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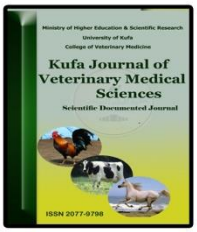

Detection of Cattle Foot and Mouth disease Virus by RT-PCR and ELISA

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Summary

This study was conducted with73 cattle in different region of Basrah governorate with typical clinical symptoms of FMD from April to July 2013 in Basrah ,Iraq**.** Blood ,saliva and vesicle fluid from the affected animals were collected and tested by ELISA and RT-PCR. Collected sera were tested for antibodies against FMDV using a commercial ELISA. Antibodies to FMDV were detected at an overall prevalence rate of **94.5** %. There was no significant seropositivity ratio variation in relation to sex and age.RT-PCR base detection of FMDV for primary and serotype specific diagnosis was done with(universal primer sets 1F / 1R, A- 1C612 and O- ARS4) with expected band of(328 ,865 and1301 bp respectively). Universal primer pair 1F/1R detected FMD in 55 out of 73(75.3%), of these 37 (**92.5**%) Females and 18 (**62.1**%) males samples, with high significant statistical difference **(p<0.01)** between males and females in the PCR positivity ratio and there was no positive samples of FMDV Universal primer in suspect seropositive cases . The tested samples with positive universal primer were amplified with specific primers **A-IC612** in **15(27.3**%) and nil for serotype O-**ARS4**.,. The circulation of Serotype A was higher in the males compared to the females with a ratio of **33.3**% (6/18) but this difference was not statisticaly significant(p > 0.05) .The statistical analysis showed high significant differences (P>0.01) between animals that tested for Universal primer in relation to age. Out of all animals that tested positive for Universal primer the age group <1-4 year realized a ratio of **58.6** %while ratio of age group >4-8 year **was 95**%. Serotype A circulation was not significantly differed (P>0.05) between the tested animals in relation to age with overall ratio **27.3**% (15/55) and higher ratio (**31.6**%, 12/38) was observed in the older animals compare with (**17.7**%, 3/17) in younger.

Key word: ELISA. Rt-PCR. Bovine. FMD

الكشف عن فايرس مرض الحمى القلاعيه بالابقار بواسطة ELISAوPCR

عدنان موسى الروضان

كلية الطب البيطري / جامعة البصرة

الخلاصه

اجريت هذه الدراسه في محافضة البصره عاى (73) ماشيه ذو اعراض سريريه نموذجيه لمرض الحمى القلاعيه وذلك من شهر نيسان الى شهر تموز لعام 2013.جمعت عينات الدم واللعاب و سائل الحويصلات من الحيوانات المصابه وفحصت بواسطة اختبار الممتز المناعي المرتبط بالانزيم و اختبار سلسلة البلمره المنعكس.

فحصت المصول بعد جمعها باستخدام اختبار الممتز المناعي المرتبط بالانزيم التجارى للكشف عن الاجسام المضاده لفايرس مرض الحمى القلاعيه حيث كانت نسبة الايجابيه المصليه الكليه لهذه الاجسام المضاده % **94.5**ولا توجد صله للجنس و العمر بالاختلاف المعنوى لنسبة الايجابيه المصليه.

استخدم اختبار سلسلة البلمره المنعكس للكشف عن فايروس مرض الحمى القلاعيه من حيث التشخيص الاولي وتحديد الانماط المصليه تم باستخدام البادئات(universal primer sets 1F / 1R و1C612 -A و **ARS4** -O)وبحزمه متوقعه (328و865و 1301 bpعلى التوالي).من ثلاثه وسبعون عينه تم الكشف عن مرض الحمى القلاعيه فى (75.3%) 55 عينه بواسطة البادئ العام وكانت (**92.5%**) 37 منها اناث و 18ذكور(**62.1%**) و كان الفرق الاحصائ بين الاناث والذكور عالي المعنويه ($P < 0.01$).ولا توجد اى عينه موجبه للبادئ العام لفايرس مرض الحمى القلاعيه فى الحالات ذات الايجابيه المصليه. ضخمت(**15(27.3%**) عينه من العينات الموجبه للبادئ العام مع البادئ**A-IC612** ولا نتائج موجبه للبادئ -O **ARS4**

ان انتشار النمط المصلى **A** كان عاليا فى الذكور بالمقارنه مع الاناث مع نسبة (6/18)**33.3%** لكن هذا الاختلاف لم يكن ذو دلاله احصائيه ($p > 0.05$).فيما يتعلق بالعمر. اظهر التحليل الاحصائي اختلاف ذو معنويه عاليه بين الحيوانات التي فحصت ($P<0.01$)بالبادئ العام.من مجموع الحيوانات التى فحصت على انها موجبه حققت الفئه العمريه4-1> سنه نسبة% **58.6** بينما كانت نسبة الفئه العمريه8-4> سنه**95%**. ا لم يكن انتشار النمط المصلى **A بين** الحيوانات المفحوصه ذو دلاله احصائيه ($p > 0.05$).فيما يتعلق بالعمر و كانت النسبه الكليه(15/55) **27.3%** ولوحظت اعلى نسبه(12/38, **31.6%**) في الحيوانات المتقدمه في السن بالمقارنه مع (3/17, **17.7%**)بالحيوانات صغيرة السن.

Introduction

The virus of foot and mouth disease(FMD) exists in seven distinct serotypes: O, A, C, Asia-1,SAT 1, SAT 2 and SAT 3 (1). Within the serotypes, multiple subtypes can also be identified that sometimes fail to induce total cross protection against other viruses of the same serotype. Genetic heterogeneity within FMDV may arise as a result of normal genetic drift (2), due to selection pressure(3,4) or as a result of recombination between different FMDV genomes (5; 6). Serotype A is considered to be the most diverse of the Eurasian serotypes both genetically and antigenically (7) and 26 regional genotypes within three continental toptypes have been identified globally (8).This high level of divergence makes it difficult to prevent the disease by vaccination (9). In addition, control of this disease is constantly challenged by the emergence of new virus strains. FMD is endemic in large parts of Africa, Asia and South America. The virus can readily cross international boundaries and hence cause epidemics in previously free areas.

Foot-and-mouth disease (FMD) is the economically most important animal viral disease worldwide (10). It classified as a List A disease by "Office International des Epizooties (OIE; World Organization for Animal Health)(11). The List A diseases have the potential for rapid and extensive spread within and between countries. (12,13). The effected countries cannot participate in international trade of animals and animal products (10). FMD has ability to cross international boundaries and causes epidemics in previously free areas including Europe (14). Currently, the high capacity of this disease spread is probably favorable by the growing globalization of trade, which facilitates dissemination of the disease (15).Foot-and-mouth disease (FMD) is a viral infectious disease of cloven-hoofed animals. This disease is caused by foot-and-mouth disease virus (FMDV). The prominent clinical signs are fever, vesicles in mouth and feet (16). Although the mortality rate is very low, it is fatal in young animals. Surviving cattle are subsequently carry virus for up to two years (16).These animals will be reservoir of a following outbreak (15).The infected animals can be discriminated from vaccinated by non-structural proteins(NSP) which can be detected only in infected animals.

There are several specific ELISA to detect non-structural proteins (17). For example, a Mab trapping (MAT) ELISA for detecting antibody to 3ABC and blocking ELISAs for detecting antibody to 3AB or 3ABC. Currently, the Enzyme-linked immunoelectro transfer blot assay (EITB) has been widely applied for sero surveillance and risk assessment associated with animal movement..In addition to the classical techniques of virus isolation in tissue culture and antigen detection by enzyme-linked immune sorbent assay (ELISA),

RT-PCR has become established as a reliable, fast and sensitive method of early FMD diagnosis (18). Rapid identification of the serotype of the virus that is responsible for an outbreak is essential to speed up diagnosis, for

selection of an appropriate emergency vaccine. A rapid and sensitive RT-PCR method for FMDV detection, which differentiated FMDV from other genetically and/or symptomatically related viruses and, more importantly, comprised a multiplex-PCR method that differentiated between common serotypes, has been reported (19).

The aim of this study was to document the seropositivity of Foot and Mouth Disease (FMD) and to use a RT-PCR for the detection of FMDV in clinical samples obtained from FMD symptomatic cattle in Basrah province.

Materials and methods

Animals

This study include 73 head of cattle in different region of Basrah governorate with typical clinical symptoms of FMD as lameness , high fever and presence of some vesicles in the oral cavity and feet, from April to July 2013 in Basrah,Iraq**.** Sampled animals were divided into 2 groups based on age**(<1-4 and >4-8 years)** and sex(males and females).

Measurement of the antibodies

Five ml of blood were drawn from 73FMD symptomatic cattle for separating sera. ELISA CHEKIT FMD-3ABC Bo-Ov ELISA Test had been used to determine and measure the antibodies in the serum samples against non-structural protein 3ABC of the FMDV which is present in ELISA reaction micro-plate according to manufacturer instructions(Bommeli Diagnostics, Switzerland).Briefly,each sample and control were prediluted 1:100 in a tube using CHEKIT-FMD-3ABC Sample Diluents, that 5 µl of sample and control were added to 495 µl CHEKIT-FMD-3ABC sample diluent. The CHEKIT-10x wash concentrate 1:10 was diluted with D.W (1 part concentrate with 9 parts of D.W under sterile conditions and stored at (4°C).One hundred µl of prediluted samples and controls were dispensed into the appropriate wells of the Microtiter plate.The Microtiter plate was covered with a lid and incubated for 60 minutes at 37°C in humid chamber .Each well was washed with approximately 300 µl CHEKIT wash solution three times. One hundred µl of the CHEKIT-FMD-3ABC-Anti-Ruminant-lgG conjugate was dispensed into each well. The microtiter plate was covered and incubated for 60minutes at 37°C in humid chamber then step of washing was repeated. One hundred µl CHEKIT-TMB Substrate was dispensed into each well. The Substrate was incubated at room temperature for 15 minute. The color reaction was stopped by adding 100 µl CHEKIT-Stop Solution TMB per well. In the same order and at the same seed as the substrate. The results were read using a Micro-titer plate reader / ELISA(Germany)at a wave length of 450 nm and the OD of the positive control (ODpos) and the OD of the samples (ODsample) were corrected by subtracting the OD of the negative control (ODneg)**.**

$$\text{Value\%} = \frac{\text{OD sample} - \text{OD neg}}{\text{OD pos} - \text{OD neg}} \times 100$$

Interpretation of the results: if a %OD of less than 20% is negative, 20-30% is ambiguous and greater than 30% is positive.

RT-PCR of vRNA

Total RNA was extracted from saliva ,serum or vesicular fluid (140 µl) by using RNeasy kits (QIAamp Viral RNA Mini Kit of Qiagene,USA), according to the manufacturer's instructions, and resuspended in 60 µl of Buffer AVE . This RNA (5 µL) was used as the template in a QIAGEN- One Step RT_PCR Premix mixture (USA): in addition to 1µL of one of the forward primers and reverse primers , RNase free water (Variable µl), 5x QIAGEN one step RT-PCR buffer (10.0 µl) , dNTP Mix (2.0 µl) and QIAGEN One Step RT-PCR Enzyme Mix (2.0 µl) in a 50 µL reaction volume.

RT-PCR that targeted VP1 of Universal, A and O serotypes (Universal Primer F1/R1, A-1C612F/EUR-2B52R, and ARS4/ EUR-2B52R; Table 1) generated a single band corresponding to the size expected (328 ,865 and1301 bp respectively).(20; 21;22)

Table (1**) FMDV Oligonucleotide primers used for RT-PCR** .

Primers	primer sequence **(5′ → 3′)** '	
Universal	1F	GCCTGGTCTTTCCAGGTCT
	1R	CCAGTCCCCTTCTCAGATC
A-serotype Specific	A- C612F	TACCAAATTACACACGGGAA
	EUR-2B52 R	GACATGTCCTCCTGCATCTGGTTGAT
O - serotype Specific	ARS4F	ACCAACCTCCTTGATGTGGCT
	EUR- 2B52 R	5'- GACATGTCCTCCTGCATCTGGTTGAT-3'

Amplification reaction was done by using the following thermal profile one cycle of 50°C for 30 min, : one cycle of 95°C for 15 minutes followed by 30 cycles of 94
°C for 1 min,58 °C or 54°C for 1 min(58 °C for A- 1C612 or O- ARS4 Primer while 54°C was used for the Universal Primer), 72 °C for1.5 min and finally, one cycle of 72 °C for 10 min and a subsequent hold temperature of 4°C using aTechnethermocycler,(UK). PCR products were analyzed by electrophoresis on a 1.5% agarose-Tris-borate-EDTA gel containing 0.5 µg/mL ethidium bromide. DNA markers(Bioneer,Korea) were run alongside the samples to facilitate product identification .

Statistical Analysis

Statistical analysis is done by using SPSS software version 11, the chi square was used to assess Statistical significance

Results

FMDV seropositivity according to age and gender.

Table 2,3 showed that the overall FMDV seropositivity among the 73 FMD symptomatic cattle was **94.5**% using CHEKIT–3ABC bo-ov ELISA Test Kit. Cattle suspected as positive to anti-FMDV antibodies were **4** of the **73 (5.5**%).No significant ($p > 0.05$) difference between the two age groups, with cattle of age group **<1-4 years**

recording the highest seropositivity rates (**96.7**%) and **>4-8 year cattle** the lowest (**93.02**%).On the other hand adults recorded the highest prevalence of suspected seropositivity (**6.98**%), followed by young cattle (**3.3**%). (Table-2). No significant ($p > 0.05$) difference was noted also between the seroprevalence rates of females (**97.6**%) and males (**90.6**%).In contrast seropositive males had the highest suspected seroprevalence (**9.4**%), followed by females(**2.4**%)., with statistical evidence to suggest that FMDV suspected **Seropositivity** was not significant($p > 0.05$) (Table- 3).

Table(2). Seropositivity and significant test analysis for foot and mouth disease in cattle of different age groups.

Age groups (years)	FMD symptomatic n.(%)	ELISA result n.(%)		
		+Ve	suspected	-Ve
<1-4	30(41.1)	29(96.7)	1(3.3)	0
>4-8	43(58.9)	40(93.02)	3(6.98)	0
Total	73(100)	69(94.5)	4(5.5)	0

χ^2=0.023 p > 0.05

Table(3). Seroprevalence and significant test analysis for foot and mouth disease seropositivity in cattle of different gender.

Sex	FMD symptomatic n.(%)	ELISA result n.(%)		
		+Ve	suspected	-Ve
Males	32(43.8)	29(90.6)	3(9.4)	0
Females	41(56.2)	40(97.6)	1(2.4)	0
Total	73(100)	69(94.5)	4(5.5)	0

χ^2=0.599 p > 0.05

RT-PCR assays

FMD clinically positive 73 tissue samples (mouth and hoof epithelium and vesicle fluid) and secretion samples (saliva and serum,) were evaluated by RT-PCR for the diagnosis of FMD with different pair of universal,A and O serotype-specific primers.RT-PCR samples producing a band of the expected size ((328 ,865 and1301 bp respectively).) were considered positive (Fig.1,2). Results were shown in tables(4,5,6,7)

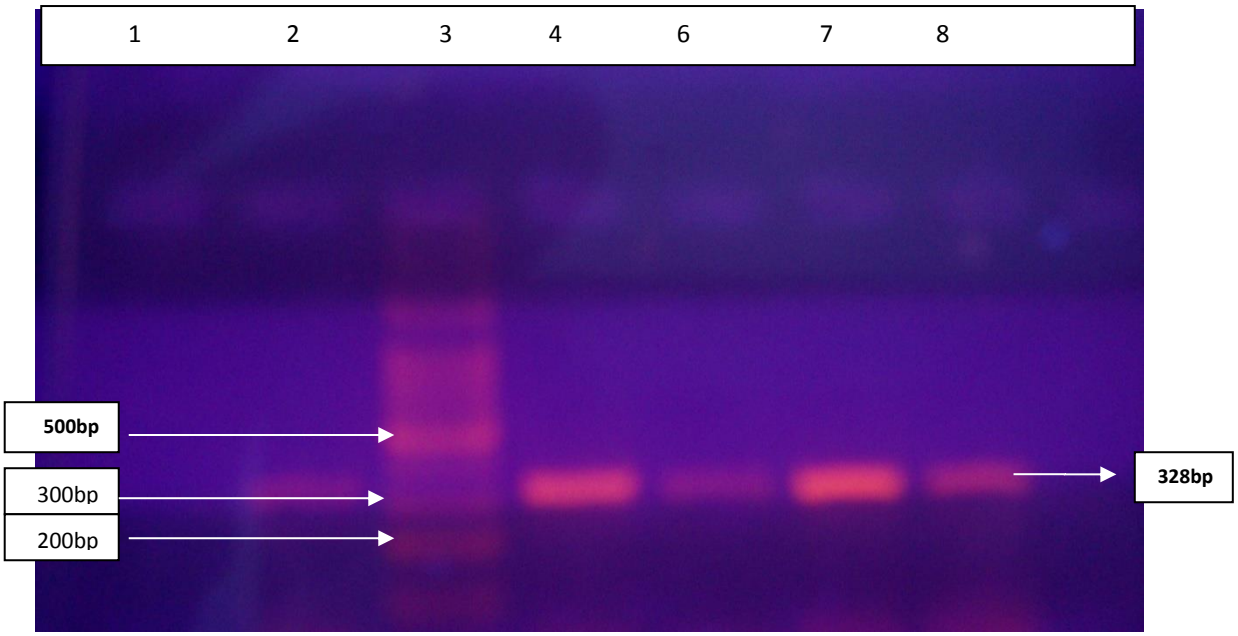
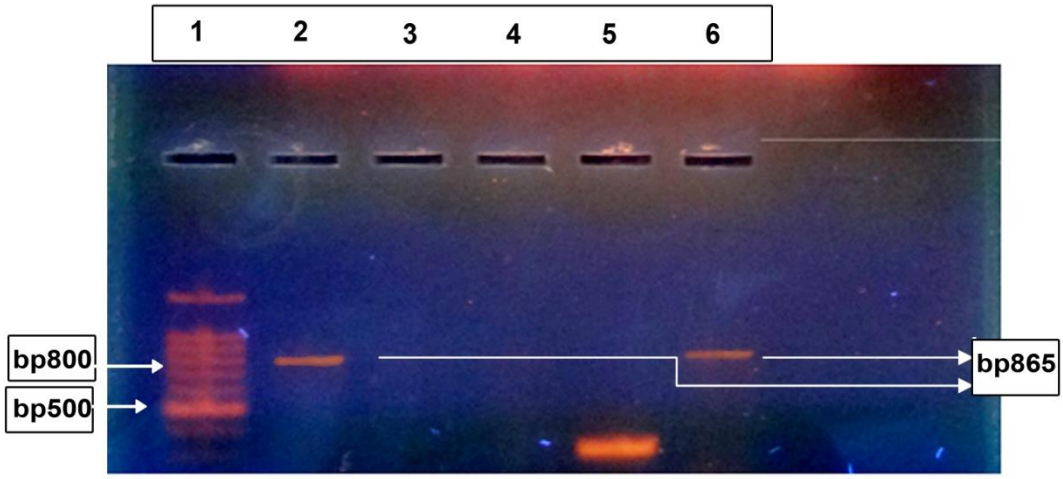


Figure (1) positive and negative samples of vesicles fluid , serum and saliva according to RT-PCR amplification of (328bp) of universal primer .Lane (1 ,8)are negative , lane (2 ,4 , 5 , 6, 7) are positive , lane (3) is 100 bp DNA ladder marker.



ding to RT-PCR amplification. Lane (2,6) are positive serotype A(865bp), lane (3,4,5) are O serotype negative and lane (1) is 100bp DNA ladder marker.

Universal primer pair 1F/1R from 5'UTR region detected FMD in 55 out of 73(75.3%) of these 37 (**92.5**%) females and 18 (**62.1**%) males samples, with high significant statistical difference **(p<0.01)** between males and females in the PCR positivity ratio and there was no positive samples of FMDV Universal primer in suspect seropositive cases (**Table-4**) . Of these, 15(27.3%) were detected with

A-1C612/ EUR- 2B52, primer pair and there was no positive case of serotype O in seropositive cases. The circulation of serotype A was higher in the males as compared to the females with a ratio of **33.3**% (6/18) with statistical evidence to suggest that FMDVRT- PCR positivity ratio was not significant($p > 0.05$) (**Table-5**).

Table(4) RT-PC R results of Universal gene according to sex of Cattle

Sex	ELISA seropositivity results	Tested animals n.(%)	Universal gene n.(%)	
			+ Ve	- Ve
Males	seropositive	29(39.7)	18(62.1)	11(37.9)
	suspected	3(4.1)	0	3(100)
Female	seropositive	40(54.8)	37(92.5)	3(7.5)
	suspected	1(1.4)	0	1(100)
Total		73	55 (75.3)	18(24.7)

χ^2=21.311 p<0.01

. **Table(5) RT-PC R results of A- 1C612 and** O-**ARS4 FMDV serotypes according to sex of cattle**

Sex	Tested animals n.(%)	Serotypes n.(%)			
		A- IC612			O-ARS4
		+Ve	-Ve	+Ve	-Ve
Males	18 (32.7)	6(33.3)	12(66.7)	0	18(100)
Female	37(67.3)	9(24.3)	28 (75.7)	0	37(100)
Total	55 (100)	15(27.3)	40 (72.7)	0	55(100)

χ^2=0.145 p>0.05

The statistical analysis showed high significant differences (P<0.01) between tested samples in relation to age with the older animals showing a higher risk of infection with FMD virus compared to younger animals and no positive sample of FMDV Universal primer in the suspected animals . Out of all animals that tested positive for Universal primer pair 1F/1R from 5'UTR region <1-4 year age realized a prevalence of **58.6** while >4-8 year age realized prevalence of **95**% (Table 6). Serotype A circulation was not significantly differed between the tested animals in relation to age with overall ratio **27.3**% (15/55).Although the prevalence of serotype A (**31.6**%, 12/38) in the older animals was

higher than that of younger (**17.7**%, 3/17) but the difference was not statistically significant (P>0.05) (Table 7).

Table(6) Rt- PC R results of Universal gene according to age of Cattle

Age groups (years)	ELISA seropositivity results	Tested animals n.(%)	Universal primer n.(%)	
			+Ve	-Ve
<1-4	seropositive	29 (39.7)	17 (58.6)	12(41.4)
	suspected	1(1.4)	0	1(100)
>4-8	seropositive	40(54.8)	38(95)	2 (5)
	suspected	3(4.1)	0	3(100)
Total		73 (100)	55(75.3)	18 (24.7)

χ^2=29.907 (P<0.01)

Table(7) RT-PC R results of A-I C612 and O- **ARS4 FMDV serotypes according to sex of cattle**

Age groups (years)	Tested animals n.(%)	FMDV Serotypes n.(%)			
		A-I C612		O- ARS4	
		+Ve	-Ve	+Ve	-Ve
<1-4	17 (29.1)	3 (17.7)	14(82.4)	0	17(100)
>4-8	38(63.6)	12(31.6)	26 (68.4)	0	38(100)
Total	55 (100)	15(27.3)	40 (72.7)	0	55(100)

χ^2=1.149 p>0.05

Discussion

Measurement of the antibodies by ELISA

The potential use of measuring antibody against NSP of FMD virus to differentiate infection from vaccination was first demonstrated by adioimmunoprecipitation (23). (24) described an electroimmunotransfer-blot (EITB) assay in which sera are examined for the presence of antibodies to several NSPs simultaneously by immunoblotting. However, ELISA is more suitable than immunoblotting for screening large numbers of sera, considerable effort has been focused on developing sensitive, specific and reproducible ELISA's for the detection of antibodies to NSPs. A number of such assays were also described (25, .26, 27, 28, 29). In animals seropositive for antibody to structural proteins, the detection of antibody to the polyprotein 3ABC is the most reliable single index of infection (30, 31, 24, 32). The high sensitivity of this assay was confirmed by using sets of sera from naive and vaccinated cattle as previously described by (25,26 ,33).

The overall seropositivity of FMD in the Basrah was found to be high at **94.5**%. In comparison, a similar survey by (34.) in the same region found a seropositivity of 72.7%. The seropositivity finding is higher than the overall seropositivity of 62, 61.4, 34.09%and (33.3 and 66.7 %) reported by (35; 36; 37;38) respectively in cattle in middle and south of Iraq, respectively.Other study in the neighbouring country of Saudi Arabia (39) also found low prevalence of 16% compared with the current study. The sero-positivity in the present study is higher than the overall sero-positivity of 14.6,21,26.5 and 8.18% reported by. (40), (41) and (42) in other parts of world respectively.

On the contrary, (38) in his study to the seroprevalence of FMD among cattle in middle part of Iraq documented a higher rate of seroprevalence (100,75 and75%)in Karbala,Al-Najaf and Al-Diwaniyah respectively.

A higher sero-positivity of FMD in this study could be attributable to the nationwide prevalence of circulating FMD virus and fact that all tested sera has been collected from FMDS symptomatic cattle.

No significant ($p > 0.05$) difference between the two age groups, with cattle of age group <1-4 years recording the highest seropositivity rates (96.7%) and >4-8 year cattle the lowest (93.02%).the present result is in agreement with the previous reports of (42) ; (43) who found no significant association between seropositivity of FMD and age of cattle. In contrast with the current study, the significantly ($P < 0.05$) higher seropositivity rates of FMD in adult cows than in calves observed in the other studies. (44);(45) ;.(46);. (38); (41) and(34). Similarly the effect of sex on the FMDV seropositivity was not significant ($p>0.05$) in this study. This finding was consistent with the previous finding of (37;38) in middle of Iraq documented no significant ($p>0.05$) association between sex of cattle and seropositivity. The non significant ($p>0.05$) association between sex of cattle and seropositivity also reported by many studies conducted in other parts of world (41 ;43; 47) .But the current resulte was in contrast with the previous studies of (48;34) who documented significant ($p<0.05$) association between sex of cattle and seropositivity with higher rate of incidence in female cattle (16.63 and78.6% respectively) than that of male cattle (1.37 and 60.4% respectively.

RT-PCR assays

Serotyping of FMDV is done using the antigen capture ELISA that has replaced the complement fixation test as the routine method of choice (49). Samples sometimes have to be inoculated into cell cultures for the production of sufficient antigen, which may require several days, before typing can be undertaken. Molecular biology is providing extremely sensitive and specific tools for identifying and characterizing FMDV strains in clinical samples (18). The molecular biological technique is rapid, accurate, highly sensitive and only small quantities of material are needed to do the test.

In this study RT-PCR was performed on73 clinical samples.In order to detect FMD viral RNA, regardless of their serotypes the universal primer pair IF and IR was used in the first step of RT-PCR.This primer was used by many previous local and international studies(50; 37;34).

In the present study universal primer IF when used individually with the reverse primer IR, 328 bp DNA fragment was amplified in**55(75.3**%**)** out of 73 tested clinical samples. Being RNA in nature,FMD genome is very sensitive to degradation by RNAses and other degradative enzymes. If a sample is collected during the early phase of the infection (especially from vesicles), chances of viral amplification by RT-PCR are higher,but if the lesions get invaded by bacteria, or lesions start healing, the probability of obtaining the intact viruses from samples decreases drastically. Some samples were collected in the late phase of infection and the viral genome may have been degraded by bacterial RNAses and other degradative enzymes resulting in either weak or no detectable signals by RT-PCR.

The results of primer pair IF and IR successfull amplification by RT-PCR were in contrast with other Iraqi studies (37;34) who reported that universal primer pair IF and IR amplicon of 328 bp was observed in 100 and 81.9% of tested samples respectively.

Detection of the primer pair IF and IR was used by(50) and designed with reference to the conserved sections of the 5 ′UTR of the FMD virus genome and was intended for the diagnosis of all seven serotypes. They found that the universal primer (IF/IR) located in the 5 UTR of the FMD virus genome successfully detected serotypes O, A, Asia-1 and C in clinical samples,so that this finding supported the present results in which the tested samples with positive universal primer were amplified with specific primers **A- IC612** of 865 bp DNA fragment in **15(27.3**%) and nil for serotype O- **ARS4**.A relatively low frequency finding of FMDV serotypes circulated in the areas which were reported in this study , may however increase because of unrestricted movement of animals within the region and across borders. On the other hand the present result had been supported by other local and international studies (37; 38; 51;34) which confirmed the presence of serotype A in middle part of Iraq.and neighboring countries IRAN and TURKEY during 2011 , 2012.

On the contrary (38) found that serotype O was the predominant serotype in middle of Iraq while, serotype A was the second serotype. Also he demonstrated that percentages of A and O serotypes in cattle under the study, in AI-Diwaniyah province were (11.1 and 66.6% respectively), in Baghdad (12.5 and 62.5% respectively), in Babil (0 and 100% respectively), in Al- Najaf (14.2 and 71.4% respectively) and in Karbala (0 and 87.5% respectively).Also the current results were in contrast with (34) who reported that 19(22.9%) samples were found to be serotype A-1C562 positive and 26(31.3%) were serotype O-1C272 positive. But the difference between the present results and other local results may be due to multiple samples collected from the same epidemics or may reflect sampling bias within a relatively limited number of samples.

According to sex, universal primer pair IF and IR amplicon of 328 bp was observed in 37 Females and 18 males samples, with PCR positivity ratio(**92.5** and **62.1**% respectively).High significant statistical difference **(p<0.01)** was found between males and females concerning the universal gene PCR positivity ratio. Nearly similar result was reported by other local study (34) who reported that universal primer pair IF and IR amplicon of 328 bp was observed in 49(87.5%) Females and 19(70.4%) males samples and there was significant statistical difference **(p<0.05)** between males and females concerning the universal gene PCR positivity ratio.

The significantly (**P < 0.05**) higher PCR positivity ratio of universal gene in adult cows **38(95**%)than in calves**17 (58.6**%**)** observed in the current study is in agreement with the previous study of (34) who reported that High significant statistical difference **(p<0.01)** in concern to PCR positivity ratio of universal gene in adult cows (90.7%)than in calves (65.5%).

Non significant effect (p>0.05) for the sex and age of cattle was observed on the PCR positivity ratio of A serotype in this study. Although the prevalence of serotype A (**31.6**%, 12/38) in the older animals was higher than that of younger (**17.7**%, 3/17) but the difference was not statistically significant (P>0.05) . The circulation of Serotype A was higher in the males as compared to the females with a ratio of **33.3**% (6/18) with statistical evidence to suggest that PCR positivity ratio was not significant(p > 0.05) This finding was consistent with the previous finding of (34)who mentioned that non significant (p>0.05) association between sex and age of cattle and circulation of Serotype A, although the males and the animals at age group(**>4-8 year**) showed higher rate of PCR positivity (31.6 and34.7% respectively) .

The explaination for the non significant effect for the sex and age of cattle on the ELISA sero-positivity and PCR positivity ratio of A serotype in this study could be attributable to the sampling bias within a relatively limited number of samples.As the all tested sera in case of ELISA screening collected from 73 FMD symptomatic cattle and only tested samples with positive universal primer were amplified with specific primers **A- IC612** serotype O-ARS4

In conclusion the sero-positivity of FMD was found to be high at **94.5**% and different levels of frequency of FMD virus serotype A

and universal gene but not serotype O were detected in the Basrah in south of Iraq.

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