CHAPTER II

LITERATURES REVIEW

Canine distemper disease is a highly contagious disease in dogs which resulted in a high morbility and high mortality rates. CDV infection causes multisystemic pathological lesions, mainly in respiratory, gastrointestinal and central nervous systems. CDV has worldwide distribution and wide host range; not only animals in family Canidae (dogs, wolves, coyotes) but animals in family Mustelidae (ferrets, minks), Viverridae (Binturong) and Procyonidae (raccoons) also are susceptible (Jones et al., 1997). Furthermore, CDV was the cause of death in Felidae (lions, leopards and tigers) in American zoos in 1990s, in lions, leopards and hyenas of Serengeti national park, Tanzania in 1994, and the death of seals in Lake Baikal in 1987-1988, and in Caspian seal in 2000 (Banyard et al., 2006).

CDV is susceptible to ultraviolet light, although proteins or antioxidants protect it from inactivation. It is extremely susceptible to heat and drying, CDV is destroyed by temperatures greater than 50°C to 60°C for 30 minutes. In excised tissues or secretions, it can be survive for at least an hour at 37°C and for 3 hours at 20°C (room temperature). In warm climates, CDV does not persist in kennels after infected dogs have been removed. Storage and survival times of CDV are longer at colder temperatures. At near-freezing (0°C to 4°C), it survives in the environment for weeks. Below the freezing temperature the virus is stable, surviving at -65°C for at least 7 years (Greene and Appel, 2005).

In natural infection, CDV spreads by aerosol droplets and contacts to upper respiratory of the dogs, beside that, dogs gregarious social behavior also the important way to be infected. Then virus replicates in respiratory epithelium and alveolar macrophages. After multiplying itself in tissue macrophages, it is still in these cells and spreads via local lymphatic ducts to tonsils and bronchial lymph node, 2-4 days later, the virus number highly increase in these lymphoid organs. Multiplication occurs again in lymphoid follicles of spleen, lymphoid tissues of lamina propria in the stomach and the small intestine, mesenteric lymph nodes and kuffer's cells in the liver. The virus

proliferated in lymphoid organs causes body temperature rises and leucopenia which is result from viral damage to lymphoid cells, both T and B cells. Eight to nine days after infection, viremia begins by cell-associated virus (mostly mononuclear cells) circulating through bloodstream to other organs, including most of epithelial tissues and central nervous system, in this phase virus can be found in bloodstream (Jones et al., 1997; Greene and Appel, 2005).

Common clinical signs of CDV variously show from the first to the fourth week after infection. After viremia, the oculonasal discharge, pharyngiitis and tonsilar enlargement become evident. Later, signs of depression, anorexia, coughing, vomiting and diarrhea are showed. Nervous signs including myoclonus, chewing movements, excessive salivation, incoordination, circling, nystagmus and convulsions are commonly seen in day 21 post infection. Therefore, clinical signs of infected dogs are influenced by the immunity. Dogs with adequate immune response can clear the virus from most tissues within 15-16 days after infection without obvious clinical signs. These are due to specific immunoglobulin (Ig) G-CDV antibody that effective in neutralizing extracellular and inhibiting intercellular spread of CDV. In dogs with low immune response, virus can spread to their epithelial tissues and show clinical signs. If antibody titer increases, viruses will be cleared from most tissues but may persist in uveal tissues, neurons and integument such as footpads. Dogs with inadequate immune response, virus can be spread in many tissues; skin, epithelial cells of the gastrointestinal, respiratory, genitourinary tracts and central nervous system. The dogs show severe clinical signs and virus usually persists in those tissues until the dog death. Although, the treatment is only supportive and nonspecific but they beneficially reduce mortality. The treatment is including broad spectrum antibiotic such as ampicillin, to control complication of respiratory infection by *Bordetella bronchiseptica*. Supplementary with polyionic isotonic fluids such as lactated Ringer's solution and vitamin A, B, C, E can be used for replace the lost from anorexia as a nonspecific treatment (Greene and Appel, 2005).

Pathological lesions are mainly found in respiratory system, lymphatic tissues, gastrointestinal tract and central nervous system. In upper respiratory tract, purulent or catarrhal exudates may be found over conjunctival, nasal and pharyngeal mucosa, purulent bronchopneumonia is clearly showed. In microscopic feature, eosinophilic

intracytoplasmic and intranuclear inclusion bodies can be found in upper respiratory epithelium. Lung lesions show purulent bronchopneumonia, bronchi and alveoli are filled with mononuclear cells, multinucleated giant cells, neutrophils, mucin and tissue debris, or diffuse interstitial pneumonia with thickening of alveolar septa and proliferation of alveolar epithelium, inclusion bodies also can be found in these cells and in respiratory epithelium. The inclusion bodies can be found in urinary tract epithelium, stomach and intestine's epithelium and probably in the liver. Gross lesion of gastrointestinal is catarrhal enteritis. In lymphoid organs; lymph nodes and spleen show necrosis area and depletion. The necrosis of both T and B cells and inclusion bodies are present. In central nervous system, the gross lesions including; meningeal congestion, ventricular dilation, and brain edema are occasionally found. Microscopically, destruction of neurons, demyelination and encephalomyelitis or meningoencephalitis is commonly found. The inclusion bodies can be found in microglias and neurons, while, perivascular cuffing is often found in chronic infection. In additional, acute encephalitis occurs in early infection of young or immunosuppressed animals. The non - inflammatory demyelination associates with the infection of microglial and astroglial cells rather than oligodendroglial cells, the myelin – production cells. However, functions of oligodendroglial cells were decreased. The demyelination is occurred by bystander effect; the infected macrophages in brain lesions are activated and release reactive oxygen radicals. This activity can lead to destruction of oligodendrocytes and myelin (Jones et al., 1997; Greene and Appel, 2005).

The viruses are most concentrated in respiratory exudates; however, it can be present in all tissue secretion including urine. Virus can be excreted up to 60 to 90 days after infection, although shorter periods of shedding are more typical. Prevalence rate of spontaneous distemper is highest between 3 and 6 months of age, correlating with the loss of maternal antibodies in puppies after weaning. Furthermore, the minor genetic variation of CDV isolates are serological homogeneous. However, strains differ in their pathogenicity which may affect the severity and extent or type of clinical disease. Certain isolates, such as Snyder Hill, A75/17 and R252 strains, are highly virulent and neurotropic. In additional, the target of antibody response in dog has been separated into envelope and core determinants of the virus. Only dogs producing anti-envelope

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antibodies appear to be able to prevent persistent viral infection of the central nervous system (Greene and Appel, 2005).

In necropsy cases, CDV can be diagnosed by presence of eosinophilic intracytoplasmic and intranuclear viral inclusion bodies in infected cells using routine hematoxylin and eosin staining (H&E). By immunohistochemistry staining, CDV antigens can be observed within tissue mononuclear cells and epithelial cells of infected tissues in the sections of brain, lung, mucosal epithelium and other susceptible tissues (Greene and Appel, 2005). In clinical cases, CDV can be diagnosed from the unique neurological signs that mentioned above, but some dogs showed only anorexia, depression, diarrhea and oculonasal discharge which was difficult to diagnose from other infectious diseases. Complete blood count and serology cannot show an obvious abnormality but inclusion bodies may found in epithelium or peripheral blood smear in early infection phase (Greene and Appel, 2005). Hence, tools for diagnosis clinical cases of CDV have been developed for several years. Several techniques such as Reverse transcriptase-polymerase chain reaction (RT-PCR) was developed and modified for its sensitivity, specificity and rapidity that able to detect small amounts of virus. The method appears to be efficient to detect the virus from clinical specimen, such as clinical swab and urine (Elia et al., 2006). The limitation of RT-PCR is due to small amount of virus, virus titer should be at least $4x10^3$ $TCID_{50}$ and the specimens should be collected from correlated lesions within virus target organs (Tiwananthakorn et al., 2000).

However, the question is not end with what the cause of illness or death is, but also why the vaccinated dogs are still infected and died? These are resulted in the invention of more specific method for identifying the virus.

CDV is a member of genus Morbillivirus in family Paramyxoviridae, CDV closely related viruses are Measles virus (MV) which cause an important disease in human, Cetacean morbilliviruses (including porpoise morbillivirus (PMV) in seals and dolphin morbillivirus (DMV) in marine mammals), phocine distemper virus (PCV) in seals, Peste des petits ruminants virus and Rinderpest in cows (Lamb and Kolakofsky, 1996; Banyard et al., 2006).

Morbillivirus is an enveloped, negative-sense, single stranded RNA virus, composed of 6 protein genes. The helical nucleocapsid cores consists of 3 proteins; nucleocapsid protein (N), phosphoprotein (P) and large (L) protein, follow by 2 envelope proteins; hemagglutinin glycoprotein (H) and fusion protein (F) genes and the last is matrix protein (M) gene, places between envelope and nucleocapsid cores (Figure1). The N protein is the most abundant and the first transcript from the genome, it encapsidates the viral RNA and serves as a template for transcription and replication. The L and P proteins are transcriptase-associated proteins; L protein is a RNA-dependent RNA polymerase (RdRp) which is assumed to carry all the activities necessary for genomic RNA transcription and replication. P protein is more abundant than L protein; its duty is to binds with N and L proteins, and acts as a co-factor for the replication. M protein serves as a bridge between the external surface viral proteins and internal nucleocapsid and plays an important role in the formation of new virus particles. In the other hand, M has an ability to bind with nucleocapsid and inhibit RNA transcription, which is often found in persistent infection. F protein is a highly conserve region, it enables the virus to penetrate the cell by mediating the fusion of the viral and cellular membranes. While, H protein is the most variable part of CDV, enables the virus to bind to the cell receptor (Griffin and Bellini, 1996; Barrett et al., 2006). The first process of infection begins with H protein binds with host cell receptor and F protein mediates cell entry by inducing fusion between viral envelope and the cell membrane (Von Messling et al., 2001). As a result, it implies that H is the main target of host's humoral immune response. H protein also plays important roles in fusogenetic activity, growth characteristics and growth tropism (Griffin and Bellini, 1996).

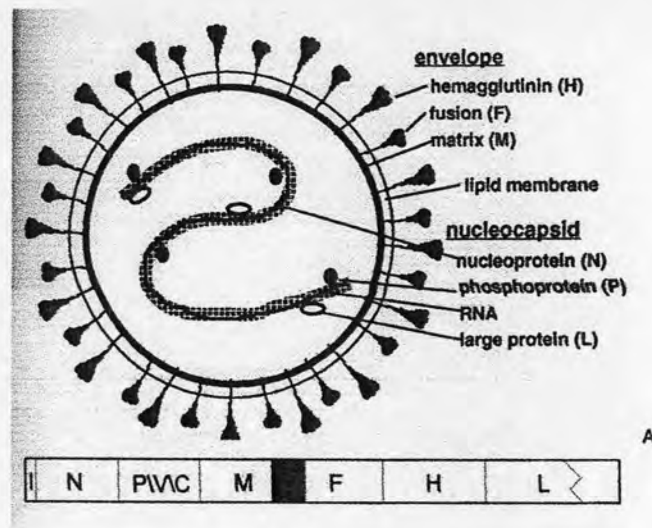

Figure 1: Morbillivirus structure (Griffin and Bellini, 1996).

The techniques that use in the study of CDV are mostly modified from measles virus (MV). One of that is virus isolation in a particular cell line. After CDV infected into the cells, it produces syncytial formation which is an important cytopathogenic effect (CPE) of Paramyxovirus. The syncytial formation results from fusion of infected cells with uninfected cells and may produce syncytial with 50 or more nuclei bounded by a single cytoplasmic membrane. Another form of CPE is the infected cells change from a normal polygonal shape to a stellate, dendritic or spindle shape appearance with increased refractivity to light (Griffin and Bellini, 1996). CDV commonly inoculated in African green monkey kidney epithelial cells or "Vero cell" (Greene and Appel, 2005). There was a research which compared the inoculation of CDV in Vero, MDCK (canine kidney epithelial cell), MV1LU (Mink lung) cells. The result showed CDV can be detected earliest in MDCK, about 1 month post-inoculation (p.i.), about 40 days p.i. in Vero cell, and 45 days p.i. in MV1LU cell. None of the viruses caused an obvious CPE in MV1LU, one of viruses caused CPE in MDCK and all of the viruses caused CPE in vero cell, but shape and size of syncytial formation were varied (Lednicky et al., 2004). Mammoset B lymphocyte cell line (B95a) was also developed for inoculating of MV and CDV. In CDV, B95a show CPE in 4-6 days (Kai et al., 1993). Later, signaling lymphocyte activation molecule (SLAM, also called CD150) was found to be an efficient cellular receptor.

SLAM is a member of immunoglobulin superfamily which is signaling together with T cell receptor engagement, regulates the production of interleukin (IL)-4 and IL-13. SLAM is expressed on the cells of immune system, such as, immature thymocytes, memory T cells, a portion of B cells and mature dendritic cells (Yanagi et al., 2006). Other researcher compared the inoculation of CDV on B95a, Vero cell and Vero expressing dog SLAM tag (Vero-DST) cell. The result showed that CPE found in Vero-DST within 24 hour after inoculation and lesser samples caused CPE in B95q cell within 7 days and none of the sample showed CPE in Vero cell within 21 days p.i. (Seki et al., 2003). Nowadays, Vero-DST is the most effective cell line for inoculating of CDV because its early detected of CPE and its stability. The Vero-DST stability resulted from the P, L and M gene regions of 4 strains virus showed no nucleotide sequences differences between original viruses and viruses after 20 passages in Vero-DST cell, and only 2 non specific amino acids of H gene were different between both viruses. These made Vero-DST cell a suitable cell line for isolation, inoculation, titration and further biological research (Lan et al., 2006[b]).

Phylogenetic analysis is a classification system for separating of the organisms into groups; the classification mostly depends on different percentages of the nucleotide or amino acid sequences. CDV H protein was the most frequently used for phylogenetic analyses, due to H gene showed its highest antigenic variation, whereas F and P genes were affected much lower extent (Bolt et al., 1997). Lately, CDV had been briefly divided in to 6 lineages by H gene (Matella et al., 2007). The first lineage is the America-1 or vaccine lineage which all of the available vaccines were grouped in. The second is America-2 lineage, isolated from infected dogs and zoo animals including raccoon, the A75/17 strain which is usually used in the experiment was also grouped in this lineage. The third is Europe lineage which is contains 2 minor lineages; Europe and Europe wildlife. The Europe lineage was isolated from dogs in European countries, including Denmark, Germen and Italy, whereas, the Europe wildlife had been isolated from minks and ferrets (Martella et al., 2006). The forth is Asia-1, which contains the virus from China, Japan and Taiwan. The fifth is Asia-2 lineage, viruses in this lineage are only found in Japan. The last lineage is Arctic lineage, viruses in this lineage was isolated from dogs in Greenland and seals in Siberia (Matella et al., 2007). In addition,

there is another lineage, which was not often used to compare with the other new isolated strain, the Africa lineage. This lineage was isolated from domestic dogs, bat-eared foxes, lions and hyenas in the area of Serengetti national park, Tanzania (Carpenter et al., 1998). Therefore, the distribution of CDV is not depending on its geographic origin (Bolt et al., 1997). However, all the presence lineages cannot divide into different genotypes because the percentage of homologous was at least 90% in H gene and 94% in P gene.

The P gene region is selected next to H gene in the phylogenetic analyses. Some researches analyzed both H and P genes, and used P gene as a control, due to it's highly conserved character. P gene can be divided into; Vaccines, North America, Europe, Asia-1 and Asia-2 lineages (Lan et al., 2006[a]). Africa isolated also had been analyzed in this region (Carpenter et al., 1998). However, the nucleotide sequences of P gene in the Genbank database was not as much as H gene, that made it can not be used for comparing as wide as H gene.

Examples of previous phylogenetic analyses were included the following researches. In Japan, new isolated strains from infected dogs, showed at least two genotypes, Asia1 and Asia2, circulating among dogs in Japan (Mochizuki et al., 1999; Lan et al., 2005). In Europe, there are at least three different CDV lineages present, the first one isolated from infected dogs which was the main Europe lineage. Second, the strains that were isolated from European wildlife such as foxes, minks and ferrets and the third also isolated from dogs but clustered along with CDVs of the Arctic lineage (Martella et al., 2006). The recently isolated strain in North America also showed that new CDV stains were genetically distinct from viruses previously detected in the continental of United States and most closely related with strains from Asia or possibly Europe (Prado et al., 2005).

In Thailand, 13 strains of CDV were revealed by used N protein and concluded that CDV Thai isolates could be divided in two groups, one was closely related to vaccine strain (Onderstepoort strain) and another group was extensive homology to various virulent reference strains (Keawcharoen et al., 2005). However, some isolates were distantly homologous to the vaccine strain, which was suggested that the vaccine strains have apparently disappeared in last five decades. The strains that related to the

Ondersterpoort strain were analyzed by N gene and reported in Thailand and Poland (Matella et al., 2006).

Nowadays, there are 5 available attenuated live vaccines. The most common used in phylogenetic comparisons is Onderstepoort strain, which isolated from the 1930s outbreak in red ranch foxes in North America, and attenuated in ferrets and embryonic eggs. The second is Snyder Hill strain, isolated from dog's brain in North America since 1950s. The third is Rockbom strain, isolated from mink that infected CDV and grown in canine kidney cells. The forth is Convac strain. The last is Lederle which is only used in other susceptible animals, such as ferret (Greene and Appel, 2005; Martella et al., 2006).

In addition, the prevention of CDV is still possible. Firstly, puppies received the maternal antibody in utero and via colostrum. The maternal antibody decrease with a half-life of 8.4 days. Maternal antibody for CDV transfers in utero only 3 % and in colostrum 97%. A puppy which has not had colostrum is probably protected for at least 1 to 4 weeks. Immunity to natural CDV infection or booster vaccination is considered long term. This protection may be adequate unless the dog is exposed to a highly virulent strain or large quantities of viruses or become stressed or immunocompromised. In comparison with modified live-virus vaccine, inactivated canine distemper whole-virus vaccines do not produce sufficient immunity to prevent infection after a challenge exposure, but vaccinated dogs show less severe disease than unvaccinated controls. Therefore, modified live vaccines (MLV) have superior protection against CDV. MLV viruses have not reversed to virulence under natural conditions and do not spread to other dogs. However, different virus strains produce different levels of protection, increased potency of protection means higher vaccine virulence. Unfortunately, the most potent vaccines have been associated with induced illness, especially in certain wild or immunocompromised domestic carnivores. For example, the onderstepoort strain may produce lower measured levels of humoral immunity but no postvaccinal complication. On the other hand, the Rockbom strain induces high titer of neutralizing antibody and longer term protection but occasionally produces postvaccinal encephalitis in dogs and more commonly in exotic carnivores (Greene and Appel, 2005). Therefore, the explanations of the disease in vaccinated dogs are including

vaccine failure, reversion of attenuated CDV vaccine strains to virulence, or the emergence of new strains that are sufficiently divergent to evade immune protection elicited by vaccine used (Prado et al., 2005).