BIOAFTOGEN® SAT1

HETEROLOGOUS CHALLENGE AGAINST SAT1 TOPOTYPE III ISOLATE

The current SAT1 FMD epidemic shows an unusual expansion of SAT1 outside Africa into the Eastern Mediterranean, the Near East, and Europe. While initial outbreaks were associated with SAT1 topotype I, subsequent outbreaks in Turkey and Azerbaijan were caused by a genetically distinct lineage identified as SAT1 topotype III. This lineage has continued to spread to Cyprus, Greece, Lebanon, Iran, and Israel. More recently, SAT1 outbreaks in cattle were reported in northwestern China (unofficially reported as SAT1 topotype III), followed by a SAT1 incursion in Mongolia, indicating continued geographic expansion of this emerging lineage. Genetic characterization of SAT1/III strains from Turkey, Iran, Azerbaijan, and Israel showed 98–99% genetic homology, confirming their close relationship within the same phylogenetic cluster.

BIOAFTOGEN® vaccines are formulated with the SAT1 2020 vaccine strain, belonging to topotype I. Previous studies demonstrated that vaccine matching studies using bovine sera collected 30 days post-vaccination (dpv) after a single dose showed limited antigenic matching against several SAT1 topotype III isolates, with r_1-values <0.3. However, heterologous virus neutralization titers remained above the protective threshold (≥1.5 log10) (Gubbins et al., 2022), ranging from 1.5 to 1.7 $\log_{10}$. Additionally, a booster dose at 30 dpv significantly enhanced antibody responses, increasing titers against these isolates to approximately 3 $\log_{10}$.

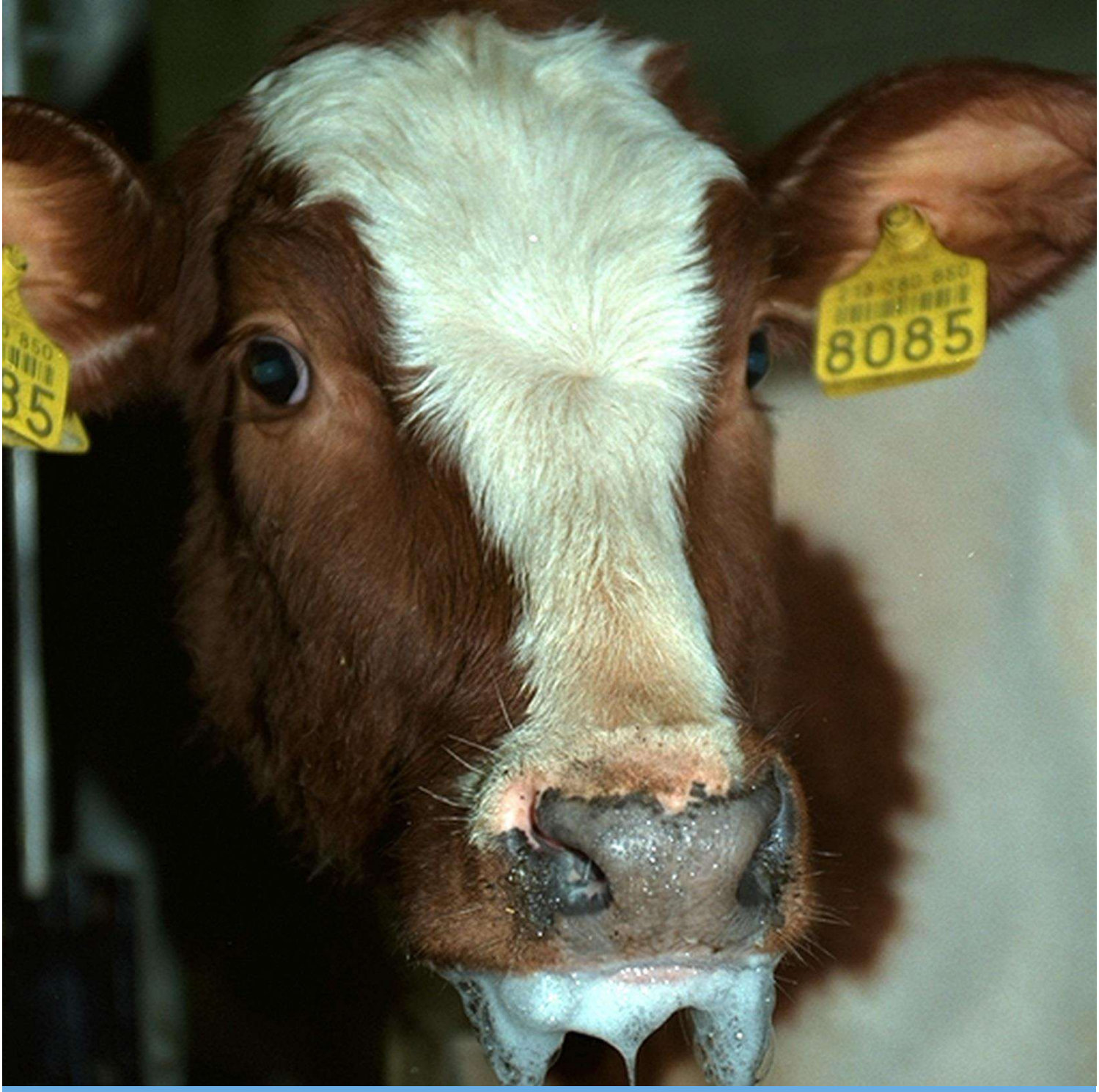
Although the r_1-value is useful for assessing antigenic similarity between vaccine and field viruses, it does not necessarily predict protective efficacy in animals. Vaccine efficacy is influenced not only by antigenic match but also by formulation-related factors, including antigen payload, adjuvant composition, manufacturing quality, and adherence to appropriate dosing schedules. In particular, high-potency vaccines, such as Bioaftogen formulations (>32 PD_{50} per dose), may compensate for limited antigenic matching and enhance heterologous protection (Brehm et al., 2008; Dekker et al., 2018). In this regard, the in vivo challenge test remains the gold standard method for directly evaluating the protective capacity of a vaccine.

To directly evaluate protection against circulating SAT1 topotype III viruses, an *in vivo* cross-protection study conducted at Wageningen Bioveterinary Research (Lelystad, The Netherlands), an FAO Reference Centre for FMD, assessed BIOAFTOGEN® SAT1 against the Lebanese field isolate SAT1/LEB/8/2025 (SAT1/III). This strain was selected because of its low r_1-value in the vaccine matching assay (r1 = 0.11). Two vaccination regimens were evaluated to compare the effect of a single vaccination versus a prime-boost schedule. One

group of seven cattle received a single 2 mL dose of BIOAFTOGEN® SAT1 (batch AFT 426/25) and were challenged 21 days later, while a second group received a booster dose 21 days after the first vaccination and was challenged 21 days later**. Following challenge, 6/7 animals receiving a single dose were protected against generalized foot infection (86%), whereas all boosted animals were protected (7/7; 100%). Unvaccinated controls developed generalized lesions affecting all four feet, confirming the validity of the challenge model (official report attached).**

The results obtained in the present challenge study confirms that r_1-values should not be interpreted in isolation but rather considered together with virus neutralization titers, vaccine potency, in vivo protection data, and overall vaccine formulation quality.

The results support that high potency BIOAFTOGEN® SAT1 vaccine provides effective cross-protection after one dose against recently emerging SAT1 topotype III strains circulating in the Middle East and Asia, despite the antigenic distance suggested by r_1-values alone.



Wageningen Bioveterinary Research

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Heterologous challenge study using FMD monovalent BIOAFTOGEN SAT1 vaccine against strain SAT1/LEB/8/2025 (summary report)

Aldo Dekker

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1 Objective

The objective of the study was to determine the performance of BIOAFTOGEN SAT1 FMD vaccine containing SAT1 2020 strain, against challenge with the heterologous SAT1/LEB/8/2025 strain belonging to SAT1/topotype III.

2 Sponsor of the study

Biogenésis Bagó S.A. Ruta Panamericana Km 38,5; GARIN Prov. de Bs. As. Argentina

3 Name and batch number vaccine used

Name of the vaccine	BIOAFTOGEN SAT1
Batch number, manufacturing and expiry date	Batch No.: AFT 426/25 Mfg. date: 06/2025 Exp. date: 06/2027
Route of injection	Intramuscular (in the neck)

4 Date of vaccination, challenge and final bleeding

Arrival of the cattle group 1	20 April 2026
Vaccination of the cattle group 1	27 April 2026
Arrival of the cattle group 2 and 3	11 May 2026
Booster vaccination of the cattle group 1	18 May 2026
Vaccination of the cattle group 2	18 May 2026
Challenge of the cattle (group 1, 2 and 3)	08 June 2026
Initial check for lesions	11 June 2026
Final reading in post-mortem room	16 June 2026

5 Vaccination

Group (date)	Number of cattle	Volume applied
1 (27 April 2026)	7 cattle (3223 - 3229)	2 ml (right side of the neck)
1 (18 May 2026)	7 cattle (3223 - 3229)	2 ml (left side of the neck)
2 (18 May 2026)	7 cattle (3230 - 3236)	2 ml (left side of the neck)

The challenge control group 3 consisted of 2 cattle (3237 and 3238)

6 Challenge

All cattle (group 1 to 3) were challenged on 8 June 2026 by intradermal injection in the tongue on 2 sites, 0.1 ml per site. The challenge virus SAT1/LEB/8/2025 (Serotype SAT1, Topotype III) was produced in cattle tongue in experiment LVZ 368. The initial titration resulted in $10^{7.68}$ PFU/ml. A 1:955 dilution was made in Minimal Essential Medium containing Hanks salts supplemented with 2% Foetal Bovine serum and 2% antibiotic cocktail to obtain 10^4 PFU/0.2 ml. Back titration confirmed that the dose was correct. For Group 1 the challenge was performed 42 days after the first vaccination (21 days after the second vaccine dose), whereas for Group 2, challenge was conducted 21 days after the first vaccination.

7 Lesions score

Group (date)	Number of cattle	Protected
1 (2 doses, 2 ml/dose)	7 cattle (3223 - 3229)	7
2 (1 dose, 2 ml/dose)	7 cattle (3230 - 3236)	6
3 (control)	2 cattle (3237 and 3238)	0

The trial was valid as both control cattle showed lesions on at least three feet. Vaccinated cattle presenting FMD lesions on at least one foot were classified as non-protected.

8 Conclusion

A single dose of the BIOAFTOGEN SAT1 FMD vaccine provided 86% heterologous protection against generalized foot infection following challenge with SAT1/LEB/8/2025. Administration of two vaccine doses resulted in complete protection (100%) against generalized foot infection.