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# AN OUTBREAK OF FOOT AND MOUTH DISEASE IN EGYPT IN 1998 THE NEED OF REEVALUATION OF VACCINATION PROGRAM AGAINST FOOT AND MOUTH DISEASE IN EGYPT

(With One Table and One Figure)

By

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## وباء مرض الحمى القلاعية فى مصر عام ١٩٩٨
## الحاجة إلى إعادة تقييم برنامج التحصين ضد مرض الحمى القلاعية فى مصر

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تمت دراسة وباء مرض الحمى القلاعية الذى انتشر فى الحيوانات المحصنة والغير محصنة. كان الهدف الأساسى لهذا البحث هو تحديد سبب فشل برنامج التحصين فى حماية الحيوانات المحصنة ومنع ظهور أوبئة لمرض الحمى القلاعية. تم فحص الحيوانات (١٠٠ بقرة و ١٠٠ جاموسة ) من الناحية الإكلينيكية و السيرولوجية (باستخدام اختبار الاليزا) والتكنولوجيا الحيوية (باستخدام التفاعل التبلمرى المتسلسل) . تم فحص الحيوانات مرتين متتاليتين يفصل بينهما ثلاثة أشهر. فى اختبار الاليزا الأول تم تشخيص المرض فى ٤٦ بقرة (٤٦%) و ١٦ جاموسة (١٦%) بينما لم يتم التعرف على أى حالة ايجابية فى اختبار الاليزا الثانى. باستخدام التفاعل التبلمرى المتسلسل الأول تم التعرف على الحامض النووى لفيروس مرض الحمى القلاعية فى ٥٦ بقرة (٥٦%) و ٣١ جاموسة (٣١%) بينما ٨ بقرات (٨%) و ١١ جاموسة (١١%) أعطت نتائج إيجابية مع الاختبار الثانى . الحيوانات التى أعطت نتائج إيجابية مع اختبار التفاعل التبلمرى المتسلسل الثانى كانت مستمرة الإصابة بالرغم من أنها تبدو سليمة ظاهريا. تم التعرف على ثلاثة أنواع سيرولوجية للفيروس وهى O(٦٤،٣%) و A(٢١،٤%) و C (١٤،٣%). نتائج هذا العمل أثبتت أن اختبار التفاعل التبلمرى المتسلسل سريع وحساس فى تشخيص و تصنيف فيروس مرض الحمى القلاعية فى الحيوانات المصابة ومستمرة الإصابة التى تبدو سليمة ظاهريا ويصعب التعرف عليها باستخدام طرق التشخيص التقليدية وهى من أخطر صور المرض من الناحية الوبائية كما أن هذا الاختبار أشد حساسية من اختبار الاليزا. من أهم نتائج هذا البحث التعرف على النوعين السيرولوجيين (A) و (C) اللذان لم يسبق تسجيلهما فى مصر من قبل. من خلال هذه النتائج نعتقد انه من المستحيل منع

وباء مرض الحمى القلاعية فى مصر باستخدام اللقاح الحالى المحضر من النوع السيرولوجى (O) فقط لهذا يجب إعادة النظر فى برنامج التحصين ضد مرض الحمى القلاعية فى مصر.

## SUMMARY

An outbreak of foot and mouth disease (FMD) that spread between vaccinated and nonvaccinated cattle and buffaloes was investigated. This investigation was carried out to determine the cause of apparent failure of vaccination program to protect vaccinated animals and prevent outbreaks of FMD. Clinical, serological (using indirect enzyme linked immunosorbent assay "ELISA"), and biotechnological (using reverse transcription polymerase chain reaction "RT-PCR") examinations were performed. The animals were examined by ELISA and RT-PCR two successive times three months apart. Clinical examination revealed great variations in clinical signs among infected animals. ELISA was positive with 46 cattle (46%) and 16 buffaloes (16%) in the first examination while no positive results were detected in the second examination. FMD virus RNA was detected in 56 cattle (56%) and 31 buffaloes (31%) with the first RT-PCR assay while 8 cattle (8%) and 11 buffaloes (11%) were positive with RT-PCR in the second examination. The animals which gave positive result with second RT-PCR examination seemed to be persistently infected with FMD virus without showing any clinical signs. Three FMD virus serotypes O (64.3%), A (21.4%) and C (14.3%) were detected and differentiated by RT-PCR using serotype specific primers. The result of this investigation proved that RT-PCR is rapid and sensitive assay in diagnosis and typing of FMD virus serotypes in diseased and apparently healthy persistently infected animals which could not be detected by other methods of diagnosis and seemed to be the most dangerous form of the disease from epidemiological point of view.RT-PCR is more sensitive than ELISA.The most important result of this work is detection of FMD virus serotypes A and C which are not recorded in Egypt before this study. This result may explain the cause of failure of vaccination program in Egypt in prevention of FMD outbreaks. We suggest that it may not be possible to prevent FMD outbreaks using currently available vaccine prepared from serotype O only, therefore vaccination program against FMD virus in Egypt should be reevaluated.

***Key words:*** *Foot and Mouth Diesease, vaccination, ELISA, RT-PCR.*

# INTRODUCTION

Foot and mouth disease (FMD) is one of the most economically important diseases of the cloven-hoofed animals (Kitching, 1992; Hutber et al., 1999; Suryanarayana et al., 1999). The speed with which it spread and its ability to change its antigenic identity makes it very threating to the beef and dairy industries of many countries (Stram et al., 1995).

The causative virus of this disease belongs to family Picornaviridae, genus Aphthovirus (Hofner et al., 1993: Vangrysperre and De Clercq, 1996). The virus exists in 7 immunologically distinct serotypes (Pattnaik et al., 1997); its genome is an 8.5 kilobase, single strand of RNA (Stram et al., 1995). The virion consists of four proteins, VP1 to VP4, of which VP1 is the main protein determining antigenic identity of the virus (Archarya et al., 1989 and Logan et al., 1993).

This highly contagious important disease is characterized by the appearance of vesicles located in and around the mouth and on the feet, and has a very high morbidity but low mortality rate in adults. The death of young animals, the loss in meat and milk yield and the severe secondary complications lead to important economic losses (Vangrysperre and De Clercq, 1996).

Because of the highly contagious nature and rapid spread of infection substantial progress has been made in control of FMD (Suryanarayana et al., 1999). The impotent requirements for the control of FMD are rapid laboratory diagnosis and epidemiological investigation (Hofner et al., 1993). A fast and sensitive diagnosis of FMD that clearly differentiates it from other related diseases is of great importance in case of suspected outbreak and is needed to limit the spread of the disease. The quick identification of the type of virus will be useful when emergency vaccination is required, and it is necessary to track the origin of an outbreak (Stram et al., 1995; Vangrysperre and De Clercq, 1996).

At present, detection and typing of FMDV are carried out mostly by serological tests (Rweyemamau, 1984; Hamblin et al., 1986; Kitching et al., 1989). However, a significant number of samples fail regularly to give serological positive reaction and thus, virus isolation in primary culture still to be attempted for serology negative samples (Suryanarayana et al., 1999). Virus isolation is sensitive method, but it is laborious, slow and expensive. In addition, amplification of FMDV in tissue culture involves the risk of disseminating the virus in environment (Kitching et al., 1989; Moss and Hass, 1999). At the same time, these methods do not provide complete information on the epizootiological

tracing of the viruses and the ability of vaccines to protect against circulating virus (Kitching et al., 1989; Rodriguez et al., 1994). In order to avoid the passaging of the virus in tissue culture, several other tests based on the detection of the virus genome have been evolved, of which reverse transcription polymerase chain reaction (RT-PCR) is most sensitive (Suryanarayana et al., 1999).

A strategy for RT-PCR was developed to detect and differentiate the serotypes of FMD virus quickly and accurately (Callens and De Clercq, 1997) in different clinical samples including vesicular fluids (Rodriguez et al., 1992), tissue and oropharyngeal fluid (Amaral-Doel et al., 1993), organ extract (Lochar et al., 1995), tongue and foot epithelia (Niedbalski et al.,1998) and nasal swabs (Moss and Hass 1999). The use of RT-PCR technique to amplify specific nucleic acid regions offer the unique possibility of combining swift viral detection with the production of genetic material suitable for sequencing and other methods of molecular epidemiological analysis (Hofner et al., 1993). Various serotype specific primer sets have been used to amplify FMD virus sequences in order to type the virus (Rodriguez et al., 1992; Suryanarayana et al., 1999).

In Egypt, vaccination is the only pragmatic approach to control FMD. Strict quality control measures are essential to supply potent vaccine to the field application (Suryanarayana et al., 1998).There are a number of elements should be achieved in the production of FMD vaccine such as the selection of appropriate strain (s), which have a direct bearing on the quality of the immune response. Equally, development of effective immunity depends on application of suitable vaccine (Doel, 1999).

In last few years, many FMD outbreaks were recorded in vaccinated and nonvaccinated cattle and buffaloes in Egypt. There is no accurate explanation of cause of reoccurrence of these outbreaks inspite of vaccination of animals. Here we investigated outbreak of FMD occurred in Egypt in summer of 1998 trying to explain the cause of outbreak reoccurrence .We report on the very fast and sensitive detection and differentiation of FMD virus serotypes using RT-PCR. In addition, comparison between RT-PCR as a recent method and indirect ELISA as a traditional method for diagnosis of FMD virus was recorded.

## MATERIALS and METHODS

**Animals:** 100 cattle and 100 buffaloes were used in the study, some of them were suffered from clinical signs of FMD and others were apparently healthy.

**Nasal swabs:** 2 nasal swabs were collected three months apart from each examined animal for extraction of viral RNA.

**Blood:** 5 ml blood was collected twice three months apart from each examined animal to obtain serum.

**Reference viruses:** 3 FMD virus serotypes O, A and C (used in ELISA) were kindly provided by Dr. M Taniyama, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

**1- Clinical examination:**

Clinical examination of all investigated animals was performed three times weekly, body temperature, respiratory and heart rates as well as pulse were recorded, any abnormal clinical signs were reported. After serotyping of the virus the relation between the serotype and the clinical signs were recorded.

**2- Indirect ELISA:**

All the reagents were used in 50 UL. Sets of plates (96-well flat bottomed high binding EIA microplates "Greiner 655061") were coated with reference FMD virus, then coating buffers (carbonate-bicarbonate buffer "0.05M pH 9.6", phosphate buffered saline "0.04M pH 7.4" and tris-NaCl buffer "0.02M pH 7.4") were assessed. EIA plates were incubated at 37°C for 80 min. After that the coated plates were washed (3X in washing buffer). The tested sera were diluted to 1:500 in ELISA diluent (phosphate buffered saline supplemented with 20.75 g/litre sodium chloride and 0.1% v/v Tween 20 containing 3%w/v lactalbumin hydrolysate, 5% v/v healthy calf serum and 5% healthy rabbit serum) and were added to the coated plates, incubated at 37°C for 1 hour. The plates were washed (3X in washing buffer). Anti-guinea pig Ig-conjugate (Dakopatts P141) diluted to 1:2500 in ELISA diluent was added to all wells and allowed to react for 45 min at 37°C. The plates were washed and substrate (O-phenylenediamine "OPD" dihydrochloride, substrate solution was prepared in 0.01M phosphate citrate buffer pH 5.0 just before dispensing into the wells, the substrate solution was activated by 30% hydrogen peroxide "8ul/15 ml substrate solution") was dispensed to all wells. Substrate reaction was allowed to take place at 37°C in dark for 10-15 min and then stopped by the addition of stopper solution. The OD

was read at 490nm using microplate reader model Σ 960 (Metertech Inc). Indirect ELISA were performed according to Bhattacharya et al. (1996).

**3- RT-PCR:**

**(a)** Synthetic oligonucleotide primers:

The primers for FMD serotypes (Pharmacia Biotech.) were designed by comparison of published sequences of FMD virus.4 primers were used in the present study. More specifications of these primers are listed in Table (1).

**(b)** RNA extraction:

Total RNA was extracted with TRIzol Reagent (Gibco BRL). 100 ul sample (nasal swab extracted in phosphate buffer saline) was mixed with 900 ul TRIzol Reagent.The samples were incubated for 5 min at room temperature, addition of equal amount of chloroform, precipitation of RNA with isopropanol and washing of RNA with 75% ethanol. The purified RNA was resuspended in 20ul Rnase free-water and redissolved by incubating for 10 min at 55 °C.

**(c)** Reverse transcription:

First strand cDNA synthesis was performed for 15 min at 37°C in a 10ul reaction mix containing the followings:- (1)50mM Tris-Hcl (pH 8.3), (2)75mM KCl, (3)3mM MgCl2, (4)10mM dithiothreitol, 0.125 mM (each) dNTP, (5)5uM ( each ) downstream primer, (6) 100 units Moloney murine leukemia virus reverse transcriptase (GibcoBRL) and (7)0.5 ug RNA . This was followed by heating for 5min at 95°C, in order to denature RNA-cDNA hybrid. Samples were chilled and stored at -20°C.

**(d)** PCR amplification:

A PCR mix (10ul) consists of the following: 10mMTris-Hcl (pH8.8), 50mM KCl, 1.5mM MgCl2, 0.1% Triton X-100, 0.05mM (each) dNTP, 0.6um (each) primer, 0.25 units Taq polymerase (Promega) and 2% cDNA reaction mix. Amplification and reverse transcription was performed on a gene Amp CR system 9600 (Perkin Elmer) using program as follow: (1) 1 min at 95°C (2) 15 sec at 95°C (3) 1 min at 60°C (4) 6 min at 60°C, repeating steps (2) and (3) for 35 cycles.

**(e)** Gel electrophoresis:

The samples were electrophoresed at 100 V for 30 min in TAE buffer on 1.5% agarose gels, stained with ethidium bromide (0.6ug/ml). DNA molecular weight marker type 100bp DNA ladder (Gibco BRL) was applied to identify the size of the PCR products.Negative control for RT-PCR were performed by adding of distilled water instead of the

primers. RT-PCR was performed as described by Vangrysperre and De Clercq (1996) and Moss and Hass (1999).

## RESULTS

**1- Clinical examination:**

Clinical examination of investigated animals revealed great variation in clinical signs among infected animals varied from slight rise of body temperature up to typical FMD symptoms with vesicles in the mouth and on the feet.

**2- Indirect ELISA:**

**(a)** First examination: 46 cattle (46%) and 16 buffaloes (16%) were positive.

**(b)** Second examination: No positive results were detected.

**3- RT-PCR:**

**(a)** First examination: FMD viral nucleic acid (RNA) was detected in 56 cattle (56%) and 31 buffaloes (31%).

**(b)** Second examination: FMD viral RNA was detected in 8 cattle (8%) and 11 buffaloes (11%). All these animals were apparently healthy.

**(c)** By using combining of four primers sets (P33-P38, P33-P87 and P33-P40 three FMD virus serotypes (Figure 1) O (36 out of 56 "64.3%"), A (12 out of 56 "21.4%") and C (8 out of 56 "14.3%") were detected among infected cattle while only serotype O was recorded in buffaloes. All persistently infected cattle and buffaloes were infected with serotype O.

The most severe signs were observed in animals infected with serotype A or C and in animals in contact with animals have persistent infection.

## DISCUSSION

A great variation in clinical signs was observed among infected animals investigated in the present study. FMD may appear as an acute, mild or subclinical infection, dependent upon the immune status of the herd, the level of challenge and the efficacy of vaccine used (Hutber et al., 1999). From the result of the present work we can add that the serotype of the causative virus play a very important role in determination of clinical signs of the disease. Severe clinical signs were observed in animals infected with serotypes A and C and animals in contact with animals persistently infected with FMD virus, the same observation have been recorded by Hutber et al. (1999).

FMD was observed in cattle and buffaloes investigated in our study but the percentage of infection among cattle (56%) was higher than that of buffaloes (31%) while persistent infection in buffaloes (11%) was higher than that observed in cattle (8%). Studies on buffaloes have shown that this species is susceptible to FMD and that the virus persists in it for long periods. The buffaloes act as true reservoirs of the virus and transmit it to cattle (Dawe et al., 1994; Gomes et al., 1997). However, in our study we could not confirm this observation as in some areas the cattle and buffaloes found to be infected with the same serotype whereas in other areas the serotypes detected in both are different.

Indirect ELISA was used for diagnosis of FMD in this study. Within the world reference laboratory for FMD, the ELISA has replaced the complement fixation and virus neutralization tests (Samuel et al., 1991; Kitching, 1992). The advantages of ELISA have been well documented (Hamblin et al., 1986; Ferris and Dawson,1988; Bergmann et al., 2000) . ELISA detected antibodies against any of the seven serotypes of FMD virus (Mackay et al., 1998; Sorensen et al., 1998). However, the result of the present work proved that ELISA is less sensitive than RT-PCR in diagnosis of FMD as ELISA failed to detect 10 infected cattle and 15 infected buffalo which were positive with RT-PCR and could not detect any of persistently infected animals.Failure of ELISA in diagnosis of some cases of FMD have been reported (Kitching et al., 1989; Bhatacharya et al., 1996). RT-PCR assay is 500-fold more sensitive than a conventional indirect ELISA (Rodriguez et al., 1994).

Fast and accurate detection of FMD outbreaks is needed to limit spread of the disease (Stram et al., 1995) through directing vaccine selection and epidemiological studies (Vangrysperre and De Clercq, 1996). Current detection systems are usually more time demanding and less specific (Vangrysperre and De Clercq, 1996). Therefore, we used RT-PCR in diagnosis and typing of FMD virus in our investigation. A strategy for RT-PCR was developed to detect and to differentiate the seven serotypes of FMD virus simultaneously, quickly and accurately (Meyer et al., 1991; Amaral-Doel et al., 1993; Prato Murphy et al., 1994; David et al., 1995; Lochar et al., 1995; Callens and De Clercq, 1997; Callens et al., 1998). The value of the RT-PCR is that it can rapidly facilitate the molecular analysis of field isolates and thus provide important epidemiological information regarding the source of outbreak (Reid et al., 1998).

Serotyping of FMD virus was an important aim of our work. In countries where FMD is endemic, large number of samples are to be

serotyped regularly (Suryanarayana et al., 1999).Serotype-specific primers selected from VP1 genome were used for typing of FMD virus in our study. Selected specific primers in VP1 gene region when used permitted the identification of serotypes of FMD virus (Laor et al., 1992; Lochar et al., 1995; Vangrysperre and De Clercq, 1996) as capsid protein VP1 contains major antigenic determinants and is highly variable among different serotypes (Rodriguez et al., 1992). Therefore, the differences in VP1 sequences are the basis for developing RT-PCR tests to identify different serotypes (Rodriguez et al., 1992; Stram et al., 1995). Three serotypes O, A and C were detected in this study using RT-PCR. A RT-PCR assay for the specific detection and identification of viral sequences that correlate with established serotypes of FMD virus has been developed (Rodriguez et al., 1994; Stram et al., 1995; Niedbalski et al., 1998; Nunez et al., 1998a). Genetic distance observed between complete sequences of different serotypes of FMD virus (Domingo et al., 1990; Martin et al., 1998). Concerning the typing of FMD virus, Rodriguez et al. (1994) mentioned primers could differentiate serotypes O. A and C. Different serotypes of FMD virus could be identified and differentiated using primers selected from the VP1 genome region based on the different lengths of the respective amplified segments (Vangrysperre and De Clercq, 1996;Nunez et al., 1998b). Specific, rapid and highly sensitive detection was achieved for FMD virus serotypes O, A and C using serotype specific primers RT-PCR (Suryanarayana et al., 1999). Since the primers used in the assay amplify selectively the variable region of VP1 of FMD virus the molecular product might reveal strain variation (Tulasiram et al., 1997).

The result of RT-PCR was achieved within 4 hours after collection of our samples. Using RT-PCR, viral genomes were detected in less than 24 hours (Marqardt et al., 2000).

Animals persistently infected with FMD virus are propaply more important in the transmission of FMD than infected animals as these animals fail to show clinical signs and could not detected by currently available methods of diagnosis (Kitching, 1992) therefore we used RT-PCR in detection of persistently infected animals successfully in the present investigation. The advantage of RT-PCR has made possible to detect the virus at a very low concentration in sample (Laor et al., 1992; Suryanarayana et al., 1999). Detection of small amounts of FMD virus is especially important for the identification of persistently infected animals (Rodriguez et al., 1994). Usefulness of the primers have been reported in detecting inapparent or persistent FMD infection in susceptible animals

by RT-PCR (Pattnaik et al., 1997). RT-PCR are superior for detecting of persistent FMD virus infection (Donn et al., 1994) and also applies to prodromal stages of the disease as well as subclinical cases (Moss and Hass, 1999).

The results of this study revealed that the highest percentage of infection was caused by serotype O (64.3%). A stable genome associated with poor virus immunogenicity may be responsible for the prevalence of FMD virus type O in the nature (Razdan et al., 1996).

FMD could be under control by means of blanket vaccination (Chen et al., 1999) and safe protection can obviously achieved only if vaccine and challenge strain are homologous (Straub, 1995) as differences were seen between antibodies acting against different FMDV serotypes (Muller et al., 1989). Control of the disease by vaccination is complicated by antigenic diversity of the virus which is reflected in the existence of 7 distinct serotypes and continuous surveillance of the field situation (Jadhao et al., 1996), it is necessary to choose a vaccine that contains a strain antigenically similar to the outbreak strain (Kitching, 1992). Therefore, the ban on vaccination did not result in an decrease of FMD outbreaks (Leforban, 1999). From the results of the present investigation, it is clear that FMD occurs in single, multiple vaccinated as well as nonvaccinated animals. It was concluded that a single vaccination does not protect cattle against FMD virus (Muller et al., 1989; Straub, 1990). In multivaccinated animals, our data indicated that the virus from the outbreak in vaccinated animals was variant from used vaccine virus which could escape neutralization by antibodies against vaccine virus. As control of FMD in Egypt is based only on vaccination, any variation between serotype of outbreak virus and serotype of vaccine virus leads to appearance of outbreak. Despite vaccination FMD outbreaks continue to occur within vaccinated animals (Hafez, 1990; Hafez, 1991; Gleeson et al., 1995; Woolhouse et al., 1996; Hutber et al., 1998; Suryanarayana et al., 1998).

From the result of our work we can conclude that RT-PCR is rapid and very sensitive assay in diagnosis of FMD and typing of FMD virus and can detect both clinically ill and apparently healthy persistently infected (which could not be detected by other methods of diagnosis and considered the most dangerous form from epidemiological point of view) animals. RT-PCR standardized here can be employed for diagnosis and typing of FMD virus directly from field samples and proved to be more sensitive than indirect ELISA. Three serotypes of FMD (O, A and C) virus are exist in Egypt, therefore, the currently available used vaccine

prepared from serotype O only could not protect the animals and prevent FMD outbreaks and so, vaccination program against FMD in Egypt should be reevaluated.

## REFERENCES

*Amaral-Doel,CM;Owen,NE;Ferris,NP;Kitching,RP and Doel,TR (1993):* Detection of foot and mouth disease viral sequences in clinical specimens and ethylene-inactivated preparations by the polymerase chain reaction. Vaccine, 11(4): 415-421.

*Archarya,R; Fry,E; Stuart,D; Rowlands,D and Brown,F (1989):* The three dimensional structure of foot and mouth disease at 2.9 A resolution. Nature, 337: 709-716.

*Bergmann, IE; Malirat,V; Neitzert,E; Beck,E; Panizzutti,N; Sanchez,C and Falczuk,A (2000):* Improvement of a serodiagnostic strategy for foot and mouth disease virus surveillance in cattle under systematic vaccination: a combined system of an indirect ELISA-3ABC with an enzyme linked immunoelectrotransfer blot assay. Arch.Virol.,145 (3): 473-489.

*Bhattacharya,S; Pattnaik,B and Venkatramanan,R (1996):* Development and application of sandwich enzyme liked immunosorbent assay (ELISA) for type identification of foot and mouth disease (FMD) virus in direct field materials. Ind.J.An.Sci., 66 (12): 1201-1209.

*Callens,M and De Clercq,K (1997):* Differentiation of the seven setrotypes of foot and mouth disease virus by reverse transcriptase polymerase chain reaction. J.Virol.Methods, 67 (1): 35-44.

*Callens, M; DeClercq, K; Gruia, M and Danes, M (1998) :* Detection of foot and mouth disease by reverse transcription polymerase chain reaction and virus isolation in contact sheep without clinical signs of foot and mouth disease. Vet.Q., 20 Suppl 2: s37-40.

*Chen,BJ;Sung,WH and Shieh,HK (1999):* Managing an animal health emergency in Taipei China: foot and mouth disease. Rev.Sci.Tech., 18 (1): 186-192.

*David,,D; Stram,Y; Yadin,H; Trainin,Z and Becker,Y (1995):* Foot and mouth disease virus replication in bovine skin langerhans cells under in vitro conditions detected by RT-PCR . Virus Genes, 10 (1) :5-13.

*Dawe,PS; Sorensen,K; Ferris,NPBarnett,IT; Armstrong,RM and Knowles,NJ (1994):* Experimental transmission of foot and mouth disease virus from carrier African buffalo (Syncerus caffer) to cattle in Zimbabwe.Vet.Rec., 134: 211-215.

*Doel,TR (1999):* Optimization of the immune response to foot and mouth disease vaccines. Vaccine, 17 (13-14): 1767-1771.

*Domingo,E;Muteu,MG;Martinez,MA;Dopaze,J;Moya,A and Sobrino,F (1990):* Genetic variability and antigenic diversity of foot and mouth disease virus. Applied virology research , 2:233-266. (Eds.) Drustak,E;Marusky,P;Murphy,F and Van Regenmorted, M Plenum Press, New York.

*Donn,A;Martin,LA and Donaldson,AI (1994):* Improved detection of persistent foot and mouth disease infection in cattle by polymerase chain reaction. J.Virol.Methods, 49 (2): 179-186.

*Ferris,NP and Dawson,RS (1988):* Routine application of enzyme linked immunosorbent assay in comparison with complement fixation for diagnosis of FMD and SDV. Vet. Microbiol., 16: 201-209.

*Gleeson, LJ; Chamnanpood, P; Cheunprasert, S; Srimasartitkol, N and Trisasarom,A (1995):* Investigation of an outbreak of foot and mouth disease in vaccinated dairy cattle. Aust.Vet.J., 72 (1): 21-24.

*Gomes,I;Ramalho,AK and Auge de Mello,P (1997):* Infectivity assays of foot and mouth disease virus: contact transmission between cattle and buffalo (Bubalus bubalis) in the early stages of infection. Vet.Rec., 140: 43-47.

*Hafez,SM (1990):* Studies on the control of foot and mouth disease in Saudi dairy farms. Report I, Riyadh, Ministry of Agriculture and water , King Abdul Aziz ,City of Science and Technology. 22-24.

*Hafez,SM (1991):* Studies on the control of foot and mouth disease in Saudi dairy farms. Report II, Riyadh, Ministry of Agriculture and water , King Abdul Aziz , City of Science and Technology. 29-31.

*Hamblin,C;Barnett,ITand Crowther,JR (1986):* A new enzyme linked immunosorbent assay (ELISA) for the detection of antibodies against foot and mouth disease virus. II.Application. J.Immunol. Methods, 93: 123-129.

*Hofner, MC; Carpenter, WC and Donaldson, AI (1993):* Detection of foot and mouth disease virus RNA in clinical samples and cell culture isolates by amplification of the capsid coding region. J.Virol.Methods, 42 (1): 53-61.

*Hutber,AM;Kitching,RP and Conway,DA (1998):* Control of foot and mouth disease through vaccination and the isolation of infected animals. Trop.Animal health and production, 30 : 217-227.

*Hutber,AM;Kitching,RP and Conway,DA (1999):* Predicting the level of herd infection for outbreaks of foot and mouth disease in vaccinated herds. Epidemiol.Infect., 122 (3): 539-544.

*Jadhao, SJ; Pattnaik, B; Venkataramana, R; Rai, DV and Mukhopadhyay, AK (1996):* Studies on trypsin-sensitive antigenic site of type O foot and mouth disease virus with monoclonal antibodies. Ind.J.An.Sci., 66 (12): 1226-1233.

*Kitching,RP (1992):* The application of biotechnology to the control of foot and mouth disease virus. Br.Vet.J., 148 (5): 375-388.

*Kitching,RP;Knowles,NJ; Samual,AR and Donaldson,AI (1989):* Development of foot and mouth disease strain characterization review . Tropical Anim.Hlth.Prod.21: 153-166.

*Laor,O;Torgersen,H;Yadin,H and Becker,Y (1992):* Detection of FMDV RNA amplified by the polymerase chain reaction (PCR). J.Virol.Methods, 36(3): 197-207.

*Leforban,Y (1999):* Prevention measures against foot and mouth disease in Europe in recent years. Vaccine, 17 (13-14): 1755-1759.

*Lochar, F; Suryanarayana, VV and Trantchin, JD (1995):* Rapid detection and characterization of foot and mouth disease virus by restriction enzyme and nucleotide sequence analysis of PCR products. J.Clin.Microbiol., 33 (2): 440-444.

*Logan,D; Abu Ghazaleh ,M and Blackmore,W (1993):* Structure of a major immunologic site on foot and mouth disease virus. Nature, 362: 266-268.

*Mackay,DK; Forsyth,MA; Davies,PR; Berlinzani,A;Belsham,GJ;Flint,M and Ryan,MD (1998):* Differentiating infection from vaccination in foot and mouth disease using a panel of recombinant, non-structural proteins in ELISA. Vaccine, 16 (5): 446-459.

*Marquardt,O;Rahman,MM and Freiberg,O (2000):* Genetic and antigenic variance of foot and mouth disease virus type Asia 1. Arch.Virol., 145 (1): 149-157.

*Martin,MJ;Nunez,JI;Sobrino,F and Dopazo,J (1998):* A procedure for detecting selection in highly variable viral genomes: evidence of positive selection in antigenic regions of capsid protein VP1 of foot and mouth disease virus. J.Virol.Methods,74 (2):215-221.

*Meyer,RF;Brown,CC;House,C;House,JA and molitor,TW (1991):* Rapid and sensitive detection of foot and mouth disease virus in tissues by enzymatic RNA amplification of the polymerase gene. J.Virol.Methods, 34 (2): 161-172.

*Moss,A and Haas,B (1999):* Comparison of the plaque test and reverse transcription nested PCR for the detection of FMDV in nasal swabs and probang samples. J.Virol.Methods, 80 (1): 59-67.

*Muller,HK;Villinger,F;Griot,C;Ackermann,M;Bruckner,L and Kihm,U (1989):* The effectiveness of foot and mouth disease vaccines in Switzerland. I.Screening tests and herd immunity. Schweiz.Arch . Tierheilkd., 131 (7): 379-386.

*Niedbalski, W ;Adam, KH and Marquardt, O (1998):* Quantitation of foot and mouth disease virus genomes in bovine tissue by competitive RT-PCR. J.Virol.Methods,72 (2): 237-242.

*Nunez,JI;Blanco,E;Hernandez,T;Dopazo,J and Sobrino,F (1998a):* RT-PCR in foot and mouth disease diagnosis. Vet.Q., 20 Suppl 2: S34-36.

*Nunez,JI; Blanco,E; Hernandez,T; Gomez-Tejedor,C; Martin,MJ; Dopazo,J and Sobrino, F (1998b):* A RT-PCR for the differential diagnosis of vesicular virus diseases of swine. J.Virol. Methods., 72 (2): 227-235.

*Pattnaik, B; Sanyal, A; George, M; Tosh, C; Hemardi, D and Venkataramana,R (1997):* Evaluation of primers for PCR amplification of RNA polymerase gene sequences of foot and mouth disease virus. Acta Virol.,41 (6): 333-336.

*Prato Murphy,ML; Meyer,RF;Mebus,C;Schudel,AA and Rodriguez,M (1994):* Analysis of sites of foot and mouth disease virus persistence in carrier cattle via the polymerase chain reaction. Arch.Virol.,136 (3-4): 299-307.

*Razdan,R;Sen,AK;Rao,BV and Suryanarayana,VV (1996):* Stability of foot and mouth disease virus, its genome and protein at 37 degrees C. Acta.Virol., 40 (1): 9-14.

*Reid, SM; Forsyth, MA; Hutchings, GH and Ferris, NP (1998):* Comparison of reverse transcription polymerase chain reaction, enzyme linked immunosorbent assay and virus isolation for the

routine diagnosis of foot and mouth disease. J.Virol.Methods, 70 (2): 213-217.

*Rodriguez, A; Martinez,L; Salas,E; Dopazo,J; Davila,M; Saiz,JC and Sobrino,F (1992):* Primer design for specific diagnosis by PCR of highly variable RNA viruses: typing of foot and mouth disease virus. Virology 189 (1): 363-367.

*Rodriguez,A; Nunez,,J; Nolasco,G; Ponz,F; Sobrino,F and De Plas,C (1994):* Direct PCR detection of foot and mouth disease virus.J.Virol.Methods, 47:345-349.

*Rweyemamu,MM (1984):* Antigenic variation in foot and mouth disease: studies based on the virus neutralization reaction. J.Biol.Stand.12: 323-337.

*Samuel,AR;Knowles,NJ;Samuel,GD and Crowther,JR (1991):* Evaluation of a trapping ELISA for the differentiation of foot and mouth disease virus strains using monoclonal antibodies. Biologicals19: 299-310.

*Sorensen,KJ; Madsen,ES; Salt,JS; Nqindi,J and Mackay,DK (1998):* Differentiation of infection from vaccination in foot and mouth disease by the detection of antibodies to the non-structural protein 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. Arch.Virol.,143 (8): 1461-1476.

*Stram,Y ;Molad,T; Gelman,B and Yadin,H (1995):* Detection and subtyping of foot and mouth disease virus in infected cattle by polymerase chain reaction and amplified VP1 sequencing. J. Vet. Diagn. Invest., 7 (1): 52-55.

*Straub,OC (1990):* Foot and mouth disease. Infection trial in cattle after a single vaccination. Dtw. Dtsch.Tierarztl.Wochenschr., 97 (5): 213-214.

*Straub,OC (1995):* Foot and mouth disease. Challenge of cattle after multiple vaccinations. Comp . Immunol.Microbiol.Infect.Dis., 18 (4): 253-257.

*Suryanarayana,VV; Tulasiram,P; Prabhundas,KS; Misra,LD and Natarajan,C (1998):* The foot and mouth disease virus type O outbreak of 1992 is not related to vaccine strain (O/R2/75). Virus genes, 16 (2): 167-172.

*Suryanarayana,VV; Madanamohan,B; Bist,P; Natarajan,C and Tratschin,JD (1999):* Serotyping of foot and mouth disease virus by antigen capture reverse transcriptase polymerase chain reaction. J.Virol. Methods, 80 (1) 45-52.

*Tulasiram,P; Mudit,T; Srinivas,K; Prabhudas,K; Natarajan,C and Suryanarayana,V (1997):* Antigenic variation in foot and mouth disease virus type Asia1 isolates circulated during 1993-1995 in India.Virus Genes, 15 (3): 247-253.

*Vangrysperre,W and De Clercq, K (1996):* Rapid and sensitive polymerase chain reaction based detection and typing of foot and mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses. Arch.Virol., 141 (4): 331-344.

*Woolhouse,ME;Haydon,DT;Pearson,A and Kitching,RP (1996):* Failure of vaccination to prevent outbreaks of foot and mouth disease. Epidemiol.Infect., 116 (3): 363-371.

**Table (1): Specification of used primers.**

| Primer | Sequence 5` - 3` | Expected serotype | Position |
|---|---|---|---|
| P33 | AGCTTGTACCAGGGTTTGGC | FMDV | 3832-3851 |
| P38 | GCTGCCTACCTCCTTCAA | FMDV-O | 3450-3467 |
| P87 | GTCATTGACCTCATGCAGACCCAC | FMDV-A | 3124-3147 |
| P40 | GTTTCTGCACTTGACAACACA | FMDV-C | 3259-3279 |

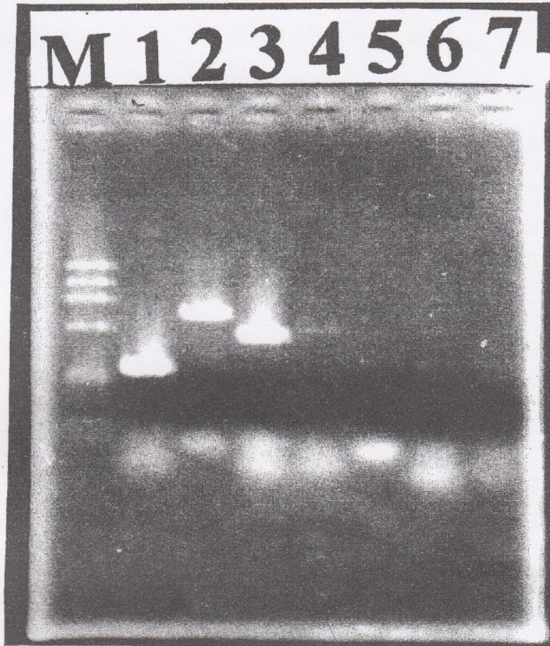


**Fig. 1:** Agarose gel electrophoresis of PCR products. Lane M (100 base pair DNA ladder), Lane 1 (FMD virus serotype O), Lane 2 (FMD virus serotype A), Lane 3 (FMD virus serotype C) and lanes 4-7 (negative control of RT-PCR).