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DETECTION OF VIRUS STRAIN THAT CAUSED FOOT AND MOUTH DISEASE OF BASRAH MARSHES CATTLE BY USING PCR TECHNIQUE

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ABSTRACT

Although, the disease has been controlled successfully in many parts of the world by regular vaccination of susceptible animals and slaughtering of infected animals, no country has been considered safe, because of the highly contagious nature and rapid spread of the infection for the effective control of the disease, outbreaks should be detected at an early stage and persistent infections should also be recognized to prevent further transmittance**.** The purpose of this study was to determine the virus strain that caused FMD in cattle of Basrah marshes by amplified VP3gene in seven strains of FMDV deposited in the Gen Bank database. The results revealed that the O strain type was appear in a total of cases (100%) of the virus strain that caused foot and mouth disease in cattle of Basrah marshes by amplified VP3gene from seven serotype of FMDV. From the total cases 8% were ASIA1 serotype , 4% SAT1, 2% SAT2, while the other strains A, C and EUR were 0% . The results showed interaction among the strains in appearance of FMD, the interaction between O and ASIA1strain was 7%; O and SAT1 was 4%; O and SAT2 was 2%; O, ASIA1 and SAT1 were 4%; O, ASIA1 and SAT2 were 2%; O, ASIA1, SAT1 and SAT2 were 2%. The interaction between ASIA1 and SAT1 was 4%; ASIA1 and SAT2 was 2%; ASIA1 , SAT1 and SAT2 were 2%.

Keyword : FMD, Bsrah marshes, PCR, Viruse strains of Foot and Mouth Disease

INTRODUCTION

Foot-and-mouth disease or hoof-and-mouth disease (*Aphtae epizooticae*) is an infectious and sometimes fatal viral disease that affects cloven-hoofed animals, including domestic and wild bovids. The virus causes a high fever for two or three days, followed by blisters inside the mouth and on the feet that may rupture and cause lameness. Foot-and-mouth disease is a severe plague for animal farming, since it is highly infectious and can be spread by infected animals through aerosols, through contact with contaminated farming equipment, vehicles, clothing or feed, and by domestic and wild predators (CFIA, 20011). The FMD virus is a member of the genus *Aphthovirus* in the family Picornaviridae. There are seven immunological distinct serotypes O, A, C, SAT1, SAT2, SAT3 and Asia 1and over 60 strains within these serotypes(Knowles *et.al*., 2003). The virus responsible for the disease is a picorna virus, the prototypic member of the genus *Aphthovirus*. Infection occurs when the virus particle is taken into a cell of the host. The cell is then forced to manufacture thousands of copies of the virus, and eventually bursts, releasing the new particles

in the blood. The virus is highly variable(Martinez-Salas *et.al*.,2008). FMD occurs throughout much of the world, and whilst some countries have been free of FMD for some time, its wide host range and rapid spread represent cause for international concern. After World War II, the disease was widely distributed throughout the world. In 1996, endemic areas included Asia, Africa, and parts of South America(FMD, 2007). FMD generally involves mortality rates below 5%, but even so it is considered the most important disease of farm animals since it causes huge losses in terms of livestock productivity and trade. Although FMDV rarely causes death in adult animals, the virus can cause severe lesion in the myocardium of young animals, leading to high mortality rates (Sharma and Das, 1984; Domingo *et.al.,* 1990 and Woodbury, 1995)

Aim of study:

The purpose of this study was to determine the virus strain that caused FMD in cattle of Basrah marshes by amplified VP3gene in seven strains type of FMDV deposited in the GenBank database**.**

MATERIALS AND METHODS

Sampling fluid from vesicles and saliva:

One hundred cases of cattle(cows and buffalo) infected with FMD were used to collect the fluid from vesicle and saliva using a sterile tubes, needles and syringes. The fluid kept in transport medium (normal saline at pH 7-8.in 4-10 °C) and transport to laboratory within 24 hours .

RNA Extraction:

RNA samples were extracted using the total RNA Mini kit (tissue) following the manufacturer's instructions. Briefly, 400 µl of the RB buffer that is included in the kit added to the tubes that containing the FMD fluid ,then 4 µl of ß-mercaptoethanol followed by 400 µl of 70% ethanol, then transferred to a Mini RNase column previously inserted into a 2ml collecting tube. RNA was immobilized in the column by centrifugation, sequentially washed, and diluted in 50 µl of RNase free water.

Reverse transcription polymerase chain reaction:

The following primers were used F5'-ACTGGGTTTTACAAACCTGTGA-3' and R5'-GCGAGTC CTGCCACGGA-3' along with the probe 5'-TCCTTTGCACGCCGTGGGAC-3'in the one-step RT-PCR amplification started with reverse transcription for 1 hr at 60°C, followed by PCR with the following parameters: 55 cycles of 2 sec at 95 °C and 30 sec at 60°C. (Knowles *et al* ,2005). The amplified PCR products (672 bp) of the expected length were subjected to electrophoresis in a 1% agarose gel and visualized by staining with ethidium bromide under UV light. The other RT-PCR protocol for VP3 gene amplification by used a kit of green master mix, the reaction mix include green master mix 12.5µl, forward primer and reverse primer(modified from)(Gelagay et.al.,2009). (table:1) each 1µl, DNA 5µl, D.W. 5.5µl, then PCR amplification according to the following thermal profile: initial denaturation at 95°C for 5 min; 94°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 2.5 min, for 30 cycles. The final extension step of 72°C for 10 min. The products were 320bp analyzed by 1% agarose gel electrophoresis and visualized under UV light after staining with ethidium bromide

Table:1 shows the sequences of the primers that used to detected the FMD virus strains

Strains types	primers
Asia1 VP3	F5'-CATCGCCCTTGGACTACGA-3' R5'-CACGATTTAGCGATCAGTCAGAG-3'
OVP3	F5'-TBGCRGGNCTYGCCCAGTACTAC-3 'R5'--GACATGTCCTCCTGCATCTGGTTGAT -3'
A VP3	F5'-TACCAAATTACACACGGGAA -3' R5'-GACATGTCCTCCTGCATCTGGTTGAT -3'
C VP3	F5'-TACAGGGATGGGTCTGTGTGTACC -3' R5'-GACATGTCCTCCTGCATCTGGTTGAT -3'
SAT 1 VP3	F5'-GTGTATCAGATCACAGACACACA -3' R5'-ACAGCGGCCATGCACGACAG -3'
SAT2 VP3	F5'-TGGGACACMGGIYTGAACTC -3' R5'-ACAGCGGCCATGCACGACAG -3'
EUR VP3	F5'-GAAGGGCCCAGGGTTGGACTC -3' R5'-GACATGTCCTCCTGCATCTGGTTGAT -3'

RESULTS AND DISCUSSION

The primary diagnosis according to the case history and the cardinal signs and symptoms of FMD which are wobble between increase the body temperature ,salivation, anorexia, laminas and appear of vesicles in mouth and foot(between digits space of hooves); the FMD was determine (figure.1-8).

Figure.1

Figure.2

Figure.3

Figure.4

Figure. 5

Figure.6

Figure.7

Figure.8

The laboratory diagnosis depended on PCR technique by amplified of the 3AB gene of FMDV which is a member of the genus *Aphthovirus* in the family Picornaviridae. The whole volume of gene were 672 bp (Fig 9,10) which fixed that the infections were FMD not another diseases. The results revealed that the O strain type was appear in a total of cases (100%) of the virus strain that caused foot and mouth disease in cattle of Basrah marshes by amplified VP3gene from seven strain type of FMDV(figure 11.table 2).From the total cases

8% were Asia1 strain type(figure 12. table 2) 4% SAT1, 2% SAT2, (figure 13,14.table 2)while the other strains A, C and EUR were 0% (figure.15-17.table 2). The results showed interaction among the strains in appearance of FMD, the interaction between O and ASIA1strain was 7%; O and SAT1 was 4%; O and SAT2 was 2%; O, ASIA1 and SAT1 were 4%; O, ASIA1 and SAT2 were 2%; O, ASIA1, SAT1 and SAT2 were 2%. The interaction between ASIA1 and SAT1 was 4%; ASIA1 and SAT2 was 2%; ASIA1 , SAT1 and SAT2 were 2% (table. 2).

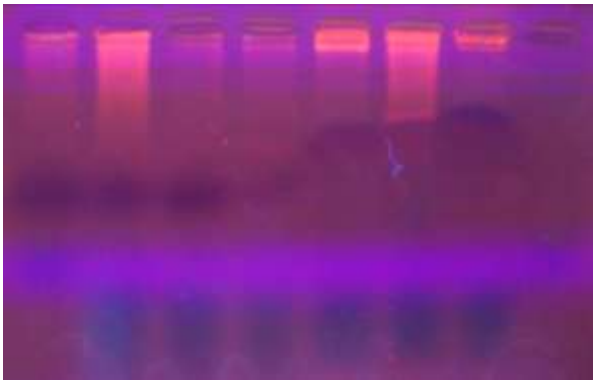

Figure.9:Agarose gel showing high molecular weight of virus RNA.

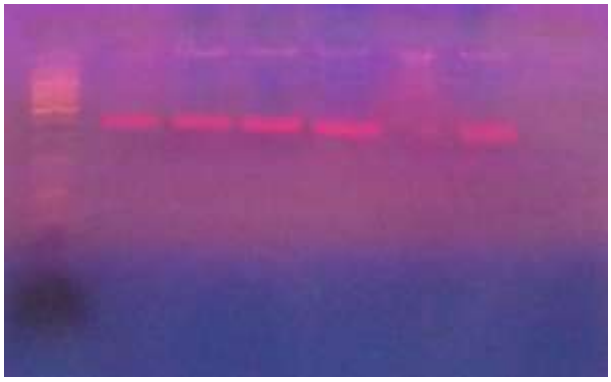

Figure.10:Amplified of the VP3gene of FMDV (672bp) Lane 1 ladder, lane 2, 3, 4, 5, 6, 8 PCR product.

Table:2 shows the ratio of VP3 gene of FMD virus strain in Basrah marshes cattle

Strains of FMD virus in100 cases	VP3gene %	OR	95% (CI=)
O	100	-	-
ASIA1	8	12.5	(6.75-14.91)
SAT1	4	0.5	(0.002-1.01)
SAT2	2	0.5	(0.008-0.9)
A	0	-	-
C	0	-	-
EUR	0	-	-
O+ASIA1	7	-	-
O+SAT1	4	0.57	(0.04-1.05)
O+SAT2	2	0.5	(0.06-0.8)
O+ASIA1+SAT1	4	-	-
O+ASIA1+SAT2	2	0.5	(0.009-1.3)
O+ASIA1+SAT1+SAT2	2	1	(0.3-2.8)
ASIA1+SAT1	4	-	-
ASIA1+SAT2	2	0.5	(0.01-0.9)
ASIA1+SAT1+SAT2	2	1	(0.1-3.6)

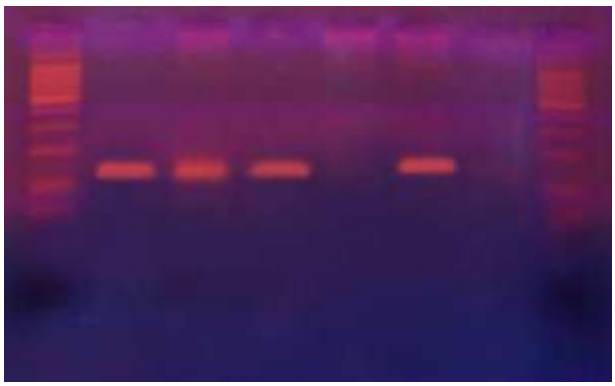

Figure .11:Amplified of the VP3 gene of O strain Lane 1,8 ladder , lane 5,7(Negative) lane 2, 3, 4, 6 PCR product. PCR (320bp)

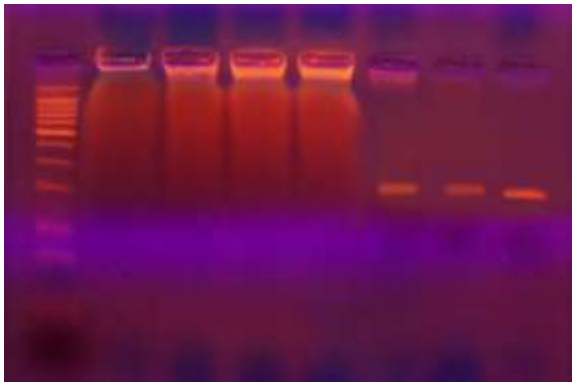

Figure.12:Aamplified of the VP3 gene of ASIA1strain Lane 1 ladder,lane2, 3, 4, 5 (Negative) lane7,8 product(320bp).

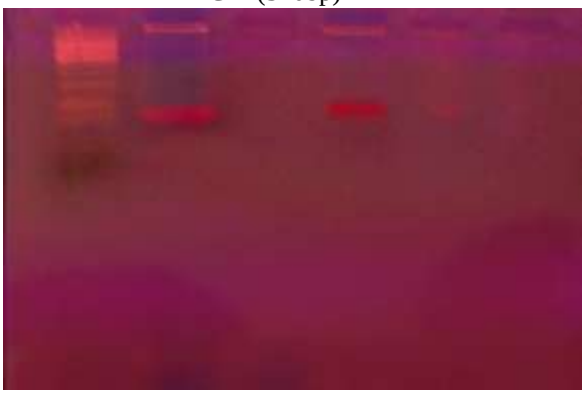

Figure.13:Aamplified of the VP3 gene of SAT1 strain Lane1 ladder,lane3, 5, 6, 7 (Negative) lane 2, 4 PCR product (320bp)

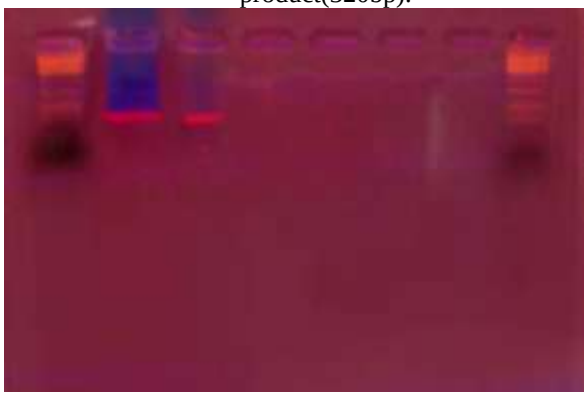

Figure.14: amplified of the VP3 gene o f SAT2 strain Lane 1, 8 ladder ,lane 4, 5, 6, 7(Negative) lane 2, 3 PCR product(320bp).

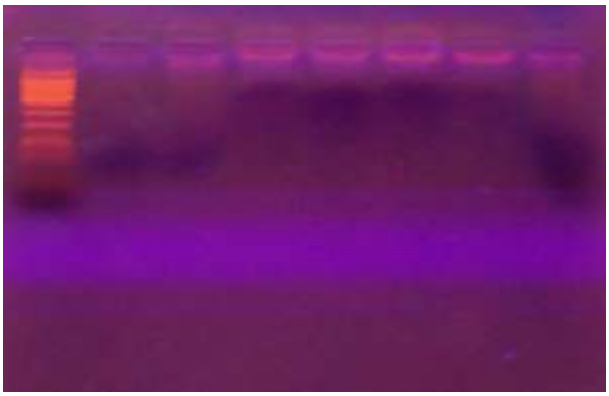

Figure.15:Aamplified of the VP3 gene of A strain Lane 1 ladder,lane2, 3, 4, 5, 6, 7, 8 (Negative)PCR product.

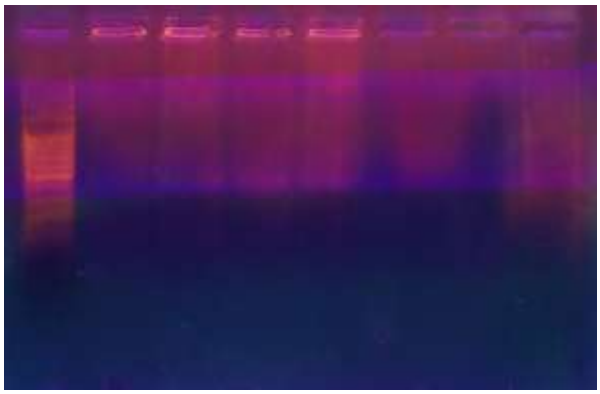

Figure.16:Amplified of the VP3 gene of C strain Lane1 Ladder ,lane 2, 3, 4, 5, 6, 7, 8 (Negative) PCR product.

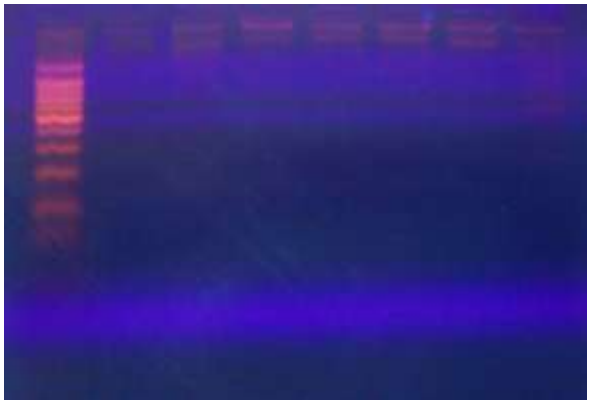

Figure. 17:Aamplified of the VP3 gene of EUR strain Lane 1 Ladder , lane 2, 3, 4, 5, 6, 7, 8(Negative) PCR product.

Infection with foot and mouth disease tends to occur locally, the virus is passed on to susceptible animals through direct contact with infected animals or with contaminated pens or vehicles used to transport livestock. The clothes and skin of animal handlers such as farmers, standing water, and uncooked food scraps and feed supplements containing infected animal products can harbor the virus as well. The absent of control measures such as quarantine and destruction of infected livestock, and export bans for meat and other animal products to countries not infected with the disease. Almost the viral disease laboratory diagnosed by use the ELISA assay, but this way of laboratory lack of the high sensitivity, so the PCR is the standard laboratory assays which applied to detect FMDV by detect DNA/RNA. Although the program of the vaccination against FMD is in continue in Iraq but this disease become endemic specially in marshes cattle. The failed in immunization may be due to lack the athletic with the virus strain that endemic in regions or due to activate the strain which is occurred by the vaccine not originally present in the region; Also one of the difficulties in vaccinating against FMD is the huge variation between and even within serotypes. The lose of cross-protection between strain types which meaning that a vaccine for one serotype won't protect against any others and in addition, two strains within a given serotype may have nucleotide sequences that differ by as much as 30% for a given gene; so the FMD vaccines must be highly specific to the strain involved. As we know the vaccination only provides temporary immunity that lasts from months to years and this idea agree with Tamilselvan *et.al*., (2009) the main constraints in controlling this disease and why it is considered as the most dreaded viral disease are its high contagiousness, wide geographical distribution, broad host range, ability to establish carrier status, antigenic diversity leading to poor cross-immunity, and relatively short duration of immunity. Poor surveillance and diagnostic facilities as well as inadequate control programs are major problems in control of this disease in the country. Although, the disease has been controlled successfully in many parts of the world by regular vaccination of susceptible animals and slaughtering of infected animals, no country has been considered safe, because of the highly contagious nature and rapid spread of the infection for the effective control of the disease, outbreaks should be detected at an early stage and persistent infections should also be recognized to prevent further transmittance. These can be achieved when vaccination is regular and effective and when diagnostic tools available are specific and sensitive and at the

same time rapid(Bruner and Gillespie, 1973). Analysis of the viral genome is importance to monitor the field isolates in areas where the disease is endemic The virus particle which sediments at 146S consists of a single stranded positive sense RNA molecule of about 8.5 kb with a molecular weight of 2.6 ´ 10^6 daltons enclosed in a capsid which is composed of 60 copies each of four structural proteins named VP1, VP2, VP3 and VP4. VP1 is exposed on the surface of the virion and has immunogenic property. (Suryanarayana *et.al.,* 1999). The results of the current study secure the O strain is the main strain that causes FMD in Iraq marshes cattle depend on genetic diagnosis of VP3 gene. Also the results showed that the other strain such as ASIA1 ,SAT1 and SAT2 respectively are combined with strain O in FMD accident. While the genetic diagnosis that used in the current study discovered the strains A,C and EUR not have any role in FMD occurrence. According to Global Animal Health–International Disease Monitoring Preliminary Outbreak Assessment(2009) the middle east specially Iran was endemic in FMD strain type O and Iraq not endemic with this disease, so we think the main causes that make Basrah marshes cattle become endemic in FMD from 2009-2011 is as a result of animal contraband between Iran and Iraq , random greasing with neighbouring countries, contaminated of marshes water that are link and sharing between Iran and Iraq and used the vaccine that prepare in Iran which contain different strain.

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