



Seroprevalence of Foot and Mouth Disease in apparently healthy beef cattle in Uganda post the 2021 outbreak

¹SENTE C., ²ROSADO-RAMOS B., ³KERFUA S., ⁴TUWANGYE I., ²BROOKSHIRE C., ¹KALUMBA P., ¹NAKABUYE R S., ¹NAMIRIMU S., ¹TAMALE A., ²REICHLEY S., ^{1,5}DRILEYO G., ²KHAITSA M

¹College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, P. O. Box 7062, Kampala, Uganda.

²College of Veterinary Medicine, Mississippi State University, USA.

³National Agricultural Research Organization (NARO).

⁴National Animal Genetics Resources Centre & Data Bank (NAGRC&DB), Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Uganda.

⁵Wildlife Conservation Society (WCS)

*Corresponding author: csente37@gmail.com ; celsus.sente@mak.ac.ug

Abstract

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals caused by a picornavirus with 7 known serotypes (O, C, A, SAT1, SAT2 and Asia) and more than 60 subtypes. FMD causes enormous economic losses, including reduced animal productivity and restrictions on international trade in animal products. In Uganda, FMD is endemic and caused by mainly O, A, SAT 1, and SAT2 serotypes. The outbreaks are mainly controlled by ring vaccination and restriction of animal movement, which methods are sometimes inefficient due to a lack of regular surveillance. FMDV seroprevalence information in animal herds post-outbreak response is not available to guide policymakers on when to lift quarantine in affected districts. This study assessed the effectiveness of the 2021 FMD outbreak response in Western Uganda following the lifting of quarantine restrictions. As part of routine herd health checks, blood samples were extracted from sixty-seven apparently healthy beef cattle (42 from Nshaara Ranch and 25 from Mbarara City abattoir). The samples were tested for the presence of specific FMDV antibodies using Solid-Phase Competitive Enzyme-Linked Immunosorbent Assay. Overall, out of 67 samples, 26 (38.8%) tested positive for FMDV. Of 42 samples from Nshaara Ranch, 19 (45.2%) were positive while 7 of 25 (28.0%) from Mbarara City abattoir were positive. No statistical difference was noted in FMD seroprevalence by animal place of origin, sex, or body condition score. However, younger animals had higher FMD prevalence than older ones ($P=0.0289$). The study revealed a concerning high seropositivity of apparently healthy animals to FMDV antibodies, possibly due to early lifting of the quarantine, weak enforcement of control measures, or lack of incentive and resources to control the disease. Therefore, the Uganda national outbreak preparedness and response unit should institute a coordinated, effective, and functional preparedness and response mechanism to control such disease outbreaks.

Keywords: Beef cattle, Foot and Mouth Disease; Seroprevalence; Uganda

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Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals, including cattle, pigs, sheep, and many wildlife species (Jamal and Belsham, 2013; Belsham *et al.*, 2021). The disease can cause enormous economic losses, including reduced animal productivity and restrictions on international trade in animal products (Jamal and Belsham, 2013; Knight-Jones and Rushton, 2013). It is caused by a picornavirus with seven different serotypes: O, A, C, Asia 1, SAT (South-African-Territories) 1, 2, 3, and numerous variants (Bari *et al.*, 2014; Lloyd-Jones *et al.*, 2017; Abdel-Aziz *et al.*, 2020). Due to the existence of several viral strains, FMD remains a challenge for cattle farmers and the international community, posing a danger of transboundary transmission (Namatovu *et al.*, 2015; Mesfine *et al.*, 2019). The diverse nature of Foot and Mouth Disease Virus (FMDV) and its hosts results in a complex epidemiology, with clinical manifestations varying from asymptomatic cases, as documented in the African buffalo, to severe symptoms and occasional fatalities (Miguel *et al.*, 2017; Abdel-Aziz *et al.*, 2020; Velazquez-Salinas *et al.*, 2020). Although morbidity due to FMD outbreaks in animals is not easily measured, especially in countries that are normally FMD-free, because they have stringent control measures, FMD is still considered highly contagious (Ilbeigi *et al.*, 2018; WOAHI, 2024). In infected animals, FMD clinical findings include fever with vesicles on the feet and in and around the mouth, which erode or ulcerate and heal in three weeks (WOAH, 2024). Death commonly occurs in the young stock after developing myocarditis (Sobhy *et al.*, 2018; Deka *et al.*, 2024).

Foot and Mouth Disease is endemic in Uganda, with outbreaks occurring almost every year (Namatovu *et al.*, 2015; Velazquez-Salinas *et al.*, 2020). Outbreaks are mainly controlled by ring vaccination and restriction of animal movement or quarantine (Velazquez-Salinas *et al.*, 2020; Hwang *et al.*, 2021; Lazarus *et al.*, 2021). However, these control measures have not

successfully controlled FMD in Uganda, and outbreaks keep reoccurring (Muleme *et al.*, 2013) because of uncontrolled animal movements, inadequate surveillance, and delayed reporting of the outbreaks (Namatovu *et al.*, 2015). Recurrence of FMD outbreaks in Uganda is also due to insufficient vaccination of susceptible animals resulting from high costs associated with importing FMDV vaccines, the “fire brigade” response of vaccinating animals after outbreaks, and not matching serotypes/sub-serotypes of FMDV vaccines used with the field strains circulating (Muleme *et al.*, 2013). To enhance the control of FMD in Uganda, there is a need for efficient and timely determination of outbreak virus strains/serotypes and vaccine matching (Namatovu *et al.*, 2015). The main FMD serotypes circulating in Uganda are mainly O, A, SAT1, and SAT2 serotypes (Dhikusooka *et al.*, 2015, 2016; Mwiine *et al.*, 2019).

In April 2021, authorities of Kiruhura District in western Uganda issued a total ban on the sale and movement of livestock and their products in a bid to stop the spread of FMD (Ainebyoona, 2021; Atwine, 2021; Okello *et al.*, 2022). This same control measure is applied whenever there is an outbreak, and occasionally it is not efficient to completely wipe the disease out of the herds. There is a possibility that even after quarantine, ring vaccination, and using the common measures applied in Uganda, some animals will test positive for FMD. However, due to a lack of regular surveillance, there is no documented information showing the seroprevalence of FMD after outbreak response, a gap that the study focused on filling.

Materials and methods

Study area

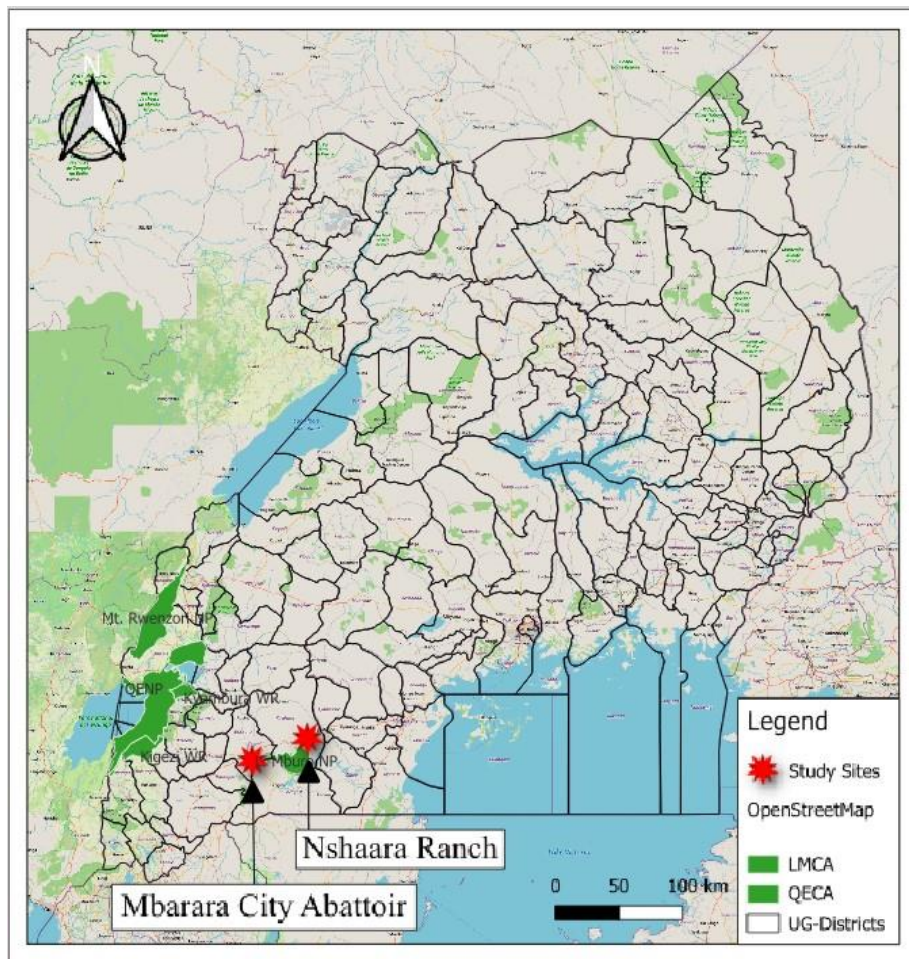
The areas of study were Nshaara Ranch and the Mbarara City Abattoir (Figure 1). Nshaara government Ranch is located approximately

53km on the Mbarara-Masaka road in Akageeti cell, Nyakahita parish, Nyakashashara Subcounty, Nyabushozi County, Kiruhura District. The ranch is 7110 ha in size, subdivided into blocks. Kiruhura District (latitude 0° 11' 47.04", longitude 30° 50' 39.12") situated in western Uganda and is bordered by Kamwenge District and Kyegegwa District to the north, Sembabule District to the northeast, Lyantonde District to the east, Rakai District to the southeast, Isingiro District to the south, Mbarara

District to the southwest and Ibanda District to the northwest. Mbarara City Abattoir is one of the main abattoirs in Mbarara found in Mbarara District, and hosts dozens of butchers that supply meat to city and its neighbourhoods. Mbarara is the largest city in the Ankole subregion and the second largest in Uganda after Kampala. It is located at latitude 0° 36' 25.92" and longitude 30° 39' 16.2".

Figure 1

Location of Nshaara ranch and Mbarara slaughterhouses in Uganda



Sample Collection

Cattle were restrained in the crush, and coccygeal venipuncture was done. Ten milliliters

of blood samples were collected from each animal and dispensed into plain vacutainer bottles. The samples were well labelled, stored

in a cool box at 4 degrees Celsius and transported to the National Agriculture Research Organization (NARO) laboratory in Nakyesasa for analysis.

Laboratory analysis

The serum samples were harvested from the blood into cryovials after spinning for 10 min at 1200g, divided into aliquots, labelled, and kept at -20°C until use. Detection of antibodies against FMDV Nonstructural Proteins (NSPs) by ELISA was performed according to the manufacturer's instructions (PRIOCHECK® FMD-3ABC NS protein ELISA) for detection of antibodies to the nonstructural polypeptide 3ABC of FMDV in serum, which detects infected animals regardless of their vaccination status and the FMDV serotype that caused the infection (Ulamu *et al.*, 2017). Detection of FMDV-specific antibodies using Solid-Phase Competitive Enzyme-Linked Immunosorbent Assay was done as described (Ulamu *et al.*, 2017).

Data analysis

Data obtained were analysed using Microsoft Excel 2019 (Microsoft Corporation, USA) and SAS version 9.4. Descriptive statistics were presented in form of tables and a bar chart. To compare animal origin, age, sex, and body condition score by FMD status, the non-parametric alternative wilcoxon rank sum test was used due to small sample size. All statistical tests were conducted at $\alpha = 0.05$ significance level. The analysis included 67 observations, with 27 missing values excluded from the age, sex, and body condition score analysis.

Results

Demographic characteristics

Some demographic characteristics of the sampled animals at Nshaara Ranch and Mbarara City Abattoir are presented in Table 1. The animals sampled were mainly cattle (94%), with more coming from Nshaara Ranch (63%). The animal's age ranged between 7 months and 8 years, and the body condition score (BCS) was 2-3.

Table 1

Animals sampled, Age, Sex, BCS FMD status of animals sampled at Nshaara Ranch, Uganda

Variable	Frequency	Percent
Animal type (n=67)		
cow	63	94
goat	2	3
sheep	2	3
Animal Origin (n=67)		
Mbarara City Abattoir	25	37
Nshaara Ranch	42	63
Age (years) (n=40)		
0.7	1	2.5
1	5	12.5
1.2	4	10
1.4	1	2.5
1.5	3	7.5
1.6	2	5
1.7	3	7.5
1.8	1	2.5
2	2	5

6	3	7.5
7	9	22.5
8	6	15
<hr/>		
Sex (n=40)		
Female (F)	27	67.5
Male (M)	13	32.5
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Body Condition Score (BCS) (=40)		
2	3	7.5
2.3	3	7.5
2.4	1	2.5
2.5	10	25
2.6	3	7.5
2.7	2	5
2.75	8	20
2.8	2	5
3	8	20

Body condition score (1-5): 5-Excellent, 4-very good, 3-Good, 2-fair, 1-Poor

Sex: F-female, M-male

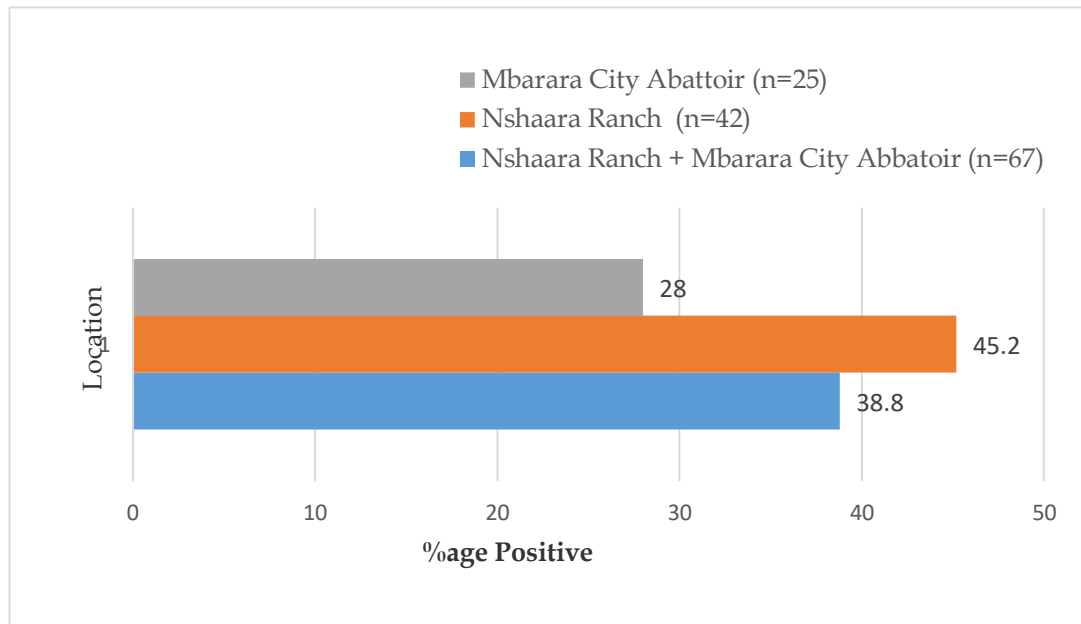
Seroprevalence

The total number of FMDV positive samples from the 67 animal samples tested were 26 (38.8%). From the 42 sampled animals at Nshaara Ranch, 19 (45.2%) were positive for FMDV, while from Mbarara City's abattoir, out of 25 animals, 7 (28%) were positive (Figure 2). The comparison of animal origin, age, sex, and

BCS with FMD status is presented in Tables 2, 3, and 4. We noted no statistical difference in FMD seroprevalence by animal place of origin, sex, or BCS. However, younger animals had higher FMD prevalence than older ones ($P=0.0289$) (Table 4).

Figure 2

Site specific FMD seroprevalence in Nshaara Ranch and Mbarara City Abattoir, Uganda



Comparison of animal origin, age, sex, and BCS with FMD status

Table 2

Comparison of animal origin, sex, age, and body condition score by FMD status (n=67)

Animal origin	FMD			OR (95 % CL)	P Value
	Positive	Negative	Total		
Nshaara Ranch	19	23	42	2.12(0.73 - 6.15)	0.1614
Mbarara City Abattoir	7	18	25		
Total	26	41	67		

Table 3

Comparison of sex of the animal by FMD status – Animals from Nshaara Ranch only (n=40)

Sex	FMD			OR (95 % CL)	P Value
	Positive	Negative	Total		
M	7	6	13	1.46(0.37 - 5.51)	0.557
F	12	15	27		

Total	19	21	40
Frequency Missing = 2			

Table 4

Comparison of age, and body condition score by FMD status – Animals from Nshaara Ranch only (n=40)

FMD								
Nshaara	Positive				Negative			P Value
	Label	N	Mean	Std Dev	N	Mean	Std Dev	
	BCS	19	2.7	0.4	21	2.6	0.2	0.1345
	Age	19	3.1	2.9	21	4.7	2.8	0.0289
Frequency Missing = 2								

Discussion

Following the 2021 FMD outbreak in Uganda and the evident prior vaccination of animals in this endemic area, the data indicated that numerous ostensibly healthy animals were testing positive for FMDV antibodies. This was possibly due to early lifting of the quarantine, weak enforcement of control measures, or lack of incentive and resources to control the disease. Previous studies have cited various reasons for recurrence of FMD outbreaks in Uganda, including; uncontrolled animal movements, inadequate surveillance, delayed reporting of the outbreaks (Namatovu *et al.*, 2015), insufficient vaccination of susceptible animals due to the high costs associated with importing FMDV vaccines, the “fire brigade” response of vaccinating animals after outbreaks, and not matching serotypes/sub-serotypes of FMDV vaccines used with the field strains circulating (Muleme *et al.*, 2013; Namatovu *et al.*, 2015; Mesfine *et al.*, 2019; Velazquez-Salinas *et al.*, 2020).

A notable disparity existed in the prevalence rates between the two study areas (Nshaara Ranch-45.2%; Mbarara City abattoir – 38.8%). This discrepancy could have arisen from the possibility that certain animals slaughtered

in the Mbarara abattoir came from FMD-free areas. The 2020/2021 FMD outbreak was reported all over the country, with most cases reported in eastern (Bukedea) Uganda and western Ugandan (Kirihera, Mbarara, Kasese) (Atwine, 2021; Okello *et al.*, 2022). The ranch animals at Nshaara, living in a closed environment and frequently sharing pasture and water, could potentially account for many of the sampled animals testing positive. This aligns with other research demonstrating the rapid transmission of FMD, a highly contagious disease within a cattle ranch, through direct contact, respiratory droplets, and contaminated items (Belsham *et al.*, 2021; WOA, 2024). Moreover, the constant interaction with wild ungulates at Nshaara Ranch, situated adjacent to Lake Mburo National Park, presents another risk factor. Previous researchers have indicated wild animals are major reservoirs of FMD (Jamal and Belsham, 2013; Miguel *et al.*, 2017; Udahehuka *et al.*, 2022).

Highly contagious diseases of livestock, such as FMD, have the potential to have a significant impact not only on the agricultural industry but also on the wider economy and society. The foot-and-mouth disease virus spreads easily and can survive in the environment, so control

methods need to stop it from spreading through surfaces as well as direct contact (Jamal and Belsham, 2013). The disease has the potential for substantial societal impact (Knight-Jones and Rushton, 2013). Control strategies rely not only on mandatory slaughter of infected and in-contact animals and restrictions on movement and trade of susceptible livestock species (Hwang *et al.*, 2021; Lazarus *et al.*, 2021) but may also require restrictions on the activities of non-susceptible animals and people, who may transmit the virus mechanically.

Also notable among the cattle at the ranch was the low body condition score (BCS) ranging from 2 to 3 with a mean of 2.7. The low BCS in the ranch cattle was likely attributable, among other variables, to insufficient feed availability, as the grasslands were dry, short, and exhibited minimal forage growth during the research period. This study is agreeable with the finding of other researchers that low BCS is attributed to inadequate feed availability (Jordan, 2021), as well as negative energy balance, lactation, and insulin resistance (Pires *et al.*, 2013; Jordan, 2021). Younger animals were more likely to test positive compared to the older animals. This is consistent with what has been reported in the literature (Deka *et al.*, 2024) that younger animals are more susceptible to FMD and have a higher mortality rate than older animals, partly due to their underdeveloped immune systems compared to older animals. Also, younger animals have higher susceptibility to myocarditis caused by FMD virus (Deka *et al.*, 2024).

Conclusion

There was a higher prevalence of FMDV cattle at Nshaara Ranch than those sampled at the Mbarara City abattoir.

The seemingly vaccinated, healthy beef cattle at Nshaara Ranch, which had not reported any cases of FMD during and after the 2020/2021 FMD outbreak, tested positive for FMDV in 45.2% of the sampled animals.

Following the recommended period of quarantine and the lifting of movement restrictions in the district, many of the sampled animals at Nshaara Ranch (45.2%) and Mbarara City Abattoir (28%) still tested positive. Infectious livestock diseases can affect the agricultural industry, economy, and society.

Recommendations

To reduce the risk of potential FMD transmission, Nshaara Ranch should erect effective, impassable fences that prevent cattle or wildlife from crossing and increasing the risk of disease transmission.

Apply effective vaccine strategies, such as early vaccination, to control FMD. When vaccinating against FMD, it's important to consider other factors such as selecting the correct vaccine type, carefully matching it to the prevailing field virus strains, adhering to the vaccination schedule, vaccinating at least 90% of the population, revaccinating, and administering emergency vaccinations.

Quarantine should not be lifted unless there is real evidence that FMD has completely been controlled.

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Ethics approval and consent to participate

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