

INAUGURAL LECTURE

**AVIAN RESPIRATORY AND IMMUNOSUPPRESSIVE
DISEASES - A FATAL ATTRACTION**

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ABSTRACT

The avian respiratory system is an efficient system for gaseous exchange but unfortunately, it is also a direct route to many infections. The structure of the lungs is inflexible, and the mucociliary blanket along the respiratory epithelial lining, the first line of defence, is easily damaged in any infection. A well developed immune system is the next line of defence. However, many pathogens can cause damage to the immune system, resulting in immunosuppression, which in turn, will predispose the host usually to respiratory disease outbreaks. There is definitely a fatal attraction between respiratory and immunosuppressive diseases, leading to severe and/or prolonged problems. They are responsible for high mortality and large economic losses to poultry producers. Advances made in the molecular biology of these pathogens and increased knowledge on the role of the immune system, give new opportunities towards the control of avian diseases, by vaccination. While there is a need for more work to determine the most appropriate way to produce a vaccine for a given disease, genetic engineering will without doubt, play a significant role in vaccine technology. Researchers in UPM are moving towards this new technology besides improving the conventional vaccines.

INTRODUCTION

There has been a tremendous expansion of the poultry population in Malaysia and worldwide. Not only has there been an increase in the number of poultry farms but also a remarkable growth in the flock size. Economic pressure has led to all sorts of systems of housing, the trend being towards intensive systems and large units. Modern technology makes it possible to increase the intensity and unit size, and at the same time increasing production risks. Disease risk is one of the important factors limiting production. The economic impact of disease on the poultry industry comes not only from the direct loss through death and interference with productivity but also from the cost of medications and vaccines, and the expense on veterinary manpower. The most common and important conditions affecting the poultry industry are those caused by respiratory and immunosuppressive diseases. A fatal attraction between these two groups of diseases results in huge economic losses to the poultry industry.

MALAYSIAN POULTRY INDUSTRY

The Malaysian poultry industry has come a long way, starting with a backyard rearing of indigenous chickens. Among the early exotic breeds brought into Malaysia by English sailors in the early 1930s were White Leghorn, Rhode –

Island Red, New Hampshire and Barred Plymouth Rock. This was the beginning of the expansion of the poultry industry in Malaysia. The structure of the industry changed dramatically over the past 60 years, not only in Malaysia, but also worldwide. The development in Malaysia evolved almost on a similar pattern as those in the advanced countries. The backyard poultry and small family farm flocks have been mostly replaced by modern intensive commercial farms with many farms practising closed housing system. The introduction of superior breeds, vaccines for disease control, high quality feed, advanced technology and favourable government policy, contributed to the transformation of subsistence poultry farming to commercialised and advanced poultry industry, with high breeding efficiency and high productivity (Aini, 1993). Peninsular Malaysia has been self-sufficient in chicken meat production since 1960 and became a nett exporter in 1983. Poultry meat production increased from 21,300 tonnes in 1960 to 678,000 tonnes in 1997. Egg production showed a similar growth pattern, with 12,800 tonnes in 1961 to 360,000 tonnes in 1997 (Watt's Poultry Statistical Year Book, 1998).

POULTRY DISEASES

As the commercial poultry industry continues to grow due to increasing domestic consumption and export opportunities, the economic pressure is to increase stocking density on farms which give rise to large concentrations of stock, resulting in more productive problems. Among those problems are non-infectious and infectious diseases. Due at least in part to the globalisation of the poultry industry, almost all known major poultry diseases have been reported in Malaysia (Aini, 1990a). The worldwide movement of poultry breeding stock, poultry products, biologics, pet birds, free-flying migratory birds and waterfowls, are the additional means of introduction of disease agents from one country to another. Poultry diseases do not respect international borders.

The definition of **disease** in the broad concept is, any deviation from the normal healthy state, when the normal body functions are impaired. The degree of impairment determines the severity of the disease. Poultry diseases are generally manifested by specific clinical signs, increased or high mortality or morbidity, poor feed conversion, retarded growth, reduced egg roduction, with or without changes in egg quality, low fertility, poor hatchability and increased cost of treatment or vaccination. Diseases, whether overt or subtle, are therefore an obvious deterrent to productivity.

Non-infectious diseases are associated with nutritional deficiencies or imbalances, toxic factors, poor management or environmental stress. **Infectious diseases** are the consequence of harmful action of infectious agents such as virus, bacteria, fungi and parasites. Complex diseases which result from the interaction of two or more factors (infectious and/or non-infectious)

are common in poultry flocks, and for some diseases, is a rule, rather than an exception. Respiratory and immunosuppressive diseases present a continuing potential threat for economic loss to the poultry industry. Some diseases are important because of their public health significance.

AVIAN RESPIRATORY SYSTEM - WHY IS IT IMPORTANT ?

The respiratory system in poultry has the principal function of delivering oxygen to the lungs so that it can be incorporated into the blood for subsequent use in the body. The other key role of the respiratory system is to disseminate the waste gas, carbon dioxide, from the chicken's body. In addition, the respiratory system also plays an important role in the maintenance of body temperature, using the phenomenon of panting as a way of dissipating excess body heat. It is also involved in phornation.

The respiratory system in poultry begins at the nares and has passages in the head that lead inhaled gas to the larynx. The trachea extends from the larynx, branches into two extra pulmonary primary bronchi, each of which goes to a lung and its associated airsacs. There are seven to nine lobes of airsacs. The lungs are relatively rigid structures that do not expand and retract with breathing. Their function is to provide a large surface area for gas exchange with the blood and they do this in a very small space. The air sacs function as bellows whose change in volume causes pressure differences across the lungs, that result in gas movement during inspiration and expiration.

The respiratory tract also, unfortunately, provides a direct route by which airborne pathogens can enter the host. However, there are various defence mechanisms that await to counter such invasions. The key among these, is the local immune system that can produce protective immunity. Failing this defence, the pathogen invades the respiratory tract resulting in disease problems.

RESPIRATORY DISEASES

Respiratory diseases continue to be one of the major causes of economic losses to the poultry industry worldwide. In many cases, respiratory diseases observed in a flock, maybe a component of a multisystematic disease or it may be the predominant disease with lesser involvement of other organ systems (Glisson, 1998).

Various pathogens may initiate respiratory disease in poultry, including a variety of viruses, bacteria, fungi and helminths. Environmental factors may augment these pathogens to produce the clinically observed signs and lesions. In many cases, the bacterial component of a respiratory disease, colonises the respiratory system only after a primary viral or environmental insult. In other cases, the

bacterial component of the respiratory disease is the primary initiating cause of the disease.

Viral Respiratory Diseases

Avian viruses, which have a predilection for the respiratory system include, Newcastle disease virus (NDV), infectious bronchitis virus (IBV), fowl pox virus (FPV), infectious laryngotracheitis virus (ILT), avian influenza virus (AIV) and avian pneumovirus (APV). These viruses are considered as primary pathogens of the respiratory tract, whereas adenovirus and reovirus are the secondary invaders of the upper respiratory tract of chickens (Villegas, 1998). ILTV, FPV and APV are primarily found in the tissues of the respiratory tract, whereas other viruses invade other tissues as well, such as the gastrointestinal tract (NDV, IBV, AIV), kidneys and the reproductive system (IBV), and the central nervous system (NDV, AIV). Most of the viral diseases are exacerbated by intercurrent infection with bacteria and mycoplasmas, and occasionally other viruses. Respiratory viruses, such as NDV, AIV and ILT are also very important, since they can infect the cells of the immune system.

Bacterial Respiratory Diseases

Bacterial pathogens such as *Pasteurella multocida* (PM), *Haemophilus paragallinarum* (HP), *Ornithobacterium rhinotracheale* (OR), *Bordetella avium* (BA), and *Escherichia coli* (EC) are associated with respiratory diseases. Most of these pathogens are considered as primary pathogens of the respiratory tract, whereas *E. coli* is an important secondary pathogen.

Fungal Respiratory Diseases

Aspergillosis caused by *Aspergillus fumigatus* is a major cause of respiratory infection in chicks. It can be due to poor hatchery or unfavourable farm conditions. Aspergillosis has also been reported in other species of poultry (Jalila *et al.* 1996).

Parasitic Respiratory Diseases

Syngamus trachea and *Cryptosporidium meleagridis* are two parasites associated with respiratory problems. *Syngamus trachea* parasitises the trachea of chickens, physically blocking the airways, leading to dyspnoea, typified by an outstretched neck with open mouth. The disease is usually seen in chickens reared in outdoor pens. *Cryptosporidium* parasitises the margin of epithelial cells, producing a variety of clinical signs, depending on the particular sites involved. There may be airsacculitis, pneumonia, sinusitis or conjunctivitis, with coughing, dyspnoea, nasal discharges and mortality.

Other Respiratory Diseases

Mycoplasma gallisepticum (MG) and *M. synoviae* (MS) are associated with respiratory disease in poultry. MG is a primary cause of respiratory disease, but MS affects respiratory tract, as well as other organs. Mycoplasmas are also well known for their interactions with other infectious agents, and environmental factors in producing clinical disease. Strains of MG vary widely in virulence, serological response and tissue tropisms and similar variability is known to occur with MS (Kleven, 1998). The most important recent finding with MG is that the strains have been shown to be able to vary the expression of major surface antigens and therefore present continually changing profile to the immune system. This variation occurs among strains and within clones of a single strain, and is a likely explanation as to how mycoplasma infections persist in birds for long periods, despite the strong immune response. Also it may be the reason for unusual serological reactions in infected flocks. The variability in antigen expression has a strong bearing on the preparation of antigens for serological tests (Jones, 1998).

Chlamydia psittaci (CP) is an important disease to be on the look-out. Besides causing respiratory disease, it is also of public health importance. The disease can result in conjunctivitis, pericarditis, air sacculitis, pneumonia, lateral nasal adenitis, peritonitis, hepatitis, splenitis and enteritis, depending on chlamydial serovar and avian host. Generalised infections often result in fever, anorexia, lethargy, diarrhoea, occasionally shock and death. Chronically infected birds show no clinical signs until stressed. These birds often shed chlamydia intermittently and serve as a source of infection for human and other birds (Phong *et al.* 1996; Phong *et al.* 1997).

AVIAN IMMUNE SYSTEM – THE BACKBONE TO DISEASE PROTECTION

The immune system is a highly efficient, early warning system of the body, that is able to recognise foreign material in the body, and to trigger a range of responses. It inactivates the microorganism, restrains its replication, destroys infected host cells, and removes debris from the body, in a highly specific manner. The immune system helps in the recovery from an infection and provides resistance to reinfection with the same or a closely related organism. However, the immune system by itself is not perfect to handle some organisms appropriately, thus induction of immune response is necessary.

Lymphoid and nonlymphoid systems constitute two broad structural categories of the avian immune system. They may be divided into a primary (or central) and secondary (or peripheral) system. Among lymphoid components, the primary system consists of the bursa of Fabricius, a site of B-lymphocyte

development and differentiation, and the thymus, a site of T-lymphocyte development and differentiation. The bursa was first described by an Italian anatomist and embryologist, Hieronymus Fabricius Ab Aquapendente, in 1590, thus the name Fabricius. Bursa-derived or B lymphocytes migrate from the bursa via the blood to the peripheral lymphatic organs, such as spleen, caecal tonsils, bone marrow and aggregates of lymphoid cells in various organs and tissues (lymph nodes, Harderian glands). B cells differentiation is antigen independent, whereas plasma cell differentiation is antigen dependent. The B cell component is responsible for humoral immunity. T cells are able to recognise many antigens existing in nature, initiating a cell-mediated immune response.

The non-lymphoid components of the immune system include cells that provide a non-specific immunological defense to the host. The most important nonspecific defense systems are the epithelial surface of the body, the skin, the mucous linings of the gastrointestinal, urinary and reproductive tracts and the epithelium of the respiratory tract, which provide mechanical barriers to invading agents (Stitz, 1994).

HOW DOES THE HOST REACT TO INVADING AGENTS?

Protective mechanisms are divided into an “**innate**” or “naturally occurring” and an “**adaptive**” or “anticipatory/acquired” defense. The innate defense is nonimmunologic and is important in the initial infection. If the innate defense system is not able to prevent the invading microorganisms, they can propagate and cause disease. If it is able to prevent the invasion, the chickens will recover, and an adaptive (immunologic) defense system will be stimulated. Both the non-immunologic and immunologic defence systems are responsible for preventing subsequent infection of the same pathogens (Figure 1).

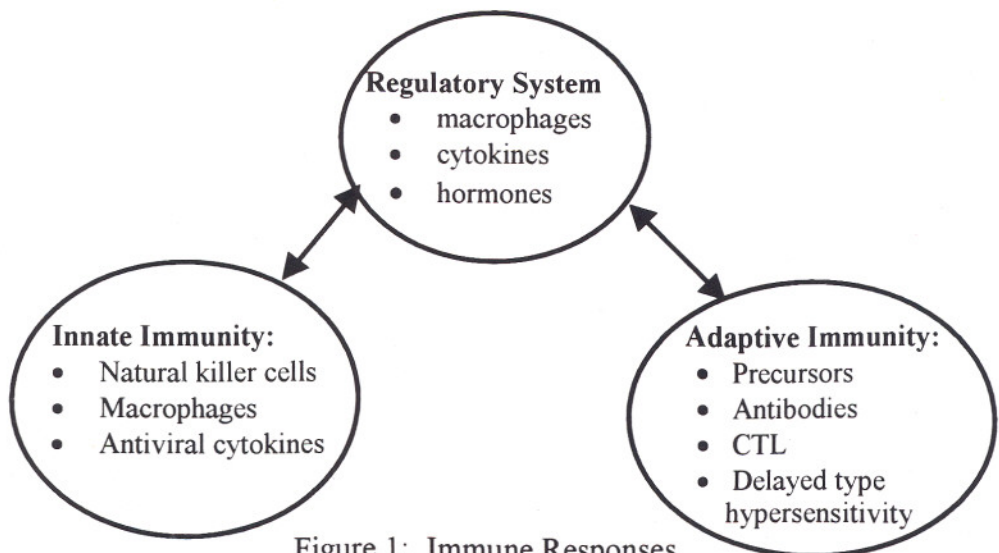


Figure 1: Immune Responses

The initial antibody response consists mostly of IgM, after primary antigen encounter, whereas the response following a secondary challenge is mostly IgY (IgG). Because of its size (19S) IgM is normally confined to the peripheral blood stream and is more active than IgY in opsonization, agglutination, virus neutralization and complement activation. IgY is the most common antibody in the serum, and due to its small size (7S) it can penetrate into tissue spaces and across body surfaces. IgY can opsonize, agglutinate and precipitate antigen.

IMMUNOSUPPRESSION

The damage to the immune system can be due to many intrinsic and extrinsic factors resulting in reduced effectiveness of the immune system. It can take several forms which vary in degree, direction and duration.

Immunosuppression is defined as: "a state of temporary or permanent dysfunction of the immune response resulting from damage to the immune system, and leading to increased susceptibility to disease" (Dohms and Saif, 1984), and "often leading to a suboptimal antibody response" (Lutticken, 1997).

A lower than expected antibody response after vaccination is probably the most frequently observed sign of immunosuppression, besides an increased incidence of secondary infections. The term immunosuppression is often used as an excuse for poor performance in a flock, when the actual cause is not known. How can immunosuppression be determined? Besides the clinical signs observed, diagnosis of immunosuppression is usually done by histological techniques to establish depletion or degeneration of lymphoid tissues, which are important signs of generalised immune unresponsiveness. Further specialised investigations may reveal other important parameters, such as decreased white blood cells (WBC) counts or decreased lymphocyte transformation responses. However, these techniques are not easily applicable for chickens on a large scale or for flock monitoring. This leaves antibody response and histopathological investigation of bursa, thymus, liver and spleen, as the most important ways to investigate immunosuppression in chickens (Lutticken, 1997).

IMMUNOSUPPRESSIVE AGENTS

Factors that influence the immune system of a chicken can be divided into those that have a **direct** effect, such as disease, nutrients, drugs, toxins, environmental contaminants, and those that have an **indirect** effect, such as heat and cold stress, water deprivation, trauma, over-crowding, and vaccination procedures. The latter factors are usually considered to act via the adrenal

gland and the production of glucocorticosteroids (Fahey, 1983). If a chicken does not eat or is deprived of feed for extended periods of time, its immune system will definitely suffer. Although in most poultry farms, malnutrition would not be expected, it should be remembered that concurrent infections can turn border-line nutrition into malnutrition and result in mild infections to become more severe.

Mycotoxins are the toxic metabolites of fungi usually associated with contaminants of feed grains. Disease may be acute or chronic, depending on the type and amount of mycotoxin consumed. Economic loss results from subtle effects of the toxin, such as a decrease in growth rate or egg production, or interference with immunity. Gliotoxin is thought to be an immunosuppressive virulence factor that enhances the severity of certain mycosis (Krunke and Richard, 1998).

The above non-infectious causes of immunosuppression may interact synergistically with immunosuppressive pathogens. Organisms that have direct tropism for any of the primary or secondary lymphoid organs would cause alterations in immune system functions. The most important viruses associated directly with immunosuppression in chickens and turkeys are listed in Table 1 (Schat, 1998).

Table 1: Viruses causing generalised immunosuppression in chickens and turkeys

	Virus Group	Virus	Species	Main target cells
DNA	Herpesvirus	Marek's disease herpesvirus	Chickens	B lymphocytes, T lymphocytes
	Circovirus	Chicken infectious anemia virus	Chickens	T lymphocytes, haemopoietic cells
	Adenovirus	Haemorrhagic enteritis virus	Turkeys	B lymphocytes, macrophages
RNA	Birnavirus	Infectious bursal disease virus	Chicken	B lymphocytes
	Reovirus	Reovirus	Chickens	Macrophages
	Retrovirus	Avian leukosis virus subgroup B	Chickens	Macrophages
	Retrovirus	Reticuloendotheliosis virus	Chickens	Lymphocytes

VIRUS-INDUCED IMMUNOSUPPRESSION

Virus infection can interfere directly and/or indirectly with the immune response. Direct effects occur when a virus replicates in, and causes lysis of cells involved in innate immunity, such as macrophages, natural killer cells, and lymphokine-activated killer cells, or effector cells involved in the acquired immunity, such as cytotoxic T cells, immunoglobulin producing cells and their precursors. Indirect effects can occur if the virus interferes with the regulatory cells of the immune system, such as macrophages, cytokine-producing cells and perhaps with the hormonal balance. A deregulation of the immune system at this level can have negative consequences for the development of antigen-specific antibodies, cytotoxic T cells, natural killer cells and other immune responses (Schat, 1994).

Viruses causing neoplastic diseases such as avian leukosis virus (ALV), reticuloendotheliosis virus (REV) and Marek's disease virus (MDV), induce immunosuppression as part of the condition they are causing. Chicken infectious anaemia virus (CIAV) and infectious bursal disease virus (IBDV) can cause generalised impaired immune responsiveness, leading to increased susceptibility to other diseases, thus regarded as the most important viral diseases of the immune system. Both viruses are small, nonenveloped viruses, which are very stable and can resist relatively high temperatures (Mc Ilroy *et al.* 1992).

ROLE OF VIRUSES CAUSING IMMUNOSUPPRESSIVE DISEASES

Marek's disease virus (MDV)

The disease is caused by a widespread, highly contagious, cell-associated, oncogenic herpesvirus. Chickens infected with pathogenic MDV result in a progressive debilitating disease that can cause mortality, reduced egg production, and immunosuppression. The disease is characterised by proliferation of lymphoid cells in various tissues and organs, including peripheral nerves. Solid lymphoid tumours may be seen in a variety of visceral organs, particularly spleen and gonads, skeletal muscles, and skin (Sharma, 1998).

Early phases of MDV infection involve extensive proliferation and subsequent cytolysis of lymphocytes in the bursa of Fabricius and thymus, causing depletion of lymphocytes. Morimura *et al.* (1996), reported that the induction of apoptosis caused the decrease of cells in the thymus.

Chicken infectious anaemia virus (CIAV)

CIAV is vertically transmitted to the progeny. Infected chickens are depressed and weak and they may be stunted. The disease is characterised by aplastic anaemia, lymphoid depletion in the thymus, atrophy of hematopoietic tissues, subcutaneous and intramuscular haemorrhages, and increased mortality. Skin lesions, in the form of ecchymotic haemorrhages, which commonly occur on the wings, are prone to secondary bacterial infection leading to gangrenous dermatitis. Though outbreaks of clinical disease are rare, subclinical infection interferes with the immune response.

Infection with CIAV not only results in a transient destruction of erythroblastoid cells but also in a transient depletion of cortical thymocytes. This is because haemocytoblasts in the bone marrow and cortical thymocytes are the first target cells, resulting in thymic atrophy. Thus, cell-mediated immune functions are impaired in chickens infected with CIAV. Jeurissen *et al.* (1989) demonstrated that the transient depletion of thymocytes from the cortex is a result of CIAV – induced apoptosis. VP3 of CIAV has been shown to be a strong inducer of apoptosis.

However, it is difficult to accept that induction of apoptosis is the only mechanism involved in the sometimes complete destruction of the thymus, specially because the percentage of the virus-positive cells detected by *in situ* hybridization is relatively low (Jeurissen *et al.* 1989). It is thought that perhaps, VP3-induced apoptosis of some cells results in changes in the production of cytokines, which in turn may lead to additional, non-viral stimuli for the induction of apoptosis. Infection with CIAV also affects cytokine production in addition to the induction of apoptosis in thymocytes.

Infectious bursal disease virus (IBDV)

Infectious bursal disease is an acute, highly infectious lymphocidal disease of young sexually immature chickens, caused by birnavirus. The clinical form of the disease typically occurs in 3 to 6 weeks old chickens, which can result in significant mortality. Affected chickens may be depressed and anorexic, have ruffled feathers, and excrete a watery, green-tinged, urate containing diarrhoea. The subclinical form of the disease occurs in chickens less than 2 weeks old. Lymphoid tissues are affected with severe atrophied bursa. Microscopic lesions include lymphoid necrosis in the bursa, spleen, thymus, Harderian gland, and caecal tonsils. The bursa is most severely affected and is characterised by a medullary lymphoid cell necrosis followed by replacement with heterophils and macrophages. Subclinical IBD is considered to be very important because it results in severe immunosuppression (Lukert and Saif, 1997).

Infection of susceptible chickens with IBDV causes immunosuppression by selective proliferation in B-lymphocytes, particularly those lymphocytes bearing surface immunoglobulin class M (Hirai *et al.* 1981) causing depletion of B-cells in bursal follicles and bursal necrosis, with subsequent immunosuppression, especially when infection occurs within the first 2 to 3 weeks of age. The main reason for the depletion is the induction of apoptosis by VP2 gene of IBDV (Fernandez-Arias *et al.* 1997). Infection causes a decrease in antibody responses against many antigens, but not against IBDV. Therefore infected birds can produce very high levels of IBDV - specific antibodies. The reasons for the normal to enhanced immune response to IBDV antigens in immunosuppressed chicks are not clear. Infection with IBDV can also cause lesions in other lymphoid tissues including the thymus, but in the absence of viral replication in thymocytes, cell-mediated immune responses are apparently not affected. However, there is increasing evidence of possible involvement of cellular immune response.

Signs of immunosuppression caused by IBDV infection include the inability to respond to vaccines with adequate antibodies (Allan *et al.* 1972), and an increased susceptibility to a range of viral infections (Saif, 1991).

Avian leukosis virus subgroup B (ALV-B)

ALV causes lymphoid leukemia (big liver disease/visceral lymphomatosis). It is the most common naturally occurring neoplastic disease of chickens induced by leukemia/sarcoma group of avian oncornaviruses. Stress or immunosuppression can increase the susceptibility of chickens to ALV infection and shedding.

ALV from chickens are classified into six subgroups, A, B, C, D, E and J. Recently, a novel subgroup-J of ALV was found to be associated with a relatively high incidence of myelocytomatosis in meat-type chickens (Payne *et al.* 1997). Most ALV subgroups have not been associated with immunosuppression, except for ALV-B. The virus replicates in lymphocytes within the bursa of Fabricius, inducing malignant transformation of one or more lymphoid follicles, accompanied by metastasis later to visceral organs. ALV-B can cause a persistent infection in macrophages, which leads to deregulation of T cell mitogenesis.

Reovirus

Infection with reovirus is characterised by decreased antibody production, and T cell mitogenesis, resulting in increased susceptibility to infection (Pertile *et al.* 1996). Destructive and abortive infection of macrophages during reovirus replication results in deregulation of production of several cytokines.

Reticuloendotheliosis virus (REV)

Infection of chickens with REV is moderately prevalent, but clinical disease is rarely recognised. The disease is characterized by bursal and thymic atrophy, retarded growth, abnormal feather development, and severe immunosuppression.

Replication of REV in the bursa of Fabricius, spleen and thymus, results in impaired cell-mediated and humoral immune responses. These appeared to be mediated through the viral glycoproteins expressed on the surfaces of REV-infected cells inducing a host-derived population of suppressor cells (Rup *et al.* 1979). However, it is still not known which REV proteins are actually responsible for the induction of immunosuppression.

RESPIRATORY DISEASES AND IMMUNE RESPONSE

The replication of the respiratory viruses in the target cells of respiratory epithelia causes the destruction of the local defense mechanisms. In addition, some respiratory viruses can damage the immune system, by either a direct effect on lymphocytes like NDV and AIV, and macrophages like ILTV and reovirus, or indirect effects by interferon and other cytokine modulations. The affected flocks, will show poor performance and give unexpected low antibody response to vaccination.

It is well known that infections of the respiratory tract are significantly affected by environmental factors. Temperature, humidity, atmospheric ammonia and dust, all have important interactions with infectious agents in producing respiratory disease (Kleven, 1998). This is an example of factors causing **indirect** effects on the immune systems.

Newcastle Disease (ND)

Newcastle disease without doubt, is the most important respiratory disease of poultry worldwide (Jones, 1998), since first described in 1926. In spite of considerable advances in our understanding of the virus, the molecular basis of pathogenicity of strains and diagnostic methods, it continues to be a serious threat to poultry health. Alexander and Gough (1997) reported recent outbreaks of ND in Western Europe, which were formerly considered free. ND outbreaks in domestic chickens and turkeys were also reported in Great Britain, during 1997 (Alexander *et al.* 1998). In Asian, African, Central and South American countries, severe forms of ND outbreaks are among the most common diseases reported (Alexander and Gough, 1997; Aini, 1993).

The clinical signs of ND include respiratory distress, diarrhoea, cessation of egg production, depression, oedema of head, face and wattles, nervous signs,

and death. Depending on the pathotypes of virus involved, some, all or none of these signs may be present.

NDV is capable of infecting most avian species, providing a constant potential threat from wild birds. The causative virus paramyxovirus 1, has a single antigenic type, although fine differences between strains can be demonstrated using monoclonal antibody panels. The uniform antigenicity means that control by vaccination is relatively simple. But why is the disease still a problem? The right vaccination strategies may be the answer to the problem.

Avian Influenza (AI)

Avian influenza viruses, like NDV, have a very wide avian host range (Aini *et al.* 1998). The essential of the epidemiology of avian influenza are that, the virus is maintained in vast pools in wild birds, especially migratory birds of the orders Anseriformes (ducks, geese, swans). The worldwide distribution of these birds means that there are occasional introductions of strains of low virulence for chickens, into the bird populations of most countries with regular or occasional spread to domestic birds, including poultry reared outdoors, such as ducks, turkeys and more recently, ostriches (Jones, 1998). In Malaysia, AIV strains H4N3, H4N6, and H3N6, have been reported (Ibrahim *et al.* 1990; Aini and Ibrahim, 1986b).

Influenza viruses (Orthomyxovirus) are subtyped on the basis of haemagglutinin (H) or neuraminidase (N) antigens, which are important in protective immunity and show great variations. There are 15H and 9N subtypes. Some H5 and H7 strains are highly pathogenic for poultry (Swayne *et al.* 1998).

Outbreaks of influenza in poultry due to highly pathogenic strains are fortunately very rare, sporadic and unpredictable. Only five outbreaks have been recorded in the 1990s. Two of these were in Australia (Westbury, 1997), one outbreak in Pakistan (Naeem, 1997), another in Mexico (Senne *et al.* 1996) and the latest (1997, 1998), in Hong Kong, where mortalities in human were also recorded due to H5N1 strain.

Avian Pneumovirus Infection

Avian pneumovirus is an acute, highly contagious upper respiratory tract infection of turkeys and chickens. Signs in young turkey poult include snicking, rales, sneezing, nasal discharge, foaming conjunctivitis, swelling of infraorbital sinuses and submandibular oedema. Severe respiratory distress may occur in broiler chickens particularly when exacerbated by secondary pathogens such as IBV, mycoplasmas, and *E. coli*. The clinical signs associated with this are swelling of the periorbital and infraorbital sinuses, torticollis, incoordination and depression (Gough *et al.* 1998).

The first disease due to avian pneumovirus infection was reported in South Africa in turkeys in the late 1970s (Buys and Du Preez, 1980). The disease was called turkey rhinotracheitis (TRT) because of its main manifestation. It causes loss of egg production in breeder turkeys. In chickens, infection is sometimes associated with swollen head syndrome (SHS), where the head and mandible are swollen, because of gelatinous exudate beneath the skin (Pattison *et al.* 1989). However, SHS does not always result from APV infection.

APV has an almost worldwide distribution, with the notable exceptions of the USA (although recently an APV-like pneumovirus has been isolated) and Australia. In Malaysia, SHS has been reported (Jasni *et al.* 1998). The syndrome began with sneezing and conjunctivitis followed by swelling of lachrymal glands, around the eyes, over the head, sub-mandibular region and finally subcutaneous oedema of the head.

Infectious Bronchitis (IB)

Infectious bronchitis, caused by coronavirus, has been known for more than 60 years. Similar to ND, it is still a problem despite the widespread use of vaccines. This is chiefly due to the appearance of different serotypes. IBV is highly host specific, with chickens being the only natural host. While considered primarily a respiratory pathogen, different strains of infectious bronchitis virus may show variable tissue tropisms and affect the oviduct of neonatal or adult female chickens or the kidneys, with serious consequences (Cavanagh and Naqi, 1997).

IBV infects chickens of all ages. Chicks display acute respiratory signs and lesions in the trachea. In layers, egg production and quality are reduced. Lymphoid cell infiltration and epithelial cell degeneration of the oviduct wall have been observed. Nephropathogenic strains produce enlarged kidneys with distended tubules and ureters, containing uric acid crystals. Diarrhoea, dehydration, depression, and death may occur in affected chickens. Some strains replicate in the intestine and persist there longer than in the respiratory tract, but apparently without significant pathological changes (Jones and Ambali, 1987).

Fowl Pox (FP)

Avian pox is a common worldwide viral disease of domestic birds (chickens, turkeys, pigeons and canaries) and has been reported in more than 60 species of wild birds representing 20 families. It is a relatively slow-spreading viral disease characterized by the development of proliferative skin lesions (cutaneous form) and/or upper digestive and respiratory tract lesions (diphtheritic form).

The cutaneous form is characterised by the development of nodular lesions on various parts of unfeathered skin, such as the comb, wattle, corner of the mouth, around the eyelids, the angle of the beak, the ventral surface of the wings and vent. In the diphtheritic form, lesions occur on the mucous membrane of the mouth, nares, pharynx, larynx, oesophagus or trachea. Death occurs in cases with the generalized infection or diphtheritic form of the disease.

Infectious Laryngotracheitis (ILT)

ILT is another disease which has been known for more than six decades. It is caused by Gallid herpesvirus 1, which has only one serotype and naturally infects only chickens and sometimes pheasants. The virus which appears to affect only the respiratory tract (Jones, 1998) is characterised by anorexia, depression and severe respiratory distress with coughing, sneezing, gurgling, gasping and rales. In the peracute form, there may be high mortality.

The important epidemiological feature of the disease is the ability of the virus to become latent after the acute phase of infection (Bagust and Johnson, 1995). Chickens with latent virus may appear completely healthy but reactivation of the virus can occur when they reach maturity or under stress.

ILTV strains are antigenically homogeneous, so cross-protection occurs between all strains. Immunity to ILTV is cell-mediated, thus maternal antibody will not interfere with the vaccination of very young chickens or *in vivo*. Eradication for ILTV is suggested to be possible, but would depend on the availability of a recombinant ILTV vaccine, since the available attenuated vaccines perpetuate virus infection (Bagust and Johnson, 1995).

CONTROL OF DISEASES – A CONTINUING CHALLENGE

Control of poultry diseases involves a complex interactions of management, nutrition and flock health programme. Biosecurity is an important component of management and a vaccination programme is the most important component of flock health programme. Biosecurity encompasses management practices needed to prevent the spread of pathogens between farms and between buildings within a farm. It is difficult and expensive to maintain a high level of biosecurity at all times. So far, mechanisms involved in the defense towards viral infections have been shown to involve pathways of immunological reactions. Since important respiratory and immunosuppressive diseases are due to viral infections (Aini, 1990b), this paper will only focus on prevention by vaccination.

The cornerstones of vaccination are of course B cells which should synthesise and secrete antibodies that could neutralise invading agents or serve as anchor molecules for cellular cytotoxicity. T cells are necessary for an optimal B cell response. Though B cells are able to respond to free antigen, an optimal response will only be achieved if at the same time an efficient T helper response is induced. Therefore, every successful vaccination should also aim at T cell activation (Stitz, 1994). The goal of vaccination is to induce an immune response that will protect chickens directly by the induction of antibody and cell mediated immunity, and result in the induction of immunological memory. A subsequent infection by virulent organisms either fails to cause disease in the face of active immunity or induces a rapid memory response that clears the infection without loss of performance.

Passive immunity, such as maternal antibody from an actively immunised hen, can also provide protection. This antibody is short lived and provides protection while the chick's immune system is developing. However, this type of chick protection is only useful where antibody is sufficient to protect against infection.

Ideally, vaccines should induce lifelong protection against a pathogen without producing any sign of disease, and reduce morbidity and subclinical infections. Vaccines must also be safe, apathogenic, stable and easily delivered to large numbers of birds. The ability to vaccinate at day-old in the face of maternal antibody and to induce rapid humoral and cellular responses at mucosal surfaces are desirable.

Currently, three types of vaccines are available to the commercial poultry industry: The classical live attenuated and killed vaccines and the new generation of recombinant vaccines.

Conventional Vaccines

Live vaccines

Conventional live vaccines contain infectious agents that have been attenuated, usually by passage *in vitro*. Vaccination using attenuated viruses represents one of the most efficient protection because of the extensive immunogenic potential due to the induction of both B (humoral) and T (cellular) cell responses. This effect is the result of the limited replication of the attenuated agent which mimics a natural infection. Live vaccines are cheap and suitable for mass vaccination. However, while often very effective at stimulating a protective immune response, they have the potential to revert to virulent form. Live vaccines also provide a source of organisms for the selection of virulent variant mutants, after a long usage.

Killed vaccines

Inactivated or killed vaccines comprise whole organisms that have been treated to make them non-infectious. There is a requirement for larger doses to stimulate an immune response comparable to those induced by live vaccines, since replication in the host is not possible. These vaccines are safer but tend to be more expensive, usually require the use of adjuvants. They require individual injection, inducing good humoral immunity but not cellular immunity.

Genetically Engineered Vaccines

Genetic engineering has made it possible the production of apathogenic organisms in a more predictable and stable manner. It is also possible to produce vaccines that can be readily distinguished from wild type organisms. This will greatly aid in diagnosis and possible eradication programmes. The other advantage is the ability to insert multiple genes of protective antigens into a vector (Boyle and Radford, 1992). The major advantage of genetically engineered vaccines is that there is no clinical disease nor does the process involve the introduction of or reliance upon, an infectious organism.

Recombinant vaccines

Recombinant vaccine modification of the vectored organisms enables the expression of foreign gene(s) encoding protective antigens from pathogenic organisms. Whether a bacterial or a viral vector, it is now possible to produce vaccines with the advantages of both live and killed vaccines. This type of vaccine combines the safety of a killed vaccine, with the efficacy of a live vaccine. They can also be used in mass administration, either by spray or aerosol. Polyvalent vaccines can also be produced using this method. The commercially available recombinant vaccines are fowlpox virus expressing NDV antigen (F & HN proteins), IBDV (VP₂ and VP₃ proteins), Marek's disease virus expressing NDV and IBDV proteins.

Subunit vaccines

It is also possible to express the genes encoding protein antigens *in vitro* for use as subunit vaccines. However, the application is still difficult due to high cost of production. Further improvements in gene expression systems and protein fractionation will result in cost effective production of subunit vaccines for poultry.

Synthetic peptide vaccines

Using the current technology it is possible to synthesise an immunogenic epitope for vaccine purposes. Due to the present high cost of production and administration, the use of this approach by the poultry industry is not likely in the near future.

DNA vaccines

There has been a great deal of interest recently in the use of naked DNA encoding antigens for immunization. Some researchers proved that DNA vaccines are capable of eliciting both humoral and cellular immunity. However, the mechanism of DNA uptake as well as the identity of the antigen presenting cells (APC) to the immune system is still not clear. This knowledge is necessary for the best formulation and delivery of DNA vaccine.

Plant - derived vaccines

The vaccine delivery systems are more or less decisive whether vaccines are not only useful but will also be used. The novel method is of course the oral route for mass administration. Currently several groups around the world are working on vaccine-producing plants. They hope that it might eventually be possible for animals to be vaccinated by eating leaves, fruits or grains from these plants.

Routes of Administration

Proper application is critical to a successful vaccination. There are six methods that are commonly used in poultry, with the addition of several new technologies. It must be remembered, however, that success for all vaccination methods is dependent on following the correct protocols.

Drinking water

Live vaccines are often administered via drinking water. It has the advantage of being fast and cost effective. However, time and water quality are important to avoid vaccine inactivation and to ensure that every bird gets the right vaccine dose.

Spray

This method of vaccination is cost effective, and able to induce mucosal immunity if used correctly. It is the most popular method for day-old vaccination and for chickens kept in closed houses. However, this method is applicable to certain live vaccines only.

Intraocular or intranasal

Among all the methods of administration of live vaccines, these two methods are probably the most effective, but labour intensive. Accuracy is important and the vaccine must be absorbed after a blink (intraocular) or inhalation (intranasal), before the bird is released.

Injection

This is done via intramuscular or subcutaneous routes. Though live vaccines may also be administered by injection, it is usually used for killed vaccines. It is time consuming and accuracy is important to avoid swelling, granulomas, liver punctures or lameness, depending on the site of injection.

Wing web stab

This route of vaccination is used mainly for fowl pox virus, though some farmers used it for reovirus and live fowl cholera vaccines. It is also time consuming.

In-ovo

In-ovo vaccination technology has gained popularity in some parts of the world, but not others, due to high investment costs. In this method, vaccination is given to embryos, thus chicks need not be individually handled at the hatchery. Widespread adoption will depend upon clear improvements in productivity and economic benefits.

Feed

Feed vaccination method has similar advantages and disadvantages as drinking water vaccination. However it is very suitable for chickens kept under free-range system. UPM has successfully developed food based ND vaccine.

Another new technology in feed vaccine is the oral capsular vaccine which is aimed at a single dose vaccination, to last the life of the chicken. It is still a long way towards commercialisation.

RESEARCH IN THE CONTROL OF AVIAN RESPIRATORY AND IMMUNOSUPPRESSIVE DISEASES AT UNIVERSITI PUTRA MALAYSIA (UPM)

Occasionally, new diseases appear in poultry populations, sometimes through cross species transmission or from contaminated biological products. Sometimes due to changing husbandry conditions, the importance of a disease

change, such as the role of respiratory conditions when intensive housing was introduced. The pathogenicity of prevalent field viruses may change too, possibly by cycling through immune or partially immune hosts. It is important that vaccine development keeps pace with changing circumstances.

Our interests are in the development of vaccines for disease control. Before any vaccine can be produced the characteristics and the pathogenicity of the virus must be known. Two very important respiratory viruses (Newcastle disease, fowl pox) and two immunosuppressive viruses (chicken infectious anaemia, infectious bursal disease) were chosen as models for our research. The research areas are designed towards understanding the characteristics of the viruses isolated, with the ultimate aim of future development of vaccine from local virus isolates. The research involves the following areas:

Newcastle Disease

1. *Pathogenicity of the virus, involving pathological and electron microscopic studies.* The effects of the virulent NDV in the respiratory tract and the bursa of Fabricius of infected chickens were determined in order to relate to prevention of disease by vaccination (Fauziah *et al.* 1993; Aini *et al.* 1997; Fauziah *et al.* 1998).
2. *Improvement of vaccines through cloning techniques utilising limiting dilution method and plaque purification.* These studies involved the selection of highly immunogenic clones from the parent virus. Three vaccines were developed (Ibrahim and Aini, 1994; Aini *et al.* 1992; Aini *et al.* 1990a; Jarra *et al.* 1991; Bell *et al.* 1991; Ibrahim *et al.* 1983; Aini *et al.* 1990b) of which one live vaccine (UPM-V4) has been commercialised. Another killed vaccine is planned for upscaling work.
3. *Molecular epidemiology and characterisation of the selected local isolates.* Extensive work on the local isolates are ongoing. Promising results have been obtained in the development of recombinant vaccines.
4. *Development of rapid diagnostic methods.* A quick method using immunoperoxidase staining directly onto infected tissues was developed. This method is now extended for use in other diseases as well (Awang *et al.* 1992). Another method, using polymerase chain reaction directly onto infected tissues has also been established.
5. *Transgenic plant vaccine.* The work in this area is still at infancy stage.

Fowl Pox

1. *Characterisation and pathogenicity studies of avian pox virus isolated from chickens, turkeys and pigeons* (Aini and Ibrahim 1986a; Sivasothy *et al.* 1996; Sivasothy *et al.* 1998).
2. *Improvement of vaccine through tissue culture adaptation and cloning techniques.* The research has successfully produced fowl pox vaccine which has also been commercialised (Aini *et al.* 1994).

Infectious Bursal Disease

1. *Characterisation and pathogenicity of local virus isolates, involving pathological and electron microscopic studies, in all the target organs* (Hair *et al.* 1996).
2. *Development of conventional live vaccine using selected local virus isolate.* The efficacy studies showed that the selected clone is capable of inducing high immune response comparable or better than imported vaccines (Hair *et al.* 1995, Sharifah *et al.* 1994).
3. *Molecular characterisation of the local isolates* (Sharifah *et al.* 1993) *with the objective of developing recombinant and/or DNA vaccine.* The research towards genetically engineered vaccine is still at an early stage.

Chicken Infectious Anaemia

Serological evidence of chicken infectious anaemia has been reported in late 1980s (Rozanah *et al.* 1995; 1996). It is therefore suggested that the presence of this important immunosuppressive virus is not "new" in Malaysia, but just previously undiscovered. This is probably because the virus is usually difficult to grow in culture. In 1997 and 1998 researchers at Veterinary Research Institute and UPM have isolated this virus from poultry farms in the northern and southern regions of Malaysia. Ongoing research involves:

1. further isolation of the virus from broiler, layer and breeder farms.
2. pathogenicity studies and molecular characterisation of all the isolates for in-depth understanding of the virus, for future selection in the production of conventional and genetically engineered vaccines.

CONCLUSION

Successful poultry production depends to a significant degree on the prevention of infectious diseases, the most important being respiratory and immunosuppressive diseases. Infections by many of the pathogens, especially virus infections, can result in damage to the immune system. Even subclinical infection with some of these pathogens can cause immunosuppression. Immunosuppression can be antigen specific or generalised unresponsiveness. Obviously, the impact of the later on poultry production is far greater than the former. However, many viruses can cause both types of immunosuppression. Immunosuppressed chickens usually are more prone to respiratory disease outbreaks, resulting in higher mortality and morbidity, respond poorly to vaccination and medication and exhibit increased post-vaccination reactions.

The prevention of infectious diseases requires the implementation of strict biosecurity and vaccination programmes. There is no doubt that vaccines are powerful tools in disease control, and the research in this area is rapidly evolving. The development of vaccine-induced immunity depends on a complex set of interactions between macrophages, antigen-presenting cells, lymphocytes and cytokines. Immunosuppression can interfere with these interactions resulting in negative impact on the success of vaccination programmes.

Advances in vaccine technology can be used to expand the range of current veterinary vaccines. This can be done either by improving the efficacy of available vaccines or developing new vaccines. In the case of existing vaccines, new vaccination techniques should qualitatively and quantitatively improve the immune response. There should also be increased duration of immunity, improved consistency, lower costs and easier delivery. Further developments will be primarily achieved through recombinant DNA technology, new adjuvants and new vaccine delivery technologies (Prowse *et al.* 1993). However, to be acceptable, the genetically engineered vaccines must offer advantages over available conventional vaccines, in terms of efficacy, cost, administration, production, safety, shelf life, and acceptance by regulatory bodies and public. It seems likely that for the present, most vaccines used for respiratory and immunosuppressive diseases will continue to be of the conventional type. Research on conventional and genetically engineered vaccines are ongoing in UPM.

Although many viruses have been well characterised, our knowledge on how they affect the immune system is still very limited. Advances in avian immunology, in particular in the field of avian cytokines will specifically help us assess and understand immunosuppressive factors better in the near future.

Finally, satisfactory disease control will never be achieved unless attention is paid to good management, specific disease control and appropriate biosecurity measures in order to minimise early exposure of the flock to infectious agents, and to allow proper development of the immune system.

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