THE ROLE OF MOLECULAR APPROACH IN FOOT AND MOUTH DISEASE ERADICATION PROGRAM

(Peranan Pendekatan Molekular dalam Program Eradikasi Penyakit Mulut dan Kuku)

Maria Aega Gelolodo

Bagian Penyakit Hewan dan Kesehatan Masyarakat Veteriner, Fakultas Kedokteran Hewan, Universitas Nusa Cendana, Kupang, Email: *gelolodo.m@staf.undana.ac.id*

ABSTRAK

Penyakit Mulut dan Kuku (PMK) adalah salah satu penyakit penting yang menginfeksi hewan sapi, kambing, domba dan babi serta beberapa jenis hewan liar. Penyakit ini penting secara ekonomi karena selain mengakibatkan angka mortalitas yang tinggi pada hewan muda, penurunan produksi susu maupun bahan asal hewan lainnya serta dapat mengakibatkan pembatasan perdagangan internasional bagi negara yang terinfeksi PMK. Selain dampak langsung dari penurunan produksi peternkan dan pembatasan perdagangan internasional, wabah PMK juga memberikan dampak yang serius bagi aspek sosial ekonomi dan industri pariwisata. Sampai saat ini, penyakit ini menyebar luas di Amerika selatan, Asia dan Africa. Mengingat arti pentingnya penyakit ini dan dampaknya secara global, maka penting untuk menyusun langkah strategis pencegahan dan eradikasi penyakit ini. Tulisan ini akan membahas beberapa langkah strategis penting yang dapat dimplementasikan dalam program eradikasi PMK khususnya melalui kegiatan-kegiatan yang berbasis teknologi molecular mulai dari penyiapan vaksin, tes diagnostic sampai kegiatan monitoring status penyakit.

Kata kunci: PMK, molecular, eradication, DIVA

INTRODUCTION

Foot and mouth disease (FMD) is a severe vesicular infection that mainly infected cloven-hoofed animals, several domesticated ruminants, swine and large number of wildlife animal (Alexandersen *et al.*, 2003b; Jamal and Belsham, 2013). FMD known for its abilities to

infect the healthy animal in minimal doses with a rapid replication and a high level of viral excretion (Alexandersen *et al.*, 2003a). This unique characteristic has placed FMD as one of the important infectious disease in the world.

FMD endemic in many countries of Asia, Africa, South America and Europe and has shown an impressive ability to pass international boundaries. Though, it once eradicated from Europe during the 1960—1980, the severe epidemic in the UK in 2001 has showed that this disease can be re-introduced into free countries that have been free for more than a decade (Brehm *et al.*, 2008). During it epidemic in UK, FMD has caused a huge economic loses at around £2.75 billion. Furthermore, other indirect effects in the agricultural and tourism sectors are still difficult to measures (Alexandersen *et al.*, 2003b).

FMD is characterised by the formation of vesicles and erosions in the cutaneous mucosae and hairless area of the skin such as mouth and the hoofs. In endemic countries, FMD causes the losses of young animal and the decline of adult animal productivities (Brehm et al., 2008). Although, FMD cause a low rate of mortality, this infection is one of high cost disease that difficult to control and eradicated (Alexandersen *et al.*, 2003b).

Regarding those devastating effects of FMD, it is urgently need a tools and strategies that capable to early recognise the infection, prevent the outbreaks of the disease and eradicate the disease. The development of a molecular-based techniques and strategies for rapid identification and characterization of

FMD play the vital roles in control and eradication programs (Le et al., 2012). Thereby, the mixture of molecular biology, epidemiology and microbiology in molecular epidemiology of infectious diseases are a powerful tool to improve and enhance FMD control strategies. Due to its reasons, this literature review will present several molecular approach applications and its role in FMD eradication program.

DESCRIPTION OF THE DISEASE

a. The virus

FMD is caused by foot and mouth disease virus (FMDV), a small single-stranded and positive-sense RNA virus (Abdul-Hamid *et al.*, 2011). This virus is a non-enveloped virus with an icosahedral structure which belongs to genus *Aphthovirus* and *Picornaviridae* family (Alexandersen *et al.*, 2003b). The RNA consists of three parts, the 5′ untranslated region (5′ UTR), a long coding region and the 3′ untranslated region (3′ UTR)(Jamal and Belsham, 2013). The viral RNA has been translated into a polyprotein during the replication in the cytoplasmic and causing the formation of 12 structural and non-structural proteins (Alexandersen *et al.*, 2003b). The RNA of the virus is surrounded by a protein capsid that consists of 60 copies of the capsomers (Jamal and Belsham, 2013). Each of the capsomer is composed by four structural protein, VP1, VP2, VP3 and VP4 (Klein, 2009). The VP1, VP2 and VP3 are located at the surface of the virus and associated with the antigenic factor of the virus while VP4 is located in the internal part of the virus (Jamal and Belsham, 2013). Among these four structural polypeptides, VP1 has

been recognised for its important role in virus attachment, protective immunity, and serotype specificity (Alexandersen *et al.*, 2003b; Ma *et al.*, 2011). The VP1 consists of two vital immunogenic sites which is the G-H loop (at amino acid positions 141–160) and the C-terminus (residues 200–213). The G-H loop contains of an arginine-glycine-aspartic acid (RGD) motif that important in viral attachment into the host cell via an integrin receptor (Jamal and Belsham, 2013). The attachment of a region in the G-H loop of the VP1 protein on the surface of the viral capsid to the surface of host cells is considered as the primary initiation of the virus infection (Alexandersen *et al.*, 2003b; Klein, 2009). Due to the vital role of VP1 in virus attachment, the nucleotide sequences of the VP1 coding region have been used for recognising the characterisation of FMDV strains. The phylogenetic analyses based on VP1 sequencing have been used also to identify the epidemiological relationships among FMDV genetic lineages and in the tracing of the original strains and movement of outbreak cases (Jamal and Belsham, 2013).

FMDV has a wide range of antigenic variable that can be grouped into seven serotypes such as Southern African Territories (SAT) 1, SAT 2, SAT 3, O, A, C and Asia 1 (Abdul-Hamid *et al.*, 2011). The phylogenetic studies of the VP1 gene sequence of FMDV show that there are at least 10 genotypes of serotype A, 10 topotypes of serotype O such as Europe-South America (Euro-SA), Middle East-South Asia (ME-SA), Southeast Asia (SEA), Cathay (CHY), West Africa (WA), East Africa 1 (EA-1), East Africa 2 (EA-

2), East Africa 3 (EA-3), Indonesia-1 (ISA-1), and Indonesia-2 (ISA-2) and 6 genotypes of serotype Asia 1 (Le et al., 2012).

b. Hosts

As a disease with a wide range of hosts, FMD can infect various different animals, such as cattle, swine, sheep, goats, buffaloes and 70 wild ruminants (Alexandersen and Mowat, 2005).

c. Transmissions

Typically, FMDVs spread through direct contact with infected animals such as through aerosolised droplets, saliva or fomites and the movement of infected animals (Alexandersen and Mowat, 2005). Transmission through the contaminated food and other indirect transmission such us human movements, contaminated farming tools, transportation vehicles, winds or wild animals and birds are the other alternatives in FMDV transmission pathways (Alexandersen *et al.*, 2003b; Alexandersen and Mowat, 2005).

d. Epidemiology

Jamal and Belsham (2013) report that approximately 100 countries have been infected by this disease. In general, it can be seen that the spreading of seven FMD serotypes are not uniformly. For instance, in Africa there are five FMD serotypes that have been spread around the continent, like O, A, SAT-1, SAT-2 and SAT-3, in Asia there are three FMD serotypes O, A and Asia-1 and in South America there are two serotypes which are O and A serotype (Rweyemamu *et al.*, 2008; Jamal and Belsham, 2013). However, Abdul-Hamid *et al.* (2011) reports that in Middle East there is invasions of SAT-1 and SAT-2 from Africa, periodically.

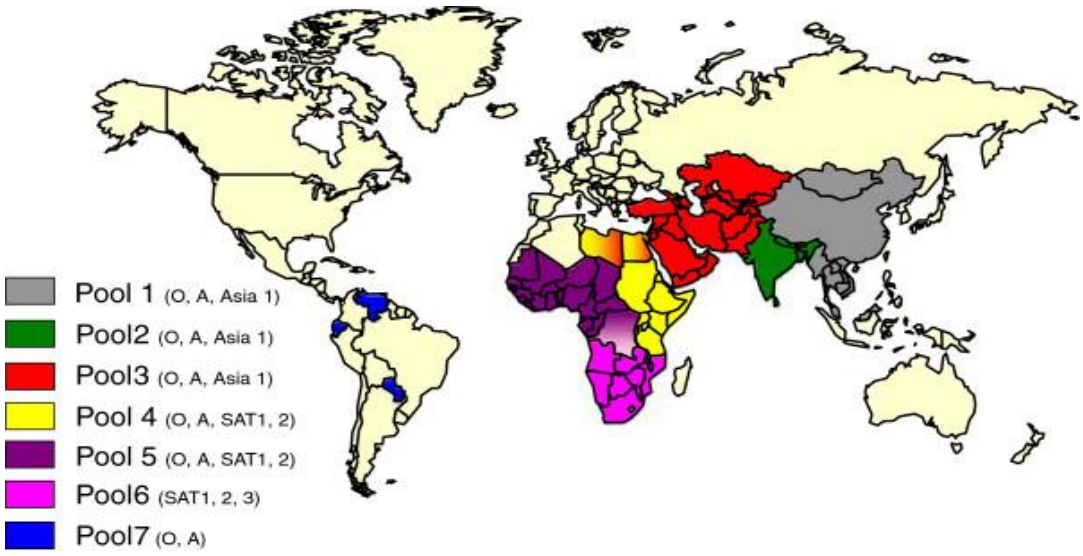

Figure 1. Geographical distribution of FMDV serotypes (Jamal and Belsham, 2013)

The FMD geographical map has shown that serotype O and A have widest range of distribution and have been proved as the major causes of FMD outbreaks in Europe, America, Asia and Africa. While serotype C that was infected Ethiopia in 2005, nowadays no longer exist at outside of the laboratory environments (Abdul-Hamid *et al.*, 2011).

e. Pathogenesis

The incubation period of FMD is very variable and rely on the host species, transmission pathways, serotype and its dose and the condition of the farming environment (Alexandersen *et al.*, 2003b).

The pharyngeal area especially the epithelial cells on the dorsal soft palate, the roof of the pharynx and the tonsil are the primary site of

FMD primary infection. In these areas, the virus can be survived for 1 until 3 days before the viraemia can be recognised. After 2-3 days of viral replication in the epithelium of the primary sites, the virus will invade the local lymph nodes before they enter the blood circulation and caused the viraemia. Viraemia usually happens for 4–5 days and through this circulation, the virus travel around the body and invade the targeted cells.

During the infections, all the body secretions and excretions become infectious and can produce significant doses of virus. Eventually, saliva, nasal droplet and fluid, lachrymal fluid and milk and expired breath serve as infectious substrates that can spreading the virus and infected other susceptible animals. This process usually happens before the infected animal shows the clinical symptoms (Alexandersen *et al.*, 2003b). Thereby, the spreading of the virus during this period become the critical phase in FMD transmission.

f. Clinical symptoms and lesions

Typically, the prominent characteristics of FMD are an acute febrile response and the formation of vesicles in the mouth and feet areas. The behavioural symptoms like lameness, a tucked up stance and reluctance to stand or moving around and Inappetence can be the early signals of FMD infections (Alexandersen et al., 2003b). Alexandersen *et al.* (2003b) also reported that in 1-2 days before the presence of vesicular lesions, the general symptoms like fever and pain may be detected from the animals. Afterwards, the vesicles can be seen on the snout or muzzle, teats,

mammary gland, prepuce, vulva and other sites of the skin, especially in the area around the mouth and the feet**.** At the end, the lesions on the ruminal pillars can be found in the post-mortem examination.

The FMD infection in Bovine is characterized by the increase of the body temperature until 40.8°C, hypersalivation, lameness, depression and the decreasing of milk production. The most severe lesions can be observed in the mucosa of the lips, dorsum of the tongue, and the dental plate. Myocardial necrosis mostly happens in young animal and cause a low number of mortality rate (Kitching, 2002; Alexandersen et al., 2003a; Gulbahar et al., 2007).

In sheep and goats, the clinical signs are less severe than in the bovine. Mild lesions such as the vesicle formations rarely observed in the mouth of sheep and goats. The signs commonly superficial and transient and heal rapidly (Alexandersen et al., 2003). However, lameness through aphthae and inflammation at the cloves are two clinical manifestation that also can be seen from the infection (Alexandersen and Mowat, 2005).

In contrast with the mild symptoms in sheep and goats, swine usually shows more severe clinical signs which mostly affect the feet region like, formation of vesicles in the epidermis of the feet (coronary band, interdigital clefts, and bulbs) and the oral region. Moreover, clinical signs like acute lameness, reluctance to stand, a dog-sitting posture, depression, loss of appetite, hypersalivation and fever also can be observed in the early-middle phase of the disease (Alexandersen *et al.*, 2003b). The hoof separation and

secondary infection on disrupted aphthae (fluid-filled blisters) which causes purulent arthritis of the pedal joint also doubled the complication of FMD infection in swine (Kitching and Alexandersen, 2002).

MOLECULAR APPROACH

a. Diagnostic techniques

Regarding the rapid spreading of FMDV and the damaging effects on the economic sector caused by this disease. The sensitive and specific laboratory diagnostic test has been urgently needed in order to early recognise the original serotype of FMDV. Since the diagnosed based on clinical signs like high temperature, excessive salivation, formation of vesicles on the oral mucosa, on the nose plus the inter-digital spaces and coronary bands on the feet can be confused with other diseases, it is important to diagnose the disease based on laboratory examinations.

For a long period of time, the virus neutralization test (VNT) has considered as the "gold standard" FMDV identification (OIE, 2012). However, this test is slower, and requires restrictive biocontainment facilities.

Recently, the reverse transcription-polymerase chain reaction (RT-PCR) assays have been developed as the diagnostic test of FMDV infection (Alexandersen et al., 2003b). As a nucleic acid recognition test, RT-PCR able to amplify genome fragments of FMDV samples such as epithelium, milk, serum and oropharynx materials (Reid *et al.*, 2003). Compare with other test**,** such as antigen-detection which faster but have lower sensitivity, RT-PCR has been proven as a faster, reliable, and sensitive technique for the rapid and sensitive identification of FMDV (Le

et al., 2012). A different RT-PCR assays have been developed to early recognise RNA of FMDV in epithelium, cell culture isolates and other tissues using universal primers for all seven serotypes of FMDV (Jamal and Belsham, 2013).

Several specific serotype primers have been formed to identify of all seven serotypes of FMDV by RT-PCR assay (Jamal and Belsham, 2013). Generally, primers that have designed for these tests target various regions of the FMDV genome like the 5′ UTR, the open reading frame and the 3′ UTR. Nonetheless, the evaluation for universal and serotype-specific diagnosis of FMDV on a wide range of field samples that representing all the seven FMDV serotypes have reported that there are no single primer sets are capable to identify the disease or typing of the virus. Regarding these reasons, multiplex assays which are incorporating more than one set of primers have been developed in order to gain better sensitivity of the test (Giridharan *et al.*, 2005; Bao *et al.*, 2008). However, this conventional RT-PCR still could only serotyping particular groups of serotypes or individual isolates (Jamal and Belsham, 2013).

Currently, real time RT-PCR (rRT-PCR) assay have been developed as the high throughput test that capable to quantify the genetic material of FMD starting sample. Two types of rRT-PCR TaqMan assays that commonly use are one targeting the internal ribosomal entry site (IRES) within the 5′ UTR and the other that targeting the 3D (RNA polymerase) coding sequence (Reid *et al.*, 2002).

Although, rRT-PCR assays are commonly used as a routine test for

FMD identification and quantification in many developed countries, these tests are still cannot differentiate the variety serotypes of FMDV**.** Moreover, the assays also unable to recognise a small number of FMDV isolates. As a result, it can be concluded that there is no single test that has an ability to detect FMDV with highly sensitivity degree.

b. Molecular epidemiology

FMD molecular epidemiology is based on the genetic differences among the FMD virus. The differentiation and comparison of whole viral genome sequencing have been used as the basic principle to distinguish the FMD virus that closely related. The genomic comparison of FMDV is the main product of RT-PCR amplification and nucleotide sequencing. Dendrograms by Knowles and Samuel (2003) that have shown the genomic relationship between FMDV field strain and the vaccine products based on the 1D gene sequencing is an example of molecular epidemiology application. Furthermore, molecular epidemiology also helps to identify the transmission routes of the FMD outbreaks. To perform this study, OIE (2012) has been suggested 3 methods based on VP1 analysis, first, the extraction of RNA directly from epithelial suspensions or from a low cell culture passage, second, performing an RT-PCR of the complete 1D gene and third, define the nucleotide sequence of the PCR product at the 3’ end of the gene (OIE, 2012).

c. Vaccine selections and matching

Nowadays, there are many FMD-free countries have used free-vaccination strategic to declare their FMD-freedom status. Those countries

prefer to use the slaughter strategy, movement regulations and zoo-sanitary measures as a FMD control strategies. Their just apply vaccination in certain situations such as in a outbreaks cases (Barnett and Carabin, 2002). However, a mass routine vaccination has still applied in several countries or zones that have been recognised as FMD-free and endemic countries.

Recently, FMD vaccines are produced by growing FMD live virus in BHK-21 cells. Afterwards, the growing infected cells are harvested, concentrated and inactivated with binary ethyleneimine, eliminated the cellular debris and mixed for use with a buffer and adjuvant or with oil either aluminium hydroxide and saponin (Clavijo *et al.*, 2004; Kitching *et al.*, 2007). The protection of these vaccines is mainly produced by antibodies against FMDV structural proteins (SPs). The high response of the antibodies are the indicator of the high protection vaccines (Doel, 2003).

Based on basic selection of FMD vaccine, Paton *et al.* (2005) state that there are two important factors that should be afforded by a good vaccine which are how strong it can induce a strong immunity response (potency) and how closely related its serotype to the field serotype (antigenic match).

FMD vaccines can be classified in two types of potency which are ‘standard’ and ‘higher’ potency vaccines. Standard potency vaccines are vaccines that contain of minimum potency required with sufficient antigen and appropriate adjuvant. This vaccine usually uses in routine vaccination programs. While, higher potency vaccines commonly use in naïve populations along FMD

outbreaks. This type of vaccine capable to induce a rapid onset of protection (OIE, 2012).

The major problems of FMD vaccination are its antigenic match and the cross-protection problem. As it mentions before, FMD has seven antigenically distinct serotypes which each serotype has a different variation of intratypic variants. This antigenic variation of FMD brings a major problem in FMD control strategies, as an vaccination with one FMD serotype cannot protect the animals from different serotypes and even the vaccination may not fully protect the animals from other subtypes within the same serotype (Parida, 2009). Thereby, in some areas, it is suggested to vaccinated the animals with more than one FMD strain per serotype in order to ensure broad antigenic coverage against prevailing viruses (OIE, 2012).

Regarding the antigenic diversity of FMDV, the selection of FMDV strain plays a crucial role in in vaccine production (Kitching, 2005). The choice of the most suitable FMDV strain for the vaccine products become the vital part in vaccine production. The matching strain between field strain and vaccine strain can be confirmed by epidemiology molecular studies, for instance by the collection from different stages of an outbreak, different geographical areas, or from different hosts. Moreover, the field evidence of a suspected lack of vaccine potency, also can be used as a consideration in FMDV strain selection. Several matching tests like ELISA and VNT also can be done to ensure the matching strain (OIE, 2012).

Another issue associate with the FMD vaccination is the fact that FMD

vaccines unstable outside the range of 2–8°C (Kitching et al., 2007) also bring a challenge to FMD vaccination program in tropical areas. Eventually, the combination of these vaccination problems causes an ineffective vaccination program in mostly endemic areas which located at tropical regions.

d. Molecular approach to differentiate between vaccinated and convalescent animals

For many years, vaccination is widely used to control the incident of the disease. Vaccination has considered as the most effective protocol to tackle FMD cases. However, some cases show that the vaccination program can be an obstacle in the FMD eradication program since it become a difficult to differentiate vaccinated animals and infected animals (Ma et al., 2011). Moreover, several studies also indicate that the vaccinated animals that were exposed by the FMDV can serve as FMDV carrier animals and spread the virus to the environment (Sariya et al., 2011; Sharma et al., 2012).

In the FMD control program, it is important to recognise and differentiate the infected animals and the vaccinated animals because both groups have the neutralizing antibodies in their serum (Jamal and Belsham, 2013). Thereby, it is urgently need the diagnostic test that can distinguish between infected and vaccinated animals**.**

Nowadays, the antibodies to non-structural protein (NSP) of FMDV has been used by the scientist to develop diagnostic tests that able to differentiate the infected and vaccinated animals (Sariya *et al.*, 2011; Sharma *et al.*, 2012). This

principle are based on fact that along the FMD natural infection, the viral replications can produce both immunogenic proteins which are structural (SP) and non-structural (NSP) proteins (Jamal and Belsham, 2013). On the contrary, vaccines just consist of purified preparations of inactivated 146S virions that exclusively able to induce antibodies to structural protein (SP) (Jamal and Belsham, 2013). Thereby, it can be possible to distinguish the infected and vaccinated animals based on the presence of antibodies to NSPs.

Previously, the radioimmunoprecipitation and enzyme linked immunoelectrotransfer blot assays had been used as the detection of anti-NSP antibodies. However, those assays are not effective in outbreak cases which have a large number of serum samples, moreover both test could not be done as rapid examinations (Jamal and Belsham, 2013). Regarding these reasons, presently, the scientists are using Differentiation of Infected from Vaccinated Animals (DIVA) as the main test to distinguish the infected and vaccinated animals.

Recently, an important effort has been constructed to develop tests that can differentiate infected and vaccinated animals based on the varieties of NSPs (3ABC, 3AB, 3A, 3B, 2A, 2B and 2C) (Uttenthal *et al.*, 2010). At this moment, tests based on the presence of antibodies for the polyprotein 3ABC have been considered as the most important tests to identify the FMD infection in vaccinated populations (Uttenthal *et al.*, 2010). The OIE has standardised the test system that mixes the 3ABC indirect ELISA (Panaftosa) for screening and an immunoblot test for antibodies against the 3A, 3B, 2C, 3D

and 3ABC NSPs as the confirmatory tests (Jamal and Belsham, 2013). Currently, several researches has been conducted to develop multiplex ELISA using different NSPs and peptides in order to enhance the sensitivity and specificity of FMD DIVA tests (Dundon *et al.*, 2010).

In addition, Dundon *et al.* (2010) also assert that there are two alternative principles of DIVA tests beside the DIVA based on the NSPs antibody detection, namely, DIVA tests based on mucosal antibody detection and DIVA based on cell-mediated immune responses. DIVA tests based on mucosal antibody detection is based on the presence of mucosal IgA antibody. A fact that the FMD vaccine has a minimum effect on mucosal IgA antibody while in cattle with persisting oropharyngeal FMDV infection, a salivary IgA antibody has reached the highest level of the antibody has been used as alternative DIVA test to identify carrier animals of FMDV (Parida *et al.*, 2006a; Parida, 2009). This DIVA test also has been carried out to detect the different species of FMDV carrier animals. Moreover, the test also has the ability to recognise the low-level contamination of NSPs in vaccine productions (Parida *et al.*, 2006a). Moreover, DIVA test based on cell-mediated immune responses has been used as a diagnostic test of FMD and as test to measure the post-vaccination protection (Dundon *et al.*, 2010). The test is based on the level of IFN-gamma that usually emerge after the vaccination. This test also can be used to confirm the infection in vaccinated populations (Parida *et al.*, 2006b). Nonetheless, this test should be verified with other FMD vaccine serotypes due to its effects to initiate cell-mediated immune

responses along the vaccination period which can be misinterpreted with FMD infection in the vaccinated animals (Dundon *et al.*, 2010).

e. Roles of DIVA in FMD eradication program

Generally, the main purpose of vaccination program is to prevent and diminish the clinical manifestations of the infectious diseases. The vaccination also has been used as control management in eradication program of certain diseases in some particular areas. In the viral vaccination program, vaccination has an ability to trigger the immune system of the hosts. However, several studies prove that sometimes, the vaccination cannot serve a fully protection to the hosts and in some cases vaccinated animals can act as carriers of the disease that able to spread the virus into the environment (Dundon *et al.*, 2010). Consequently, it is important to differentiate vaccinated animals among the infected animals in outbreaks incidents and eradication programs.

In FMD eradication program, the detection of infected and vaccinated animals is the crucial point to control the disease. Moreover, to prove the freedom status of certain areas or countries from FMD infections, differentiating the FMD infected animals from the vaccinated populations plays a vital role (Muller *et al.*, 2010). This is because almost 50% of FMD infected animals can act as FMD carriers in environment (Jamal and Belsham, 2013). The fact that FMDV can stay for more than 28 days post-infection in the oropharynx of infected animals are the major threaten in control and eradication programs. Furthermore, the ability of the virus to spread in a long period

along asymptomatic phase also increases the dangerous of this infection. Thus, the differential tools to determine the infected and vaccinated animals are urgently needed.

Currently, DIVA has been used as the preferred tests to recognise the disease status of some regions. DIVA that combined with competition ELISA (C-ELISA) has designed to identify the antibodies of NSP 3ABC which is an indicator of FMD infection (Clavijo *et al.*, 2004; Foord *et al.*, 2007). 3ABC of FMD NSP has recognised as the most immunogenic proteins that can trigger the formation of long duration of antibody responses (Bruderer *et al.*, 2004). NSP cloning and expression has brought new alternatives in FMD diagnostic approaches. As a result, FMD identification based on the detection of NSP antibodies is commonly accepted as a new diagnostic marker system. For example, DIVA has been used by South America government to monitor and evaluate the success of FMD eradication programs and to legitimise the status of "FMD-free with vaccination" in order to increase the export of livestock products (Dundon *et al.*, 2010). In 1997, DIVA also had been used to promote FMD eradication program in pig populations in Taiwan (Chung *et al.*, 2003).

INDONESIA AND POTENSIAL THREAT OF FMD

There are three FMD serotypes that establish in south-east Asia, such as serotype O, A and Asia 1. These serotypes have infected seven countries such as, Cambodia, Laos, Malaysia, Myanmar, the Philippines, Thailand and Vietnam while other three countries are free from the

disease (Brunei, Indonesia and Singapore) (Gleeson, 2002; Rweyemamu *et al.*, 2008). Although, Indonesia has sustained its freedom for more than two decades, it still important to protect the areas from external and internal threats.

Regarding its position that near with FMD infected countries such as Malaysia and Thailand. It is crucial to protect the animal and human movement from those countries especially in the border areas. To minimise the risk of FMD reinfection, the strict regulation and policy in animal trade and movement, biosecurity and regular surveillance should be taken by the government. Moreover, the continually campaign to raise public awareness due to the dangerous of infectious disease also should be considered as the prevention strategies.

CONCLUSION

FMD is a highly contagious disease that also causes the devastating effect to the economic sector. This disease can spread rapidly by a multitude of routes and infected a wide range of animal. Symptoms of the disease has been characterised by the formation of vesicles and erosions in the cutaneous mucosae and hairless area of the skin such as mouth and the hoofs. In order to prevent the outbreaks of the disease and to eradicate the disease, several molecular approaches should be developed and implemented as a part of infectious disease control program.

REFERENCING

Abdul-Hamid, N.F., Hussein, N.M., Wadsworth, J., Radford, A.D., Knowles, N.J. and King, D.P. (2011) Phylogeography of foot-and-mouth disease virus types O and A in Malaysia and surrounding countries. *Infect Genet Evol* **11**: 320-8

Alexandersen, S. and Mowat, N. (2005) Foot-and-mouth disease: host range and pathogenesis. *Curr Top Microbiol Immunol* **288**: 9-42

Alexandersen, S., Quan, M., Murphy, C., Knight, J. and Zhang, Z. (2003a) Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *J Comp Pathol* **129**: 268-82

Alexandersen, S., Zhang, Z., Donaldson, A.I. and Garland, A.J.M. (2003b) The Pathogenesis and Diagnosis of Foot-and-Mouth Disease. *Journal of Comparative Pathology* **129**: 1-36

Bao, H.F., Li, D., Guo, J.H., Lu, Z.J., Chen, Y.L., Liu, Z.X., Liu, X.T. and Xie, Q.G. (2008) A highly sensitive and specific multiplex RT-PCR to detect foot-and-mouth disease virus in tissue and food samples. *Arch Virol* **153**: 205-9

Brehm, K.E., Kumar, N., Thulke, H.H. and Haas, B. (2008) High potency vaccines induce protection against heterologous challenge with foot-and-mouth disease virus. *Vaccine* **26**: 1681-1687

Bruderer, U., Swam, H., Haas, B., Visser, N., Brocchi, E., Grazioli, S., Esterhuysen, J.J., Vosloo, W., Forsyth, M., Aggarwal, N., Cox, S., Armstrong, R. and Anderson, J. (2004) Differentiating infection from vaccination in foot-and-mouth-disease: evaluation of an ELISA based on recombinant 3ABC. *Veterinary Microbiology* **101**: 187-197

Chung, W.B., Liao, P.C., Yang, P.C., Chen, S.P., Jong, M.H. and Sheu, T.W. (2003) Surveillance of FMD virus non-structural protein antibodies in pig populations involved in an eradication programme. *Vet Rec* **152**: 595-7

Clavijo, A., Wright, P. and Kitching, P. (2004) Developments in diagnostic techniques for differentiating infection from vaccination in foot-and-mouth disease. *The Veterinary Journal* **167**: 9-22

Doel, T.R. (2003) FMD vaccines. *Virus Research* **91**: 81-99

Dundon, W.G., Haas, B., Parida, S., Paton, D.J., Rasmussen, T.B. and Uttenthal, A. (2010) Strategies for differentiating infection in vaccinated animals (DIVA) for foot-and-mouth disease, classical swine fever and avian influenza. *Expert Review of Vaccines* **9**: 73+

Foord, A.J., Muller, J.D., Yu, M., Wang, L.F. and Heine, H.G. (2007) Production and

application of recombinant antibodies to foot-and-mouth disease virus non-structural protein 3ABC. *J Immunol Methods* **321**: 142-51

Giridharan, P., Hemadri, D., Tosh, C., Sanyal, A. and Bandyopadhyay, S.K. (2005) Development and evaluation of a multiplex PCR for differentiation of foot-and-mouth disease virus strains native to India. *J Virol Methods* **126**: 1-11

Gleeson, L.J. (2002) A review of the status of foot and mouth disease in South-East Asia and approaches to control and eradication. *Rev Sci Tech* **21**: 465-75

Jamal, S.M. and Belsham, G.J. (2013) Foot-and-mouth disease: past, present and future. *Vet Res* **44**: 116

Kitching, P., Hammond, J., Jeggo, M., Charleston, B., Paton, D., Rodriguez, L. and Heckert, R. (2007) Global FMD control--is it an option? *Vaccine* **25**: 5660-4

Kitching, R.P. and Alexandersen, S. (2002) Clinical variation in foot and mouth disease: pigs. *Rev Sci Tech* **21**: 513-8

Klein, J. (2009) Understanding the molecular epidemiology of foot-and-mouth-disease virus. *Infect Genet Evol* **9**: 153-61

Knowles, N.J. and Samuel, A.R. (2003) Molecular epidemiology of foot-and-mouth disease virus. *Virus Research* **91**: 65-80

Le, V.P., Lee, K.N., Nguyen, T., Kim, S.M., Cho, I.S., Khang, D.D., Hien, N.B., Van Quyen, D. and Park, J.H. (2012) A rapid molecular strategy for early detection and characterization of Vietnamese foot-and-mouth disease virus serotypes O, A, and Asia 1. *J Virol Methods* **180**: 1-6

Ma, L.N., Zhang, J., Chen, H.T., Zhou, J.H., Ding, Y.Z. and Liu, Y.S. (2011) An overview on ELISA techniques for FMD. *Virol J* **8**: 419

Muller, J.D., Wilkins, M., Foord, A.J., Dolezal, O., Yu, M., Heine, H.G. and Wang, L.-F. (2010) Improvement of a recombinant antibody-based serological assay for foot-and-mouth disease virus. *Journal of Immunological Methods* **352**: 81-88

OIE. (2012) Foot and mouth disease. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*

Parida, S. (2009) Vaccination against foot-and-mouth disease virus: strategies and effectiveness. *Expert Review of Vaccines* **8**: 347+

Parida, S., Anderson, J., Cox, S.J., Barnett, P.V. and Paton, D.J. (2006a) Secretory IgA as an indicator of oro-pharyngeal foot-and-mouth disease virus replication and as a tool for post vaccination surveillance. *Vaccine* **24**: 1107-16

Parida, S., Oh, Y., Reid, S.M., Cox, S.J., Statham, R.J., Mahapatra, M., Anderson, J., Barnett, P.V., Charleston, B. and Paton, D.J. (2006b) Interferon-gamma production in vitro from whole blood of foot-and-mouth disease virus (FMDV) vaccinated and infected cattle after

incubation with inactivated FMDV. *Vaccine* **24**: 964-9

Paton, D.J., Valarcher, J.F., Bergmann, I., Matlho, O.G., Zakharov, V.M., Palma, E.L. and Thomson, G.R. (2005) Selection of foot and mouth disease vaccine strains--a review. *Rev Sci Tech* **24**: 981-93

Reid, S.M., Ferris, N.P., Hutchings, G.H., Zhang, Z., Belsham, G.J. and Alexandersen, S. (2002) Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay. *Journal of Virological Methods* **105**: 67-80

Reid, S.M., Grierson, S.S., Ferris, N.P., Hutchings, G.H. and Alexandersen, S. (2003) Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. *Journal of Virological Methods* **107**: 129-139

Rweyemamu, M., Roeder, P., Mackay, D., Sumption, K., Brownlie, J., Leforban, Y., Valarcher, J.F., Knowles, N.J. and Saraiva, V. (2008) Epidemiological Patterns of Foot-and-Mouth Disease Worldwide. *Transboundary and Emerging Diseases* **55**: 57-72

Sariya, L., Thangthumniyom, N., Wajjwalku, W., Chumsing, W., Ramasoota, P. and Lekcharoensuk, P. (2011) Expression of foot and mouth disease virus nonstructural polyprotein 3ABC with inactive 3Cpro in Escherichia coli. *Protein Expression and Purification* **80**: 17-21

Sharma, G.K., Mohapatra, J.K., Pandey, L.K., Mahajan, S., Mathapati, B.S., Sanyal, A. and Pattnaik, B. (2012) Immunodiagnosis of foot-and-mouth disease using mutated recombinant 3ABC polyprotein in a competitive ELISA. *Journal of Virological Methods* **185**: 52-60

Uttenthal, A., Parida, S., Rasmussen, T.B., Paton, D.J., Haas, B. and Dundon, W.G. (2010) Strategies for differentiating infection in vaccinated animals (DIVA) for foot-and-mouth disease, classical swine fever and avian influenza. *Expert Rev Vaccines* **9**: 73-87