



## Serological Detection of SAT-2 Serotype Foot and Mouth Disease Virus in Buffalo, Iraq

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**Submitted:** July 24, 2025  
**Revised:** December 9, 2025  
**Accepted:** December 26, 2025

**Abstract** This study aims to determine the SAT-2 serotype causing FMD outbreak in buffalo in Wassit and Dhi-Qar governorates, Iraq. This was done by clinical and serological methods. ELISA technology, which measures antibody levels in buffalo serum samples. 92 blood samples were collected from infected buffalo for antibody screening against FMDV serotype SAT-2 in infected animals which develop antibodies against the non-structural proteins (NSP) of the virus. Affected animals showed various clinical signs, such as fever, decreased appetite, mouth and foot vesicles, and lameness. According to the results of serological tests, the infection rate reached 85/92 positive samples (92.39%). The results showed, Age and sexspecific investigations of FMDV seropositivity did not reveal any statistically significant differences. The age groups (6-18 months and 18-36 months) had lowest infection rate (91.42%) and (92%) respectively, whereas the age group 36 months, had highest rate of infection at (93.75%). Also, the results revealed, the seroprevalence rates infection for males were (90.6%), which had lowest than females (93.33%). This study, confirms a widespread outbreak of FMD caused by the SAT2 serotype in buffalo populations across the Wassit and Dhi-Qar governorates of Iraq. The study's findings may offer helpful information about how FMD protection measures are established and carried out in Iraq.

**Keywords:** *Non-structural proteins, ELISA, SAT-2, Buffalo, FMD*

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**Introduction** One of the most important infectious diseases that can harm all animals with cloven hooves is FMD(1). This is because epidemics typically have significant economic impacts, including both direct livestock losses and indirect losses due to the high cost of containment and trade restrictions that often occur in the aftermath of an FMD outbreak, which is caused by the aphthovirus of the Picornaviridae family (2,3). The disease impairs the international transport traffic of live animals and their by-products, presenting a serious global danger.

Seven serotypes of FMDV are known to be present:” A, O, C, Asia1, SAT-1, SAT-2, and SAT-3’(4,5)Each genotype exhibits distinct subtypes that differ

antigenically as a result of a high mutation rate (4,6). FMD is widespread in numerous countries on the African continent, Asia, and Latin America (7,8). In Iraq, FMDV serotypes Asia 1, A, and O are constantly present and lead to occasional epidemics (9,10). In 2023, significant outbreaks caused by FMDV serotype SAT-2 occurred in the provinces of Iraq (11). All serotypes of FMD virus induce the same clinical symptoms(12,13), but immunization to one serotype does not protect others due to their broad antigenic variability (14). Through either direct or indirect contact with sick animals,

contaminated materials, or through the air, the disease can spread rapidly (15). Buffalo that are affected typically exhibit fever and discontinue ruminating. Saliva was shaped like a ruby. blisters on the lips, mouth, tongue, nose, and between the toes. The amount of milk produced has decreased (16,17). The infected animals can be discriminated from vaccinated by non-structural proteins (NSP) which can be detected only in infected animals (18). ELISA is an easy and efficient technique compared to virus isolation and RT-PCR. for high-throughput testing that does not require extensive technical expertise or specialised equipment (19,20). Early in 2023, animals all over Iraq were affected by a large FMD outbreak (21). The primary aim of the research was to determine the FMD virus SAT-2 Serotype present in Iraqi buffalo.

#### **material and Methods**

##### **Ethical approvals**

This study has been accepted by the Scientific Committee of the College of Veterinary Medicine at Al-Qadisiyah University in Iraq.

##### **Collection of samples**

Samples for this investigation were taken from 92 water buffalo that were suspected to have FMD. After a clinical examination, the buffalo displayed certain clinical signs. Using a sterile syringe, from each sick animal, 10 milliliters of blood was taken from the jugular vein, using a gel tube, and the sample was left for 30 minutes to clot. at 3000 rpm for 10 minutes collect the serum, transfer it to Eppendorf tubes (22), and store the samples at -20°C (23). The samples were properly labeled and transported to the laboratory under refrigeration for further processing.

#### **Sandwich Enzyme-Linked Immunosorbent Assay (ELISA)**

A total of 92 animals who exhibited symptoms of FMD underwent a ten-milliliter blood sample collection procedure, it was tested using an ELISA reaction to see if the non-structural (NSP) antibody was specific to FMDV serotype SAT-2.

#### **Result**

##### **Clinical Results**

In our study, we observed clinical signs suggestive of FMD, such as fever, dullness, and loss of appetite. Also, the result of clinical study revealed the infected animals suffering from, drooling saliva and hanging in long ropy strings up to the ground (**Figure 1-A**). Erosions, and ulcers on the nostrils and muzzle (**Figure 1-B**). The mouths of infected buffalos suggestive lesions of FMD. The gums, dental pad, hard palate, tongue, and

interior of the oral cavity contained ruptured vesicles, erosions, or ulcers (**Figure 2-A**), and skin lesions that were develop in the coronary region and interdigital cavity of the hoof (**Figure 2-B**).

**Figure 1: Shows clinical signs of FMD, A: saliva hanging**



**in long ropy strings, B: nostril lesion**



**Figure 2: Shows clinical signs of FMD, A: The dental pad, hard palate, and interior of the oral cavity contained ruptured vesicles, erosions, or ulcers B: lesion around the coronary band.**

##### **Serological Results**

The prevalence of FMD seropositivity in buffalo of both sexes and ages. Of the 92 buffalo shown 85 had positive FMDV SAT-2 antibodies, Figures 2 and 3, indicating that 92.39% of them were infected. According to the results of ELISA, the age groups of buffalo (6-18 months and 18-36 months) had lowest rate of infection affected with FMD respectively, with 32/35 (91.42 %), 23/25 (92%). as shown as in Figure (2) FMD, whereas the highest incidence was found among third group of age at 30/32 (93.75) but does not have statistical significance differences at ( $p > 0.05$ ).

The effect of sex on FMDV seropositivity was examined. Figure (3) shows the

seropositivity rates in males and females. The results showed that males had a seropositivity rate of 29/32 (90.6%) while females had a higher seropositivity rate of 56/60 (93.33%). However, the disparity in seropositivity rates among males and females did not reach significance in statistics ( $p > 0.05$ ).

Differentiation between vaccinated and infected animals is important for prevention planning. ELISA is used to detect infected animals by detecting non-structural proteins such as 3AB, 3ABC, 2C, and other targets. Antibody occurred on days 5–10 post-infection in all animals with no history of prior exposure to FMDV antigens(26,27).

Infected animals develop antibodies against nonstructural proteins (NSP) of the virus. In contrast, because vaccines are inactivated and purified, recipient animals only receive antibodies against structural proteins from vaccines(28)(29,30)

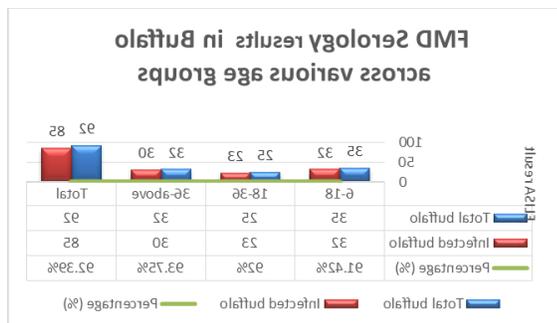
The results of serological tests showed the presence of antibodies to FMDV virus in 92.39% of the samples, indicating a high prevalence of the disease among the study population.

The high seropositivity in our results from this study is consistent with the high seropositivity reported in Al-Qadisiyah and Basrah (31,32). while the rates of infection in Al-Najaf and Diyala were (34%, 25.33%)(33,34). The high seropositivity rate observed in our study may be because all samples were collected at the optimal time from animals infected with a novel strain not previously registered in Iraq Moreover, the inclusion of animals lacking vaccination against this particular strain may have further contributed to the observed high seropositivity rate.

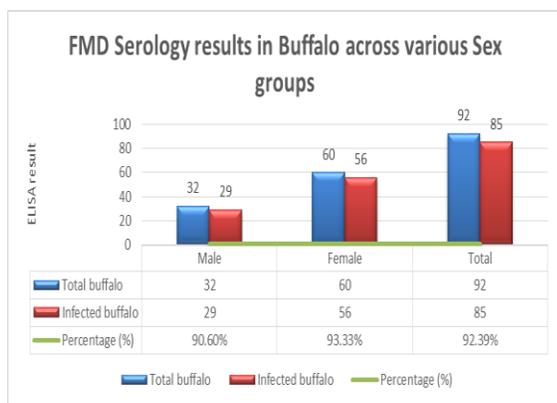
The infection rate of FMD according to age the results of this study, no statistically significant findings were discovered regarding the age of identified cases that agreed with other studies(35,36), that showed statistically no significant difference between age in seroprevalence of FMD.

The lack of significant differences in the high incidence of infection between different age groups might be due to the attribute of rearing by not separating young animals from their mothers as well as the communal grazing practices(34).

Although higher incidence of females than males, no statistically significant findings were discovered regarding the gender of identified cases. The results of this study agreed with many other epidemiological studies on FMD The results of this study agreed with many other epidemiological studies on FMD which found a slight increase in female infection (37,38) This may be attributed to physiological factors like lactation, pregnancy, and estrus(32,39)



**Figure (2).** FMD serology results in Buffalo across various Age (months)groups



**Figure (3)** FMD Serology results in Buffalo across various Sex groups

The clinical examination of the buffalo in this study revealed the presence of typical signs of FMD, including fever, reduced appetite, ulcers in the mouth and foot, and impaired mobility. These signs are consistent with previous studies and provide further evidence of the presence of FMDV in the studied population(24,25). This serological investigation aimed to determine the serotype of the FMD virus responsible for the recent epidemic in livestock in Iraq.



The gender prevalence of FMDV appears to contradict previous reports from others that showed increased seropositivity in male animals compared with female animals.

Identifying serotypes is very important, as it allows the implementation of appropriate prevention and control strategies(42).

The results of our study are important for developing and implementing plans to manage FMD in Iraq because the high prevalence of SAT-2 serotype in buffalo emphasizes the need for vaccination campaigns targeted against serotype spread throughout the country.

In this study, the use of ELISA as a diagnostic tool to detect FMDV antibodies in buffalo serum samples showed its effectiveness and reliability(43).

In summary, this investigation has shown that the present FMD outbreak in Iraq is caused by serotype SAT2 and this serotype's high prevalence highlights the need for targeted immunization.

### Conclusion

The results of our studies have proven the spread of the Sat -2 serotype for the first time in Iraq. The high prevalence of FMDV antibodies in the buffalo studied indicates the need for Iraq. The high prevalence of FMDV antibodies in the buffalo studied indicates the need for targeted vaccination campaigns, specifically the SAT-2 serotype. The use of ELISA as a diagnostic tool proves to be effective and reliable in identifying FMDV antibodies in serum samples from buffalo.

### Conflict of interest

There is no conflict of interest in this study as stated by the authors.

### Acknowledgment

Not applicable.

### Funding source

This research had no specific fund; however, it was self-funded by the authors.

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