as IL-10 and IL-13 which are involved in the Th2 response indicating that the chicken also induced Th1 and Th2 like responses following infection. Even cytokine genes which were previously thought to be mammalian-specific such as IL-3, IL-7, IL-9 and IL-26 were found in chickens.

The advancement in the genomic approach has also enhanced our understanding on the chicken MHC gene cluster which included the B-complex and Y-complex, previously known as Rfp-Y (Kaufman, 2008). Similar to the mammalian MHC genes, the chicken MHC Class I genes encode for surface receptors in all nucleated cells whilst MHC Class II genes encode receptors but are restricted to antigen presenting cells. However, in contrast to the mammalian MHC, chicken has a MHC genome segment that simpler

but densely packed with genes (Kaufman, 2008). Characterization of the chicken MHC cluster has been of considerable interest due to the strong and reproducible of disease resistance with a particular MHC haplotype. Inbred chickens of different MHC haplotypes have been known to show different susceptibility to viral and bacterial infections ie. chickens of B21 and B19 haplotypes are resistance and susceptible to MDV infection, respectively (Kaiser, 2010). In the case of MDV, numerous studies have indicated that the genetic resistance towards MDV in chickens is associated with differences in NK cell and T cell functions and productions of various cytokines and nitric oxide (Omar *et al*., 1996; 1997; Omar and Schat, 1998; Schat *et al*., 2004)

Studies are also underway in further characterization of chicken CD4+ T cells beyond Th1 and Th2 subsets since in mammals, CD4+ T cells can be classified into at least 4 different types based on surface markers and functions (Zhu *et al*., 2010). Recent studies have shown the presence of Treg cells in chickens (Shanmugasundaram and Selvaraj, 2010) however, the functions of this cell is not clearly understood. Further studies are required to identify whether the presence of other distinct CD4+ T cells such as Th2, Tr1, Th9 and Th17 cells do really exist in chickens.

New Generation Vaccines and Adjuvants

Most of the advances in the development of the so called genetically engineered vaccines against poultry diseases using recombinant/ subunit protein, synthetic peptide, viral/bacterial vector and DNA vaccine technologies and reverse genetic approach are made in the past 20 years (Figure 5). However, the use of these vaccines in the poultry industry is very limited due to several reasons such as high cost to produce compared to the conventional vaccines, difficult

to administer the vaccine for mass vaccination, unclear regulatory issues, hence difficult or late for approval and difficult to integrate with the current conventional vaccines.

Table 2 shows some of the recombinant vaccines that have been licensed in the USA. Most of them used a poxvirus vector based vaccine ie. fowlpox virus vaccine vector containing the F and HN genes of NDV and MDV (Omar *et al*., 1998; Omar, 2012; 2013; Jackwood *et al*., 2008). Other virus vector systems which have been developed are based on MDV serotype 3, herpesvirus of turkey (HVT) and adenoviral systems. Through the advancement in reverse genetic technology, researchers are exploring the use of RNA viruses such as AIV (Shi *et al*., 2007), NDV (Hu *et al*., 2009), IBV (Casais *et al*., 2001) and IBDV (Mundt *et al*., 2003) as viral vector vaccines. Using this technology, it is possible to construct avirulent or low virulent viral vaccines that express antigens of virulent field strains such as highly pathogenic H5N1 (Shi *et al*., 2007) and velogenic genotype VII NDV (Hu *et al*., 2009). This has been shown in case of reverse genetic LaSota virus vaccine expressing the F and HN genes of genotype VII that able to confer superior protection against mortality and virus shedding in SPF and commercial chickens following challenged with velogenic genotype VII NDV (Kiarash *et al*., submitted).

The most extensively studied technology to produce recombinant/subunit proteins as vaccines is by using baculovirus expression system. This system have been used to express several avian viral proteins such as HN and F proteins of NDV (Ong *et al*., 1999a, b; Salih *et al*., 2000a), VP2 protein of IBDV, S1 protein of IBV, various glycoproteins of MDV, viral proteins of CAV and HA and NA proteins of AIV (reviewed by Omar, 2005). Beside the baculovirus system, other systems that are available include yeast expression and prokaroytic expression systems (Rabu *et al*., 2002;

Abubakar *et al*., 2011). Prokaryotic expression systems such as *E. coli* and other bacteria are not suitable especially for expression of proteins that require post-translational modification to become functional and immunogenicity in the immunized host (Sodoyer, 2004). The yeast expression system is another attractive approach to express recombinant protein, however, the system require extensive optimization and only work for certain types of protein (Macauley-Patrick *et al*., 2005). Studies have also shown that plant is also a suitable system for expression of heterologous proteins where several different proteins as diagnostics and/or vaccines have been tested experimentally (Sharma and Sharma, 2006; Teen *et al*., 2013). The application of recombinant protein based vaccine for poultry may not be feasible due to inherent challenge to produce the protein in mass amount in functional and stable manner for field testing. Nevertheless, the expressed proteins are suitable to be used for the development of diagnostic for rapid detection of avian pathogens based on various serology based assay. Recombinant proteins such as HN and F of NDV, HA, NA and NS1 of AIV, SI of IBV, VP2 of IBDV are proteins with diagnostic potentials for the developed of diagnostic kits (Abubakar *et al*., 2011; Hosseini *et al*., 2007; Balamurugan *et al*., 2010).

Another important advancement in vaccine development is DNA vaccine (Figure 6). DNA vaccines against important poultry diseases namely NDV (Loke *et al*., 2005), IBV (Kapczynski *et al*., 2003), IBDV (Chang *et al*., 2003), CAV (Moeini *et al*., 2011a, c) and H5N1 (Jazayeri *et al*., 2012a, b) have been recently studied by several groups. Although plasmid DNA-based vaccines have huge potentials over traditional vaccines, one of the major setbacks is that the vaccine is unable to induce strong immune responses (Redding and Weiner, 2009). Additionally, their applications in mass vaccination of livestock animals including poultry are poorly

studied. Despite extensive research, no licensed DNA vaccines are available commercially against poultry diseases. Currently, only 4 DNA vaccines have been approved for veterinary use, namely against West Nile virus in horses, infectious haematopoietic necrosis in salmon, the treatment of canine melanoma in dogs and for growth hormone releasing hormone (GHRH) products in swine (reviewed by Omar, 2012).

Table 2 Currently licensed recombinant poultry vaccines (*Source*: Jackwood *et al*., 2008)

Company	Pathogen	Vector
Lohmann Animal Health	*Salmonella typhimurium*	Double-deletion-mutant
Merial	AIV, FPV	FPV
Merial	NDV, FPV	FPV
Merial	IBDV, MDV	HVT
Intervet	MDV	HVT
Intervet	MDV, NDV	MDV
Bioimune Co.	AEV, FPV, LTV	FPV
Bioimune Co.	NDV, FPV	FPV
Bioimune Co.	FPV, MG	FPV
Bioimune Co.	AEV, FPV, MG	FPV
Bioimune Co.	FPV, LTV	FPV

AIV, avian influenza virus; NDV, Newcastle disease virus; IBDV, infectious bursal disease virus; MDV, Marek's disease virus; AEV, avian encephalomyelitis virus; FPV, fowl poxvirus; LTV, laryngotracheitis virus; HVT, turkey herpesvirus; MG, Mycoplasma gallisepticum

Besides the advancement in development of vaccines, researchers are also characterizing new adjuvants and vaccine delivery systems to be used together with avian viruses (Rahman and Eo, 2012). Vaccine adjuvants can be defined as a component that enhances the immune responses to an antigen and/or modulates it towards the desired immune responses, hence, adjuvants are used primarily in inactivated vaccines. Most of the adjuvants that are used in poultry vaccines comprises of oil based adjuvants primarily water in mineral oil formulation. Beside oil based, aluminum based (aluminum hydroxide) adjuvants have also been tested for different poultry vaccines namely inactivated polyvalent vaccines. Development of new adjuvants able to broaden the induce immune response including cellular immune response as well as to extend the vaccinal cross- protection against different viral strains or serotypes. Some of the newer adjuvants that have been tested for poultry vaccine such as Montanide ISA 71 VG able to enhance the cellular responses (Liu *et al.,* 2011). Since most of the adjuvants for poultry vaccine are oil based, they are able to induce side-effects such as inflammation, resulting in the down-grading of meat quality, alternative adjuvants must be developed. Some progress have been made in the development of new adjuvants which has been recently licensed such as monophosphoryl lipid A (MPL) and AS03 (a squalene-based adjuvant) (Rahman and Eo, 2012).

The use of recombinant cytokines as adjuvants in poultry has been explored by several investigators (reviewed by Rahman and Eo, 2012). Studies have shown that recombinant type I IFN (α and β) administered orally or through intravenous route inhibits replication of several avian viruses such as IBV, IBDV, NDV and AIV. Meanwhile type II IFN (γ) play important role in chickens infected with *Eimeria acervulina*. Studies have also shown that the protective effects of γ-IFN are further enhanced following the administration

of IL5 or IL-18 during intracellular bacterial infections. Although numerous studies have demonstrated the potential use of cytokines as vaccine adjuvant and therapeutic in poultry, their application in commercial chickens are problematic due to challenges such as high cost to produce in large scale production and retaining protein stability and bioactivity when administered in chickens.

Table 3 Newer generations of diagnostic, vaccines and therapeutics against poultry pathogens

No	Research Innovation	References	Patents
	DNA Vaccine		
1.	DNA vaccine expressing F and HN genes of NDV	Loke *et al*., 2005	-
2.	DNA vaccine expressing H5 of AIV co-formulated with MDP-1 as genetic adjuvant	Jalilian *et al*., 2010	-
3.	DNA vaccine expressing H5 of AIV co-formulated with HSP70 as genetic adjuvant	Rasoli *et al*., 2010	-
4.	DNA vaccine expressing H5 of AIV co-formulated with ESAT-1 as genetic adjuvant	Oveissi *et al*., 2010	-
5.	DNA vaccine expressing VP1 and VP2 of CAV co-formulate with MDV VP22 as genetic adjuvant	Moeini *et al*., 2011a, c	-
6.	*Lactobacillus acidophilus* as a live vehicle for oral immunization against chicken anemia virus.	Moeini *et al*., 2011b	-
7.	Live attenuated salmonella as carrier for DNA vaccine expressing H5 of AIV	Jazayeri *et al*., 2012a, b	-
8.	Green synthesis silver nanoparticles nanoencapsulated H5 DNA vaccine	Jazayeri *et al*., 2012c, 2013	-

9.	DNA vaccine expressing H5 formulated with chicken IL-15 and IL-18 as genetic adjuvant	Lim *et al*., 2012	-
10.	DNA vaccine expressing N1 formulated with chicken IL-15 as genetic adjuvant	Lim *et al*., 2013	-
11.	DNA vaccine expressing NP formulated with chicken IL-15 as genetic adjuvant	Lim *et al*., 2013	-
	Molecular based Diagnostics		
1.	Detection and distinguishing IBDV strains by molecular biology method	-	US7,858,768 EP1666608 B1 MY143506A
2.	Molecular differentiation of IBDV strains	Kong *et al*., 2009	PCT/ MY2010/000002
3.	Assay for detection and quantification of NDV by real-time PCR	Tan *et al*., 2009	PI20070884
4.	Detection of avian influenza virus	Ong *et al*., 2007	PI20062120
5.	The nucleocapsid protein of NDV as a carrier for immunogens	-	PI20021709
6.	A novel antiviral peptide against AIV H9N2	Rajik *et al*., 2009a, b	PI20082061
7.	A method for detection and differentiation of *Mycoplasma gallispeticum*	-	PI2010000637
	Others		
1.	Circular antiviral RNA	Oii *et al*., 2014	PCT/ MY2013/000088

Due to the challenges working with recombinant cytokine as adjuvant for poultry vaccine, our group is exploring the use of immunomodulatory genes including cytokine as genetic adjuvant in enhancing efficacy of DNA vaccine against viral infection in chickens. Table 3 shows some of the different genes both cytokines and non-cytokine genes that have been used as genetic adjuvant in enhancing the efficacy of DNA vaccine against different pathogens in chickens. In one study, it was found that chicken IL-15 and IL-18 are able to enhance the antibody and T cells function following the vaccination with H5, N1 and NP DNA vaccines in SPF chickens (Lim *et al*., 2012; 2013). Similar results were also demonstrated in the case of chickens immunized with H5 DNA vaccine co-administered with immunomodulatory genes such as Esat-1 (Oveissi *et al*., 2010), MDP-1 (Jalilian *et al*., 2010) or HSP70 (Rasoli *et al*., 2010) genes. Previous studies have shown that Esat-1 and MDP-1 genes of *Mycobacterium tuberculosis* and HSP70 exhibit strong adjuvant and immunomodulatory potential in mouse models (Wang and Singh, 2011).

Even though the constructed H5 DNA vaccines when administered or co-expressed with the different genetic adjuvants are able to induce higher immune responses compared to vaccination with DNA vaccine alone, the vaccines still need to be administered parentally via intramuscular injection. To address this limitation, we have demonstrated that attenuated *Salmonella typhimurium* is a safe and effective vehicle for oral delivery of H5 DNA vaccine in chickens (Jazayeri *et al*., 2012a, b) (Figure 7 and 8). In that study we showed that SPF chickens inoculated once with 10^9 Salmonella colony-forming units carrying H5 DNA vaccine developed HI titer > 50, elicited both CD4+ and CD8+ T cells with enhanced pro-inflammatory cytokine (IL-1β, IL-12β, IL-15 and IL-18) expressions in spleen at 4 weeks post inoculation.

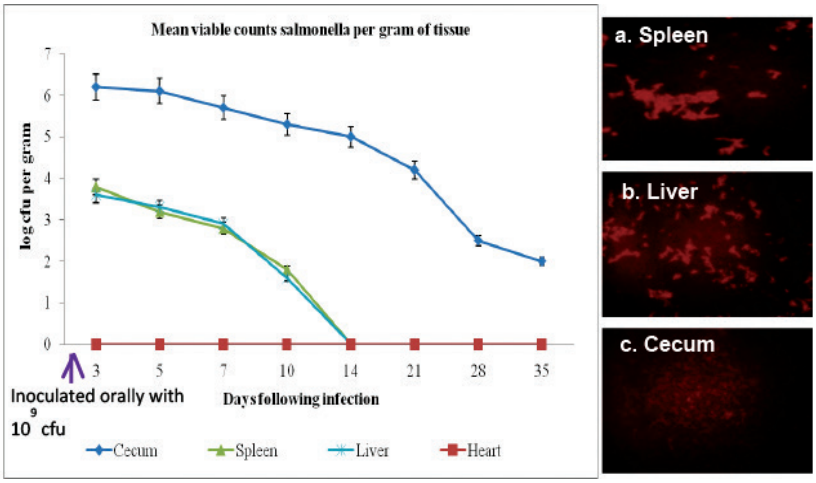


Figure 7 Colonization of chicken organs by attenuated *Salmonella typhimurium* carrying H5 DNA vaccine. The transfected bacteria were detected in cecum beyond day 35 post inoculation

Hence, single oral administrations of the attenuated *S. typhimurium* containing pcDNA3.1/H5 showed antibody, T cell and Th1-like cytokine responses against AIV in chickens. Very recently, we managed to show that inactivation of *S. typhimurium* using novel approach are equally effective in delivering H5 DNA vaccine. Besides using bacteria we have also developed green synthesized silver nanoparticles as carrier for H5 DNA vaccine for rapid and effective expression (Jazayeri *et al*., 2012c; 2013). In addition, chickens immunized with H5 DNA vaccine encapsulated with the developed nanoparticle induced both antibody and T cells responses (Jazayeri *et al*., 2012c) (Figure 9, 10, 11, 12, and 13). Besides the development of vaccines, research is also in progress in the development of novel strategies in controlling pathogens using novel molecules such as antiviral peptide (Rajik *et al*., 2009a, b), RNA (Oi *et al*., 2014) and microRNA (unpublished results) which have huge potential as effective therapeutic agents.

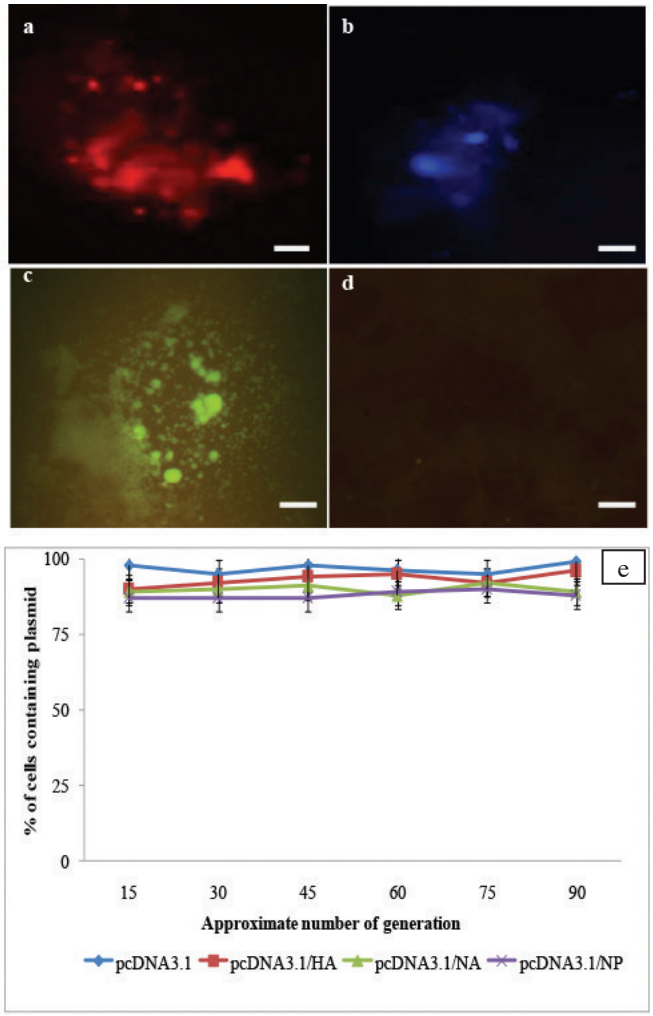


Figure 8 Stability of DNA plasmids with HA, NA, NP of H5N1 in *S. typhimurium***.** Fluorescence in situ hybridization detection of (a) HA, (b) NA, (c) NP and (d) pcDNA3.1 as negative control after transfected into *S. typhimurium*. Probes were labeled with Cy5 (red), Alexa 350 (blue) and Fluo (green) fluorescent dye. (e) In vitro stability of pcDNA3.1/HA, NA, NP and pcDNA3.1. *S. typhimurium* containing transfected plasmids were passaged for approximately 100 generations

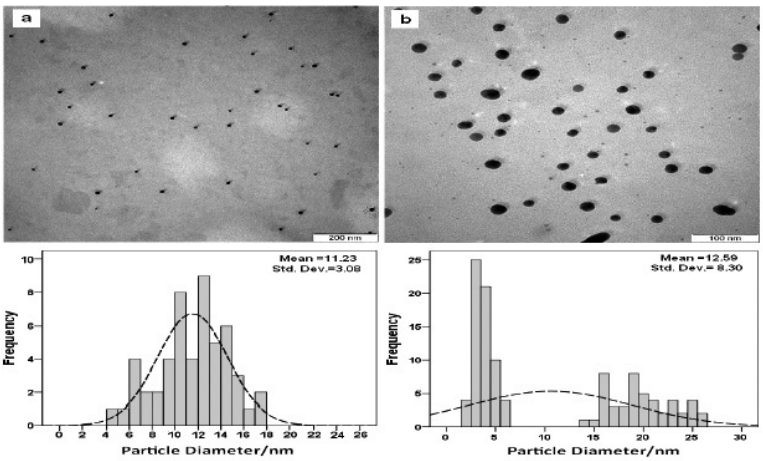


Figure 9 Characterization of the nanoencapsulated H5 DNA vaccine. TEM image and corresponding particle size and distribution of a) AgNP and b) AgNP/H5 show uniform shape, with maximum size of AgNP is 18 nm and AgNP/H5 is 25 nm

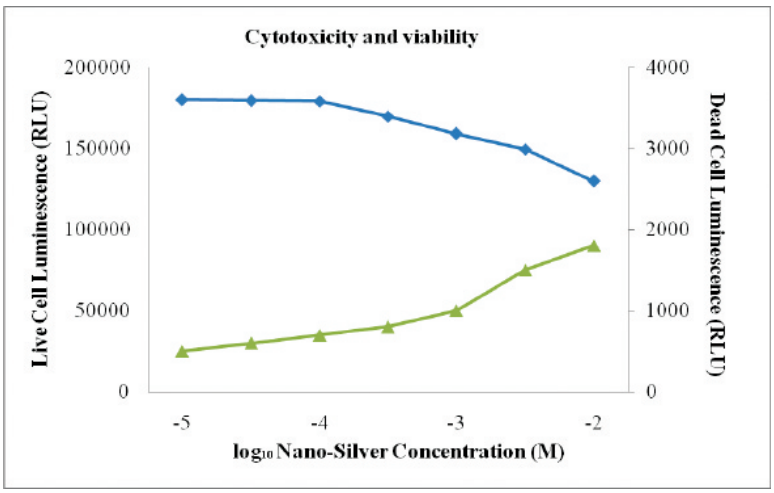


Figure 10 Viability and cytotoxicity assay of the nanoencapsulated H5 DNA vaccine. At the lowest concentration of AgNP (-5 $\log_{10}$) there were less than 10% dead cells. Increasing the dosage of AgNP as high as -2 $\log_{10}$ the cells did not reach into EC50 ratio (45%)

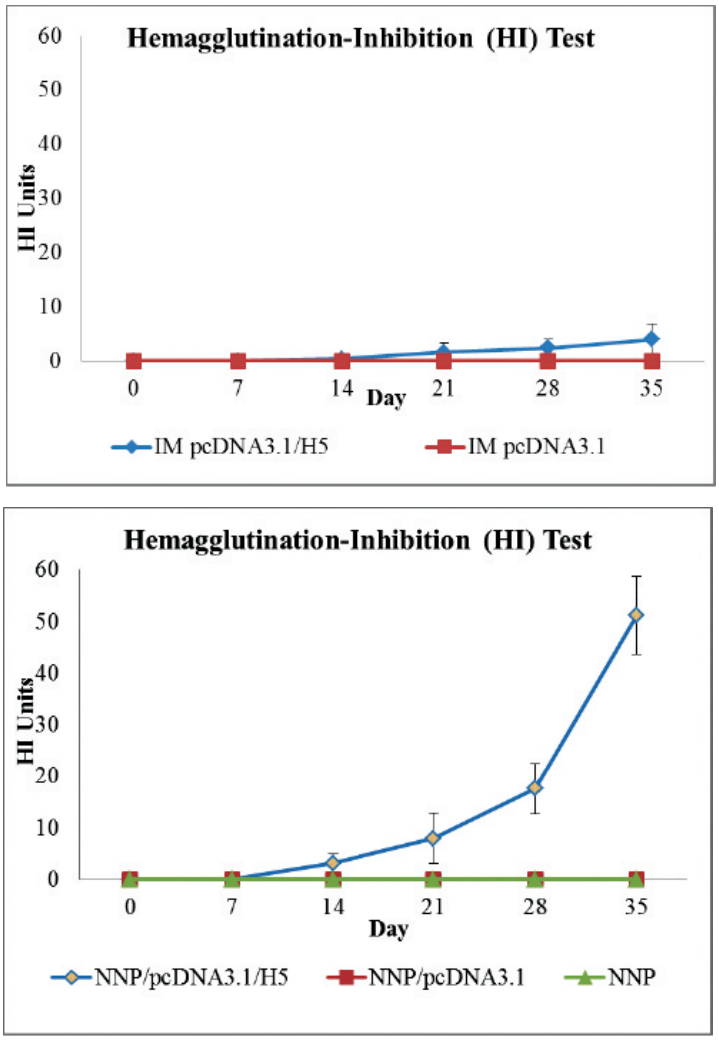
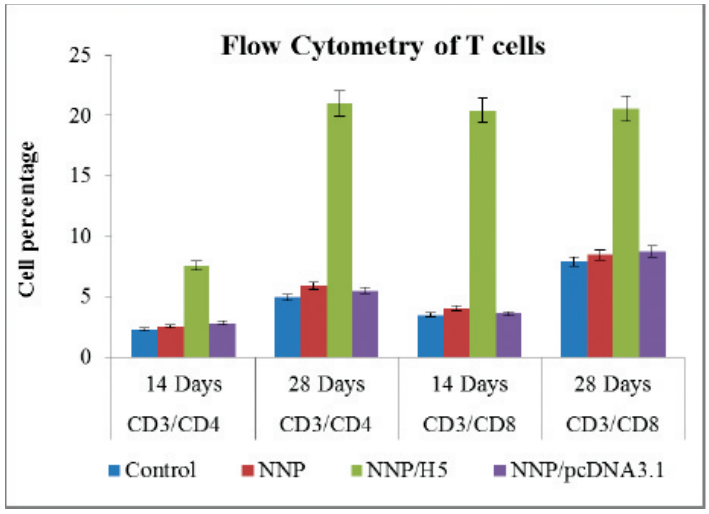


Figure 11 Antibody titer following oral immunization with nanoencapsulated H5 DNA vaccine. Each day-old chick received oral inoculation of 20 ng of pcDNA3.1/H5 nanoencapsulated with AgNP (3.7×10^{-2} ug of Ag). Chicken immunized with nanoencapsulated H5 DNA vaccine developed significantly higher HI antibody responses ($p< 0.05$) compared to IM/H5 and nanoparticle inoculations



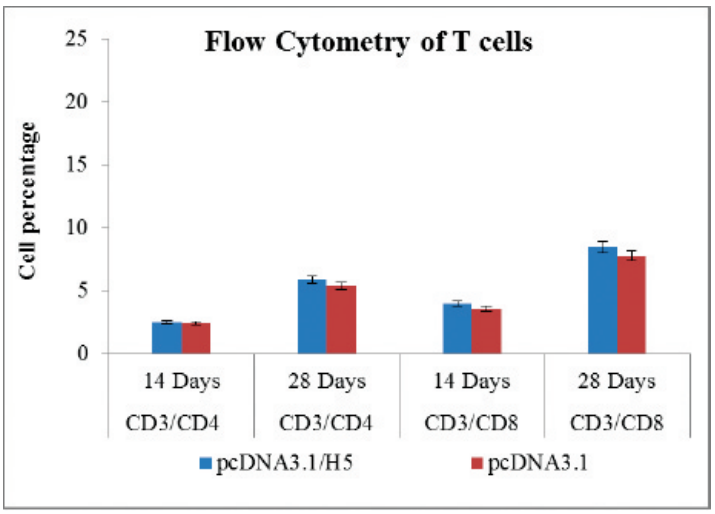


Figure 12 T cell responses following oral immunization with nanoencapsulated H5 DNA vaccine in chickens. Chicken immunized with nanoencapsulated H5 DNA vaccine developed significantly higher CD4+ and CD8+ T cell responses ($p < 0.05$) compared to IM/H5 and nanoparticle inoculations

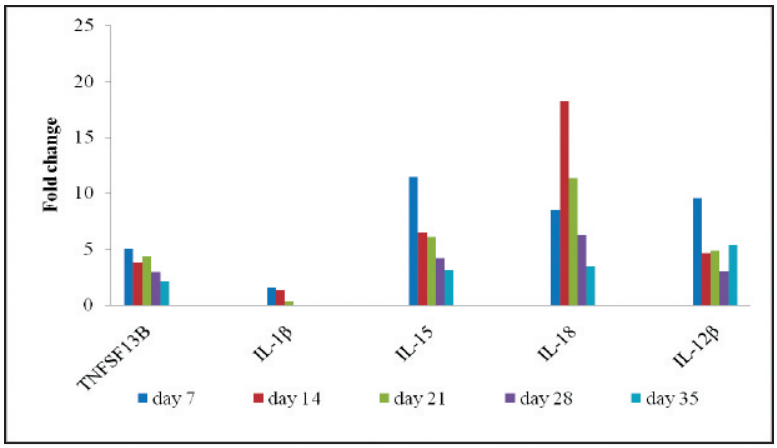


Figure 13 Th1-like cytokine responses following oral immunization with nanoencapsulated H5 DNA vaccine. Increased in cytokine expression levels (5 to 20 fold) of splenic lymphocytes obtained from chickens immunized with nanoencapsulated H5 DNA vaccine in comparison with nanoencapsulated empty vector. The cytokine expressions were measured using GeXP system (Beckman Coulter, USA).

Prospects of Genetically Engineered Vaccines

The ultimate goal in developing genetically engineered vaccine is to develop an ideal vaccine through the advancement of innovative technology. Based on the guidelines by the Royal Society's Report on Infectious Disease of Livestock in 2002, UK, the characteristics of an ideal vaccine is

- provides broad spectrum protection against all isolates of the virus in all the affected species, preventing virus carriage and the possibility of shedding and transmission,
- stimulates the level of immunity necessary to drive an effective and long-lasting immune responses,

- inexpensive to manufacture and simple to administer,
- safe to use, in the case of live vaccines, is safely attenuated and reversion to virulence is avoided,
- long shelf life and is heat stable,
- allows discrimination between infected and vaccinated animals, and
- provides strong levels of maternal immunity.

Nevertheless, there is no single vaccine that has all the above characteristics. The use of vaccines to control disease is all about assessing the risk and evaluating the benefit following vaccination. Several promising leads have been made in the development of recombinant vaccines using viral and bacterial vectored vaccine technology and reverse genetic technology. In addition, through the use of genetically engineered vaccine, it will be possible to differentiate between animal that are vaccinated from infected, an important requirement that needs to be considered in disease eradication program. Furthermore, this requirement is also crucial in vaccination against animal diseases with public health implication.

Vaccines have been proved to be effective against the widespread of diseases with the successful control and/or eradication of major animal diseases where vaccines are regularly used prophylactically to build flock immunity so that exposure with the disease agent does not result in an outbreak. Through the advancements of genetic engineered technology, vaccines may become increasingly important as an alternative therapeutics which will have a positive impact on the production and food safety by reducing or eliminating residues from the use of drugs and antibiotics. Furthermore, vaccines which induce rapid immunity are needed in the case of emergency vaccinations against endemic viral diseases. Hence, the development of multivalent vaccines would reduce the number of

inoculations to animals, reduce the handling cost and avoid animal sufferings.

Effective delivery of genetically engineered vaccine via novel mass vaccination strategies including *in ovo* vaccination and compatible with the other current vaccines may further increase the acceptance of these new generation vaccines in poultry. However, the potential of new vaccines cannot be fully exploited if there is no public acceptance of the technologies used when safety and ethical concerns are involved. Efforts need to be made to educate the stakeholders as well as the public on the benefits and risks of new technologies.

"OMIC" TECHNOLOGY ON AVIAN DISEASES AND HEALTH

"Omic' technologies can be defined as a comprehensive approach in the characterization of cell or organism based on high throughput profiling of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics). The advancement in "omic" technologies has enhanced research in the area of systems biology (systeomics), an emerging area in biomedical and biological research which uses holistic approach rather than the traditional reductionism approach to study the dynamic interactions between the biological components of the complex pathogen-host system (Aderem *et al*., 2011). Hence, rapid progress has been made in systems biology approach in elucidating host-pathogen interaction of several human diseases such as AIDS, influenza and tuberculosis. However, the use of system biology approach in avian disease is still at infancy. Nevertheless, some progress has been made in the characterization of the transcriptional regulatory networks between human, macaque and mouse model systems after infection with a highly pathogenic AIV H5N1 subtype (McDermott

et al., 2011). In that study it was found that the transcriptional regulations between in vitro and in vivo infection across different species are well conserved.

In the past, expression study was done through RNA detection based on Northern blot analysis, a low throughput technique utilizing radioactivity and requiring large amounts of input RNA. The advancement in real-time PCR has significantly improved the quantitative expression study of know gene (Espy *et al*., 2006). The advances in high throughput sequencing technology have facilitated the full genome sequencing of various pathogens and other organisms including animals and plants. Sequencing of the chicken genome and pathogens of related importance has increased the development of other tools with diagnostic potential to investigate gene expression based on microarray technology. Currently, at least 3 different commercially available microarrays namely a 20K Roslin/ARK CoRe Array, a 33K Affymetrix GeneChip and a 44K Agilent microarray have been developed for whole genome transcriptome profiling in chicken (Gheyas and Burt, 2013). Hence, microarrays have made invaluable contributions in avian genomic research in various fields including embryology, immunology, oncology, virology and evolution. Chicken microarray is also a powerful tool to study immune response in avian species where little genome information is available. For instance, this has been shown in a study that characterized the pathogenicity of H5N1 in chickens is comparable to other avian species namely duck, goose and starling (Crowley *et al*., 2009).

One of the main challenges in microarray study is in analysis of the expression profiles which may lead to false results and interpretation (Freyhult *et al*., 2010). Furthermore, the development of microarray tools has been slow primarily due to cost for fabricating custom-made array. The future of microarrays is now

being questioned with the advent of massively parallel next-generation sequencing (NGS) technologies, which promise to overcome some of the limitations of microarray platforms. NGS refers to a group of new sequencing technologies that can process millions of sequence reads in parallel, thus, able to generate enormous volume of sequencing data in a very cost effective way. Currently, at least 4 different platforms, 454 Life Sciences, Illuminia Solexa, Life Technologies and Pacific Biosciences are available commercially (Harun *et al*., 2013). NGS platforms have been used in transcriptome profiling, miRNA profiling, DNA-protein interaction studies using chromatin immunoprecipitation (ChIP) and DNA methylation studies, thus challenging the use of microarrays.

The advancement in NGS technologies has driven rapid progress on full genome projects of various microbes, plants and animals including different avian species. Furthermore, many bird species are important to agriculture and providing enormous worldwide food source, therefore the characterization of the genome of bird species has both social-economic and ecological importance. Hence, the genomes of several avian species such as chicken (Burt, 2005), duck (Huang *et al*., 2013) and turkey (Dalloul *et al*., 2010) have been sequenced. From these studies, genomic approaches are proving crucial information for studying traits that affect yield and disease resistance, behavior along with many factors affecting productivity. NGS has also been used to analyze host-pathogen interactions in poultry following infections with various avian pathogens such as MDV and AIV (Cheng *et al*., 2012, The SJCEIRS H9 Working Group 2013), the expression of chicken microRNA (Buermans *et al*., 2010) and the structural variation within different chicken lines (Kerstens *et al*., 2011). Studies have shown that certain inbred chicken lines can be classified as resistant and susceptible breeds

towards infection with MDV, IBDV and Salmonella (Kaiser, 2010). Recently, we have explored the use of strand-specific sequencing of Illuminia Hiseq technology to characterize the transcriptome of bursal tissue following infection with very virulent strain of IBDV (Noor Farhanah *et al*., 2012). Works are currently underway in elucidating the differential expression of various set of immune-mediated genes between the different lines of these inbred chickens. Using NGS based technology, our group has also started to sequence and characterize several different animal pathogens of interest in the effort to develop new strategies to control the diseases, this include complete genome sequencing of several NDV strains (Murulitharan *et al*., 2013, unpublished results), bacteriophage (Gan *et al*., 2013) and *Pasteurella multocida* (Yap *et al*., 2013). Various studies at transcriptional level (Figure 14, Rasoli *et al*., 2014) and complete genome sequencing of genotype VII NDV (unpublished results) are currently underway to address the reason why the current genotype VII NDV is difficult to be controlled using conventional LaSota vaccine.

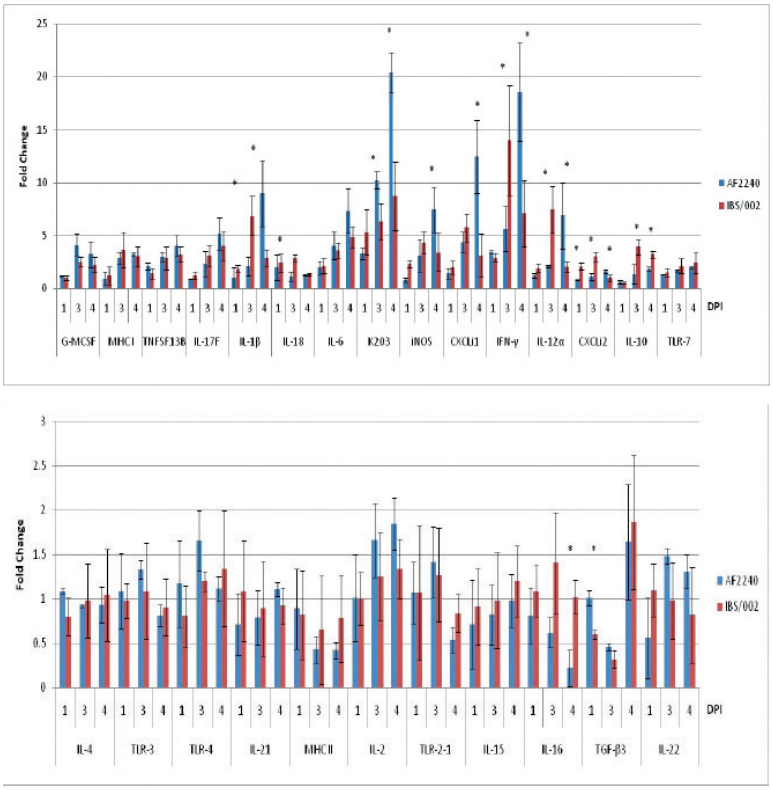


Figure 14 Modulation in the expressions of chemokines, cytokines and other immune-related genes in spleen of NDV genotype VIII AF2240 and genotype VII IBS002 infected chickens. Both NDV genotypes induced strong proinflammatory and Th1 cytokines but show different expression patterns

ECONOMIC AND SOCIAL IMPACT OF ANIMAL DIAGNOSTICS AND VACCINES

Global Animal Vaccine Market

The global animal vaccine market which was valued at USD$5.4 billion in 2012 is expected to reach US$8 billion by 2018. This is primarily driven by the accelerated growth of the livestock sector than any other agricultural sub-sector, the growing prevalence of animal diseases, increasing incidences of zoonotic diseases in humans, increasing investments by government bodies and animal welfare associations, as well as continuous innovations and introductions of new products. The large share of this segment is attributed to their major use of animals related to the food industry (meat, chicken, eggs, and dairy products) where according to Food & Agriculture Organization, global meat production is projected to more than double from 229 million tonnes in 1999/2001 to 465 million tonnes in the year 2050.

Among the biggest challenges in livestock industry are management and control of infectious diseases through the use of various biosecurity approaches including the use of diagnostics, vaccines and other therapeutics. The burden of infectious diseases in livestock and other animals continues to be a major constraint to sustain agricultural development, food security and the participation of developing and in-transition countries in the economic benefits of international trade in livestock commodities.

The world market for animal vaccines is largely for cattle, pet/small animals and poultry industry (Figure 15). These products are mainly produced by large pharmaceutical companies, such as Pfizer/Zoetis (USA), Merck (USA), Sanofi-Aventis (France), Bayer HealthCare (Germany), Virbac (France), Novartis (Switzerland), Boehringer Ingelheim (Germany), Heska Corporation (USA),

Bioniche Animal Health (Canada), and Ceva (France), which are the key players in the global animal vaccines market. These large multinational companies have R&D and manufacturing facilities in different parts of the world.

National Scenario

Besides research on conventional animal diagnostics and vaccines for the immediate and changing needs at national and global levels, most of the current researches at UPM are in line with the advancements in technology and science of infectious diseases and immunology for the development of new generation vaccines and improving the quality of existing diagnostics and vaccines. Furthermore, researchers are actively engaging in the area of vaccine delivery systems using novel carriers as well as adjuvants in an effort to enhance vaccine induced responses and to develop vaccine which are able to induce the required immune responses against an infectious agent. As described previously, the animal vaccines market comprised of live attenuated vaccines, inactivated vaccines, subunit vaccines, recombinant vaccines and DNA vaccines. It is envisioned the DNA vaccines technology will be the fastest-growing technology due to its cost effectiveness and easy production, rapid development against emerging diseases, and added thermo-stability between 4°C to 20°C in comparison with traditional vaccines (http://www.marketsandmarkets.com/Market-Reports/animal-veterinary-vaccines-market-1233.html). Hence, our group has developed several DNA vaccines against poultry disease with novel mass applications that do not require multiple injections of individual animals (Table 3).

Research and innovation in the areas of animal diseases and health towards the development of diagnostics and vaccines/therapeutics have been the main research focus of UPM due to

the unique combinations of experts from different fields such as veterinary, marine science, biomedical, biotechnology, agriculture and engineering. Hence, over the past 15 years, numerous achievements in the form of high impact publications, prestigious awards, industry-relevant innovations and research outputs, and commercialised products have propelled UPM to be at the forefront of research and discovery. The achievements are as follows;

- More than 15 experts (Professors and Associate Professor) in the field of poultry disease, vaccine and related research.
- More than 200 post-graduate students specializing in poultry diseases, vaccine and related research have graduated.
- Patents in poultry vaccine and health related innovations/ products
  - » 3 international patents (2 USA and 1 EU) on avian viruses and vaccine application
  - » 10 patent pending on avian pathogens and vaccine/diagnostic applications
- Three poultry vaccines, NDV, FPV and IBDV have been commercialized. It is anticipated at least 3 more poultry vaccines are to be commercialized in the next 2 to 3 years.
- ISO17025 accredited laboratory tests for the detection of animal pathogens
- WHO/CDC compile BSL-3 laboratory

The government, through relevant Ministries, has identified UPM as the torch bearers of excellence in agricultural based research and innovation. Researchers from various faculties especially Faculty of Veterinary Medicine, Faculty of Agriculture and Faculty of Biotechnology and Biomolecular Science are actively

engaging research in animal vaccines and related technology. Furthermore, Institute of Bioscience has been recognized as one of the six Higher Institution Centres of Excellence (HICoE) in the country by Ministry of Higher Education in 2010 to spearhead excellence in animal vaccine and therapeutic research to the next level of international repute meeting the changing needs of the nation and the world. Currently, Malaysia imports vaccines and pharmaceuticals worth RM650 million annually. Department of Veterinary Service (DVS) is looking into ways of producing at least 30% of total import of vaccines as alternative to imported ones (New Straits Times / BERNAMA -01/03/2010). Currently, 31 vaccines have been produced locally by DVS and Malaysian Vaccine Pharmaceuticals (MVP) (Table 4).

The looming of highly pathogenic AIV H5N1 in this region and the emergence of new AIV strains such as H7N9 and H10N8 also requires our researchers to develop new strategy including diagnostics and vaccines against the respective viruses. The development of effective AIV diagnostics and vaccines are essential since during the outbreak of H5N1 in 2004, Malaysia lost more than RM1 million each day due to the inability to export poultry meat and byproducts to other countries. Further losses were incurred due to depopulation of infected and suspected infected birds. The investment in R&D in the development of improved or new animal diagnostics, vaccines and pharmaceuticals is expensive and currently being carried out primarily in public universities/ RI. Besides grant for research and innovation, emphasis must also be given to build suitable containment facilities for both basic research and clinical trials. Currently, no single organization has an overview to ensure an integrated and coordinated R&D program in Malaysia. Availability of this information would lead to effective use of resources, reduce duplication of effort, encourage collaboration

and identify key gaps in research. One of the ways to move forward is to establish more industry driven research via high degree of industry and academia collaboration as it will improve access to expertise and results in shorter lead time to market the products.

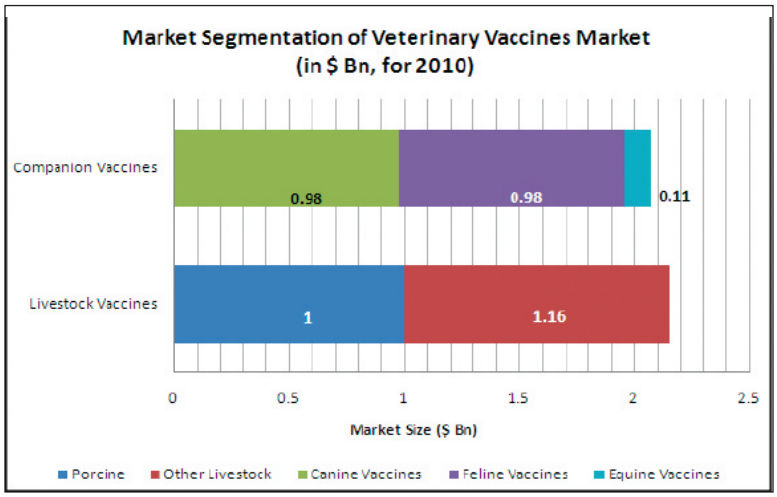


Figure 15 Global veterinary vaccines demand in 2010
(Source: Dolcera Analysis)

Table 4 Animal vaccines produced in Malaysia

Producer	No. of products	No. of animal type	Remarks
Dept. of Veterinary, Malaysia (DVS)	13	6 (Chicken, Duck, Goat, Sheep, Cattle, Buffalo)	Locally developed products for local market.
Malaysian Vaccines & Pharmaceuticals S/B (MVP)	18	3 (Chicken, Swine, Duck)	Locally developed products for both local and export market.
Foreign manufacturers (Imported products)	53	All types (Avian, Porcine, Canine, Feline, Equine, Aquatic, Caprine)	29 local importers sourcing products from 35 foreign manufacturers.

Source: Malaysian Livestock & Veterinary Industries Directory 2003/2004 and Malaysia Agricultural Directory & Index 2006

AVIAN VIRUSES AS HUMAN VACCINES AND THERAPEUTICS

Oncolytic Virotherapy

It has been well established that several animal viruses preferentially infect and kill human cancer cells, hence they are collectively known as oncolytic virotherapy (OV) (Kelly and Russell, 2007). Currently, there are extensive evidences indicating that OV is effective in treating cancer in both preclinical animal study and human patient clinical trials. Among OVs which have been used are adenovirus, herpes simplex virus, reovirus, measles virus and NDV (Lam *et al*., 2011). These viruses preferably kill cancer cells while leaving normal cells unharmed. Therefore, patients who have gone through clinical trials using oncolytic viruses have reported minimal or no side effects, and have shown an increased survival rate and enhanced tumour regression.

Poultry Viruses as Cancer Therapy

Several different poultry viruses such as NDV, IBDV and CAV have been showed to selectively destroy human cancer cells. Unlike other avian viruses, studies from as early as in the 1940s have shown the potential of NDV as an anti-cancer agent. NDV mediated cancer therapy can be divided into two distinct types based on the characteristics of the virus. The non-lytic NDV strains, e.g. Ulster, infect tumour cells and produce non-infectious viral particles which lead to an abortive viral replication, whilst the infection of tumour cells by the lytic NDV strains, e.g. MTH-68, lead to a multi-cyclic replication as infectious viral particles are produced that can infect other tumour cells, thereby amplifying the viral load (Zamarin and Palese, 2012). It is envisaged that NDV as an effective cancer therapy since it is able to remove tumor effectively by inducing

selective cancer apoptosis as well as activation of effective cancer immune responses for complete removal of cancer cells (Figure 16, Nakajima *et al*., 2013).

Several Malaysian NDV strains have been screened for their oncolytic effect on different cancer cell lines such as as CEM-SS (T-lymphoblastic leukemic cells), MCF-7 and MDA-231 (breast cancer), HT29 (colorectal cancer) and HL60 (acute promelocytic leukemia) (Abdul Rahman et al., 2003). However, most of the study focuses on NDV strain AF2240, a velogenic NDV isolated in 1960s. In vitro studies have indicated that the AF2240 is a lytic NDV that selectively induces apoptosis of MCF-7, an estrogen receptor positive human breast cancer cells (Ghrici *et al*., 2013a, b; Jamal *et al*., 2012).

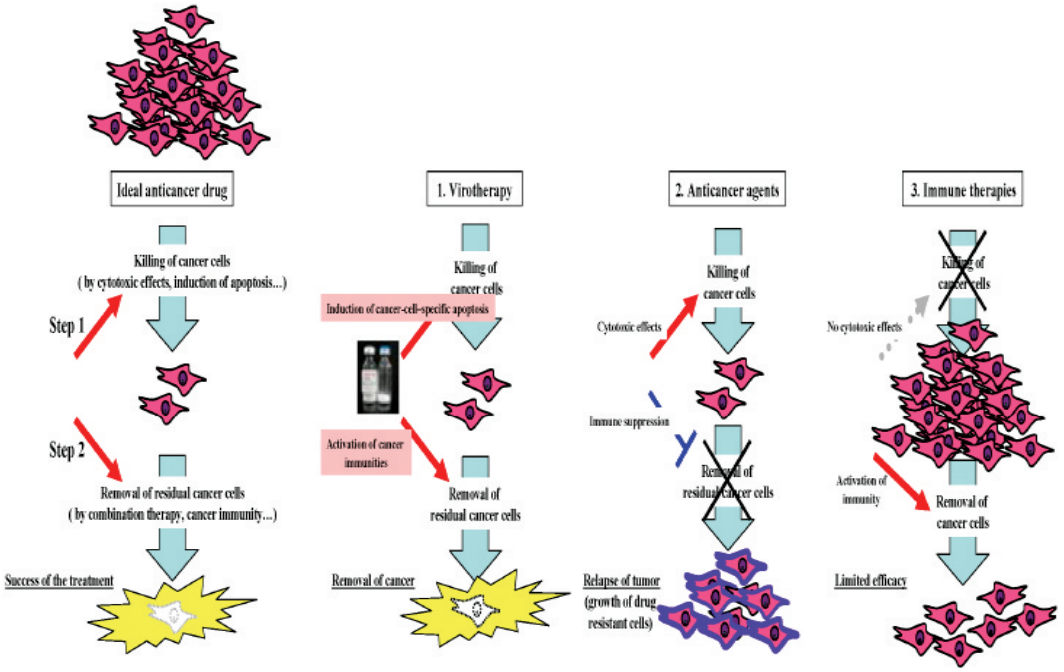


Figure 16 Problem with conventional therapies and 2-step strategy for cancer treatment. Conventional anticancer therapies are problematic and a 2-step therapeutic strategy is proposed for effective cancer treatment. Virotherapy possesses ideal characteristics for a 2 step therapy

In addition, NDV AF2240 also induces apoptosis of other cancer cells, such as the WEHI-3B leukaemia cells (Alabsi *et al*., 2011; 2012), anaplastic astrocytoma U-87MG (Ali *et al*., 2011) and the cervical cancer cells, HeLa (Molouki *et al*., 2011).

The mechanisms of NDV-induced oncolysis are not well understood, although phase II/III clinical trials on humans are currently underway (Zamarin and Palese, 2012). Understanding the mechanisms of NDV induced oncolysis will facilitate the use of NDV as oncogenic reagents together with other therapies in human. Studies have shown that the involvement of both the activation of intrinsic and extrinsic caspase-dependent apoptosis pathways as well as the type 1 IFN dependent and independent mechanisms in NDV mediated oncolysis (Mansour *et al*., 2011). Recently, we have found that AF2240 induced oncolysis of MCF-7 cells associated with activation of caspase-8 and mitochondrial transition pore opening that is independent of both virus replication and protein synthesis (Ghrici *et al*., 2013a). In addition, the expression of the HN gene alone was able to induce apoptosis in MCF-7 cells but it was a less potent apoptosis inducer compared to the parental NDV AF2240 strain (Ghrici *et al*., 2013b). Hence, mitochondrial-related pathway may be the central activator in NDV strain AF2240-induced apoptosis. AF2240 also caused significant cell death towards MCF-7 cells compared to normal breast cancer cells, MCF-10A cells, therefore analyzing the differential expression of genes in both cell lines may provide valuable information on mechanisms of NDV induced oncolysis. Recently, we have performed *in silico* analysis to predict and analyse the protein-protein interactions and identify potential pathways involved in the interactions between NDV strain AF2240 and MCF-7 breast cancer cell line. In that study, we found a total of 26 NDV-interacted proteins which were recognized as potential candidates might be responsible for inducing the selective

destruction of the MCF-7 breast cancer cell line (Khairiyah *et al*., submitted). Studies are currently underway in validating the functions of these genes in modulating ability of NDV induced oncolysis of MCF-7 cells.

Besides NDV, limited studies have also showed the potential of other avian viruses such as AIV and IBDV in selectively destroying human cancer cells (Voon *et al*., 2005). One of the recent advances in the use of avian viruses as cancer therapy in humans is the application of VP3 protein (apoptin) of CAV in the destructions of human cancer cells (Shen *et al*., 2013). Studies have shown that the expression of VP3 of CAV in cancer cells associated with the induction of apoptosis through caspase-3 and caspase-9 dependent intrinsic mitochondrial pathways. Interestingly, the majority of cancer cells which are susceptible to VP3 induced apoptosis are Bcl-2 dependent but are independent of p53 related pathway. Hence, apoptin is an attractive gene therapy for human cancer since many anticancer therapies require wild-type p53 expressions for effective destruction (Backendorf *et al*., 2008).

Recombinant Avian Viruses as Vectored Vaccines

Emerging and re-emerging infectious diseases pose significant threats to global human health and require the development of new diagnostics, therapeutics and vaccines. Various vaccine technologies, in particular, recombinant virus as vaccine vector have shown great promise and play an important role in the development of new generation vaccines. An ideal vaccine is a vaccine that is able to mimic natural infection without showing the overt infection.

With the advent of reverse genetic technology, several different animal viruses have been used as recombinant viral vector vaccines for humans. In the case of poultry viruses, NDV has been showed to have valuable potential as human viral vector vaccine. Why is

NDV an attractive recombinant viral vector vaccine for human? Newcastle disease virus is a unique avian virus that is highly attenuated in humans and other primates because of a strong host-range restriction. NDV infects the respiratory tract of non-human mammals and appears to remain restricted to that site (Bukreyev and Collins, 2008). In addition, NDV is antigenically distinct from common human viruses, able to express stable foreign proteins and easily propagated in different cell lines for human product development. Studies have shown the potential applicability of live attenuated lentogenic NDV as recombinant vaccine against influenza, severe acute respiratory syndrome, highly pathogenic H5N1, human immunodeficiency virus, rabies, measles, Nipah disease, Rift Valley fever and Ebola (Wen *et al*., 2013). Hence, these studies indicated that NDV is a safe and effective viral vectored vaccine in various animal models such as mice, dogs, pigs, cattle and sheep including humans.

CONCLUSION

Diagnostic tests and vaccines are valuable tools in the diagnosis and control of poultry diseases. Most of the vaccines currently in use for the control of animal diseases are conventional vaccines whilst, serological based tests are routinely used to determine the effectiveness of control program and as an aid in the diagnosis of diseases. The use of molecular diagnostics especially PCR based tests are making its way as important rapid and accurate tools in the detection of avian pathogens. However, new and emerging infections will continue to pose risks to the human and animal population. Continuous research and development are required to develop new innovations for poultry diagnostics, vaccines and pharmaceuticals. Innovative technologies offer new opportunities through real-time PCR, microarray, adjuvant and reverse genetic technologies and

sequencing which have allowed the identification of the genes in viruses that are responsible for disease and stimulating immune responses. This will enable the development of safe and effective live recombinant vaccine strains that mimic natural infection without showing any overt infection. Improved diagnostic tests must be developed to enable the early diagnosis and detection of outbreaks along with tests to determine the effectiveness of disease control program. In addition, a proactive approach for the detection of new virus which seems to be lacking needs to be established through research collaborations with universities/research institutes and the relevant industries. This is crucial in order to respond rapidly to new and emerging threat especially zoonotic diseases. Strengthening innovative research via multi-disciplinary approach is required to leverage the full potential of avian viruses as therapy for deadly human diseases. Although Malaysia and many countries in this region have relatively strong scientific research but the translation of scientific discoveries into innovative products with high commercial values is a complex process involving various factors including economic, regulatory and social issues.

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BIOGRAPHY

Abdul Rahman Omar was born in Kluang, Johore on 22nd October 1967. He received his formal education at Sekolah Kebangsaan Tunku Mahmood 1, Kluang and Sekolah Tinggi Kluang, Johore. In 1986, he enrolled in the Doctor of Veterinary Medicine (DVM) program at Universiti Putra Malaysia. Upon completion of his DVM in 1991 with distinction he was awarded the Chancellor Gold Medal award for his highest overall achievement as a student. He was also the proud recipient of the Tunku Medal from the Tunku Abdul Rahman Foundation. In September 1991, he was offered a post as a tutor in the field of immunology in the Faculty of Veterinary Medicine (FVM), UPM. A year later, he attended Cornell University, USA to pursue his PhD study in the field of cellular immunology. His PhD dissertation focused on the role of cytotoxic T cells following infection with oncogenic and vaccine strains of Marek's disease virus (MDV) under the guidance of Prof. Dr. Karel Schat. He was awarded the doctorate in 1997 majoring in immunology and with minor in virology and biochemistry. Upon returning from the USA, he was appointed as a lecturer at Department of Veterinary Pathology and Microbiology in May 1997 where he was given the responsibility to coordinate Vaccine Laboratory, presently known as Viro 3 @Biologics Lab at the FVM. His training in the USA has equipped him with a solid background in the areas of immunology and molecular virology to spearhead biotechnology related researches at FVM. He was promoted to Associate Professor in 2002 and became a full Professor in 2008. He served as the Head of Laboratory of Molecular and Cell Biology, Institute of Bioscience (IBS), UPM on October 2004, subsequently becoming the Deputy Director at Institute of Bioscience from February 2006 to May 2011 before he was promoted to Directorship.

His research interests progresses from cellular immunology to immunogenomics of animal pathogens where he works in molecular characterization of animal pathogens for the development of new diagnostics, vaccines and therapeutics. Particularly noteworthy is his work on molecular characterization of avian viruses such as NDV, IBDV AIV and CAV. His group was the first to characterize the genome of CAV followed by other pathogens such as IBDV and AIV H5N1 for the developed of real-time PCR to distinguish different strains of viruses. Recently the group has also successfully sequenced the complete genome of several NDV strains and *Pasteurella multocida.* Throughout his research, he has secured a total of 15 research grants as principal investigators amounting to more than RM7 million from various ministries. He is also actively engaged in industry driven research with animal health and genomic related companies such as Nutripharmax Sdn. Bhd., Rhone ma Malaysia Sdn. Bhd. and Ceva Animal Health Malaysia Sdn. Bhd, Science Vision Sdn. Bhd., Codon Genomics Sdn. Bhd., Sengenic Malaysia Sdn. Bhd. and Beckman Coulter Malaysia Sdn. Bhd. To date, he has published more than 150 publications in refereed journals and more than 250 citations in proceedings and abstracts, and also filed for 8 patents in Malaysia and 2 patents overseas in USA and Europe. The majority of his research findings have been published in international referred journals such as *Acta Virologica, Avian Pathology, Avian Diseases, Archives of Virology, Comparative Immunology Microbiology & Infectious Diseases, Immunology, Journal of Virological Methods, Veterinary Immunology and Immunopathology* and *Virology Journal* and he have supervised a total of 38 graduate students (15 PhD and 23 MSc), 26 of them have graduated. He is also actively involved in co-supervising numerous MSc and PhD students. Besides, research collaborators from local institutional, he has also collaborated with researchers from Razi

Vaccine Institute, Iran, Imperial College London, The Pirbright Institute and The Roslin Institute, UK on avian immunogenomics project. Apart from his poultry health related projects, he is also involved in biomedical-related research on the potential use of animal viruses as model for infectious study and treatment of cancer in humans. The potential of local strains of Newcastle disease virus (NDV) as anti-tumor agent for cancer in humans is currently being studied alongside with his colleagues from different faculties in UPM. His knowledge and skills in biotechnology related research have attracted interest from numerous researchers within and outside UPM in co-joint and collaborative researches.

Abdul Rahman Omar works very closely with all his graduate students. He has established a vibrant research culture among the students working in the lab. Through collaborations with colleagues from local and international universities/institutes, his lab has the facilities and trained lab members to conduct both basic and applied researches in immunology and molecular biology using techniques such as polymerase chain reaction, cloning, sequencing, expression of genes in various expression systems, mutagenesis and transfection technologies and serological techniques. The laboratory also has the capabilities to carry out researches in cells and cytokines profiling, receptor-ligand work, lymphocytes effector function studies, flow cytometry analysis, monoclonal antibody production, infectious/challenge studies and vaccine safety and testing studies. His main aspiration is to become a world-class scientist and educator. He is highly devoted and loyal toward the advancement of science and technology which manifested by the advancement and success of his graduate students who have gone on to realize their dreams and have risen to the occasion to apply their recently acquired teachings.

ACKNOWLEDGMENT

Syukur Alhamdulillah, I am most grateful to Allah SWT for the faith and blessings in my life. I am greatly indebted to my family, friends, colleagues, teachers and mentors whom have directly and indirectly inspired me to strive for greater heights.

Firstly, I would like to take this opportunity to express my sincere appreciation and gratitude to Prof. Dato' Dr. Mohd Fauzi Hj. Ramlan, the Vice Chancellor of UPM for his kindness and support in my career as an academician.

My heartfelt appreciation to all my mentors, especially among the former Deans of Faculty of Veterinary Medicine, Prof. Emeritus Dato' Dr. Abdul Latiff Ibrahim and Prof. Emeritus Dato' Dr. Sheikh Omar Abdul Rahman for the opportunity and guidance in pursing my career in the academic world. To all the other former Deans of Faculty of Veterinary Medicine, thank you very much for the support and encouragement. My deepest appreciation to the Dean of Faculty of Veterinary Medicine, Prof. Dr. Mohd Hair Bejo for his support and the opportunity to work with him as a co-researcher in numerous projects. I am forever grateful to Prof. Datin Paduka Dr. Aini Ideris for her endless support, trust and encouragement throughout my career at UPM, without her I will not be standing here. My sincere appreciation to Prof. Datin Paduka Dr. Khatijah Yusoff for including me in her group and enriching my experience working in biotechnology related projects. My heartfelt gratitude to Prof. Dr. Karel Schat who has guided and supervised me in my PhD at Cornell University. My sincere thanks goes to all my colleagues and staff especially at the Faculty of Veterinary Medicine and Institute of Bioscience who have been supporting my career in many ways.

I would also like to acknowledge all my co-researchers for the stimulating discussions and sharing their knowledge and

experiences with me. My deepest appreciation and thanks to all my students and post-doctoral fellows for their continued support and for making the research culture and environment stimulating and enjoyable for all. I also would like to express my sincere gratitude to all the funding agencies for their support in pursuing my research.

I am greatly indebted to my parents, Omar Hj Ibrahim (Allahyarham) and Hjh Hadijah Abdullah for their undying love, support, prayers and sacrifices. To my sisters Dr Zoharah Omar, Associate Professor Dr. Asiah Omar, brother Najeeb Omar and to all relatives for their support and love, without them I would not be who I am today. Last but not least, heartfelt thanks to my wife Sophia Jusoff and daughters Ain, Farheen, Adyln and Nuha for putting up with me and completing this blessed life.

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