

Available Online at EScience Press

# **Journal of Zoo Biology**

ISSN: 2706-9761 (Online), 2706-9753 (Print) https://esciencepress.net/journals/JZB

# SURVEILLANCE STUDY OF FOOT-AND-MOUTH DISEASE IN UNGULATE SPECIES IN DUBAI SAFARI PARK 2020-2023

### Abirami Kumar, Alaa Al Saleh, Anjan Talukdar, Jameela H. Ghazaly, Jayadevan Malathi, Muna A. Alhajeri, Murad B. M. Mustafa, Varun Anandan

Veterinary Hospital, Dubai Safari Park Section, Dubai Municipality Dubai, U.A.E.

# ARTICLE INFO

#### **Article History**

Received: July 02, 2024 Revised: October 19, 2024 Accepted: November 29, 2024

# Keywords

FMDV Foot and mouth disease Aphthovirus Family *Picornaviridae* Serotypes (O, A, C, SAT 1, SAT 2, SAT 3, Asia 1) Dubai Safari Park Ungulates Cloven-hoofed animals Immunochromatography

# INTRODUCTION

Foot-and-Mouth Disease (FMD) poses a significant challenge to the global livestock industry due to its highly contagious nature and the substantial economic losses it incurs. The FMD virus (FMDV), classified under the Picornaviridae family and the genus Aphthovirus, is characterized by a single-stranded RNA genome encapsulated within an icosahedral protein capsid composed of four structural proteins (VP1, VP2, VP3, and VP4) and several non-structural proteins (NSPs) essential for its replication and pathogenesis (Velazquez-Salinas et al., 2020; Bertram et al., 2020). The virus's ability to persist in wild ungulates, which often exhibit no clinical signs of infection, complicates control efforts, as these animals can shed the virus through various bodily fluids, facilitating its transmission to domestic livestock (Gunasekara et al., 2021; Omondi et al., 2020). This persistence in wildlife reservoirs underscores the need

Foot-and-Mouth Disease (FMD) is a highly contagious viral disease affecting clovenhoofed animals, posing significant economic and ecological challenges. This study presents a surveillance analysis of FMD in ungulate species at Dubai Safari Park from 2020 to 2023. A total of 572 diagnostic tests, including lateral immunochromatographic assays (373) and polymerase chain reaction (PCR) tests (199), were conducted across 32 species. All test results were non-reactive, indicating the absence of FMDV infections during the study period. These findings highlight the effectiveness of biosecurity measures and routine vaccination in maintaining a disease-free status within the park. The study underscores the necessity of ongoing surveillance, robust preventive strategies, and periodic vaccine evaluations to mitigate potential future outbreaks.

ABSTRACT

\*Corresponding Author: Murad Basheer Mustafa Email: mbmustafa@dm.gov.ae © The Author(s) 2024.

for effective surveillance and control strategies that consider both domestic and wild populations (Wekesa *et al.*, 2015).

Various wild species, primarily within the order *Artiodactyla*, have been reported to be susceptible to FMD infection. This includes families such as Bovidae, Cervidae, Suidae, Tayasuidae, Camelidae, Giraffidae, and Antilocapridae. Additionally, infection has been observed in species from other orders, including *Proboscidea*. The pathogenicity of FMD in wildlife varies widely, ranging from asymptomatic cases to fatal outcomes (Grubman and Baxt, 2004).

This study aimed to assess the prevalence, transmission dynamics, and genetic variation of FMDV in ungulate species at Dubai Safari Park from 2020 to 2023. A comprehensive surveillance program was implemented, focusing on immunochromatographic and molecular diagnostic assays for effective FMDV detection. A total of 572 tests were conducted, including 373 lateral immunochromatographic assays and 199 polymerase chain reaction (PCR) tests (Figure 1). The study's findings provide insights into the effectiveness of current vaccination campaigns and inform strategies to mitigate FMD outbreaks in the region.



Figure 1. Number of rapid and PCR tests performed during 2020-2023.

The use of immunochromatographic assays targeting NSP antibodies is crucial for detecting prior exposure to FMDV, while PCR testing allows for the molecular detection of FMDV RNA, specifically targeting the 5' untranslated region (5'UTR) Hwang et al. (2021). These methodologies are vital for differentiating between vaccinated and infected animals, which is essential for informing vaccination strategies and control. The evaluation of the specificity and sensitivity of these diagnostic tests is paramount to ensure reliable surveillance outcomes. Comparative analyses of the diagnostic performance of immunochromatographic assays and PCR testing provide insights into their respective utilities and limitations in detecting FMDV infections and antibody responses among ungulate populations (Armson et al., 2020; Wong et al., 2020).

Understanding the genetic variation of FMDV strains is essential for elucidating the molecular mechanisms underlying virulence and vaccine efficacy, which is critical for developing effective control strategies (Lee *et al.*, 2024; Sente<sup>1</sup> *et al.*, 2023). The assessment of vaccination protocols within Dubai Safari Park is also crucial in determining the effectiveness of vaccination campaigns in providing protective immunity against prevalent FMDV strains in the region. The ongoing evolution of FMDV strains necessitates continuous monitoring and adaptation of vaccination strategies to mitigate the risks associated with FMD outbreaks (Kim and Pak, 2020; Kim *et al.*, 2017). Overall, the integration of robust surveillance methodologies and thorough evaluation of vaccination strategies is essential for the effective management of FMD, thereby safeguarding the health and welfare of livestock and the economic stability of the livestock industry (Wong *et al.*, 2020; Colenutt *et al.*, 2025).

Susceptibility among wildlife species raises several concerns, distinct from those associated with domestic livestock. The primary concern for FMD-free nations is the potential complication of wild animal infections during an outbreak. Among wild Bovidae, the African (Cape) buffalo (*Syncerus caffer*) has been the most extensively studied for FMD pathogenesis. This is due to its susceptibility to persistent infection with South African strains (SAT 1, 2, and 3) of FMDV, which can persist for up to five years in an individual and up to twenty years in an isolated herd. Natural transmission from buffalo to cattle and impala (*Aepyceros melampus*) has also been documented (Bastos *et al.*, 2000).

FMD infection is typically subclinical, with an incubation period of 2–14 days. In domesticated species, clinical signs include fever (lasting 2–3 days), vesicles followed by erosions or ulcers on the tongue, lips, oral mucosa, teats, and between the hooves, as well as excessive stringy saliva in cases of significant oral involvement (Mahy, 2005). Additional symptoms may include decreased appetite, lameness, abortion, and, in severe cases, sudden death due to myocarditis in newborns. While rare, sudden death has also been observed in some severely affected wildlife species. The severity of illness varies between species, with some, such as sheep, exhibiting minimal clinical signs. Viral shedding may occur before the onset of symptoms.

The primary site of FMDV infection and replication is the mucosa of the pharynx (Figure 2), though the virus can also enter through skin lesions or the gastrointestinal tract. Once it spreads through the lymphatic system, the virus replicates in the epithelial tissues of the mouth, muzzle, teats, feet, and damaged skin, leading to vesicle formation. These vehicles typically rupture within 48 hours. More than 50% of ruminants that recover from illness—or vaccinated individuals exposed to the virus— can carry viral particles in the pharyngeal region. Cattle may become persistently infected in the pharynx, though there is no evidence of transmission. In contrast, African buffalo can serve as long-term viral shedders and

contribute to transmission (Thurber *et al.*, 2023; Aiello *et al.*, 2016).



Figure 2. Simplified pathogenesis of FMDv.

# **MATERIALS AND METHODS**

### **ONE STEP Foot and Mouth Disease NSP Antibody Test**

The Food and Mouth Disease NSP antibody test was conducted using Rapid FMD NSP Ab Test Kit which is an immunochromatographic assay for the qualitative detection of FMDV antibody in whole blood, plasma, or serum from cattle, pigs, goats, and sheep.

The Rapid FMD NSP Ab Test Kit has two letters: test line (T) and control line (C) on the surface of the device. The test line and control line in the result window are not visible before applying any samples. The control line is a reference line indicating that the test is performing properly. The control line must appear every time the test is performed. A purple test line would appear in the result window if the FMD NSP antibodies were present in the sample. The highly selective recombinant nonstructural protein (NSP) antigens are used as a capture material in the assay. These can detect FMDV antibodies with high accuracy and differentiate infected animals from those that have been vaccinated (BIONOTE, 2019).

# Limitation of the Test

The Rapid FMD NSP Ab Test Kit can detect the presence of antibodies against field-infected Foot-and-Mouth Disease Virus. Although the test kit demonstrates high accuracy in detecting Foot-and-Mouth Disease NSP antibodies, a low incidence of false results can occur. In cases of questionable results, additional clinical and/or laboratory tests may be required for confirmation. As with other diagnostic assays, a conclusive clinical diagnosis requires comprehensive evaluation of all clinical and laboratory findings by a qualified veterinarian. The reading window may exhibit a light pink background coloration, which does not compromise the accuracy of the results (BIONOTE, 2019).

# **Animals Sample Collection**

Samples from different animal species were collected, including Addax, Axis Deer, Arabian Gazelle, Arabian Oryx, Sand Gazelle, Barbary Sheep, Indian Blackbuck, Bongo, Asiatic Water Buffalo, Cameroon Sheep, Common Eland, Holstein Friesian Cow, Gemsbok, Greater Kudu, Hog Deer, Impala, European Mouflon Sheep, Mountain Gazelle, Nadji Sheep, Nilgai, Nubian Ibex, Pygmy Goats, Sable Antelope, Sambars, Scimitar Horned Oryx, Springbok, and Eastern White Bearded Wildebeest.

# **Collection Of Blood Sample from Ungulate Species**

Blood samples were collected by trained personnel proficient in the required techniques. Proper animal restraint was ensured to minimize stress and prevent injuries to both the animal and the handler. In cases where the procedure caused more than momentary discomfort, anesthetics were administered as needed. The blood collection kit used in the procedure is shown in Figure 3.



Figure 3. Sample collection kit.

To facilitate blood aspiration, the animal was positioned correctly for shearing. The designated area was shaved to approximately 4 inches wide by 8 inches long to ensure clear visibility of the vein. If assistance was required, an additional handler supported the procedure by turning the animal's head 30 degrees to the side while holding it under the jaw, allowing easier vein access. In some cases, body restraint was also necessary, as illustrated in Figures 4–7, which depict blood sampling from various species.



Figure 4. Sample collection from an Impala-note the physical restraining technique.



Figure 5. Sample collection from a Nubian ibex.



Figure 6. Sample collection from a Springbok.



Figure 7. Sample collection from a Springbok.

The vein was located by drawing an imaginary line from the middle of the animal's eye down to the side of its neck. To enhance visibility, pressure was applied below the midpoint of the shaved area using the thumb or fingers, causing the vein to become more prominent. Once identified, a small amount of alcohol was poured sprayed over the area to disinfect the site.

Before inserting the needle, the area was sanitized to prevent bacterial contamination by spraying alcohol over the site. Care was taken to avoid wiping over an already cleaned area, preventing recontamination.

For blood collection, a 20-gauge needle attached to a 5 mL syringe was used. Both the syringe and needle were sterile and securely attached to prevent contamination. The needle cap was removed carefully to avoid accidental injury. The needle was inserted at the lowest possible point on the exposed vein, allowing for alternative puncture sites if initial attempts were unsuccessful.

To confirm vein penetration, the syringe plunger was gently pulled back. If no blood appeared, the needle was repositioned slightly to locate the vessel. Once blood flowed into the syringe, collection continued until the required volume was obtained.

Following collection, pressure on the vein was released, the needle was withdrawn, and gentle pressure was applied to the insertion site to minimize bleeding. The collected sample was immediately transferred into the appropriate blood collection tube using the needle stopper (Figures 5–6).

# **Collection and Preparation of Sample**

Whole blood, serum, or plasma was used as the sample for this test. Whole blood was collected into an anticoagulant tube, and if not tested immediately, it was refrigerated at 2–8°C and used within 24 hours. For serum collection, blood was drawn into a plain collection tube without anticoagulants such as heparin, EDTA, or sodium citrate, left undisturbed for 30 minutes to allow coagulation, and then centrifuged to obtain the serum supernatant. Plasma samples were obtained by collecting whole blood into a tube containing heparin, EDTA, or sodium citrate, followed by centrifugation. If serum or plasma samples were not tested immediately, they were refrigerated at 2–8°C, and for long-term storage, freezing was recommended. Before use, frozen samples were brought to room temperature (15–30°C). Samples containing precipitates, which could cause inconsistent test results, were clarified before analysis (BIONOTE, 2019).

# **Serum Preparation**

Whole blood was collected in a clot activator vacutainer tube. After collection, the blood was allowed to clot by leaving it undisturbed at room temperature for 15-30 minutes. The clot was then removed by centrifuging at  $1,000-2,000 \times g$  for 10 minutes in a refrigerated centrifuge. For immunochromatographic testing, specific precautions were taken: animals were fasted to prevent lipemic serum, and appropriate sampling materials were used to avoid hemolysis by ensuring the correct syringe size.

# RT-qPCR Assay for the Diagnosis of Foot and Mouth Disease

Nucleic acid amplification and detection techniques have become essential tools in modern biological research. Polymerase chain reaction (PCR) is a widely used molecular biology technique for amplifying and detecting DNA and RNA sequences. PCR is highly sensitive and requires minimal template material for the detection and amplification of specific sequences. Quantitative PCR (qPCR) is commonly employed to detect, characterize, and quantify nucleic acids. In RT-qPCR, RNA transcripts are first reverse transcribed into complementary DNA (cDNA), as described earlier, before proceeding with qPCR. Like standard PCR, DNA amplification occurs in three repeating steps: denaturation, annealing, and elongation. However, in qPCR, fluorescent labeling enables real-time data collection throughout the PCR process. Fluorescence is measured during each cycle, increasing proportionally with the amount of replicated DNA, allowing for precise quantification. The change in fluorescence over time is used to determine the amount of amplicon produced in each cycle.

Foot-and-mouth disease (FMD) is caused by an *Aphthovirus* of the *Picornaviridae* family, with seven known strains (A, O, C, SAT1, SAT2, SAT3, and Asia1) endemic in different regions worldwide. The genetic material of *Aphthovirus* is stored as RNA. In FMD PCR analysis, the viral RNA is first converted to its complementary DNA sequence through reverse transcription (RT). The detection of amplified DNA is facilitated using a fluorophore targeting the FMD virus, while VIC dye is used to measure the internal extraction control.

At Dubai Safari Park, a thermal cycler was utilized for PCR analysis. This six-channel real-time PCR instrument, featuring five fluorescence detection colors and one FRET channel, combined advanced optical technology with precise temperature control to ensure sensitive and reliable detection for both single-plex and multiplex reactions (Genesig Advanced Kit, 2018).

# **Sample Quality**

Strict sample standards were followed to ensure highquality PCR analysis. EDTA blood samples were used for FMD PCR analysis, and hemolyzed samples were identified as a potential threat to sample integrity. To prevent hemolysis, appropriately sized needles and syringes were selected based on the animal species and the blood collection site. Using a small needle with a larger syringe could create excessive pressure, leading to cell rupture and hemolysis. After collection, samples were transferred to appropriately sized EDTA blood tubes, ensuring the correct anticoagulant-to-blood ratio. The samples were then mixed properly—at least ten times—to prevent clot formation (Genesig Advanced Kit, 2018).

# **Genetic Identification Details**

The PCR kit for FMD virus (FMDV) genome detection was designed for *in vitro* quantification of FMDV genomes. The kit had a broad detection profile, with primers showing 100% homology with over 95% of the reference sequences available in the NCBI database at the time of design. The target sequence within the 5' untranslated region (5' UTR) had previously been established as a reliable genetic marker for all FMDV serotypes in clinical real-time PCR-based studies (Donald P. King et al., 2006). A comprehensive bioinformatics analysis confirmed that the primers and probe sequences in this kit exhibited 100% homology with more than 95% of clinically relevant reference sequences in the NCBI database (Genesig Advanced Kit, 2018).

# Rapid Test for FMD NSP Antibodies Serum and Plasma Sample

All reagents and samples were equilibrated to room temperature  $(15-30^{\circ}C)$  before testing. The test kit was removed from the foil pouch and placed on a flat, dry surface. Using a disposable capillary tube,  $10 \ \mu L$  of serum or plasma was added to the sample hole (marked "S" on the test device) and allowed to incubate for 1 minute (Figure 8). Subsequently, three drops of developing buffer were dispensed into the designated developing buffer hole. The test was monitored for 15 minutes to observe sample flow across the result window. If no sample flow was observed within 1 minute, an additional drop of developing buffer was added. Results were interpreted at the 15-minute mark (BIONOTE, 2019).



Figure 8. Immunochromatographic assay analysis.

### Whole Blood Sample

All reagents and samples were brought to room temperature (15–30°C) before testing. The test kit was removed from the foil pouch and placed on a stable surface. Three drops of whole blood diluent were added into a test tube, followed by the addition of 30  $\mu$ L of whole blood using a disposable dropper. The sample was mixed for 1 minute, and 10  $\mu$ L of the mixture was transferred to the sample hole using a capillary tube. After 1 minute, three drops of developing buffer were dispensed into the designated buffer hole, and the test was allowed to develop for 15 minutes. If no sample migration was observed, an additional drop of developing buffer was added (BIONOTE, 2019).

# **RT-PCR for FMD Virus Detection**

# Nucleic Acid Extraction

Between 2020 and 2021, rapid test techniques were

employed in the laboratory. Subsequently, PCR-based methods were developed to enhance diagnostic accuracy (Figure 9). RNA extraction was performed using 200 µL of whole blood, which was transferred to the sample well (1st and 7th well) of a 96-well plate (Figure 10). To prevent contamination, filter tips were used, and all procedures were conducted in a Class II biosafety cabinet. The extraction plate was processed using an automated nucleic acid extraction machine, with two strip tip combs assigned per plate. The extraction process was completed within approximately 30 minutes. The extracted RNA was either stored at - 20°C for up to one month or processed immediately for amplification.



Figure 9. Rapid testing and PCR testing performed during 2020-2023.



Figure 10. Loading the samples in the extraction machine.

# **RT-qPCR Protocol for FMD Virus**

RT-PCR was performed following the kit protocol (Table 1). The amplification program was set as follows:

			0
Stage	Temperature	Time	Cycle Number
1	55°C	10 min	1
2	95°C	2 min	1
3a	95°C	10 sec	50
3b	60°C	60 sec	50

Table 1. Thermal cycler reference settings for FMD virus.

As shown in Table 1, the amplification process was programmed in three stages. Stage 1 was set at 55°C for 10

minutes in cycle 1. Stage 2 was set at 95°C for 2 minutes. Stage 3 consisted of two substages: the first substage was set at 95°C for 10 seconds, followed by the second substage at 60°C for 60 seconds. Additionally, 50 repeated cycles were programmed in Stage 3, resulting in a total of 50 cycles (one initial cycle followed by 49 repeated cycles). The total reaction volume was 20  $\mu$ L, comprising 5  $\mu$ L of the sample and 15  $\mu$ L of the supermix. After verifying all parameters, the program was saved in the system (Figure 11).



Figure 11. Thermal cycler programming setup.

# **RT-PCR Plate Setup**

Following the kit protocol, a new plate setup was initiated. Sample wells were assigned as "Unknown," and fluorophores were selected according to the kit instructions. FAM and VIC fluorophores were designated for target and internal control detection, respectively (Figure 12). The last three wells were reserved for negative control, positive control, and No Template Control (NTC). The finalized setup was saved in the system (Figure 13).

# Sample Amplification and Result Interpretation

Extracted nucleic acids were processed in a biosafety cabinet. The detection kit was thawed from -20°C storage and allowed to reach room temperature. All reagents,

including enzyme mix, RT-PCR reaction buffer, positive control, and negative control, were vortexed and briefly centrifuged to ensure homogeneity. A master mix was prepared in a sterile, RNase/DNase-free microcentrifuge tube, vortexed, and centrifuged before being aliquoted into PCR wells. A total of 15  $\mu$ L of supermix was added to each well, followed by 5  $\mu$ L of extracted sample. Control wells contained either the FMDV synthetic positive control, negative control, or NTC. After sealing the wells, amplification was initiated using the pre-programmed FMDV protocol. The samples were labeled in the system and results were generated (Figure 14).

Select Fluorophores				×	
	Channel	Fluorophore	Selected	Color	
	1	FAM	<b>v</b>		
		SYBR			
	2	HEX			
		TET			
		Cal Orange 560			
		Cal Gold 540			
		VIC	<b>v</b>		
	3	ROX			
		Texas Red			
		Cal Red 610			
		Tex 615			
	4	Cy5			
		Quasar 670			
	5	Ouasar 705			-
				OK Cano	cel

Figure 12. Fluorophore setup.



Figure 13. Positive, negative and no-template control plate setup.



Figure 14. Loading samples in the thermal cycler. **RESULTS** 

The rapid test for FMD NSP antibodies was successfully implemented on serum, plasma, and whole blood samples, providing results within 15 minutes. Positive samples exhibited distinct test bands, while negative samples showed only the control band. The test demonstrated a sensitivity of over 90%, with a low rate of false-negative results. The specificity was also high, reducing the likelihood of cross-reaction with other viral infections.

RT-PCR results confirmed the presence of FMD viral RNA in positive samples. The amplification of the target gene (FAM) was observed in all positive samples, with a Cq value  $\leq$ 30 indicating a strong positive quantitative result. Internal controls (VIC) were consistently amplified, validating the integrity of the test. The interpretation criteria are summarized in Table 2.

Table 2. Results interpretation for FMD virus.					
Target	Internal	Positive	Negative	Interpretation	
(FAM)	Control (VIC)	Control	Control		
≤30	+ / -	+	-	POSITIVE QUANTITATIVE RESULT (copy number	
				calculated)	
>30	+	+	-	POSITIVE QUANTITATIVE RESULT (copy number	
				calculated)	
>30	-	+	-	POSITIVE QUALITATIVE RESULT (copy number not	
				reported due to potential poor sample extraction)	
+ / -	+	-	-	NEGATIVE RESULT	
+ / -	+	+	≤35	EXPERIMENT FAILED (contamination detected)	
-	-	-	-	SAMPLE PREPARATION FAILED	

Further analysis showed that the positive control templates exhibited expected amplification between Cq 16–23, confirming assay performance. The experiment failed when negative control amplification was observed

at Cq >35, suggesting possible contamination or technical errors. Overall, RT-PCR demonstrated a high level of specificity and sensitivity, making it a reliable diagnostic tool for FMD detection. The combination of rapid testing and RT-PCR provided a comprehensive diagnostic approach, allowing for both immediate screening and confirmatory molecular analysis. This methodology significantly reduced the time required for FMD diagnosis, enabling faster response and management strategies in affected populations.

### DISCUSSION

The findings of this surveillance study provide valuable insights into the prevalence and risk of Foot-and-Mouth Disease (FMD) among ungulate species in Dubai Safari Park from 2020 to 2023. A total of 572 tests were conducted, including both lateral immunochromatographic assays and polymerase chain reaction (PCR) tests, to ensure comprehensive disease monitoring. Fortunately, our results during that period were all non-reactive. The lateral immunochromatographic assays and molecular assays were both negative. Around thirty-two species were tested, and they were all proven to be negative. This confirms that our animals are free from disease in relation to the Foot and Mouth virus. Table 3 lists all the details of our animals.

Table 3. Species tested for both immunochromatographic assays and PCRs.

Sr.	Spacies	No. of Rapid	No. of	Total no. of tests performed
	Species	tests	PCR tests	for each species
1	Addax	19	3	22
2	Axis Deer	6	20	26
3	African elephant	0	1	1
4	Arabian gazelle	6	0	6
5	Arabian Oryx	33	1	34
6	Sand gazelle	38	10	48
7	Bannur sheep	0	3	3
8	Barbary sheep	19	15	34
9	Indian Blackbuck	38	16	54
10	Bongo	2	1	3
11	Asiatic water buffalo	1	0	1
12	Dromedary	0	2	2
13	Cameroon sheep	12	27	39
14	Common eland	5	2	7
15	Holstein Friesian cow	1	1	2
16	Donkey	0	1	1
17	Gemsbok	4	1	5
18	Greater kudu	4	7	11
19	Hog deer	12	0	12
20	Impala	31	27	58
21	European mouflon sheep	4	14	18
22	Mountain gazelle	5	0	5
23	Najdi sheep	7	0	7
24	Nilgai	4	2	6
25	Nubian ibex	38	2	40
26	Pygmy goat	16	2	18
27	Sable antelope	3	1	4
28	Sambar	3	0	3
29	Scimitar horned oryx	28	7	35
30	Springbok	29	27	56
31	Common warthog	0	2	2
32	Eastern white bearded Wildebeest	1	4	5

Combined with that, is our annual vaccination program that ensures that these ungulates get their sufficient immunity (Aslam and Alkheraije, 2023; Grubman and Baxt, 2004). Moreover, Figures 14 and 15 provide information regarding the total number of tests performed in each species and the type of tests performed in each species.



Figure 14. Total number of tests performed in each species.



Figure 15. Types of tests performed in each species.

The negative results observed throughout the study period suggest that the biosecurity measures and vaccination protocols implemented within Dubai Safari Park have been effective in preventing FMD infections. Routine surveillance, coupled with annual vaccinations, plays a crucial role in mitigating potential disease outbreaks. Given that FMD can spread rapidly among cloven-hoofed animals and has significant economic and ecological consequences, continuous monitoring remains a key strategy for ensuring the health and welfare of the park's animal population (Ijaz *et al.*, 2022; Eltahir *et al.*, 2024).

Comparative analysis of immunochromatographic assays and PCR testing demonstrated the reliability of these diagnostic techniques in detecting FMDV infections. The lateral flow assays targeting non-structural protein (NSP) antibodies provided a rapid means of screening for previous exposure, while PCR allowed for the direct detection of viral RNA. Both approaches proved effective in confirming the absence of FMDV in the tested species (Anjum *et al.*, 2004; Bronsvoort *et al.*, 2008). The use of such complementary diagnostic techniques enhances the robustness of surveillance programs and enables early detection should an outbreak occur in the future.

In light of these findings, the importance of continued surveillance cannot be overstated. Although no cases of FMD were detected during the study period, ongoing monitoring is necessary to identify any potential threats and to ensure timely intervention measures. Additionally, while the park's vaccination program has contributed to maintaining disease-free status, periodic assessments of vaccine efficacy should be undertaken to account for potential antigenic variation in circulating FMDV strains (Navid *et al.*, 2018; Metwally *et al.*, 2024).

The results of this study also highlight the significance of stringent quarantine measures and biosecurity protocols when introducing new animals into the park. Given that wildlife species can act as reservoirs for FMDV, preventing the introduction of infected individuals is paramount. The negative test results reaffirm that such precautions have been successfully implemented.

Future research efforts should focus on genetic characterization of regional FMDV strains to further enhance vaccine strategies and improve diagnostic capabilities. Additionally, serological studies can be expanded to include a broader range of wildlife species to evaluate potential exposure risks beyond the park's confines (Ijaz *et al.*, 2022; Aslam and Alkheraije, 2023).

# CONCLUSION

the surveillance study of Foot-and-Mouth Disease in ungulate species at Dubai Safari Park (2020-2023) underscores the importance of proactive monitoring and effective disease control measures in safeguarding both domestic and wild animal populations. While this research aimed to enhance the understanding and containment of Foot-and-Mouth Disease, the lack of significant data highlights the complexities of disease surveillance, particularly within wildlife ecosystems. Despite the absence of conclusive findings, the study reinforces the need for sustained surveillance efforts, robust prevention protocols, and collaborative initiatives to protect animal health and well-being. Moving forward, advancing research methodologies and strengthening surveillance strategies will be crucial for gaining deeper insight into infectious disease dynamics in wildlife, ultimately enabling more effective control and mitigation measures to protect both animal and human populations. ACKNOWLEDGEMENT

We extend our sincerest gratitude to the management of Dubai Safari Park for their steadfast support and guidance, which have been instrumental throughout this research endeavor. Our deep appreciation also goes to the dedicated hospital team—including veterinarians, nurses, and hospital keepers—as well as the professionals in the Zoology Department for their expertise and collaboration. Finally, we sincerely thank all the committed employees of Dubai Safari Park whose collective efforts made this surveillance study possible. Without their unwavering dedication and support, this research would not have been accomplished.

# REFERENCES

- Aiello, S. E., M. A. Moses and D. G. Allen. 2016. The Merck veterinary manual. Merck & Company: White Station, NJ, USA. p. 630-631.
- Anjum, R., M. Hussain, A. Zahoor, H. Irshad and U. Farooq. 2004. Epidemiological analyses of foot and mouth disease in Pakistan. Econ Survey, 5: 648-51.
- Armson, B., S. Gubbins, V. Mioulet, I. A. Qasim, D. P. King and N. A. Lyons. 2020. Foot-and-mouth disease surveillance using pooled milk on a large-scale dairy farm in an endemic setting. Frontiers in Veterinary Science, 7: 264.
- Aslam, M. and K. A. Alkheraije. 2023. The prevalence of footand-mouth disease in Asia. Frontiers in Veterinary Science, 10: 1201578.
- Bastos, A. D. S., C. I. Boshoff, D. F. Keet, R. G. Bengis and G. R. Thomson. 2000. Natural transmission of foot-andmouth disease virus between African buffalo (Syncerus caffer) and impala (Aepyceros melampus) in the Kruger National Park, South Africa. Epidemiology & Infection, 124: 591-98.
- Bertram, M. R., S. Yadav, C. Stenfeldt, A. Delgado and J. Arzt. 2020. Extinction dynamics of the foot-and-mouth disease virus carrier state under natural conditions. Frontiers in Veterinary Science, 7: 276.
- BIONOTE. 2019. BIONOTE, Doc. No.: I2802-6EC, Issued date: Jul.01, 2019, BIONOTE.
- Bronsvoort, B., S. Parida, I. Handel, S. McFarland, L. Fleming, P. Hamblin and R. Kock. 2008. Serological survey for foot-and-mouth disease virus in wildlife in eastern Africa and estimation of test parameters of a nonstructural protein enzyme-linked immunosorbent assay for buffalo. Clinical and Vaccine Immunology, 15: 1003-11.
- Colenutt, C., A. Shaw, S. N. Esemu, A. J. Kfusi, B. Willington Ojong, E. Brown, J. Wadsworth, N. J. Knowles, D. P.

King and L. M. Ndip. 2025. Detection and genomic characterisation of foot-and-mouth disease virus serotypes circulating in Cameroon using environmental sampling. Scientific Reports, 15: 2834.

- Eltahir, Y. M., H. Z. A. Ishag, J. Wadsworth, H. M. Hicks, N. J. Knowles, V. Mioulet, D. P. King, M. S. Mohamed, O. K. Bensalah and M. F. Yusof. 2024. Molecular Epidemiology of Foot-and-Mouth Disease Viruses in the Emirate of Abu Dhabi, United Arab Emirates. Veterinary Sciences, 11: 32.
- Genesig Advanced Kit. 2018. Quantification of Foot & Mouth Disease Virus genomes. Primerdesign Ltd. Place Published.
- Grubman, M. J. and B. Baxt. 2004. Foot-and-mouth disease. Clinical microbiology reviews, 17: 465-93.
- Gunasekara, U., M. R. Bertram, D. H. Dung, B. H. Hoang, N. T. Phuong, V. V. Hung, N. V. Long, P. Q. Minh, L. T. Vu and P. V. Dong. 2021. Use of slaughterhouses as sentinel points for genomic surveillance of foot-and-mouth disease virus in southern vietnam. Viruses, 13: 2203.
- Hwang, Y.-J., K.-K. Lee, J.-W. Kim, K.-H. Chung, S.-J. Kim, W.-S. Yun and C.-S. Lee. 2021. Effective diagnosis of footand-mouth disease virus (FMDV) serotypes O and A based on optical and electrochemical dual-modal detection. Biomolecules, 11: 841.
- Ijaz, M., M. M. Ali, F. Awan, M. Ishaq and A. Ahmad. 2022. FMD virus spillover from domestic livestock caused outbreak in captive wild ungulates: first report from Pakistan. Acta Tropica, 231: 106439.
- Kim, E., T. Carpenter, S. Rowanowski and N. Cogger. 2017. Criteria and indicators for foot and mouth disease control strategy decision-making in Asia–Oceania countries. Rev Sci Tech, 36: 867-78.
- Kim, E. and S. Pak. 2020. Multi-criteria decision analysis to evaluate foot and mouth disease control strategies with the perspectives of Chief Veterinary Officers in the Asia-Oceania region. Revue Scientifique et Technique (International Office of Epizootics), 39: 1003-15.
- Lee, M. J., S. Shin, H. W. Kim, M.-K. Ko, S. H. Park, S.-M. Kim and J.-H. Park. 2024. Oral Administration of Zinc Sulfate with Intramuscular Foot-and-Mouth Disease Vaccine Enhances Mucosal and Systemic Immunity. Vaccines, 12: 1268.

- Mahy, B. W. J. 2005. Introduction and history of foot-andmouth disease virus. Foot-and-Mouth Disease Virus: 1-8.
- Metwally, S., J. Drewe, G. Ferrari, J. Gonzales, M. McLaws, M. Salman and B. Wagner. 2024. Practical surveillance guidelines for the progressive control of foot-andmouth disease and other transboundary animal diseases.
- Navid, M., U. Farooq, A. Latif, M. Awais, M. Anwar, M. Akhtar and A. Zahur. 2018. Prevalence of foot and mouth disease virus in apparently healthy buffaloes brought to Islamabad slaughterhouse in Pakistan.
- Omondi, G. P., F. Gakuya, J. Arzt, A. Sangula, E. Hartwig, S. Pauszek, G. Smoliga, B. Brito, A. Perez and V. Obanda. 2020. The role of African buffalo in the epidemiology of foot-and-mouth disease in sympatric cattle and buffalo populations in Kenya. Transboundary and emerging diseases, 67: 2206-21.
- Sente<sup>1</sup>, C., B. Rosado-Ramos, S. Kerfua, I. Tuwangye, C. Brookshire, P. Kalumba<sup>1</sup>, R. S. Nakabuye<sup>1</sup>, S. Namirimu<sup>1</sup>, A. Tamale<sup>1</sup> and S. Reichley. 2023. Seroprevalence of Foot and Mouth Disease in apparently healthy beef cattle in Uganda post the 2021 outbreak.
- Thurber, M., R. R. Sim and R. Sadler. 2023. Infectious diseases of concern to captive and free ranging wildlife in North America. The Infectious Disease Committee of the American Association of Zoo Veterinarians (AAZV). Place Published. pp.606-09.
- Velazquez-Salinas, L., F. N. Mwiine, Z. Ahmed, S. Ochwo, A. Munsey, J. J. Lutwama, A. M. Perez, K. VanderWaal and E. Rieder. 2020. Genetic diversity of circulating foot and mouth disease virus in Uganda cross-sectional study during 2014–2017. Frontiers in Veterinary Science, 7: 162.
- Wekesa, S. N., A. K. Sangula, G. J. Belsham, K. Tjornehoj, V. B. Muwanika, F. Gakuya, D. Mijele and H. R. Siegismund.
  2015. Characterisation of recent foot-and-mouth disease viruses from African buffalo (Syncerus caffer) and cattle in Kenya is consistent with independent virus populations. BMC veterinary research, 11: 1-15.
- Wong, C. L., C. Y. Yong, H. K. Ong, K. L. Ho and W. S. Tan. 2020. Advances in the diagnosis of foot-and-mouth disease. Frontiers in Veterinary Science, 7: 477.

Publisher's note: EScience Press remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License. To view a copy of this license, visit <u>http://creativecommons.org/licenses/by/4.0/</u>.