Iraqi Journal of Biotechnology, 2025, Vol. 24, No. 2, 13-23

Study of Cytopathic and Histopathic Effect for the Novel Serotype SAT2 of Foot and Mouth Disease in Iraq

[1]Nagham J. Al-Kalabadi , [2]Amina N. Al-Thwani

[1,2] Institute of Genetic Engineering and Biotechnology for post graduate studies, University of Baghdad, Baghdad, Iraq.

Received: January 14, 2024 / Accepted: June 11, 2024 / Published: July 5, 2025

Abstract: Superinfection of bovine continuously infected with foot-and-mouth disease virus (FMDV) that bring about economic losses because the livestock around the globe is the major trade barrier, in addition to decrease milk production and weight gain. Biological samples (101) as epithelial tissue of tongue and gum of infected animal with FMD were collected from, 47 cattle and 54 buffalo during the period extended between the 15th May- 2022 to end of April-2023 of several regions in some Iraqi governorates. This work aimed to study the cytopathic and histopathic effect of diagnosed FMDV/SAT2. Because virus isolation is the gold stander in diagnostic procedures of this disease, so one step polymerase chain reaction (RT-qPCR) was used to diagnose the virus. Then investigate serotype SAT2 using QIAGEN® One-step reverse-transcription polymerase chain reaction (RT-PCR) Kit. The result revealed that fifty-six sample (35 buffalo and 28 cattle) were positive for FMDV/SAT2. Vero cell lines, primary lamb kidney cell, primary lamb testis cell and primary fetal lamb kidney applied in study of cytopathic effects (CPE) compared with monolayer control after 24-72hours to find out changes in the morphological of infected cells. Epithelial tissue of tongue used for detection the pathological lesions, the result of histopathic study appeared necrosis and hydropic degeneration are the most important features compared with control.

Keywords: Cytopathic effect, Histopathic effect, FMD, FMDV/SAT2 and RT-PCR

Corresponding author: (E-mail: fatimajawad. nj2000@gmail).

Introduction

Foot-and-mouth disease (FMD) has been recognized as one of the most serious infectious diseases of livestock globally that causes huge economic losses, so that global efforts are being undertaken to control and eliminate the disease in many countries (1, 2). This disease considers as one of the most significant diseases threatening the international trade of animals and their products according to Office International des Epizooties (3). The FMDV is a small single-strand and non-enveloped RNA virus with contagious style and a vast range of host predilection (70 species of cloven-footed) (4, 5, 6).

The clinical impact of the FMDV noticed usually in oral cavity (tongue, lips, dental pad and gum) and feet in addition to udder, the lesions are

appeared as vesicles then developed remarkable ulcer. The microscopical examination showed hydropic degeneration in the lining epithelium with vacuoles formation in the tongue mucosa which may be opened with each other forming bullae of different size and shapes (7).

The cytopathic effects (CPE) were investigated to detect the impact of the virus on different live cells including, Vero cell lines, baby hamster kidney cells (BHK), Bovine kidney cells, lamb testicle cells, lamb kidney cells, primary and secondary thyroid cells of calf and ovine, a fetal goat tongue cell line, a fetal porcine kidney cell line, and bovine kidney cell. These cells are more preferable for FMD virus isolation and propagation (8, 9, 10, 11).

Cell cultures are the most sensitive system for the isolation of FMDV, but some types used are not widespread because of the difficulties obtaining tissue, the time and expense required to prepare the cells, and the fact that the cells have a relatively short life span.

In the present study the cytopathic effects on Vero cell lines and lamb kidney cell in addition to pathological lesions of epithelial cells were investigated to know about the virulence of the recent FMD virus serotype SAT2 cause of the current outbreak.

Materials and methods

Samples- collecting and processing

One hundred and one (101) (biological samples) as epithelial tissue of tongue and gum were collected from 47 cattle and 54 buffaloes appeared significant symptom as of confirmed infected animal with FMD were collected from, 46 cattle and 55 buffalo during the period extended between the 15th May- 2022 to end of March- 2023. Specimens were treated with PBS (pH 7.4-7.6) then centrifugation was carried for 4000 rpm at 4°C between 10-15 min. The supernatant was taken then penicillin +Streptomycin was added to it and kept at-80°C.

Detection of FMD/ serotype SAT2

The RNA extraction from collecting samples was done, then Taq® Probe One-Step Real Time quantitative polymerase chain reaction (RT-qPCR) was used to identify positive samples of FMDV, which were 34 buffalo and 22 cattle biological samples. The final step was taken to check serotype SAT2 using One-step reverse-transcription polymerase chain reaction (RT-PCR) as mentioned by (12).

According to the procedure of manufacture company QIAGEN® the reaction reagents were prepared and mixed, then amplification was carried out by the following – program as shown in (Table 1).

Table (1): Protocols of standard thermocycling amplification for the region of the vp1 of FMDV used for reverse-transcription polymerase reaction.

Step	Number of cycles	Time	Temp
1-Reverse transcription	One	Half an hour	50°C
2-Inactivation	One	A quarter hour	95°C
3-Denaturation	Repeat steps 3 to 5 45 times	1 min.	95°C
4-Primer annealing		1min.	50°C
5-Extension		2 min.	72°C
6-Final extension	One	5 min.	72°C

The PCR product was analyzed by electrophoresis on agarose- Tris-borate-EDTA gel (1.5%) and examined under UV light.

Clinical signs

The most important sign of FMDV of saliva is in the form of threads or ropes, fever also the mouth lesion appeared very sever in cattle's and buffaloes as various area of vesicle, ulceration, and erosion of different shape and size were usually formed on the upper surface of the tongue, gum, dental pad, the lip from the inside and outside, the area below and around the muzzle, in these cases the animal is in pain and has difficulty to eat which conflict the loss of weight. When the infected in udder the outer surface has blisters in different size, especially in the teat, consequently it affects it's the quality and quantity of milk means decline the milk production in some cases suffered from mastitis. During visits through sample collecting of infected animals with FMDV/SAT2 to one of the large fields, one of the workers has vomited, suffer from stomach pain and was uncomfortable after drinking the milk from the infected udder. Whereas when the infected was interdigital space and the hooves from behind it will be very painful because of the presence of vesicles there which leads to their separation, the animal was lameness and immobile due to pain in some cases animal lies there and doesn't move (recumbency) especially when the infection occurred in older cattle and buffaloes as 13-15 years. Young age and newborn without clinical signs only two cases were founding vesicle on the lip and gum but not sever, while sudden death occurs when playing or suckling, this was very cruel and painful for the breeders or owner because of impact on their economic state. According to the veterinarians and the owners of the animal flock, the mortality rate in this study was 100% for calves under four months old especially newborn in both cattle and buffalo (Figure 1).

A **B**

Figure (1): Grossly lesion observed on buffalo infected with FMD/SAT2.
A-Hemorrhagic area of sub mucosa of oral commissure and ulcer formation of dental pad
B- Heavy salivation like a rope of infected buffalo FMD/SAT2

The clinical signs of FMD mostly similar and many studies described the clinical signs as, Gab-Allah *et al.* (21) in Egypt observed vesicle, ulceration and erosion on the upper surface of the tong, cheeks, gum and dental pad in animals infected with FMD serotypes. Also, Faruk and Das (22) in Bangladesh exhibited many salivations like ropey strings and saliva hanging in long, vesicles appeared on the tongue of oral mucosa, and then inter-digital space of the feet form ulcer after this ruptured. different shape and size were usually in the mouth cavity and inter digital space

Furthermore Abd-Ellatieff (7) in Egypt demonstrated that FMDV serotype O on the oral cavity and the space of interdigital of the adult buffalo and cattle as, Vesicles, erosions/ulcers irregular-shaped and size, were found typical on the anterior third of the tongue, torus lingua and more frequently on the lip, gingiva, and dental pad.

Histopathological Study

The histopathological study was conducted on samples of epithelial tissue of the tongue obtained from infected animals (Buffalo) that kept in neutral buffered formalin10%. The samples proceed and slide prepared according to (13, 14).

Tissue cultures

A- Reagents of tissue culture: The reagents were prepared according to (15, 10, 11).

B-Types of cells of tissue culture: Four types of cells were used in this study as:

1-Monolayer Vero Cell lines

These cells supply by Al-Kindy Company for production vaccines and drugs, ready for injection with the virus, they are as monolayer and maintaining at temperature 37°C. Then cells injected by filtrate of confirmed FMDV/SAT2 positive samples.

2- Primary fetus lamb kidney Cell

These cells were manual prepared in Central Veterinary Laboratories and Research/Veterinary Directorate, which obtain from fetus of 3-4 months of age. The confluent monolayers cells after preparation were infected with the positive sample of the epithelial tissue as described by Plowright and Ferris (16, 9)

3-Primary lamb kidney cell and Primary lamb testis cell

These cells types (3) were manual prepared in Department of Biology and Medical Supervision, in the virus division /Veterinary Directorate, which obtain from lamb of 3-4 months of age. The confluent monolayers cells after preparation were infected with the positive sample of the epithelial tissue.

Virus preparation

Epithelial tissue specimens of sloughing tongue were cultured in Vero cell lines. And fetus lamb kidney. In one word, in sterile sand tissue specimens were ground, mortar and pestle containing tissue culture medium containing specific antibiotics (17). When, the tissue specimen's become homogenate was centrifuged for 15 min for explanation at 4000 rpm then with a pore size of 0.22 µm filtered through membrane filter paper Subsequently, the prepared samples were incubated

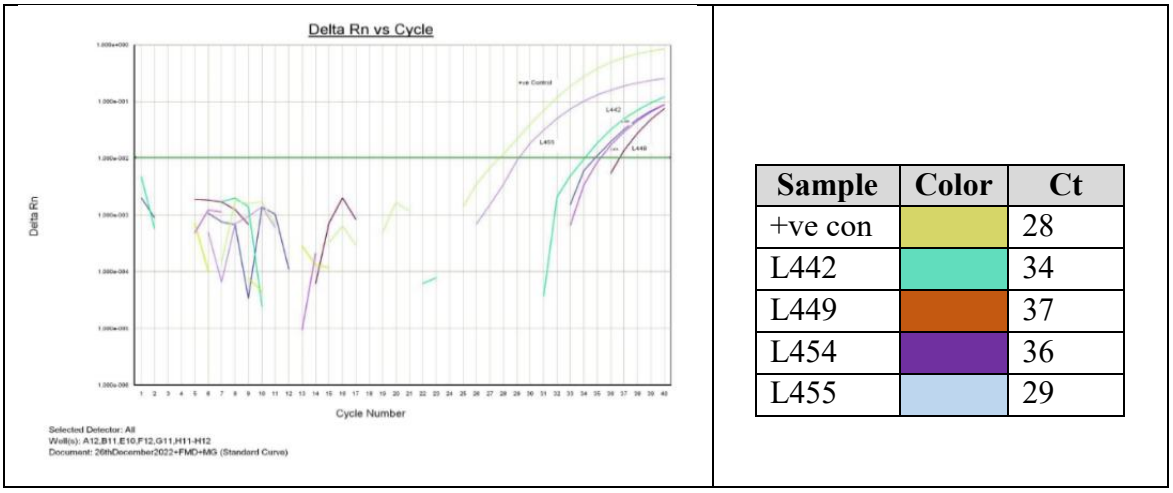
with Confluent monolayer cell cultures, 10% fetal calf serum, and minimum essential medium (MEM). Normal non-infected cells served as control. 0.2–0.4 mL filtered epithelium samples were inoculated to lamb kidney cell and Vero cell lines to investigate the cytopathic effects (CPE) monitored for 24–72 hours for Vero cell lines, but 48-72 hours for lamb kidney according to (18, 19, 20).

Results and discussion

This study was conducted to determine the cytopathic and histopathic effects of FMDV /SAT2 as a new serotype in Iraq.

The first step of diagnosis depends on clinical signs The most important sign of FMDV of saliva is in the form of threads or ropes, fever(40-41°C) also the mouth lesion appeared very sever in cattle's and buffaloes as various area of vesicle, ulceration, and erosion in the mouth cavity and in the foot.

Second step is diagnosis the FMDV using real time polymerase chain reaction the positive infected animals were 63/101 sample in some Iraq governorate reach to 62.37%. The positive cases for cattle were 28 out of 63 and positive cases for buffalo were 35 out of 63 Figure (2) some positive samples for FMDV in some Iraqi governorate which displays four positive samples with positive control.



Sample	Color	Ct
+ve con		28
L442		34
L449		37
L454		36
L455		29

Figure (2): The RT-qPCR detection the Logarithmic fluorescence plots versus cycle number resulting from the determination of FMDV, two positive samples of C_T value 29 and36 from buffalo in Al Fadhiliya village/Baghdad in addition CT value of 37 from buffalo in Al Qasim/Babi l, and C_T value of 36 from buffalo in Khan bani Saad/Diyala with positive control of 28 C_T value.

Then checked serotype SAT2 using conventional one step RT-PCR all samples were positive for FMDV are positive for SAT2 some positive samples for FMDV/SAT2 in some Iraqi governorate.

Gross pathology

While collecting samples their variable degree of sloughing or abrasions and ulcer distributed in different area in the super facial upper layer of the tongue in different sizes, shapes, the edges are irregular and

swollen. On the feet vesicle appeared specially on the clefts and on the inter-digital space, coronary band and behind the hooves, the region is red due to inflammation consisting of the of vesicle ruptured to form ulcer.

Histopathology

The histopathological changes of sloughing epithelial tissues (mouth, tongue, coronary bands) were described as, hydropic degeneration, their cytoplasm takes intensely eosinophilic, acute cellular swelling, inter cellular spinosum of epithelial, inter cellular prickles are lost, the epithelial cell become round and detached from one another, edematous fluid contain bands of fibrine accumulate between and separate the cells with pyknotic nuclei vesicular formations and intraepithelial bullae were plainly visible, as shown in Figure (5) comparing with control Figure (4).

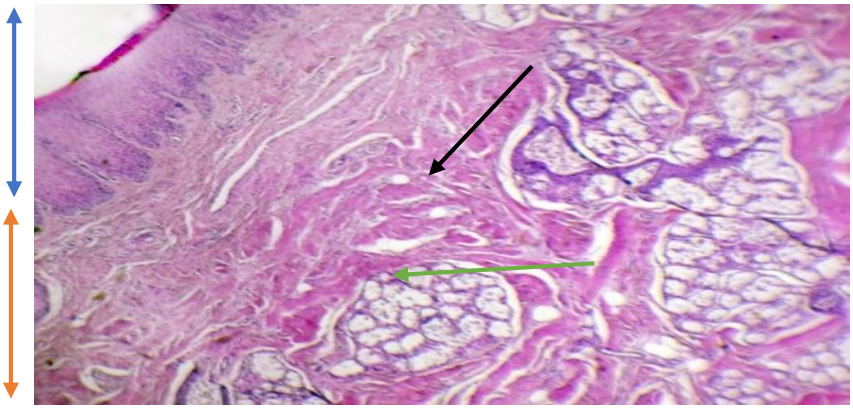


Figure (4): Histopathological section of normal buffalo tongue control Shows (green arrow) sebaceous gland ,(black arrow) connective tissue,(blue Arrow) normal epidermic and (orange arrow) dermic.

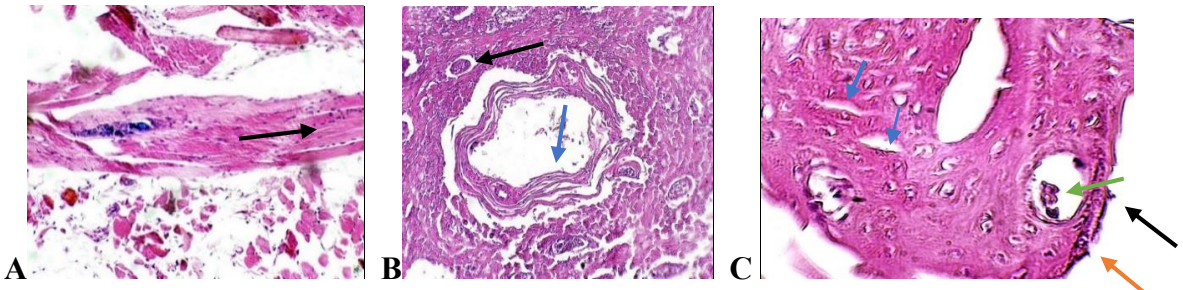


Figure (5): Histopathological section of buffalo tongue with infected of foot and mouth disease (H and E stain 400x).
A- Shows necrosis of nuclei(pyknosis) black arrow
B- Shows debris necrotic tissue (black arrow), and fibrous of connective tissue in (blue arrow)
C- Shows necrosis of taste buds (black arrow), necrosis of tongue gland in (green arrow), all papillae of tongue ulcer in (orang arrow) and vesicle hydropic degeneration in (blue arrow).

Study by Gab-Allah *et al.* (21) found clear vacuolar in the tongue and hydropic degeneration in the lining epithelium with vesicles formation in the tongue mucosa that forming bullae of different size and shapes. Also, the

sub epithelial tissue was widened by homogenous eosinophilic material mixed with number of inflammatory cells especially lymphocyte and neutrophil.

Abd-Ellatieff *et al.* (7) in Egypt describe the lesion of FMDV as hydropic degeneration, increase of cytoplasmic eosinophilia, and mononuclear cell, hyperkeratosis and infiltration granulocyte in the cells of the stratum spinosum, vesicular formations and intraepithelial bullae were plainly visible in addition to micro-abscess formation. Vesicles formations with elevation of superficial epithelium in the stratified squamous epithelium of dermis.

Also, vesicular stomatitis of the dental pad of infected calf described by leukocytic infiltrations of mucosal and submucosal layer of cornified epithelial tissue.

Faruk and Das. (22) were observed the tongue epithelium composed of ballooned epithelial cells of stratum spinosum seeing acantholysis, eosinophilic cytoplasm, nucleus (pyknotic) with infiltration of granulocyte and or dissolution of stratified squamous epithelial surface or complete necrosis and the filiform papillae infected cattle at the stage of advanced in comparison with control.

In the second step of this research of the cytopathic effect (CPE) detected study on four types of cells, Vero cell lines, lamb kidney cells, fetus lamb kidney cell and lamb testis cell.

The visible cytopathic effect result from inoculation virus on four types of cells appeared after 24-72 hours as disorganization of internal cellular structure, cell rounding, swelling, clumping of the cell, change in cell size and detachment from cell monolayer due to cell death (Figure 4) contain four types of TC cells when compared with control which didn't show any CPE and seemed as elongated cells attached to the surface falcon culture.

The period of appearance of CPE varied depended on the type of the cells, the cytopathic effect on Vero cells lines appeared after 24hr post inoculation when on lamb testis cell observed after 36-48hr, whereases cytopathic effect on lamb kidney and fetus lamb kidney noticed after70-72hr post inoculation. It was found that the Vero cell the best for study the effect of SAT2 because the CPE appear earlier than other three types.

The primary fetal lamp kidney that give clear picture for the effect of this serotype was recommended by OIE reference laboratory in Botswana which employs primary lamb kidney cells for the virus isolation procedure for diagnosing FMDV(10).

The endemic countries should be joining the OIE/FAO supported regional networks and take advantage of new cheap technologies being rolled out to collect isolates and submit them to the World Reference Laboratory.

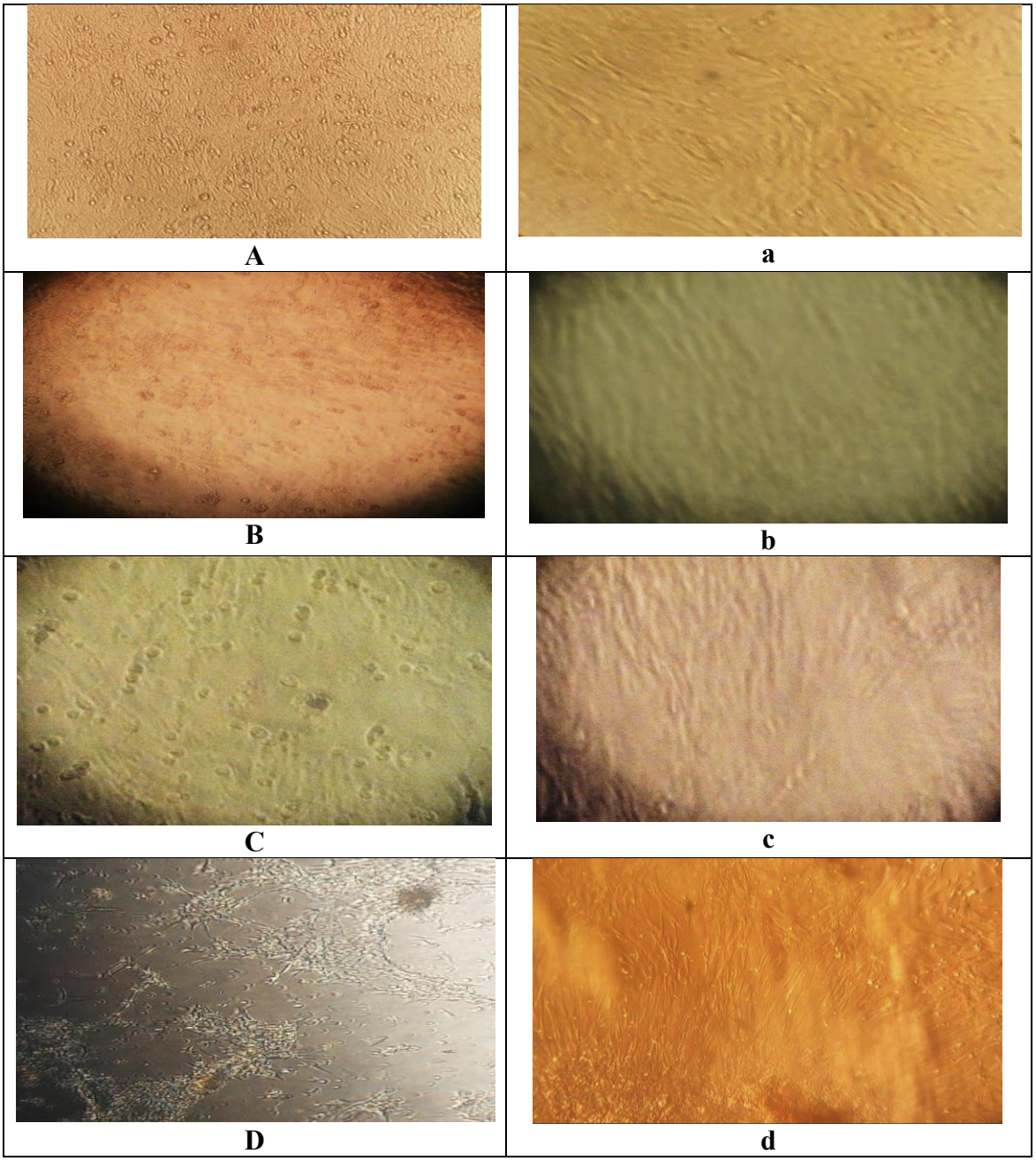


Figure (6): Tissue culture of four types of cells. A-Culture of Vero cell lines infected with FMDV\ SAT2 10X a-Culture of Vero cell lines (control) 10x. B- Culture of lamp kidney cell infected with FMDV\ SAT2 10X. b- Culture of lamp kidney cell (control) 10x C- Culture of lamp Testis cell infected with FMDV\ SAT2 10X. c- Culture of lamp Testis cell (control) 10x D- Culture of fetus lamp kidney cell infected with FMDV\ SAT2 10X. d- Culture of fetus lamp kidney cell (control) 10x

Paprocka (23) explain that lamb cells culture is possible a good tool in diagnostic studies of FMDV. But Chukwuedo and Gowalk (8) demonstrated highest serotype SAT 2 CPE in BK-21 followed by Vero cells but lowest effect observed on L.K. cell. The CPE for the positive samples of FMDV/SAT2 cultured on baby hamster kidney (BHK-21) by Dubie and Amare (24) who described as a rapid sloughing of these monolayer cells then became

roughly round, swelling, and found singly in shape, as time advance, the monolayer cell that sloughed separation from the wall of the cell-culture falcon and even several cells within 72 hrs. were severely damaged, and in the end cell death due to the presence of virus. While Kabelo *et al.* (10) used primary lamb kidney cell (RM), baby hamster kidney cell (BHK-21) and a derivative of female pig kidney cells (IR-P1) to check the Southern African Territories (SAT) serotype, the outcome indicated that sensitivity of IR-P1 and RM cells were high, but BHK-21 low. Concerning the Iraqi study of Sabar *et al*. (9) that demonstrated the CPE of FMD/A, O, and Asia-1 on (all of fetal origin) primary bovine thyroid, ovine thyroid, lamb kidney, bovine kidney, bovine tongue, sheep and bovine testis,

They found that CPE appears after 24 hours on fetal lamb kidney (FLK) cells, 48 hours on the fetal bovine kidney (FBK), 30 hours on primary fetal bovine thyroid cell (BTY) and 20 hours on the primary fetal ovine thyroid cells (OTY) It was found that the cell culture of primary bovine thyroid (BTY) cells most sensitive for FMDV isolation, but these cells are seldom used due to the cost, time wanted to prepare batches of cell orderly and the challenges connected with thyroid sources from calves' negatives to FMDV (11). Kamal *et al.* (25)reported that FMDV infected cells exhibit morphological changes termed cytopathic effect (CPE), commonly characterized by disorganization of internal cellular membranes, cell rounding and detachment from cell monolayer due to cell death.

In 2022 Qureshi *et al.* (26) tested many local serotypes A, O, and Asia-1of FMDV by using baby hamster kidney-21 cell line, they found that serotype A virulence is high as compared to serotype O and Asia-1.

The FMDV/ SAT2 was spread for first time in Iraq and cause an outbreak in cattle and buffalo with high morbidity even the elderly animals, and high mortality in young animals especially in new born (Suden death due to myocarditis) which reach to 100% in some fields. So, the cytopathic and histopathic study was carried out to investigate the effects of this serotype which enter Asia countries recently and reach Iraq as a neoteric led to huge losses different from the previous outbreak of the serotype A, O, and Asia 1. The outcome of this study confirmed that this serotype was virulent because, clear lesions were observed as a result from the influence of recent FMDV/Sat2 serotype.

This result suggests higher virulence and aggressiveness of the FMDV field circulating serotype, which may be reached Iraq via movements and importation of animals from FMD-infected areas with this serotype in addition continuous viral mutations leading to severe histopathological lesions scoring, especially in elder ages of cattle and buffalo.

It is worthy to mention that in last year's many viruses' disease outbreak emerge in Iraq either in human as COVID-19, Hepatitis B Virus, Epstein Barr Virus (27,28,29,30) or in animals as FMD (31).

Conclusion

FMD is endemic disease in Iraq which responsible for many outbreaks, but current outbreak cause by new serotype SAT2 enter the Iraq for first time.

Refrences

1. Ali, I.; Rehman, A.; Mushtaq, M. H.; Ijaz, M.; Khaliq, M. S.; Khan, M. S. U., *et al*. (2022). Outbreak investigation and identification of risk factors associated with the occurrence of foot and mouth disease in Punjab, Pakistan. Preventive Veterinary Medicine, 202: 105613.
2. Jones, G.; Heuer, C.; Johnson, W.; Begg, D.; McFadden, A.; Sutar, A., *et al*. (2023). Evaluating serological tests for foot-and-mouth disease while accounting for different serotypes and uncertain vaccination status. Preventive Veterinary Medicine, 214, 105889.
3. Shaban, A. K.; Mohamed, R.H.; Zakaria, A. M. and Baheeg, E. M. (2022). Detection of foot-and-mouth disease virus in raw milk in Menofia Governorate and its effect on reproductive hormones and physiochemical properties of milk. Veterinary world, 15(9), 2202.
4. Paton, D. J.; Gubbins, S. and King, D. P. (2018). Understanding the transmission of foot-and-mouth disease virus at different scales. Current opinion in virology, 28, 85-91.
5. Garner, G.; Vosloo, W.; Tapsuwan, S.; Bradhurst, R.; Seitzinger, A. H.; Breed, A.C. and Capon, T. (2021). Comparing surveillance approaches to support regaining free status after a foot-and-mouth disease outbreak. Preventive Veterinary Medicine, 194, 105441.
6. Bukhari, W.; Naz, A.; Meiran, F. and Umair, A. (2023). Evaluation of SAT-2 Serotype Foot and Mouth Disease and Use of One Health Tripartite as Solution. ACTA Scientific Veterinary Sciences, 5, 7.
7. Abd-Ellatieff, H. A.; Hegazy, A. A.; AbouRawash, A. R. A.; Tohamy, H. G.; Al-Shehri, M.; Bazh, E. K., *et al*. (2023). Pathological and genetic characterization of foot and mouth disease viruses collected from cattle and water buffalo in Egypt. Plos one, 18(10), e0291970.
8. Chukwuedo, A. A. and Gowalk, N. E. (2005). Growth comparison of Nigerian strains of foot and mouth disease virus (FMDV) types SAT 1 and SAT 2 in BHK, BK, Vero and LK cell culture systems. Nigerian Journal of Biotechnology, 16(1), 112-120.
9. Sabar, A. A.; Al-Banna, A. S.; Abdul-Rasoul, L. M. and Abood, B. K. (2012). Diagnostic study of FMD virus in different area in Iraq. In Proceeding of the Eleventh Veterinary Scientific Conference (55).
10. Kabelo, T.; Fana, E. and Lebani, K. (2020). Assessment of the sensitivity of primary cells and cell lines to the Southern African Territories (SAT) serotypes in the diagnosis of foot-and-mouth disease virus. Heliyon, 6(5).
11. Gray, A. R.; Wood, B. A.; Henry, E.; Azhar, M.; King, D. P. and Mioulet, V. (2020). Evaluation of cell lines for the isolation of foot-and-mouth disease virus and other viruses causing vesicular disease. Frontiers in Veterinary Science, 7, 426.
12. Al-Kalabadi and Al-Thwani (2024) submitted for publication
13. Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology. In Manual of histologic staining methods of the Armed Forces Institute of Pathology (pp. xii-258).
14. Suvarna, K. S.; Layton, C. and Bancroft, J. D. (2018). Bancroft's theory and practice of histological techniques. Elsevier health sciences.
15. Bachrach, H. L.; Hess, W. R. and Callis, J. J. (1955). Foot-and-mouth disease virus: its growth and cytopathogenicity in tissue culture. Science, 122(3183), 1269-1270.
16. Plowright, W. and Ferris, R. D. (1958). The growth and cytopathogenicity of sheep pox virus in tissue cultures. British Journal of Experimental Pathology, 39(4), 424.
17. Hedger, R. (1968). The isolation and characterization of foot-and-mouth disease virus from clinically normal herds of cattle in Botswana. Epidemiology and Infection, 66(1):27–36.
18. OIE. Foot and mouth disease. Chapter 3.1.8. Terrestrial Animal Health Code 2021. World Organization for Animal Health, Paris, France. https://www. oie. int/ filea dmin/ Home/ eng/ Health_ stand ards/ tahm/3. 01. 08_ FMD.pdf. 2021.
19. Arzt, J.; Baxt, B.; Grubman, M.; Jackson, T.; Juleff, N.; Rhyan, J., *et al*. (2011). The pathogenesis of foot-and-mouth disease II: viral pathways in swine, small ruminants, and wildlife; myotropism, chronic syndromes, and molecular virus–host interactions. Transboundary and emerging diseases, 58(4):305–26.

20. Sulayeman M, Dawo F, Mammo B, Gizaw D, Shegu D. Isolation, molecular characterization and sero-prevalence study of foot-and-mouth disease virus circulating in central Ethiopia. BMC veterinary research. 2018;14(1):1–10
21. Gab-Allah, M. S.; El-Mashad, A. B. I.; Moustafa, S. A. and El-maghraby, I. A. (2018). Pathological Studies on Foot and Mouth disease at Kaluobia Governorate. Benha Veterinary Medical Journal, 34(1), 195-208.
22. Faruk, M. A. Z. and Das, S. K. (2021). Comparative Histomorphological Changes of Bovine Foot and Mouth Disease (FMD) at different Clinical Stages in Bangladesh. Int Journal Curr Microbial App Sci, 10(06), 29-39.
23. Paprocka, G. (2008). Applying primary lamb kidney cell culture in diagnosing foot-and-mouth disease. Medycyna Weterynaryjna, 64(3), 332-334.
24. Dubie, T. and Amare, T. (2020). Isolation, serotyping, and molecular detection of bovine FMD virus from outbreak cases in Aba'ala district of Afar region, Ethiopia. Veterinary medicine international, 2020.
25. Kamal, T.; Naeem, K.; Munir, A.; Ali, M. and Ullah, A. (2014, January). Comparative study for the sensitivity of BHK-21 and bovine kidney cell line for the isolation of FMD viruses. In Proceedings of 2014 11th International Bhurban Conference on Applied Sciences and Technology (IBCAST) Islamabad, Pakistan, 14th-18th January, 2014 (pp. 59-64). IEEE.
26. Qureshi, S. S.; Khan, B.; Khan, S.; Ur Rahman, H. and Qureshi, M. S. (2022). Comparative Study of the Virulency of Different Serotypes of Foot and Mouth Disease Virus by Using Baby Hamster Kidney-21 Cell Line. Sarhad Journal of Agriculture, 38(3): 778-783.
27. Fadia, M.M.; Al-Thwani, A. N. and Kareem, S. J. (2022). Interleukin-1 Beta (IL-1β) Persistence in Post SARSCoV-2 Infection and Vaccination: A Double Case Control Study. Iraqi Journal of Biotechnology, 21(2): 561-567
28. Ahmed, D. and Al-Thwani, A. N. (2022) Determination of Angiotensin-Converting Enzyme 2 (ACE2) Receptor Level in Samples of Iraqi Patients Infected with COVID-19. Iraqi Journal of Biotechnology, 21(2): 178-182.
29. Gharbi, A. J. H. W. A. and Razzaq, S. A. A. (2022). Estimation of Liver Enzymes in Patients Infected with Hepatitis B Virus in Baghdad Hospitals. Iraqi journal of biotechnology, 21(2).
30. Abd, N. Q.; Al-Ahmer, S. D. and Gha four, K. H. A. (2021). Detection of Epstein Barr Virus in Some Iraqi Women Patients with Invasive Ductal Carcinoma Using Immunohistochemistry Technique. Iraqi journal of biotechnology, 1(20).
31. Sheikh, M. B.; Rashid, P. A.; Raheem, Z.; Marouf, A. S. and Amin, K. M. (2021). Molecular characterization and phylogenetic analysis of foot and mouth disease virus isolates in Sulaimani province, Iraq. In *Veterinary Research Forum*. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. 12(2): 247.