



Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Shatrah
College of Veterinary Medicine
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Molecular Identification and Some Biochemical Parameters Assessment in Isolated Serotypes of FMD Infected Calves in Thi-Qar Province

A Thesis

Submitted to the Council of Veterinary Medicine College/University of Shatrah, in Partial Fulfillment of the Requirements for a **Master's Degree (M.Sc.)** in Veterinary Sciences (**Microbiology**).

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2025 A.D

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Dedication

To nation's Mustafa and its leader Muhammad (God bless him and his family),

who shone a light on the road of the darkness.

To the pure and eternal souls of my beloved mother and father, whose love and prayers continue to guide me.

To my dear husband and my supportive family, whose encouragement has been my strength.

To my brothers and sisters, and their families, for their endless love and unwavering support

Zainab Abd Al ameir

AcKnowledgegment

All praise is due to Allah, by whose grace good deeds are accomplished, and by whose guidance challenges are overcome.

I would like to express my sincere thanks and deep appreciation to my esteemed supervisors: **Professor Dr. Basim Jarullah, and Professor DR. Ashwaq Jabbar**, for their invaluable support, continuous guidance, and constructive feedback throughout the course of this study. Their encouragement and insightful supervision played a crucial role in the successful completion of this research.

I would also like to thank **Assistant Prof. Dr. Nabil Mahdi, Assistant Prof. Dr. Abdulaziz Salih and Assistant Prof. Dr. Dhurgham Ali** for their valuable support and guidance during the research period.

I also extend my gratitude to the College of Veterinary Medicine – University of Shatrah, and the Department of Microbiology, for providing the academic environment and research facilities that made this work possible.

My heartfelt thanks go as well to **Assistant Prof. Dr. Adeeb Abd Al-aali Alazbejee** for his cooperation in this research.

My thanks also extend to my friends and colleagues for their support, patience, and motivation throughout my academic journey.

May Allah bless you all.

Zainab Abd Al-Ameir

Summary

Foot-and-mouth disease virus (FMDV) is one of the highly contagious viral pathogens that can affect cloven-hoofed animals, such as cattle, pigs, sheep, goats, and deer. These viral particles cause foot-and-mouth disease, which is of major economic concern in agriculture due to its rapid spread and the severe trade restrictions it can trigger. This study aimed to diagnose virus by PCR technique, Gene sequences of serotypes, identification relation of age, sex, serotype to virus infection, to evaluate the activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA) levels in healthy and infected calves with FMD virus and to determine the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) activities in healthy and infected calves.

This study was gained bloods and swabs samples from 150 FMD infected calves from different regions, ages and sexes of Thi-Qar province with 20 healthy calves for control during the period extending from November (2024) to March (2025).

The molecular detection and characterization of Foot-and-Mouth Disease Virus (FMDV) serotypes were performed using reverse transcription polymerase chain reaction (RT-PCR) followed by gel electrophoresis analysis. RT-PCR is a highly sensitive and specific technique that enables the amplification of viral RNA by first converting it into complementary DNA (cDNA) using reverse transcriptase, followed by targeted PCR amplification.

The gene that encodes the polyprotein in FMDV is critically important because it contains the entire coding capacity of the viral genome. The genetic variation in this gene can possess multiple implications in viral phylogenesis and classification. Based on the genetic variants of the polyprotein sequences, the present study was conducted to assess the pattern of biological diversity of the FMDV in 111 isolates collected from calves. Sequencing results showed that the amplified PCR products of the fragment A locus exhibited the highest numbers of

serotypes due to the discovery of four serotypes (Asia 1, SAT 2, O, and A) in the investigated fragments. Analysis of fragment A revealed fourteen SNPs distributed across the 866 bp region, of which a subset produced non-synonymous substitutions predicted to impact the structure and function of the analyzed polyprotein. Translation placed these within approximately 12 % of the full 2329 amino-acid polyprotein, highlighting both silent and missense effects.

The Neighbor-Joining tree partitioned isolates into four well-supported clusters, reflecting serotypes A, O, Asia 1, C, and the SAT types. Whereas the amplified products of fragment B exerted three serotypes (A, A/O, and O). Despite identifying 23 SNPs over its 275 bp length, fragment B demonstrated lower discriminatory capacity among the identified serotypes. Although it could delineate a coherent A/O clade and separate SAT 1–3 clades, it failed to resolve serotypes A, O, and C without overlap, yielding a large mixed clade of 20 leaves.

The limited number of variable sites in this short amplicon constrained its phylogenetic resolution. Despite that the amplified products of the fragment C indicated the discovery of only one serotype (serotype O), the 1301 bp fragment C exhibited superior performance. Fifteen SNPs defined three distinct serotype O subclades, each supported by both sequence identity and phylogenetic clustering. The C-based tree clearly segregated all FMDV serotypes into non-overlapping clades and robustly traced the Asian origins of the outbreak isolates.

Results of antioxidant parameters revealed significant decrease ($P \leq 0.05$) in levels of SOD, CAT and GSH in all affected groups as compared to healthy animals in control group, but SOD levels in groups A, O serotypes showed a significant decrease when compared with SOD levels of mixed group (A&O) . Levels of MDA were highly and significant increased ($P \leq 0.05$) in all affected groups as compared with healthy animals.

Results of liver function test revealed a highly significant increase ($P \leq 0.05$) in (ALT) and (AST) levels in groups of calves that were diagnosed with serotype O of FMDV when compared with control group and infected animals in other groups (serotypes A and mixed A&O).

However, ALP levels in affected groups were not significantly difference from each other and those in control group. GGT were significantly increased in animals affected with serotype A as compared with other groups including control.

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List of Abbreviation

Abbreviation	Symbol
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase

BLASTn	Basic Local Alignment Search Tool Nucleotides
CAT	Catalase
cDNA	Complementary DNA
DF	Degrees of Freedom
DNA	Deoxy Ribonucleic Acid
EDTA	Ethylene Diamine Tetraacetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FMD	Foot and Mouth Disease
GGT	Gamma Glutamyl Transferase
GPx	Glutathione Peroxidase
GSH	Glutathione
HRP	Horseradish Peroxidase
IAH	Institute for Animal Health
iTol	Iterative Tree of life
MCF	Malignant Catarrhal Fever
MDA	Malondialdehyde
MOP	Myeloperoxidase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center for Biotechnology Information
NFKB	Nuclear Factor Kappa B
NK	Natural Killer Cell
NSPs	Non-structure Proteins
OD	Optical Density
OIE	Office International Epizooties
OSI	Oxidative Stress Index
PCR	Polymerase chain reaction
PDGF	Platelet-Derived Growth Factor
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive oxygen species
ROS	Reactive Oxygen Species
RT-PCR	Reverse Transcription PCR
SOD	Superoxide dismutase
TAC	Total Antioxidant Carbon
TAS	Total Antioxidant Status
TBE	Tri-borate-EDTA
TE	Tris-EDTA
TGF- β	Transforming Growth Factor Beta
Th1	T helper 1
TOC	Total Oxidant Carbon
TOS	Total Oxidant Status

Tris	Trihydroxy methyl amino methane
V	Virus
VP	Viral Polypeptides (protein)
VSI	Veterinary Surveillance Initiative
VTM	Viral Transport Media
WOAH	World Organisation for Animal Health
XOD	Xanthino Oxidase

Chapter One

Introduction

1.1. Introduction

Foot-and-mouth disease (FMD) is a contagious disease that impacts up to 70 species of cleft hoofed mammals' thorough cattle, goat, sheep, and pigs (Jarullah and Sameer, 2014). The viral agent of Foot and Mouth Disease (FMD) belongs to the genus *Aphthovirus* and family *Picornaviridae*. The virus of FMD is a small, non-enveloped, positive-sense, single-stranded RNA virus, with genome of approximately 8500 bases ringed four structural proteins which form an icosahedral capsid. There are seven immunologically distinct serotypes of FMDV (A, C, O, Asia 1, and Southern African Territories 1, 2, and 3), with a wide spectrum of antigenically and epidemiologically different subtypes within each serotype. The major variety is seen a result of the high mutation rate, nearly-kinds dynamics, and recombination (Knowles *et.al.*, 2012; Ularamu, 2017; Brito *et.al.*, 2018).

There are 7 different varieties (serotypes) of FMDV, all give rise to a similar disease and infection but one serotype does not confer immunity against another (OIE and FAO World, 2024). Each serotype has different subtypes, resulting from the virus exhibiting a significant mutation rate (Shin *et al.*, 2024). FMD is endemic in several regions including south America, Asia and Africa (Junior *et al.*, 2024; Nishi *et al.*, 2024). The serotypes Asia 1, A and O of the FMDV continue to circulate in Iraq with occasional epidemics (Sheikh *et al.*, 2021; Aslam and Alkheraije, 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 (Amir *et al.*, 2023; Zewdie *et al.*, 2023). The serotype A was advertised officially for the first time in 1952 according to FAO /FMD reference laboratory in Iraq (IAH, Pirbright Laboratory).

The virus hits cattle, sheep and pigs and many cleft hoofed wildlife types. The contagion results in vesicular injuries in and round the mouth and on the feet,

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reaching in the unwillingness of an animal to eat or move (Aslam and Alkheraije, 2023).

Animals infected with FMD present severe clinical symptoms, generally they turn into febrile and promote vesicular injuries on the tongue, gums, soft palate, dental pad and nostrils. Vesicles on the tongue burst speedily and leave a raw, aching surface which recovers in about one week. The infected calf is incapable to eat, related with copious salivation, the saliva pending in long rope-like threads. A perfect smacking of the lips then shows and the animal gums hardly. The vesicles contain a thin wall including a hay-colored fluid (knight and Rushton, 2013). In Iraq 2009 outbreaks samples were missioned to Pirbright in subtypes as an associated with Turkish isolations. The most closely linked virus isolate A / IRQ /24 / 2009 was agreed A /IRQ /10/2009 with 99.69% of identity and to A / KUW/6/ 2009 isolate with 99.69% and to A/IRN/1/2005 with 95.46% and A/SAU/41/91 isolate with 84.75% and finally to A22/IRQ/24/64 vaccine strain with 82.16% identity (Reid *et al.*, 2002).

The field of oxidative stress in ruminant medicine is still in the early stages of development. There is a great deal to be discovered about its role in ruminant health and production (Celi, 2010). In the last few years the detection of free radicals damage and the body's defenses it have become increasingly important in clinical medicine as complementary tool in the evaluation of metabolic status of the animals (Castillo *et al.*, 2005).

Reduced antioxidant concentrations in inoculated animals can result from both anaphylactic shock and heat stress (Rajendram *et al.*, 2021). The damaging effects of free radicals are repelled by antioxidants. Living cells are harmed by molecules with an unshared electron (Verhagen *et al.*, 2006). Particularly dynamic, unshared electrons react quickly with oxygen to produce reactive oxygen species (ROS). In addition to being exposed to free radicals from the environment, such as air pollution and UV radiation from the sun, the body produces ROS naturally when it breaks down food into energy. ROS are a component of cell-to-cell signaling (Sies and Jones, 2020).

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The liver is an important metabolic organ and is abundant in enzymes, that play a crucial role in the metabolic processes and synthesis of proteins all over the body (Zhang *et al.*, 2022). Particularly, application on degrading the toxic substances, secretion of bile, storage of glycogen, and drug metabolism (Kradtap Hartwell, 2012). Liver functions can be interrupted by liver diseases and/or drugs use (Musazadeh *et al.*, 2022; Roa *et al.*, 2022).

Liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamine transferase (GGT) and alkaline phosphatase (ALP), are highly affected by liver injury and dysfunction (Younossi, 2019; Cotter and Rinella, 2020).

AST is represented in both cytoplasm and mitochondria of hepatocytes, myocardium and skeletal muscles. ALT is a cytoplasmic enzyme present exclusively in the hepatocytes. GGT catalyzes transfer of glutamyl group from glutathione to amino acid. ALP is a family of zinc metallohydrolase enzymes present in liver, bone, intestinal epithelium, kidney, placenta and germ cells.

High levels of ALT and AST are suggested to be in liver disease (Kim *et al.*, 2004; Lee *et al.*, 2008) and mortality rate related to liver diseases, as well as ALT high levels are associated with diabetes mellitus (Fraser *et al.*, 2009). Elevated levels of GGT in serum indicates to biliary injury. ALP is attached to biliary canaliculi; elevated ratio indicates cholesteric liver disease.

1.2. Aim of Study

Investigation of FMD virus serotypes predominant in cattle herds in Thi-Qar province by:

Diagnosis of virus by PCR technique.

Gene sequences of serotypes.

Identification relationship of age, sex, serotype to virus infection.

Evaluate the activities of SOD, Cat, GSH, and MDA level in healthy and infected calves with FMD virus.

Determine the levels of AST, ALT, ALP, and GGT activities in healthy and infected calves.

ChapterTwo

Literature Review

2. Literature Review

2.1. Background

Foot and Mouth Disease (FMD) is a strongly infectious disease affecting cleft hooved, mostly cattle, swine, sheep, goats, and many species of wild hooved mammals (Brooksby, 1982). FMD infects spacious regions worldwide and is inclusive in the list of diseases notifiable to the World Organization for Animal Health (http://www.oie.int/eng/en_index.htm). It is known as a serious epidemic disease menacing the cattle industry since the sixteenth century and till date it is a major global animal health problem.

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).

Frosch and Loeffler in 1897 first described FMDV as the etiological agent of FMD, laying the foundation of the science of virology. Initial details about the characteristic symptoms of the disease in cattle were given by Hieronymus Fracastorius in Venice in 1514 (Wang and Liu, 2021). These specifics tolerate a similarity to the disease which reported in the United Kingdom (1839) and Germany (1751). This disease was prevalent throughout the world at the time, with the exception of New Zealand, and it is currently common in a number of South American, Asian, and African nations (Wang and Liu, 2021). This discovery represents a major advance in the field of animal virology. Subsequently, a highly contagious viral infection known as FMD showed a significant degree of spread, affecting many countries covering diverse geographic regions, most notably Asia, Africa, and specific regions within South America (Paton *et al.*, 2021). Due to the development of transportation in the century and the increase in cross-border animal trade led to an increase in the

spread of FMD, causing major economic consequences (Björnham *et al.*, 2020). The lack of a suitable animal for experimentation to study this condition was a major obstacle until 2022 when guinea pigs were used successfully and vaccinations against FMD were also developed during the same time frame (Al-Hammadi, 2025). Currently, certain regions in Europe, Central America, North America, and Australia have achieved elimination of FMD, leading to their status as disease-free areas. In response to the serious impact of FMD on Europe, the Food and Agriculture Organization of the United Nations (FAO) created the European Committee for the Control of FMD (EU-FMD) in 1954 (Clavijo *et al.*, 2017).

In 2005, a novel subtype of FMDV serotype A was discovered in Iran, according to the World Reference Laboratory for FMD. Significant disease outbreaks in the Middle East caused by this serotype spread quickly in Turkey in 2005, Iran and Saudi Arabia in 2006, and Jordan in 2007. According to Geering and Lubroth (2002), this serotype, designated A/IRN/2005, was also reported in Iraq from samples taken from FMD outbreaks in different parts of the country in 2009, 2010, 2011, 2012, and 2013. The samples were sent to the FAO/FMD reference laboratory (IAH, Pirbright Laboratory) for genotyping and phylogeny reconstruction. Different FMDV serotypes were determined, namely FMDV-A ASIA Iran05 BAR-08, FMDV-O ME-SA PanAsia2 ANT-10, FMDV-A ASIA Iran05 AFG-07, FMDV-A Asia 1 Sindh-08, and FMDV-A Asia1 Iran05SIS-10 (Donald King/Head of WRLFMD, The Pirbright Institute, UK, 2016) (King, 2016).

2.2. Epidemiology

2.2.1. Distribution

Foot and Mouth Disease is a serious transboundary disease that has a significant economic impact on livestock worldwide (Capozzo *et al.*, 2023). Buffaloes, sheep, goats, deer, African buffaloes, and other cleft-footed livestock and wildlife species are among those affected (Bekere *et al.*, 2023). FMD is present on most

continents, with the exception of Iceland, New Zealand, Greenland, and some small islands in Oceania. Several countries, including the United States, Mexico, Canada, and Australia, haven't experienced outbreaks since the mid-20th century (Woldemariyam *et al.*, 2023).

Foot and Mouth Disease (FMD) is divided into three continental epidemiological groups: Africa, Asia, and South America. Many countries in Africa and Asia are endemic to FMD. Worldwide, FMD is divided into seven virus groups, each serotype multiple sub-serotypes. Historically, Africa, has serotypes six “A, O, C, SAT1, SAT-2, and SAT3”, whereas Asia has four “A, O, Asia1, C”, and South America has three “A, O, C” serotypes, respectively (Maree *et al.*, 2014). Serotype O is widely spread worldwide among the seven FMDV serotypes, although serotype C has a low report rate (Ali *et al.*, 2024). Globally, over one hundred countries are still impacted by FMD, and it's estimated that the illness still exists in approximately two-thirds of all countries (Raoof, 2024).

2.2.2. Status of FMD in Iraq

In Iraq, FMD is widespread and leads to animals’ owners to incur significant financial loses each year. With the presence of three separate strains (A, Asia1, and O), the illness has spread and grown entrenched in Iraq over the past seventy years (Salim *et al.*, 2020). Iraq has continuously harbored the FMD virus, which has occasionally caused massive epidemics with protracted, severe economic consequences. Specifically, serotypes O, SAT 1, and Asia1 were documented in the years 1957, 1962, and 1975, respectively (FAO World Reference/Laboratory for Foot and Mouth Disease).

Eleven of the 202 calves in the group at the Sulaymanyha / Bashder checkpoint on the Iraqi edge showed early signs of the disease in 1937, which was the first instance in Iraq that could be recorded. In 1938, the first known FMD was identified in cattle and buffalo in Basra, Missan, and Diala, which are located on the borders between Iraq and Iran. An important factor in the virus's spread and

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the disease's transfer from Iran to Iraq is the presence of wild pigs in the border swamplands of Basra and Missan (VSI Reports, 2009).

In 1938, there was a reported case of FMD in bovine and buffalo in the Iraqi-Iranian borderlands towns of Basra, Missan, and Diala. Following that, there were widespread outbreaks of FMD in 1957 that affected the majority of the Iraqi governorates and affected buffalo, cattle, sheep, and goats. Records for the illness persisted into the 60s, 70s, 80s, and 90s. The epidemic of 1999 is regarded as the worst case of FMD to have struck Iraq during the economic sanctions period of 13 governorates in Iraq, it resulted in a significant loss of cattle and small ruminants (El Idrissi, 2003).

The FAO/FMD reference laboratory (IAH, Pirbright Laboratory) officially reported serotype A in Iraq for the first time in 1952. Several serotypes were later identified, including A IRN 96 in 2000 and 2002; O1 in 1985 and 1993; Asia 1 in 1975, 1983, and 1984; and O Manisa in 1998 and 1999 (Mohammed, 2013).

Since 2010, the FMD epidemic has persisted. While the number of affected animals decreased, FMD outbreaks reappeared in 15 governorates in Iraq in 2012. Additionally, the veterinary services conducted a vaccination campaign in 2012, immunizing 7,105,941 small ruminants and 1,798,074 cattle and buffalo, respectively (Raoof, 2024). The Sulaymaniyah province reported a serotype of FMD in 2013 (Rashid *et al.*, 2014). The investigation conducted in 2017 was regarded as the first molecular characterization of serotype Asia1 in Iraq (Sheikh *et al.*, 2017). Molecular characterization and prevalence rates of FMD have been measured in 20 from outbreaks in the Iraqi province of Nineveh through the use of ELISA and RT-PCR technologies. The prevalence rates were 46.95% (Salim *et al.*, 2020). In Sulaymaniyah province, cattle were found to harbor FMDV serotype O (Sheikh *et al.*, 2021).

Table (2-1): FMDV serotypes have been detected in Iraq:

FMDV Serotype	Years
O	1957, 1959,1961-1964, 1966, 1969-1970. 1973, 1977, 1985,1994, 1999-2001, 2010
A	1952, 1955,1963-1964, 1970, 1978, 1985, 2000, 2002, 2009-2010, 2012-2013
SAT 1	1962
Asia 1	1975, 1985, 2011-2012

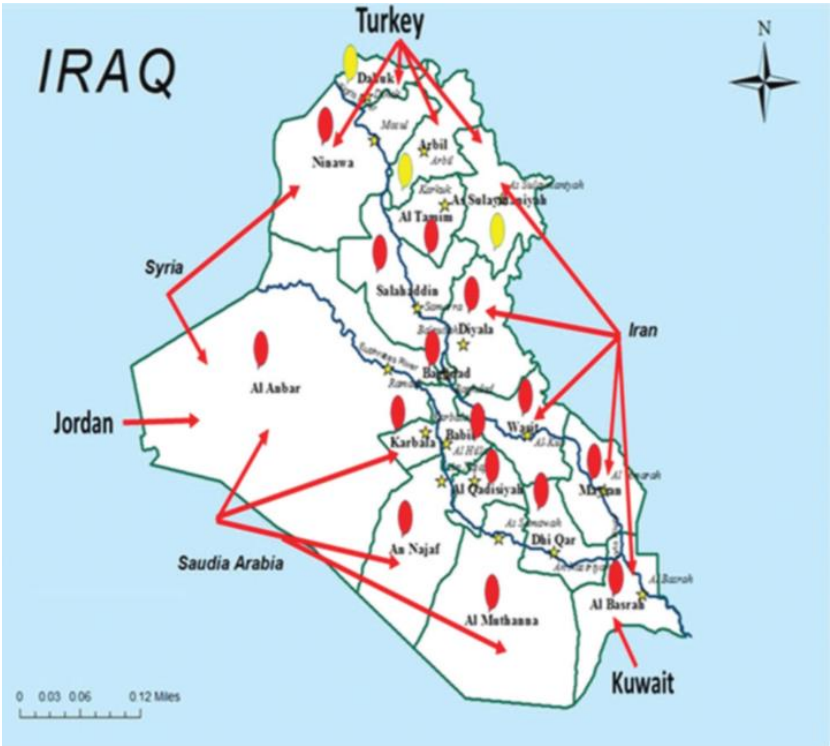


Figure (2-1): Map of Iraq depicting the foot-and-mouth disease (FMD) outbreaks in 2016. Red balloons indicate FMD-infected foci in various governorates, yellow balloons indicate excluded governorates, and red arrows indicate the anticipated source of the spread of FMD virus infection from the surrounding nations. Yellow stars represent provincial capital.

2.3. Etiology

2.3.1. FMDV Classification

The FMDV is classified within the picornavirus family. FMDV the most typical *aphthovirus* (Francisco-Velilla *et al.*, 2022). The *Picornaviridae* family consists of 68 genera, 158 species grouped (Cui *et al.*, 2024; Xu *et al.*, 2024), but the established species known so far in the *Picornaviridae* family (*Enterovirus*, *Cardiovirus*, *Aphthovirus*, *Hepatovirus*, *Parechovirus*, *Erbovirus*, *Kobuvirus* and *Teschovirus*) (Zhang *et al.*, 2023). The FMD virus in Iraq has been studied by many researchers on cattle (Mansour *et al.*, 2018; Abd Hatem *et al.*, 2022), sheep (Saleh *et al.*, 2013; Dawood and Alsaad, 2018), and camels (Al-Husseiny *et al.*, 2020). However, there is no adequate investigation of FMD in buffaloes and there is no study on the SAT-2 strain in Iraq.

RNA polymerase leads to a high The taxonomic classification of FMDV is aided by the relatively recent field of molecular epidemiology. Identifying the new topotype and origin of new FMDV lineages, as well as comprehending FMDV dynamics, are all made possible by this crucial instrument (Yirdaw and Abera, 2023a). Thus, the virus be divided into seven varieties that differ in serology and genetics: Asia1, SAT1, SAT2, SAT3, O, A, and C, with more than 60 subtypes with its inability to proofread, FMDV's RNA-dependent rate of mutations. Cross-immunity across strains does not exist. Serotypes do not differ in their clinical manifestations; nevertheless, the immune response does because each serotype has a different antigenic structure (Rodrigues-Habibe *et al.*, 2020).

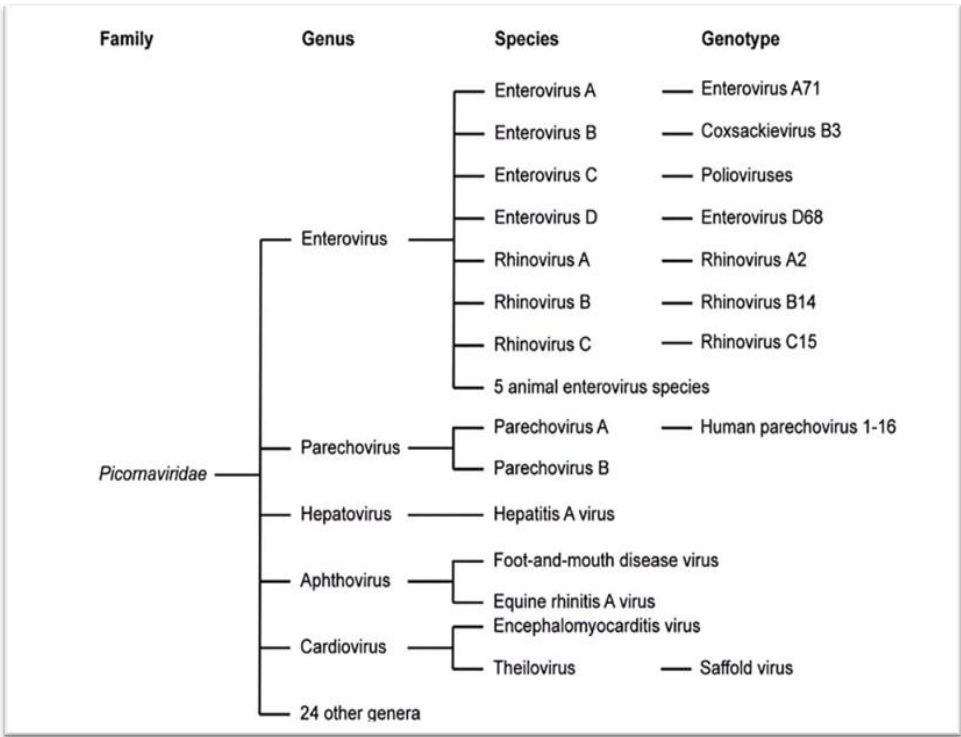


Figure (2-2): The Picornaviridae family (van der Linden *et al.*, 2015)

2.3.2. Viral Structure

2.3.2.1. Morphology

The FMD virion exhibits a spherical morphology characterized by 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 FMDV capsid contains 60 copies of each of the four structural viral polypeptides, namely VP1 (1D), VP2 (1B), VP3 (1C), and VP4 (1A), arranged in highly complex clusters (Njihia, 2022). VP1, VP2, and VP3 proteins are found on the outside of the capsid, whereas VP4 is located entirely on the inside. Genomic RNA is poly A tail at 3' end and covalently bound to VPg protein at its 5' end (Lozano *et al.*, 2018). During capsid construction, the polyprotein precursor encoded by the FMDV genome is split into VP2 and VP4 as well as 10 non-structural proteins (NSPs) after initially being cleaved into three structural proteins, VP1, VP3, and

VP0 (Kumar *et al.*, 2015). The surface of the three structural proteins (VP1, VP2, and VP3) is exposed. Whereas VP4 exhibits significant serotype conservation (Ashkani *et al.*, 2016). Because it contains the B-cell epitope that produces antibody-mediated immunity, the highly antigenic protein VP1 is primarily used for epidemiological investigations (García-Briones *et al.*, 2004).

2.3.2.2. Genome

FMDV is covered within a protein capsid. FMDV capsid is spherical around the genome. The virion is composed of 70% protein, 30% RNA. The 3'untranslated region (3'UTR), the longer coding region, and the 5' untranslated region (5' UTR) are three distinct parts of the RNA (Kazemi *et al.*, 2022). Virions are made of 60 copies of the four capsid proteins, VP1, VP2, VP3, and VP4, twelve proteins (NSP) and the genome protein Vpg. The VPg is a small protein of 24 or 25 amino acids, encoded by the 3B portion of the viral genome region, and is covalently linked to the 5' end of the genome. The 5' UTR is about 1300 nucleotides in length (He *et al.*, 2021; Li *et al.*, 2024). The four structural proteins exhibit varying degrees of conservation. VP1 exhibits high variability, whereas VP2 and VP3 are relatively conservative. On the other hand, VP4 is highly conserved across all serotypes. VP1 and VP3 play an important role in the inhibition of the synthesis of host interferons, thereby reducing the host defense against infection. However, VP2 plays a role in autophagy initiation (Peng *et al.*, 2020). Sequencing of VP1 gene is a gold standard method for genetic characterization of FMDV and it is used to study virus evolution, outbreaks, and virus strain relationships (El Nahas and Salem, 2020; Gulholm *et al.*, 2022). Can identify the difference between each serotype of the virus by identifying the specific proteins that make up the outer capsid of the virus. The unique sequence of GHloop region on VP1 protein can be used to identify an origin of FMDV outbreak. Through genetic testing, researchers can determine the origins and relationships of FMDV (Islam *et al.*, 2021).

2.3.2.3. Chemical Properties of The Virus

The FMDV are non-enveloped viruses surrounded by a protein shell. Capsids are polypeptides without lipo- proteins and are therefore resistant to chloroform and ether, two lipid solvents (Chepkwony, 2023). When the pH falls below 6.5 or rises over 10, the virus becomes dormant and vulnerable. In general, picornaviruses are stable between pH 3 and pH 9. However, FMDV is differentiated from other picornaviruses due to its limited pH stability range of pH 7-9 (Moens *et al.*, 2020). The virus is also susceptible to alkaline solutions, with a 90% reduction in infectivity at pH 10. Therefore, Sodium Hydroxide 2% and Sodium Carbonate 4% are utilized as cost-effective and efficient disinfectants for decontaminating objects affected by FMD (Onodera *et al.*, 2023).

2.4. Serotypes

Seven strains of FMDV have been found in different parts of the world these type of serotype A, O, C, Asia1, SAT-1, SAT-2, and SAT-3 (Aslam and Alkheraije, 2023). All seven forms of the disease exhibit similar clinical signs but differ in immune response. Exposure to one serotype does not confer immunity to another, even if infection occurs naturally or through vaccination (Paton *et al.*, 2021). FMDV has high antigenic variation. This is evidenced by the presence of many topotypes, which can be converted into 61 reported genotypes (Abd El-Rhman *et al.*, 2020). FMDV has considerable genetic variability, due to errors during its manufacture and recombination of RNA genes (Nishi *et al.*, 2017). The extent of cross-protection conferred between different virus serotypes is contingent upon the degree of antigenic similarity exhibited by their respective strains.

To be effective, it is important to ensure that each inoculation is exactly matched to the relevant field strain (Gubbins *et al.*, 2022). The FMDV RNA is

prone to mistakes and, consequently, genetic variation because it lacks proof-reading activity (Morelli *et al.*, 2013).

Worldwide outbreaks have been triggered by serotype O. All six serotypes have been recorded from Africa, with the exception of Asia-1. Three serotypes (A, O, and Asia-1) and three (A, O, and C) have been responsible for epidemics in South America and Asia, respectively. Prior reports of the two serotypes, SAT 1 and SAT 2, have come from the Middle East and Africa. Notably, Serotype C is now extinct and was last documented from Brazil and Kenya in 2004 (Paton *et al.*, 2021).

In Iraq, two further outbreaks of FMD type SAT2 were reported in sheep on 13 June 2023 at tal samer-aljana (Al Ba'aj, Ninawa) and in cattle on 11 June 2023 at Jorf alnadaf (Adhamiya, Baghdad). (WOAH World Animal Health Information System (event IDs4856).

2.5. Pathogenesis

In FMDV-infected animals, the virus causes a primary infection in the nasopharyngeal epithelium and then replicates extensively in alveolar epithelial cells, within 1 to 2 days post infection, the virus reaches the bloodstream and undergoes secondary replication in multiple organs and tissues, resulting in apparent infection (Azeem, 2020; Rodrigues-Habibe *et al.*, 2020; Stenfeldt and Arzt, 2020). Viremia happens within 1-3 days, hyperthermia within 1-4 days. Clinical indications include salivation, serous nasal discharge, mouth lesions, and foot lesions (Arzt *et al.*, 2017). Furthermore, diverse vesicular lesions manifest in the oral cavity, pharynx, and nostrils of the livestock, resulting in impaired mastication and excessive salivation (Rawaa *et al.*, 2021).

Viruses invade the lymphatics from the primary lesions and enter the blood circulation, producing viremia. Again, from the bloodstream, the virus goes to the site of preference, i.e. mouth lining (epithelium), dental pad, udder, and feet.

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While the lesions themselves heal quickly, bacterial infections can complicate things. These secondary infections can induce significant tissue damage. In the feet, this may manifest as the loss of the hard outer layer (horny covering) and even the detachment of the hooves. While the primary blisters on the udder do not directly cause mastitis (inflammation), bacterial infections that arise as a complication of these blisters can (Arzt *et al.*, 2011).

The inflammatory response triggers vascular dilatation, enhancing permeability of cell membranes. This facilitates the movement of substances across these membranes, subsequently leading to inflammation in the surrounding tissue. Plasma proteins and leukocytes enter the infected tissue as a result of inflammation. Vesicles form when blood vessel inflammatory cells, cellulose, cytokines, and other proteins seep into the tissue, resulting in oedema (Zhang *et al.*, 2022). Vesicles develop in secondary sites of replication, mainly the oral mucosa and the hoof junction, causing cell damage and severe inflammation. However, the primary site of replication is the nasopharyngeal epithelium, which remains unaffected. The specific mechanism by which the virus replicates in the nasopharyngeal without leading to cell lysis remains unidentified, and this explains why lesions are more common in the mouth, feet, and tongue in the clear (Kouato, 2017). Heart muscles may show acute degeneration of the myocardial cell fibers in the calves. Musculature will have a striped appearance which is termed a “tiger heart”. There is heavy mortality in young calves due to cardiac damage with the absence of appreciable vesicular eruption. Some indigenous buffalo show superficial brown necrotic lesions of the epithelium with a tendency for rapid healing (Chepkwony, 2023).

2.6. Clinical Signs

The clinical signs of FMD often occur within a period of 3 to 5 days after entry of the virus (Azeem, 2020). At first signs include a nasal discharge and an excessive production of saliva, accompanied by of smacking the lips (Dickmu *et al.*, 2022). Originally, the discharge is mucus-like, but later it develops a mixture of mucus and pus. Affected animals show signs of fatigue, may rapidly lose physical condition, and may experience gradual or sudden significant reductions in milk output (Perez-Martin *et al.*, 2022a). Skin lesions form in the coronary region and the interdigital cavity of the hoof. Foot lesions cause shaking and a strong stamping of the feet in addition can't stand (Zewdie *et al.*, 2023).

Foot and Mouth Disease (FMD) is characterized by pyrexia and lesions on the gums, oral mucosa, and mammary glands. The vesicles can appear in various locations of the oral cavity, including the dental pad, gums, soft palate, and tongue, as well as the nostrils and muzzle. These vesicles rupture rapidly, leading to erosion, ulceration and causing pain and discomfort from wounds. Although FMDV does not cross the placenta, but it can cause abortions in pregnant animals due to pyrexia (Abd-Ellatieff *et al.*, 2023). Mastitis might result from a subsequent bacterial infection caused by teat lesions.

The animals rapidly lose their ability to produce milk and condition after developing vesicular lesions, and this loss may last for a longer period of time (El Bagoury *et al.*, 2022). In severe cases, the hooves can begin sloughed. Most adults fully recover within two to three weeks, although the time needed for recovery may be longer due to the presence of a secondary infection (Sahoo *et al.*, 2023). The post complications infected include temporary or persistent decreases in milk production or mastitis, weight loss, and chronic lameness (Salim *et al.*, 2020). Death usually occurs in young animals, resulting from multifocal myocardial infarction. Some disease outbreaks can cause a significant increase in the mortality rate of young animals. Acute illness can also cause sudden death in older animals, but this is rare (El Damaty *et al.*, 2021).

2.7. Diagnosis of FMD

Fast and precise identification is essential for treating afflicted livestock and preventing sickness in order to manage FMD effectively (Amir *et al.*, 2023). Accurate and verified diagnoses are essential for the control and elimination of FMD. Clinical symptoms or the presence of FMD antibodies or nucleic acids could serve as their foundation (Yirdaw and Abera, 2023b).

2.7.1. FMD Diagnosis Clinically

According to the severity of the symptoms, FMD can be diagnosed early on in order to effectively treat it. This is because FMD symptoms are similar to those of other vesicular diseases in cattle, such as infectious bovine rhinotracheitis, bluetongue, vesicular and necrotic stomatitis, and bovine popular stomatitis.

To diagnose FMD, a history of outbreaks, a physical exam, and biomarkers can all be used (Ismail *et al.*, 2023).

The first sign is a rise in temperature. Next, the animal is less energetic and may refuse to eat because sores are showing up in its mouth (on the dental pad, gums, soft palate, and tongue), nose, and snout. Blisters on the tongue often merge, burst quickly, and cause significant pain, making the animal refuse to eat (Humadi, 2024). Common symptoms include excessive drooling and nasal discharge, which at first appears clear and mucus-like before thickening and containing pus. Animals with the infection become listless, may lose weight quickly, and their milk supply may gradually or abruptly and severely diminish. Skin sores develop between the hoof's toes and on the coronary band. The animal may shiver and stomp its feet, and foot lesions make it reluctant to stand. Blisters may not even form before calves die from cardiac failure, and pregnant animals may abort (Nielsen *et al.*, 2021).

2.7.2. Conventional Polymerase Chain Reaction (PCR)

A standard PCR system, as described in the preceding table, was used to configure the PCR thermocycler parameters.

One-step reverse transcription RT-PCR's heat cycling conditions are engineered to guarantee effective reverse transcription, DNA amplification, and product stability. The synthesis of complementary DNA (cDNA) from RNA is made possible by reverse transcription (RT-activation), which starts the process at 50°C for 20 minutes. To completely denature RNA secondary structures and activate the DNA polymerase, an initial denaturation phase is then performed for three minutes at 94°C. All PCR products are guaranteed to be fully elongated with a final extension at 72°C for five minutes. The enhanced products are then preserved for further examination by keeping the reaction at 4°C indefinitely. High specificity, efficiency, and repeatability in RNA detection and research are guaranteed by this designed cycling process.

2.8. Gene Sequences

2.8.1. Nucleic Acid Sequencing of PCR Amplicons

In accordance with the sequencing company's instruction manuals, the resolved PCR amplicons were commercially sequenced in both forward and reverse orientations (Macrogen, South Korea). The virtual locations and other features of the obtained PCR fragments were determined by comparing the returned nucleic acid sequences with the observed nucleic acid sequences of the local virus samples.

2.8.2. Analysis of Sequencing Data

Using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA), the sequencing results of the PCR products of the targeted sample were modified, aligned, and compared with the corresponding sequences in the reference database. Both the PCR amplicons and their corresponding positions within the referring genome were used to number the observed variations in the sequenced sample. Both in the PCR amplicons and at the corresponding locations within the reference genome, the detected nucleic acids were numbered.

A unique accession number for the examined sample was assigned to each of the observed variations, which were then entered into the national center biotechnology information NCBI database.

2.8.3. Translation of Nucleic Acid Variations into Amino Acid Residues

Targeted proteins' amino acid sequences were obtained from the Protein Data Bank. All of the nucleic acid sequences of the examined samples were converted to their corresponding amino acid sequences using the Expasy translate suite in order to evaluate the effect of the detected nucleic acid variations found in the coding regions on the encoded protein.

2.8.4. Deposition of Sequences to GenBank

Every sequence that was examined and evaluated was uploaded to the NCBI portal, and the server followed all of the guidelines provided by the site. In order to get a distinct GenBank entry number for the examined viral sequences, the submitted sequence was submitted as nucleic acid sequences to the NCBI.

2.8.5. Comprehensive Phylogenetic Tree Construction

The NCBI-BLASTn service was used to compare the detected variations with their neighbor homologous reference sequences prior to creating a phylogenetic tree (Zhang *et al.*, 2000a). To guarantee comprehensive coverage, the reference sequences retrieved from the NCBI BLASTn program were meticulously matched with the viral query sequences. In order to prevent redundancy, GenBank accession numbers that shared the same homology were also eliminated. The obtained nucleic acid sequences were aligned using the ClustalW program. The MEGA7 program was utilized to create a phylogenetic tree from the aligned sequences (Kumar *et al.*, 2016). The neighbor-joining approach was used to derive the evolutionary history from the aligned sequences (Tamura *et al.*, 2004).

Branch lengths are expressed in terms of the number of substitutions per site, and the tree is depicted to scale. In the produced tree, all places with gaps and incomplete data were removed. The produced tree was exported to iTOL software, where it was annotated and displayed with distinct colors for each clade (Letunic and Bork, 2021).

2.9. Transmission of FMD Virus

The FMDV has the potential to exhibit high transboundary disease, with the ability to spread rapidly among a limited number of individuals. The main method of transmitting this virus is through direct contact between animals that are infected and those that are susceptible. Direct Contact is the most common route, where the virus spreads through saliva, nasal secretions, or blisters of infected animals. Respiratory aerosols are the primary means of viral transmission in animals. Being the principal location of viral replication, the respiratory tract releases a significant amount of virus particles into the air (Tolawak and Pal, 2022). Nevertheless, it has been observed that the transmission of the virus can also occur through indirect contact, such as the interaction between susceptible animals and contaminated feed products or equipment (Brown *et al.*, 2022).

FMDV can persist for long periods in the environment under cool, and moist conditions. The virus can then be transmitted mechanically by fomites (contaminated objects) such as clothing, farm equipment, vehicles, hay, and bedding. People who handle infected animals can become inadvertent carriers, spreading the virus to susceptible animals (Bjornham *et al.*, 2020). Under optimal conditions of humidity and temperature, the FMD virus has the potential to be transmitted over significant distances, spanning up to 200 kilometers over of water and up to 50 km across terrestrial environments (Brown *et al.*, 2022).

The virus spreads easily during an acute infection because it leaks out of ruptured vesicles and through bodily fluids like breath, milk, and sperm (Oak and Khillare., 2021).

Handlers of infected animals may be exposed to contamination on clothing, hands, or nasal passages that carry live FMD (Udahemuka *et al.*, 2020). Human contact with infected animals can become infected with the virus up to 24 hours after the initial infection. As a result, they can inadvertently spread the virus to vulnerable animals through direct contact, acting as vectors who come into contact with infected animals (Paton *et al.*, 2018).

2.10. Prevention and Control

The control for this disease and preventive practice are used to stop the transmission of disease among animals (Garner *et al.*, 2021). The validation of control strategies may differ between countries depending on the state of the disease and their respective economic (Tadesse *et al.*, 2017). To stop the spread of the virus, susceptible animals must be isolated from the risk of a pathogen, by vaccination or by restricting movement (Yadav *et al.*, 2022). The disease is complicated to manage because of its high contagiousness, ability to infect a range of hosts, virus instability, multiple antigens or subtypes, and short immune responses. There are no reliable methods for controlling disease because if one serotype of FMDV is used in vaccination, it does not protect against other serotypes (Sutmoller *et al.*, 2003). The controlling is frequently carried out utilizing a well-equipped laboratory, quick and perfect diagnosis, emergency measures, continual tracking or monitoring, immunization, isolation of infected animals, quarantine, and restrictions on animals' movement (Yirdaw and Abera, 2023b). In endemic countries, FMD prevention and control are essentially contingent on recurrent immunization, skilled veterinary personnel, effective governance, movement of animal restriction, and the physical division of wildlife mixed livestock (Gunasekera, 2021). The most widely used method of disease prevention worldwide, both in endemic and disease-free regions, is vaccination. Based on the method of manufacture, there are numerous types of vaccination; DNA vaccine, genetically modified attenuated strain vaccine, protein vaccine,

proteins fragment vaccine, and vaccine made of viral subunits (Medina *et al.*, 2023).

Most of the viral diseases, including FMD, default known treatments. Symptom at therapy as opposed to specialized treatment may be appropriate depending on how the condition presents clinically (Windsor *et al.*, 2020). Vaccines utilized in the control of FMD in endemic regions are mostly used for collective protection application. Such vaccines are multivalent to provide prophylactic against multiple serotypes (Rweyemamu, *et al.*, 2008).

2.11. The vaccination of FMD in Iraq

FMD is a highly contagious viral disease affecting cloven-hoofed animals and poses a threat to Iraq's food security (WOAH, 2023). Unregulated cross-border animals' movement (especially with Iran and Turkey) and multiple circulating virus serotypes (A, O, Asia1) (Muhammed *et al.*, 2024). Iraq's Ministry of Agriculture conducts vaccination campaigns, but coverage rarely exceeds 60 % due to funding shortages and logistical barriers (World Bank, 2023). Vaccines are imported from Turkey, Egypt and Iran, primarily bivalent or trivalent formulations (FAO, 2023). Detection of serotype SAT2 in 2022, not covered by existing vaccines (Muhammed *et al.*, 2024). Some livestock owners refuse vaccination due to fear of side effects or lack of compensation for losses (FAO, 2023). The varieties of FMD vaccinations used in Iraq throughout the last five years (2020–2025) are shown in the following parts, along with the sources for each section:

2.11.1. Conventional Inactivated Vaccines (Predominant Vaccine Type)

Comprising inactivated (killed) whole-virus vaccinations, the great majority of vaccines are given with an adjuvant (often oil-based or aluminum hydroxide

/saponin) to boost the immune system. These vaccinations target certain serotypes of the FMD virus that are prevalent in Iraq and the surrounding area.

Serotypes Covered: The core composition has consistently included serotype O (O PanAsia-2 strain) and serotype A (An Iran-05 strain). Inclusion of serotype Asia-1 (Asia-1 Sindh-08 strain) in trivalent vaccines has become increasingly common since around 2022-2023, moving beyond the earlier focus on mainly O and A (Quarterly Report, October-December 2021; Annual Report for West Eurasia 2022; FAO, 2023).

2.11.2. Shift Towards Trivalent Vaccines (Vaccine Formulation)

Composition: While bivalent (O, A) vaccines were still used, especially in routine vaccination or for specific campaigns targeting known serotypes in certain areas, trivalent vaccines (O, A, Asia-1) became the dominant formulation recommended and procured for large-scale national vaccination campaigns from approximately 2022 onwards. This shift responded to confirmed circulation and outbreaks caused by Asia-1 in neighboring countries and within Iraq. **Serotype Strains:** The specific strains within the trivalent vaccines remained consistent with the regionally recommended ones: O PanAsia-2, A Iran-05, and Asia-1 Sindh-08 (FAO, 2023; WOA, 2024).

2.11.3. Potential Use of Bivalent Vaccines and Regional Variations

Composition: Bivalent vaccines (typically O PanAsia-2 + An Iran-05) were likely still used in some contexts, such as: Routine vaccination programs in areas perceived as lower risk for Asia-1. Some private doctors or smaller farms obtaining various vaccines may conduct follow-up vaccinations or focused campaigns when recent monitoring showed just O and A circulation. The use of trivalent formulations in official national mass immunization programs has grown. Variations in the type and quality of vaccinations actually provided on the ground may result from the difficulty of obtaining consistent, high-quality

vaccines in all locations, particularly in rural or conflict-affected areas (FAO, 2021).

2.11.4. Key Considerations Regarding Vaccine Sources and Strains

Composition Specifics: The vaccines used are inactivated and polyvalent, which protect against numerous serotypes, such as trivalent or bivalent. There is no live FMD virus in them. The adjuvant is essential to effectiveness.

Procurement Sources: International tenders were the main method used by Iraq to acquire vaccinations. Turkey, Iran, and occasionally European or foreign manufacturers (such as Merial/Boehringer Ingelheim, MSD Animal Health, and certain Turkish/Iranian firms like Veteriner Ilac San-VILSAN Biotech, Razi Institute) are major providers of vaccines. Because of cost, logistics, and strain appropriateness, there is a considerable reliance on regional manufacturers (Iran, Turkey).

Strain Matching: O PanAsia-2, A Iran-05, and Asia-1 Sindh-08 continue to be the most suitable strains for use in Iraqi vaccines, according to ongoing strain characterisation by reference laboratories (e.g., WRLFMD, Pirbright; regional labs sponsored by FAO/EuFMD) (FAO, 2025). There are few detailed, publicly accessible, year-by-year breakdowns of the precise vaccination brand names and dosages utilized across Iraq. WOAHP epidemic data, technical reports, FAO project documentation, regional organization updates (EuFMD), and producer information are used to put together the information. Even though trivalent vaccines were now the norm for nationwide campaigns, supply chain problems, funding hold-ups, or local choices may have caused actual field use to lag or differ.

Using inactivated vaccines with the appropriate strains for the circulating serotypes (O, A, and Asia-1) has been the main focus; these vaccines are mostly obtained through international tenders from both domestic and foreign producers. The most significant change in composition over the past few years has been the move toward common trivalent inclusion.

2.12. General Overview

Endemic Pools comprise separate ecosystems that maintain independently circulating and evolving FMDV genotypes. In the absence of specific reports, it should be assumed that the serotypes indicated below are continuously circulating in parts of these pools and would be detected if sufficient surveillance was in place.

Table (2-2) Serotypes of FMD in different countries

Group	Countries & Regions	Serotypes
1	SOUTHEAST ASIA/CENTRAL ASIA /EAST ASIA China (Hong Kong), Taiwan province, Indonesia, the Democratic People's Republic of Korea, the Lao People's Democratic Republic, Malaysia, Mongolia, Myanmar, the Russian Federation, Vietnam and Thailand. China	A, Asia1 and O
2	SOUTH ASIA India, Mauritius ¹ , Bangladesh, Bhutan, Nepal, and Sri Lanka	A, Asia1 and O
3	WEST EURASIA & NEAR EAST The Islamic Republic of Iran, Afghanistan, Armenia, Azerbaijan, Bahrain, Georgia, Iraq, Israel, Jordan, Kazakhstan, Kuwait, Kyrgyzstan, Lebanon, Oman, Pakistan, Palestine, Qatar, Saudi Arabia, Syria, Tajikistan, Turkey United Arab Emirates, Uzbekistan, and Turkmenistan	A, Asia1 and O(SAT2)
4	EASTERN AFRICA Egypt, Eritrea, Ethiopia, Kenya, Rwanda, Somalia, Burundi, Comoros, Djibouti, South Sudan, Sudan, Uganda, United Republic of Tanzania, and Yemen NORTH AFRICA² Morocco, Tunisia, Algeria, and Libya	O, A, SAT1, SAT2 and SAT3 A and O
5	WEST/CENTRAL AFRICA Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Benin, Burkina Faso, Cabo Verde, Cameroon, Central African Republic, Chad, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Senegal, Togo, Sierra Leone, Nigeria, and Sao Tome and Principe	O, A, SAT1 and SAT2
6	SOUTHERN AFRICA South Africa, Zambia, Namibia, Botswana, Malawi, Mozambique, Angola, and Zimbabwe	SAT1, SAT2 and SAT3(O4, A)
7	SOUTH AMERICA Venezuela (Bolivarian Republic of)	A and O

1. The O/ME-SA/Ind-2001-caused FMD epidemics in 2016–21 show strong epidemiological ties between Group 2 and Mauritius. 2. Since there is no evidence of long-term preservation of FMDV lineages in the North African Maghreb countries, this region does not represent an Endemic Group; yet, data is separated here since the movement of FMD in this region offers a

particular risk to Southern European countries that do not have FMD. 3. Egypt is situated at the intersection of the Near East (Group 3) and East Africa (Group 4). NB: In this nation, serotypes SAT1 and SAT3 have not been found. 4. The discovery of O/EA-2 in Namibia (2021), Malawi (2022), Mozambique (2022), and southern/western Zambia (2018–2021) marks a new entry into Group 6.

FMD is a serious and extremely infectious virus that affects ruminants with cloven hooves. An aphthovirus belonging to the family Picornaviridae, which causes FMD, has seven strains: A, O, C, SAT1, SAT2, SAT3, and Asia1. These strains are prevalent in many regions around the world, including much of Africa and the Middle East, as well as numerous portions of Asia (Constable *et al.*, 2017). All secretions and excretions from acutely infected animals include the causal agent, which spreads quickly through a variety of direct and indirect interactions as well as airborne pathways. Clinical signs of FMD in cattle include high fever, copious salivation, and vesicles on the mammary gland and in the interdigital fissure in the oral cavity (Poonsuk *et al.*, 2018). Adult animals seldom die from the disease, while young animals frequently die from myocarditis at high rates—up to 50% (Admassu *et al.*, 2015). The pathophysiology of FMD in domestic animals has not been extensively studied (Arzt *et al.*, 2011; Stenfeldt *et al.*, 2016).

These investigations often focus on the kinetics of the virus in the host rather than the formation of tissue lesions and the aftermath of organ failure. However, in vitro research conducted in well regulated cell culture settings provides a large portion of the fundamental understanding of FMDV virus–host interactions. The intricacy of the virus-host interaction at the tissue, organ, and systemic levels in the natural hosts cannot be adequately addressed by these investigations, despite their importance (Arzt *et al.*, 2011).

Furthermore, the serum biochemical results from cattle that are naturally infected with the FMD virus are not well documented in the literature (Khoshvaghti *et al.*, 2014).

Serum biochemical analysis aids in a better understanding of the disease's pathophysiology and can be a helpful technique for evaluating the health of animals.

The first-line defensive antioxidant enzymes include SOD, CAT, and GSH. According to (Ighodaro and Akinloye, 2018), these enzymes convert superoxide radicals, hydrogen peroxides, and hydroperoxides into innocuous molecules (H_2O_2 /alcohol and O_2). The depletion of the antioxidant defense system, which results from the overproduction of free radicals brought on by a variety of circumstances, including viral infections, has been proposed as the reason of reduced antioxidant enzyme activity (Strycharz-Dudziak *et al.*, 2019).

Oxidative stress causes the oxidative destruction of macromolecules such as proteins, lipids, and nucleic acids in tissues by producing more highly reactive oxygen species (ROS) than antioxidant defense systems can scavenge. Numerous studies have shown that the pathophysiology of inflammatory disorders is significantly influenced by excessive ROS generation. For instance, When influenza and bovine herpes virus-1 are present, ROS plays a role in viral multiplication and inflammatory cytokine activation (Ye *et al.*, 2015).

2.13. Oxidant and Antioxidant Parameters Function in Infectious Diseases

High-energy atoms or molecules with one or more unpaired electrons in their outer orbitals are known as free radicals. Examples of these radicals include nitric oxide (NO), nitrogen dioxide (NO_2), superoxide (O_2^-), hydroxyl ($\cdot\text{OH}$), lipid peroxy ($\text{LOO}\cdot$), peroxy ($\text{ROO}\cdot$), and alkoxyl ($\text{RO}\cdot$) (Hoffmann and Solter., 2008). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are two types of these free radicals that originate from oxygen and nitrogen, respectively (Valko *et al.*, 2006).

Under healthy settings, mitochondria are the primary source of intracellular reactive products. Large amounts of these reactive products are generated by monocytes, neutrophils, and macrophages.

Many enzymes, including xanthine oxidase (XOD), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, neutrophil myeloperoxidase (MPO), cyclooxygenase, and lipoxygenase, produce endogenously generated free radicals in addition to being generated by the mitochondrial electron transfer system (ETS) chain and phagocyte activation. By (Kalyanaraman 2013).

Exogenous ROS can be caused by UV, X-ray, gamma, and microwave radiation; air pollutants like asbestos, benzene, carbon monoxide, formaldehyde, ozone, and toluene; chemicals like glue, paint, thinner, pesticides, and cleaning products; sudden and excessive oxygen entry; medical hyperbaric oxygen exposure; an increase in catecholamines; an increase in lactic acid in muscles and blood; an increase in lytic enzyme activities like lactate dehydrogenase and creatine phosphokinase; various and challenging physiological conditions like exercise, pregnancy, and old age; extreme stress; and long-term consumption of diets high in polyunsaturated and easily peroxidable fats. The oxidant-antioxidant equilibrium is upset in favor of oxidants when the antioxidant system is deficient or surpasses the defense wall, leading to oxidative stress (Karabulut and Gulay 2016). The most often utilized metrics are NO, the lipid peroxidation marker malondialdehyde (MDA), total oxidant capacity (TOC), which is simple to detect using kits, and 8-hydroxydeoxyguanosine, which is a marker of ROS in DNA. Oxidative stress indicators have been the subject of several published research in the veterinary sector.

2.14. Radicals Physiological States

Free radicals are dangerous, yet depending on their concentrations, they can also serve certain physiological purposes. For instance, Free radicals also play a role in vascular tone and signal transmission, protein phosphorylation, transcription factor activation, cell differentiation, apoptosis, oocyte maturation, embryonic development, steroidogenesis, spermatogenesis, pregnancy, and the

immune system. NO is a key mediator in the relaxation of smooth muscles and regulation of microcirculation as an intracellular messenger (Murad 2006).

Leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis, and the ability of vascular smooth muscles to control blood pressure all depend on NO leukocyte adhesion, which is used by endothelial cells. In addition to these, NO, which is a transmitter molecule that is crucial for brain plasticity, is generated by neurons. However, NO, which macrophages generate, is a crucial mediator in the development of an immune response. H₂O₂ and superoxide can function as second messengers (Bogdan, 2001). In defense against tumor cells, parasites, fungi, protozoa, helminths, and mycobacteria, NO is a chemical with a strong cytotoxic impact; nevertheless, it is ineffective against external pathogens. Through fibroblast proliferation, (O₂⁻) may promote collagen production, and H₂O₂ plays a part in Nuclear Factor kappa B cells (NFκB) activation (Kinnula and Crapo, 2004).

The cytotoxic lymphocytes and macrophages that kill cancer cells during phagocytosis in viral, parasitic, and microbial infections; the detoxification of xenobiotics using p450; the activation of ROS and RNS nuclear transcription factors; the release of calcium from intracellular stores; the activation of tyrosine phosphorylation amino acid; the activation of non-receptor tyrosine kinase; the activation of certain cytokines and growth factor signals are all considered to play significant roles in cellular signals (Prakriya and Lewis, 2015). Both phagocyte activation and the activation of several enzymes, including XOD, NADPH oxidase, myeloperoxidase MPO, cyclooxygenase and lipoxygenase, culminate in the production of the mitochondrial electron carrier (ETS) chain. In addition, ROS promotes the growth of these activities and takes part in the manufacture of chemicals like thyroxin and prostaglandin. During the oestrus cycle's luteal phase andThe amount of oxidation is high during the follicular (oestrous) phase.

Dissolved in cells, reactive oxygen species are utilized in essential processes including gene transcription and the control of guanylate cyclase activity (Ni *et al.*, 2020).

2.15. Viral Infections and Oxidative Stress

It has been reported that oxidative stress, reactive oxygen/nitrogen species, lipid peroxidation, and low antioxidant reserves are all strongly linked to parvovirus infection in dogs. In a research by (Aydogdu *et al.*, 2018). Total Oxidant Status (TOS) rose, but Total Antioxidant Status (TAS) remained same, and the Oxidative Stress Index (OSI) was normally high. Additionally, there have been instances when oxidants like MDA and H₂O₂ have been shown to boost SOD and GPx activity while decreasing CAT activity (Elsayed *et al.*, 2020). MDA was shown to rise in sheep infected with the sheeppox virus, but GSH and albumin were found to fall (Bozukluhan *et al.*, 2018). The inhibition of the antioxidative system and negative acute phase reactant brought on by infection causes this drop in albumin. A recurrent finding throughout research on sheep, cattle, and bulls with FMD is a drop in GSH and Total Antioxidant Carbon (TAC) levels and a rise in MDA, NO, and Total Oxidant Carbon (TOC) levels (Abou-Zeina *et al.*, 2019).

According to research, oxidative stress is significant in this type of viral illness. Oxidative stress indicators provide identical outcomes in viral infectious diseases, regardless of the disease's modifications. Cattle with malignant catarrhal fever (MCF) had lower GSH levels despite higher MDA and NO levels (Cenesiz, 2020).

MDA was elevated in the goat pneumonia table, although SOD, GPx, and GSH were reduced (Jarikre 2017). Increased ROS generation from low GSH leads to inflammation, an imbalanced immunological response, and increased vulnerability to infection. In viral infections, the inducible NO synthase enzyme is induced, which increases RNS synthesis. Th1-related cytokines cause the generation of ROS and RNS in infected host tissues. An imbalance between the generation of ROS and RNS and their elimination causes oxidative/nitrosative stress, which can accelerate the rate of viral RNA mutation and virus multiplication, increasing host tissue damage (Molteni *et al.*, 2014).

Reactive oxygen species produced by neutrophil and kupffer cells in response to the infection are the primary mechanism that causes cell death in viral hepatitis. The pathophysiology of the development of fibrosis involves the control of collagen gene transcription in fibroblasts and the stimulation of profibrinogenic cytokines like Transforming Growth Factor β (TGF- β) and Platelet Derived Growth Factor (PDGF), which results in an increase in collagen synthesis. An indicator of hepatocarcinogenesis is elevated 8-Hydroxy-2-deoxyguanosine (8-OHdG) with infection (Ivanov *et al.*, 2017). Herpes virus infections have been shown to reduce Glutathione peroxidase (GPx) and SOD activity, whilst Bovine Herpes Virus (BHV-1) infections have been shown to boost GPx activity and inhibit SOD activity (Fu *et al.*, 2019). Oxidative stress can be discussed in the pathophysiology of zoonotic viral illnesses like ecthyma contagiosum because of the rise in MDA and NO and the reduction in GSH (Deveci *et al.*, 2017).

Free radicals are the weapon that the body uses to phagocytize the pathogen as a defense mechanism to stop bacterial, viral, and parasitic diseases from entering the cell and infecting the host. The rise in oxidants in biological fluids including serum, tissue, etc., and an increase in oxidative stress due to a lack or inhibition of the antioxidative defense mechanism were similar to all of the disorders listed above. When the antioxidant defense system is not strong enough to combat free radical products, oxidative stress (OS) results. Lipid, protein, and DNA changes occur if this stress is sustained over an extended period of time. Base or sugar lesions, single and double strand breaks, abasic areas, DNA-protein, or cross-linking across strains are all examples of oxidative damage to DNA. Both nuclear and mitochondrial DNA acquire damaged nucleosides. When this redox equilibrium is upset, cell signals shift, which leads to apoptosis, aging, tissue inflammation, loss of fundamental biological processes, and eventually tissue destruction(Chiurchiu *et al.*, 2011).

2.16. Utilizing Antioxidants in Viral Illness

Reactive oxygen species (ROS), which are produced during physiological and metabolic activities, are eliminated via enzymatic and non-enzymatic antioxidant systems.

Under some conditions, the oxidant-antioxidant balance shifts in favor of oxidants, and it is impossible to prevent the increase in oxidants and decrease in antioxidants. One of the most detrimental effects of oxidative stress is the production of reactive oxygen species, such as peroxides and free radicals (Erel 2005). Glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) are among the many antioxidant molecules present in blood that prevent or lessen the harmful effects of free radical reactions (Cemek *et al.*, 2006; Işık *et al.*, 2007).

Malondialdehyde (MDA) and other oxidant concentrations in plasma or serum may be determined independently in a lab (Nath *et al.*, 2014; Sangha and Kalra, 2016; Manat *et al.*, 2017). The involvement of reactive oxygen species in the pathophysiology of viral infections has been the subject of an increasing number of research (Ivanov *et al.*, 2017). Additionally, in order to incorporate antioxidant medications into their treatment regimen, physicians may need to assess oxidative stress. The knowledge about the ruminants' oxidative stress is still not well developed. It is very interested to be discovered about their role model in ruminants' health and production (Celi, 2010). In recent years the invention of cellular damage causes by free radicals and body defenses it has become important issue especially in the clinical medicine as an integral agent in the estimation of metabolic condition of the animals (Castillo *et al.*, 2005). Cells are shielded by antioxidants from the damaging effects of free radicals.

According to Verhagen *et al.*(2006) Free radicals-molecules with an unshared electron-damage cells and may contribute to the onset of cancer and cardiovascular diseases. Particularly dynamic, unshared electrons react quickly with oxygen to produce reactive oxygen species (ROS). In addition to being exposed to free radicals from the environment, air pollution, and UV radiation from the sun, the body produces ROS naturally when it transforms food into energy. ROS are a component of the cell-to-cell communication system. It has been well-established over past 20 years that a Varsity of infections cause reactive oxygen species to be produced (Ivanov *et al.*, 2017).

Although oxidant's role in virus inactivation was first demonstrated in 1970, their metabolic role in viral infection only recently became clear. Because oxidant regulate both host metabolism and viral replication, their role in viral diseases is more complicated than that of microbial diseases and autotoxic effects (Peterhans 1997). The oxidant-antioxidant balance shifts in favor of oxidants under specific circumstances, and the rise in oxidants and fall in antioxidants cannot be stopped. The generation of reactive oxygen species, such as free radicals and peroxides, is a particularly harmful effects of oxidative stress (Erel, 2005).

An established mechanism of cellular damage, lipid peroxidation serves as a marker of oxidative stress in tissues and cells (Berger *et al.*, 2012). Malondialdehyde (MDA) is the most prevalent lipid peroxide by product (Heidarpour *et al.*, 2013). According to Kandemir *et al.*, (2011), it serves as an inductive indicator of oxidative damage. By producing antioxidants, which are depleted as ROS production increases, the body reduces the cellular effects of ROS (Zalba *et al.*, 2006). The changes in clinical and biochemical parameters in cattle with FMD have not been extensively studied (El-Saied *et al.*, 2007). Regarding the alterations of SOD, GPx, CAT, and MDA in animals infected with FMD, no published reports exist. Thus the current investigation was carried out to assess the actions of SOD, GSH, CAT, and MDA levels in FMD infected and healthy cattle. Additionally, an investigation was conducted into the correlation between the measured parameters. The body uses SOD (superoxide dismutase), CAT (catalase), MDA (malondialdehyde), and GSH (glutathione) to fight off oxidative stress, which is caused by reactive oxygen species (ROS) damaging cells. GSH is an essential antioxidant that aids in cell protection, MDA is a byproduct of lipid peroxidation, a process that can be brought on by oxidative stress, SOD and CAT are enzymes that neutralize ROS.

- Superoxide dismutase or SOD, is an essential part of the antioxidant system that breaks down superoxide radicals (O_2^-) into oxygen and peroxide (H_2O_2). this aids in eliminating a dangerous kind of ROS.

- CAT (catalase): catalase breaks down hydrogen peroxide into oxygen and water. This enzyme aids in preventing H₂O₂ buildup.
- Malondialdehyde or MDA is a byproduct of lipid peroxidation, as series of events that can harm various components, including cell membrane. It is frequently employed as an indicator of oxidative stress.
- Glutathione or GSH: A tripeptide consisting of glutamic acid, glycine, and cysteine, glutathione functions as direct antioxidant and aid in the detoxification of toxic substances. For other antioxidants enzymes, it also a cofactor.

GSH aids in cell protection and repair, while MDA levels can reveal the degree of oxidative(Yener *et al.*,2024).

2.17. Liver Enzymes

An essential metabolic organ, the liver is rich in enzymes that are vital to the body's protein synthesis and metabolic functions (Zhang *et al.*, 2022). Specifically, use in the degradation of harmful chemicals, drug metabolism, glycogen storage, and bile secretion (Hartwell 2012). Drug use and/or liver illnesses can disrupt liver functioning (Musazadeh *et al.*, 2022; Roa *et al.*, 2022).

Liver damage and dysfunction have a significant impact on liver enzymes such as alkaline phosphatase(ALP), gamma-glutamine transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Younossi, 2019; Cotter and Rinella, 2020). Hepatocytes, the heart, and skeletal muscles all contain AST in their cytoplasm and mitochondria. The cytoplasmic enzyme ALT is only found in hepatocytes. Glutamyl group transfer from glutathione to amino acid is catalyzed by GGT. The liver, bone, intestinal epithelium, kidney, placenta, and germ cells all contain the zinc metallohydrolase enzymes known as ALP. In addition to the mortality rate associated with liver diseases, high levels of ALT and AST are also linked to diabetes mellitus and liver disease, Serum GGT levels above normal are indicative of biliary damage.

Biliary canaliculi are connected to ALP; an elevated ratio denotes cholesteric liver disease (Kim *et al.*, 2004; Lee *et al.*, 2008; Fraser *et al.*, 2009).

Hepatocyte involvement in Foot and Mouth Disease Virus: Before spreading via the circulation, FMDV multiplies in the pharynx. Viral localization in the liver due to viremia can result in either direct pathogenic effects on hepatocytes or indirect harm through oxidative stress, inflammation, and hypoxia brought on by myocarditis.

Hepatocellular degeneration, necrosis, and inflammatory infiltration are hepatic abnormalities associated with FMD that contribute to the release of enzymes into the bloodstream (Li *et al.*, 2024).

2.15. Alterations in Individual Liver Enzymes

2.15.1. Aspartate Aminotransferase (AST)

Hepatocytes, heart muscle, and skeletal muscle all contain large amounts of AST. Elevations in cattle infected with FMDV indicate either concurrent myocardial damage or hepatocellular injury (Kumar *et al.*, 2020), naturally sick cattle had 3.5 times higher AST levels than healthy controls. 92% of acute FMD cases had elevated AST, which was correlated with the severity of the disease, (El-Tholoth *et al.*, 2021).

2.15.2. Alanine Aminotransferase (ALT)

In cattle, ALT is unique to the liver and is a more accurate indicator of hepatocellular injury than AST. (El-Ansary *et al.*, 2025) found that cattle infected with FMDV had a 2.8-fold increase in ALT, which is indicative of hepatocyte necrosis. ALT decreases when vesicles heal after peaking during the viremic period (days 4–7 post-infection) (Sharma *et al.*, 2023).

2.15.3. Alkaline Phosphatase (ALP)

Elevation of ALP indicates biliary epithelial damage or cholestasis. It may result from inflammation-induced intrahepatic cholestasis in FMD. Ethiopian cattle with FMD showed a two-fold rise in ALP (Abay *et al.*, 2019). ALP elevation has recently been linked to anorexia and decreased bile flow in acute FMD (El-Tarabany, 2023).

2.15.4. Gamma-Glutamyl Transferase (GGT)

GGT is a biomarker that is sensitive to biliary obstruction and chronic liver disease. Bile duct damage may be indicated by its rise in FMD (El-Ansary *et al.*, 2025), cattle with FMD had a three-fold rise in GGT. In FMD convalescence, linked secondary bacterial cholangitis to persistent GGT elevation (Ghanem *et al.*, 2022).

ChapterThree

Materials and

Methods

3.1. Materials**3.1.1. Instruments and Equipment**

The instruments and equipment utilized in this study are detailed in Table (3-1).

Table (3-1): Instruments and equipment:

No.	Instrument / Equipment	Company / Country
1	Autoclave	Stermite / Germany
2	Centrifuge	Netherlands / Holland
3	Compound light microscope	Olympus / Japan
4	Container	BBL/ USA
5	Cover slide	Superstar / India
6	Digital camera	Samsung / Korea
7	Exispin vortex centrifuge	Bioneer/ Korea
8	Hot plate stirrer	Labtech /Korea
9	Incubator	Memmert (Germany)
10	Plastic plates	BDH /England
11	Refrigerator	Concord/ Lebanon
12	Sensitive balance	Sartorius /Germany
13	Slide	Superstar / India
14	Catton Swab	BDH /England
15	Test tube	BDH /England
16	Vortex	Cyan/ Belgium
17	Water bath	Kottermann / Germany
18	Clod centrifuge	Nuve/ Turkey
19	Gell Electrophoresis	Rovigo/ Italy
20	Eppindrof tube	Sigma/ England
21	Electrical oven	Memmert/ Germany
22	Conventional PCR	Bio-Rad/Signapore
23	Micropipettes (different volume)	Ependrof/ Germany
24	Tips	Sterellin Ltd./UK

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25	Shaker	Lab Tech/ Kore
26	Ultraviolet	Elettofor/ Italy
27	Water distillator	Polar Bear/ Canada

3.1.2. Chemicals and Reagents

The constituents, both chemical and biological materials that were used in this study with their remarks listed below in table (3-2):

Table (3-2): Chemical and Biological reagents with their origin:

No.	Chemicals	Company's Origin
1	Agarose	BioBasic /Canada
2	Distilled water	Al-Raphedane / Iraq
3	Ethanol	BDH /England
4	Iodine solution	BDH / England
5	Iodine crystals	Difco / USA
6	Loading dye buffer	Bioneer /Korea
7	Trans Zol UP	Tran/ Chine

Table(3-3) Kits used in present study

	Kit	Company / Country
1	Bovine Super Oxidase Dimutase (SOD) ELISA Kit	Sun Log Biotech/India
2	Bovine Glutathione (GSH) ELISA Kit	Sun Log Biotech/India
3	Bovine Catalase (CAT) ELISA Kit	Sun Log Biotech/India
4	Bovine Malondialdehyde (MDA) ELISA Kit	Sun Log Biotech/India
5	Alkaline Phosphatase SL Kinetic Method Kit	Giese Diagnostics srl /Italy
6	Aspartate Amino Transferase/ GOT Kinetic Method UV Kit	Giese Diagnostics srl /Italy
7	ALT/GPT SL Kinetic Method UV Kit	Giese Diagnostics srl / Italy
8	GAMMA GT SL Kinetic Method UV Kit	Giese Diagnostics srl / Italy
9	RNA Extraction Kit Contents	Magic Pure/ Chine

3.1.3. Molecular Techniques

3.1.3.1. Kits for RNA Extraction from Blood and Swabs Samples

Magic Pure® Up Viral DNA/RNA Kit Protocol.

3.1.3.2 Reverse Transcription PCR (RT-PCR)

Reverse Transcription PCR (RT-PCR) Using One-Step Master Mix

The kit and material which used in this study are listed below in table (3-4):

Table (3-4): RT-PCR Master mix contents

Component	Quantities
TransScript ® II One-Step RT/RT Enzyme Mix	80µl
2x One-Step Reaction mix	2×1 ml
RNase-free Water	2×1 ml

3.1.4. ELISA techniques:

3.1.4.1. Bovine Catalase (CAT)

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to CAT. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody.

Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for CAT is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain CAT and HRP conjugated CAT antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of CAT.

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Man can calculate concentration of CAT in the samples by comparing the OD of the samples to the standard curve.

3.1.4.2. Bovine Malondialdehyde (MDA)

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to MDA. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for MDA is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain MDA and HRP conjugated MDA antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of MDA. You can calculate the concentration of MDA in the samples by comparing the OD of the samples to the standard curve.

3.1.4.3. Bovine Super Oxidase Dimutase (SOD)

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to SOD. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP) conjugated antibody specific for SOD is added to each Microelisa stripplate well and incubated.

Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain SOD and HRP conjugated SOD antibody will appear blue in color and then turn yellow after the addition of the stop solution.

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The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of SOD. You can calculate the concentration of SOD in the samples by comparing the OD of the samples to the standard curve.

3.1.4.4. Bovine Glutathione (GSH)

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to GSH. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for GSH is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain GSH and HRP conjugated GSH antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at awavelength of 450 nm. The OD value is proportional to the concentration of GSH. You cancalculate the concentration of GSH in the samples by comparing the OD of the samples to the standard curve.

3.1.5. Primers

The primers set which used in this study with their references in table (3-5):

Table (3-5): Primes sets used in this study with their references:

Primer name	Sequences 5'-'3	Tem.	PCR Product	Reference
FMD virus serotype A	F: TACCAAATTACACACGGGAA R:GACATGTCCTCCTGCATCTGGTTGAT	52.3°C	865bp	Al-Rodhan (2014)
FMD virus serotype O	F: ACCAACCTCCTTGATGTGGCT R:GACATGTCCTCCTGCATCTGGTTGAT	60.1°C	1301bp	
FMD virus - FMDV (Ba)	F: GACAAAGGTTTTGTTCTTGGTC R: CAWCGCAGGTAAAGTGATCTG	56°C	275bp	Fernández <i>et al.</i> ,(2007)
FMD virus - SAT1, 2, 3	F:CCACATACTACTTTTGTGACCTGGA R: ACAGCGGCCATGCACGACAG	56°C	715–730 bp	Reid <i>et al.</i> ,(2000)

3.1.6. Preparation of Primer

The primer preparation method followed the manufacturer's instructions. Lyophilized primers were dissolved in TE (Tris-EDTA) buffer to achieve a stock solution concentration of 100 pmole/ML. This stock solution was then diluted with TE buffer using the concentration-volume formula $C_1V_1=C_2V_2$ after centrifugation and an overnight incubation at 4°C. As a result, the final concentration of each primer in the working solution was adjusted to 20 pmole/ML.

3.1.7. Preparation of Buffers, Solutions

All buffers and solutions were prepared following the guidelines outlined in Sambrook and Russell (2001).

3.1.7.1. TBE Buffer

Tris-borate-EDTA (TBE) is commonly utilized as the preferred buffer solution in nucleic acid electrophoresis. used in molecular biology for nucleic acid electrophoresis. Tris buffers are utilized because they may keep the pH of the solution slightly alkaline, which is ideal for DNA electrophoresis. At this pH level, DNA may move toward the positive electrode and pass through a gel matrix because it remains in solution and allows for deprotonation. TBE buffer also includes ethylenediaminetetraacetic acid (EDTA), a chelating agent that shields nucleic acids from enzymatic degradation, in addition to Tris. EDTA binds to the divalent cations that nucleases need as cofactors to stop them from digesting the DNA sample. But because magnesium functions as an enzyme cofactor for DNA polymerase.

3.1.7.2. Tris-borate EDTA Buffer (TBE-10X)

To prepare the electrophoresis buffer solution, dissolved 3.8 grams of Tris-HCl, 2.7 grams of boric acid, and added 2 milliliters of a 0.5M EDTA solution to

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50 milliliters of distilled water. Adjust the pH to 8 by heating if necessary and stored the solution in the refrigerator until needed for electrophoresis experiments.

3.1.7.3. TBE (1X)

To prepare the solution, 10 mL of the stock TBE-10x was diluted with 90 mL of distilled water, and then the mixture was refrigerated at 4°C until it was needed for electrophoresis experiments.

3.1.7.4. Ethidium Bromide (0.5%)

A stock solution was created by adding 10 milliliters of distilled water to 0.05 grams of ethidium bromide powder in an opaque, sterile container. The combination was thoroughly mixed, and the solution completely dissolved by vortexing it. Sambrook and Russell (2001).

3.1.7.5. Preparation of 1% Agarose Gel:

In flask containing 100ml of 1XTBE buffer, one gram of agarose was added and dissolved in a microwave oven until the boiling appearance started." Swirl the agarose mixture inside the flask after removing it from the microwave, and then return it to warm until it starts to boil. This is what you should do till there are no more flicks. Once the agarose reaches a temperature of 50 to 65 degrees Celsius, mix 2µl of a stock solution containing 10 mg/ml with every 100 milliliters of agarose gel.

3.1.6. Gel Electrophoresis

The PCR products from various genes were analyzed using agarose gel electrophoresis. The procedure involved the following steps:

- Preparation of a 2 % agarose gel: Agarose was dissolved in 1X TBE buffer, heated in a water bath at 100°C for 15 minutes, and then cooled to 50–60°C (adapted from Sambrook and Russell, 2001).
- Addition of ethidium bromide dye: 2µL of ethidium bromide dye was added to the agarose gel solution.

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- Gel casting: The agarose gel solution was poured into a casting tray, ensuring proper comb placement, and left to solidify at room temperature for fifteen minutes. Subsequently, each well received 10 μ L of PCR product and 2 μ L of a 1500 bp ladder after carefully removing the comb.
- Electrophoresis: The gel tray was placed in an electrophoresis chamber filled with 1X TBE buffer. Electrophoresis was conducted at 110 volts for the first 30 minutes and then at 75 volts for an additional 60 minutes.
- Using a UV transilluminator, the PCR products were detected.

3.2. Study Design

3.2.1. Study Design for Molecular

One hundred and fifty samples (blood and swabs) were collected from infected calves in many difference places in Thi-Qar province with taking information about age, sex, places and signs. Five ml of blood was put in a EDTA tube (1ml blood+2ml Trizol) and swab with VTM (1 ml swab + 2 ml Trizol). Directly transported of samples into virology laboratory for at diagnose the virus by PCR and serotypes, in addition to conducting a sequence test. Viral RNA was extracted by using a special RNA extraction kit, and complementary DNA (cDNA) was synthesized from the extracted RNA, and then (cDNA) was amplified using the polymerase chain reaction technique (PCR).

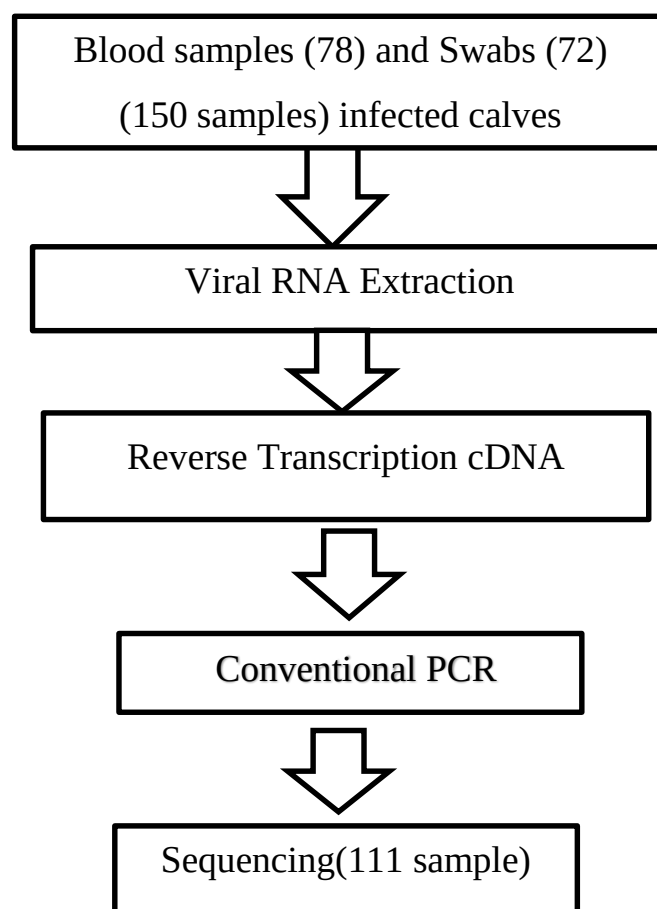


Figure (3-1): Study design for Molecular study

3.2.2. Study Design for Biochemical

Antioxidant Parameters and Enzymatic Assays :

Analysis of sera to detect the antioxidant activity by estimation the following antioxidant agents: Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH) and Malondialdehyde (MDA) activity by using ELISA technique.

Analysis of sera for alanine aminotransferase (ALT); aspartate aminotransferase (AST), alkaline phosphatase (ALP); and Gamma - Glutamyl Transferase (GGT); effectiveness using kits for standard enzymatic assay.

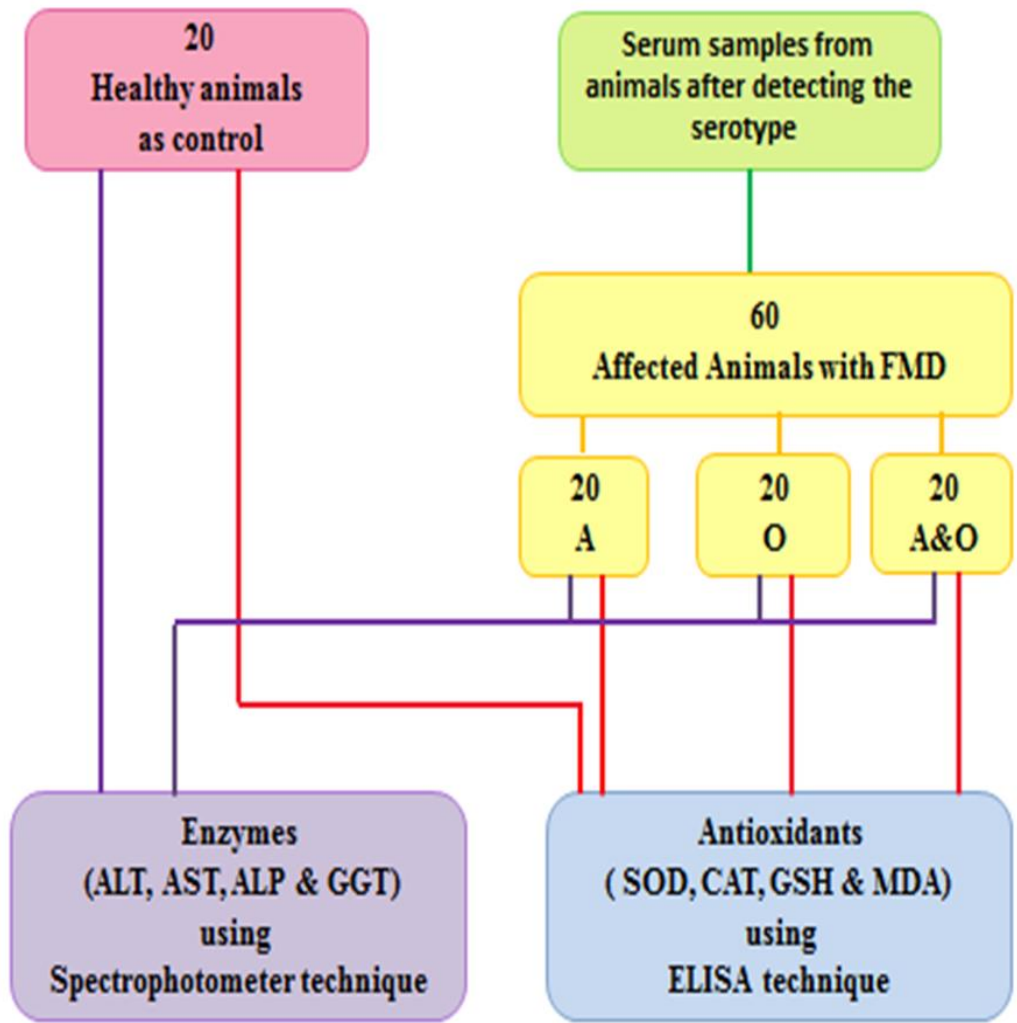


Figure (3-2):Study design for Biochemical study

3.3. Methods

3.3.1. Samples Collection:

Blood Samples and Swabs

Samples for this study were collected from 150 calves (FMD infected calves) from different regions, ages and sexes of Thi-Qar province with 30 calves (healthy) for control during the period extending from November (2024) to March (2025) as showed. Blood samples (15 ml) were collected from the jugular vein of each calf by using aseptic syringes and needles (18G). The venipuncture site was cleaned by 70% ethanol and dropped out to dry before sample collection.

Blood sample (15 ml) was collected (10 ml) into gel tube (non-anticoagulant) and (5ml) into EDTA tub (anticoagulant) and stored in a cooling box before processing in the laboratory.

Ages of infected calves were ranged between 4 to 18 months and from both sexes (Female 64 and Male 86). Gel tube with 10 ml blood was centrifuged at speed 3000-4000 rpm for 10-15 minutes, serum was separated. The serum collected in Eppendorf tubes (1.5ml) and stored at -20 °C until an enzyme-linked immunosorbent assay was produced and enzymatic assays. These methods were used according to Nielsen *et al.*, (2021).

EDTA tube with 5 ml of sample was mixed with Trizol (phenol and guanidine thiocyanate) (Amirouche *et al.*, 2021). Added 2ml Trizol to 1 ml blood sample with mixed gently, all samples were then frozen at -20 °C for the time of the PCR test. Swab sample was taken from mouth lesions and interdigital lesions by VTM (viral transport media) after mixed by vortex, added 2ml Trizol to 1ml from sample and mixed gently. All samples were saved then frozen at -20 °C for the time PCR test.

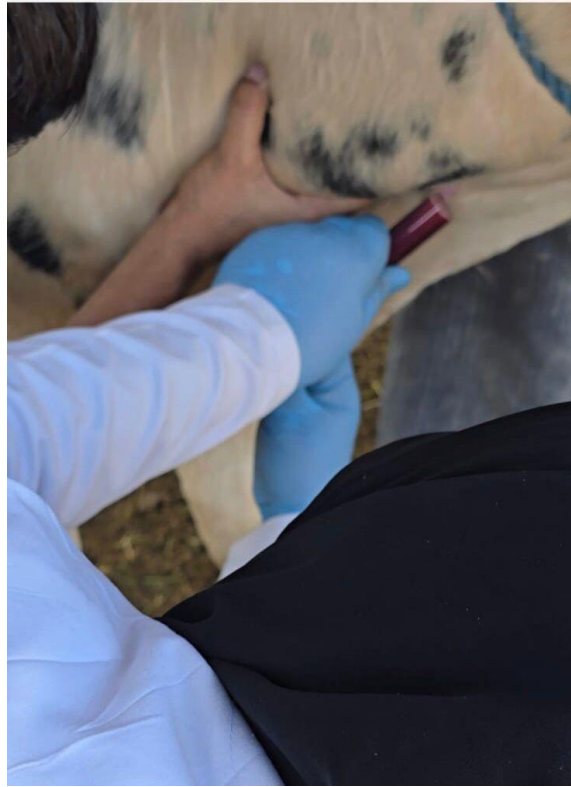


Figure (3-3): Blood Sample Collection.



Figure (3-4): Swab Samples Collection, A: Interdigital Swab, B: Mouth Swab.

3.3.2. Viral RNA Extraction

This kit is designed for rapid lysis of aquatic and livestock samples, releasing viral DNA/RNA, followed by purification of viral DNA/RNA using silica-based magnetic beads. It is suitable for extracting viral DNA/RNA from $\leq 200 \mu\text{l}$ whole blood, plasma, serum, oral/nasal fluids, urine, tissues, feces, feed, and environmental samples. The resulting product is highly pure and suitable for experiments such as PCR, RT-PCR, qPCR, and qRT-PCR.

3.3.3. Reverse Transcription-PCR

One-step RT-PCR is a streamlined method that combines reverse transcription and PCR amplification into a single reaction, eliminating the need for separate cDNA synthesis and PCR steps. This is achieved using a specially formulated one-step master mix, which contains all the necessary reagents for both reverse transcription and PCR amplification in a single tube.

The PCR master mix reaction was prepared following the manufacturer's instructions provided by **TransGen Biotech Inc., China.**"

Table (3-6): Reaction components for PCR reaction:

Component	20 μl reaction
Total RNA	1pg~1 μg
Forward Primer	0.4 μl
Reverse Primer	0.4 μl
2x One-step Reaction Mix	10 μl
TransScript [®] II One-Step Enzyme Mix	0.4 μl
RNase-free Water	Variable Up To 20 μl
Total volume	20 μl

3.3.4. Reaction Setup**One-Step RT-PCR Procedure**

The one-step RT-PCR reaction was prepared in a 20 μ L total volume, optimized for efficient reverse transcription and amplification of RNA targets. The reaction included 1 pg to 1 μ g of total RNA, ensuring sensitivity for detecting both low- and high-abundance transcripts. Forward and reverse primers were each added at 0.4 μ L, facilitating sequence-specific amplification. The 2 $\times$ One-Step Reaction Mix (10 μ L) provided essential components, including dNTPs, buffer, and optimized salts, ensuring robust enzyme activity. The TransScript® II One-Step Enzyme.

Mix (0.4 μ L) contained both reverse transcriptase for cDNA synthesis and DNA polymerase for PCR amplification, streamlining the workflow in a single tube. The final reaction volume was adjusted to 20 μ L using RNase-free water, minimizing contamination risks. This composition allowed for high specificity, sensitivity, and efficiency in RNA detection and quantification, making it ideal for gene expression analysis, pathogen detection, and other molecular applications.

3.3.5. PCR Thermocycling Conditions

PCR thermocycler conditions were set using a conventional PCR system, detailed in the accompanying table. The reaction components are listed in the table below." The thermal cycling conditions for one-step RT-PCR are designed to ensure efficient reverse transcription, DNA amplification, and product stability. The reaction begins with reverse transcription (RT-activation) at 50°C for 20 minutes, allowing the synthesis of complementary DNA (cDNA) from RNA.

This is followed by an initial denaturation step at 94°C for 3 minutes to fully denature RNA secondary structures and activate the DNA polymerase. The amplification phase consists of 35 cycles, each including denaturation at 94°C for 30 seconds, annealing at a variable temperature (determined by the primer's melting temperature) for 30 seconds, and extension at 72°C for 1 minute to synthesize the DNA strand. A final extension at 72°C for 5 minutes ensures the

Chapter Three.....Materials and Methods

complete elongation of all PCR products. The reaction is then held at 4°C indefinitely, preserving the amplified products for downstream analysis. This optimized cycling protocol ensures high specificity, efficiency, and reproducibility in RNA detection and studies.

Table (3-7): PCR Amplification Program:

Step	Temperature(°C)	Time	No.of cycles cycle
RT-activation	50 °C	20 min	1
Initial denaturation	94 °C	3 min	1
Denaturation	94 °C	30sec	35
Annealing	Variables according to (primer's melting TM)	30sec	
Extension Step	72 °C	1 min	
Final extension	72 °C	5 min	1
Hold temperature	4 °C	∞	-

3.3.6. Sequencing Methods

3.3.6.1. Nucleic Acid Sequencing of PCR Amplicons

The resolved PCR amplicons were commercially sequenced from forward and reverse directions, following to instruction manuals of the sequencing company (Macrogen, South Korea). By comparing the observed nucleic acid sequences of the local viral samples with the retrieved nucleic acid sequences, the virtual positions and other details of the retrieved PCR fragments were identified.

3.3.6.2. Interpretation of Sequencing Data

The sequencing results of the PCR products of the targeted sample were edited, aligned, and analyzed with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed variations in the sequenced sample were

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numbered in PCR amplicons as well as in their corresponding position within the referring genome.

The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. The observed variations were respectively deposited in the NCBI database under a unique accession number for the analyzed sample.

3.3.6.3. Translation of Nucleic Acid Variations into Amino Acid Residues

The amino acid sequences of the targeted proteins were retrieved from the protein data bank. To assess the impact of the identified nucleic acid variations located in the coding regions on the encoded protein, all nucleic acid sequences of the investigated samples were translated to their corresponding amino acid sequences using the Expasy translate suite.

3.3.6.4. Deposition of Sequences to GenBank

All the investigated and analyzed sequences were submitted to the NCBI portal, and all the instructions described by the portal were followed as described by the server. The submitted sequence was provided as nucleic acid sequences in the NCBI to get a unique GenBank accession number for the investigated viral sequences.

3.3.6.5. Comprehensive Phylogenetic Tree Construction

Before generating a phylogenetic tree, the observed variants were compared with their neighbour homologous reference sequences using the NCBI-BLASTn server (Zhang *et al.*,2000b). The viral query sequences were carefully matched to ensure complete coverage with the reference sequences obtained from the NCBI BLASTn tool. Additionally, GenBank accession numbers with identical homology were removed to avoid redundancy.

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The ClustalW tool was used to align the retrieved nucleic acid sequences. The aligned sequences were used to generate a phylogenetic tree using the MEGA7 tool (Kumar *et al.*, 2016).

The evolutionary history was inferred from the aligned sequences using the neighbour-joining method (Tamura *et al.*, 2004). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated in the generated tree. The generated tree was exported to iTOL software and annotated and visualized in such away each specific clade was represented by a different colour (Letunic and Bork, 2021).

3.3.7. ELISA Methods

(Al Hayat Lab./ Al Nassiriya)

3.3.7.1. Bovine Catalase (CAT)

After collection of the whole blood, allowed the blood to clot by leaving it undisturbed at room temperature. This usually took 10-20 minutes. Removed the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appeared during reservation, the sample should be centrifugated again.

In the Microelisa stripplate, leaved a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubation: incubated 30 min at 37°C after sealed with Closure plate membrane.

4. Dilution: diluted the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).

5. Carefully peeled off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

6. Added 50 µl HRP-Conjugate reagent to each well except the blank control well.

7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Added 50 μ l Chromogen Solution A and 50 μ l Chromogen Solution B to each well, mix with gently shaking and incubated at 37°C for 15 minutes. Please avoid light during coloring.
10. Termination: added 50 μ l stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microliter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

3.3.7.2. Bovine Malondialdehyde (MDA)

Procedure

1. Dilution of Standards:

Ten wells were set for standards in a Microelisa stripplate. In Well 1 and Well 2, 100 μ l standard solution and 50 μ l Standard Dilution buffer were added and mixed well. In Well 3 and Well 4, 100 μ l solution from Well 1 and Well 2 are added respectively. Then 50 μ l Standard Dilution buffer were added and mixed well. 50 μ l solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 μ l solution from Well 3 and Well 4 were added respectively. Then 50 μ l Standard Dilution buffer were added and mixed well. In Well 7 and Well 8, 50 μ l solution from Well 5 and Well 6 were added respectively. Then 50 μ l Standard Dilution buffer were added and mixed well. In Well 9 and Well 10, 50 μ l solution from Well 7 and Well 8 were added respectively. Then 50 μ l Standard Dilution buffer are added and mixed well. 50 μ l solution was discarded from Well 9 and Well 10. After dilution, the total volume in all the wells were 50 μ l and the concentrations were 300 ng/ml, 200 ng/ml, 100 ng/ml, 50 ng/ml and 25 ng/ml, respectively.

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2. In the Microelisa stripplate, leaved a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mixed well with gentle shaking.
3. Incubation: incubated 30 min at 37°C after sealed with Closure plate membrane.
4. Dilution: diluted the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peeled off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeated the washing procedure for 5 times.
6. Added 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Added 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mixed with gently shaking and incubated at 37°C for 15 minutes. Please avoided light during coloring.
10. Termination: added 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well was set as zero. Assay should be carried out within 15 minutes after adding stop solution.

3.3.7.3. Bovine Super Oxidase Dimutase (SOD)

Procedure

1. Dilution of Standards

Ten wells are set for standards in a Microelisa stripplate. In Well 1 and Well 2, 100µl Standard solution and 50µl Standard Dilution buffer are added and mixed well. In Well 3 and Well 4, 100µl solution from Well 1 and Well 2 are added

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respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50µl solution from Well 3 and Well 4 are added respectively.

Then 50µl Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50µl solution from Well 5 and Well 6 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50µl solution from Well 7 and Well 8 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 9 and Well 10. After dilution, the total volume in all the wells is 50µl and the concentrations are 1800 pg/ml, 1200 pg/ml , 600pg/ml, 300 pg/ml and 150pg/ml, respectively.

2. In the Microelisa stripplate, leaved a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample were added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mixed well with gentle shaking.

3. Incubation: incubated 30 min at 37°C after sealed with Closure plate membrane.

4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).

5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeated the washing procedure for 5 times.

6. Added 50 µl HRP-Conjugate reagent to each well except the blank control well.

7. Incubation as described in Step 3.

8. Washing as described in Step 5.

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9. Coloring: Added 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mixed with gently shaking and incubated at 37 °C for 15 minutes. Please avoided light during coloring.

10. Termination: added 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well was set as zero. Assay should be carried out within 15 minutes after adding stop solution.

3.3.7.4. Bovine Glutathione (GSH)

Procedure

1. Dilution of Standards

Diluted the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube used two wells, total ten wells.

Table (3-8): Dilution of Standard (GSH):

360ng/L	Standard No.1	300µl Original Standard + 150µl Standard diluents
240ng/L	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
120ng/L	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
60ng/L	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
30ng/L	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

2. In the Microelisa stripplate, leaved a well empty as blank control. In sample wells, 40µl sample dilution buffer and 10µl sample were added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mixed well with gentle shaking.

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3. Incubation: incubated 30 min at 37°C after sealed with Closure plate membrane.
4. Dilution: diluted the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peeled off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeated the washing procedure for 5 times.
6. Added 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Added 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mixed with gently shaking and incubate at 37°C for 15 minutes. Please avoid light during coloring.
10. Termination: added 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well was set as zero. Assay should be carried out within 15 minutes after adding stop solution.

3.3.8. Kinetic Method UV:

(Dr. Adeeb Lab./ Al Shatrah)

3.3.8.1. Alkaline Phosphatase

3.3.8.1.1. Intended Use

Quantitative determination of alkaline phosphatase (ALP) in serum and plasma using an optimized method according to DGKC recommendations.

3.3.8.1.2. Principle

The enzyme alkaline phosphatase hydrolyzes p-nitrophenylphosphate (4-NPP),

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releasing p-nitrophenol (4-NP). The rate of 4-NP formation is measured spectrophotometrically at 405 nm to quantify the enzyme activity in the sample.

Sample Types: Serum, heparinized plasma

Kit Component

Reagent(A) ALP Volume= 40/80 ml	Buffer DEA Magnesium chloride	1 mol/l 0.5 mmol/l
Reagent(B) ALP Volume=10 ml	P-nitrophenylphosphate	10 mmol/l

Procedure

Wavelength: 405 nm (410 nm)

Light Path: 1 cm

Temperature: 37°C

Blank: Distilled water

Monoreagent Method:

Pipette:

Working solution (A+B): 1000 µl

Sample: 20 µl

Mix, incubate at 37°C for 1 minute, and read initial absorbance.

Take 3 readings at 60-second intervals.

Calculate mean $\Delta A/\text{min}$.

Bireagent Method:

Pipette:

Reagent (A): 800 µl

Sample: 20 µl

Mix, wait 1 minute, then add Reagent (B): 200 µl.

Follow steps 2–4 as above.

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References:

J. Clin. Chem. Clin. Biochem. 8:658 (1970).

Vassault et al. Ann. Biol. Clin. 44:686 (1986)

3.3.8.2. AST/GOT

(Tietz *et al.* , 1995;Young, 1995; Burtis, 1999; Young *et al.*, 2001)

3.3.8.2.1. Intended Use

Quantitative determination of aspartate aminotransferase (AST) in serum and plasma in accordance with IFCC recommendations.

3.3.8.2.2. Principle

In the presence of α -ketoglutarate, AST/GOT in the sample transforms aspartate into oxalacetate and glutamate. Oxalacetate is then converted into malate and NAD in the presence of NADH and malate dehydrogenase. The consumption of NADH per unit of time, measured at 340 nm, is proportional to the concentration of AST/GOT in the sample.

Kit Components

Reagent(A) AST Volume=40/80 ml	Tris buffer PH 7.8 L-aspartate, MDH	50 mmol/l, 300 mmol/l, 400 U/l
Reagent(B) AST Volume=10/40/80 ml	NADH α -ketoglutarate	0.18 mmol/l 9 mmol/l

Procedure

General Parameters

Wavelength: 340 nm

Lightpath: 1 cm

Temperature: 37°C

Reading: Against distilled water

Method: Decreasing Kinetic

Monoreagent Method

Pipette:

Working solution (A+B): 1000 μ l

Sample: 100 μ l

Mix and incubate at 37°C for 1 minute.

Read initial absorbance against water.

Perform 3 readings at 60-second intervals.

Calculate the average $\Delta A/\text{min}$.

Bireagent Method

Pipette:

Reagent (A): 800 μ l

Reagent (B): 200 μ l

Mix and wait 30 seconds.

Add sample: 100 μ l.

Mix and incubate at 37°C for 1 minute.

Read initial absorbance against water.

Perform 3 readings at 60-second intervals.

Calculate the average $\Delta A/\text{min}$.

Method Performance

Sensitivity and Linearity

Sensitivity: 1 U/L

Linearity: Up to 450 U/L. For higher values, dilute samples 1:10 with saline and multiply results by 10.

3.3.8.3. ALT/GPT

3.3.8.3.1. Intended Use

Quantitative determination of alanine aminotransferase (ALT/GPT) in serum and plasma according to IFCC recommendations.

3.3.8.3.2. Principle

In the presence of α -ketoglutarate, alanine is converted into pyruvate and glutamate by ALT/GPT in the sample. Pyruvate is then transformed into lactate and NAD in the presence of NADH and lactate dehydrogenase. The rate of NADH oxidation, measured at 340 nm, is proportional to the ALT/GPT concentration in the sample.

Kit Components

Reagent(A) ALT Volume=40/80 ml	TamponeTris PH 7.8, L-alanine, LDH	50 mmol/l, 300 mmol/l 800 U/l
Reagent(B) ALT Volume=10/40/80 ml	NADH α -ketoglutarate	0.18 mmol/l 9 mmol/l

Procedure

General Parameters

Wavelength: 340 nm

Lightpath: 1 cm

Temperature: 37°C

Reading: Against distilled water

Method: Decreasing kinetic

Monoreagent Method

Pipette:

1000 μ l of working solution (A+B)

100 μ l of sample

Mix and incubate at 37°C for 1 minute.

Read initial absorbance against water.

Take 3 readings at 60-second intervals.

Calculate the average $\Delta A/\text{min}$.

Bireagent Method

Pipette:

800 µl of Reagent (A)

100 µl of sample

Mix and wait 30 seconds.

Add 200 µl of Reagent (B).

Mix and incubate at 37°C for 1 minute.

Read initial absorbance against water.

Take 3 readings at 60-second intervals.

Calculate the average $\Delta A/\text{min}$.

Method Performance:

Sensitivity and Linearity

Sensitivity: 4 U/L

Linearity: Up to 450 U/L. For higher values, dilute samples 1:10 with saline and multiply results by 10.

3.3.8.4. GAMMA GT

3.3.8.4.1. Intended Use

Quantitative determination of glutamyltransferase (γ -GT) in serum. Produced by Giese Diagnostics company with management system certified by DNV ISO 9001, ISO 13485.

3.3.8.4.2. Principle

The γ -GT in the presence of glycyl-glycine, splits the L- γ -glutamyl-3-carboxy-4-nitroanilide (carboxi-glupa) in L- γ -glutamyl-glycine and 5-amino-2-nitrobenzoate. The absorbance change in time unit measured at 450 nm is proportional to the enzyme activity in the sample.

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Kit components

Reagent(A) γ -GT	Tris buffer	50 mmol/l
Volume=10/40/80 ml	Glycyl-glycine	125 mmol/l
Reagent(B) γ -GT	L- γ -glutamyl-3-carboxy-4-nitroanilide	6 mmol/l
Volume=10/20 ml		

Procedure

Wavelength	405 nm
Lightpath	1 cm
Temperature	37°C
Reading	Against distilled water
Method	Increasing kinetic

Use as Monoreagent

Pipette

Working solution (A+B) 1000 μ l

Sample 100 μ l

Mix, incubate at 37°C for 1 minute, read the initial absorbance against water.

Make 3 readings at a distance of 60 seconds. Calculate the average value of the absorbance variations per minute.

Use as Bireagent

Pipette

Reagent (A) 1000 μ l

Sample 100 μ l

Mix and after 1 minute add:

Reagent (B) 250 μ l

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Mix, incubate at 37 °C for 1 minute, read the initial absorbance against water.

Make 3 readings at a distance of 60 seconds. Calculate the average value of the absorbance variations per minute.

Performance

Sensitivity: the sensitivity of the method is: 2 U/L.

Linearity: the method is linear up to 1200 U/L. For higher values, dilute the sample 1:10 and multiply the result by 10.

3.3.9. Statistical Analysis

The SPSS program was used to do the statistical analysis. The relationship between age, sex, serotypes, months, regions, clinical symptoms and type of sample (blood or swab) of FMD infection was assessed using the chi-square test.

Chapter Four

Results

Results

4.1. Molecular Analysis

From 150 sample taken from vesicular lesions of cattle herds that exhibited signs of FMD in different districts of Thi-Qar province, we estimated that(150)samples were positive by PCR technique :

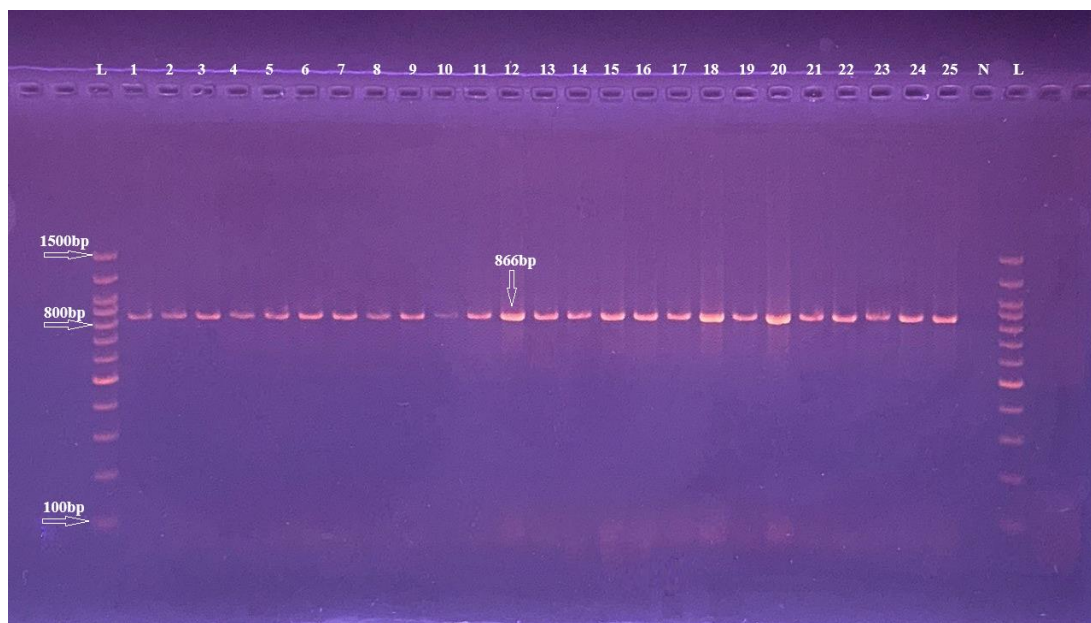


Figure (4-1): - Gel electrophoresis for PCR product of (FMDV serotype A gene primer) used for detection of serotype A, which show 865 bp Primer TM at (52.3°C), (Agarose 1%, 15min. at 110 voltages and then lowered to 75 volts, 60min). Visualized under U.V light after staining with ethidium bromide Lane L: DNA ladder (100-1500) bp, Lanes (1 -25) and Lane (N) represented Negative control.

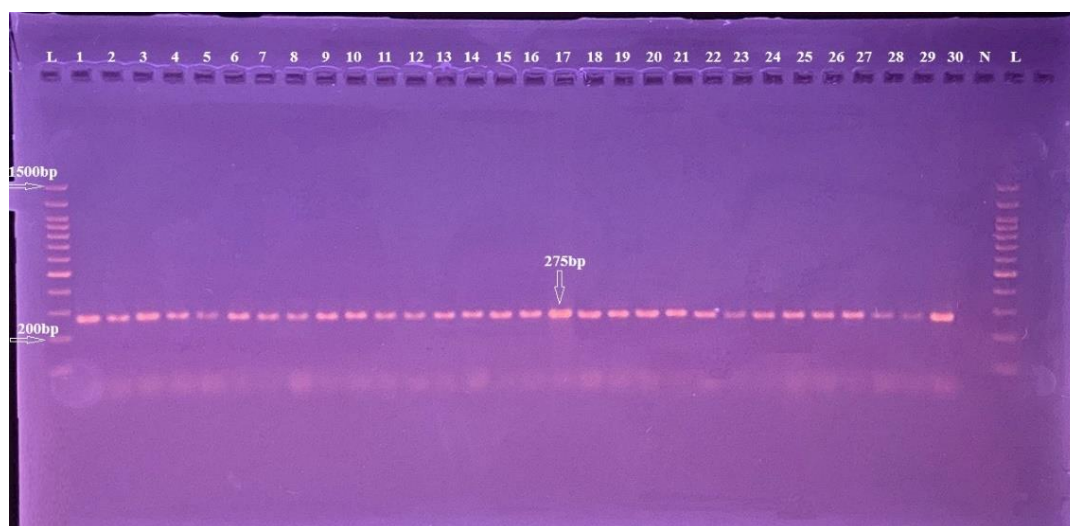


Figure (4-2): - Gel electrophoresis for PCR product of (Foot-and-mouth virus serotype BA gene primer). used for detection of serotype Asia ,SAT, which show 275 bp Primer TM at (56°C), (Agarose 1.5%, 15min. at 110 voltage and then lowered to 75 volts, 60min.). Visualized under U.V light after staining with ethidium bromide Lane L : DNA ladder (100-1500)bp. All Lanes (1 -64) represented positive results , NO Negative result , and Lane (N) represented Negative control.

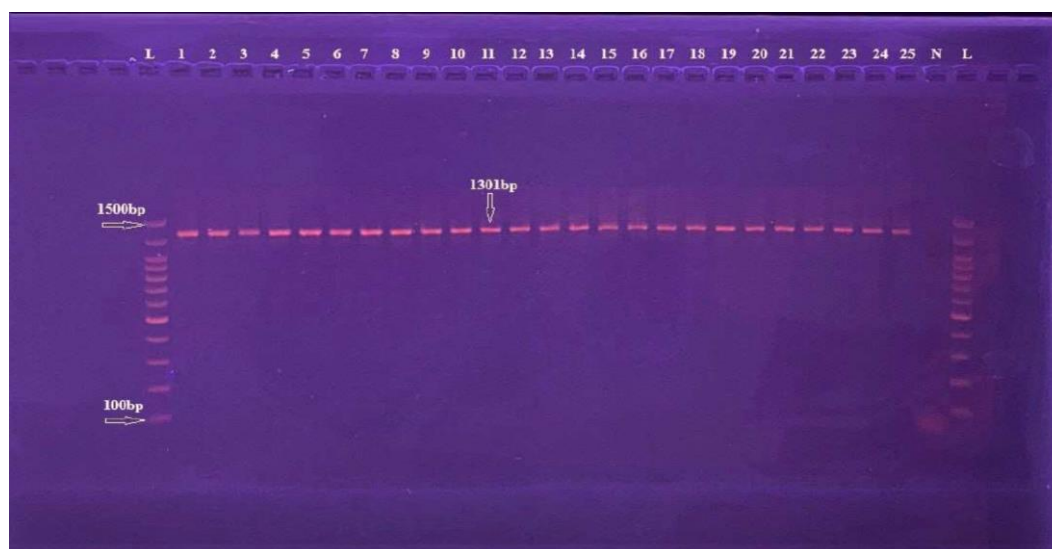


Figure (4-3): - Gel electrophoresis for PCR product of (Foot-and-mouth virus serotype O gene primer). which show 1301 bp Primer TM at (60.1°C), (Agarose 0.8 %, 15min. at 110 voltage and then lowered to 75 volts, 60min.). Visualized under U.V light after staining with ethidium bromide Lane L : DNA ladder (100-1500)bp, Lanes (1,9,12,17,19,20,23,25, 27,33,39,50 and 53) represented positive results.

4.2. Sequencing

Using the sequencing reactions, the exact identity of the investigated samples was resolved after performing NCBI blastn for PCR amplicons (Ye *et al.*, 2012). Regarding fragment A, twelve samples were included in the present study. These samples were screened to amplify an 866 bp region of the FMDV polyprotein gene. Sequence similarity analysis using the NCBI BLASTn tool revealed approximately 99% nucleotide identity between the sequenced samples and the most closely related reference sequences available in the GenBank database. Among these, two samples showed the highest sequence similarity to the Asia 1 serotype (GenBank accession no HQ113233.1), while one sample exhibited the greatest similarity to the SAT 2 serotype (GenBank MT602090.1). Two additional samples were most closely related to the A serotype (GenBank OM455481.1). The remaining samples were attributed to the O serotype, with the closest match to GenBank accession OP957418.1 (table 4-1a) .

Regarding fragment B, a total of 79 samples were analyzed in this study. These samples were screened to amplify a 275 bp region of the FMDV polyprotein gene. Sequence similarity analysis using the NCBI BLASTn tool revealed nucleotide identities ranging from 98% to 100% when compared to the closest reference sequences in the GenBank database. Eight of the samples exhibited the highest sequence similarity to the O serotype, with GenBank accession number MN983153.1. Two samples aligned most closely with a different variant of the same serotype, corresponding to GenBank accession OK422489.1. An additional fourteen samples showed the highest similarity to another form of serotype O (GenBank PQ153286.1), while three samples were most similar to yet another O serotype variant (GenBank KC440883.1). One sample displayed the closest sequence similarity to the O serotype strain MG983733.1, and another matched most closely with the variant represented by GenBank OK318523.1. Furthermore,

Chaptar Four..... Result
 two samples showed the highest level of sequence identity with the O serotype variant registered under GenBank KJ144929.1.

In contrast, twenty samples demonstrated the highest sequence similarity to the A serotype (GenBank HQ832585.1). One additional sample was closely related to another variant of serotype A, identified as GenBank AY593752.1. Twenty-six of the analyzed samples were found to be most similar to a recombinant or intermediate serotype A/O (GenBank MT340219.1). Finally, a single sample showed the closest sequence similarity to the Asia 1 serotype, corresponding to GenBank AY593798.1 (table 4-1b).

In contrast to the amplified samples of fragments A and B, the BLASTn analysis of the 20 PCR amplicons from fragment C revealed that all samples belonged to the serotype O, with varying degrees of sequence similarity to different known strains. Nine samples showed high sequence similarity to the PAK/12/2021 isolate, with GenBank accession number PQ153287.1, exhibiting similarity ratios ranging from 99.46% to 99.69%. Six samples closely matched the BAN/MY/My-466/2021 strain (GenBank OP957418.1), with similarity values reaching up to 99.77%, indicating a strong relationship with this strain among several isolates. In addition, five samples displayed the highest similarity to the O/jk-1 strain from India, corresponding to GenBank OP189368.1, consistently showing a 99.69% identity.

These findings suggest the presence of at least three distinct but closely related viral lineages within serotype O in the analyzed samples: those aligning with PAK/12/2021, BAN/MY/My-466/2021, and O/jk-1/IND (table 4-1c).

Table (4-1): Sequence similarity of FMDV fragments A (branch A), B (branch B), and C (branch C) amplicons with reference strains based on BLASTn analysis.

PCR amplicon	Total No.	Fragment No.	Serotype	Isolate/strain details	GenBank	Similarity ratio
A)		1.	Asia 1	Asia1/BAM/AFG/L-590/2009	HQ113233.1	99.77%
		2.	Asia 1	Asia1/BAM/AFG/L-590/2009	HQ113233.1	99.77%
		3.	SAT 2	ETH/16/2015	MT602090.1	99.77%

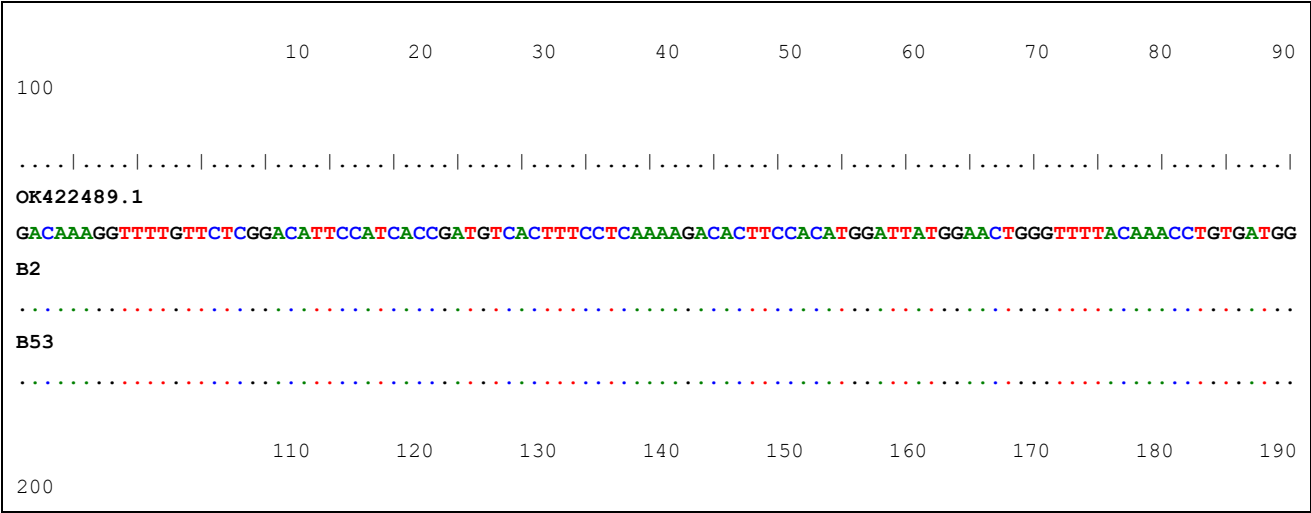
B)	4.	O	BAN/MY/My-466/2021	OP957418.1	99.74%
	5.	O	BAN/MY/My-466/2021	OP957418.1	99.74%
	6.	O	BAN/MY/My-466/2021	OP957418.1	99.65%
	7.	O	BAN/MY/My-466/2021	OP957418.1	99.65%
	8.	A	A/PAK/ICT/276/2012	OM455481.1	99.54%
	9.	A	A/PAK/ICT/276/2012	OM455481.1	99.54%
	10.	A	A/PAK/ICT/276/2012	OM455481.1	99.54%
	11.	O	BAN/MY/My-466/2021	OP957418.1	99.31%
	12.	O	BAN/MY/My-466/2021	OP957418.1	99.31%
	1.	O	O/IND/121/2001	MN983153.1	98.91%
	2.	O	FMDV/Goat/India/2013	OK422489.1	99.27%
	3.	O	O/IND/121/2001	MN983153.1	99.27%
	4.	O	O/IND/121/2001	MN983153.1	99.27%
	5.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	6.	O	O/IND/121/2001	MN983153.1	99.64%
	7.	O	O/IND/121/2001	MN983153.1	98.91%
	8.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	9.	A	IND 26/2006	HQ832585.1	99.27%
	10.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	11.	O	PAK/9/2021	PQ153286.1	99.64%
	12.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	13.	O	O/IND/121/2001	MN983153.1	99.64%
	14.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	15.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.27%
	16.	O	PAK/9/2021	PQ153286.1	99.64%
	17.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	18.	A	IND 26/2006	HQ832585.1	100%
	19.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	20.	A	IND 26/2006	HQ832585.1	99.27%
	21.	A	IND 26/2006	HQ832585.1	99.27%
	22.	A	IND 26/2006	HQ832585.1	98.91%
	23.	O	PAK/9/2021	PQ153286.1	99.64%
	24.	A	IND 26/2006	HQ832585.1	99.27%
	25.	A	IND 26/2006	HQ832585.1	98.91%
	26.	A	IND 26/2006	HQ832585.1	99.27%
	27.	A	IND 26/2006	HQ832585.1	98.91%
	28.	O	PAK/9/2021	PQ153286.1	99.64%
	29.	A	IND 26/2006	HQ832585.1	99.27%
	30.	A	IND 26/2006	HQ832585.1	99.64%
	31.	O	EGY 10/2011	KC440883.1	99.27%
	32.	O	EGY 10/2011	KC440883.1	99.27%
	33.	A	IND 26/2006	HQ832585.1	99.27%
	34.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.64%
	35.	Asia 1	asia1leb-89 iso89	AY593798.1	99.27%
	36.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	37.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.27%
	38.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.27%
	39.	A	IND 26/2006	HQ832585.1	98.91%
	40.	A	IND 26/2006	HQ832585.1	99.64%
	41.	A	IND 26/2006	HQ832585.1	99.27%
	42.	A	IND 26/2006	HQ832585.1	99.27%
	43.	A	IND 26/2006	HQ832585.1	98.91%
	44.	O	SRL/28/2014	MG983733.1	99.27%
	45.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.64%
	46.	A	a12valle 119 iso20	AY593752.1	99.64%
	47.	O	PAK/9/2021	PQ153286.1	99.64%
	48.	O	PAK/9/2021	PQ153286.1	99.64%
	49.	O	PAK/9/2021	PQ153286.1	99.64%
	50.	O	PAK/9/2021	PQ153286.1	99.64%
	51.	O	PAK/9/2021	PQ153286.1	99.64%
	52.	O	PAK/9/2021	PQ153286.1	99.64%
	53.	O	FMDV/Goat/India/2013	OK422489.1	99.27%

	54.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	55.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	56.	O	PAK/9/2021	PQ153286.1	99.64%
	57.	O	PAK/9/2021	PQ153286.1	99.64%
	58.	A	IND 26/2006	HQ832585.1	99.64%
	59.	O	PAK/9/2021	PQ153286.1	99.64%
	60.	O	EGY 10/2011	KC440883.1	99.27%
	61.	A	IND 26/2006	HQ832585.1	99.27%
	62.	A	IND 26/2006	HQ832585.1	99.27%
	63.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	64.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	65.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.27%
	66.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	67.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.27%
	68.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	69.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	98.91%
	70.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.64%
	71.	O	O/IND/121/2001	MN983153.1	98.91%
	72.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.27%
	73.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	100%
	74.	O	O/VIT/18F207/2018	OK318523.1	99.27%
	75.	O	O/IND/121/2001	MN983153.1	99.27%
	76.	O	O/ETH/03/96 P2/P3	KJ144929.1	99.64%
	77.	O	O/ETH/03/96 P2/P3	KJ144929.1	99.64%
	78.	A/O	A/VIT/DL-P-164-4/2018	MT340219.1	99.64%
	79.	O	PAK/9/2021	PQ153286.1	99.64%
C)	1.	O	PAK/12/2021	PQ153287.1	99.46%
	2.	O	o/jk-1/ind	OP189368.1	99.69%
	3.	O	o/jk-1/ind	OP189368.1	99.69%
	4.	O	o/jk-1/ind	OP189368.1	99.69%
	5.	O	PAK/12/2021	PQ153287.1	99.46%
	6.	O	PAK/12/2021	PQ153287.1	99.46%
	7.	O	BAN/MY/My-466/2021	OP957418.1	99.69%
	8.	O	BAN/MY/My-466/2021	OP957418.1	99.69%
	9.	O	PAK/12/2021	PQ153287.1	99.69%
	10.	O	BAN/MY/My-466/2021	OP957418.1	99.69%
	11.	O	PAK/12/2021	PQ153287.1	99.46%
	12.	O	BAN/MY/My-466/2021	OP957418.1	99.77%
	13.	O	o/jk-1/ind	OP189368.1	99.69%
	14.	O	o/jk-1/ind	OP189368.1	99.69%
	15.	O	PAK/12/2021	PQ153287.1	99.62%
	16.	O	PAK/12/2021	PQ153287.1	99.54%
	17.	O	PAK/12/2021	PQ153287.1	99.54%
	18.	O	BAN/MY/My-466/2021	OP957418.1	99.77%
	19.	O	BAN/MY/My-466/2021	OP957418.1	99.77%
	20.	O	PAK/12/2021	PQ153287.1	99.54%

To unravel the details of the amplified sequences of the A, B, and C amplicons, the positions of the forward and reverse primers, alongside the total lengths of the amplified products, were determined as per the cataloged sequences within the representative segments of the FMDV polyprotein gene. For each amplicon, GenBank accession numbers are provided, along with the precise

Chaptar Four..... Result genomic coordinates, corresponding nucleotide sequences, and the calculated amplicon length in base pairs (bp). Importantly, the positions of the forward and reverse primers are visually represented in green and red, respectively, where the green marks the 5' end of the forward primer, and red indicates the position of the reverse primer (in reverse complement orientation) (Table4-1). As per the amplified fragments cataloged in the NCBI server, it has been known that the amplicon A spans approximately 866 bp and is conserved across multiple FMDV serotypes, including Asia 1, SAT 2, and serotype A (Table 4-1a). The amplified fragment of the amplicon B encompasses only a 275 bp region that appears highly conserved among multiple isolates of serotypes O, A, and Asia 1, with minimal sequence variation noted in the respective GenBank entries (Table 4-1b). Meanwhile, the amplicon C is made up of 1301 bp in length, covering a broader region of the polyprotein gene (table 4-1c).

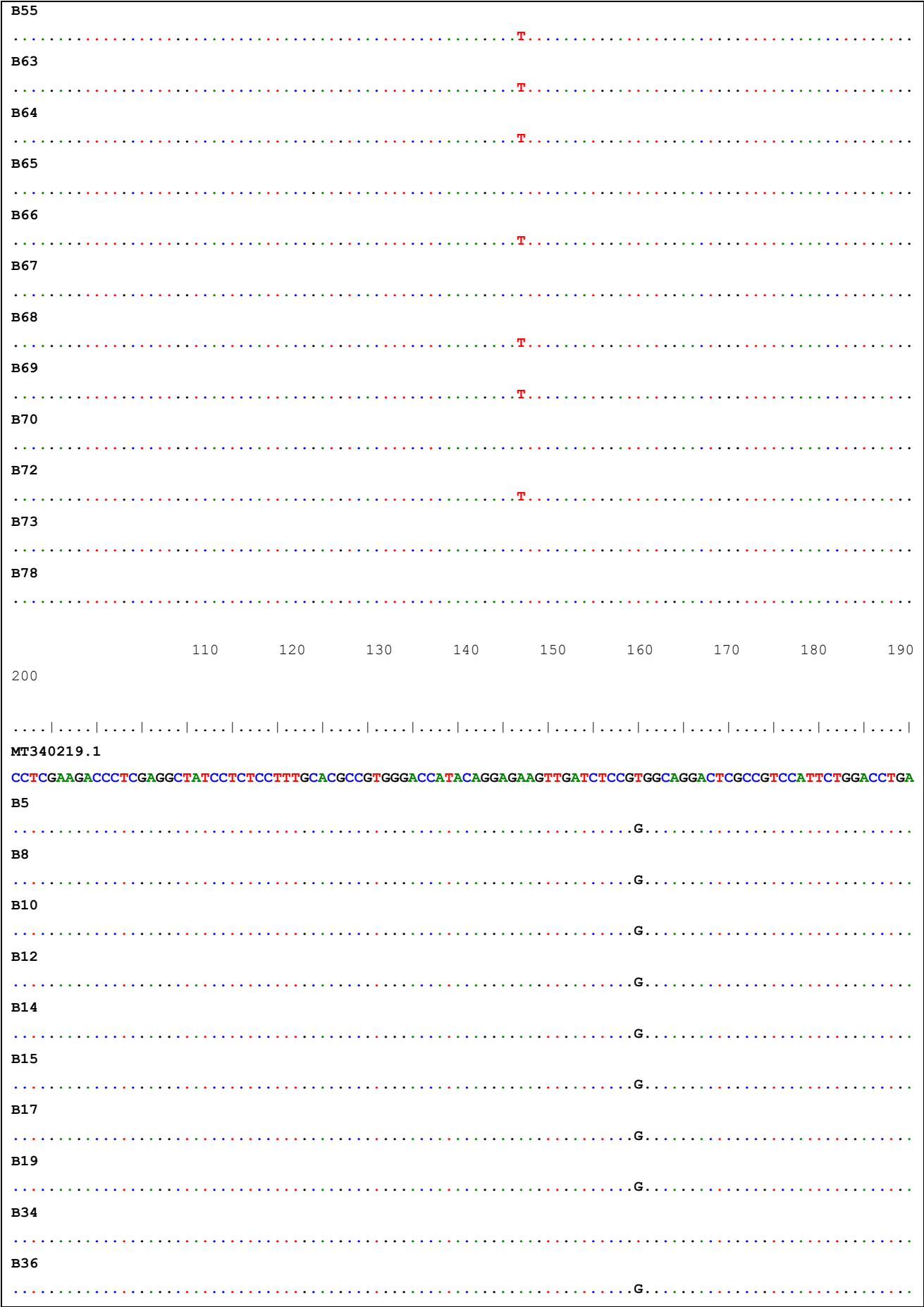
Table (4-1): The position and length of the PCR amplicons that are used to partially amplify the polyprotein gene within the FMDV genomic sequences, which are made of 866 bp, 275 bp, and 1301 bp in branches A, B, and C, respectively. Green and red colors refer to the positions of the forward and reverse primers, respectively. The green refers to the position of the forward primer (placed in the forward direction). The red colour refers to the position of the reverse primer (placed in reverse complement direction).

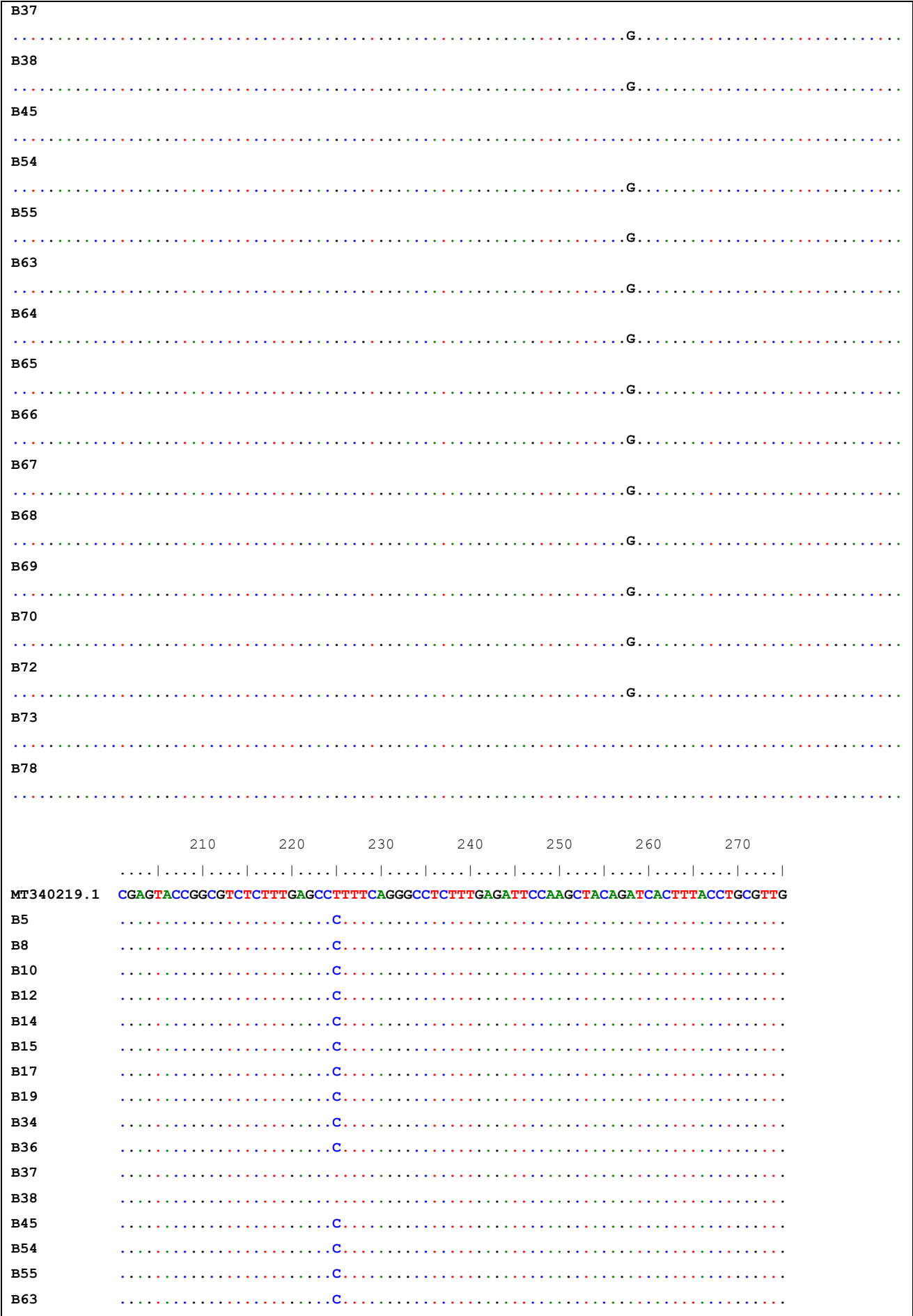


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OK422489.1
CCTCGAAGACCCCTCGAGGCTATTCTCTCCTTTGCACGCCGTGGGACCATACAGGAGAAGTTGATCTCCGTGGCAGGACTCGCCGTCCACTCCGGACCAGA
B2
.....
B53
.....
210220230240250260270
.....
OK422489.1
CGAGTACCGGGCTCTCTTTGAACCCCTCCAGGGCCTCTTTGAGATTCCAAGCTACAGATCACCTTACCTGCGTTG
B2
B53

B3)

102030405060708090
100
.....
MT340219.1
GACAGAGGTTTGTCTTTGGTCACTCCATTACCGATGTCACCTTTCCTCAAAGACACTTCCACATGGATTATGGAAC TGGGTTTTACAACCTGTGATGG
B5
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B8
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B10
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B12
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B14
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B15
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B17
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B19
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B34
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B36
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B38
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B45
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B54
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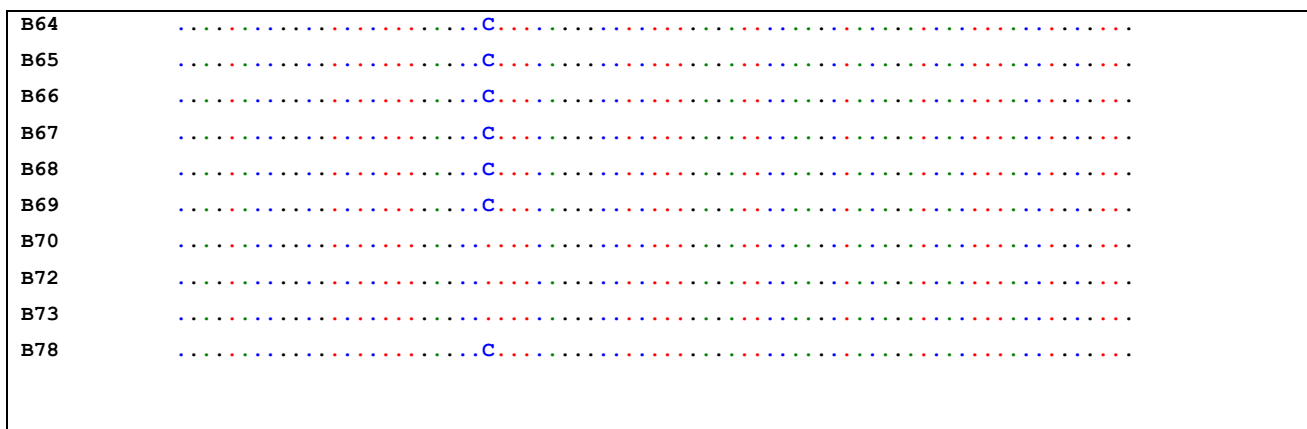


Figure (4-4): Multiple nucleic acid sequences alignment of the PCR amplicons that are used to partially amplify the polyprotein gene within the FMDV genomic sequences that are made of 12 samples of 866 bp (A1 – A12), 79 samples of 275 bp (B1 – B79), and 20 samples of 1301 bp (C1 – C20) in branches A, B, and C, respectively. The symbol “ref” refers to the NCBI referring sequence.

After analyzing the detected nucleic acid substitutions on the investigated viral samples of the polyprotein gene, the consequences of these SNPs on their corresponding positions in the polyprotein should be revealed. For this reason, the nucleic acid sequences were translated into amino acid sequences to assess whether the identified SNPs exert a synonymous (silent) or non-synonymous (missense) effect on the resulting protein. With regard to the amplicons A, the amplified nucleic acid sequences of 866 bp have been translated into their corresponding amino acid sequences with the FMDV polyprotein sequences. The results indicated that these sequences accounted for 288 amino acids within the full 2329-amino-acid FMDV protein structure, representing approximately 12% of the entire protein.

Based on the GenBank HQ113233.1, both identified SNPs of 771T>C and 774T>C exhibited, respectively, two silent effects on the protein, p.945D= and p.946V= (Fig. 4-5a, 1). Based on the GenBank MT602090.1, the identified 524G>C SNP exhibited a missense effect on the protein, p.869R>P (Fig. 4-5a, 2). The R869→P amino acid change replaces a positively charged arginine with proline, a residue known to disrupt α -helices and impose backbone rigidity.

This sort of amino acid substitution could compromise proper folding of a critical protease or polymerase domain of the viral protein. Based on the GenBank OP957418.1, the identified 339A>G, 408C>T, 435G>A, and 681C>T caused four respective silent effects of p.803A=, p.826=, p.835P=, and p.917A= (Fig. 2a, 3). Meanwhile, the detected 348T>C and 523G>A caused two missense effects of p.806H>Q and p.865A>T, respectively. The amino acid substitution of histidine by glutamine residue at position 806 (H806→Q) removes an ionizable side chain, potentially altering pH-dependent interactions in an active site or receptor-binding loop. Whereas the introduction of a hydroxyl group through the substitution of alanine with tyrosine (A865→T) may create a new site for hydrogen bonding, subtly modulating domain stability of the altered viral polyprotein.

Based on the GenBank OM455481.1, the identified 673C>T SNP caused a silent effect of p.916L= on the viral FMDV polyprotein (Fig. 4-5a, 4). Meanwhile, the detected 109A>G, 119A>G, 337G>T, and 673C>T SNPs caused three missense effects of p.728T>A, p.731E>G, and p.804V>L, respectively.

The amino acid substitution of T728→A eliminates a hydroxyl-bearing threonine, which could destabilize a turn or loop if that –OH had been forming intramolecular hydrogen bonds. The E731→G mutation removes a negatively charged side chain and replaces it with glycine's exceptional flexibility, likely increasing local backbone mobility but at the cost of electrostatic interactions. On the other hand, the V804→L substitution is relatively conservative because both amino acid residues are hydrophobic. However, the slightly larger leucine side chain could alter packing within the hydrophobic core of its structural domain, with possible ripple effects on overall FMDV protein stability.

With regard to the amplicons B, the amplified nucleic acid sequences of 275 bp have been translated into their corresponding amino acid sequences with the FMDV polyprotein sequences. The results indicated that these sequences accounted for 91 amino acids within the full 2332-amino-acid FMDV protein structure, representing only 1% of the entire viral protein.

Based on the GenBank MN983153.1, the four nucleic acid substitutions of 119C>T, 152A>G, 170T>G, and 222G>C exerted four missense SNPs are distributed across non-structural regions that govern polyprotein processing and genome replication, namely p.2272A>V, p.2283Q>R, p.2289V>G, and p.2306E>D (Fig. 4-4b,1).

The A2272to V change introduces greater hydrophobicity, which may strengthen core packing but could also affect local flexibility of the viral protein. Q2283 to R exchanges an uncharged polar side chain for a positively charged guanidinium group, potentially forming new salt bridges or disrupting existing hydrogen bonds, with consequences for enzymatic activity.

The recurrent V2289 to G mutation occurs in multiple isolates and, by replacing a branched aliphatic side chain with glycine, likely increases backbone freedom in a motif that could be critical for protease dimerization or substrate recognition. Conversion of glutamate to aspartate at position 2306 (E2306 to D) preserves negative charge but shortens the side chain, which might fine-tune interaction distances in the catalytic center of the FMDV protein.

Based on the GenBank OK422489.1, both nucleic acid substitutions of 137G>C and 189C>T exerted two respective missense SNPs of p.2250R>P and p.2267H>L (Fig. 4-4b, 2). The amino acid substitutions p.2250R>P and p.2267H>L in the FMDV polyprotein likely result in structural and functional alterations of this viral protein. The replacement of the positively charged, flexible arginine residue with proline at position 2250 introduces a rigid kink in the polypeptide chain, which can disrupt local secondary structures or hinder proper folding. Similarly, the amino acid substitution of a histidine residue with a leucine residue at position 2267 replaces a polar residue with a nonpolar, hydrophobic one, which may affect protein stability or interactions with the receptors.

Based on the GenBank MT340219.1, both nucleic acid substitutions of 57C>T and 225T>C exerted two respective silent SNPs of p.2251H= and p.2307P= (Fig. 4-4b, 3).

Whereas the 170T>G SNP exerted a missense effect of p.2289V>G in the viral protein. The p.2289V>G substitution in the FMDV polyprotein replaces valine, a hydrophobic amino acid with a branched side chain, with glycine, the smallest and most flexible amino acid. This change can increase local flexibility and potentially destabilize secondary structures. Based on the GenBank HQ832585.1, both nucleic acid substitutions of 189C>T and 213T>C exerted two respective silent SNPs of p.2296H= and p.2304R= (Fig. 4-4b, 4). Whereas the 100G>C SNP exerted a missense effect of p.2267A>P in the FMDV protein.

The amino acid substitution of p.2267A>P in the FMDV polyprotein replaces alanine, a small, nonpolar amino acid that typically supports flexible, compact structures, with proline, which introduces rigidity due to its cyclic structure. Proline often disrupts alpha-helices and beta-sheets because it lacks the backbone hydrogen necessary for stabilizing these structures. Based on the GenBank PQ153286.1, the nucleic acid substitution of 222A>G exerted a silent effect of p.2306E= on the entire structure of the FMDV polyprotein (Fig. 4-4b, 5). Based on the GenBank entry KC440883.1, the nucleotide substitutions 127T>C and 170T>G each result in distinct missense mutations within the FMDV polyprotein, specifically p.S2275P and p.V2289G, respectively (Fig. 4-4b, 6).

Both of these missense SNPs have the potential to alter the overall structure and function of the polyprotein. The S2275P substitution in the FMDV polyprotein replaces serine, a small polar residue capable of forming hydrogen bonds, with proline, a rigid, nonpolar residue that can disrupt local secondary structures due to its constrained conformation.

Whereas the p.V2289G substitution replaces valine with glycine, removes a bulky hydrophobic side chain, and introduces increased backbone flexibility.

Based on the GenBank entry AY593798.1, both nucleotide substitutions 199G>A and 202G>A each result in distinct missense mutations within the FMDV polyprotein, p.2296D>N and p.2297E>K, respectively (Fig. 4-4b, 7).

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The p.2296D>N substitution replaces aspartic acid, a negatively charged, acidic residue, with asparagine, a polar but uncharged residue.

This change eliminates a negative charge, potentially disrupting ionic interactions or salt bridges that stabilize the protein's structure or mediate interactions with other molecules. Whereas the p.2297E>K substitution replaces glutamic acid, also negatively charged, with lysine, a positively charged residue. This represents a charge reversal at that position, which can significantly affect electrostatic interactions, potentially altering local structure, disrupting functional motifs of the viral protein. Based on the GenBank entry MG983733.1, the nucleotide substitution 183T>A exerted a silent effect within the FMDV polyprotein, p.2293A= (Fig. 4-4b, 8). Whereas another nucleic acid substitution, p.222G>C, caused a missense effect of p.2306E>D. The p.2306E>D substitution in the FMDV polyprotein involves the replacement of glutamic acid with aspartic acid, both of which are negatively charged, acidic residues.

While this is a conservative substitution, the shorter side chain of aspartic acid may slightly alter the local geometry or the ability to form salt bridges or coordinate metal ions. Based on the GenBank entry AY593752.1, the nucleotide substitutions 162G>T exerted a missense effect of p.2287L>F within the FMDV polyprotein (Fig. 4-4b, 9).

Such amino acid substitution in the FMDV polyprotein replaces leucine, a nonpolar, aliphatic amino acid, with phenylalanine, a bulkier nonpolar residue containing an aromatic ring. Although both are hydrophobic, phenylalanine's larger side chain and aromatic structure may influence packing within the protein core or alter local hydrophobic interactions. Based on the GenBank OK318523.1, the nucleic acid substitutions of 192T>G exerted a silent effect of p.2296S= (Fig. 4-4b, 10).

Whereas the 185T>G SNP exerted a missense effect of p.2294V>A in the FMDV protein. The p.2294V>A substitution in the FMDV polyprotein replaces valine, a medium-sized, branched hydrophobic amino acid, with alanine, a smaller and less bulky nonpolar residue. Based on the GenBank KJ144929.1, the nucleic

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acid substitutions of 180T>G exerted a silent effect of p.1357L= within the structural component FMDV protein (Fig. 4-4b, 11).

With regard to the amplicons C, the amplified nucleic acid sequences of 1301 bp have been translated into their corresponding amino acid sequences with the FMDV polyprotein sequences.

The translation results indicated that these sequences accounted for 434 amino acids within the full 2332-amino-acid FMDV protein structure, representing about 19% of the whole viral protein. Based on the GenBank PQ153287.1, five nucleic acid substitutions of 105G>A, 138G>A, 156T>C, 273G>C, and 453G>A exerted five silent SNPs of p.Q580=, p.S591=, p.S597=, p.P636=, and p.G696=, respectively. Whereas the other two nucleic acid substitutions of 722T>G and 751T>A SNPs exerted two missense SNPs of p.786V>G and p.796Y>N, respectively (Fig. 4-4c, 1).

The p.786V>G substitution in the FMDV polyprotein replaces valine, a hydrophobic, branched-chain amino acid, with glycine, the smallest and most flexible amino acid residue. This substitution reduces hydrophobicity and introduces greater conformational flexibility, which can disrupt local secondary structures of the viral protein. Meanwhile, the p.796Y>N substitution replaces tyrosine, a bulky aromatic amino acid with a polar hydroxyl group, with asparagine, a smaller polar residue lacking aromatic character.

This change can affect hydrogen-bonding patterns and eliminate potential π -stacking or aromatic interactions. Based on the GenBank OP189368.1, three nucleic acid substitutions of 375T>C, 399C>T, and 514C>T exerted three silent SNPs of p.125D=, p.133T=, p.172L=, respectively.

Whereas the nucleic acid substitutions of 990G>T exerted one missense of p.330L>F (Fig. 4-4c, 2). The p.330L>F amino acid substitution replaces the nonpolar and aliphatic leucine residue with the larger hydrophobic phenylalanine residue with an aromatic ring.

While both residues are hydrophobic, the larger size and aromatic nature of phenylalanine could lead to changes in local packing, potentially affecting the stability of nearby secondary structures of the viral FMDV polyprotein. Based on the GenBank OP957418.1, all four nucleic acid substitutions of 328t>c, 399c>t, 594t>a, AND 672A>G exerted four silent SNPs of p.655L=, p.678T=, p.743G=, and p.769k=, respectively (Fig. 4-4c, 4). Although the many silent SNPs in this fragment do not alter the polypeptide sequence, they could potentially influence co-translational folding kinetics or RNA–protein interactions of the nascent polypeptide (Chamary *et al.*, 2006).

To summarize all the identified SNPs in the current study, the details of each detected variant are listed in Table (4-2). In this table, the position of the identified 52 SNPs in the PCR products, amino acid sequences, and their effect on the FMDV polyprotein were annotated, and their effects were also described. These SNPs were distributed across the investigated samples into three categories. The first one is represented by 14 SNPs, which were detected in fragment A. The first set of SNPs is represented by 23 SNPs, which were detected in the fragment B. While the third set of SNPs is represented by 15 SNPs in the amplified fragment C. The list of the identified SNPs in the partially amplify the polyprotein gene within the FMDV genomic sequences that are made of twelve samples of 866 bp (A1 – A12), 79 samples of 275 bp (B1 – B79), and 20 samples of 1301 bp (C1 – C20) in branches A, B, and C, respectively. The symbols “>” and “=” referred respectively to the “missense” and “silent” effect of the detected SNP. As showed in table(4-2).

Table (4-2): The list of the identified SNPs in the partially amplify the polyprotein gene within the FMDV genomic sequences:

Frag- ment	No. of SNP	position in the PCR amplicon	SNP	Sample no.	Position of the amino acid residue	SNP type	Nomenclature	GenBank
	1	771	T>C	1,2	D945	Silent	p.945D=	HQ113233.1
	2	774	T>C	1,2	V946	Silent	p.946V=	HQ113233.1

A	3	524	G>C	3	R869	Missense	p.869R>P	MT602090.1
	4	339	A>G	4,5,6,7,11,12	A803	Silent	p.803A=	OP957418.1
	5	348	T>C	4,5,6,7,11,12	H806	Missense	p.806H>Q	OP957418.1
	6	408	C>T	4,5,11,12	T826	Silent	p.826=	OP957418.1
	7	435	G>A	4,5,11,12	P835	Silent	p.835P=	OP957418.1
	8	523	G>A	4,5,11,12	A865	Missense	p.865A>T	OP957418.1
	9	681	C>T	4,5,6,7,11,12	A917	Silent	p.917A=	OP957418.1
	10	783	C>T	4,5,11,12	N951	Silent	p.951N=	OP957418.1
	11	109	A>G	8,9,10	T728	Missense	p.728T>A	OM455481.1
	12	119	A>G	8,9,10	E731	Missense	p.731E>G	OM455481.1
	13	337	G>T	8,9,10	V804	Missense	p.804V>L	OM455481.1
	14	673	C>T	8,9,10	L916	Silent	p.916L=	OM455481.1
B	15	119	C>T	1,3,4,71	A2272	Missense	p.2272A>V	MN983153.1
	16	152	A>G	1,7,71,75	Q2283	Missense	p.2283Q>R	MN983153.1
	17	170	T>G	1,7,13,71,75	V2289	Missense	p.2289V>G	MN983153.1
	18	222	G>C	3,4,6,7,13	E2306	Missense	p.2306E>D	MN983153.1
	19	137	G>C	2,53	R2250	Missense	p.2250R>P	OK422489.1
	20	189	C>T	2,53	H2267	Missense	p.2267H>L	OK422489.1
	21	57	C>T	5,8,10,12,14,17,19,36,37,38,54, 55,63,64,66,68,69,72	H2251	Silent	p.2251H=	MT340219.1
	22	170	T>G	5,8,10,12,14,15,17,19,36,37,38, 54,55,63-70,72	V2289	Missense	p.2289V>G	MT340219.1
	23	225	T>C	5,8,10,12,14,15,17,19,34,36,45, 54,55,63-69,78	P2307	Silent	p.2307P=	MT340219.1
	24	100	G>C	20,22,25,27,28,33,39,41-43	A2267	Missense	p.2267A>P	HQ832585.1
	25	189	C>T	9,20,21,22,24,25-27,29-31,33, 39-43,58,61,62	H2296	Silent	p.2296H=	HQ832585.1
	26	213	T>C	9,21,22,24-27,29,31,39,43,61,62	R2304	Silent	p.2304R=	HQ832585.1
	27	222	A>G	11,16,23,28,47,48,56,57,59,79	E2306	Silent	p.2306E=	PQ153286.1
	28	127	T>C	31,32,60	S2275	Missense	p.2275S>P	KC440883.1
	29	170	T>G	31,32,60	V2289	Missense	p.2289V>G	KC440883.1
	30	199	G>A	35	D2296	Missense	p.2296D>N	AY593798.1
	31	202	G>A	35	E2297	Missense	p.2297E>K	AY593798.1
	32	183	T>A	44	A2293	Silent	p.2293A=	MG983733.1
	33	222	G>C	44	E2306	Missense	p.2306E>D	MG983733.1
	34	162	G>T	46	L2287	Missense	p.2287L>F	AY593752.1
	35	185	T>C	74	V2294	Missense	p.2294V>A	OK318523.1
	36	192	T>G	74	S2296	Silent	p.2296S=	OK318523.1
	37	180	T>C	76,77	L1357	Silent	p.1357L=	KJ144929.1
C	38	105	G>A	1,5,6,9,11,15-17,20	Q580	Silent	p.Q580=	PQ153287.1
	39	138	G>A	1,5,6,9,11,15-17,20	S591	Silent	p.S591=	PQ153287.1
	40	156	T>C	1,5,6,9,11,15-17,20	S597	Silent	p.S597=	PQ153287.1
	41	273	G>C	1,5,6,11,15-17,20	P636	Silent	p.P636=	PQ153287.1
	42	453	G>A	1,5,6,11	G696	Silent	p.G696=	PQ153287.1
	43	722	T>G	1,5,6,11,16,17,20	V786	Missense	p.786V>G	PQ153287.1
	44	751	T>A	1,5,6,9,11,15-17,20	Y796	Missense	p.796Y>N	PQ153287.1
	45	375	T>C	2,3,4,13,14	D125	Silent	p.125D=	OP189368.1
	46	399	C>T	2,3,4,13,14	T133	Silent	p.133T=	OP189368.1
	47	514	C>T	2,3,4,13,14	L172	Silent	p.172L=	OP189368.1
	48	990	G>T	2,3,4,13,14	L330	Missense	p.330L>F	OP189368.1

49	328	T>C	7,8,10,19	L655	Silent	p.655L=	OP957418.1
50	399	C>T	7,8,10,12,18,19	T678	Silent	p.678T=	OP957418.1
51	594	T>A	7,8,10,12,18,19	G743	Silent	p.743G=	OP957418.1
52	672	A>G	7,8,10,12,18	K769	Silent	p.769k=	OP957418.1

The observed variations were respectively deposited in the NCBI database to get a unique accession number in the NCBI database for all investigated samples. For the A1 – A12, the accession numbers PV630875- PV630886 were received. For the B1 – B79 samples, the accession numbers PV630887 – PV630965 were received. Whereas for the C1 – C20 samples, the accession numbers PV630966 - PV630985 were received. To give a phylogenetic understanding of the actual distances between our investigated sample and the other related species, a comprehensive phylogenetic tree was generated in the present study according to nucleic acid variations observed in the amplified polyprotein amplicons.

This phylogenetic tree contained the A1 – A12 samples alongside other related nucleic acid sequences of FMDV sequences. Furthermore, several outgroup species from other related species were also incorporated within the same tree to assess the actual phylogenetic distances between the observed serotypes and the closest related serotypes within the viral sequences.

The total number of aligned nucleic acid sequences in this tree was sixty. The evolutionary distances were computed using the Neighbour-joining method and are in the units of the number of base substitutions per site.

The tree was constructed based on an alignment comprising 1049 nucleotide sites. Among these, 142 sites were conserved, which indicates that these positions are identical across all sequences. The number of conserved sites suggests regions of evolutionary stability, possibly linked to essential functions in the viral genome. The remaining 759 sites are variable, indicating the level of differences across the sequences.

This variability contributes to the genetic diversity observed among the sequences. Of the variable sites, 550 were parsimony-informative. The number of parsimony-informative sites is critical for constructing the phylogenetic tree, as they provide an assessment of evolutionary relationship patterns among the incorporated species. Only 201 were singleton sites, representing unique variations since this number does not support shared evolutionary relationships among multiple sequences.

The high number of parsimony-informative sites compared to singleton sites indicates that the sequences have a robust level of shared evolutionary information, making the phylogenetic tree reliable.

The phylogenetic tree reconstructed by the Neighbor-Joining method reveals a clear partitioning of FMDV isolates into the seven recognized serotypes of O, A, Asia 1, C, SAT 1, SAT 2 and SAT 3, each one demarcated by distinct clades and supported by bootstrap values that range from moderate (0.63) to maximal confidence (1.00). At the base of the tree, the scale bar (0.1 substitutions per site) indicates that the longest branch lengths correspond to approximately ten percent sequence divergence, underscoring the deep evolutionary split among serotypes. The serotype O clade, comprising a diverse array of isolates from Turkey (TUR/1003/2010), Bulgaria (BUL/HS018-1/2011), and multiple Pakistani field samples (e.g., O/PAK/KCH/38/2011 through O/PAK/KCH/39/2011, PAK/4/2017, and PAK C8/2018), forms a well-supported monophyletic group with bootstrap support consistently above 0.88.

This suggests both geographic spread and relative genetic cohesion among these O-type viruses. Within this clade, our investigated samples of A4 – A7 and A11 and A12 were positioned in the immediate vicinity of O isolate BAN/MY/My-466/2021 that was also isolated for the Bangladeshi cattle (GenBank OP957418.1).

Accordingly, the Bangladeshi origin is a potential origin from which our A4 – A7 and A11 and A12 samples might have originated. On the other hand, the

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serotype A clade and the Asia 1 clade lie on neighboring branches, highlighting their closer relationship to one another than to serotype O.

The serotype A branch has been shown to be mainly anchored by various Pakistani sequences (A/PAK/ICT/276/2012, A/PAK/ICT/168-2/2012, A/BAD/AFG/L-2813/2009). As well, it was also extended to incorporate isolates from Turkey (A/TUR/11/2013) and Iraq (type A isolate 4258). Within these details, A8 – A10 are positioned in the vicinity of one Pakistani strain (A isolate A/PAK/ICT/276/2012). In parallel, the Asia 1 isolates, including various reference strains, including Asia1/HKN/5/2005, BAN/DH/Sa-318/2018, and IND 63/72, alongside the two investigated field samples (A1 and A2), aggregate with bootstrap values at or above 0.75.

On the other hand, serotype C, which is represented by the isolate CGC GER/26c, has resided on a comparatively long branch supported by a moderate bootstrap value (~ 0.63). This sort of positioning captures its relative rarity and slower evolutionary rate in contrast to the other serotypes.

The terminal region of the tree is dominated by the African Territories types: SAT 2 sequences that are deposited from Ethiopia (ETH/16/2015) and Egypt (EGY/1/2018), and one strain deposited from Iraq (SAT2/IRQ/3/2023) in a tightly clustered position (bootstrap ≈ 0.88). Though our studied A3 sample has been positioned in the vicinity of the Ethiopian strain, all SAT 2 serotype strains, including the cataloged Iraqi one were originated from the same phylogenetic ancestor. Whereas the clade of SAT 1 that is represented by the isolate NIG/1/2015 was found to occupy a separate but related branch (bootstrap ≈ 0.75). The deeply divergent SAT 3 serotype that is represented by the SAT 3/ZIM/4/81 isolate extends on the longest branch of all, reflecting its historical isolation and substantial genetic distance from contemporary FMDV lineages (Fig. 4-5).

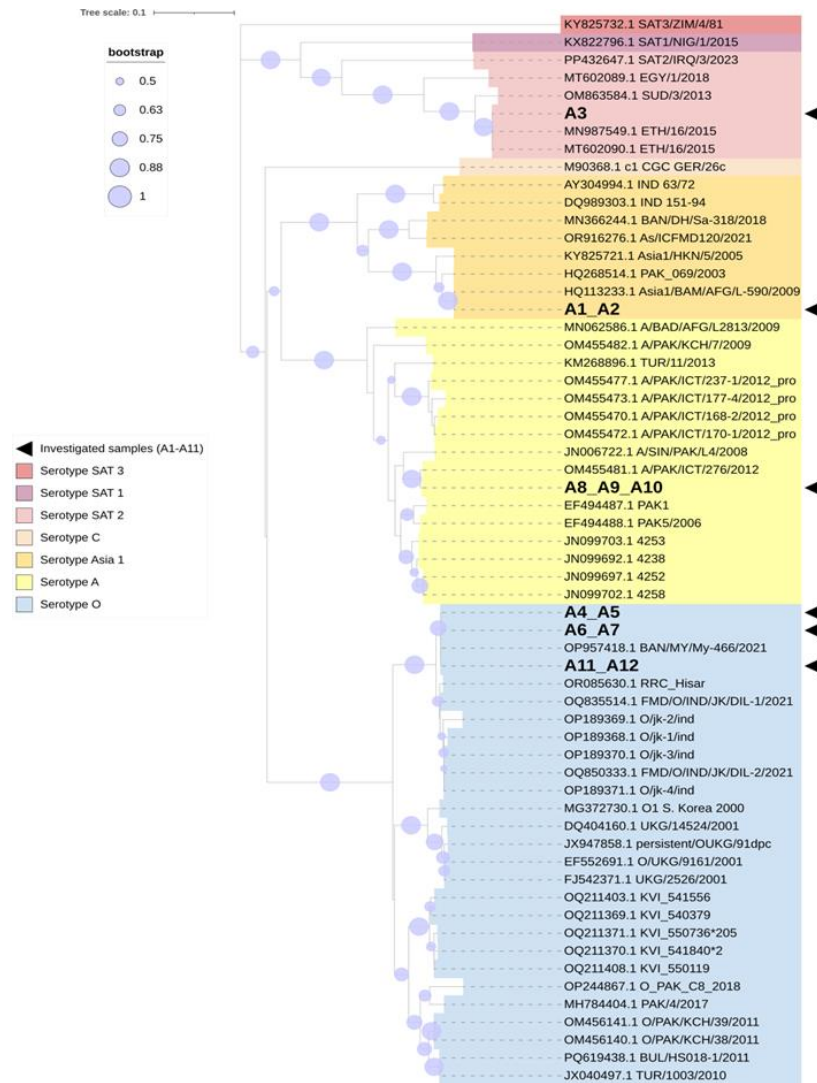


Fig. (4-5). Evolutionary relationships of the FMDV taxa using the Neighbor-Joining method. The optimal tree with the sum of branch length = 3.02958374 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 61 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7. The number “0.1” at the upper left portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. A rectangular and circular tree were shown in branches A and B, respectively.

Similar to the approach taken with fragment A amplicons, a phylogenetic tree was also constructed for the fragment B amplicons to evaluate their phylogenetic positioning relative to corresponding reference sequences. To this end, a

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comprehensive phylogenetic tree was generated to assess the evolutionary relationships and distances among the investigated samples and their closest homologous sequences.

The tree was constructed based on an alignment comprising 275 nucleotide sites. Among these, 207 sites were conserved, which indicates that these positions are identical across all sequences. The number of conserved sites suggests regions of evolutionary stability, possibly linked to essential functions in the viral genome. The remaining 68 sites are variable, indicating the level of differences across the sequences.

This variability contributes to the genetic diversity observed among the sequences. Of the variable sites, 50 were parsimony-informative. The number of parsimony-informative sites is critical for constructing the phylogenetic tree, as they provide an assessment of evolutionary relationship patterns among the incorporated species. Only 18 were singleton sites, representing unique variations since this number does not support shared evolutionary relationships among multiple sequences.

The high number of parsimony-informative sites compared to singleton sites indicates that the sequences have a robust level of shared evolutionary information, making the phylogenetic tree reliable. The resulting tree comprises 52 terminal leaves, which are organized into six principal clades (Fig. 4-6). Notably, the clades corresponding to the SAT 1, SAT 2, and SAT 3 serotypes are clearly separated from the remaining groups, suggesting a distinct divergence of the fragment B amplicons associated with these outgroup sequences from those representing other FMDV serotypes.

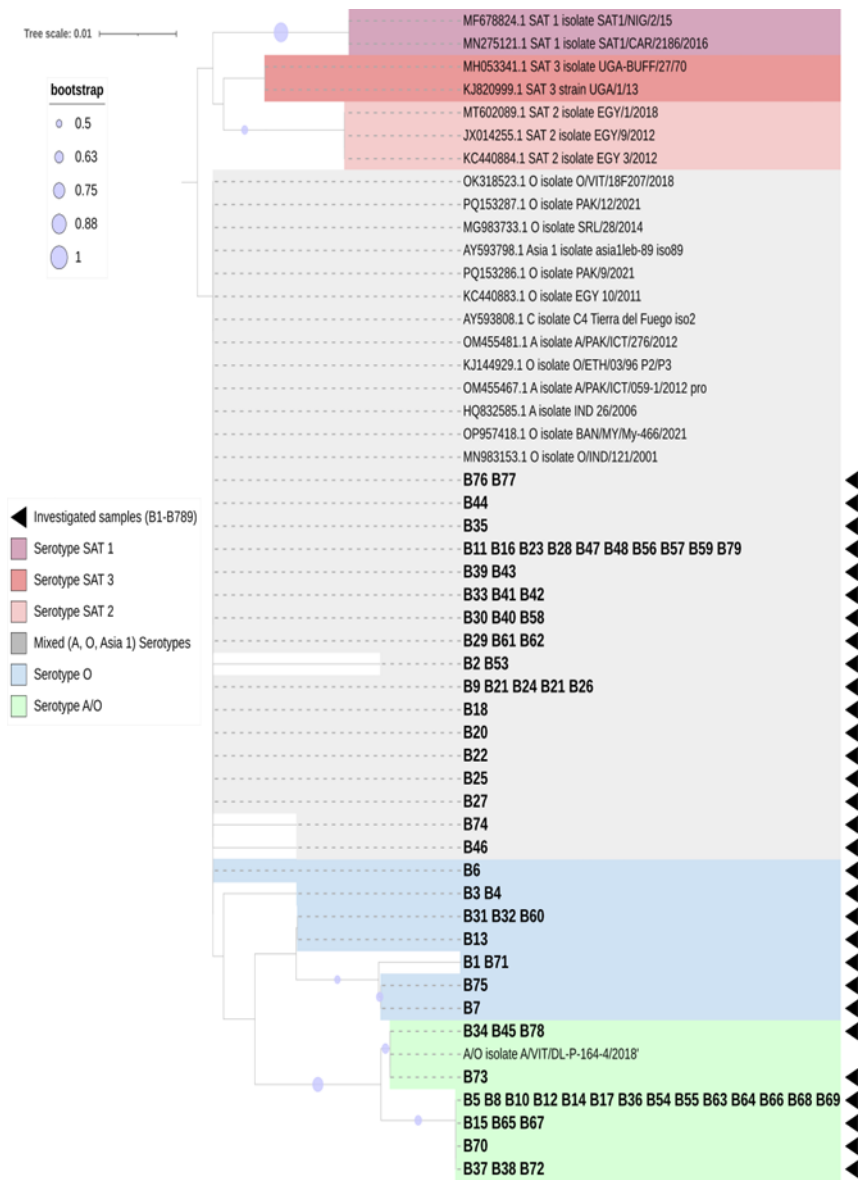
In addition to the clear phylogenetic separation of the SAT serotypes from the other clades, the A/O serotype clade was also distinctly delineated. This separation is attributed to the capacity of the amplified fragment B to discriminate effectively between the A/O serotype and other common FMDV serotypes. Within the A/O clade, 25 B fragment amplicons clustered closely around the strain

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A/O isolate A/VIT/DL-P-164-4/2018, previously reported from Vietnam (GenBank accession: MT340219.1).

Among these isolates, four samples (B73, B34, B45, and B78) exhibited a particularly high level of sequence similarity to the reference strain, suggesting greater homology compared to the remaining 21 samples in the clade. Adjacent to the A/O clade, the O serotype clade was positioned, comprising 11 B fragment amplicons with varying phylogenetic placements within the clade. However, none of these samples showed close similarity to previously cataloged O serotype sequences.

This divergence may be attributed to the high number of SNPs identified in these samples, which likely influenced their distinct phylogenetic positioning. One of the main sources of ambiguity in the current phylogenetic tree is the overlapping of three serotypes A, O, and C within a single clade. This overlap resulted in the formation of a major clade comprising 30 leaves, which reflects the limited discriminatory power of the B fragment in differentiating among these serotypes.

This limitation is primarily attributed to the short length of the B amplicons (275 bp), in contrast to the longer A (866 bp) and C (1301 bp) amplicons used in earlier analyses. The reduced sequence length of the B amplicons diminishes their resolution, making them inadequate for clearly distinguishing between serotypes A, O, and C without phylogenetic overlap. The widespread distribution of isolates from multiple serotypes within the same phylogenetic branch further emphasizes the shortcomings of the current B fragment-based tree in accurately resolving serotype identity. Consequently, the 275 bp B amplicons demonstrated substantially lower discriminatory capacity compared to the 866 bp A amplicons employed previously.



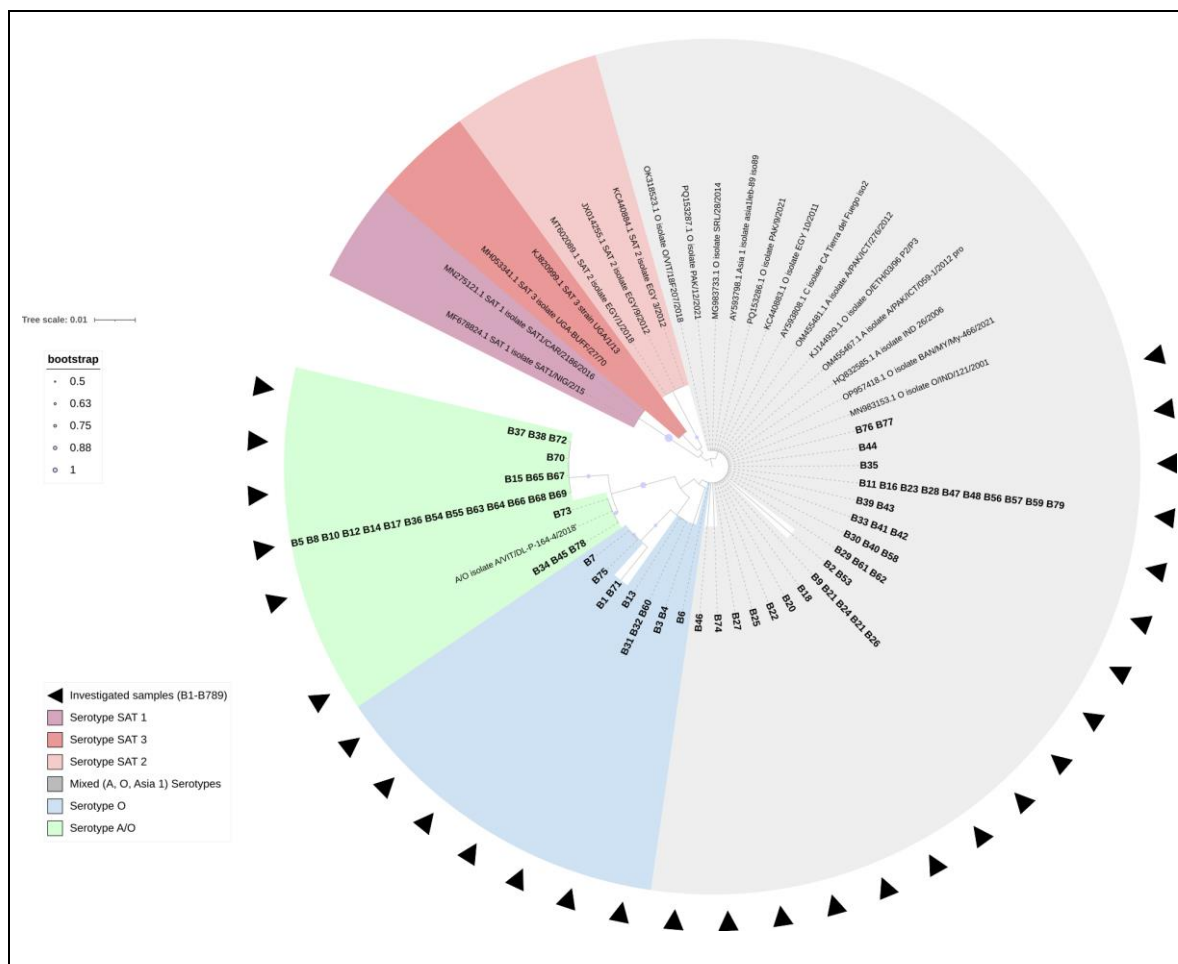


Fig (4-6). Evolutionary relationships of the FMDV taxa using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.15760870 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 54 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7. The number “0.01” at the upper left portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. A rectangular and circular tree were shown in branches A and B, respectively.

The phylogenetic tree constructed from the C-based fragment comprises a total of 35 terminal leaves. These leaves represent all seven major serotypes of the Foot-and-Mouth Disease Virus (FMDV).

The tree was constructed based on an alignment comprising 1345 nucleotide sites. Among these, 499 sites were conserved, which indicates that these positions

Chaptar Four..... Result are identical across all sequences. The number of conserved sites suggests regions of evolutionary stability, possibly linked to essential functions in the viral genome. The remaining 836 sites are variable, indicating the level of differences across the sequences.

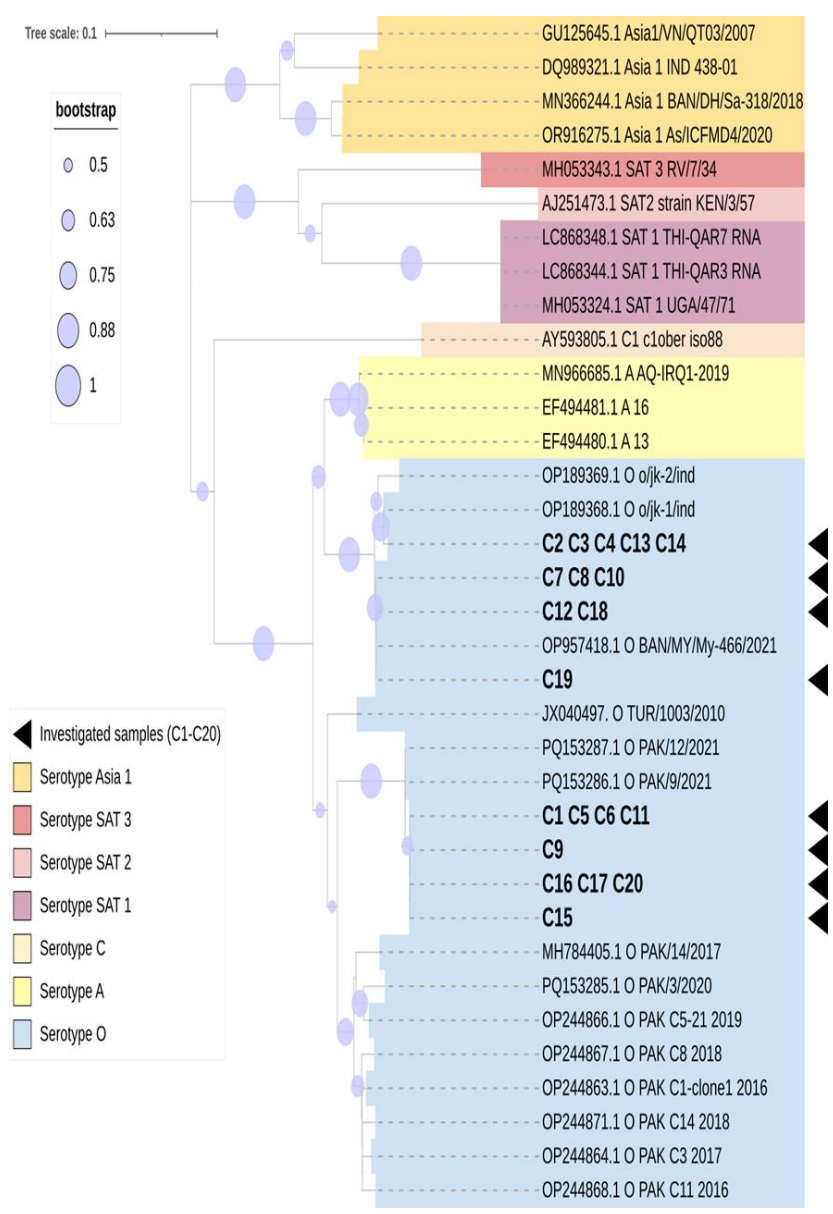
This variability contributes to the genetic diversity observed among the sequences. Of the variable sites, 695 were parsimony-informative. The number of parsimony-informative sites is critical for constructing the phylogenetic tree, as they provide an assessment of evolutionary relationship patterns among the incorporated species. Only 140 were singleton sites, representing unique variations since this number does not support shared evolutionary relationships among multiple sequences.

The high number of parsimony-informative sites compared to singleton sites indicates that the sequences have a robust level of shared evolutionary information, making the phylogenetic tree reliable. Notably, all analyzed C amplicon samples were clustered within the serotype O clade, while the remaining serotypes were positioned as outgroups relative to this clade.

The distinct phylogenetic separation among the serotypes highlights the strong discriminatory capability of the C fragment. This is evidenced by the clear and non-overlapping placement of each serotype-specific clade within the tree, demonstrating the ability of the C amplicons to effectively differentiate between FMDV serotypes. Clear phylogenetic out grouping of the Asia 1, SAT 1, SAT 2, SAT 3, and serotype C clades was observed in comparison to serotypes A and O. The C amplicon-based phylogenetic tree revealed that the Asia 1 clade is distinctly separated from the neighboring SAT clades, suggesting a divergent evolutionary origin and potentially a different ancestral lineage. Adjacent to the Asia 1 clade, the SAT 1, SAT 2, and SAT 3 serotypes were grouped under a single common ancestor, reflecting the C fragment's capacity to accurately discriminate among closely related clades. In contrast, the C, A, and O serotypes were found to share a different common ancestor, separate from the Asia 1 and SAT

Chaptar Four..... Result groupings, further supporting the discriminatory potential of the C fragment. Notably, the serotype most closely related to serotype O was identified as serotype A, represented by three isolates: strain A/AQ-IRQ1-2019, isolated from a bovine nasal swab in Iraq (GenBank: MN966685.1), and isolates A/16 and A/13, both obtained from Denmark (GenBank: EF494480.1 and EF494481.1, respectively). Within the serotype O clade, the investigated samples were distributed into three distinct phylogenetic groupings. The reason for this sort of distinct distribution is attributed to the differences observed among these three groups in their polymorphism intensity and distributions.

The first cluster comprised five samples (C2, C3, C4, C13, and C14) closely aligned with two Indian strains (O o/jk-1/ind and O o/jk-2/ind), both isolated from cattle in India (GenBank: OP189368 and OP189369). The second cluster included six samples (C7, C8, C10, C12, C18, and C19), which grouped with a Bangladeshi isolate (O isolate BAN/MY/My-466/2021; GenBank: OP957418.1), also originating from cattle. These two clusters shared a common ancestral lineage within the serotype O clade. In contrast, the third group consisted of nine samples (C1, C5, C6, C11, C9, C16, C17, C20, and C15) and two Pakistani isolates (O isolate PAK/9/2021 and O isolate PAK/12/2021), which together formed a separate subclade, indicating a distinct evolutionary origin from the other two groupings. Due to these sorts of grouping, the Asian origins of the investigated C amplicons are revealed.



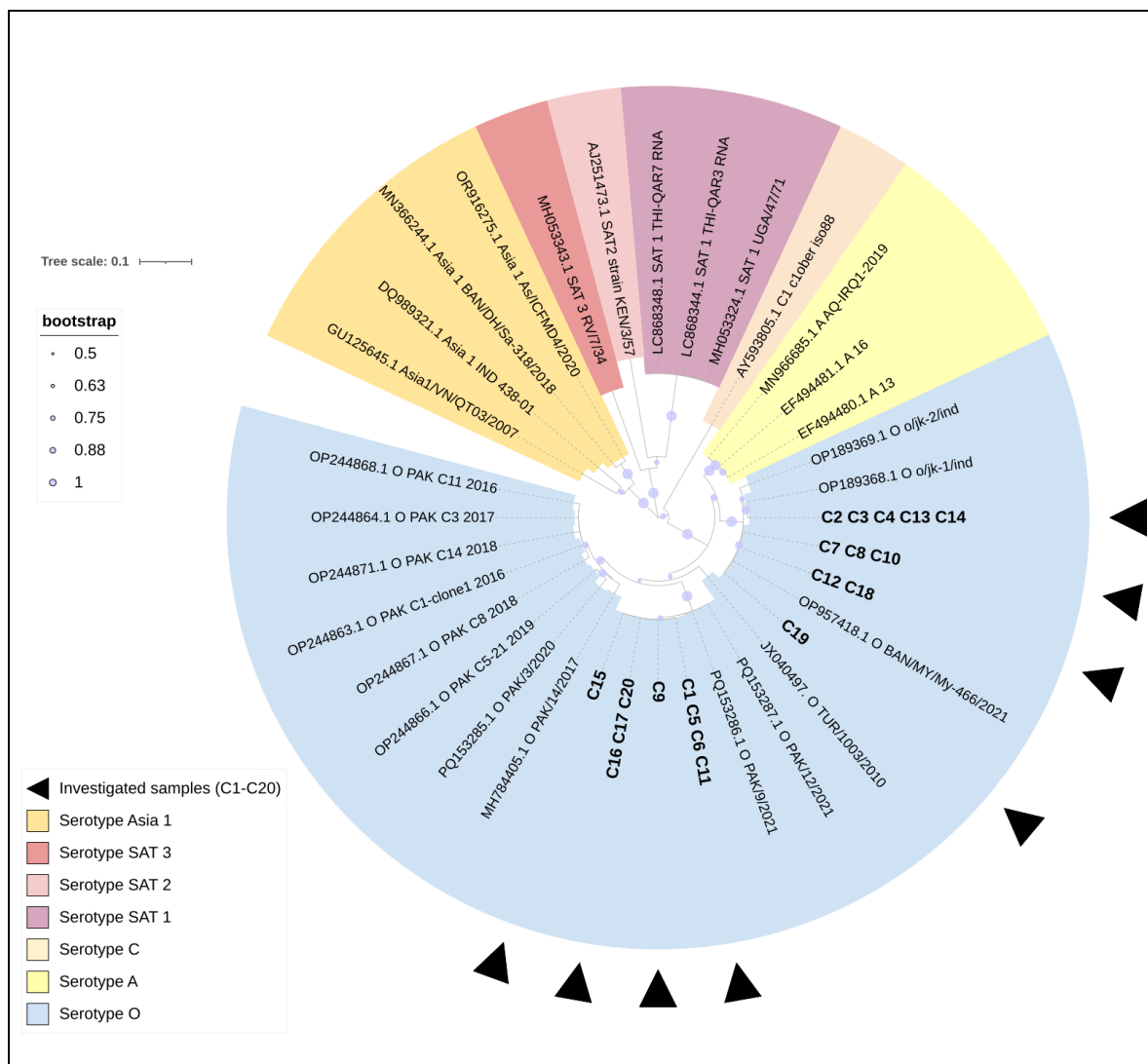
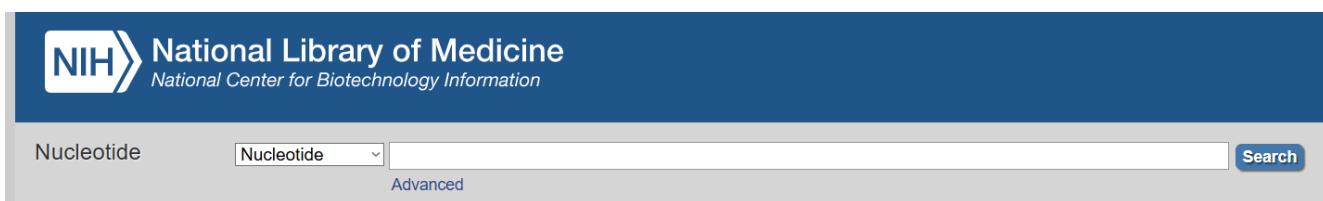


Fig. (4-7). Evolutionary relationships of the FMDV taxa using the Neighbor-Joining method. The optimal tree with the sum of branch length = 1.59571002 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 36 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7. The number “0.1” at the upper left portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. A rectangular and circular tree were shown in branches A and B, respectively.

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These findings demonstrate the high discriminatory capability of the C amplicons in differentiating among geographically diverse serotype O strains. When considered alongside the performance of the A amplicons, both fragments exhibited strong potential for serotype-level discrimination among commonly circulating FMDV strains. This level of resolution was not achieved with the B amplicons. Therefore, the A and C amplicons appear to be more reliable candidates for diagnostic marker development in FMDV molecular detection and phylogenetic analysis compared to the B fragment.

Table (4-3): Accessions number of FMDV that were submitted in Gene Bank of NCBI; PV630875:



Foot-and-Mouth Disease Virus Asia 1 Isolate Zainab- A1 Polyprotein Gene, Partial cds

LOCUS Seq1 866 bp DNA linear VRL 06-MAY-2025

DEFINITION Foot-and-mouth disease virus strain Zainab -A1.

ACCESSION Seq1

VERSION

KEYWORDS

SOURCE Foot-and-mouth disease virus

ORGANISM Foot-and-mouth disease virus

Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
Picornavirales; Picornaviridae; Caphthovirinae; Aphthovirus.

REFERENCE 1 (bases 1 to 866)

AUTHORS Alabudi, Z.A.A., Jar Allah, B.A. and Felaih, A.J.

TITLE Direct Submission

JOURNAL Submitted (06-MAY-2025) Department of Microbiology, College of
Veterinary Medicine, Al Shatrah University, Al Shatrah,
Al-Nasiriya, Al-Nasiriya 64001, Iraq

COMMENT Bankit Comment: TOTAL # OF SEQS:111

##Assembly-Data-START##

Sequencing Technology: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

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  361 acctgggtcc cgaacggggc gcccgaaaaa gccttgaca acaccacaa tccaacggct
  421 taccacaagg caccgtcac ccgacttga ctgccgtaca cggcaccca ccgtgtcatg
  481 gctactgttt acaacgggaa ctgcaagtac ggcgagagcc acgcaactag cgtgagaggt
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4.3. Epidemiological Study of FMD

4.3.1. Clinical Signs of FMD in Infected Calves

The percentage of clinical symptoms seen in calves with FMD is shown in the table. To ascertain the significance of the observed variations in clinical symptoms among the infected calves, the Chi-square (X^2) test was used to examine the data. Total infected calves were 150 and most common clinical signs were fever (88.66 %), salivation (81.33 %), oral lesions (65.33 %), interdigital lesions (52 %), lameness (46 %) and nostril lesions (24 %).

Fever and salivation were the most dominant symptoms affecting > 80 % of calves. Oral and interdigital lesions were also prevalent (> 50 %), consistent with classic FMD symptoms. Nostril lesions were the least common (24 %), suggesting they were not a primary indicator. P-value= 0.000 , the Minuscule P-value (less than 0.05) signifies that the observed variations in clinical symptoms are statistically significant. This shows that the distribution of clinical symptoms is linked to FMD infection rather than being the result of chance.

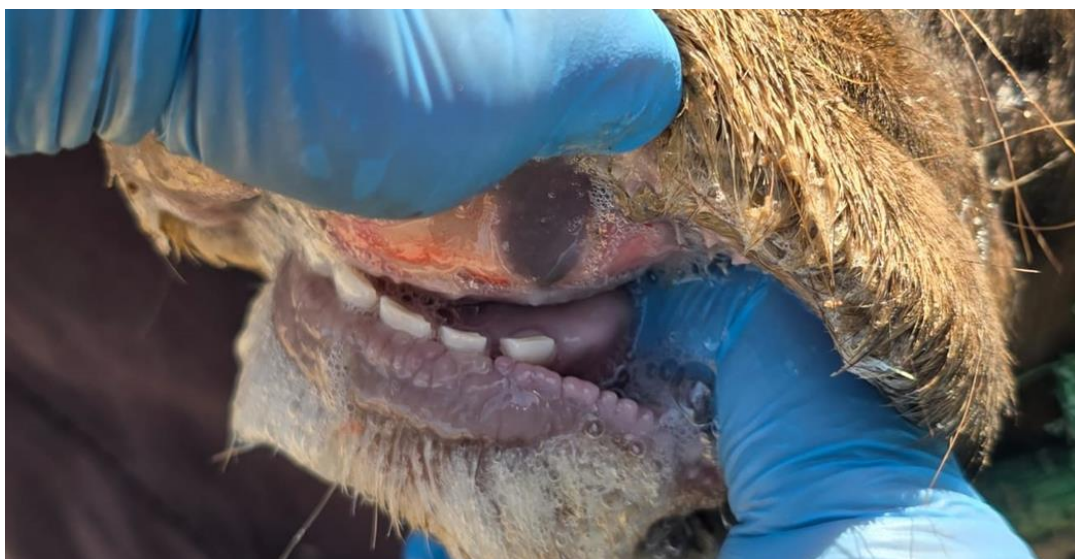


Figure (4-8):Ulceration in the mouth.

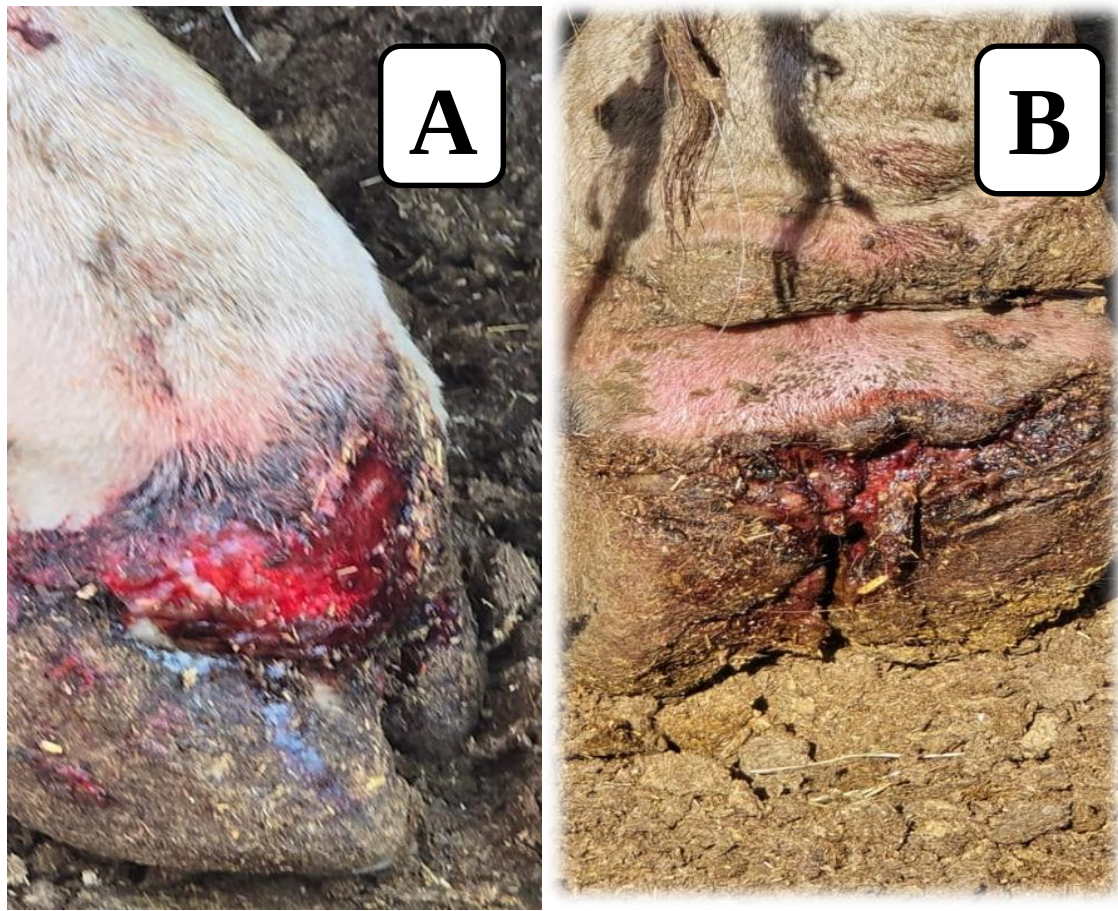


Figure (4-9): Interdigital Lesions (A & B).

Table (4-4): Percentage of Clinical Signs Result of FMD in infected Calves:

Clinical Signs	Observed Signs No.	%	No Signs No.	%	Total
Fever	133	88.66%	17	11.33%	150
Salivation	122	81.33%	28	18.66%	150
Nostril Lesion	36	24 %	114	76 %	150
Oral Lesion (vesicles- erosions)	98	65 %	52	34.66%	150
Interdigital Lesion	78	52 %	72	48 %	150
Lameness	69	46 %	81	54 %	150
$\chi^2= 117.218$	Df=5	P= 0.00			

4.3.2. The Association Between FMD Incidence and Sex

The FMD infection rates in calves by sex are shown in table (4-5). Males were 86 cases (57.33 % of total) while Females were 64 cases (42.67 %of total). Serotype O was the most prevalent in males (51.16 % of male cases while 40.62 % in females). Serotype A&O was more frequent in females (34.37 % while 18.6 % in males.SAT2 was only detected in males (2.33 % of male cases).

Chi-square Test Results: Chi-square (χ^2) was 6.48, Degrees of Freedom (DF) was 4, P-value was 0.165. The P-value ($0.165 > 0.05$) indicating no statistically significant association between sex and FMD serotypes distribution. Despite observed differences (e.g. higher Serotype O in males). Sex does not significantly influence FMD serotype infection rates in this dataset.

Table (4-5): FMD infections rate with serotypes according to sex.

Sex	Serotype O	Serotype A	Serotype A & O	SAT2	Asia1	Total
Male	44	21	16	2	3	86
%	51.16	24.41	18.6	23.43	3.48	100
Female	26	15	22	0	1	64
%	40.62	23.43	34.37	0	1.56	100
Total	70	36	38	2	4	150
$\chi^2 = 6.48, Df=4, P=0.165$						

4.3.3. Association Between FMD Infection Rates and Age

Foot and mouth disease infection rates in calves by age are shown in table (4-6). Total infections were 150 calves (sum of all serotypes). Serotype O was 70 cases (most prevalent, 46.7% of total). Serotype A & O (mixed) was 38 cases (25.3 %). Serotype A was 36 cases (24 %). Rare Serotypes were SAT2 (2 cases, 1.3 %) and Asia1 (4 cases, 2.7 %). In the 4-6 Months age (Youngest Calves) were dominated by Serotype O (41.4 %) and Serotype A (50 %). No cases of SAT2 or Asia 1. In the 7-11 months age were Serotype O (37.1 %) remains dominant,

Chaptar Four..... Result
 Serotype A & O (mixed infections) increase to 42.1 %. First appearance of Asia1 (1 case, 25 % of Asia1 total). In the 12-18 months age (Oldest Calves) were Serotype O (21.4 %) still prevalent but less dominant. Rare serotypes emerge were SAT2 (100 % of SAT2 cases) and Asia1 (75% of Asia1 cases).

The P-value (0.0485) was statistically Significant ($p \leq 0.05$), indicating that the distribution of FMD serotypes varies significantly by age group. Serotype A & O were more common in younger calves (4-11 months). Rare serotype (SAT2, Asia1) were exclusive to older calves (12-18 months). Mixed infection (A&O) peak in 7-11 months olds. Small sample sizes for SAT2 (2) and Asia1 (4) limit generalizability.

Table (4-6): FMD infection with serotypes according to age:

Age	Sero. A	%	Sero. O	%	Sero. A & O	%	Sero. SAT2	%	Sero. Asia 1	%
4-6 m.	18	50	29	41.42	12	31.57	0	0	0	0
7-11m.	12	33.3	26	37.14	16	42.10	0	0	1	25
12-18m.	6	16.66	15	21.42	10	26.31	2	100	3	75
Total	36	100	70	100	38	100	2	100	4	100
$X^2=15.595$ $Df=8$ $P=0.0485$										

4.3.4. Association Between FMD Infection Rates with Different Regions of Thi-Qar Province

The table (4-7) displayed the distribution of FMD Infections across different regions of Thi-Qar province, including infection count and percentages. Infected calves were 150 cases, distributed unevenly across region ALShatrah was 26 cases (17.33), ALNasir was 21 cases (14 %), ALRifae was 20 cases (13.33%). Lowest infection rates: ALQalaa was 13 cases (8.66%) and Sooq ALShuokh was 17 cases (11.33%).

The P-value (0.06) was greater than the significance level ($P \leq 0.05$), indicating no statistically significant difference in FMD infection rates among the regions. The observed distribution of infections is likely due to random variation rather than regional differences. While ALShatrah and ALNasir hade the highest infection counts, the chi-square test confirms that geographic region does not significantly influence FMD infection rates in Thi-Qar province.

Table (4-7): Distribution of FMD infection according to residence area of Thi-Qar province:

Region	Infected calves	%
AL Nassiriya	19	12.66
AL Shatrah	26	17.33
Sooq AL Shuokh	17	11.33
AL Qalaa	13	8.66
AL Fajer	16	10.66
AL Rifaee	20	13.33
AL Garaf	18	12
AL Nasir	21	14
Total	150	100
X²	5.52	
Df	7	
P	0.06	

4.3.5. The Relationship Between FMD Incidence and The Types of Samples

The table (4-8) presented the FMD incidence across two types of samples Blood and swabs. Bloods were 78 samples (52 % of total) and swabs were 72 samples (48 % of total). Total samples were 150 samples (100%).

The P-value (0.6242) was greater than the significance level (0.05), indicating that there was no statistically significance in FMD infection rates between the two sample types (Bloods and Swabs).

Table (4-8): FMD Infection rate according to types of samples:

Type of Sample	No. Samples	%
Bloods	78	52 %
Swabs	72	48 %
Total	150	100%
X ² (0.24)	Df=1	P=0.6242

4.3.6. Association Between FMD Infection Rate and Months of The Year

According to the study's months, the distribution of calves with FMD is shown in table (4-9). Total infected calves were 150 cases, highest infection rate was observed in March (41 cases, 27.33%), foolowed by January (36 cases, 24%), while the lowest rate was in November (14 cases, 9.33%).

The P-value (0.008) was less than the significance level of 0.05, indicating that there was a statistically significant diference in the FMD infection rates among the months studied. The results suggest that the month of the year had a significant association with the FMD infection rate in calves.

Table (4-9): FMD infection rate according to months.

Month	Calves	%
November	14	9.33 %
December	30	20 %
January	36	24 %
February	29	19 %
March	41	27.33 %
X ² =13.79	Df=4	P=0.008

4.4. Antioxidant Parameters

Results of table (4-10) revealed significant decrease ($P \leq 0.05$) in levels of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in all affected groups as compared to healthy animals in control group, but SOD levels

in groups A, O serotypes showed a significant decrease when compared with SOD levels of mixed group (A&O) , This indicates the animals were seriously affected with these virulent serotypes A and O, while levels of Malondialdehyde (MDA) were highly and significant increased ($P \leq 0.05$) in all affected groups as compared with the healthy one, this indicated those animals- in affected groups- were suffered from massive oxidative stress.

Table (4-10): Antioxidant parameters in groups of calves affected with FMD-V of (A, O and (mix A & O)) serotypes compared with healthy calves. N=20

Groups	SOD (pg/ml) Mean $\pm$ SD	CAT(ng/ml) Mean $\pm$ SD	GSH (ng/L) Mean $\pm$ SD	MDA (ng/ml) Mean $\pm$ SD
A	275.33 $\pm$ 10.69 c	0.58 $\pm$ 0.60 b	39.75 $\pm$ 23.19 b	131.16 $\pm$ 30.62 a
O	276.07 $\pm$ 12.11 c	0.91 $\pm$ 0.72 b	45.48 $\pm$ 27.12 b	136.44 $\pm$ 52.39 a
A & O	332.08 $\pm$ 19.14 b	0.68 $\pm$ 0.76 b	35.17 $\pm$ 21.10 b	121.55 $\pm$ 54.83 a
Control	981.46 $\pm$ 74.06 a	2.86 $\pm$ 1.61a	261.69 $\pm$ 127.57 a	48.71 $\pm$ 19.75 b
LSD	35.46	0.91	60.85	38.15

4.5. Enzymatic Parameters:

Results of table (4-11) revealed a highly significant increase ($P \leq 0.05$) in (ALT) and (AST) levels in groups of calves that were diagnosed with serotype O of FMDV when compared with healthy animals in the control group and infected animals in other groups (serotypes A and mixed A&O). However, ALP levels in affected groups were not significantly difference from each other and those in control group. GGT were significantly increased in animals affected with serotype A as compared with other groups including control.

Table (4-11): Enzymatic parameters-as Liver Function Test- in groups of calves affected with FMDV of (A, O and mix A & O) serotypes compared with healthy calves. N=20

Groups	ALT (U/L) Mean ± SD	AST (U/L) Mean ± SD	ALP(U/L) Mean ± SD	GGT(U/L) Mean ± SD
A	24.1 ± 11.06 bc	67.1 ± 17.01b	304.11 ±82.07	257.4 ± 60.89 a
O	59.4 ± 20.72 a	233 ± 163.31 a	342.31 ±90.83	220.1 ± 59.58 ab
A & O	26.9 ± 5.04 b	101.5 ± 28.9 b	345.04±142.27	200 ± 78.41 b
Control	18 ± 1.63 c	51.5 ± 9.81 b	287.86 ± 52.33	70 ± 44.79 c
LSD	10.92	75.74	88.36	56.30

Chapter five

Discussion

Discussion

5.1. Epidemiological Study of FMD

5.1.1. Clinical Presentation

A different hierarchy of FMD symptoms in calves has been demonstrated by the data: Most prevalent: Fever (88.66%) and salivation (81.33%), indicating systemic and oral involvement. Moderately prevalent: Oral lesions (65.33%) and interdigital lesions (52%), aligning with classic FMD pathology. Least common: Nostril lesions (24%) and lameness (46%), suggesting these are less reliable diagnostic markers.

The majority of scientists agree that the two main signs are fever and salivation: Fever: Indicates early viremia, or the virus's entrance into the bloodstream. According to Alexandersen *et al.*, (2003), fever is the first symptom in over 90% of ruminants infected with FMD.(Grubman and Baxt, 2004) indicate saliva includes large viral loads, explaining 81.33% salivation. Oral Lesions and Salivation: Caused by viral replication in pharyngeal epithelium. Foot lesions are listed as cardinal signs in young cattle by the OIE (World Organization for Animal Health, 2022). Interdigital Lesions common in calves because of weaker hoof tissue.

Interdigital lesions illustrate epithelial tissue tropism, while high fever/salivation rates confirm the acute phase of FMD. Low Nostril Lesion (24%), (Thomson *et al.*, 2013) identified nasal lesions in fewer than 30% of young cattle, attributing this to less frequent aerosol exposure. Nostril lesions are inconsistent in calves. (Hughes *et al.*, 2002) contend that if strains enter through the respiratory system, nose lesions should be more prevalent, indicating that this group may have experienced unusual exposure.

Lesions in the mouth at 65.33%:(Rasmussen *et al.*, 2024) suggested oral vesicles rupture quicker in calves, making lesions difficult to see.

Some FMDV serotypes (such O) induce lesser oral symptoms; strain-dependent variation is noted by Arzt *et al.* (2011).

Lameness (46%) surprisingly low Lameness often correlates with foot lesions, yet here it is less frequent (46%) despite 52% interdigital lesions. (Dunn *et al.*, 2014) suggested calves may mask lameness due to reduced mobility.

The test provides considerable confirmation of symptom heterogeneity ($p < 0.001$). But Cramer (2016) cautions that chi-square could not tell if symptoms differ significantly without testing. Subclinical infections are highlighted by the 11–54% of symptoms that show no indications, which complicates field diagnosis (Pacheco *et al.*, 2010). In agreement: Salivation and fever are universal indicators of FMD, and interdigital lesions validate epithelial tropism. Age, stress, or the time of the examination may be the cause of the lower rate of mouth lesions and the rarity of nostril lesions.

5.1.2. The Correlation Between Sex and The Prevalence of FMD Infection

Serotype distribution disparities between male and female calves are shown by the data: Male calves had a higher frequency of serotype O (51.16% vs. 40.62%). Females had a greater rate of co-infection (A&O) (34.37% vs. 18.6%). The detection of SAT2 was limited to males (2.33%). Consent to the non-significant sex association.

The majority of researchers agree that biological sex alone rarely affects FMD epidemiology: Grubman and Baxt (2004) emphasize that the main determinant in FMD transmission is local interaction, such as sharing feeding and watering, rather than basic sex differences. According to OIE (2022), there is no reliable proof of sex-based susceptibility in cattle, which is compatible with the non-significant p -value. (Paton *et al.*, 2009): Sex accounted for less than 2% of the probability of transmission, according to a meta-analysis of 32 outbreaks, confirming chance variation.

Management practices (e.g., grouping by sex, movement patterns) likely explain observed differences more than biology. Disputed remarks males have higher serotype O (51.16% vs. 40.62%) assisting perspective: connected to behavioral elements. According to Alexandersen *et al.*, (2003), males may exhibit greater oral exploration and aggressiveness, hence increasing exposure. (Knight-Jones *et al.*, 2016) contend that was not actual susceptibility but rather sampling bias (e.g., more males in high-risk markets).

(Arzt *et al.*, 2011) propose that hormonal variations may allow for multiple infections by prolonging virus shedding in females. (Dunn *et al.*, 2014) ascribe this to secondary infections that occur because of later illness detection in females. Exclusively for males, SAT2. SAT 2 is frequently associated with specific vectors (e.g., tainted equipment), which is an implausible explanation. Tasks that are dominated by men may expose men more than women (Thomson *et al.*, 2013). The fact that there are only two SAT2 examples raises the possibility that this is a sampling artifact (Pacheco *et al.*, 2010).

The distribution of FMD serotypes in calves is not primarily influenced by sex. Additional research on behavioral and immunological causes is necessary in light of observed trends (Serotype O in males and co-infections in females).

5.1.3. Relationship Between Age and FMD Infection Rates

The age-serotype distribution has a significant ($\chi^2=15.595$, $DF=8$, $p=0.0485$). Rare serotypes (SAT2/Asia1) exclusively appear in calves older than 12 months (SAT2 100%, Asia1 75%). Serotype O predominates in younger calves (4-6 months: 41.4% of O cases; 7-11 months: 37.1%), according to observed patterns. The 7–11 month group accounts for 42.1% of all mixed infections (A&O) cases. Only the oldest calves (12–18 months: 100% of SAT2 cases, 75% of Asia1 cases) have rare serotypes (SAT2, Asia1). The youngest group had a greater prevalence of serotype A (50 percent of A cases in the 4-6 month range).

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Non-significant connection : When serotypes were probably grouped into more general categories , a second chi-square test ($X^2=4.025$, $DF=2$, $p=0.133$) revealed no significant connection. This emphasizes how crucial serotype-specific analysis. The reliability of conclusions regarding these particular serotypes is limited by the extremely small sample sizes for Asia1 ($n=4$) and SAT2 ($n=2$).

There is strong evidence for a true relationship between age and FMD serotype distribution in this population, as evidenced by the statistically significant chi-square result ($p=0.0485 < 0.05$) and the obvious and biologically plausible pattern. Maternal antibodies (colostrum) frequently provide protection for younger calves (4-6 months).

Over time, these antibodies may diminish, making patients first vulnerable to the most common local serotype or serotypes (in this case, O and A). This is consistent with O and A dominance in the youngest group. (Knight-Jones and Rushton, 2013).

The adaptive immune system of calves develops with age. Increased exposure and/or the growing immune system's capacity to manage co-infection prior to acquiring stronger, maybe more serotype-specific immunity could be the cause of the peak in mixed infections (A&O) in the 7–11 month group (Lyons *et al.*, 2015). The uncommon SAT2 and Asia1 are only seen in this group because older calves (12–18 months) have had more opportunity to be exposed to a greater variety of serotypes that are circulating in the environment or from other animals. It's possible that partial protection from earlier exposure accounts for the reduced relative occurrence of O and mixed infections. (Alexandersen *et al.*, 2003; Arzt *et al.*, 2011).

Even though there aren't many unusual serotypes, the more frequent serotypes (A, O, and A&O) have enough sample sizes to generate the significant chi-square result across age groups. Although the small n necessitates careful interpretation, the trend for uncommon serotypes is remarkable and in line with the general connection. Due to cumulative exposure and the deterioration of maternal immunity, studies frequently discover age-related patterns in FMD susceptibility

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and serotype circulation(Paton *et al.*, 2009; Knight-Jones and Rushton, 2013; Lyons *et al.*, 2015).

Although statistically significant, the link may not be generalizable or may be over interpreted. Conclusions about SAT2 and Asia1 are very questionable because to the relatively tiny samples and the slightly significant p-value (0.0485). Combining serotypes yielded a non-significant result, indicating that the effect is unique to serotype distribution rather than the total infection incidence by age. Critique of Rare Serotypes: It is statistically dubious to attribute SAT2 and Asia1 only to older calves based on two and four instances, respectively. Even if there was no real age correlation for these serotypes, the observed pattern may have happened by accident given the small numbers. There is no need to make generalizations about them. (Bland and Altman, 1996; Kirkwood and Sterne, 2003). With a p-value of 0.0485, the arbitrary 0.05 criterion is nearly reached. To lower the possibility of Type I error (false positive), some researchers recommend a more cautious approach (e.g., $p < 0.01$), particularly when numerous comparisons or modest anticipated cell counts are involved. If predicted frequencies in some table columns were too low (< 5), the chi-square test's validity may be called into doubt (McHugh, 2013).

Age is the only confounding factor examined in this study. Other important considerations factors (such as the immunization status of the dams and calves, herd density, movement patterns, location, and season) that affect serotype exposure and susceptibility are not taken into consideration. These could make the observed age association less clear. (Dill *et al.*, 2017; Knight-Jones and Rushton, 2013).

The results are unique to this time period, place, and calf population. Regional differences in serotype prevalence are significant. The absence of unusual serotypes in younger calves may just be a reflection of their lower total circulation, and the dominance of O and A here may not hold elsewhere. (Knowles and Samuel, 2003; Jamal and Belsham, 2013).

Epidemiological studies and statistical textbooks frequently caution against reading too much into marginal p-values and tiny cell counts (Kirkwood and Sterne, 2003; McHugh, 2013). Regional serotype circulation and other confounding variables are crucial, according to FMD research (Knowles and Samuel, 2003; Sumption *et. al.*, 2008). The majority of researchers concur that the data shows moderately strong evidence for a correlation between the distribution of the more frequent FMD serotypes (O, A, A&O) in this particular study population and the age group of the calves.

The physiologic justifications for rising exposure and declining maternal immunity are sound. Rare serotypes (SAT2, Asia1): It is not statistically reliable or generalizable to attribute their prevalence only to aging based on examples 2 and 4. To validate this particular discovery, significantly larger research is needed. The marginal p-value and perhaps low anticipated frequencies in certain cells raise doubts regarding the overall chi-square result's robustness. Because of different serotype ecology, the results are very specific to the context and might not apply elsewhere. Results may be impacted by unmeasured elements like as herd dynamics or vaccination.

Based on believable biology, scientists would agree that age influences serotype patterns for prevalent serotypes in this dataset. They would, however, stress the context-specific character of the results and strongly advise against making definite judgments concerning the unusual serotypes based only on this study. It is also necessary to use caution when interpreting the marginally significant p-value. For more conclusive findings, greater sample sizes and confounder accounting are required, particularly for rarer serotypes.

5.1.4. Relationship Between Age, Serotypes, and FMD Infection Rates

The age group and FMD serotype distribution are highly statistically significant according to the primary chi-square test ($\chi^2 = 51.91$, $DF=4$, $P=0.0001$). Dominance of Serotype O: Serotype O is responsible for over half of all infections (49.15%) and is very prevalent in calves of all ages, but it tends to decline with age.

Serotype A: Shows a significant correlation with age ($\chi^2=16.68$, $DF=2$, $P<0.001$). frequency decreases with age, with the youngest calves having the highest frequency (50% in 4-6 months). Serotype O: Significant correlation ($p=0.035$, $\chi^2=6.656$, $DF=2$). Prevalence is lowest in older groups and highest in the youngest calves (41.42%).

Mixed serotype A&O: No significant association ($P=0.145$, $DF=2$, $\chi^2=3.852$). The fluctuation is not statistically significant, although it peaks in 7–11 months (42.1%). Results for SAT2 and Asia1 are quite significant ($P<0.001$), however they are only observed in older calves (12–18 months). In the oldest group, SAT2: 100%. Asia1: 25% in 7–11 months, 75% in the oldest group.

-No Difference in Overall Infection Rates by Age: The test on the Total row ($\chi^2=4.025$, $DF=2$, $P=0.133$) shows no significant difference in the total number of infections across the three age groups (59, 55, 36). Critically small sample sizes for SAT2 ($n=2$) and Asia1 ($n=4$) severely.

The overwhelmingly significant overall chi-square result ($P<0.0001$) provides very strong evidence that the distribution of FMD serotypes differs significantly by age group in this population. The serotype-specific tests confirm significant age-related patterns for Serotypes A and O. The exclusive finding of rare serotypes in older calves, while based on small numbers, aligns with biological plausibility and reinforces the overall significant finding.

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The function of declining maternal antibodies is clearly supported by the high incidence of serotypes A and O in the youngest calves (4-6 months). The early peak can be explained by the fact that calves that are initially protected against circulating strains (likely O and A dominant) become vulnerable as antibodies decrease. (Knight-Jones and Rushton, 2013).

The requirement for longer exposure times to meet fewer frequent strains is consistent with the occurrence of unusual serotypes (SAT2, Asia1) only in older calves (7-18 months).

Due to their increased mobility, interaction, or environmental persistence, older calves have had greater exposure possibilities. (Alexandersen *et al.*, 2003; Arzt *et al.*, 2011). Although it decreased somewhat with age, O dominance throughout may have been due to its increased transmissibility or endemic persistence in the area. Complex interactions between serotypes or evolving immune responses might be the cause of the non-significant rise in mixed infections (Lyons *et al.*, 2015).

The p-value (0.0001), which is significantly below traditional significance limits, is very persuasive. This conclusion is supported by the substantial results for the two most prevalent serotypes (A and O). Although the small number necessitates careful interpretation, the pattern for uncommon serotypes is remarkable and in line with the general relationship and biological assumption. Total Row Resolution: There is no contradiction in the non-significant finding for the total infections by age ($P=0.133$). It only demonstrates that there is no discernible difference in the overall risk of contracting any of the FMD serotypes among the age groups in this research. The main conclusion is that a calf's age has a significant impact on the type (serotype) of infection it contracts.

Source Support: The FMD literature has extensively shown age-specific serotype patterns associated with exposure and maternal immunity decline (Paton *et al.*, 2009; Knight-Jones and Rushton, 2013). There are also proven regional differences in serotype dominance (Knowles and Samuel, 2003).

Researchers Questioning the Generality or Strength of Particular Findings (Warnings/Limitations): Justification: Although the general link is statistically indisputable, much caution should be used when interpreting particular patterns, particularly when it comes to uncommon serotypes and mixed infections. Meaningful statistical tests or inferences on the age association between SAT2 and Asia1 are invalidated by their tiny samples. A possible biological expectation is contradicted by the absence of relevance for mixed infections.

5.1.5. Correlation Between FMD Infection Rates and Various Thi-Qar Province Regions

According to normal statistical principles, the p-value of 0.06 (>0.05) should be interpreted as no significant regional difference (Wasserstein and Lazar, 2016). Complete disclosure of p-value, test statistics ($X^2=5.52$, $DF=7$), percentages, and sample sizes improves repeatability. The result exhibits statistical rigor by attributing the unequal case distribution to chance rather than imposing geographic significance (Greenland *et al.*, 2016).

There is a possibility of Type II error (failing to identify actual differences) due to the small sample size and low power. There are 150 instances total throughout 8 areas. The study may be underpowered; a X^2 value of 5.52 with $DF=7$ is near to significance (Cohen, 1992).

5.1.6. The Correlation Between Sample Categories and The Incidence of FMD Infection

A conclusion that is statistically sound according to statistical best practices, $p = 0.6242$ (>0.05) should be interpreted as non-significant (Wasserstein *et al.*, 2019). In light of the evidence, the null hypothesis (no connection) is suitably maintained.

Equivalent subjects (blood: 78, swabs: 72) offer enough strength to identify moderate-to-large effects (Cohen, 1992). Because of under powering, the non-significant outcome is improbable.

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Reproducibility is supported by the clear display of test statistics ($X^2=0.24$, $DF=1$, $p=0.6242$).

It is illogical for infection rates to vary depending on the type of sample (blood versus swab). Samples are not risk factors; they are diagnostic instruments. The study mixes up infection rates and diagnostic sensitivity. OIE Manual (2023): Virus isolation from swabs (epithelium) is gold standard for acute FMD; serology (blood) indicates past exposure.

Similar infection rates are inevitable since both sample types test the same population of calves. The analysis fails to address meaningful questions:

Sensitivity/specificity of each test.

(Knight-Jones *et al.*, 2015): Sample type validity depends on infection stage. False negatives (e.g., sample timing affecting swab sensitivity).

Cross-contamination risks during swab collection (Grubman and Baxt 2004).

5.1.7. Relationship Between The Months of The Year and The Rate of FMD Infection

Reasons in Favor of the Study (Strengths): Statistically Valid Finding - Using the proper chi-square test ($X^2=13.79$, $DF=4$), a significant seasonal trend ($P = 0.008 < 0.05$) was correctly identified. The chi-square test of independence (McHugh, 2013).

Alignment with Known FMD Epidemiology Global trends are consistent with higher infections during the colder months of January through March: The FMD virus is more resilient in cold, low-humidity environments. In winter, animal overpopulation makes transmission easier. (Knight-Jones and Rushton, 2013).

Incomplete Seasonal Coverage Only 5 months (November–March) are covered by the data, leaving out 7 months. Annual patterns, such as summer breakouts, are not captured. Winter and spring are overrepresented, which skews the seasonal assertion. OIE (2023). No correction for seasonality-causing variables Data on temperature and humidity.

Movements of livestock (such as seasonal migrations or marketplaces). The timing of vaccination efforts. (Paton *et al.*, 2018).

5.2. Antioxidant Parameters

Calves infected with FMD virus, especially in cases of co-infection with (A&O) serotypes, show a marked reduction in the endogenous antioxidant parameters SOD, CAT, and GSH when compared to healthy calves. This reduction is an important factor in the pathogenesis of FMD and significantly affects tissue damage and the severity of the disease (Mishra *et al.*, 2020 ; El-Shehab *et al.*, 2022) have reported that calves infected with FMDV serotypes A and O showed significantly lower levels of SOD, CAT, and GSH compared to healthy calves. (Mishra *et al.*, 2020) pointed out that this depletion was due to increased oxidative damage (with markers like Malondialdehyde MDA) and the clinical severity of the disease. Research on mixed infections (Serotype A&O) done by Rasheed *et al.*, (2023) argues that there is a much sharper decline in these defensive antioxidants than in single-serotype infections. Al-Saeedi *et al.*, (2021) and others demonstrated that the greater the depletion of SOD, CAT, and GSH, the more severe the clinical signs and lesions (fever, vesicle formation, lameness). Decreased antioxidants with increased markers of stress indicate a worse outcome with infections. This points toward co-infection resulting in increased oxidative burden, whether added together or jointly acted upon.

This could be because the depletion of SOD, CAT, or GSH is not a simple case of absence of production but rather the result of the host and virus confrontation the primary causes include massive reaction oxygen species (ROS) generation as well as direct consumption of antioxidants . Replication of FMDV, similar to other viral infections, within target tissues and subsequently in the cytoplasm and peroxisomes of host cells causes elevated levels of ROS as a result of reprogrammed cellular metabolism and energy production required for the production of tRNA and other constituents or structure elements of viral particles (Mishra *et al.*, 2020; El-Shehab *et*

al., 2022). Besides that, FMDV has the capability of causing the body to respond with potent inflammation that is out of balance.

Responding Immunocytes such as neutrophils and macrophages recruited to infected areas particularly vesicles or other tissues respond to the infection by producing excessive superoxide anion and hydrogen peroxide), catalase (3, 4 hypochlorous acid) at large amounts. Due to the burst of ROS, superoxide dismutase is overwhelmed, and so is CAT that decomposes H_2O_2 as well as GSH which draws free radicals and enzymatically interacts together with glutathione peroxidase) (Al-Saeedi *et al.*, 2021; Rasheed *et al.*, 2023).

5.3. Enzymatic Parameters:

Infection with serotype O leads to calves having 2-3 times more ALT and AST than controls or other infected groups. Focused liver damage caused enhanced destruction of liver cells. Mixed infections and serotype A show lower increases. Improved binding of serotype O to heparin sulfate proteoglycans (HSPGs) on hepatocytes that helps in replication and entry is noted to be binding of integrin-free pathways, thus increasing the viral load in the liver which disrupts the mitochondrial membranes of the hepatocytes causing release of AST (mitochondrial isoform) into the blood (Rasheed *et al.*, 2023).

The difference in Hepatopathologies associated with O serotype shows the most damage, supporting FMDV O infection. Very significant differences ($p \leq 0.05$), also observed with ALT and AST show stronger effects for inflammatory stimuli suggest possibility for greater damage. Changes to ALT and AST by FMDV O infected calves suggest the existence of preferential presence within liver cells which may highlight a selective advantage to infection with that strain.

This is consistent with findings by Balamurugan *et al.*, (2020) that demonstrate serotype specific differences in fibrous tissue degenerative lesions and foetal tissue slicing. As evidence by the system infection's lack of significant increase in A and mixed A&O groups, suggested laparoscopic trends indicates that these types do not

cause significant liver injury; or, any injury that does occur is subclinical during the level of these enzymes in serum.

ALP is also connected to firing activity of osteosarcoma, and so can indicate pancreatitis. The lack of difference in ALP levels between any of the infected groups (O, A, A&O) and the healthy control group suggests the absence of significant cholestasis or major disease of the biliary system induced by any of the FMDV serotypes under study. This suggests that the hepatocellular damage induced by serotype O is likely centrilobular or midzonal and not periportal.

According to Karapinar *et al.*, (2022) discussion of viral hepatitis in livestock this pattern-higher transaminase without elevated ALP is consistent with acute hepatocellular injury without obstruction. In 2022, GGT is a very sensitive indicator of biliary epithelial damage and cholestasis. Additionally, some medications and hepatic insults can cause it. When compared to controls the O serotype group and the mixed A and O group the particular increase in GGT in calves infected with serotype A suggests a distinct pattern of hepatic involvement.

This suggests that cholestasis or biliary tract pathology is a more common characteristic of serotype A infection in this investigation. Although the exact causes of this serotype-specific cholestatic effect are unknown they may include variations in the livers viral replication sites the type of immune response triggered (e. g. A. focusing on bile duct epithelia) or causing particular metabolic alterations.El-Tholoth *et al.*, (2021). observed differences in tissue distribution and clinical manifestations among FMDV serotypes in 2021 which may help to explain the different enzyme patterns found in different organs. Although the lack of a discernible increase in GGT in the mixed infection group calls for more research it may indicate intricate viral interactions influencing pathology.

The idea that various FMDV are supported by these findings. Different serotypes can result in different patterns of organ damage especially in the liver. Serotype A is linked to cholestatic alterations whereas serotype O seems to be more closely linked to direct hepatocellular damage. Alongside virological and serological diagnostics monitoring liver enzyme profiles (ALT AST and GGT) may help determine the

Chapter Five..... Discussion

predominant serotype involved in an outbreak (particularly distinguishing O from A) or gauge the severity of hepatic complications in afflicted calves. Significant liver cell injury is indicated by the transaminase elevation in group O.

The distinct patterns of enzymes indicate that there may be various pathogenic mechanisms at work. Serotype O: Immune-mediated hepatocyte damage direct hepatocytolysis (e. g. G. either ischemic injury brought on by systemic inflammation or viremia or CTL activity. Serotype A: Drug-induced changes brought on by treatment procedures intrahepatic cholestasis brought on by inflammation or metabolic changes or potential involvement of bile ductless (cholangiolitis). Mixed Infection Complexity: It's interesting that the mixed A and O group did not exhibit a discernible increase in enzymes when compared to the pure O group for ALT/AST or the pure A group for GGT. It may indicate a dominant strain that is less hepatitis in the mix interference between serotypes or a regulated immune response that results in distinct pathology. This demonstrates how complicated mixed infections are and how much more research is needed.

Conclusion & Recommendations

Conclusions and Recommendations

Conclusions

1. The study showed that the FMD serotypes were A, O, A&O, SAT2 and Asia1 in Thi-Qar province.
2. Post-Sequencing analysis uncovered 52 genetic mutations prevalent in most subserotypes.
3. Infected calves were (4-18 months): The most common signs were fever & salivation, serotype O was more prevalent in males, serotypes A & O were more prevalent in females, serotype SAT2 detected only in males, younger calves (4-6 m) infected more with serotypes A, O, the rare serotypes SAT2 & Asia1 appeared exclusively in old calves (12-18 m).
4. FMDV pathogenesis is driven by oxidative injury, with single serotypes (A/O) causing more severe antioxidant suppression than mixed infections.
5. MDA elevation is a universal indicator of oxidative damage in FMDV-infected cattle, regardless of serotype.
6. The hepatic impact of FMDV serotypes in calves varies significantly according to this analysis. Profound hepatocellular damage linked to serotype O infection is demonstrated by elevated ALT and AST levels. Conversely cholestasis alterations which are indicated by elevated GGT are associated with serotype A infection.

Recommendations:

The findings of this study are expected to pave the way for further studies aimed at identifying FMDV from field outbreaks in the country .

To stop FMDV from spreading from endemic regions, proper regulation of animal movement is crucial. Before any animals are moved between locations, their FMD

status should be checked, and animals and animal products should be systematically inspected as part of this control.

Regular surveys are recommended to obtain detailed information on the incidence of FMDV serotypes and topotypes in the country during the outbreak .

Test for vaccine compatibility to determine whether FMDV field strains are compatible with the vaccinations that are already in use.

Successfully manage and prevent FMD outbreaks, a regular immunization program should be established. This technique need to be employed as a preventative measure.

Create modern, high-quality vaccines using the isolated strains.

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Zhang, C.; Zhang, C.; Zheng, S.; Qiao, Y., Li, C.; Zhang, M. and Hong, C. S. (2023). A Complete Survey on Generative AI (AIGC) Is ChatGPT from GPT-4 To GPT-5 All You Need?. *Arxiv Preprint Arxiv*, 2303:11717.

Zhang, T.; Lu, B.; Yang, B.; Zhang, D.; Shi, X.; Shen, C.; Cui, H.; Yuan, X.; Zhao, D.; Yang, J.; Hao, Y.; Chen, X.; Liu, X.; Zhang, K.; Zheng, H.; Zhang, Z.; Green, B. R. and Cavalier-Smith, T. (2000a). Phylogeny of Ultra-Rapidly Evolving Dinoflagellate Chloroplast Genes: A Possible Common Origin for Sporozoan and Dinoflagellate Plastids. *Journal of Molecular Evolution*, 51(1): 26-40.

Zhang, Z.; Schwartz, S.; Wagner, L.; and Miller, W. (2000b). A Greedy Algorithm for Aligning DNA Sequences. *Journal of Computational biology*, 7(1-2): 203-214.

Appendixes

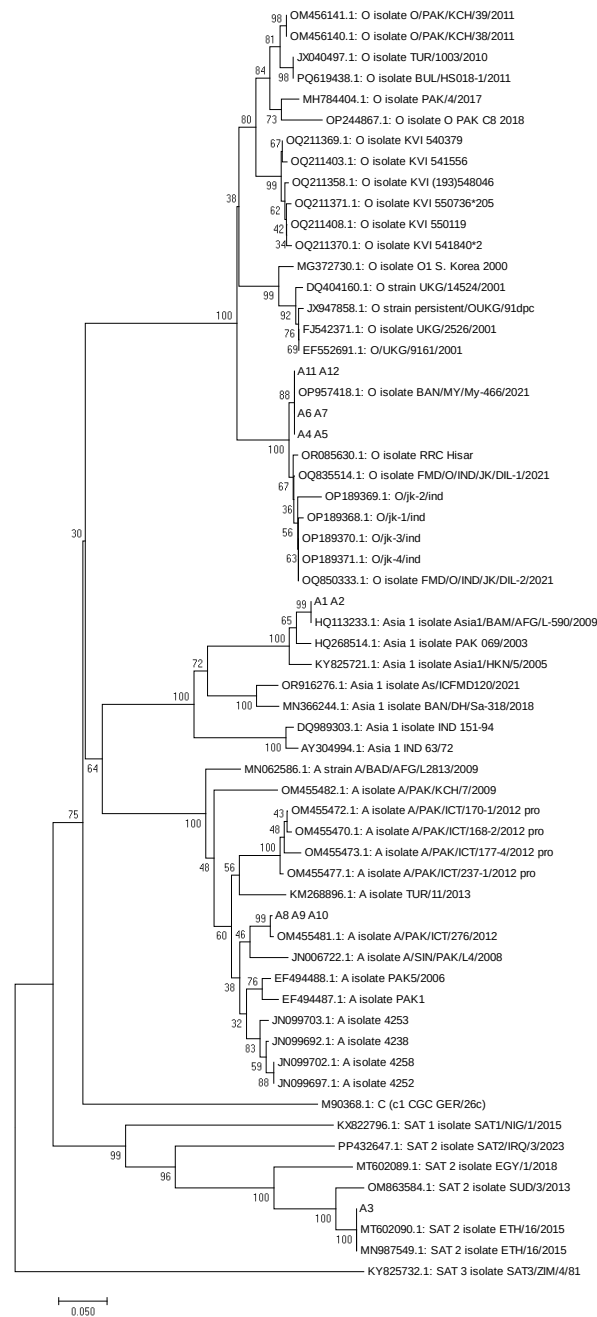


Figure (1): Evolutionary relationships of the FMDV taxa using the Neighbor-Joining method. The optimal tree with the sum of branch length = 3.02958374 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 61 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7. The number “0.05” of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms.

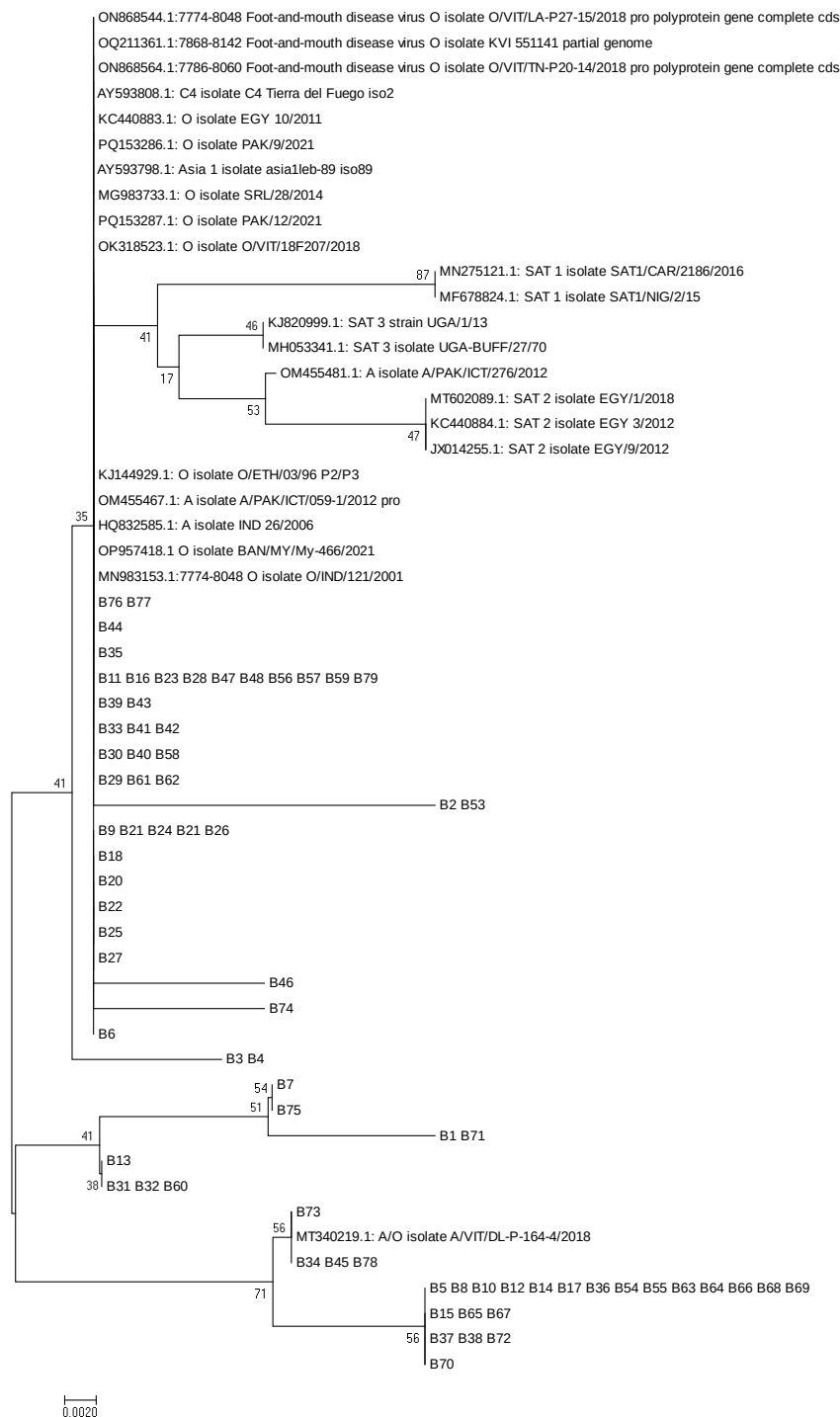


Figure (2): Evolutionary relationships of the FMDV taxa using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.15760870 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 54 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7. The number “0.002” of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms.

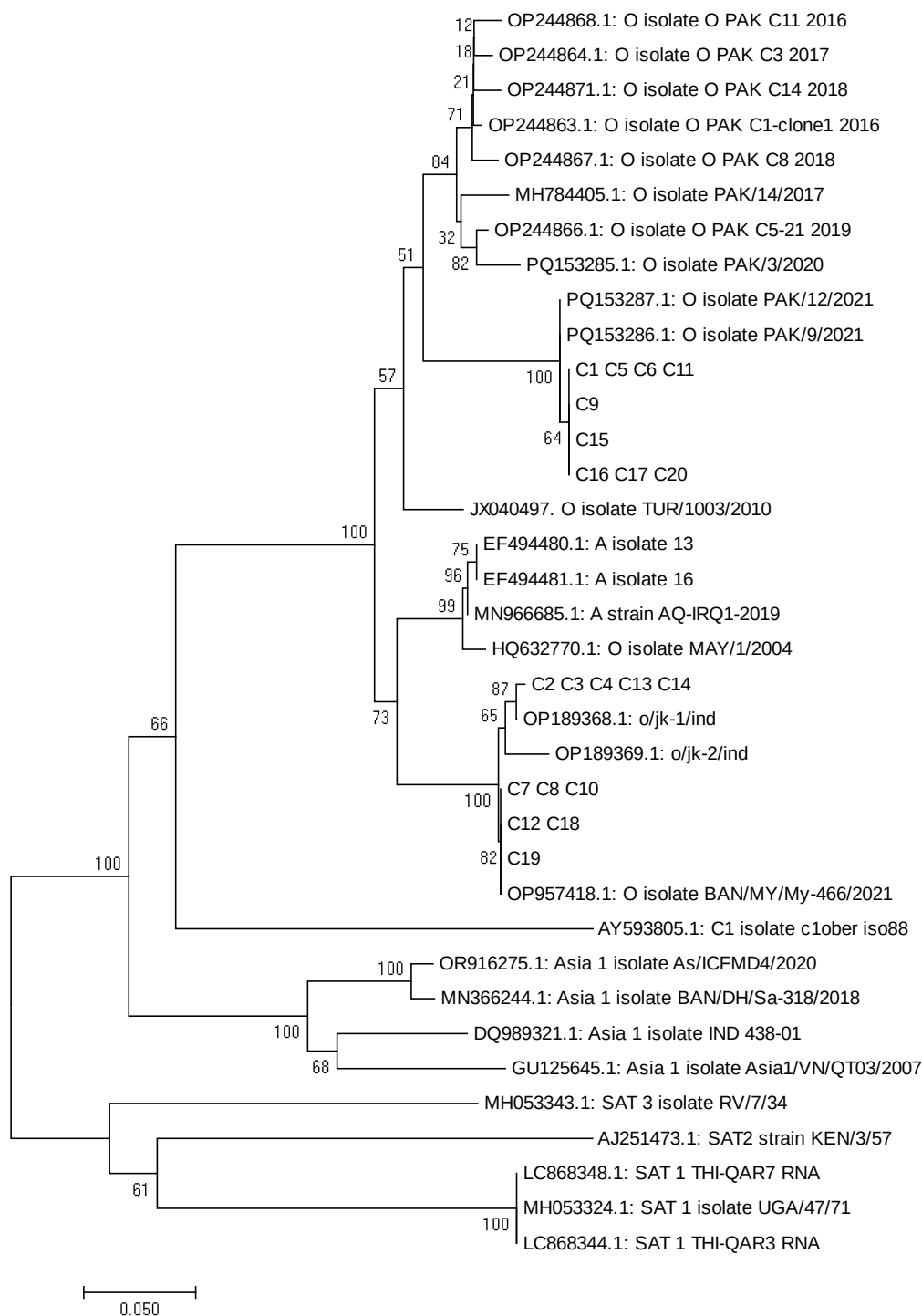


Figure (3): Evolutionary relationships of the FMDV taxa using the Neighbor-Joining method. The optimal tree with the sum of branch length = 1.59571002 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 36 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7. The number “0.05” of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms.

Appendix (1): Accessions number of FMDV that were submitted in Gene Bank of NCBI; PV630876:

National Library of Medicine
National Center for Biotechnology Information

Nucleotide

Nucleotide

Advanced

Search

Foot-and-mouth disease virus Asia 1 isolate Zainab-A2 polypeptide gene, partial cds

LOCUS Seq2 866 bp DNA linear VRL 06-MAY-2025
DEFINITION Foot-and-mouth disease virus strain Zainab-A2.
ACCESSION Seq2
VERSION
KEYWORDS
SOURCE Foot-and-mouth disease virus
ORGANISM Foot-and-mouth disease virus
Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
Picornavirales; Picornaviridae; Caphthovirinae; Aphthovirus.
REFERENCE 1 (bases 1 to 866)
AUTHORS Alabudi, Z.A.A., Jar Allah, B.A. and Felaih,
A.J. TITLE Direct Submission
JOURNAL Submitted (06-MAY-2025) Department of Microbiology, College of
Veterinary Medicine, Al Shatrah University, Al Shatrah,
Al-Nasiriya, Al-Nasiriya 64001, Iraq
COMMENT Bankit Comment: TOTAL # OF SEQS:111

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Sequencing Technology: Sanger dideoxy sequencing
##Assembly-Data-END##
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/serotype="Asia 1"

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ATVYNGNCKYGESHTTSVRGDLQVLAQKAARTLPTSFNYGAIKATRVTELLYRMKRAE
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BASE COUNT 224 a 248 c 219 g 175 t

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Appendix (2): Accessions number of FMDV that were submitted in Gene Bank of NCBI; PV630877:



Foot-and-mouth disease virus SAT 2 isolate Zainab-A3 polyprotein gene, partial cds

LOCUS Seq3 866 bp DNA linear VRL 06-MAY-2025

DEFINITION Foot-and-mouth disease virus strain Zainab-A3.

ACCESSION Seq3

VERSION

KEYWORDS .

SOURCE Foot-and-mouth disease virus

ORGANISM Foot-and-mouth disease virus

Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;

Picornavirales; Picornaviridae; Caphthovirinae; Aphthovirus.

REFERENCE 1 (bases 1 to 866)

AUTHORS Alabudi, Z.A.A., Jar Allah, B.A. and Felaih, A.J.

TITLE Direct Submission

JOURNAL Submitted (06-MAY-2025) Department of Microbiology, College of

Veterinary Medicine, Al Shatrah University, Al Shatrah,

Al-Nasiriya, Al-Nasiriya 64001, Iraq

COMMENT Bankit Comment: TOTAL # OF SEQS:111

##Assembly-Data-START##

Sequencing Technology: Sanger dideoxy sequencing

##Assembly-Data-END##

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/serotype="SAT 2"

/isolation source="Calves"

/host="Calves"

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Appendix (3): Accessions number of FMDV that were submitted in Gene Bank of NCBI; PV630878:


National Library of Medicine
National Center for Biotechnology Information

Nucleotide

Advanced

Foot-and-mouth disease virus O isolate Zainab-A4 polyprotein gene, partial cd

LOCUS Seq4 866 bp DNA linear VRL 06-MAY-2025
DEFINITION Foot-and-mouth disease virus strain Zainab-A4.
ACCESSION Seq4
VERSION
KEYWORDS .
SOURCE Foot-and-mouth disease virus
ORGANISM Foot-and-mouth disease virus

Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
 Picornavirales; Picornaviridae; Caphthovirinae; Aphthovirus.
 REFERENCE 1 (bases 1 to 866)
 AUTHORS Alabudi, Z.A.A., Jar Allah, B.A. and Felaih, A. J.
 TITLE Direct Submission
 JOURNAL Submitted (06-MAY-2025) Department of Microbiology, College of
 Veterinary Medicine, Al Shatrah University, Al Shatrah,
 Al-Nasiriya, Al-Nasiriya 64001, Iraq
 COMMENT Bankit Comment: TOTAL # OF SEQS:111

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 Sequencing Technology: Sanger dideoxy sequencing
 ##Assembly-Data-END##

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BASE COUNT 224 a 248 c 220 g 174 t
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Appendix (4): Accessions number of FMDV that were submitted in Gene Bank of NCBI; PV630879:

 **National Library of Medicine**
National Center for Biotechnology Information

Nucleotide

Advanced

Foot-and-mouth disease virus O isolate Zainab-A5 polyprotein gene, partial cds

LOCUS Seq5 866 bp DNA linear VRL 06-MAY-2025

DEFINITION Foot-and-mouth disease virus strain Zainab-A5.

ACCESSION Seq5

VERSION

KEYWORDS .

SOURCE Foot-and-mouth disease virus

ORGANISM Foot-and-mouth disease virus

Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
Picornavirales; Picornaviridae; Caphthovirinae; Aphthovirus.

REFERENCE 1 (bases 1 to 866)

AUTHORS Alabudi, Z.A.A., Jar Allah,B.A. and Felaih, A.J.

TITLE Direct Submission

JOURNAL Submitted (06-MAY-2025) Department of Microbiology, College of
Veterinary Medicine, Al Shatrah University, Al Shatrah,
Al-Nasiriya, Al-Nasiriya 64001, Iraq

COMMENT Bankit Comment: TOTAL # OF SEQS:111

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

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BASE COUNT 224 a 248 c 220 g 174 t

ORIGIN

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Appendix (5): Accessions number of FMDV that were submitted in Gene Bank of NCBI; PV630880:



Foot-and-mouth disease virus O isolate Zainab-A6 polyprotein gene, partial cds

LOCUS Seq6 866 bp DNA linear VRL 06-MAY-2025

DEFINITION Foot-and-mouth disease virus strain Zainab-A6.

ACCESSION Seq6

VERSION

KEYWORDS .

SOURCE Foot-and-mouth disease virus

ORGANISM Foot-and-mouth disease virus

Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;

Picornavirales; Picornaviridae; Caphthovirinae; Aphthovirus.

REFERENCE 1 (bases 1 to 866)

AUTHORS Alabudi,Z.A.A., Jar Allah,B.A. and Felaih,A.J.

TITLE Direct Submission

JOURNAL Submitted (06-MAY-2025) Department of Microbiology, College of

Veterinary Medicine, Al Shatrah University, Al Shatrah,

Al-Nasiriya, Al-Nasiriya 64001, Iraq

COMMENT Bankit Comment: TOTAL # OF SEQs:111

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Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

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BASE COUNT 222 a 250 c 222 g 172 t

ORIGIN

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Appendix (6): Accessions number of FMDV that were submitted in Gene Bank of NCBI; PV630881:



National Library of Medicine
National Center for Biotechnology Information

Nucleotide

Advanced

Foot-and-mouth disease virus O isolate Zainab-A7 polyprotein gene, partial cds

LOCUS Seq7 866 bp DNA linear VRL 06-MAY-2025
DEFINITION Foot-and-mouth disease virus strain Zainab-A7.
ACCESSION Seq7

VERSION
 KEYWORD
 DS .
 SOURCE Foot-and-mouth disease
 virus ORGANISM Foot-and-mouth
 disease virus
 Viruses; Riboviria; Orthornavirae; Pisuviricota;
 Pisoniviricetes; Picornavirales; Picornaviridae;
 Caphthovirinae; Aphthovirus.
 REFERENCE 1 (bases 1 to 866)
 AUTHORS Alabudi,Z.A.A., Jar Allah,B.A. and Felaih,A.J.
 TITLE Direct Submission
 JOURNAL Submitted (06-MAY-2025) Department of Microbiology,
 College of Veterinary Medicine, Al Shatrah University, Al Shatrah,
 Al-Nasiriya, Al-Nasiriya 64001, Iraq
 COMMENT Bankit Comment: TOTAL # OF SEQS:111

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 Sequencing Technology :: Sanger dideoxy sequencing
 ##Assembly-Data-END##
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 172 t ORIGIN
 1 ttccagatta cacacgggaa ggctgatggg gatgcactag ttgtactggc tagcgccggg
 61 aaggactttg agctgcgatt gccaatgac gccgcacag agacaacctc
 cacaggtgag 121 tcggctgac ccgtgactgc caccgtgaa aactacgggtg
 gcgagacaca ggtccagaga 181 cgccagcaca cggacgtctc ttcatattg
 gacagatttg taaaagtgac accaaaagac 241 caaattaatg tgttgacct
 gatgcaaacc cctgctcaca cttgggtggg tgcccttctc
 301 cgccaccgca cctactactt cgagattta gaggtggcgg tgaacacagg

ggggaacctc 361 acctgggtcc cgaacggggc gcccgaaaa gccttgaca
acaccaccaa tccaacggct 421 taccacaagg caccgctcac cgcactgca
ctgccgtaca cggcacccca ccgtgtcatg 481 gctactgttt acaacgggaa
ctgcaagtac ggcgagagcc acgcaactag cgtgagaggt 541 gacctgcaag
tgttggccca gaaggcagca aggacgctgc ctacctctt caactacggt 601
gccatcaaag ctactcgggt gactgaactg ctttatcgca tgaagagggc tgaaacatac
661 tgcctcggc ctctttggc tatccacccg agtgaagcta gacacaaaca aaagattgtg
721 gcacctgtga agcaacttct gaacttgac ctgctcaagt tggcgggaga
cgtcgagtc 781 aaccctgggc cttcttctt ctccgacgtg aggtcaaact
tcaccaagct ggtagacacc 841 atcaaccaga tgcaggagga catgtc

A1)

The figure displays a sequence alignment of HQ113233.1 against two reference sequences, A1 and A2. The alignment is presented in six windows, each showing 100 positions. The sequences are color-coded: red for mismatches, green for matches, and blue for gaps. The alignment shows high similarity between HQ113233.1 and both A1 and A2, with some mismatches highlighted in red and green.

Sequence alignment results (HQ113233.1 vs A1 and A2) across six windows (100 positions each):

Window 1 (Positions 1-100):

HQ113233.1: TGCATCTATCAGATCACCCATGGCAAGGCTGAAGGAGACGCACCTGGTCGTTTCTGTCAGCGCCGGCAAGACTTTGAGTTCGCCTGCCCGTTGACGCGC

A1: TGCATCTATCAGATCACCCATGGCAAGGCTGAAGGAGACGCACCTGGTCGTTTCTGTCAGCGCCGGCAAGACTTTGAGTTCGCCTGCCCGTTGACGCGC

A2: TGCATCTATCAGATCACCCATGGCAAGGCTGAAGGAGACGCACCTGGTCGTTTCTGTCAGCGCCGGCAAGACTTTGAGTTCGCCTGCCCGTTGACGCGC

Window 2 (Positions 110-200):

HQ113233.1: GCCGGCAAACCAACCACTACCAGGCGAGTCAGCAGACCCGGTAACAACCAACGGTCGAGAACTACGGAGGAGAACTCAGACAGCCAGGCGGCTTCACACTGA

A1: GCCGGCAAACCAACCACTACCAGGCGAGTCAGCAGACCCGGTAACAACCAACGGTCGAGAACTACGGAGGAGAACTCAGACAGCCAGGCGGCTTCACACTGA

A2: GCCGGCAAACCAACCACTACCAGGCGAGTCAGCAGACCCGGTAACAACCAACGGTCGAGAACTACGGAGGAGAACTCAGACAGCCAGGCGGCTTCACACTGA

Window 3 (Positions 210-300):

HQ113233.1: TGTTCCTTCGTTCTTGACAGGTTTGTGAAACTCACTGCACCCCAAGAACATTCAGACCCCTTGACCTCATGCAAAATCCCTCACACACGCTGGTTGGAGCA

A1: TGTTCCTTCGTTCTTGACAGGTTTGTGAAACTCACTGCACCCCAAGAACATTCAGACCCCTTGACCTCATGCAAAATCCCTCACACACGCTGGTTGGAGCA

A2: TGTTCCTTCGTTCTTGACAGGTTTGTGAAACTCACTGCACCCCAAGAACATTCAGACCCCTTGACCTCATGCAAAATCCCTCACACACGCTGGTTGGAGCA

Window 4 (Positions 310-400):

HQ113233.1: CTGCTGCGGTCTGCGACGTACTACTTCTCAGACCTGGAGGTTGCGATTGTTTACACAGGCCCAATCACCTGGGTGCCCAACGGCTCGCCCAAGGATGCTC

A1: CTGCTGCGGTCTGCGACGTACTACTTCTCAGACCTGGAGGTTGCGATTGTTTACACAGGCCCAATCACCTGGGTGCCCAACGGCTCGCCCAAGGATGCTC

A2: CTGCTGCGGTCTGCGACGTACTACTTCTCAGACCTGGAGGTTGCGATTGTTTACACAGGCCCAATCACCTGGGTGCCCAACGGCTCGCCCAAGGATGCTC

Window 5 (Positions 410-500):

HQ113233.1: TAGACAAACAGACCAACCCAACTGCTTACCAGAAGCAACCCGTCACTCGCTTGGCGCTCCCTTACACCGCTCCCCATCGTGTGCTGGCGACAGTGTACAA

A1: TAGACAAACAGACCAACCCAACTGCTTACCAGAAGCAACCCGTCACTCGCTTGGCGCTCCCTTACACCGCTCCCCATCGTGTGCTGGCGACAGTGTACAA

A2: TAGACAAACAGACCAACCCAACTGCTTACCAGAAGCAACCCGTCACTCGCTTGGCGCTCCCTTACACCGCTCCCCATCGTGTGCTGGCGACAGTGTACAA

Window 6 (Positions 510-600):

HQ113233.1: CGGGAAGACGACGTACGGGGTAACAACCGAGCGGCGTGGCGATATGGCGGCCCTTGCAAAAGACTGAGTGGGCGGTTGCCCACTCATTTAATTACGGC

A1: CGGGAAGACGACGTACGGGGTAACAACCGAGCGGCGTGGCGATATGGCGGCCCTTGCAAAAGACTGAGTGGGCGGTTGCCCACTCATTTAATTACGGC

A2: CGGGAAGACGACGTACGGGGTAACAACCGAGCGGCGTGGCGATATGGCGGCCCTTGCAAAAGACTGAGTGGGCGGTTGCCCACTCATTTAATTACGGC

A1	
A2	
	610 620 630 640 650 660 670 680 690 700
	
HQ113233.1	GCTGTAAAGGCTGAAACCATCAGCTGAGCTTTTGATTGCGCATGAAACGTGCGGAACATACTGCCCTAGGCCTTGCTAGCTCTTGACACCACTCAGGACC
A1	
A2	
	710 720 730 740 750 760 770 780 790 800
	
HQ113233.1	GCCGAAACAGAGATTATTGCACCTGAAAAGCAGATGATGAACCTTGACCTGCTCAAGTTGGCGGAGATGTTGAGTCCAACCTGGGCCCTTCTTCTT
A1	C.C.....
A2	C.C.....
	810 820 830 840 850 860
	
HQ113233.1	CTCCGACGTCAGGTCAAATTTTCCAGCTGGTTGAAACCATCAACCAGATGCAGGAGGACATGTC
A1	
A2	
A2	

الخلاصة

فايروس الحمى القلاعية (FMDV) هو أحد العوامل المرضية الفيروسية شديدة العدوى التي يمكن أن تصيب الحيوانات ذوات الاظلاف، مثل الأبقار والخنازير والأغنام والماعز والغزلان يسبب هذا الفايروس مرض الحمى القلاعية، الذي يمثل عاملاً اقتصادياً رئيسياً في مجال الزراعة نظراً لسرعة انتشاره وما يمكن أن يسببه من قيود تجارية شديدة.

هدفت هذه الدراسة إلى تشخيص الفايروس بتقنية (PCR) ، وتحديد التسلسلات الجينية للأنماط المصلية، وتحديد العلاقة بين العمر والجنس والنمط المصلي بالإصابة الفيروسية، وتقييم نشاطات إنزيمات السوبرأوكسيد ديس ميوتيز (SOD) ، والكاتاليز (CAT) ، والجلوتاثيون (GSH) ، ومستويات المالوندايالديهايد (MDA) في العجول السليمة والمصابة بفايروس الحمى القلاعية، وتحديد مستويات نشاطات إنزيمات الأسبارتات أمينوترانسفيريز (AST) ، والألانين أمينوترانسفيريز (ALT) ، والفوسفاتيز القلوي (ALP) ، وجاما-جلوتاميل ترانسفيريز (GGT) في العجول السليمة والمصابة.

جمعت عينات الدم والمسحات من ١٥٠ عجلاً مصاباً بالحمى القلاعية من مناطق وأعمار وأجناس مختلفة في محافظة ذي قار، بالإضافة إلى ٢٠ عجلاً سليماً كمجموعة تحكم، خلال الفترة الممتدة من تشرين الثاني (٢٠٢٤) إلى آذار (٢٠٢٥).

تم إجراء الكشف الجزيئي ووصف الأنماط المصلية لفايروس الحمى القلاعية باستخدام تقنية تفاعل البوليميريز المتسلسل العكسي (RT-PCR) يليه تحليل الترحيل الكهربائي. تعتبر تقنية RT-PCR حساسة ونوعية للغاية، حيث تمكن من تضخيم الحمض النووي الريبوزي الفايروسي عن طريق تحويله أولاً إلى DNA متمم (cDNA) باستخدام إنزيم النسخ العكسي، ثم التضخيم المستهدف بواسطة PCR.

يُعد الجين الذي يشفر البروتين متعدد الببتايد (Polyprotein) في فايروس الحمى القلاعية بالغ الأهمية لأنه يحتوي على القدرة الترميزية الكاملة للجينوم الفيروسي. يمكن أن يكون للتباين الجيني في هذا الجين تداعيات متعددة في التصنيف والتطور النوعي للفايروس. استناداً إلى المتغيرات الجينية لتسلسلات البروتين متعدد الببتايد، أُجريت الدراسة الحالية لتقييم نمط التنوع البيولوجي للفايروس في ١١١ عينة مأخوذة من عجول مصابة.

أظهرت نتائج التسلسل أن نواتج PCR المضخمة للجزء A أظهرت أعلى عدد من الأنماط المصلية باكتشاف أربعة أنماط (Asia 1 ، SAT 2 ، O ، A) في المقاطع المدروسة. كشف تحليل الجزء A عن وجود ١٤ شكل نيوكليوتيدي الأحادي (SNP) أي طفرة موزعة عبر منطقة طولها ٨٦٦ زوج قاعدي، وقد نتج عن مجموعة فرعية منها بدائل غير متشابهة (Non-synonymous) من المتوقع أن تؤثر على بنية ووظيفة البروتين متعدد

الببتايد المُحلَّل. وضعت ترجمة هذه الطفرات ضمن حوالي ١٢% من البروتين متعدد الببتايد الكامل (الذي يتكون من ٢٣٢٩ حمضاً أمينياً)، مسلطة الضوء على تأثيرات كل من الطفرات الصامتة (Silent) والطفرات المؤثرة (Missense) قسمت شجرة الارتباط المجاور (Neighbor-Joining) العزلات إلى أربع مجموعات مدعومة جيداً، تعكس الأنماط المصلية A, O, Asia 1, C وأنواع ال SAT. بينما أظهرت المنتجات المضخمة للجزء B ثلاثة أنماط مصلية A, A/O, O على الرغم من تحديد ٢٣ SNP (طفرة) على طوله البالغ ٢٧٥ زوج قاعدي، أظهر الجزء B قدرة تمييزية أقل بين الأنماط المصلية المحددة. فعلى الرغم من قدرته على تحديد مجموعة أحادية العرق (Clade) متماسكة لـ A/O وفصل مجموعات SAT 1-3، إلا أنه فشل في فصل الأنماط المصلية A و O و C دون تداخل، منتجاً مجموعة كبيرة مختلطة من ٢٠ فرعاً. وقد حدد العدد المحدود من المواقع المتغيرة في هذا المنتج المضخم القصير من قدرته على التحليل الوراثي.

على الرغم من أن المنتجات المضخمة للجزء C أشارت إلى اكتشاف نمط مصلي واحد فقط النمط (O)، إلا أن الجزء C بطول ١٣٠١ زوج قاعدي أظهر أداءً فائقاً. حيث حددت ١٥ SNP ثلاث مجموعات فرعية متميزة للنمط O، تدعم كل من تطابق التسلسل والتجميع الوراثي. فصلت الشجرة المعتمدة على الجزء C جميع أنماط فيروس الحمى القلاعية بوضوح إلى مجموعات غير متداخلة، وتتبع بقوة الأصول الآسيوية لعزلات الانتشار. نتائج معاملات مضادات الأكسدة كشفت النتائج عن انخفاض ذي دلالة إحصائية ($P \leq 0.05$) في مستويات SOD و CAT و GSH في جميع المجموعات المصابة مقارنة بالحيوانات السليمة في مجموعة التحكم. لكن مستويات SOD في مجموعات النمطين المصليين A و O أظهرت انخفاضاً ملحوظاً مقارنة بمستويات SOD في المجموعة المختلطة (A&O) كما سُجل ارتفاع عالٍ وذو دلالة إحصائية ($P \leq 0.05$) في مستويات MDA في جميع المجموعات المصابة مقارنة بالحيوانات السليمة.

كشفت نتائج اختبارات وظائف الكبد عن ارتفاع عالٍ ذي دلالة إحصائية ($P \leq 0.05$) في مستويات إنزيم ALT وإنزيم AST في مجموعات العجول التي شُخصت إصابتها بالنمط المصلي O من فايروس الحمى القلاعية، عند مقارنتها بمجموعة التحكم والحيوانات المصابة في المجموعات الأخرى النمط المصلي A والمختلط (A & O).

بالمقابل، لم تختلف مستويات إنزيم ALP في المجموعات المصابة بشكل ملحوظ عن بعضها البعض أو عن تلك في مجموعة التحكم. بينما ارتفعت مستويات إنزيم GGT ارتفاعاً ملحوظاً في الحيوانات المصابة بالنمط المصلي A مقارنة بالمجموعات الأخرى بما في ذلك مجموعة التحكم.



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة الشطرة
كلية الطب البيطري
فرع الاحياء المجهرية

التشخيص الجزيئي وتقييم بعض المعايير الكيموحيوية في العتر المعزولة من العجول المصابة بفايروس الحمى القلاعية في محافظة ذي قار

رسالة ماجستير مقدمة الى مجلس كلية الطب البيطري- جامعة الشطرة وهي جزء من متطلبات الحصول
على شهادة الماجستير في اختصاص علوم الطب البيطري (الاحياء المجهرية)

قدمت من قبل الطالبة:

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طب وجراحة بيطرية (١٩٩١)

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