Molecular epidemiology and serological study of FMD virus in Egypt 2014

Hala K. Abdelmegeed [1], Eman M. Abohatab [1], Khattab,O. M. [1] , Salem, S.A.H. [1] , Arafa, A. [2], Nashwa M. Helmy [3]

[1] Virology Research Dept., [2] NALQP, [3] Biotechnology Dept.
Animal Health Research Institute, Dokki, Giza, Egypt

ABSTRACT

Foot and mouth disease (FMD) is an enzootic disease among the livestock in Egypt, affecting practically all cloven-footed domesticated mammals.

In this study 161 out of 376 serum samples in percentage 42.8% were positive for FMD non structure proteins(NSP) antibodies which indicated the natural infection. ELISA kit used for FMDV antigen detection for the epithelial suspension from 42 field samples collected from various geographic locations 10 governorates are (El-Sharkia,Giza, Port Said, Assuit, Suize, Kafre El-Shake, Qina, Al-Gahrbia, Domiatte and Alexandria) Serotyping and molecular characterization by polymerase chain reaction PCR for (31) samples as FMD Serotype O , (2) Serotype A and (3) serotype SAT2 the samples collected from outbreaks in period November 2013 –July 2014 the season Autumn and winter for forming an Epidemiological map for 10 governorates of the circulating strains during this period

INTRODUCTION

Foot-and-mouth disease (FMD) is an economically devastating infectious disease of farm animals. The causative agent is FMDV, Genus aphthovirus of the Picornaviridae family (FAO,1984)

An outbreak of foot-and-mouth disease (FMD) in Egypt affected approximately 40,000 cattle and water buffaloes and killed more than 4,600 animals during February-March 2012. To investigate the etiology of the 2012 outbreak, specimens were collected from six governorates and analyzed using universal primers to amplify the 5' untranslated region (UTR) by reverse-transcription polymerase chain reaction. Only FMDV-SAT2 was detected, with an overall detection rate of 80.3 %. Complete VP1- and leader-proteinase-coding sequences, obtained from three isolates from three different governorates, were compared with previously reported sequences. Phylogenetic analysis of these sequences indicated that the circulating viruses were homogeneous and were closely related to topotype VII. Importantly, the newly emerged viruses were genetically closely related to strains isolated from Saudi Arabia, Sudan, Eritrea and Cameroon between 2000 and 2010 (Kandeil et al., 2012).

FMDV has a genome consisting of a single strand of positive-sense RNA. Consequently, the virus has a high mutation rate and may change, on a random basis, 1–8 nucleotides (nt) per replication cycle (. Domingo et al., 1995). Nucleotide sequencing of part or all of the genome region coding for the outer capsid polypeptide VP1 was first used to study the epidemiology of FMD by (Beck and Strohmaier 1987). who investigated the origin of outbreaks of types O and A in Europe over a 20-year period. Since then, genetic variability

has been used to individually characterize strains of FMDV and track their movement across international borders (Knowles and Samuel ,1997): and a large number of epidemiologic studies have been published (Knowles and Samuel ,2003) .

MATERIAL AND METHODS

1 - **Serum samples**

A total of 376 serum samples serum samples were randomly collected from vaccinated and diseased cattle in 10 Egyptian Governorates (El-Sharkia,Giza, Port Said, Assuit, Suize, Kafre El-Shake, Qina, Al-Gahrbia, Domiatte and Alexandria) for detection of NSP of FMDV antibodies to differentiation between naturally infected and vaccinate animals.

ELISA kit: The commercial PrioCHECK, FMD NS ELISA kit supplied by Prionic sweeden for **in vitro** detection of antibodies against FMDv in serum of cattle, sheep, goat and pigs. The kit is performed according to (Sorensen *et al*., 1998) instructors. Samples give percent of inhibition IP<50% considered negative and that give IP>50% considered positive.

2- Epithelial suspension:

One gram of epithelial samples per case were preserved in equal volume of glycerol – buffer saline and transported in ice- box at 4ºC to Virology Department, Animal Health Research Institute- Dokki.

The epithelial samples were prepared by grinding and centrifugation to obtain 0.2ml of the supernatant for virus detection and identification then serotyping determination of antigen according to (Kitching and Doanldson, 1987).

3-Serotyping of serum samples:

The typing of antibodies against FMD main serotypes (A , O, C,Asia1,SAT1,2 and SAT3) which were done by liquid phase **blocking** ELISA supplied by BDSL-Pirbright Laboratory (WRL) UK according to Hamblin et al., (1986 a) and Samuel et al., (1999).

4-Indirect sandwich ELISA for detection of antigens of FMD virus type A, O, SAT_2 according the protocol of OIE/FAO WRL for FMD, Pirbright Laboratory, UK according to (Hamblin et al. 1986 a, b).

5-RNA extraction

The reverse transcription - polymerase chain reaction (RT-PCR) assay was applied according to (Vangrysperre and De Clercq ,1996) for epithelial samples

Genotyping of positive FMD samples

Initial screening of tissue suspensions prepared from field cases was undertaken using real-time RT-PCR targeting the 3D region of the FMDV genome (Callahan et al., 2002). Briefly, RNA was extracted using QIAamp® viral RNA mini Kit (Qiagen),

Real Time PCR was used for detection of FMDV in (vesicular fluid and tongue epithelium tissues of infected animals) the samples gave positive results when previously tested FMDV antigen detection kit

Real Time PCR Kit QuantiTech Probe RT-PCRkit (Qiagen, Germany)

RESULTS

From the positive 161 serum samples for NSP- ELISA choose some positive samples from each governorates for tested by using LPB ELISA (liquid phase blocking ELISA) is considered to be international test for FMD virus antibodies serotyping as shown in **Table(2)**

Table (1): Serum samples results for NSP-FMD ELISA KIT

Name of Governorates	*No. of samples*	*No. of positive samples for FMD non structure proteins(+ve NSP)*
Elexandria	**20**	**7**
Al-Sharkia	**30**	**12**
Domiatte	**33**	**20**
Suiez	**36**	**10**
Kafre El-Shake	**35**	**16**
Assuit	**40**	**27**
Ei-Gharbia	**31**	**19**
Giza	**90**	**35**
Quina	**29**	**5**
Port Said	**32**	**10**
Total No.	**376**	**161**

Table (2): Serotyping of FMD virus Antibodies in serum samples using liquid phase blocking ELISA

No.	Governorates	A	SAT2	O	Priocheck NS
1	**Elexandria**	**neg**	**neg**	**+ve**	**+ve**
2	**Al-Sharkia**	**neg**	**+ve**	**neg**	**+ve**
3	**Domiatte**	**+ve**	**neg**	**neg**	**+ve**
4	**Suiez**	**+ve**	**neg**	**neg**	**+ve**
5	**Kafre El-Shake**	**neg**	**neg**	**+ve**	**+ve**
6	**Assuit**	**neg**	**+ve**	**neg**	**+ve**
7	**Ei-Gharbia**	**+ve**	**neg**	**neg**	**+ve**
8	**Giza**	**neg**	**neg**	**+ve**	**+ve**
9	**Quina**	**+ve**	**neg**	**neg**	**+ve**
10	**Port Said**	**neg**	**neg**	**+ve**	**+ve**

Indicates that LPB ELISA is specific, sensitive, quantitative, quicker to perform, antibodies serotyping as agreed with OIE (2000); Mackay *et al*., (2001) and Declerq (2002). The epithelial suspension from 42 field samples collected from infected cases in 10 governorates (El-Sharkia,Giza, Port Said, Assuit, Suize, Kafre El-Shake, Qina, Al-Gahrbia, Domiatte and Alexandria) during periodical surveillance were tested by ELISA kit used for FMDV Antigen detection as in **Table (3)**

Table (3) Relationship between field samples and identified FMD antigen serotypes by indirect sandwich ELISA:

Gov.	total	Serotype A	Serotype O	SAT2.
Alexandria	4	-	4	-
Giza	10	-	8	-
Gharbia	8	1	7	-
El-Sharkia	5	-	-	1
Domiatte	4	-	4	-
Qina	4	-	2	1
Suize	4	1	3	-
Port Said	3	0	3	-
Kafr Elshake	1	-	-	1
Total	**42**	**2**	**31**	**3**

Showed the results of identification and Typing of different samples (42) for field samples by indirect sandwich ELISA. The Results showed the positive samples were 2 samples out of 42 were serotype A, 31samples were serotype O, and 3 samples were SAT2. These results agree with (Sangula *et al.,* 2005). The detection of multiple serotype infection was possible by the use of the antigen detection ELISA. The same positive 36 samples by antigen detection ELISA submitted for Reverse transcription polymerase chain reaction (RT-PCR) which was done on FMD field samples for identification and serotyping which have to apply with large scale in veterinary diagnosis.

The procedure was used as described by (Reid *et al*., 2000) The value of the RT–PCR can rapidly facilitate the molecular analysis of field isolates for conformation the positive samples with virological traditional techniques requiring specialized equipment, expertise and refined reagents and has to be used in conjunction with current procedures for FMD diagnosis.

Details about the molecular characterization as Conventional PCR, (1),(2) and (3) and the real time RT-PCR figure (4)

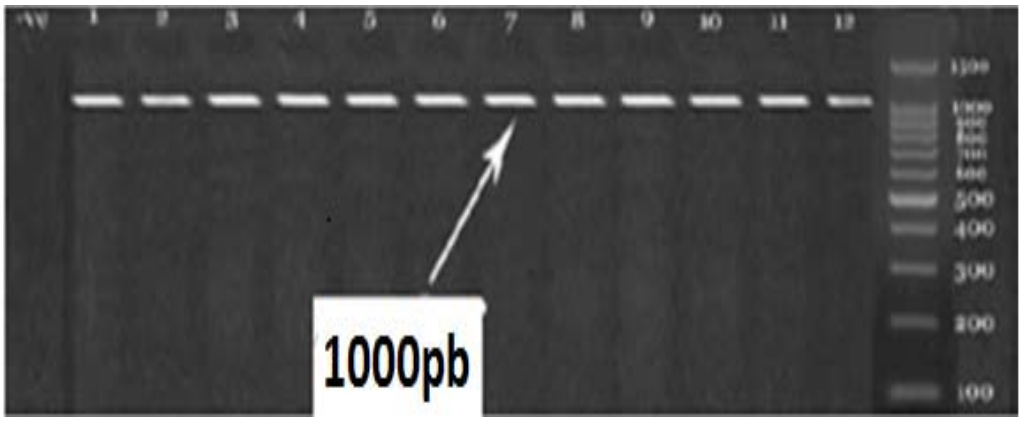

Figure (1): Molecular characterization using conventional RT-PCR for genotyping of FMD Genotyping of FMD type "O"
PCR Results of 31 clinical samples pooled in 11 groups using primers (f583+EUR) for FMD type-O.
Lane 0= Negative control,
Lane 1= Positive control of FMD-O,
lanes 2-12 (numbers 1-11) = positive clinical samples
Lane 13 = 100 bp ladder.
The positive results detected at 1000 pb.

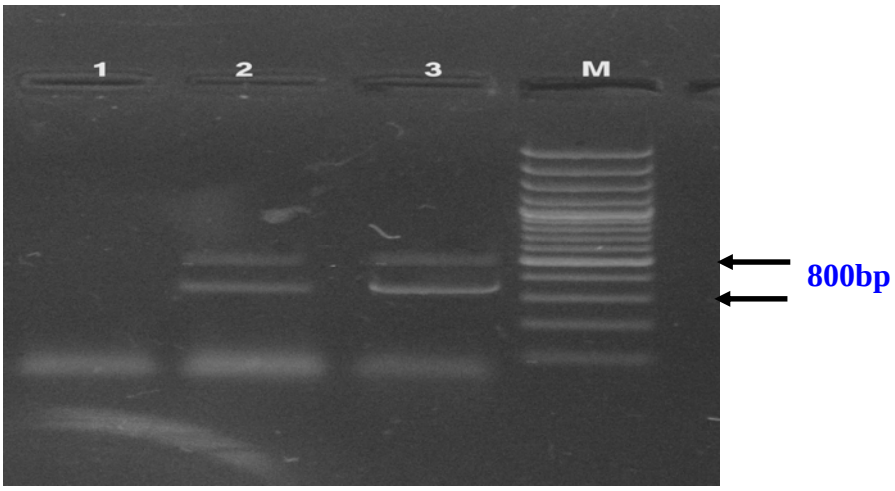

Figure (2): Genotyping of FMD type "A"

PCR Results of 2 clinical samples Pooled in one using primers (F562+NK72) for FMD type-A.
Lane 1= Negative control,
Lane 2= Positive control of FMD-A,
lanes 3 = positive clinical samples
M: Lane 4 = 1000 bp ladder.
The positive results detected at 800 pb.

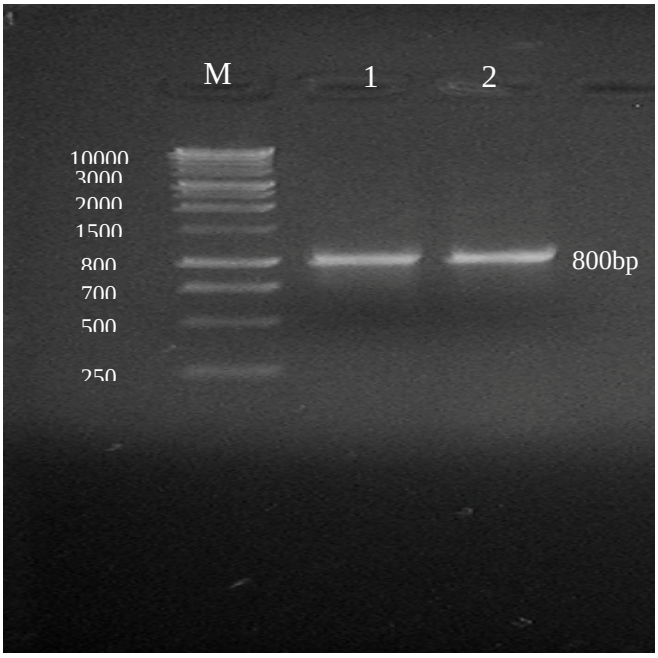

Figure (3): Genotyping of FMD type "SAT-2"

PCR Results of clinical samples using primers (209+208) for FMD type-SAT-2.
Lane M= 1000 bp ladder.
Lane 1= Positive control of FMD-SAT-2,
lanes 2 (numbers 3) = positive clinical samples
Lane 3 = Negative control,
The positive results detected at 800 pb.

The following primers and probe sets can be used for real-time PCR of FMDV:

'5UTRForward primer: CACYT YAAGR TGACA YTGRT ACTGG TAC;

Reverse primer: CAGATYCCRA GTGWC ICITG TTA

and TaqMan® probe: CCTCG GGGTA CCTGA AGGGC ATCC.

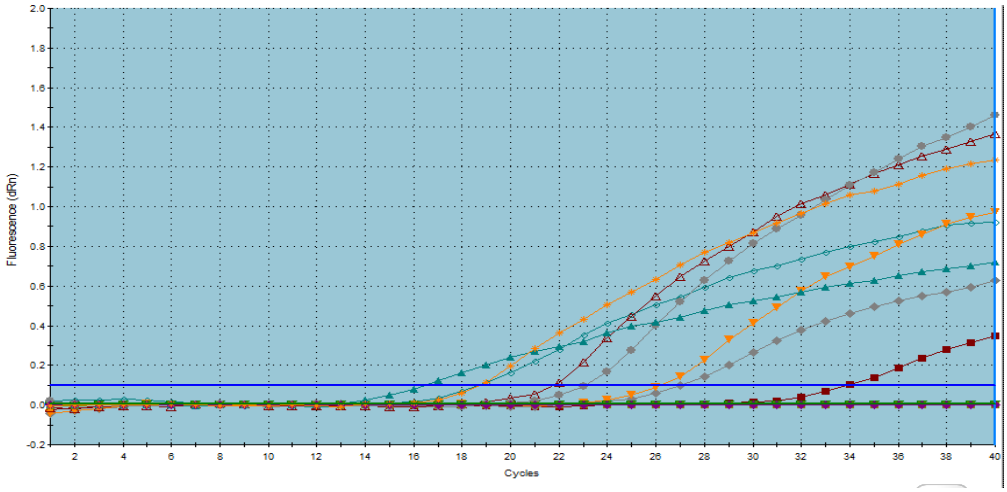

Figure (4): Molecular characterization using Real-time RT-PCR for detection of FMD Detection of FMD using Real-time RT-PCR
Field Samples tested for detection of FMD.
Positive control has (cycle threshold) Ct = 22,
there are 36 positive samples have Ct ranged from 17-34,
Negative control and Negative samples have no Ct.

CT values < 40 were defined as positive and those with Ct values from 40-50 were considered border line. Samples falling within the border range indicative of a weak FMD positive (Andrew et al. 2007)

Epidemiological Maps

The disease mapping For FMD- 2013-2014 in 10 Egyptian governorates

geographically-related risk areas this agree with risk or incidence (Ostfeld et al.,2005)

Epidemiological map of the circulating strains in Egypt during 2013-2014

DISCUSSION

The FMD is one of the most economically devastating diseases of ruminants all over the world (Rodriguez and Gay, 2011).

Effective vaccines and stringent control measures have enabled FMD eradication in most countries, which maintain unvaccinated, seronegative herds in compliance with strict international trade policies. However, the disease remains enzootic in many regions of the world, posing a serious problem for commercial trade with FMD-free countries.

A severe FMD outbreak in cattle and buffaloes in Egypt on early February 2006. and 2012 It has been established that rapid control of FMD is foremost to reduce dissemination of the causative virus to other non-infected regions (Howard and Donnelly, 2000 and Kandeil et al., 2012).. Conventional serological tests cannot differentiate FMD virus vaccinated from infected animals as there are methods such as virus neutralization and liquid phase blocking ELISA depend on detection of antibodies elicited against both structural and non structural FMD proteins which were vaccination or infections (Wieslaw and Haas, 2003, Bruderer *et al*., 2004 and Clavijo *et al*., 2004).

Consequently, In this study 161 out of 376 serum samples were positive for NSP (42.81%) collected from infected cattle in 10 governorates (El-Sharkia,Giza, Port Said, Assuit, Suize, Kafre El-Shake, Qina, Al-Gahrbia, Domiatte and Alexandria). That the 3 ABC. Based ELISA was the most reliable single indicator of infection in bovine Detection of FMD virus non Structural proteins (NSP) antibodies in cattle in different governorates (Table 1) the results agree with (Mackay *et al*., 1998b). That can't to differentiate between animals that had infected from vaccinated based on the detection of antibodies to structural proteins alone. Differentiations of these two categories of animals are important during serological surveys to detect evidence of infection in vaccinated cattle (Mackay *et al*., 1998a, b).

In table (2) Serotyping of FMD virus Antibodies in serum samples using liquid phase blocking ELISA Indicates that LPB ELISA is specific, sensitive, quantitative, quicker to perform, antibodies serotyping as agreed with OIE (2000); Mackay *et al*., (2001) and Declerq (2002).

The epithelial suspension from 42 field samples collected from infected cases in 10 governorates (El-Sharkia,Giza, Port Said, Assuit, Suize, Kafre El-Shake, Qina, Al-Gahrbia, Domiatte and Alexandria)

during periodical surveillance were tested by ELISA kit used for FMDV Antigen detection as in Table (3)shown Relationship between field samples and identified FMD antigen serotypes by indirect sandwich ELISA:showed the results of identification and Typing of different samples (42) for field samples by indirect sandwich ELISA. The Results showed the positive samples were 2 samples out of 42 were serotype A, 31samples were serotype O, and 3 samples were SAT2. These results agree with (Sangula *et al.*, 2005). The detection of multiple serotype infection was possible by the use of the antigen detection ELISA. The same positive 36 samples by antigen detection ELISA submitted for Reverse transcription polymerase chain reaction (RT-PCR) which was done on FMD field samples for identification and serotyping which have to apply with large scale in

veterinary diagnosis. The procedure was used as described by (Reid *et al*., 2000). The value of the RT–PCR can rapidly facilitate the molecular analysis of field isolates for conformation the positive samples with virological traditional techniques requiring specialized equipment, expertise and refined reagents and has to be used in conjunction with current procedures for FMD diagnosis.

Details about the molecular characterization as Conventional PCR, (1),(2) and (3) and the real time RT-PCR figure (4). The primers and probe sets can be used for real time RT-PCR of FMDV acc to OIE (2009) detection using for 36 samples were pooled as five groups and run for RNA detection. The results as shown in Fig (4) the 5 groups were positive as FMDV.

The real – Time RT-PCR method according to (Callahan *et al*., 2002) is a technique for reliable detection of FMDV in a fraction of the time required for combined virus identification by ELISA techniques (Paixao *et al*., 2008).

The disease mapping For FMD- 2013-2014 in 10 Egyptian governorates
Explore the nature of such distributions in widely used in risk modeling to identify high-risk areas or geographically-related risk areas this agree with risk or incidence (Ostfeld et al.,2005)

Conclusion

- Based on current results, it concluded that RT-PCR could be used as a sensitive and time saving tool for confirmation of FMDV antigen during outbreaks. The LPB-ELISA is more sensitive and rapid method for detection and typing of sera collected from diseased cattle**.** Using Indirect Sandwich Enzyme Linked Immunosorbant Assay (ELISA) for identification of different FMDV antigen serotypes. Then highlight on the epidemiological situation of FMD in some Egyptian governorates more researches should be applied to control the incidence of FMDV in Egypt.

REFERENCES

Andrew E. shaw, Scott M. Reid, Katja Ebert ,Geoffery H. Hutching ,Nigel p Ferris, Donald P. King (2007): institute for Animal Health Pirbright laboratory Ash Road,pirbright,woking.Survey,GU24 ONF,united Kingdom

Beck E, Strohmaier K (1987): Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. J Virol.;61:1621–9

Bruderer, V.; Swam,; Bearnd Haas; Visser, N. Brocchi, E.; Graziolo, S.; Esterhuysen, J.J.; Vosloo, W. and Anderson, J. (2004): Differentiation infection from vaccination in foot and mouth disease: evaluation of an ELISA based on recombinant 3ABC. Vet. Microbiology 101:187-197.

Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J., Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM.: Use of a portable real-Time reverse transcriptase – Polymerase chain reaction assay for rapid detection of foot and mouth disease virus. J Am. Vet. Med. Assoc 2002, 220:1636-1642

Clavijo A., Wright P., and Kitching P. (2004): Developments in diagnostic techniques for differentiating infection from vaccination in foot

and mouth disease. The vet. J. 167:9-22.

Clavijo, A.; Viera-Pereira, P. J. and Bergmann, I. (2003). Use of reverse transcription polymerase chain reaction (RTPCR)for rapid diagnosis of foot and mouth disease in South America. Vet. Res.Commun., 27: 63-71.

De Clercq, K. (2002): Overview on FMD diagnostic techniques. FMD control strategies, symposium proceeding, 2-5 June 2002, Yon, France, PP. 345-351

Domingo E, Dopazo J, Rodrigo MJ, Rodriguez A, Saiz JC, Sabrino F Aphthovirus evolution. In: Gibbs AJ, Calisher CH, Garcia-Arenal F(1995): editors. Molecular basis of virus evolution. Cambridge (UK): Cambridge University Press; p. 310–20.

FAO (Food aAgricultural Organization of the United Nations) (1984).

Hamblin, C. , Rarnett, I. T. R. and Hedger, S. (1986a): A new enzyme linked immunosorbant assay for detection of antibodies against foot and mouth disease virus. I. Development and method of ELISA. Journal of Immunological Methods 93, 115-121.

Hamblin, C. , Rarnett, I. T. R. and Hedger, S. (1986b): A new enzyme linked immunosorbant assay for detection of antibodies against foot and mouth disease virus. II. Application Journal of immunological methods 93, 123-129.

Kandeil A, El-Shesheny R, Kayali G, Moatasim Y, Bagato O,Darwish M, Gaffar A Younes A, Farag T, Kutkat MA, Ali MA. (2012): Characterization of the recent outbreak of foot-and-mouth disease virus serotype SAT2 in Egypt. Arch Virol. Mar;158(3):619-27. doi: 10.1007/s00705-012-1529-y. Epub 2012 Nov 7.

Kitching, R.P.and A. I. Doanldson (1987): Collection and transportation of specimens for vesicular virus investigation. Res. Sci. Tech. off. Int. Epiz.,6 (1): 263-272.

Knowles NJ, Samuel AR (1997): Molecular techniques in foot-and-mouth disease epidemiology. Towards livestock disease diagnosis and control in the 21st century: proceedings of an international symposium on diagnosis and control of livestock diseases jointly organized by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations. Vienna, 1997 Apr 7–11. Vienna: International Atomic Energy Agency; 1998. p. 185–201

Knowles NJ, Samuel AR (2003): Molecular epidemiology of foot-and-mouth disease virus.Virus Res. 2003;91:65–80.10.1016/S0168-1702(02)00260-5

Mackay, D. K. J.; Forsyth, M. A.; Davies, P.R. and Salt, J.S. (1998b): Antibody to non-structural proteins of foot and Mouth Disease virus in vaccinated animals exposed to infection. Vet. Q, 20:9-11.

Mackay, D. K.; Bulut, A. N.; Rendle, T.; Davidson, F. and Ferris, N. (2001): A solid phase competition ELISAfor measuring antibody to FMDV. J. virol. Methods, 97; 33-48

Mackay, D.K.; Vorsytha, M.A.; Davis, P. R.; Belizan, A.; Belsham, G. J.; Flint, M. and Ryan, M.D. (1998a): Differentiation infection from vaccination in foot and Mouth Disease using a panel recombinant,

non-structural protein in ELISA. Institute for Animal Hlth, Pirbright Laboratory, Working, Survey, UK Davio. Vaccine, 16 95): 446-459.

OIE (2009): Manual of Diagnostic tests and vaccines for terrestrial animals.

OIE (Office International des Epizooties) (2000): Foot and Mouth disease, chapter 2.1.1. In manual of standards for diagnostic tests and vaccine 4th Ed. 2000, Paris, 77-92.

OSTFELD, R. S., G. E. GLASS, AND F. KEESING. (2005). Spatial epidemiology: An emerging (or re-emerging) discipline Trends Ecol. Evol. 20: 328–336, doi:10.1016/j.tree.2005.03.009

Paixao TA, Neta AV, Paiva No, Reis JR, Barbosa MS, Serra CV, Silva RR, Beckham TR, Martin BM, Clarke NP, Adams LG, Santos RL. (2008): Diagnosis of foot – and mouth disease by real time reverse transcription polymerase chain reaction under field conditions in Brazil BMC Vet. Res. Dec. 31; 4:53.

Reid , M.A; Forsyth M.S.; ;Hutchings G.H; Ferris N.P (1998): Comparison of reverse transcription polymerase chain reaction, enzyme linked immunosorbent assay and virus isolation for the routine diagnosis of foot-and-mouth disease Methods. Volume, February 1998, Pages 213–217.

Reid SM, Ferris NP, Hutchings GH, Samuel Ar, Knowles NJ (2000) Primary diagnosis of foot – and – mouth disease by reverse transcription polymerase chain reaction. J. Virol Methods. 89 (1-2).

Reid, S. M., Ferris, N. P., Hutchings, G. H., Zhang, Z., Belsham, G. J., Alexandersen, S.,(2002). Detection of all 7 serotypes of FMDV by real-time, fluorogenic RT-PCR. J. Virol. Meths 105, 67-80.

Reid, S. M., Grierson, S., Ferris, N. P., Hutchings G. H., Alexandersen, S., 2003. Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of FMDV. J. Virol. Meths, 107, 129-139.

Rodriguez LL.; Gay CG. (2011). Development of vaccines toward the global control and eradication of foot-and-mouth disease. Expert. Rev. vaccine. 10, 377-387.

Samuel A.R. ; Knowles N.J.and Muckay D.K.J.(1999): Gentic analysis of type O virus responsible for epidemics of Foot and Mouth Disease in North Africa Epidemiology and Infection 122:529-538.

Sangula, A.K.; Wekesa, S. N.; NMg'ag' Z.W.; Wamwayi, H. M. (2005). Foot – and – Mouth Disease Laboratory, Embakasi, P. O. Box 18021, 00500 Nairobi Enter Pries Road, Kenya, Department of Zoology, Kenyatta University, P.,O. Box 43844, 00200 Nairobi city square, Kenya, National veterinary Research centre , Muguga, P.O. Box 32,00902 Kikuyu, Kenya.

Shaw A. E., Reid S. M., Ebert K., Hutchings G. H., Ferris N. P. and King D. P. (2007) :Implementation of a one-step real-time RT-PCR protocol for diagnosis of foot-and-mouth disease. Journal of Virological Methods 143 (1): 81-85.

Sorensen, K.J., K.G. Madsen, E.S. Madsen, J.S. Salt, J. Nqindi and D.K.J. Mackay, (1998) Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using

antigens expressed in baculovirus. Arch Virol., 143: 1461-1476.

Van Aarle, P., (2001) The use of a 3ABC marker-test for FMD. Proceedings of the 22nd Conference of the OIE Regional Commission for Asia, the Far East and Oceania, November 27-30, 2001, Kathmandu, Nepal, pp: 1-11.

Vangrysperre W. & DE Clercq K. (1996): Rapid and sensitive polymerase chain reaction based detection and typing of foot-and-mouth disease virus in clinical samples and S.M cell culture isolates, combined with a simultaneous differentiation with other gnomically and/or symptomatically related viruses. Arch. Virol., 141(2), 331–344.

Wieslaw Niedlask and Bernd Haas (2003): Differentiation of infection from vaccination by detection of antibodies to the non-structural protein 3ABC of foot and mouth disease virus. Bull. Vet. Inst. Pulawy 47, 51-60.