Detection of the presence Foot and Mouth Disease Virus in Al-Diwaniayah

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Abstract

This study aimed to investigate the prevalence of FMD infection, examine buffalo , sheep and goat clinical manifestations, and confirm the causative agent's using various techniques. For the FMD outbreak in Iraq, laboratory methods were employed to confirm the presence of the virus in some regions. A total of samples were129 collected from different regions conducted from January 2025 to march 2025 in Al-Diwania Provinces, Iraq. All animals underwent clinical examination, and suspected cases were identified, followed by epithelial tissue collection. Clinically, infected cattle exhibited symptoms such as pyrexia, anorexia, and erosions in the mouth and feet. Analysis of collected samples revealed that 86 out of 130 were positive for FMDV, Sample analysis indicated slightly higher detection rates in females than males, with no statistically significant differences, suggesting both sexes are susceptible to FMDV infection. Age-specific FMD infection rates suggested a potential decrease in FMD occurrence in young cattle (6-18 months vs. 19-36 months) compared to adult animals (older than 36 months), but without statistically significant differences. The study's findings on FMD prevalence in buffalo , sheep and goat indicate that virus is responsible for the recent 2025 FMD outbreak in Al-Diwaniayah in this study. Furthermore, the study demonstrates that factors such as age and sex have minimal impact on FMD occurrence.

Key words :FMD , Foot and Mouth Disease , apthovirus .

الكشف عن وجود فيروس الحمى القلاعية في الديوانية

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خلاصة

هدفت هذه الدراسة إلى تقصي مدى انتشار عدوى مرض الحمى القلاعية، وفحص المظاهر السريرية للجاموس ،الأغنام و الماعز، وتأكيد العامل المسبب باستخدام تقنيات مختلفة. بالنسبة لتفشي مرض الحمى القلاعية في العراق، تم استخدام الأساليب المختبرية للتأكد من وجود الفيروس في بعض المناطق. تم جمع 129 عينة من مناطق مختلفة أجريت في الفترة من كانون الثاني 2025 إلى آذار 2025 في محافظة الديوانية، العراق. خضعت جميع الحيوانات للفحص السريري، وتم تحديد الحالات المشتبه فيها،

تليها جمع الأنسجة الظهارية. سريريًا، أظهرت الماشية المصابة أعراضًا مثل الحمى وفقدان الشهية وتآكل الفم والقدمين. وكشف تحليل العينات التي تم جمعها أن 86 من أصل 129 كانت إيجابية لفيروس الحمى القلاعية في فحص ال pcr، وأشار تحليل العينة إلى أن معدلات الكشف لدى الإناث أعلى قليلاً من الذكور، مع عدم وجود فروق ذات دلالة إحصائية، مما يشير إلى أن كلا الجنسين عرضة للإصابة بفيروس الحمى القلاعية. أشارت معدلات الإصابة بمرض الحمى القلاعية حسب العمر إلى انخفاض محتمل في حدوث مرض الحمى القلاعية في الماشية الصغيرة (6-18 شهرًا مقابل 19-36 شهرًا) مقارنة بالحيوانات البالغة (أكبر من 36 شهرًا)، ولكن دون فروق ذات دلالة إحصائية. تشير نتائج الدراسة حول انتشار مرض الحمى القلاعية في الماشية إلى أن الفيروس هو المسؤول عن تفشي مرض الحمى القلاعية الأخير في الديوانية في هذه الدراسة عام 2025. علاوة على ذلك، توضح الدراسة أن عوامل مثل العمر والجنس لها تأثير ضئيل على حدوث مرض الحمى القلاعية.

الكلمات المفتاحية: الحمى القلاعية، مرض الحمى القلاعية، فيروس الأبثو.

Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral infection that significantly impacts cloven-hoofed animals and poses substantial economic challenges to the global livestock industry (Namatovu et al., 2015). The causative agent, Aphthae epizooticae, exhibits multiple serotypes and genotypes that vary across geographical regions and temporal periods (Soltan et al., 2017). The virus's genetic diversity is primarily driven by persistent infections and recombination events, leading to the emergence of novel strains that challenge the efficacy of existing vaccines (Malirat et al., 1994). Furthermore, simultaneous infections with different serotypes can significantly influence clinical manifestations, highlighting the disease's complexity (Arzt et al., 2021). FMD predominantly affects economically important domesticated species including, buffalo , goats , sheep , and pigs .

The virus spreads rapidly through multiple transmission routes, including contaminated meat, milk, offal, skin, and non-sterile excretions, affecting multiple organ systems in infected animals (Perry et al., 2020). The FMD virus has reached outbreak status, significantly impacting the socio-economic situation as communities gradually recover from the COVID-19 pandemic.

The FMD virion exhibits a spherical morphology characterized by a smooth outer surface. It is a small non-enveloped virus of single-stranded positive-sense RNA virus. The diameter was about 30 nm when examined using electron microscopy, ranging in length from 7.2 to 8.4 kb (Malik et al., 2017)

The disease is caused by an aphthovirus belonging to the Picornaviridae family. The RNA genome of FMD virus is single-stranded. The diameter was about 30 nm when examined using electron microscopy (Njihia, 2022). The surface of FMDV exhibits a spherical shape (Dill & Eschbaumer, 2020). Viral protein 1 (VP1) is in charge of the attachment and penetration of host cells, as well as the development of protective immunity. The nucleotide sequence of this protein is

widely regarded as the gold standard for serotyping FMDV (Metwally et al., 2023; Al-Rawahi et al., 2024;).

FMDV is the virus that causes FMD and there are seven identified serotypes: "A, O, C, Asia1, SAT-1, SAT-2, and SAT-3" (Anjume et al., 2024). Each serotype has different subtypes, resulting from the virus exhibiting a significant mutation rate (Shin et al., 2024). FMD is endemic in several including south America, Asia, and Africa (Nishi et al., 2024,). The serotypes Asia 1, A, and O of the FMDV continue to circulate in Iraq, with occasional epidemics (Aslam & Alkieaije, 2023;). In 2023, Iraq experienced a significant outbreak of FMDV, serotype SAT-2 (FAO, 2023). Although all FMD serotypes show the same signs, vaccination against a single strain does not provide immunity against other strains because of significant differences in antigens (Zewdie et al., 2023).

Monitoring FMD cases, particularly in cattle, is critically important due to their high morbidity rate. Providing not only farm products but also cattle and calves (Hutahaean, 2020). The FMD attacks the mouth and legs which makes it difficult for the animal to stand up (Belay & Muktar, 2015). While the recovery rate for cattle is relatively high, it takes considerable time for them to recover their ideal weight and milk production. Unfortunately, many farmers have suffered financial losses and depleted resources, as this outbreak has led to a decline in prices and demand for cattle products (Punyapornwithaya et al., 2022).

The FMD can be transmitted by both vector organisms and human activities (anthropic) (Naipospos, 2020).

FMDV induces vesicles on the feet, mammary glands, and oral cavity in the infected animal [Grubman,et al ,2004]. The disease may cause high mortality in young animals due to cardiac arrest succeeding myocarditis [Barnett,et al 1999]. In a fully susceptible livestock population, the morbidity rate of FMDV can be as high as 100% with a high mortality rate. However, the morbidity and mortality rates of FMDV depend on various factors, such as the animal species, breed, production type, age, immunity, virus dose, and animal movement [Jemberu,et al2020]. FMD can persist in goats and sheep for up to nine months [Donaldson ,2000]. The FMD is diagnosed using a combination of history, clinical symptoms, and laboratory tests. FMDV can be isolated in cell cultures, viral nonstructural proteins can be detected using enzyme-linked immunosorbent assays (ELISAs), and viral genomic material can be detected using polymerase chain reaction (PCR) assays [Donaldson ,et al 2000]. Anti-nonstructural protein (NSP) antibody testing is commonly used to differentiate infected animals from vaccinated animals in FMD endemic areas [OIE ,2012] and FMD-free countries [Brocchi,et al2006]. FMD outbreaks are widespread in

market-oriented systems compared to subsistence systems due to frequent movement and mixing of animals [Jamal , et al 2013]. FMDV can be transmitted directly via inhalation of virus particles through direct contact with the acutely infected animals [FAO,2021] or indirectly via a contaminated environment, as the virus can survive for a long period under favourable conditions [Paton,et al 2010], such as temperatures <50 °C, relative humidity >55%, and neutral pH [Arzt et al., 2021]. Airborne transmission has also been reported over both long and short distances [Miller et al 2018].

Foot and mouth disease (FMD) was first described by Hieronymus Fracastorius, an Italian residing in Venice in 1514. Fracastorius observed animals refusing food, accompanied by redness of the mouth mucosa. In addition, the animal exhibited the development of blisters in both their oral cavities and feet (Cole, 2020). That, majority of infected animals eventually recovered from the disease (Njihia, 2022).

Aim of study is to detection the the prevalence of FMD

FMD virus in Iraqi cattle

Methods:

Animal

The study conducted on 129 number of femal are 29 and male 14 that suspected FMD infected of different sex and ages for each group of animals(sheep ,gout and buffalo), immune status, and originating from Al-Diwaniya Province/Iraq, between January2025 to March2025. All animal were examined clinically, and clinical signs were documented.

Tissue samples:

Epithelial tissue samples and blood (n = 129) were collected from animals in. The oral epithelium samples were collated and placed in a plastic container containing Trizol® ((Amirouche et al., 2021). All samples were then frozen at -20 for the time of the PCR test.

Table 1, 2 and 3 The table above represent the species and the sex that we take sample from

sheep	Age/month		
sex	6-12	19-36	37>
female	10	8	11
Male	5	5	4
total	15	15	15
Total	43		

Buffaloes	Age/month		
sex	6-12	19-36	37>
female	10	8	11
Male	5	5	4
total	15	13	15
Total	43		

goat	Age/month		
sex	6-12	19-36	37>
female	10	8	11
Male	5	5	4
total	15	13	15
Total	43		

Table 4 represent the state of it if vaccinated or not

Vaccine state	vaccinated	Non-vaccinated	total
No.	62	67	129

Molecular Method:

Viral RNA Extraction:

The extraction steps were done according to the manufacturer's protocol. AccuPrep® Total RNA extraction kit (Bioneer, Korea) was used to extract viral RNA from epithelial tissue samples.

50 ng of epithelial samples were placed in sterile Eppendorf (1.5 ml) tubes, and 1 ml of the Trizol solution was added. The mixture was then combined

200 µl of chloroform were added and shaken the tubes for 15 seconds before incubating on ice for 5 minutes.

* These contents were centrifuged at 12,000 run per minute at 4 °C for 15 minutes.

* The supernatant containing the required RNA is then transferred to another Eppendorf tube. Then, 500 µl of isopropanol is added to the supernatant. The tube was gently mixed by inverting it 5 times then incubated at -20°C for 10 minutes

* For ten minutes at 4 °C, the mixture was centrifuged at 12,000 rpm. To prevent damaging the RNA pellet, the supernatant was gently removed.

* In each tube, 1 ml of 75 % ethanol were added and mixed vigorously.

* The tubes were centrifuged at 4 °C and 12,000 rpm for five minutes. After removing the supernatant from the RNA pellet, it was left to dry at room temperature for five minutes.

* The RNA pellet was resuspended in RNase-free water by pipetting up and down a few times. Then, it was incubated at 55-60°C for 10 minutes.

* Finally, the RNA samples were stored at -80°C in a deep freezer to maintain stability.

Extracted RNA estimation:

The quantity and purity of extracted RNA were checked using a nanodrop spectrophotometer operating at 260/280 nm wavelength at a ratio of (1.8-2) as pure. The estimation process involved the following steps:

1- First the nanodrop software was run, and the appropriate program for nucleic acid analysis, specifically RNA, was selected. Then the measuring pedestals were thoroughly cleaned using a dry wipe.

2- Subsequently, 2µl of nuclease-free water was carefully pipetted and deposited on the surface of the lower measuring pedestal. This step was essential for establishing a blank measurement for the Nanodrop.

total RNA was carefully pipetted onto the measuring pedestals for measurement.

Pcr step	Temperature	time	repeated
Initial denaturation	95℃	3 min	1
Denaturation	95℃	35 sec	30
Annealing	57℃	35 sec	30
Extension	72℃	35 sec	30
Final extension	72℃	5 min	1
Inactivation	4℃	-	Inactivation

ELISA method :

The test method was preceded by several steps. As following:

ELISA steps:

A: Prepare test wells:

1 -Add the standard solution (50 µl) to the standard wells.

Sample addition:

2 -Add 10 µl of the test sample to the sample wells, then add 40 µl of sample diluent. Do not add anything to the blank well.

B: Conjugate addition:

3 -Add HRP-conjugated reagent (100 µl) to all wells.

C: Incubation and washing:

4 -The plate Incubation at 37°C for 60 minutes.

5 -Wash the wells five times, (400 µl detergent per wash) and ensure complete water removal after each wash.

D: Substrate addition and incubation:

6 -Add 50 µl each of A and B chromogen solution to all wells.

7 -Carefully mix and incubate at 37 degrees Celsius for 15 minutes.

F: Stop reaction:

8 -Add stop solution 50 µl to all wells.

9 -Make sure the color changes from blue to yellow.

G: Measurement:

10- After 15 minutes, measure the wells optical density (O.D.) at 450 nm after the stop solution was added.

standart	Mean O.D.	Concentration (ng/ml)
ST.1	2.124	800
ST.2	1.491	400
ST.3	0.954	200
ST.4	0.621	100
ST.5	0.503	50
ST.6	0.348	0

Statistical analysis was conducted using SPSS software (version 32). The Chi-square test was utilized to assess the relationship between age, sex, and the observed clinical signs of FMD infection. Statistical significance was established for p-values of 0.05 or less.

The result

FMD Epidemiological Analysis

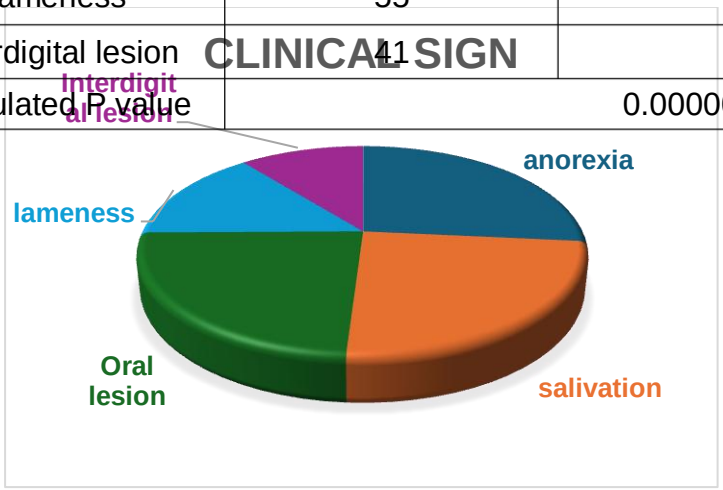
Physical Assessment

The outcomes of the clinical examination are detailed in table . Key clinical signs included excessive drooling, characterized by long, stringy saliva extending to the ground . Furthermore, lesions and open sores were present on the nostrils and muzzle . Lesions or ulcers were also noted on the gums, dental pad, hard palate, tongue, and inner surfaces of the mouth . Additionally, skin lesions were observed in the area around the coronary band and between the digits of the hoof

The table 5 represent the clinical signs of each species and number of each species that infected and non infected

The table 5 represent the clinical signs of each species and number of each species that infected and non infected

Clinical sign	Observed with signs	Observed with no signs	Total
	N0.	%	N0.
anorexia	101	77.45	29
salivation	93	71.57	37
Oral lesion	91	69.61	39
Nostril lesion	59	45.10	71
lameness	55	42.16	75
Interdigital lesion	41	31.37	89
Calculated P value	0.0000042 HS		



The table 6,7 and 8 represent the resutl of the pcr and Elisa of each species

test	Infected buffaloes no. (%)	nonInfected buffaloes no. (%)	Calculated x^2	P value
pcr	30 (70.58%)	13 (29.42%)	6.027	0.0491 S
ELISA	35 (80.39%)	8 (19.61%)		
total	43 (100%)			

test	Infected sheep no. (%)	nonInfected sheep no. (%)	Calculated x^2	P value
-pcr	25 (58.14%)	18(41.86%)	6.027	0.0491 S
ELISA	28 (65.12%)	15 (34.88%)		
total	43 (100%)			

The table 9 ,10 and 11 represent species number and the sex of each species that infected and non infected

sex	Buffaloes N	Infected buffaloes no. (%)	nonInfected buffaloes no. (%)
male	15	12(80.00%)	3(20.00%)
total	42	35(83.33%)	7(16.67%)

test	Infected goat no. (%)	nonInfected goat no. (%)	Calculated x^2	P value
pcr	24 (55.81%)	19(44.19%)	6.027	0.0491 S
ELISA	23 (53.49%)	20 (46.51%)		
total	43 (100%)			

sex	sheep N	Infected sheep no. (%)	nonInfected sheep no. (%)
femal	28	24(85.71%)	4(14.29%)
male	15	12(80.00%)	3(20.00%)
total	43	36(83.72%)	7(16.28%)

sex	goat N	Infected goat no. (%)	nonInfected goat no. (%)
femal	21	18(85.71%)	3(14.29%)

The table 12 represent the village that we take sample from and the percentage of the negative and positive infection .

village	result	Percentage
Afak	+ve	83.33%
Afak	_ve	16.67%
Nafar	+ve	25%
Nafar	_ve	50%
Al-sudair	_ve	100%
Ghammaz	_ve	100%
Outpatient clinic	_ve	100%

Conclusion

-1 Detection of the presence Foot and Mouth Disease Virus in Other provinces

2There were no significant differences in infection rates between age groups or sex.
3. The observed positive result is attributed to the owners' lack of acceptance of animal vaccination
4 Compared to ELISA, PCR demonstrated higher effectiveness and accuracy detection.

Recommendations:

1. Appropriate administration of animal movement is crucial to limit the dissemination of FMDV from regions where the disease is prevalent. This control should encompass examining animals for their FMD status before any relocation between sites, including routine inspections of both animals and their products.
2. It is suggested to conduct regular surveys to acquire comprehensive data regarding the prevalence of FMDV serotypes and topotypes across the country during periods of outbreak.
3. Establishing a consistent vaccination program is advisable with the aim of effectively managing and containing FMD outbreaks. This system should be implemented as a preventative measure for the administration and control of these outbreaks.

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