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Molecular Detection of foot and mouth disease

in Al- Qadissiyia province

A research submitted to veterinary collage /AL-Qadisiyah university its partial of fulfillment to get B.Sc.in medical and surgery of Vet Med

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بسم الله الرحمن الرحيم

((فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ

وَقُلْ رَبِّ زِدْنِي عِلْمًا))

صدق الله العلي العظيم

طه الآية ١١٤

Certificate of supervisor

I certify that asraa nabeel has completed the fulfillment of her graduation project for the year 2016/2017 under my construction

Supervisor

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Certificate of Instructor

We certify that ***Asraa nabeel Jawed*** has completed the fulfillment of her graduation project entitled **Molecular Detection of foot and mouth disease in Al- Qadissiyia province** for the year 2016/2017 under our construction.

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Summery

Foot-and-mouth Disease (FMD) is a serious contagious viral disease principally affecting cloven hoofed animals. Although serotypes A and O of Foot-and-mouth disease Virus (FMDV) has been continuously circulating in Iraq, during 2016.

The etiological agent, foot and- mouth disease virus (FMDV), is a non-enveloped, RNA virus belonging to the genus Aphtho virus within the family Picornaviridae and The virus exists in seven distinct serotypes: O, A, C, Asia-1,SAT 1, SAT 2 and SAT3.

The affected animals refused their feed, the oral mucosa showed redness and the animals had vesicles in the oral cavity and on their feet. Most of the affected animals eventually recovered

In our study we using rapid and high sensitive test reverse transcription polymerase chain reaction to detection the foot and mouth disease virus in 17 samples were positive out of 30 samples by using universal primers to detection the FMD virus as general. on conclusion the disease is distribution in Iraq and the PCR consider high sensitive test to detection small amount of DNA of virus

Introduction

The earliest description of probable foot-and-mouth disease (FMD) in cattle was made by an Italian monk, Hieronymus Fracastorius, in Venice in 1514. The affected animals refused their feed, the oral mucosa showed redness and the animals had vesicles in the oral cavity and on their feet. Most of the affected animals eventually recovered. This description, made 500 years ago, shows a strong resemblance to that of FMD when seen currently. FMD is considered one of the most important diseases of cloven-hoofed animals; it affects cattle, buffaloes, pigs, sheep, goats and about 70 wildlife species. (Fisher, 1984).

Foot-and-mouth disease (FMD) is a severe, highly contagious and economically devastating viral disease worldwide, which affects animals with cloven hooves such as cattle, pigs, deer, goats and sheep. Numerous outbreaks have been reported around the globe since first outbreak of FMD in America in 1870 (Gibbs, 2003))

The disease causes heavy economic losses to the livestock industry in terms of high morbidity in adult animals and mortality in young stock. Beside direct loses, treatment costs, reduced milk production, loss of work efficiency in draught animals remain greatest hindrances for fattening industries especially in developing countries(Sumption et al., 2008).

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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 (Domingo et al 1990).

The aims of this study are to established the PCR technology for detection of FMDV from vesicles samples and in order to improve and develop the diagnostic technique for detection of FMD Virus and detection the disease in AL-Qadisyia province .

Hiostopry of disease

The first official cases of fmd were recorded in 1937,while the first record of a specific fmd serotype in Iraq was serotype a in 1952 .other serotypes have been reported since then ,serotypes o,sat-1 and asia1 were recorded in 1957,1962 ,and 1975,respectively. (Schnepf, R. 2003)

Definition:

FMD is an acute, highly contagious viral disease of cloven-hoofed livestock characterized by vesicular lesions, erosions, and ulcers in the mouth and interdigttal areas and on the muzzle, teats, and coronary band.26.5 Natural hosts include cattle, sheep, goats, swine, water buffalo, bison, deer, elk. antelope , cattle and swine are the most susceptible, and camellds have low susceptibili ty(Knowles,*et al.*,2009*)*.

Etiology

The FMD virus is a picornavirus of the genus Aphthovirus. At least seven immunologically distinct types of FMDV have been identified: A; 0 ; C; SAT 1, 2, and 3; and Asia 1. Within the seven types at least 60 subtypes have been recognized(Reid, *et al*.,2000)

Foot-and -mouth disease is associated with an aphthovirus (family Picomaviridae) which occurs as seven major serotypes: A,0, C, Southern African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1. However, there are a number of immunologically and serologically distinct subtypes with different degrees of virulence, especially Viral diseases characterized by alimentary tract signs _ within the A and 0 types. As there is no cross immunity between serotypes, immunity to one type does not confer

protection against the others. This presents difficulties to vaccination programs. Furthermore, there can be great changes in antigenicity between developing serotypes; virulence may also change dramatically. There are also biotypical strains which become adapted to particular animal species and then infect other species only with difficulty there arestrains that are much more virulent for pigs (so-called porcinophilic strains),some for buffalo, and some even for tropical breeds of cattle, which generally react only mildly to endemic strains. Newer techniques for identifying subtypes involve enzyme-linked immunosorbent assay (ELISA), reverse transcriptasepolymerase chain reaction (RT-PCR) and nucleotide sequence analysis.(Wee, *et al*.,2001)

Transmission

Transmission occurs primarily by means of aerosols,287.294.295 anima l contact. and fomites such as shoes, tires, and equipment. The virus may be spread to farms 50 miles distant . Human beings can carry (and subsequently transmit) the virus on their shoes or clothing or in respiratory tract tissues for longer than 24 hours. Recovered cattle usually stop shedding the virus by 2 weeks, but some harbor it for 6 to 24 months Cape buffaloes can be lifelong carriers. The virus exists in milk, and it may survive pasteun zation Uncooked or partly cooked meat products or garbage scraps, hides, or other tissues contaminated with the FMD
virus from an endemic area of the world may transmit the disease long distances. The virus can persist in frozen meat for years. Besides actively infected animals, sources of infection include bedding, feed, milk, shoes and hands of humans.(Abd El-hamid, *et al*.,2011)

Clinical signs

Clinical signs of FMD include fever, depression, anorexia, listlessness, occasional shivering, excess salivation, lip smacking.. nasal d discharge, and lameness Agalactia may occur. In addition to vesicles the differential diagnosis includes BPS, blue tongu e, RP, MCF, and severe cases of infectious bovine rhinotracheitis. Teat lesions can be confused with those caused by bovine herpes mammillit is or parapoxviruses.
Vesicular lesions (blisters) 0.5 to 10 cm in diameter rupture within 48 hours, followed by a mucosal slough and large erosion . These lesions

appear to be very painful, and affected animals are reluctant to eat or move. The morbidity rate is high, but the mortality rate is low.

The disease is economically devastating because it spreads rapidly and causes weight loss, mastitis, loss of milk production .we and frequent abort ion. An outbreak of FMD in a country previously free of the disease can cost billions of dollars in trade losses over ensuing years. Deaths may occur in young calves, mainly from myocardial necrosis, and secondary bacterial pneumonia and foot infections are common. All animals with in 3 km may be destroyed during efforts to halt the disease.(Bachrach,1986)

Pathophysiology

The usual incubation period is 2 to 14 days. The usual primary sites of infection an d replication by the FMD virus are the pharyngeal and digestive mucosa- s" and alveolar epithelium of the udder. The virus replicates in the cells of the stratum sptnosum. It spreads locally and also enters the circulation and is carried to other susceptible tissues. Within 2 to 2 1 days a fever begins, and some vesicles appear. Vesicles develop in the mouth and on the ruminal pillars, and myocardial and skeletal muscle degeneration characterized by Zenker's necrosis

may occur in young cattle. The mucosal cell disruption results in separation of superficial epithelium from basal epithelium, which fills with tissue fluids. When these epithelial layers slough, erosions are left behind that take days to weeks to heal, depending on their size.(Knowles *et al*.,2007)

Clinical pathology/diagnostic

confirmation Virus isolation, serology and RT- PC R detection. Typing confirmed i n a reference laboratory Lesions Vesicular, erosive/ulcerative stomatitis and esophagitis, vesicular/ulcerative dermatitis (feet and teats) and in neonates, interstitial mononuclear and necrotic myoca rditis(**Thomson,1994).**

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Primers

The Primers and their sequences and RT-PCR product size.

Primer	Sequence		Product size
Universal FMD primer	**F**	**GCCTGGTCTTTCCAGGTCT**	**330bp**
	R	**CCAGTCCCCTTCTCAGATC**	

Samples collection:30 vesicles sample were collected from cattle that suspected infected with FMD disease . sample were collected from the different districts of AL-Qadisyia governorate (AL-hamza ,AL- seeder and affak) .

Viral RNA extraction: samples suspensions were prepared in DEPC-treated ultra-pure water to a 1:4 final dilution, clarified at 5,000xg/15min at 4ºC and the supernatant submitted to RNA extraction with TRIzol.

The genomic RNA of FMD virus were extracted by using Trizol RNA extraction Kit (Bioneer, Korea) and done according to kit instructions as following steps:

1- sample was transferred into DEPC–treated eppendorf tube, then 1ml of TRIzol® reagent added and the tube shaken vigorously for 30 seconds.
2- Chloroform (200 µl) was added to each Eppendorff tube and shaken vigorously for 15 seconds.
3- The mixture was incubated on ice for 5 minutes.
4- Spined at 12,000 rpm , 4C° , for 15 minutes.
5- Supernatant was transferred to a new Eppendorff tube, and isopropanol (500 µl) was added.
6- Mixed mixture by inverting the tube 4-5 times and incubated at 4C° for 10 minutes .
7- Spined at 12,000 rpm , 4C° for 10 minutes.
8- Supernatant was discarded.
9- Adding 80% Ethanol (1 ml) and Vortex again.
10- Spined at 12,000 rpm , 4C° for 5 minutes.
11- Supernatant was discarded and the pellet dried.
12- RNase free water (30µl) was added to the sample with vortexing until dissolving.
13- The extracted RNA sample was kept at -20.

RT- PCR Thermocycler Conditions (FMD virus)

RT PCR Thermocycler conditions were done according to method previously described by Cho *et al*., (2001)

Step	Temperature C°	Time	Number of cycles
cDNA synthesis	50	60 min	1

Predenaturation	95	5 min	1
denaturation	٩٤	30 sec	40
annealing	55	30 sec	
Extension	72	30 sec	
Final extension	72	5 min	1
Hold	4	4	

RT- PCR Product Analysis

The final RT PCR products were subjected to gel electrophoresis The electrophoresis device was run at 100

volts and 80 Ampere for 1 hour. After the samples that showed as positive bands for FMDV were visible at 330bp in the PCR product on UV light.

Detection of FMDV antigen by using RT-PCR

In the present study we used RT-PCR assay for detecting small quantities of FMDV, using a universal primer to detect a target the 330 bp fragment of the nucleocapsid envelope capsid protein gene of FMDV and amplify the FMDV. We detected the presence of FMDV samples. Figure (1).This study agreed with other reported who detection The bovine coronavirus FMDV **(**Adnan *et al*., 2014).

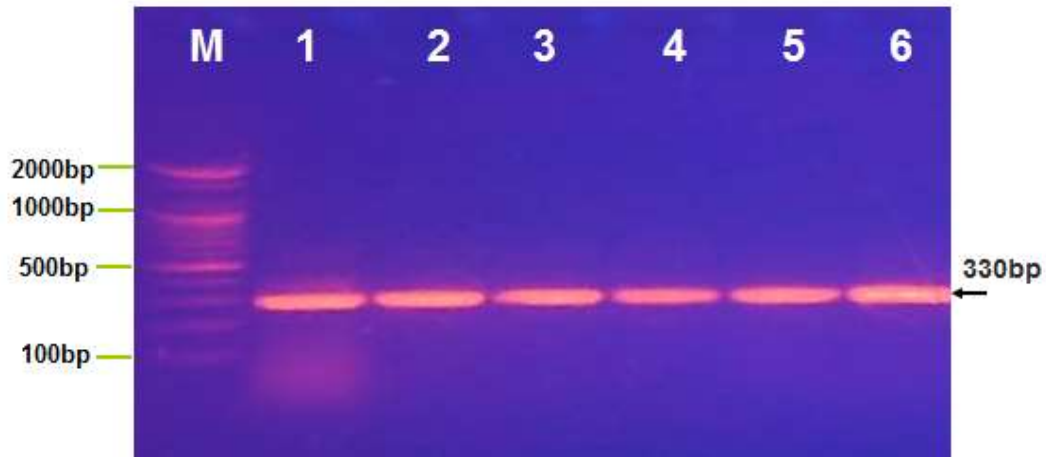

Figure 1: Agarose gel electrophoresis image that show the RT-PCR product analysis of Foot and Mouth Disease virus positive samples isolates. Where M: marker (2000-100bp), lane (1-6) positive serum samples at (330bp) PCR product.

Comparing with standard diagnostic procedures such as viral culture or serology, reverse transcription polymerase chain reaction (RT-PCR) has been proved to be extremely useful for diagnostic investigation in the detection of pathogens, especially when the detection method is time consuming, expensive or unavailable

(Zhe *et al*., 2010).

Conclusions.

1. FMD disease cause wide spread outbreaks in Iraq with economic losses due to sever lesions and high mortalities in cattle.
2. There were typical and characteristic clinical features useful in preliminary diagnosis of disease .
3. PCR technique was specific in the identification of virus and sensitive for detection of the virus.

Recommendations

1- The disease is endemic in Iraq thus we should be to use atypical vaccine to protection the cattle.
2- Do not purchased the animal from infected area .
3- Vaccination should be carried out at regular intervals using vaccine prepared from circulating strains.
4- Where an outbreak has occurred, strict Quarantines should be enforced to avoid the spread of the disease to new FMD free areas.

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