Chapter 2

Literature review

Foot and mouth disease (FMD) is a devastating disease of livestock. All species of cloven-hoofed animals are susceptible and the disease is extremely contagious. Financial losses as a result of FMD can be significant. There are direct losses due to deaths in young animals, loss of milk, loss of meat and a decrease in productive performance (James & Rushton, 2002). The costs associated with eradication or control can be high and, in addition, there are indirect losses due to the imposition of trade restrictions. For this reason countries which are free from FMD go to great lengths to maintain their disease-free status and many countries which have the disease invest large sums in eradication campaigns. A key element in the control of FMD is knowledge of the epidemiology of the disease and how best to control it. This chapter gives an overview of the agent responsible, the disease itself and methods of control.

2.1 Foot and mouth disease virus

FMD is caused by a virus of the genus *Aphthovirus*, family *Picornaviridae*. They have single-stranded positivesense RNA with approximately 8450 nucleotides that serve as messenger RNA. The RNA is initially translated as a single polypeptide which is subsequently cleaved by viral-encoded proteases to produce the structural

and non-structural proteins depicted in the genome map. There are seven serotypes of FMD virus (FMDV), namely O, A, C, SAT 1, SAT 2, SAT 3 (SAT = Southern African Territories), and Asia 1, that infect cloven-hoofed animals. There is no cross-protection between the serotypes. All serotypes have numerous subtypes, which may arise during acute or persistent infection (MurF.A. & E.P., 1999).

The FMDV has a diameter of about 25 nm. By electron microscopy, the FMDV appears to be a round particle with a smooth surface. FMDV is distinguished from other picornaviruses by the lack of a surface canyon, or pit, which has been shown to be the receptor binding site for the entero-and cardioviruses. Another feature of the virion is the presence of a channel at the fivefold axis which permits the entry of small molecules, such as CsCl, into the capsid, resulting in FMDV having the highest buoyant density of the picornaviruses. FMDV, like other members of the Picornaviridae, has a relatively short infectious cycle in cultured cells. Depending on the multiplicity of infection, newly formed infectious virions begin to appear at between 4 and 6 hours after infection. The virus is cytocidal, and infected cells exhibit morphological alterations, commonly called cytopathic effects, which include cell rounding and alteration and redistribution of internal cellular membranes. The virus also causes biochemical alterations, including inhibition of host translation and transcription (Grubman & Baxt, 2004).

Figure 2.1 Aphthovirus: Molecular surface of Foot and Mouth Disease Virus, radially depth cued, as solved by X-ray crystallography (available on: http://pathmicro.med.sc.edu/virol/fmdv.jpg)

FMD virus is temperature-sensitive and is rapidly inactivated at elevated temperatures. The virus can be preserved by refrigeration and freezing and progressively inactivated by temperatures above 50°C. It is extremely sensitive to pH. Virus survival is optimal between pH 7.2 and 7.6. At pHs above 9 and below 6 the virus is rapidly destroyed. For this reason either acids (e.g., citric acid 0.2%) or bases (e.g., caustic soda or sodium carbonate) are effective at inactivating the virus, particularly in combination with detergents to ensure penetration of organic material. But, the virus resist to iodophores, quaternary ammonium compounds, hypoclorite and phenol, especially in the presence of organic matter. At higher temperatures the effect of pH on virus survival is increased whereas at low temperatures it is reduced. FMD virus can survive for long periods of time in dark, moist conditions but is rapidly inactivated by a combination of desiccation, pH and temperature.

2.2 Epidemiology of FMD

FMD is the most contagious viral disease of animals with important economic losses. The small dose required to infect, the large amount of virus excreted, and the variety of routes of infection and routes of excretion all contribute to the extreme contagiousness of the disease. Susceptible host are Bovidae (cattle, zebus, domestic buffaloes, yaks), sheep, goats, swine, all wild ruminants and suidae. Camelidae (camels, dromedaries, llamas, vicunas) have low susceptibility

The incubation period of FMD is usually 2 to 14 days. In some circumstances longer incubation periods have been recorded, particularly if the infecting dose is low. The disease is of high morbidity and low mortality - mortality is only significant when young animals are infected, in which case neonates may die due to a peracute myocarditis.

Infection with FMD can occur via a variety of routes for example, direct or indirect contact (droplets inhalation or ingestion, entry via damaged epithelia), animate vectors (humans, etc.) inanimate vectors (vehicles, implements), airborne, especially temperate zones (up to 60 km overland and 300 km by sea) and iatrogenic

FMD virus is excreted in all the secretions and excretions of infected animals.

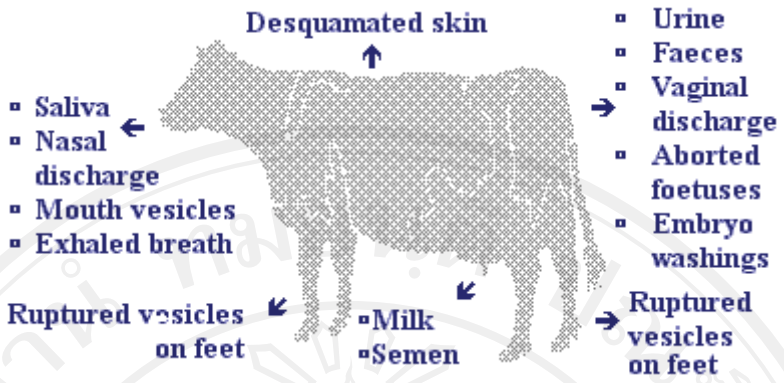

Figure 2.2 The route of FMDV excretion from cattle (Available on: http://aleffgroup.com/avisfmd/A010-fmd/tools/3-diag-virus-excretion.html)

Virus excretion declines with the appearance of circulating FMD-specific antibody at around four to five days after infection. Virus is frequently isolated from oropharyngeal fluid ('probang' fluid) for several months after infection. There are isolated reports of prolonged detection in blood, urine and milk.

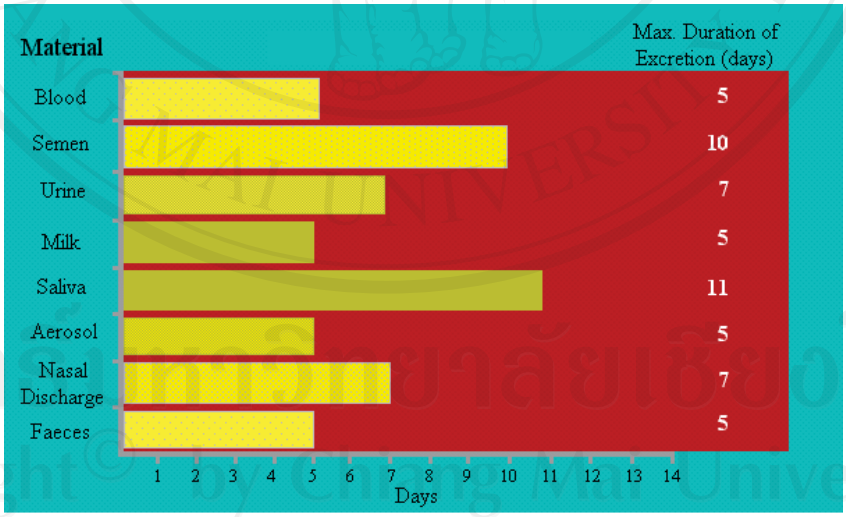

Figure 2.3 The Duration of FMDV excretion in cattle (Available on: http://aleffgroup.com/avisfmd/A010-fmd/tools/0-chrt-duration-fmdv-excretion-cattle.html)

The sources of FMDV including incubating and clinically affected animals, breath, saliva, faeces, and urine; milk, semen (up to 4 days before clinical signs), meat and by-products in which pH has remained above 6.0. Particularly cattle and water buffalo; convalescent animals and exposed vaccinates (virus persists in the oropharynx for up to 30 months in cattle or longer in buffalo, 9 months in sheep). African Cape buffalo are the major maintenance host of SAT serotypes (Salt, 1993).

More than 50 of the 162 Member Countries of the Office International des Epizooties (OIE), the World Organisation for Animal Health, have obtained recognition from the OIE for freedom from foot and mouth disease (FMD) without vaccination. The virus continues to circulate in two-thirds of the remaining countries, thus dividing the globe into two zones. This has significant effects on international trade patterns in susceptible animals and animal products. Consequently, countries that do not have FMD-free status continue to suffer a severe handicap in terms of access to international markets. This situation was highlighted by the sudden and largely unexpected resurgence of FMD in Europe, South America and Asia at the beginning of the 21st Century. This endemic situation with respect to FMD in many parts of the world is a constant threat to countries that have acquired FMD-free status at considerable cost and effort. The threat has been exacerbated over the last decade by accelerated trade and movements of people due to globalization. At the same time, developed countries have either decreased or discontinued vaccination. The dangerous cocktail of globalization and non-immunised animals exploded in 2001, first in South America and then in the United Kingdom and other countries of the European Union (Vallat et al., 2003). In 1997 an FMD outbreak was reported in Taiwan, a country that had been free of the disease for 68 years. This devastating outbreak resulted in the

slaughter of more than 4 million pigs, almost 38% of the entire pig population, at a cost of approximately U.S $6 billion and reminded the international animal health community of the severe economic consequences that a FMD outbreak could have for a previously disease-free country. Starting in late 1999 and 2000, a series of FMD outbreaks occurred in a number of countries in East Asia. This was followed by an outbreak in South Africa and culminated in the destructive outbreak in the United Kingdom, which then spread to the European continent. These outbreaks reemphasized the extreme virulence of the FMDV in a variety of animal species, the vulnerability of FMD-free countries as well as countries where FMD is enzootic to new viral strains, the efforts of globalization on increasing the risks of disease incursion, and hence the need for countries to more closely monitor for the presence of exotic disease (Grubman & Baxt, 2004).

Europe and Central Asia: In the past, the disease has ravaged European livestock, but has been gradually brought under control, at great cost, by preventive vaccination programmes, supplemented by the destruction of infected herds in most of the countries of continental Europe and, in the United Kingdom (UK) and Nordic countries, by destruction of infected herds alone. After careful evaluation of the two possible options for preventing the re-occurrence of the disease in Europe to either continue or discontinue mass vaccination the European Union decided to prohibit all vaccination after 1991. FMD remained and is still endemic in the Middle East, including Asian Turkey (Anatolia), and despite efforts of the Governments of Turkey and Europe, Anatolia appears to be a permanent source of sporadic outbreaks in the Balkans and a threat to Europe. In recent years, FMD was reported mainly in the Balkans. Despite these occasional incursions of FMD into south-east Europe, in all

cases, the control measures were efficient and the disease never spread to such an extent as to become endemic. A major outbreak, which affected 2,030 farms occurred in the UK between February and September 2001. This was the first major epidemic of FMD in Europe since preventive vaccination had been abandoned in continental Europe in 1991. The disease also spread to Ireland, France and the Netherlands although the number of outbreaks was limited in these countries (Leforbant & Gerbier, 2002).

South America: Since the signing in 1987 of the Hemispheric Plan for the Eradication of Foot-and-Mouth Disease by the countries of South America, clinical cases of foot and mouth disease have decreased significantly throughout the continent. During the early 1990s, national laboratories diagnosed an average of 766 cases per year in South America. By the late 1990s, this continent-wide average had fallen to 130. By the end of the 1990s, the international community recognized Argentina, Chile, Guyana, and Uruguay as free of FMD without vaccination. In 1999, clinical signs of FMD were absent in 60% of all cattle on the continent. These cattle represented 41% of all herds in South America and extended over 60% of the geographical area of the continent. However, in the spring of 2001, FMD re-appeared in certain countries of the Southern Cone. This wide-spread re-occurrence of the disease in Argentina, Uruguay and the State of the Rio Grande do Sul in Brazil called into question whether countries in South America can achieve and maintain FMD-free status, with or without vaccination (Melo et al., 2002).

Middle East and North Africa: Only one country in the Middle East (Cyprus) is presently included in the OIE list of foot and mouth disease-free countries. The region is regarded as that most affected by FMD in the world. FMD has been

recorded in all countries in the Middle East on numerous occasions between 1960 and 2000, serotype O being the most prevalent. In the past, exotic FMD viruses were the cause of panzootics, which spread to many areas of the region, even extending to the frontier of Europe. A remarkable example was the rapid dissemination of serotype SAT 1 virus, which occurred initially in Bahrain in December 1961. The virus spread north-westwards to reach Iraq, Jordan, Israel, and Syria by April 1962, continuing to Iran and Turkey. In September 1962, this serotype crossed the Bosporus to enter Europe for the first time, and in November, caused an outbreak further west, near the border between Turkey and Greece. Historically, epidemics mainly affected cattle and spread from east to the west in the Middle East. The slow spread of FMD from Tunisia in 1989 to Morocco in 1991 exemplifies the difficulty in controlling the disease since unregulated movements of herds of small ruminants may play an important role in spreading infection. The situation in the Middle East and North Africa constitutes a threat to other regions of the world, especially Europe (Aidaros , 2002).

East Asia: Japan regained the status of freedom from foot and mouth disease without vaccination in September 2000 and the Republic of Korea likewise obtained this status in September 20001. However, new outbreaks of FMD caused by the Pan-Asian topotype have occurred in pigs in the Republic of Korea since May 20002. Taipei China has not experienced an outbreak of FMD since February 20001 and the country is currently implementing and eradication programme. These countries had been free from FMD for many decades when in 1997, the FMD virus once again invaded the region, particularly in 2000; this resulted in widespread occurrence of the disease. The types of FMDV were investigated by genome analysis, and in each case

the virus concerned was found to be a member of the pan-Asian O lineage (Sakamoto & Yoshida, 2003).

South-East Asia: Of the ten countries in South-East Asia, FMD is endemic is seven (Cambodia, Laos, Malaysia, Myanmar, the Philippines, Thailand, and Vietnam) and three are free of the disease (Brunei, Indonesia and Singapore). Part of the Philippines is also recognized internationally as being free of FMD. From 1996 to 2001, serotype O viruses caused outbreaks in all seven of the endemically infected countries. On the mainland, three different type O lineages have been recorded, namely: the South-East Asian topotype, the pig-adapted or Cathay topotype and the pan-Asian topotype. Prior to 1999, one group of SEA topotype viruses occurred in the eastern part of the region and another group in the western part. However, in 1999, the pan-Asian lineage was introduced to the region and has become widespread. The Cathay topotype was reported from Vietnam in 1997 and is the only FMD virus currently endemic I the Philippines. Type Asia 1 has never been reported from the Philippines but was reported from all countries on the mainland except Vietnam between 1996 and 2001. Type A virus has not been reported east of the Mekong River in the past six years and seems to be mainly confined to Thailand with occasional spillover into Malaysia. The distribution and movement of FMD in the region is a reflection of the trade-driven movement of livestock (Gleeson, 2002).

Sub-Saharan Africa: Six of the seven serotypes of FMDV (i.e. all but Asia 1) are prevalent in Africa although there are marked regional differences in distribution. Three of these serotypes are unique in Africa -- the three SAT serotypes. Serotype C may also now be confined to Africa because it has not been reported elsewhere recently. In southern Africa at least, the SAT serotypes have an intimate and probably

ancient association with African buffalo (Syncerus caffer) that is instrumental in their maintenance. Within each of the six prevalent serotypes, with the possible exception of C, there are a number of different lineages with more or less defined distributions (topotypes) that in some cases are sufficiently immunologically different from one another to require specific vaccines to ensure efficient control. This immunological diversity in prevalent serotypes and topotypes, in addition to the uncontrolled animal movement in most parts of the continent, render FMD difficult to control in present circumstances. This fact, together with poorly developed intercontinental trade in animals and animal products has resulted in the control of FMD being afforded a low priority in most parts of the continent, although the northern and southern regions of the continent are an exception. As a consequence, eradication of FMD from Africa as a whole is not a prospect within the foreseeable future (Vosloo et al., 2002A)

2.3 Clinical sign

In cattle: The clincal sign of FMD in cattle can be found after the end of incubation period, normally within 2 to 14 days. Clinical signs include pyrexia up to 41^{o}C, anorexia, shivering, a dramatic drop in milk production for 2-3 days, then smacking of the lips, grinding of the teeth, drooling, lameness, stamping or kicking of the feet: caused by vesicles (aphthae) on buccal and nasal mucous membranes and/or between the claws, coronary band and also occur on the mammary glands. After 24 hours the vescicle may be rupture and leaving erosions. There may be mortality in young animals due to myocarditis. The recovery generally occurs within 8-15 days.

In cattle infected with FMD virus, a febrile response, with rectal temperatures ranging from 39.5 degrees to 41 degrees celcius is usually detectable prior to vesicle formation and continues for 3 to 4 days afterward (Musser, 2004).

Vesicles develop on the tongue, hard palate, dental pad, lips, gums, muzzle, coronary band and interdigital space. Vesicles may also be seen on the teats, particularly of lactating cows. Stamp feet as they try to relieve pressure. They prefer to lie down and resist attempts to raise them. Lactating cattle with teat lesions are difficult to milk and ruptured vesicles frequently become infected, predisposing to secondary mastitis. Vesicles in mouth rupture rapidly, usually within 24 hours, leaving shallow erosion surrounded by shreds of epithelium. Vesicles on the feet may remain intact for two or three days before rupturing. Severe form with some vaccinated cattle: the tongue swells and protrudes from the mouth and the majority of the tongue epithelium is shed (Kitching, 2002).

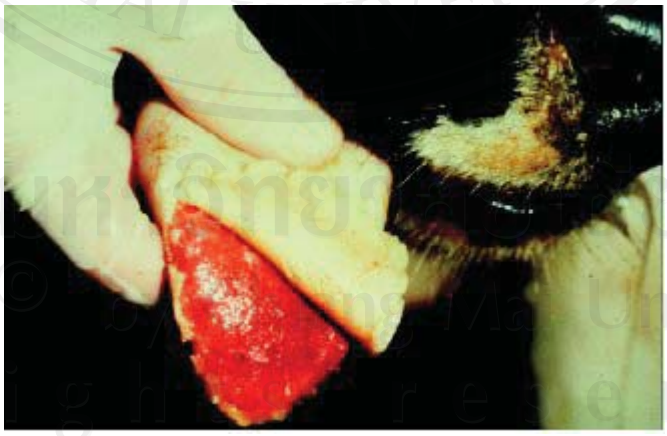

Figure 2.4 Cow with ruptured tongue vesicle, two days after start of clinical signs of foot and mouth disease (Kitching, 2002)

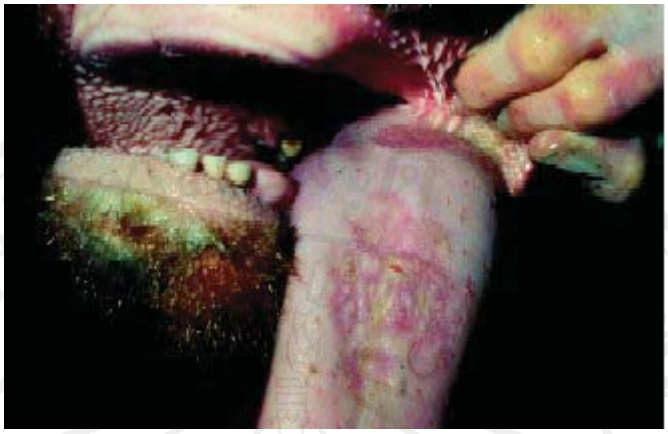

Figure 2.5 Healing tongue lesion four days after the start of clinical signs (Kitching, 2002).

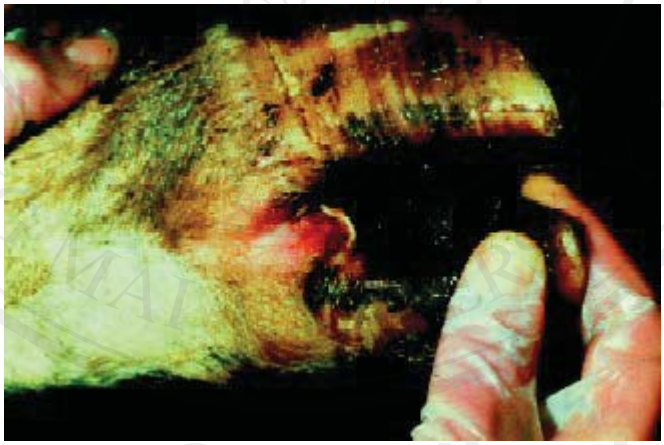

Figure 2.6 Ruptured vesicle on foot, five days after the appearance of clinical signs (Kitching, 2002).

Acutely infected cattle salivate profusely and develop nasal discharge, at first mucoid, then mucopurulent (Kitching, 2002). The quintessential clinical sign in infected cattle is excessive drooling, which typically occurs after vesicle and ulcer formation. Cattle sometimes display smacking of their lips (Musser, 2004).

Figure 2.7 Young bovine with foot and mouth disease (Kitching, 2002)

With viremia and vesicle development, lethargy and anorexia or poor food intake typically develop. Vesicles and erosions can occur on the teats, and mastitis may develop secondarily. Milk production and feed consumption may decrease gradually or precipitously depending on the virulence of the serotype and the strain of the virus (Musser, 2004).

Young calves may die before the appearance of vesicle because of the predilection of the virus to invade and destroy cells of the developing heart (Kitching, 2002C). Although mortality rates in mature cattle with FMD are usually low, they can be quite high in calves. Death in calves is due to acute myocarditis. Myocardial lesions are referred to as tiger heart (Musser, 2004).

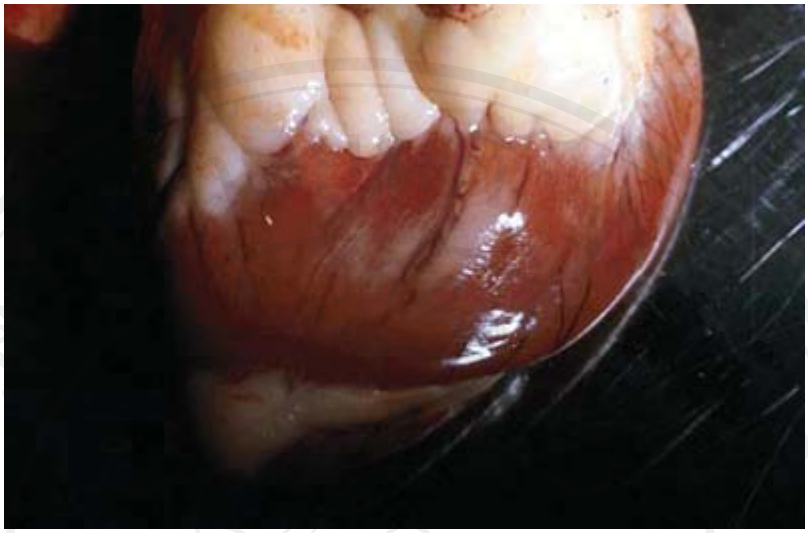

Figure 2.8 In very young animals, myocardial necrosis can occur, appearing as pale streaks in the ventricular wall. (available on: http://www.microbiologybytes.com/blog/2007/08/06/foot-and-mouth-disease/)

In pig: In intensely reared pigs, the introduction of FMD results in severe clinical disease and vesicular lesions in adult and fattening animals, and high mortality in piglets. The clinical sign can be pyrexia, anorexia and lethargy. Vesicular lesions appear rapidly on the tongue, dental pad, gums, cheek, hard and soft palate, lips, nostrils, muzzle, coronary bands, teats, udder, snout of pigs, corium of dewclaws and interdigital spaces. Pigs are extremely lame, are reluctant to move and adopt a hunched gait if forced to stand. The severity of lesions on the limbs depends on the conditions under which the the pigs are kept. Mortality in unweaned piglets due to myocarditis can be up to 100% and can precede any other signs of the disease (for example, vesicles on the teats of lactating sows)

Pig may develop a fever up to 42 ^{o}C, but most often this is in the range of 39 degrees to 40 degrees C. Temperature increase in FMD-infected pigs may sometimes

be inconsistent, short-lived or close to the normal variation seen and severely affected pigs may even have a drop in temperature to below the normal range. Local signs of inflammation such as heat and pain when touching and applying finger pressure on areas of the feet may often be detected by careful clinical examination before any increase in body temperature is apparent (Kitching & Alexandersen, 2002). Affected pigs become lethargic and remain huddled together and take reduced or little interest in food (Kitching & Alexandersen, 2002)

Vesicles develop on the coronary band and heel of the foot (including the accessory digits), on the snout, lower jaw and tongue. Lesions on the coronary band are the most consistent findings in pigs while lesions at other sites may be found less regularly. Vesicles on the tongue of pigs are most often found far back on the tongue or as tiny vesicles-erosions near the tip of the tongue. Pigs housed on rough concrete floors may show additional lesions on their hocks and elbows or other areas of previously damaged skin, and lactating sows frequently develop vesicles on the udder (Kitching & Alexandersen, 2002)

Figure 2.9 Foot and mouth disease in a pig showing lesions at day 2 after first appearance of clinical signs (Kitching & Alexandersen, 2002)

Figure 2.10 Foot and mouth disease in a pig showing loss of epithelium at day 4 after first appearance of clinical signs (Kitching & Alexandersen, 2002).

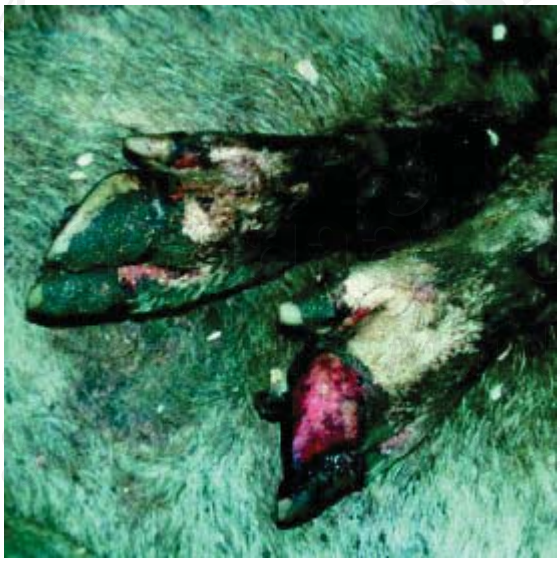

Figure 2.11 Foot and mouth disease in a pig showing loss of horn from digit (Kitching & Alexandersen, 2002)

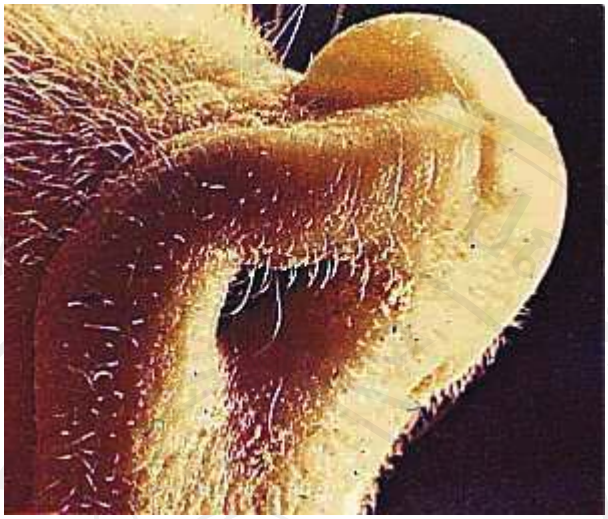

Figure 2.12 Large unruptured vesicle on nose (available on http://www.thepigsite.com/FeaturedArticle/Default.asp?AREA=FeaturedArticle&Display=305)

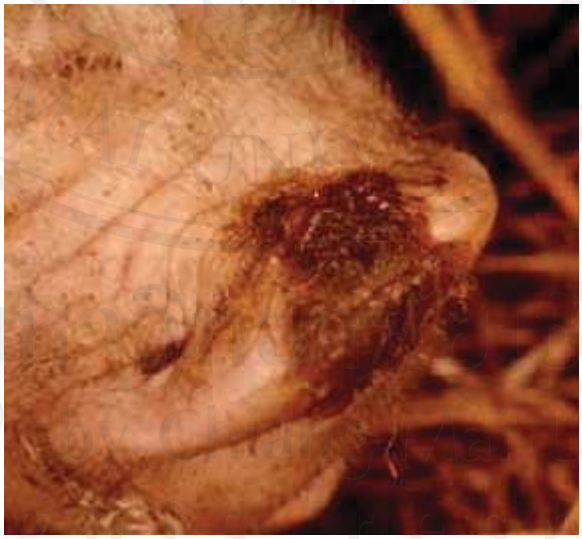

Figure 2.13 Ruptured vesicle on nose (available on: http://www.thepigsite.com/FeaturedArticle/Default.asp?AREA=FeaturedArticle&Display=305)

2.4 Diagnosis

The diagnosis of FMD in cattle usually initiate on the basis of clinical signs, with or without a history of contact between the herd and an infected animal, or report of FMD in the vicinity. In a fully susceptible herd, the clinical signs are frequently severe and pathognomonic. However, in endemic regions in cattle that have partial natural or vaccinal immunity, clinical signs may be mild and may be missed (Kitching, 2002). In pigs, the diagnosis of FMD is based initially on the appearance of clinical signs. However, these can be confused with those caused by vesicular stomatitis, swine vesicular disease virus, or, in the past, vesicular exanthema virus. For the diseases are clinically indistinguishable from each other, in countries where vesicular diseases are endemic, national animal disease diagnostic laboratories are generally available. Samples may be shipped to the World Reference Laboratory for Foot-and-Mouth Disease at Pirbright, Great Britain, or to regional vesicular disease diagnostic laboratories including the Foreign Animal Disease Diagnostic Laboratory (FADDL) at the PIADC in Plum Island, New York, United States, or the Pan American Foot-and-Mouth Disease Laboratory in Rio de Janeiro, Brazil, including Regional Reference Laboratory for Foot and Mouth Disease in South-East Asia (RRL) in Pakchong Nakhonratchasima, Thailand. The director of the laboratory should be contacted before shipment. Permits may be needed for shipment to some laboratories, and ever-changing international shipping regulations for diagnostic samples must be followed.

The laboratory diagnostic tests for FMD in pigs are the same as those used for cattle, with the additional requirement to also include SVDV reagents on the antigen detection. Currently FMD is confirmed by antigen capture enzyme-linked

immunosorbent assay (ELISA) and virus isolation. While ELISA can be obtained in three to four hours after the sample is received by the laboratory, a negative result must be confirmed by inoculation of the sample into sensitive cultures followed by confirmation of the virus serotype by ELISA. These assays can take up to four days, a time frame incompatible with the need to rapidly detect disease and initiate an appropriate disease control strategy (Grubman & Baxt, 2004).

RT-PCR methods have been used to rapidly detect and type FMDV and to detect virus infection in asymptomatic animals. However, this assay is often not superior in sensitivity to ELISA and virus isolation and, in addition, is labor-intensive. Most recently, real-time RT-PCR methods have been examined by a number of groups with the aim of developing portable on-site diagnosis. The assay is specific and as sensitive as virus isolation, and viral RNA could be detected in oral and nasal samples from experimentally infected animals 24 to 96 hours before the onset of clinical signs. In addition, the assay is rapid, results can be obtained in about 2 hours and the cycler is portable. The next steps required for assessing and validating this assay are optimization of conditions with all possible field samples (blood, milk, tissue) and testing under field conditions (Grubman & Baxt, 2004).

In 1966 Cowan and Graves identified a highly immunogenic FMDV NS antigen, called the virus infection-associated antigen (VIAA), which reacted with sera from convalescent animals but not with sera from vaccinated animals. However, in later studies, investigators found that sera from multiply vaccinated animals, and even from some animals, which had received a single vaccination, had antibodies to VIAA. The major reason that antibodies against an NS protein are present in sera from vaccinated animals is that FMD vaccines are not purified and, depending upon the

manufacturer, contain various amounts of contaminating NS proteins. Nevertheless, VIAA, which was subsequently identified as the viral RNA polymerase is currently used in an agar-gel immunodiffusion test to differentiate infected from vaccinated animals.

To improve the reliability of this diagnostic assay, investigators have targeted other NS proteins as potential diagnostic reagents. They recommend the use of NS proteins 3AB, 2C, 3C, and 2B, or their respective peptides, as antigens in an ELISA-based assay. ELISA-based assays with various NS proteins produced by recombinant baculovirus, in *E. coli*, or with synthetically produced peptides to NS proteins have been developed. Currently, these assays are being validated (Grubman & Baxt, 2004).

A minimum of 2 cm squared of epithelium from a ruptured vesicle in a 50/50 mixture of glycerine and 0.04 molar buffered phosphate (pH 7.4-7.6) should be sent to a laboratory designated for handling live FMD virus and equipped with the necessary reagents for typing a positive sample. Whole and clotted blood samples and probang samples may also be sent (Kitching, 2002). Antibodies to FMD virus can be detected in the milk of cattle that have recovered from FMD, using either the liquid phase blocking enzyme-linked immunosorbent assay (ELISA) (LPBE) or a specific isotype assay (SIA) for bovine immunoglobulin G1 (IG1). However, whereas the LPPBE would not detect antibodies derived as a consequence of vaccination, the SIA was able to identify 95 percent of cattle vaccinated up to 12 months previously, in the study reported. There was also a strong correlation between serum antibody titres and milk antibody titres, to the extent that individual and herd immunity levels against FMD could be assessed using the SIA on individual or bulk tank milk samples, respectively (Kitching, 2002).

2.5 Prevention and control

Stamping Out: Stamping-out consists of the killing and disposal of all susceptible livestock on the infected farms and their immediate contact farms that most likely infected followed by a thorough disinfection, cleaning, disinfection procedure of the premises, the first disinfection begin to prevent the production of virus aerosols during the cleaning. In traditionally FMD free countries, stamping-out is the first option to eradicate the disease. As a first line of defense it is often quite successful, at least if the disease has not yet spread too widely and if the density of the livestock in the area is relatively low. Also, during the first days of an outbreak the proper vaccine may not be available. The choice of the stamping-out option should also depend on the possibility of tracing dangerous contacts, political will and available resources (Sutmoller et al., 2003). In countries free of the disease, a policy of slaughter of all infected and in-contact susceptible animals is usually employed (Kitching, 2002). The slaughter of infected or at-risk herds should be the primary means for controlling diseases such as FMD as long as they are detected at an early stage (Leforban, 2002). This stamping out policy is standard and is recognized by the Office International des Epizooties (OIE) as the most appropriate way to break the chain of virus transmission and thus control and eradicate the disease in industrialized countries (Gibbs, 2003).

Control of the disease in FMD-free countries includes an exclusion and slaughter policy. However, stamping out of infected and contact animals alone may not be sufficient to eradicate the virus promptly and vaccination is now considered an acceptable alternative or adjunct (Clavijo et al., 2004). Cattle have been infected by entry into decontaminated premises, up to four months after culling, cleaning and

disinfection had occurred: 12 occurrences of this were reported in the winter of 1967-1968 in the UK. The mechanisms of such infection are unclear, but apparently the virus was able to survive that length of time in the environment or in some unknown other host (Sutmoller et al., 2003).

Heavy equipment used in these operations is difficult to decontaminate and might be a source of infection or contamination of roads when being driven to another job or back home Disposal of cadavers also presents a risk since virus in lesions, excrements and excretions is not rapidly destroyed after death and might be disseminated by transport of cadavers, by pyres, at burial sites or digester plants. Transport systems for carcasses are not bio-secure, neither are the handling of the carcasses at the rendering plants. The highest risk comes probably from the involvement of large numbers of contractors not trained in disease containment (Sutmoller et al., 2003). After the 2001 outbreak in the UK, public reaction, questioned the need for large-scale slaughter of susceptible animals, particularly the slaughter of vaccinated animals that were healthy (Grubman et al., 2004).

Circle Culling: So-called circle culling and culling of contiguous farms has been applied in the UK and in the Netherlands as an extension of usual stamping-out procedures. The aim of the circle is to eliminate incubating infections that may have spread from the outbreak farm(s) and create a fire break around the outbreak. The diameter of the circle was based on the analysis of spread of FMD during the outbreak using computer models. However, the calculated distance of spread must include spread due largely to the culling process itself as an additional transmission mechanism (Sutmoller et al., 2003). In countries free of the disease, a policy of slaughter of all infected and in-contact susceptible animals is usually employed

(Kitching et al., 2002C). The slaughter of infected or at-risk herds should be the primary means for controlling diseases such as FMD as long as they are detected at an early stage (Leforban et al., 2002). Although ring culling reduces the need for surveillance, it creates potentially much higher numbers of cadavers, some of which might be infected (Sutmoller et al., 2003).

Most culled farms within the circle are not infected and do not represent a risk of further spread of the disease and, therefore, are culled unnecessarily. The operation itself has a high-risk of disseminating FMDV over short and long distances. A long drawn-out campaign is very disruptive for the rural society as a whole, including sectors like tourism. The rural community may fear the control measures more than the disease, and live under this fear for several months after the last case. The consequent application of circle and of contiguous culls poses a threat to zoological collections and valuable (rare) breeding stock. Massive killing and destruction of livestock usually is not done with adequate respect for animal welfare and bio-ethical principles. The small risk represented by hobby farms and smallholdings is not taken into account. An enormous serological surveillance exercise is often required to detect residual infection since new cases could easily re-start the epidemic at its tail end, particularly if movement controls are prematurely lifted. Finally, many culls represent a human tragedy and traumatic experience not only for farmers and their families but for many veterinarians as well. The risk-avoidance behavior of farmers leads to social isolation and breakdown of the socialeconomic and trading patterns of rural communities (Sutmoller et al., 2003).

Vaccination: In endemic or epi-endemic regions, strategic or general vaccination is required with vaccine containing the FMD subtypes that are active in

the area. This could be carried out with the more classical aqueous vaccine or with oil-adjuvant vaccine (Sutmoller et al., 2003). The current FMD vaccine is an inactivated whole-virus preparation that is formulated with adjuvant prior to use in the field. A number of countries have established vaccine banks, which contain concentrated antigen stored in the gaseous phase of liquid nitrogen. Banks contain antigen against a number of virus serotypes and provide member countries with an almost immediate source of vaccine (Grubman & Baxt, 2004). Vaccination would be used to control an outbreak in an endemic area. Vaccination may also be used to surround a focal outbreak of a disease to prevent the virus from spreading and the vaccinated animals may be subsequently slaughtered to reduce the delay in re-establishing trading status. A buffer zone containing vaccinated animals may be used to separate an area within a country in which FMD is endemic from an FMD-free area, from which exports of cattle and cattle products are sourced (Kitching, 2002). Aqueous vaccines must be applied twice yearly. In general, current oil-adjuvant vaccines protect cattle of different breeds more effectively. Cattle up to 2 years should be vaccinated twice yearly. Thereafter, a yearly vaccination will maintain their immune status (Sutmoller et al., 2003).

Given the rapid generation time of pigs, it is usually considered uneconomic or impractical to attempt to maintain immunity in a national pig herd, and in vaccinating countries, usually only cattle and sometimes the sheep, are vaccinated. FMD vaccines for use in pigs must have an oil adjuvant, as the aluminium hydroxide saponin adjuvant used for cattle and sheep vaccines is not effective in stimulating good protection against FMD in pigs, although the same adjuvant appears to work well in certain other virus vaccines in pigs (Kitching & Alexandersen, 2002).

Protection can be provided to naive pigs by day 4 post-vaccination using high potency vaccines with some of the newer oil adjuvants now available (Kitching & Alexandersen, 2002).

Normally, when a number of animals are vaccinated, some animals fail to develop immunity. Should these animals become infected and develop clinical FMD, they can excrete large amounts of virus, which may overcome the vaccinal immunity of the other animals in the group (Kahn et al., 2002). Intensive vaccination does not always prevent the appearance of clinical FMD. Some very high yielding diary herds in the Middle East are vaccinated every ten weeks with vaccine produced under European standards containing eight strains of FMDV. However, because of severe challenge originating predominantly from the nomadic herds of sheep, goats and cattle, which graze freely in the area, introduction of virus into the dairies is inevitable. When these dairy cattle become infected, they frequently exhibit a very severe form of disease, in which the tongue swells and protrudes from the mouth and the majority of the tongue epithelium is shed (Kitching, 2002). Since cattle that are exposed to infection can become persistently infected, whether vaccinated or not, all seropositive animals are considered a risk, which explains, in part, the distinction established by the OIE. The objective of improved serological tests, therefore, must be to reliably detect animals that have been infected with FMD, regardless of whether they have also been vaccinated. A serological test that detects antibodies to the nonstructural polyprotein 3-ABC can be used on a herd basis to detect viral circulation in vaccinated populations. However, there is evidence that not all animals that have been vaccinated and are infected seroconvert to nonstructural proteins (Kahn et al., 2002).

The duration of immunity following a single dose of high-potency vaccine in a previously naive animal is usually less than a few months against homologous challenge, and shorter for heterologous challenge. A booster dose given 3 to 4 wk after the initial dose will prolong the immunity for up to 6 months, but this can be dependent on the level of exposure of the vaccinated animals to live virus challenge (Kahn et al., 2002).

Usually, vaccination in pig is delayed until 10 to 12 weeks of age and repeated 2 weeks later, which may provide sufficient immunity until slaughter weight is reached. Sows should be vaccinated at least twice yearly during pregnancy (Kitching, 2002). The immune response to FMD vaccine in young pigs is poor, and protection is best provided by vaccination of the pregnant sow so that immunity can be passed on in the colostrum of the sow. However, in the presence of maternally derived immunity, an effective immune response from vaccination cannot be initiated before 8 weeks of age. In the presence of clinical disease within a pig herd, vaccination is unlikely to provide sufficient protection, and even using vaccines of high potency (50 percent protective dose), vaccinated pigs in contact with clinically affected pigs commonly develop clinical signs. This is not evidence of poor vaccine quality, but of the extremely high excretion level of FMDV in pigs and of the virulence of some strains of FMDV in pigs (Kitching & Alexandersen, 2002).

Freedom from disease, as established through stamping out, allowed exports to open in 3 months, compared with 12 months when vaccine was used. While the Netherlands used emergency vaccination to control the epidemic, it subsequently culled all vaccinated animals to allow its markets to open early. With the knowledge that vaccination could significantly delay resumption of the UK export market, the

question of whether or not to vaccinate created considerable controversy during the (2001) epidemic (Gibbs, 2003)

High-containment facilities are required for the production of vaccine. Vaccinated animals develop antibody responses against the contaminating proteins, in addition to the viral structural proteins, making it difficult to reliably distinguish vaccinated from infected or convalescent animals with currently approved diagnostic tests. Most Virus preparations are concentrated cell culture supernatants from FMDV-infected cells and, depending on the manufacturer, contain various amounts of contaminating viral NS proteins. The vaccine does not induce rapid protection against challenge by direct inoculation or direct contact. Vaccinated animals can become long-term carriers following contact with FMDV (Grubman & Baxt, 2004). Interference in response to vaccination by young animals with high levels of maternally derived antibody. Only when it is below a LPBE titer of 1:45 will the calf respond to vaccination. The problem of the approximately one month time gap between susceptibility to infection and vaccination can only be managed by keeping the calves isolated from any source of FMD virus during that period (Kitching, 2002). A misconception is that vaccination causes the carrier status. This is impossible since FMD vaccine is an inactivated, safe vaccine. A vaccinated animal must be exposed to a large quantity of FMDV in order to become a carrier, for instance when vaccinated cattle come in contact with large numbers of diseased pigs. Because vaccination suppresses the amount of FMDV that is released into the environment (low morbidity!) it is very unlikely that vaccinated animals will become carriers. It is also unlikely that vaccinated animals become carriers through infection by FMDV transmitted by fomites or people and brought from infected farms. It is thus very

unlikely that new carriers will be induced in vaccinated herds. Carriers among vaccinated cattle have not caused FMD outbreaks among susceptible non-vaccinated livestock populations nor have they hampered FMD eradication efforts (Sutmoller et al., 2003).

Ring Vaccination: It has been demonstrated that early FMD vaccination of herds or flocks round the infected premise creates a cordon of protective animals that can stop effectively the diffusion of the disease. The size of the ring required depends on the rapidity of action of the vaccine and the anticipated rapidity of potential spread of infection from the IP, and location of high-risk farms, which might amplify infection for onward spread. For example, to get ahead of the disease with a vaccine would require 45 days to stimulate immunity and create an area in which farms/animals are protected before the anticipated first contact with virus. The higher the anticipated aerosol transmission, the larger the area that would be required to ensure an adequately immunised ring. Therefore, ring vaccinations should be performed without delay and should include all susceptible species. Preferably, the vaccination should be carried out from the outside of the ring towards the center of the outbreak. Simultaneously, to protect the most endangered farms as soon as possible, vaccination should proceed from the center towards the outside. In the immediate vicinity of the outbreak farm, the large (cattle) holdings should be vaccinated first because potentially, those are the largest aerosol collectors (Sutmoller et al., 2003). Outbreaks in the vaccinated zone or ring will usually cease within 10 days of effective herd immunity being reached, and frequently cease well before this (Sutmoller et al., 2003).

This control option is heavily penalized by present OIE regulations because of the 1224 months waiting period to regain the status of freedom from FMD, depending on whether or not stamping-out was applied (Sutmoller et al., 2003).

Ring Vaccination followed by Slaughter: Fear of carriers among vaccinated animals has led to suppressive vaccination. In that approach, vaccination is used to control the outbreak(s), but all vaccinated animals have to be killed before FMD free status can be regained. It was used in The Netherlands in the main outbreak area to control the recent outbreak (Sutmoller et al., 2003). Four to six days after vaccination all vaccinated animals will have sufficient protection to prevent dissemination of virus. The vaccinated animals can be killed over a more extended period, depending on incinerator capacity (Sutmoller et al., 2003).

Suppressive vaccination creates several of the problems mentioned for circle culling, with the exception of the risk of dissemination of the virus. This risk is much reduced, because 46 days after vaccination all vaccinated animals will have sufficient protection to prevent dissemination of virus. The vaccinated animals can be killed over a more extended period, depending on incinerator capacity. It is interesting to note that, although vaccinated pigs do not become carriers they still must be slaughtered as well! (Sutmoller et al., 2003)

Fencing: The establishment of wildlife conservancies has created a problem with regard to FMD because the Office International des Epizooties (OIE) presently considers any territory on which buffalo infected with FMD viruses occur as infected. Zones recognized as free of FMD by the OIE need to be separated from infected zones by a defined surveillance zone of at least 10km deep (International Health

Code, 1992). According to the OIE recommendations this means that landowners acquiring even one infected buffalo cause their land to be in an infected zone and, by implication, their neighbors to be in a surveillance zone. However, in May 1997 it was accepted by the OIE that infected and free zones may be separated by a barrier instead of a surveillance zone. It appears that modified low-maintenance buffalo control fence complemented with buffer zones or vaccination zones may be a cost-effective solution to the containment of FMD in wildlife zones (Sutmoller et al., 2002). The floods of 2000 in southern Africa damaged the Kruger National Park game fence extensively, and there were several accounts of buffalo that had escaped from the park. The VPI gene, which codes for the major antigenic determinant of the FMD virus, was used to determine phylogenetic relationships between virus isolates obtained from the outbreaks and those previously obtained from buffalo in the KNP. These results demonstrate that buffalo were most probably the source of the outbreaks, indicating that disease control using fencing as well as vaccination is extremely important to ensure that FMD does not become established in domestic livestock (Vosloo et al., 2002B).

The construction of a game fence along international borders if there are no wildlife areas in the neighboring countries would serve no purpose as far as FMD control is concerned (Sutmoller, 2002). In principle, wildlife fences should not be constructed only between wildlife zones and the farming areas. They should not run through the middle of any wildlife zones, but between them and any commercial farms or communal lands. Also from a FMD control point of view there is no need to fence through communal lands or along international borders (Sutmoller, 2002).

Fences are supposed to prevent close contact between infected animals and noninfected animals from the same species or from different species. However, other transmission mechanisms, such as intermediate hosts, must be accounted for (Sutmoller, 2002). The use of fencing has been severely criticised by conservationists, because the fences sometimes have blocked migration routes and access of wildlife to water, resulting in ecological disturbances and wildlife mortality. The necessity for fencing is increasingly questioned -- the argument being that vaccination alone should be sufficient to protect livestock from infection (Thomson et al., 2003).

FMD is the most important livestock disease in the world in terms of economic impact. The reason for this is not only due to the ability of the disease to cause losses of production, but also related to the reaction of Veterinary Services to the presence of the disease and to restrictions on the trade of animals both locally and internationally(James & Rushton, 2002). In 1997, FMD virus caused widespread outbreaks in Taiwan, which resulted in the immediate closure of the export trade to Japan and South Korea and a loss of four billion dollars, 90% of which was lost export earnings(Perry & Randolf, 2003). In 2001, the Netherlands slaughtered 200,000 animals vaccinated against FMD as part of the control program during an outbreak that had spread from UK, in order to re-establish the country's FMD trading position as quickly as possible (Pluimers, et al., 2002). Even without the loss of a significant export trade, the cost of the 2001 outbreak to the UK economy was over eight billions dollars(Thompson et al., 2002).

Factors which may enhance the outbreak including virus contamination in transport vehicle, water supplies, pets, birds, meat products and human. In 1995,

Cleland et al. studied FMD in villages in northern Thailand found that the total number of cattle and buffaloes purchased in the previous year, the number of neighboring villages which shared a common water source and whether agriculture was the most important source of cash income for the village were significantly associated with FMD outbreak in villages in northern Thailand (Cleland, et al., 1995). Gloster identified high density of livestock as a risk factor of FMD outbreak (Gloster, 1982).

The Department of Livestock Development (DLD) of the Royal Thailand government is the organization in charge of the control and possible eradication of FMD from Thailand. FMD control measures initially comprised strict control of animal movement, vaccination program, animal quarantine, sanitary control, outbreak investigation, field surveillance and slaughtering of sick animal (Chaisrisongkram, 1993).

Vaccination program is considered to be the most favorite strategies for controlling the disease (Gregory, 2001). DLD also supply officers as vaccinator, free of charge FMD vaccine to cattle farmers and a cheap FMD vaccine to pig farmers. Anyhow FMD still outbreak in Thailand. This might due to the fact that the outbreak of FMDV can spread out into the surrounding areas rapidly in the low temperature and high humidity atmosphere (Leech, 1981). The World Organization for Animal health (OIE) has recommended the control measure which should be integrated such as vaccination, surveillance, animal movement control, etc.

In prevention and control of FMD, many studies found biosecurity being one of effective strategies which could reduce disease especially FMD introduce in to the

pig farm. Pinto and Urcelay (2003) found that pig farms with low biosecurity practice theoretically have higher potential for introduction and spread of diseases (Julio Pinto & Santiago Urcelay, 2003). Boklund et,al (2004) also found that biosecurity for the transport vehicles and visitors was important in fattening pig farms (Boklund, et al., 2004). This result was similar to the study of Cleland et,al in 1996 and Sutmuller et,al in 2003 which showed that a good biosecurity practice could reduce disease outbreak in farm level, and it was an important strategy to control FMD (Cleland, et al., 1996; Sutmoller & Olascoaga, 2003).

The study of FMD status and identification the risk factors associated with FMD including biosecurity practices in pig and cattle farms, slaughter houses and live animal markets might supply the information to encourage mechanism of control and prevention FMD in study area. The present study is designed to determine the FMD status, to identify risk factors of FMD in pig and cattle farms, slaughter houses and live animal markets and FMD control strategies in pig farm in Chiang Mai and Lamphun provinces in Northern Thailand.