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# Foot and Mouth Disease Virus Strain-specific Antibody Titres in Naturally Infected or Vaccinated Bulls in Kenya

**Jemimah Simbauni [a*], Michael M. Kavuso [a,b], Joshua M. Mutiso [a], Rebeccah M. Ayako [a] and Michael M. Gicheru [a]**

*[a] Department of Zoological Sciences, Kenyatta University, Nairobi, Kenya.*
*[b] Kenya Animal Genetic Resource Centre (KAGRC), Nairobi, Kenya.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Foot-and-mouth disease (FMD) is a global viral infection that causes vesicular lesions in and around the mouth and feet, causing reluctance of animals to eat or move. In Kenya, bulls raised for AI receive vaccinations against FMD, but it is unclear if these animals experience vaccine-induced immunity. No research has been conducted to determine if animals in endemic areas develop natural immunity or whether animals in disease-free regions might be seropositive. This study aimed to determine the prevalence and levels of foot and mouth disease virus infection-triggered and vaccine-induced antibodies. A cross-sectional study was conducted on bulls farmed for AI

**Corresponding author: Email: jsimbauni@yahoo.com;*

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production and vaccinated against FMD. Antibodies were quantified using a virus-neutralization test. One-way analysis of variance (ANOVA) and the Kruskal-Wallis test with Tukey and Dunn post-tests, respectively, were used to examine the data using the GraphPad InStat program. Additionally, the Spearman test was employed for correlation analysis and the t-test for intergroup differences analysis. A statistically significant P value was defined as less than 0.05. Findings showed protective antibody levels were present in 23%, 10.3%, 2.6%, and 7.7% of the animals in the FMD non-endemic region against the FMD virus strains O, A, SAT 1, and SAT 2, respectively. The protection provided by the O strain virus was significantly greater than that of SAT 1 ($P = 0.01$). In the FMD endemic area, all sampled animals showed protection levels at 100%, 100%, 100% and 29% for virus strains O, A, SAT 1, and SAT 2 respectively with the antibody titres showing significant differences ($P < 0.05$) for all the intergroup analysis except between strains O vs SAT 1 and A vs SAT 1 ($P > 0.05$). To conclude, the current research suggests that FMD may be making a comeback in the areas where the illness is not established. Furthermore, it seems that sperm recovery upon freezing is somewhat mitigated by FMDV-specific antibodies. The study advises monitoring FMD in areas where the illness is not endemic and confirms the current findings with larger sample sizes to enable more informed decision-making.

*Keywords: Foot and mouth disease; artificial insemination; antibodies; natural immunity; vaccine.*

## 1. INTRODUCTION

Foot and mouth disease (FMD) is an acute, highly infectious disease that mostly affects ruminants in particular [1]. The disease is caused by the FMDV virus. Domestic and wild ruminants are also infected [2,3]. When livestock output is significantly impacted, it causes significant losses due to animal deaths and trade disruptions involving the afflicted animals and their products [4]. The use of vaccinations against FMD in farm animals is a practical method of disease prevention (OIE, 2018). While vaccination against the FMD virus is considered a means of preventing the virus and boosting livestock productivity, the effects of vaccination, particularly in bulls raised as breeds to produce semen for artificial insemination, have not been thoroughly studied [5]. Nevertheless, the products produced by the vaccination and immune system may have an adverse effect on the quality of semen produced by these animals. Because of this, scientific research and product development studies to raise cattle production are necessary, particularly in light of the rising costs of goods like commercial semen extenders. Furthermore, research that assessed how vaccinations affected farm animals' ability to reproduce might assist clarify any unfavorable impacts connected to particular vaccinations. According to WOAH [6], there are seven known serotypes of FMDV: O, A, C, SAT1, SAT2, SAT3, and Asia-1. According to KEVEVAPI [7], infection with a particular viral serotype does not confer immunity against other strains. This poses a problem for vaccine development and emphasizes the necessity for the creation of a cocktail vaccination that protects against many virus strains. Strains O, A, SAT 1, and SAT 2 may be the viruses that are circulating and linked to FMD in Kenya. For use in sheep, pigs, goats, and cattle, FOTIVAX TM is an inactivated vaccine against pig and sheep-related diarrhea (FMD) that is linked to infections by virus serotypes A, O, SAT 1, and SAT 2.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This study was conducted at the Kenya Animal Genetic Resource Centre (KGRC) located in Lower Kabete which is 16 kilometers west of Nairobi city center.

### 2.2 Study Animals and Samples

The proposed study used Bulls of the Friesian breed for collection of blood samples for laboratory analysis. These animals included only those bulls vaccinated against the FMD and bred specifically for semen production for AI. Blood samples were collected from the bulls for analysis of FMD vaccine IgG antibodies. Collected samples were taken to the laboratory for analysis with the help of a veterinary doctor. For animals included in the assessment of natural protection against the FMD, bulls were sampled within an area of disease endemicity as well as in an FMD non-endemic area. Blood samples were obtained from these animals for quantification of circulating antibodies against each of the four viral strains including, O, A, SAT 1, and SAT 2.

## 2.3 Study Design

For assessment of the acquisition of natural immunity, at least seven bulls were sampled from each of the FMD endemic (Wangige in Kiambu County) and non-endemic (Makueni) regions in Kenya in a pilot study. All antibody quantification will be carried out using virus-neutralization enzyme-linked immunosorbent assay-based methodology.

## 2.4 Quantification of Serum Titres from Blood Samples

Fifty µL of Eagles (MM1) media were added using a pipette into all the wells of a microtiter plate but excluding wells in row A before adding 100 µL of 1/4 dilutions of the control sera and test sera samples in row A. Fifty 50 µL of this 1/4 dilution were transferred from the row A of microtiter plate to row B and the hundred µL contents were carefully mixed several times using a pipette, while ensuring no bubbles are introduced (this resulted in a doubling dilution). Fifty µL were transferred from microtiter plate row B to C and mixing was repeated. From row C to row D, 50 µL were transferred and mixed carefully and this step was repeated down to row H. Fifty µL of the dilution were discarded from row H leaving a final volume of fifty µL (this resulted in 1/4 to 1/512 dilutions in fifty µL volumes). This step was repeated with all test samples and duplicate wells were performed both for the test and control sera. The virus antigen dilutions were then added at this stage.

Fifty µL of the 100TCID50 virus dilution were added to all the wells of the test and control plates resulting in dilutions from 1/8 to 1/1024 before incubation microtiter plates for 1 hour at 37˚C. The cell suspension was added to the microtiter wells at this stage: Fifty µL /well of the cell suspension were added at the prepared concentrations of 0.4-1.0 x $10^6$ cells/ml of LFBK, BHK-21 or IB-RS 2 cells in Eagles (MM1) media. The microtiter plates were sealed and incubated at 37˚C for upto72 hours before reading results: The microtiter plates were viewed under an inverted microscope for cytopathic effect (CPE) after 48 hours of incubation. Wells that did not show CPE were recorded as positive while those that showed CPE were regarded as negative. After 72 hours of incubation, the plates were stained with naphthalene blue-black dye. Wells staining blue-black were considered positive and those appearing colorless were considered negative. For quality control, a standard antiserum of known titre, a control cell, media control, and a virus titration are included in the test in every test and used to calculate the actual virus titre. The virus titre was then calculated and the results were interpreted. The virus titre was considered the dilution where fifty percent of the cells showed positive CPE which upon staining appeared colorless. The endpoint was reported as where there was no CPE and the cell monolayer stained blue-black, the color of the stain. This was carried out following the procedure by Kärber (1931):

The microtitre wells number shows a hundred percent CPE divided by the number of the wells per dilution before subtracting 0.5 (correction factor) and then multiplying the dilution interval of the log. The highest step of dilution with a hundred percent CPE was added to all the microtitre wells. The serum tire was then calculated: With each virus neutralization test (VNT), titration of the virus was added so that the exact titre of the virus and doses of the virus could be determined. For every dose of the virus, the corresponding titre of serum was established. The Titres of serum were expressed as the reciprocal of log10 dilution which showed fifty percent protection of cultures against infection by that virus dose. The endpoint titre of the sera was expressed as reciprocal of the log dilution which recorded protection levels of fifty percent in cultures against 100TCID50 of virus. This was carried out by using plots of doses of the virus ranging from 101.5 to 102.5 versus the corresponding titers of serum and extrapolating the final titre of serum at 100TCID50.

Mean values of data on parameters on variables between the experimental and control animal groups were analyzed by use of GraphPad InStat software for statistical data analysis: Data on antibody levels between FMD vaccinated and non-vaccinated bulls were analyzed using student-t-test statistics. Differences between more than two groups of treatments were analyzed using both parametric one-way analysis of variance (ANOVA) and the Kruskal-Wallis test with Tukey and Dunn test as post-tests respectively. Discontinuous data values involving sperm morphology for samples obtained between the two animal study groups were analyzed through descriptive statistics. All data cleaning and normality tests were carried out on each data set by the analysis software. The significance level was set at $P < \text{or} > 0.05$.

## 3. RESULTS

### 3.1 Immune Status in Bulls from FMD Non-endemic Region

For the animals sampled (n=39) from Makueni County, a region considered non-endemic for Foot and Mouth Disease, the natural protection status against the four virus strains including O, A, SAT 1, and SAT 2 indicated that the seroprevalence for the FMD-positive were 23%, 10.3%, 2.6%, and 7.7% with titre levels of 1.51±0.24, 1.40±0.07, 1.51±0 and 1.36.±0 All other FMD-negative animals had titre levels below 0.96 while the mean value for the total number of bulls in each category of the virus strain was below 0.99 (Fig. 1). Although on average, all antibody levels against each of the virus strains were below the protective value of 1.36, there was a significant difference between tire levels against virus strain O and the SAT 1 strain with antibodies against strain O, being slightly higher ($F = 3.74$; $q = 4.73$; $P = 0.01$). Antibody levels compared between any other two groups were not different ($P > 0.05$).

### 3.2 Immune Status in Bulls from FMD Endemic Region

In Wangige, Kiambu County, an FMD endemic region, antibody titres for the four viruses including strain O, A, SAT 1 and Sat 2 ranged from 1.83±0.36 to 2.41±0.21 for the seropositive animals with the highest level being associated with the A strain virus. All sampled animals were seropositive for viral strains O, A, and SAT 1 while 61% of the study subjects were seronegative for the viral strain SAT 2. On average the total number of animals recorded antibody levels of 1.83±0.36, 2.41±0.21, 1.98±0.36 and 1.09±0.36 against viral strains O, A, SAT 1 and SAT 2 respectively (Fig. 2). Comparing the mean values of the antibody titres against the four virus strains, there was a significant difference ($F = 19.22$; $q > 3.90$; $P < 0.01$) indicating varying immune statuses. Only titre levels against virus strain O vs SAT 1 and strain A vs SAT 1 were comparable ($P > 0.05$) while comparison antibody levels between any other two groups concluded a significant difference ($P < 0.05$).

### 3.3 Proportions of Bulls from Endemic and Non-endemic Regions have Acquired Natural Protection against the FMDV Infection

Comparing the proportions of bulls from FMD endemic and non-endemic regions that had acquired natural protection through possible infections with one or more of the circulating virus strains, results indicated that overall 29% of the bulls from FMD endemic region were protected from all four virus strains while none

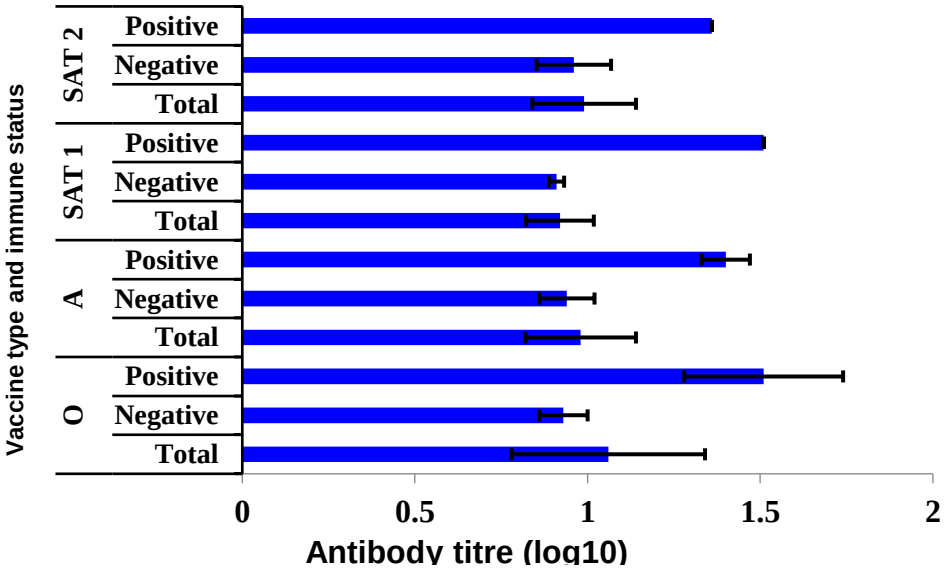


**Fig. 1. Natural protection status of cattle from FMD non-endemic region**

*Blood samples were obtained from study animals and quantified for antibody levels against virus strains O, A, SAT 1 and SAT 2 by ELISA to establish the level of naturally acquired immunity against disease. Data are presented as mean± SD (standard deviation). Animals with antibody titre levels > 1.36 are considered protected against FMDV*

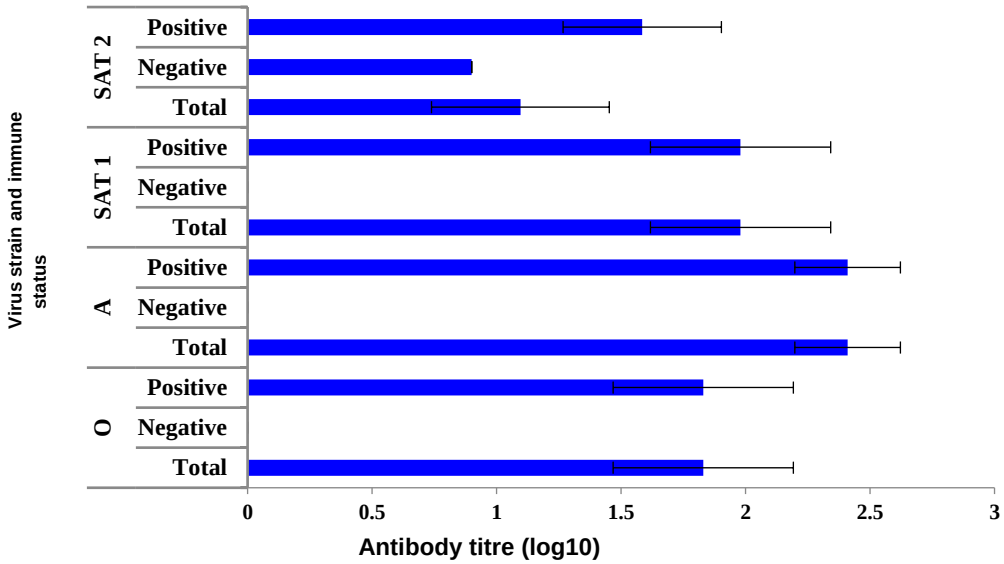


**Fig. 2. Natural protection status in bulls from FMD endemic region**

*Blood samples were obtained from study animals and quantified for antibody levels against virus strains O, A, SAT 1 and SAT 2 by ELISA to establish the level of naturally acquired immunity against disease. Data are presented as mean± SD (standard deviation). Animals with antibody titer levels > 1.36 are considered protected against FMDV*

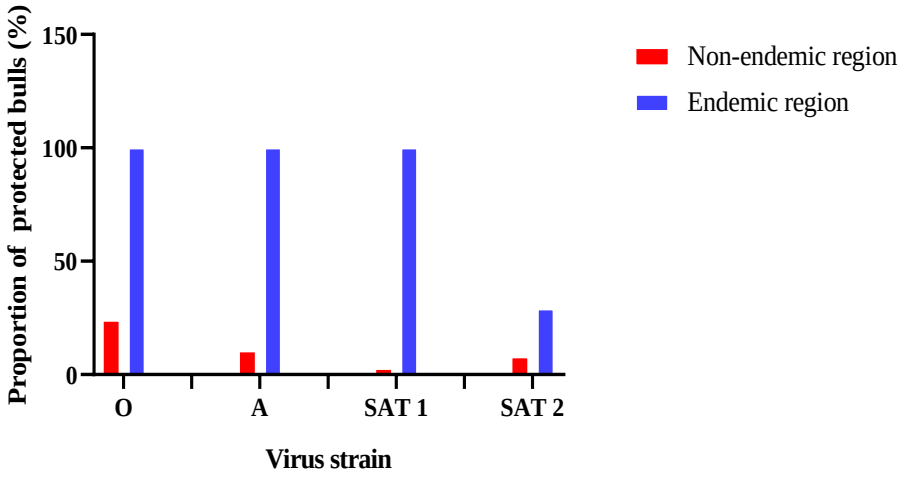


**Fig. 3. Proportions of naturally protected bulls against FMD in both disease-endemic and non-endemic regions**

*Blood samples were obtained from study animals and antibody levels against FMDV were quantified using ELISA to determine to percentages of bulls that were protected against viral strains O, A, SAT 1, and SAT 2 by having antibody titres greater than 1.36. The graph represents the proportions of bulls that are protected against FMD associated with the four virus strains*

(0%) of the bulls from non-endemic region was protected from all the four viruses. Considering individual viruses, animals from disease-endemic region were fully (100%) protected from virus strains O, A, and SAT 1 while only 29% were protected from the SAT 2 viral strain. On the other hand, 23%, 10.3%, 2.6% and 7.7% of animals from FMD non-endemic region were protected against viral strains O, A, SAT 1 and SAT 2 respectively (Fig. 3). In the FMD non-endemic region only 2.56% (1/39) of the animals were protected from a combination of three virus types including strains O, A, and SAT 1 for one bull and O, A, and SAT 2 for the other animal.

### 3.4 Serology Titre Levels against FMD Virus Strains

Following vaccination of a group of bulls with vaccines against FMD viruses' strains O, A, SAT1, and SAT 2, and assessment of protection status, results indicated that out of 12 animals, only one (8.33%) bull did not develop any protection against any of the viruses. Two other animals did not develop protection against the strain O of the viruses and developed antibody titres of 1.2 (Fig. 4). For the vaccine-induced protection, the antibody titres ranged from 1.36 against viral strain O to 2.85 for the A strain vaccination. The protection achieved a 91% level for each of the virus strains A, SAT 1, and SAT 2 while the viral strain O achieved a protection level of 75% among the vaccinated animals. Among the various vaccine categories, antibody titres were significantly different ($F = 4.89$; $q > 3.78$; $P < 0.01$). Significantly higher antibody titres were recorded for vaccines for virus strain A and SAT 2 as compared to strain O ($P < 0.01$ and $P < 0.05$). There were, however, no significant differences when antibody titres between any other two vaccine categories were compared ($P > 0.05$).

## 4. DISCUSSION

Animals are naturally affected by pathogens, and while some infected animals may have severe and/or deadly illness, others may just experience moderate symptoms or none at all [8,9]. Certain illnesses have an innate resistance in certain animals, while others cause no symptoms at all in carriers. According to Kim et al. [10] and Moonen et al., [11] the foot-and-mouth disease is a highly infectious condition that causes significant losses in afflicted cattle. Animals that have evolved antibodies against an unavailable pathogen are uncommon in disease-free areas unless they were imported from a far-off disease-endemic zone [12]. A disease might emerge if certain animals in a disease-free region are immune to the particular disease pathogen [13].

The findings of the current investigation, which show that there were variable numbers of bulls with antibody titres over the minimal values specific to each of the four FMD virus strains, suggest that the animals were likely infected as a result of recent exposure to viral replication. It is deemed extremely concerning when a novel strain of the FMD virus appears in an area where there have been no previous reports of illness cases or vaccine coverage (WOAH, 2023). It is not possible to rule out the possibility of circulating FMD virus strains in the non-endemic research region in this investigation. This could necessitate more research to determine the extent to which disease onset could be brought on by climate change [14,15].

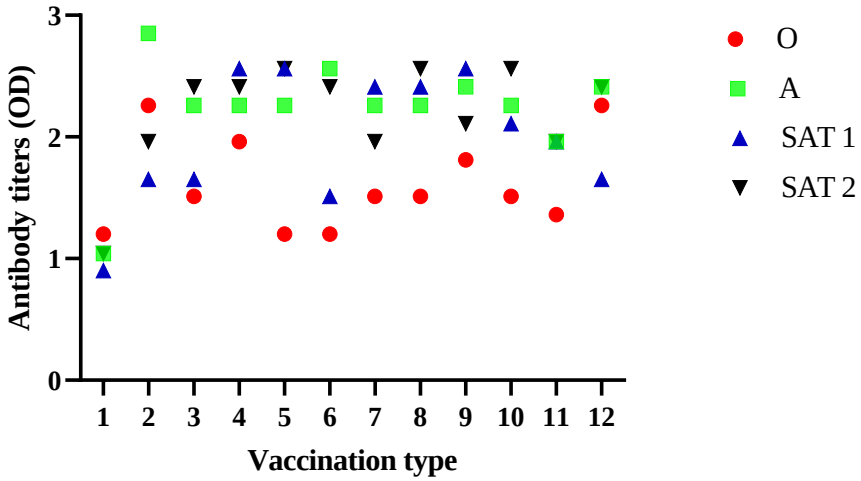


**Fig. 4. Antibody titres in bulls vaccinated against FMD viral strains O, A, SAT 1 and SAT 2**

*A group of bulls were vaccinated against four FMD viruses and antibody levels were quantified using ELISA. The Graph represents vaccinated bulls and the mean of duplicate antibody titres for each of the vaccines against viral strains O, A, SAT 1, and SAT 2.*

Since a small percentage of the bulls had protective antibody titre levels, it is clear that natural resistance in the animals is not the reason for the lack of reports of disease outbreaks in the FMD non-endemic zone. Furthermore, although virus strain O could be the most prevalent, illness monitoring and surveillance has to be started since all strains are represented by one or more instances of seroprevalence. However, with the exception of SAT 2, which had a protection level of 29%, all three other FMD virus strains had a 100% protection level. This reveals that the majority of animals in the disease-endemic region have likely contracted the infection naturally and have been able to develop immunity against infection, as shown by the high antibody titres found in sampled bulls.

The incidence of FMD in Northern Pakistan was found to be 67% in a recent study on the sero-epidemiology of the disease [16], suggesting a high degree of natural protection. Therefore, in a region where illness is prevalent, the development of natural defense may be high. The current study's results suggest that further research is needed to determine the degree of seroprevalence in a sizable sample of cattle. This is because reaching high levels of herd immunity might indicate that vaccination is not required in areas where the illness is prevalent.

Animals should be protected against all four virus strains when vaccinated against FMD using a combination vaccination that targets strains O, A, SAT 1, and SAT 2. The different FMD viral strains that cause infection do not provide cross-protection to one another [17,10] WOAH, 2023. Therefore, it presents a problem if the combination FMD vaccination fails to produce protection against every variant of the virus. The ability of the FOTIVAX TM combination vaccination to fully produce antibody titre levels above the minimum necessary threshold in at least 75% of the total vaccinated bulls in the current investigation suggests that the vaccine has strong protective status. The reason behind one bull's total failure to develop ant antibodies against any of the vaccine's viral antigens, meanwhile, was not immediately apparent. This continued to be a mystery since it is uncommon.

Certain animals may be unable to respond to vaccinations for a variety of reasons, including genetics or other variables like vaccine delivery [17-19]. More research is required to determine whether the vaccine antigens should be repackaged to improve the composition contributed by the FMD virus strain O, as evidenced by the failure of 2 (16.6%) vaccinated bulls to produce protective antibody levels against the virus strain O despite being fully protected against all other strains. By doing this, it will be guaranteed that all virus strains will have antibody levels that provide full protection. Significant efforts have been undertaken to create better FMD vaccines, and advancements have been made via the use of several strategies (Belsham, 2020). An alternative strategy in vaccine development, such as recombinant vector technology, can likely produce superior longer duration and correlates of protection, as the existing inactivated vaccinations only offer protection for six months.

## 5. CONCLUSION

Regardless of the region's endemicity or non-endemicity, several virus strains can cause FMD in bulls. All of the tested bulls in the FMD-endemic area were completely protected against the O, A, and SAT 1 virus strains, whereas only 29 bulls were protected against the SAT 2 strain. The percentage of bulls in the FMD-free zone that were protected against viral strains O, A, SAT 1, and SAT 2 was 23.8%, 10.3%, 2.56%, and 7.69%, respectively. In contrast to the disease-endemic area, where the Log10 of the titer levels varied from 1.85 to 2.41, the serology tire levels produced against the FMD viruses in the bulls grown in the non-endemic area varied from 1.36 to 1.51 at Log10.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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