2. LITERLATURE REVIEW

2.1 Background of Avian Influenza virus

2.1.1 History of Avian Influenza virus

Avian influenza (AI) caused by influenza A viruses is a disease of many kinds of poultry, wild and caged birds characterized by marked variation in morbidity, mortality signs and lesions. In addition, the infection causes periodical epidemics in humans, pigs, seals, and a variety of birds (Swayne and Halvorson, 2003). AI viruses are members of the Orthomyxoviridae family. Within the family there are three types of influenza: A, B and C. Type B and C affect only humans (Scholtissek et al., 1983). The RNA virus is enveloped, sensitive to ether, chloroform and different chemical disinfectants. Influenza A viruses are devided, on the bases of the antigenic of the antigenic relationships in the surface glycoprotein's haemagglutinin (HA) and neuraminidase (NA), into subtypes. There are at present 16 H subtypes and 9 N subtypes. The virus genome is single stranded, which multiplies in the same cell; progeny viruses may originate from the reassortment of parental genes derived from different viruses and theoretically 256 different combinations of progeny viruses may arise from two parental viruses (Murphy and Webster, 1996; Anon, 2000; Suarez, 2000).

In poultry the most virulent from of avian influenza was designated as fowl plague. In 1981, the term fowl plague was replaced with the term 'highly virulent influenza virus infection. Recently the term Highly pathogenic avian influenza-HPAI base on the surface antigen and pathogenicity is suggested to be used (Anon, 2000). Currently , only viruses of H5 and H7 subtype have been shown to cause HPAI in

susceptible species, but not all H5 and H7 viruses are virulent. However, it has been proved that highly pathogenic avian influenza (HPAI) viruses emerge in domestic poultry from low pathogenicity (LPAI) progenitors of the H5 and H7 subtypes (Garcia et al. 1996; Senne et al 1996; Perdue et al. 1997; Villarreal and Flores, 1998). Also, In 2004, highly pathogenic avian influenza was detected in commercial and backyard flocks in Thailand . Serious epidemics of HPAI have occurred in Thailand during 2004, The eradication of disease is the stamping out policy, by culling and destroying of birds. A total of about 50 million poultry have been killed with very high costs and financial losses to the community, and to the poultry industry. Recently several outbreaks of AI H5N1 have been confirmed among poultry in Cambodia, China, Hong Kong, Indonesia, Japan, Laos, South Korea, Vietnam and Thailand.

2.1.2 Taxonomy of Avian Influenza virus

Influenza viruses are enveloped single-stranded RNA viruses with a pleomorphic appearance, and an average diameter of 120 nm. Projections of haemagglutinin and neuraminidase cover the surface of the particle (Figure 1).

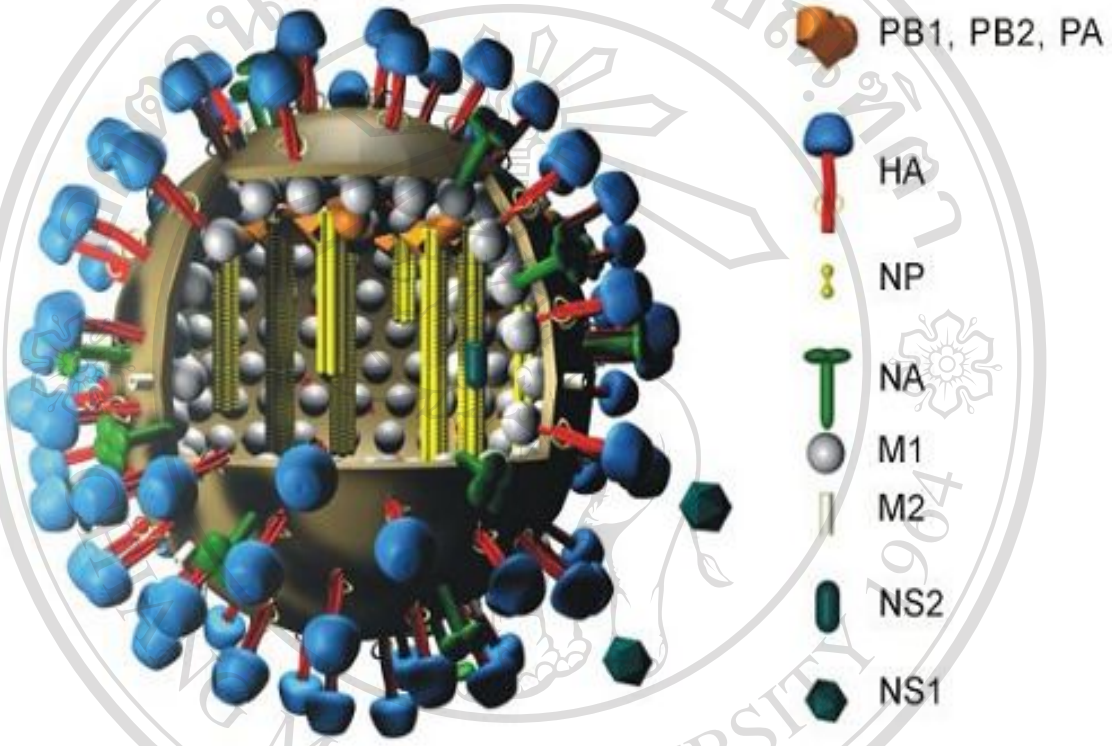

Figure 1: Structure of an influenza A virus. Image copyright by Dr. Markus Eickmann, Institute for Virology, Marburg, Germany. Used with permission. - http://www.biografix.de

The influenza A and B virus genomes consist of 8 separate segments covered by the nucleocapsid protein. Together these build the ribonucleoprotein (RNP), and each segment codes for a functionally important protein: Polymerase B2 protein (PB2).Polymerase B1 protein (PB1).Polymerase A protein (PA).Haemagglutinin (HA or H). Nucleocapsid protein (NP).Neuraminidase (NA or N).Matrix protein (M): M1 constructs the matrix; and in influenza A viruses only, M2 acts as an ion channel pump to lower or maintain the pH of the endosome. Non-structural protein (NS); the function of NS2 is nuclear export protein. The active RNA-RNA polymerase, which is responsible for replication and transcription, is formed from PB2, PB1 and PA. It has an endonuclease activity and is linked to the RNP. The NS1 and NS2 proteins have a regulatory function to promote the synthesis of viral components in the infected cell.

Avian Influenza Virus is a type A influenza virus. There are 16 subtypes of influenza virus known to infect birds. These subtypes are classified as “H” or “N”. Type A Influenza viruses can affect humans (H1N1, H1N2, H2N2), horses (H3N8, H7N7), pigs and many bird species. There are two forms of the type A Influenza viruses that affect birds, Low Pathogenic Avian Influenza (LPAI) and High Pathogenic Avian Influenza (HPAI). Avian influenza outbreaks in Asia have resulted in the death and depopulation of millions of birds and fatal disease in people with direct contact with infected birds.

2.1.3 Morphology and Nature of the Genome

Influenza particles are highly pleiomorphic: The morphological characteristics of influenza viruses are a genetic trait, but spherical morphology depends on passage in eggs or tissue culture (Subbarao et al., 1998). The majority of viruses are found to be

spherical, 80-120 nm in diameter, but many forms occur, including filamentous particles up to 2000 nm long. Different strains of virus tend to have different filamentous forms, a property which maps to the matrix protein, which lines the inside of the viral envelope (Klenk, 1974).

2.2 Avian Influenza virus

2.2.1 Epidemiology of Highly pathogenic AI of subtypes H5 and H7

Up to the end of 2003, HPAI was considered a rare disease in poultry. Since 1959, only 24 primary outbreaks had been reported world-wide. The majority occurred in Europe and the Americas. Most outbreaks were geographically limited, with only five resulting in significant spread to numerous farms, and only one which spread internationally. None of the outbreaks had ever approached the size of the Asian outbreaks of H5N1 in 2004 (WHO 2004/03/02). To date, all outbreaks of the highly pathogenic form have been caused by influenza A viruses of the subtypes H5 and H7. The original H5N1 virus, encountered for the first time in 1997, was of a reassortant parentage, including at least a H5N1 virus from domestic geese (A/goose/Guangdong/1/96, donating the HA) and a H6N1 virus, probably from teals (A/teal/Hong Kong/W312/97, donating the NA and the segments for the internal proteins), which underwent many more cycles of reassortation with other unknown avian influenza viruses (Xu, 1999, Hoffmann, 2000, Guan, 2002b). Several different genotypes of the H5N1 lineage have been described (Cauthen, 2000, Guan, 2002, 2003). The so-called genotype 'Z' has dominated the outbreaks since December 2003 (Li, 2004). A new dimension of HPAI outbreaks became evident late in 2003. From mid-December 2003 through to early February 2004, outbreaks in poultry caused by the Asian lineage HPAI H5N1 virus were reported in the Republic of Korea, Vietnam,

Japan, Thailand, Cambodia, Lao People's Democratic Republic, Indonesia, and China. The simultaneous occurrence in several countries of large epidemics of highly pathogenic H5N1 influenza in domestic poultry is unprecedented. All efforts aimed at the containment of the disease have failed so far. Despite the culling and the pre-emptive destruction of some 150 million birds, H5N1 is now considered endemic in many parts of Indonesia and Vietnam and in some parts of Cambodia, China, Thailand, and possibly also the Laos.

In January 2004, an outbreak of highly pathogenic avian influenza outbreak occurred in Thailand. HPAI was detected in a layer farm, Bangplama district, Suphanburi province, central region of Thailand. The peak of infection rate and seasonal influenza outbreaks was during the end of rainy season and winter season -it occurred in commercial poultry, backyard flocks.

2.2.2 Mode of infection and transmission

The disease can be transmitted horizontally by direct contact with infected birds or indirectly through contaminated equipments. There is little or no evidence of vertical transmission (egg-borne infection). However, eggshell surfaces can be contaminated with the virus (Cappucci et al., 1985; Tanyi and Klaczinski, 1992). Wild and domesticated waterflow are the major natural reservoir of influenza viruses. Representatives of all of the different subtypes of avian influenza A virus have been isolated from birds, particularly from aquatic species such as ducks, geese, and gulls (Hinshaw et al., 1981; Alexander, 2000). They may be infected with more than one subtypes without any clinical signs, excrete the virus for a long period and mostly do not develop detectable antibodies. A marked similarity between the subtypes'prevalence in the waterfowl population and poultry were reported several times (Bahl et al., 1979; Halvorson et al., 1983,1987). The infection can also be spread by contaminated shoes, clothing, crates, egg flats, and egg-case vehicles. The major way of the further spread of avian influenza viruses seems to be mechanical

transfer of infective feces (Utterback, 1984) through movement of man and contaminated equipment (Halvorson et al.,1980; Alexander and Spackman, 1981). The virus can survive in the contaminated environment for long periods of time at moderate temperatures and longer in frozen materials. Rodents and insects may mechanically carry the virus from infected to susceptible poultry.

Wild birds such as geese, ducks and game birds – they can be carriers of infectious agents shedding the virus in their feces without clinical signs of disease.

2.2.3 Clinical signs and Gross lesions

The severity of clinical signs, course and mortality in poultry after infection with avian influenza are extremely variable from highly acute to a very mild, or even inapparent form with few or no clinical signs and are influenced by many factor such as the virulence of the virus, the species, age of host, the immune status, concurrent diseases and management. Clinical signs include ruffled feathers, depression, diarrhea, a sudden drop egg production, cyanosis of combs, and wattles oedema, and swelling of the head, blood-tinged discharge from nostrils, respiratory distress, in-coordination and pinpoint hemorrhages mostly seen on the feet and shanks. Lesions include swelling of the face, straw- color fluid in the subcutaneous tissues. Blood vessels are usually engorged. Hemorrhage may be seen in the trachea, proventriculus and throughout the intestines. Young broilers may show signs of severe dehydration with other lesions. In turkeys, lesions consist of sinusitis, tracheitis, pericarditis, petechial hemorrhages in pericardial fat, fibrinous airsacculitis, lung congestion, pneumonia as well as enlargement of the spleen and inflammation of the pancreas (Tanyi and Klaczinski, 1992; Swayne and Harvoson, 2003).

2.2.4 Avian influenza infections in Humans

In humans, outbreaks of influenza A of subtypes H1N1, H1N2 and H3N2, appear to be the most common ones. Investigations showed that the most of human and mammal influenza viruses originated from avian sources (Webster et al., 1992). However Avian influenza A viruses do not usually infect humans (Swayne, 2000). In some cases, people with intensive contact with poultry and swine can be infected with other subtypes (Ito et al., 1998, Schotissek, 1994). Several instances of human infection and outbreaks have been reported since 1996.

In 1996 an H7N7 virus was isolated in England from the eye of the woman with conjunctivitis who kept ducks. This virus was shown to be genetically related to a virus of H7N7 subtype isolated from turkeys in Ireland in 1995 (Banks et al., 1998). Influenza A H5N1 was isolated from human in Hong Kong in 1997. This virus was identical with the HPAI H5N1 circulated in poultry (Class et al., 1998; Suarez et al., 1998). Again in Hong Kong in 1997, there were 18 confirmed human cases with 6 deaths. At this time, there are still no definite sign of human to human transmission, but even if it occurs, the efficiency of transmission is low at this time (Shortridge et al., 2000; Buxton Bridges et al., 2000). During the recent ongoing outbreaks in Asia with AI H5N1 from 2003 until 2007(up date 20 August, source World Health Organization), Vietnam has reported 95 confirmed cases in human and died 42 cases died.

Indonesia has report 102 which 81 have died. China has report 25, of which have 16 died, In Thailand has reported 25 confirmed case, of which 17 have been fatal. At this time it is believed that this case resulted from contact with infected birds or surfaces contaminated with excretion from infected birds. An investigation is ongoing to determine the source of human infection. After an incubation period of 1-3 days, the virus causes influenza-like symptoms characterized by high fever, chill,

headache, myalgia, prostration, sore throat, and cough. Children, elderly and those debilitated by chronic diseases or immunosuppression may be susceptible to infection and express severe manifestations such as difficulties in breathing and pneumonia. Risk populations includes poultry farmers, slaughterers, and handlers in contaminated areas. Avian influenza is different from human influenza, in which no transmission from person to person is evident.

2.3 Diagnosis of Avian influenza virus

2.3.1 Overview of Avian Influenza virus isolation

Although virus isolation remains the gold standard of diagnosis and is necessary for virus characterization, rapid laboratory confirmation of suspected influenza disease in routine diagnostic laboratories is usually performed by immunochromatographic or immunofluorescent detection of influenza virus antigens, or reverse transcriptase (RT). Regarding laboratory tests of avian influenza disease; many factors should be considered in deciding which tests to use. Sensitivity, specificity, turn-around-time, repeatability, ease of performance governmental regulation and costs should all be taken into account.

Clinical signs and lesions are not pathognomonic. Therefore, identification and the characterisation of the virus involved are essential.

The OIE subsequently adopted the following criteria for classifying an avian influenza virus as highly pathogenic:

a. Any influenza virus that is lethal for six, seven or eight of eight 4-8 week-old susceptible chicken within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid

b. The following additional test is required if the isolate kills from one to five chickens but is not of H5 or H7 subtype: growth of the virus in cell culture(for example primary cell such as chick embryo cells or cell lines such as MDCK cells, although most cell culture support the growth of HPAI influenza viruses or those of low pathogenicity in the presence of trypsin with cytopathic effect or plaque for mation in the absence of trypsin) If no growth is observed, the isolate is not considered to be a HPAI isolate.

c. For all H5 and H7 viruses of low pathogenicity and for other influenza viruses, if growth observed in cell culture without trypsin, the amino acid sequence of the connecting peptide of the haemagglutinin must be determined. If the sequence is similar to that observed for other HPAI isolates, the isolate being tested will be considered to be highly pathogenic.

According to the European Union legislation on avian influenza "Council Directive 92/40/EEC" the disease is defined as follows: avian influenza means an infection of poultry cause by any influenza A virus which has an intravenous pathogenicity index in six-week-old chickens greater than 1.2 or any infection with influenza A viruses of H5 or H7 subtype for which nucleotide sequencing has demonstrated the presence of multiple basic amino acids at the cleavage site of haemagglutinin. (Hafez, H.M., 2005)

2.3.1.1 PCR detection

RT-PCR is generally more sensitive than serology and culture and the combination of RT-PCR with serology more sensitive than the combination of any other two methods (Zambon, 2001). RT-PCR can only be performed in well-equipped laboratory facilities by trained personnel. These methods can either detect both influenza A and B or differentiate between types (influenza A or B). The only direct

technique that has the potential to differentiate between subtypes (i.e. on the basis of haemagglutinin and neuraminidase) is RT-PCR.

2.3.1.2 Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR is a process whereby RNA is first converted to complementary DNA (cDNA) and a section of the genome is then amplified through the use of primers that bind specifically to this target area. This allows for exponential amplification of small amounts of nucleic acid, through the action of a thermo stable DNA polymerase enzyme, which enables highly sensitive detection of minute amounts of viral genome. Not only does RT-PCR have superior sensitivity (Steininger, 2002) but it can also be used to differentiate between subtypes and conduct phylogenetic analysis (Allwinn, 2002). RNA degradation of archival samples can decrease the sensitivity of RT-PCR (Frisbie, 2004). Therefore specimens should be processed as fast as possible after collection.

2.3.1.3 Other direct detection methods

Different methods exist for direct detection of influenza viruses. Some methods such as enzyme immunoassays (EIAs) can be suitable for bedside testing, others such as direct immunofluorescence allow for the preparation of slides onsite in clinics and the posting of fixed slides to a central laboratory (Allwinn, 2002).

2.3.1.4 Isolation method

Virus isolation or culture is a technique whereby a specimen is inoculated in a live culture system and the presence of live virus infection is then detected in this culture system. Since culture amplifies the amount of virus, it is more sensitive than direct methods, with the exception of RT-PCR. Virus isolation is only of use if the

live system or cells are sensitive for the virus that one intends to isolate. Isolation requires the rapid transport of specimens to the laboratory, since delays may lead to inactivation of virus (Allwinn, 2002).

2.3.1.5 Embryonated egg culture

Specimens are inoculated into the amniotic cavity of 9 -11 day embryonated chicken eggs. High yields of virus can be harvested after 3 days of incubation (WHO, 2005). Since this technique requires a supply of fertilized chicken eggs and special incubators, it is no longer used for the routine diagnosis of influenza infection. However egg isolation provides high quantities of virus and is a very sensitive culture system. Reference laboratories therefore utilise this culture system to ensure high sensitivity and to enable the production of virus stocks for epidemiological monitoring.

2.3.1.6 Cell culture

Conventional culture: Various cell-lines are used to isolate influenza viruses, most commonly primary monkey kidney cells and Madin-Darby canine kidney (MDCK) cells. Some authors recommend the use of trypsin to aid virus entry into the cell lines (WHO, 2005). Conventional cell culture takes up to two weeks but has a very high sensitivity. Cytopathic effects such as syncytia and intracytoplasmic basophilic inclusion bodies are observed. The presence of influenza virus can be ascertained using haemadsorption using guinea pig red blood cells (Weinberg, 2005),

or immunofluorescence on cultured cells. The latter can also be used to type the isolated virus. Immunofluorescence has a higher sensitivity in detection of positive cultures than haemadsorption.

2.3.1.7 Real-time PCR

The principle of real-time PCR is based on monitoring of a fluorescent signal, which arises during the amplification process. Real-time PCR eliminates post-PCR processing of PCR products. This helps to increase throughput and reduces the chances of carryover contamination. The real-time PCR system is based on the detection and quantification of a fluorescent reporter (Heid et al., 1996). Taq Man probes are one of the main fluorescence monitoring systems for DNA amplification.

2.3.1.8 Serology

Serology refers to the detection of influenza virus-specific antibodies in serum (or other body fluids). Serology can either detect total antibodies or be class-specific (IgG, IgA, or IgM). Different serological techniques are available for influenza diagnosis: haemagglutination inhibition (HI), enzyme immunoassays (EIA) and indirect immunofluorescence. Serological diagnosis has little value in diagnosing acute influenza. In order to diagnose acute infection, an at least four-fold rise in titre needs to be demonstrate, which necessitates both an acute and a convalescent specimen. However it may have value in diagnosing recently infected patients. Serology is also used to determine the response to influenza vaccination (Prince, 2003).

2.3.1.8.1 Haemagglutination inhibition (HI)

HI assays are labour intensive and time- consuming assays that require several controls for standardization. However the assay reagents are cheap and widely available. Various red blood cells such as guinea pig, fowl and human blood group "O" erythrocytes are used. An 0.4- 0.5% red blood cell dilution is generally used. Serum is pre-treated to remove non-specific haemagglutinins and inhibitors. A viral haemagglutinin preparation that produces visible haemagglutination (usually 4 haemagglutination units) is then pre-incubated with two-fold dilutions of the serum specimen. The lowest dilution of serum that inhibits haemagglutination is the HI titre. HI is has advantage that it is more specific in differentiating between HA subtypes (Julkunen, 1985).

2.3.1.8.2 Ezyme immuno assays (EIA)

EIAs are more sensitive than HI or CF assays (Bishai, 1978, Julkunen, 1985). Various commercial EIAs are available. but are not indicative of acute infection.

2.3.1.8.3 Indirect immunofluorescence

Indirect immunofluorescence is not commonly used as a method to detect influenza virus antibodies.

2.3.1.9 Rapid Tests

The clinical value of a diagnostic test for influenza is to a large extent dependent on the particular test's turnaround time. The first diagnostic tests that were developed for influenza diagnosis were virus isolation and serological assays. At that stage it took more than two weeks to exclude influenza infection. Although shell vial tests

have reduced the turn-around time of isolation, they are not generally regarded as rapid tests. The development of direct tests such as immunofluorescence enabled the diagnosis within a few hours (1 to 2 incubation and wash steps). Immunofluorescence tests however necessitate skilled laboratory workers and the availability of immunofluorescence microscopes.