



# Complete Genome Sequence Analysis of Over 140 Foot-and-mouth Disease Viruses Isolated From Free-living African Buffalo (*Syncerus Caffer*) in Zimbabwe

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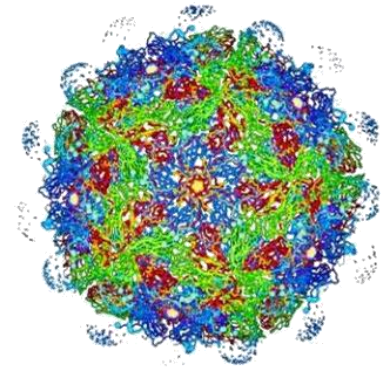
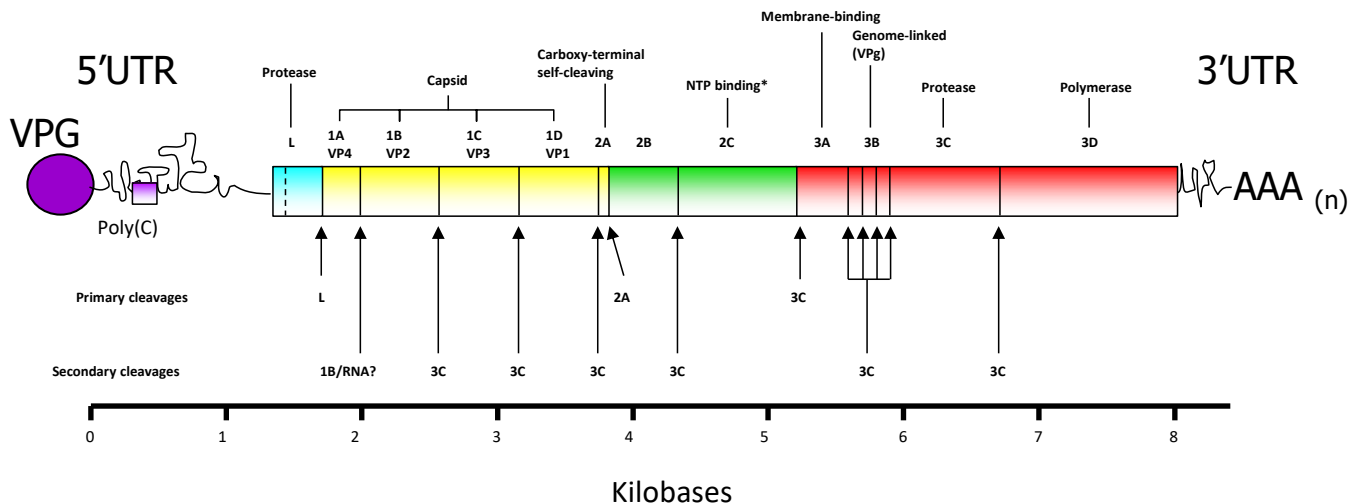


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# Foot-and-Mouth Disease Virus

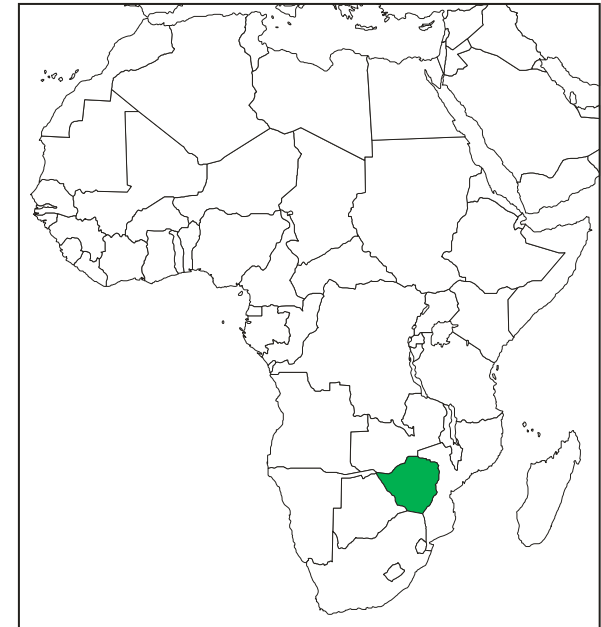
- Family *Picornaviridae*, genus *Aphthovirus*
- Causes a highly contagious disease of cloven-hoofed livestock
  - Cattle, sheep, goats and pigs
- + sense ss RNA genome ~8300nt



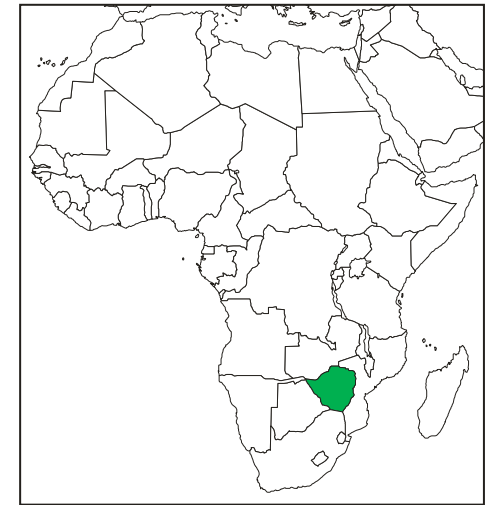
- 7 Serotypes (O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1)
  - including numerous variants
  - VP1 sequence data widely used for strain characterisation

# Introduction

- Foot-and-mouth disease virus (FMDV) causes an acute vesicular disease in domestic cloven-hooved animals. However, in the African buffalo (*Syncerus caffer*) clinical disease is rarely observed and following infection virus is persistently carried in the oesophageal-pharyngeal area of the upper respiratory tract.
- During the 1990s oesophageal-pharyngeal scrapings were collected from free-living African buffalo in multiple herds in six different geographic areas of Zimbabwe.
- Virus isolation on primary bovine thyroid cells and typing by ELISA resulted in the identification of 158 FMD viruses each belonging to one of the Southern African Territories (SAT) serotypes (89 SAT 1, 27 SAT 2 and 42 SAT 3).



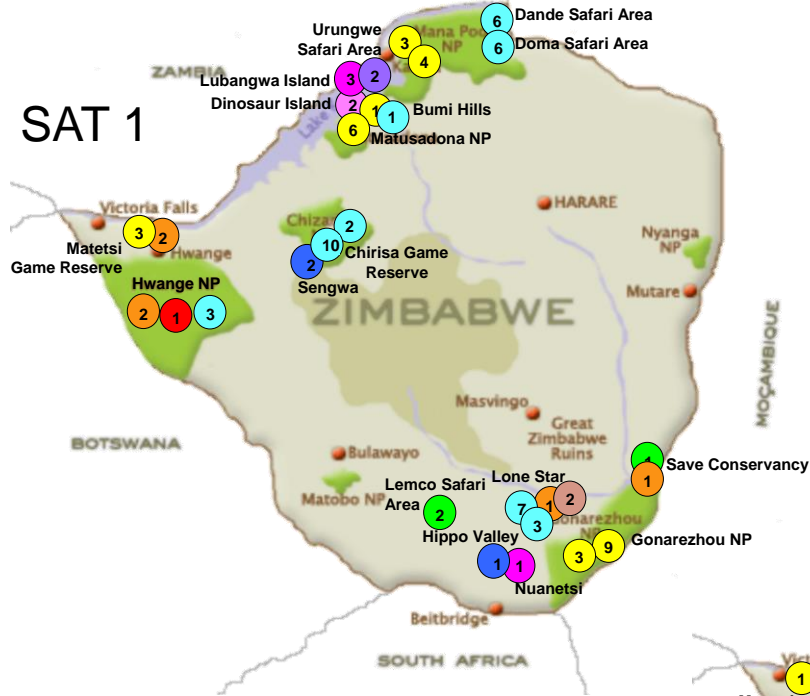
# Study Areas



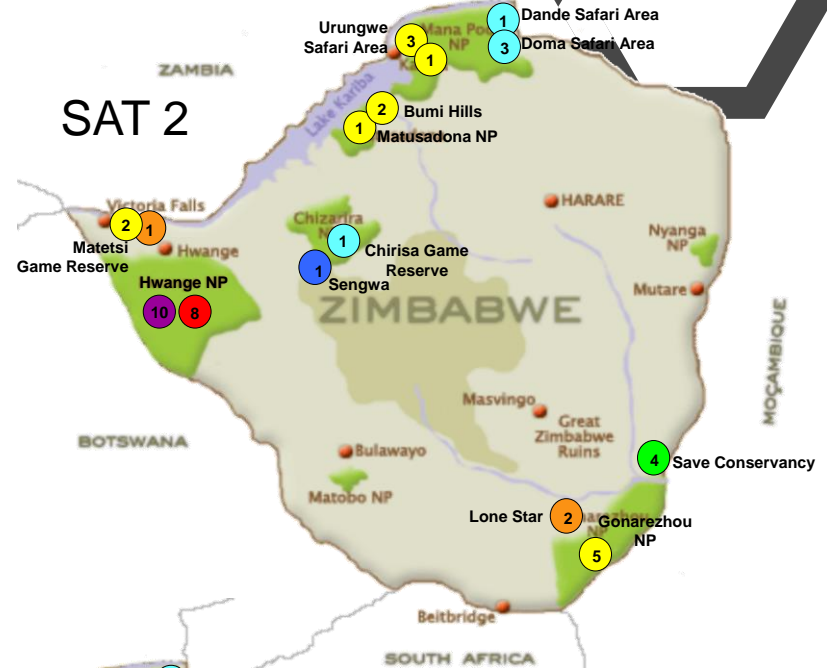
Buffalo-ID	Location
A	Hwange National Park (NP), W Zimbabwe
BC or GN	Gonarezhou NP, SE Zimbabwe
BUMI	Bumi Hills Nature Reserve, Kariba, N Zimbabwe
CHER	Chirisa Safari Area, N Zimbabwe
CK	Chikwarakwara, SE Zimbabwe
DI	Dinosaur Island, Lake Kariba, N Zimbabwe
DSA	Dande/Doma Safari Area, N Zimbabwe
HV	Hippo Valley Estates, Chiredzi, SE Zimbabwe
Hwange	Sinamatela, Hwange NP, W Zimbabwe
K	Lubangwa Island, Kariba, N Zimbabwe
LEMCO	Lemco Safari Area, SE Zimbabwe
LS	Lone Star Ranch, Chiredzi, SE Zimbabwe
MAT	Matusadona National Park, N Zimbabwe
MT	Matetsi, nr. Victoria Falls, W Zimbabwe
MJK	Mukazi Ranch, Save Conservancy, SE Zimbabwe
MWA	Mwakzi Ranch, Save Conservancy, SE Zimbabwe
N	Nuanetsi Ranch, SE Zimbabwe
SAVE	Save Conservancy (ex. Gonarezhou), SE Zimbabwe
SEN	Sengwa, Kariba, N Zimbabwe
UR	Urungwe Safari Area, N Zimbabwe

# Buffalo isolates

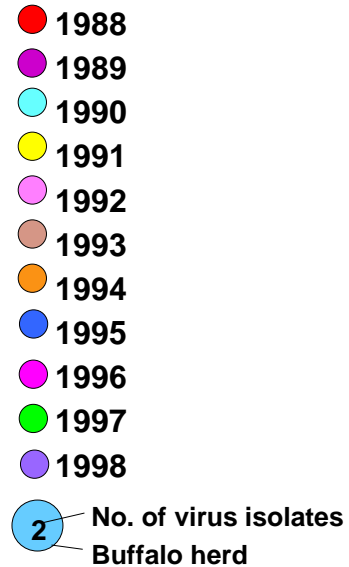
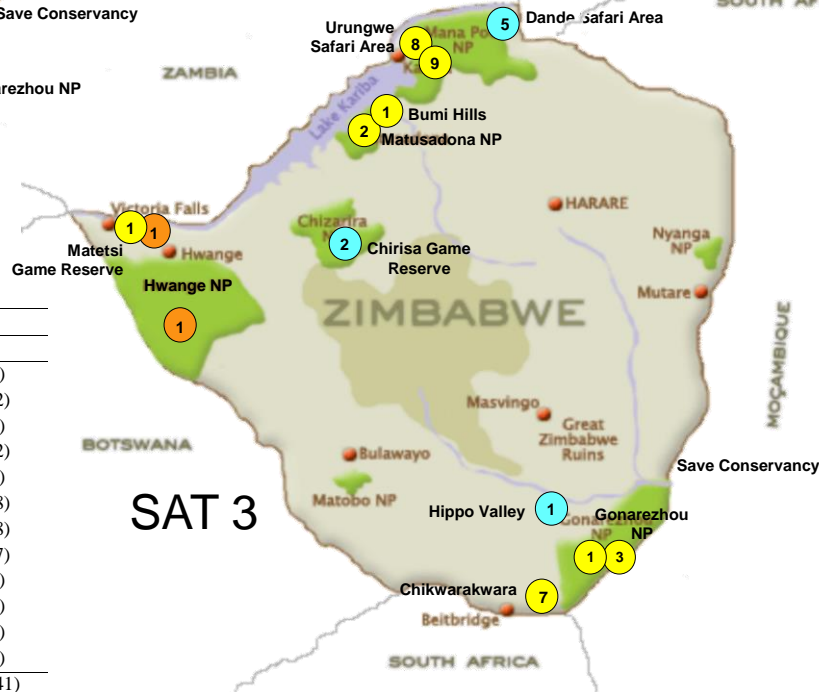
SAT 1



SAT 2



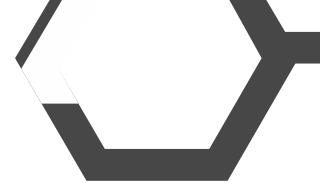
SAT 3



Location	Number positive/number sampled (% positive)			
	< 1 yr	1-3 yrs	>3 yrs	Total
Hwange NP	-	-	3/10 (30)	3/10 (30)
Hippo Valley Ranch	0/2 (0)	5/10 (50)	6/22 (27)	11/34 (32)
Doma SA	2/3 (67)	2/6 (33)	5/16 (31)	9/25 (36)
Dande SA	4/5 (80)	2/2 (100)	6/16 (38)	12/23 (52)
Bumi Hills NR	1/5 (20)	1/4 (25)	3/17 (18)	5/26 (19)
Chirisa SA	1/1 (100)	5/6 (83)	9/31 (29)	15/38 (38)
Gonarezhou NP	3/12 (25)	16/21 (76)	5/30 (17)	24/63 (38)
Urungwe SA	2/2 (100)	18/21 (86)	7/12 (58)	27/35 (77)
Chikwarakwara	1/1 (100)	3/3 (100)	3/9 (33)	7/13 (54)
Matusadona NP	3/5 (60)	-	6/12 (50)	9/17 (53)
Matetsi	-	2/4 (50)	4/14 (29)	6/18 (33)
Lubangwa Island	-	1/3 (33)	1/13 (8)	2/16 (13)
Average	17/36 (47)	55/80 (69)	58/202 (29)	130/318 (41)



# Materials and methods



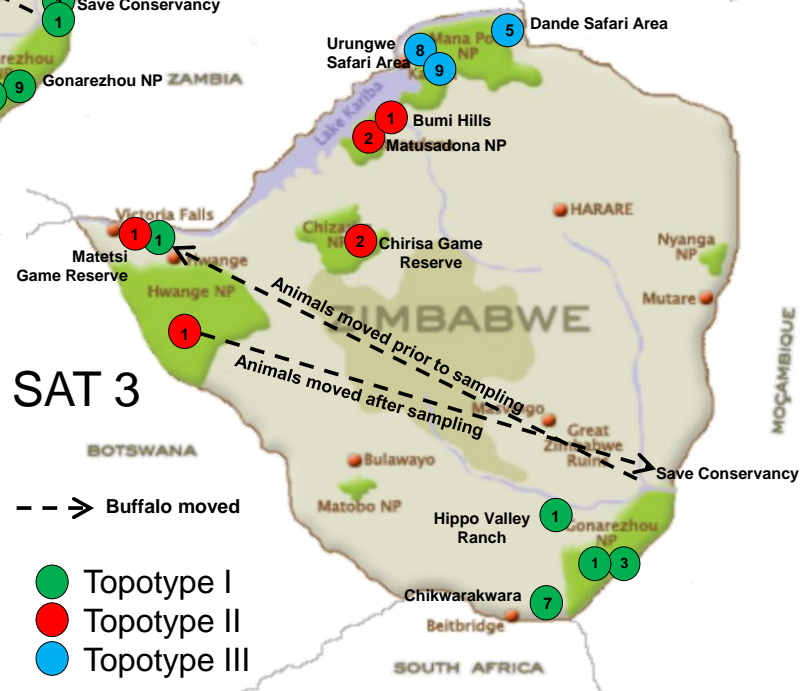
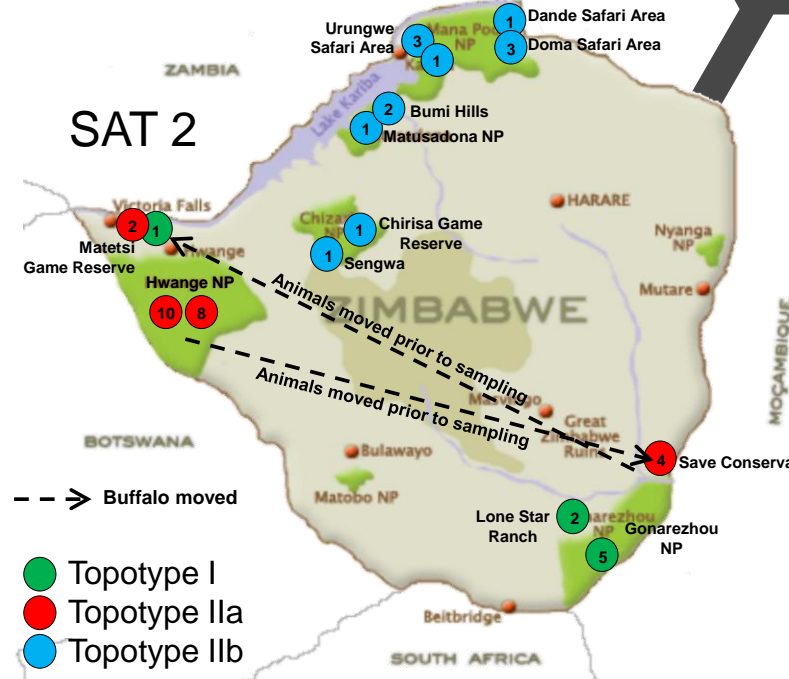
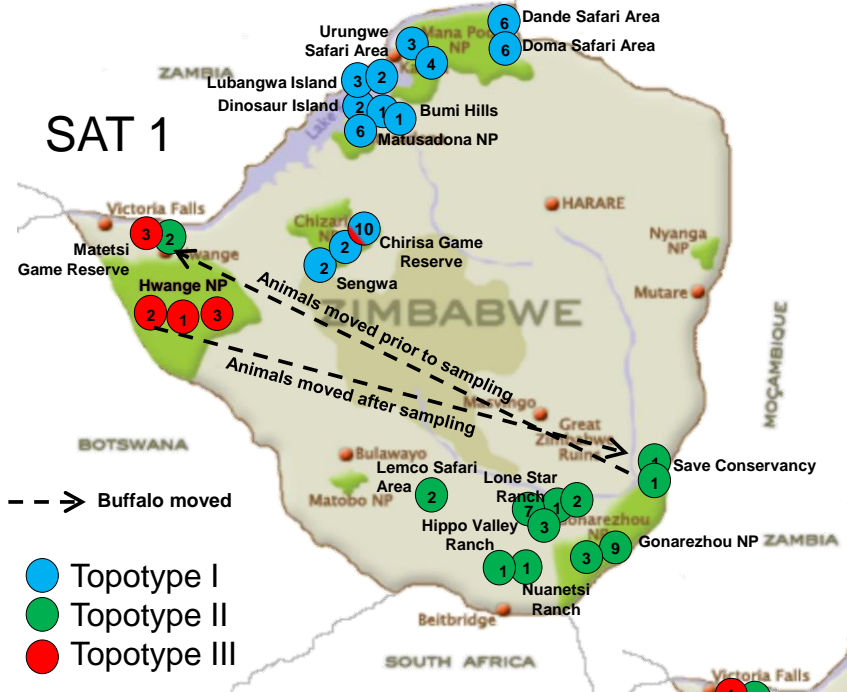
- VP1 sequences were determined by RT-PCR and Sanger sequencing (Knowles et al., 2016).
- Complete genome sequences were determined using the Illumina MiSeq platform – without RT-PCR (Logan et al., 2014).
- Genome assembly: after trimming adaptors and merging overlapping read pairs, the viral genomes were assembled in parallel with host contaminants using a novel in-house pipeline with two main components.
  - The first one enables detection and assembly of sequence, irrespective of whether viral or host, even when coverage is low.
  - The second stage scaffolds viral contigs obtained during the previous stage using a reference sequence solely as a guide, without incorporating any of the reference sequence in the final assembly.
- Phylogenetic analyses were performed using Maximum Likelihood (MEGA 7) and time-resolved Bayesian methods (BEAST 1.10.2).

# Results



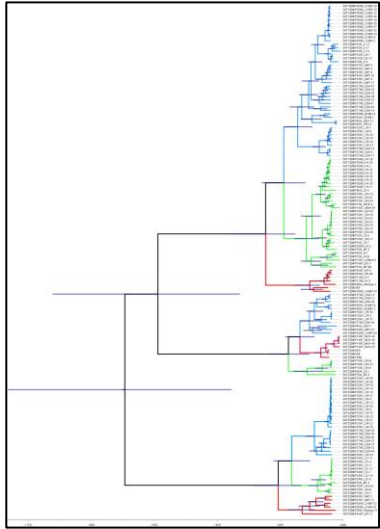
- The genome sequences of 143 FMD viruses were assembled.
- In some cases multiple serotypes were identified in a sample which had not been evident at the time of serological typing.
- For phylogenetic analyses, the polyprotein-coding region sequences were split into four parts, L, P1, P2 and P3.
- In the P1 region sequences clustered together by serotype and then by buffalo herd/geographic location, whereas in the L, P2 and P3 regions sequences clustered by buffalo herd/geographic region irrespective of serotype.

# VP1 topotyping

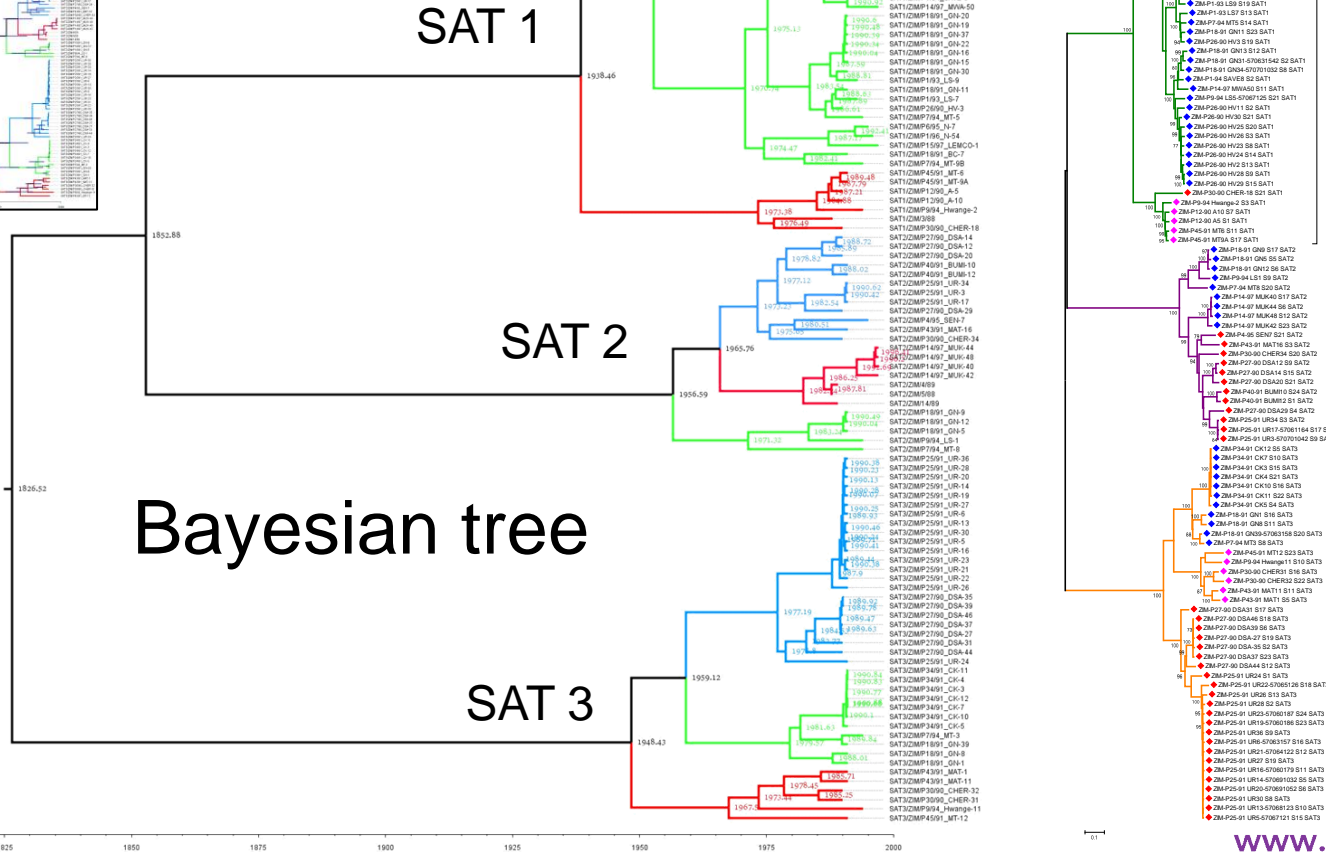




# Capsid-coding region



- NW Zimbabwe
- SE Zimbabwe
- W Zimbabwe



## Bayesian tree

SAT 1

ML tree

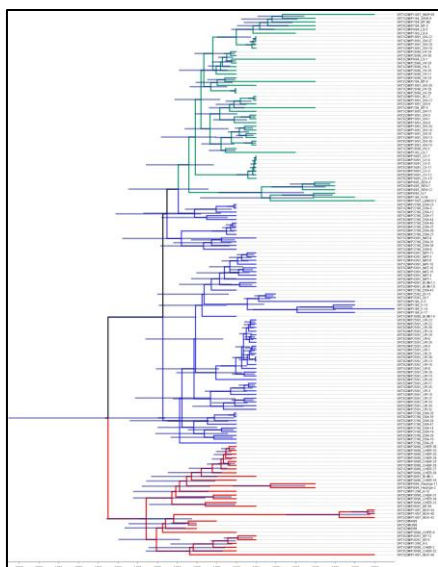
SAT 2

SAT 3

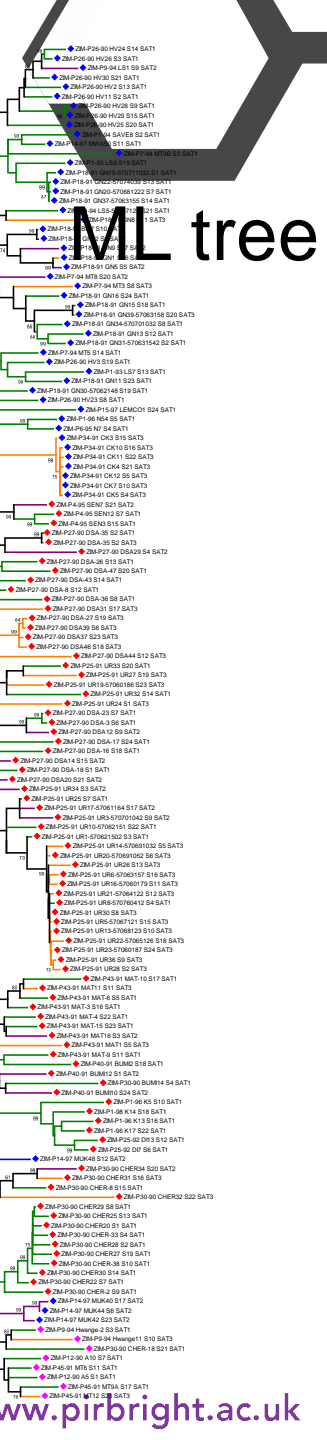
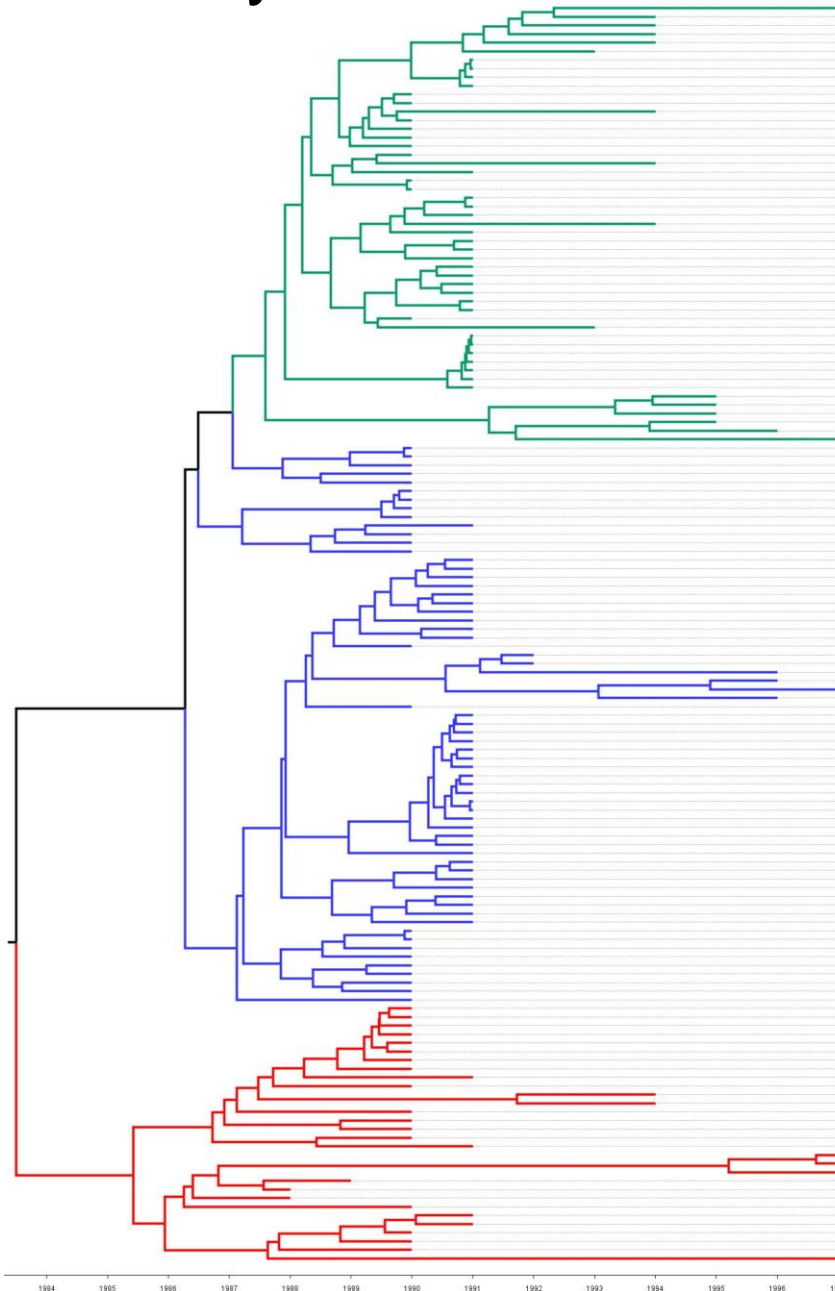
# 3Dpol

- NW Zimbabwe
- SE Zimbabwe
- W Zimbabwe

No clustering by serotype observed



# Bayesian tree

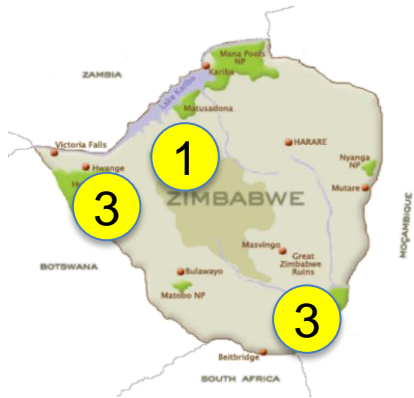


# ML tree

# SAT 1 – VP1

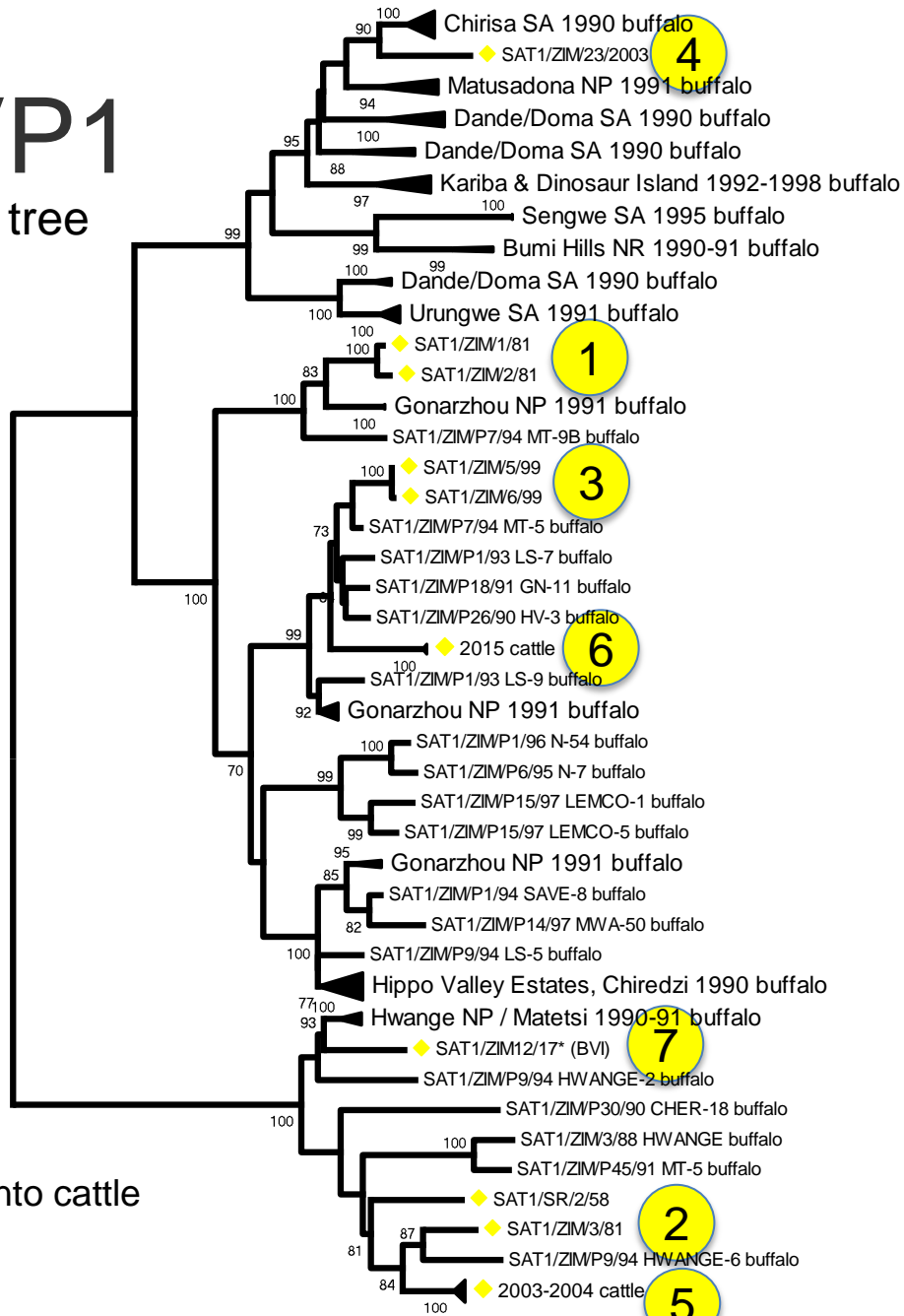
Maximum Likelihood tree

◆ cattle isolates



Origin of the introductions

□ At least 7 introductions into cattle between 1981 and 2017



Topotype I (NW Zimbabwe)

Topotype II (SE Zimbabwe)

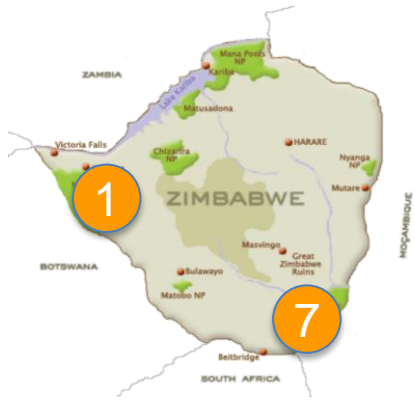
Topotype III (W Zimbabwe)

0.1

# SAT 3 – VP1

## Maximum Likelihood tree

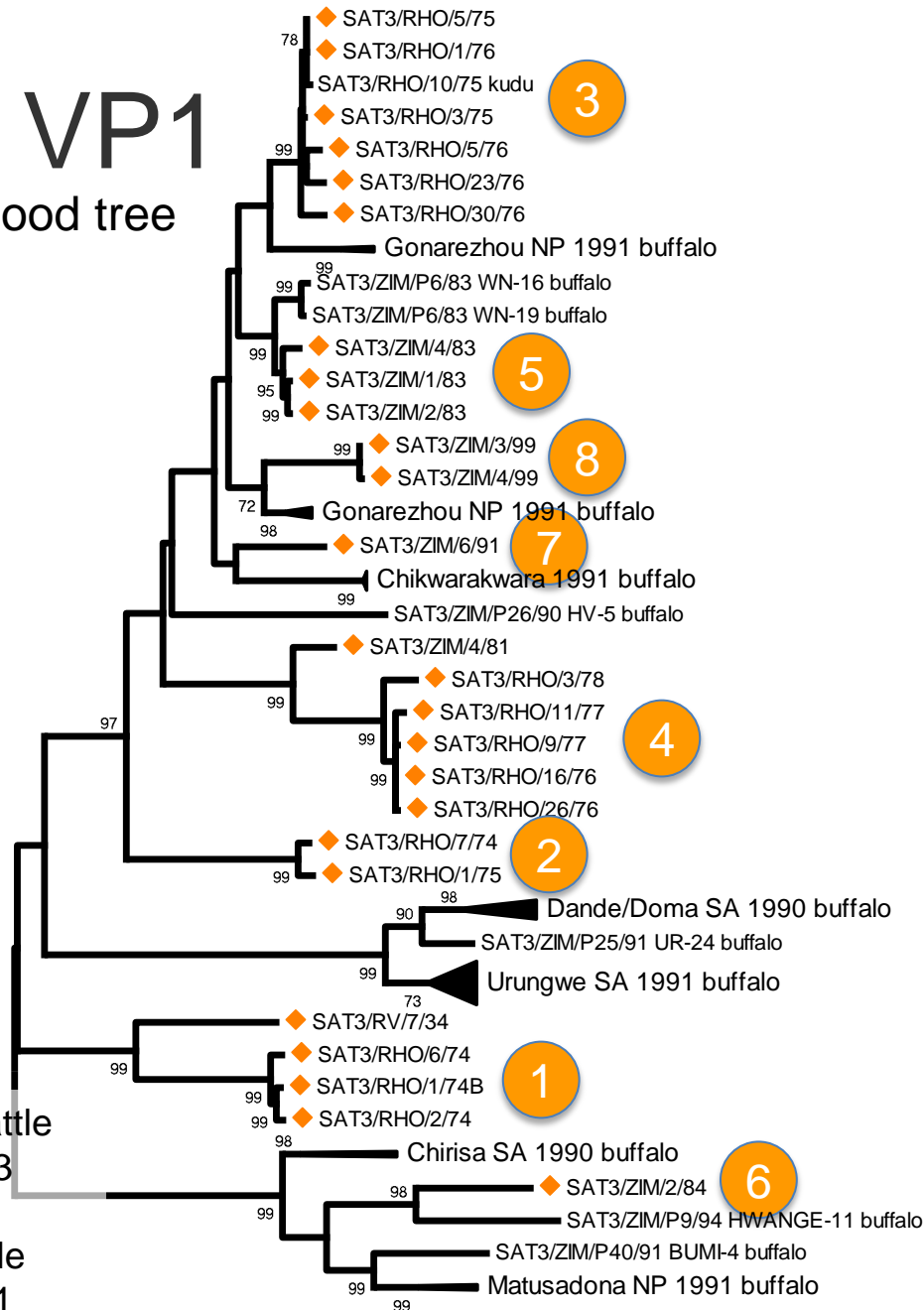
◆ cattle isolates



Origin of the introductions

□ No introductions into cattle between 1955 and 1973

□ 8 introductions into cattle between 1974 and 1991



Topotype I (SE Zimbabwe)

Topotype III (NW Zimbabwe)

novel topotype (SE Zimbabwe)

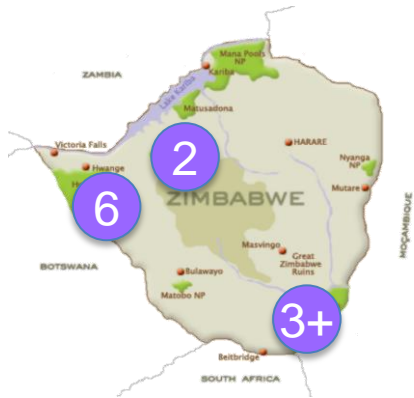
Topotype II (W Zimbabwe)

0.05

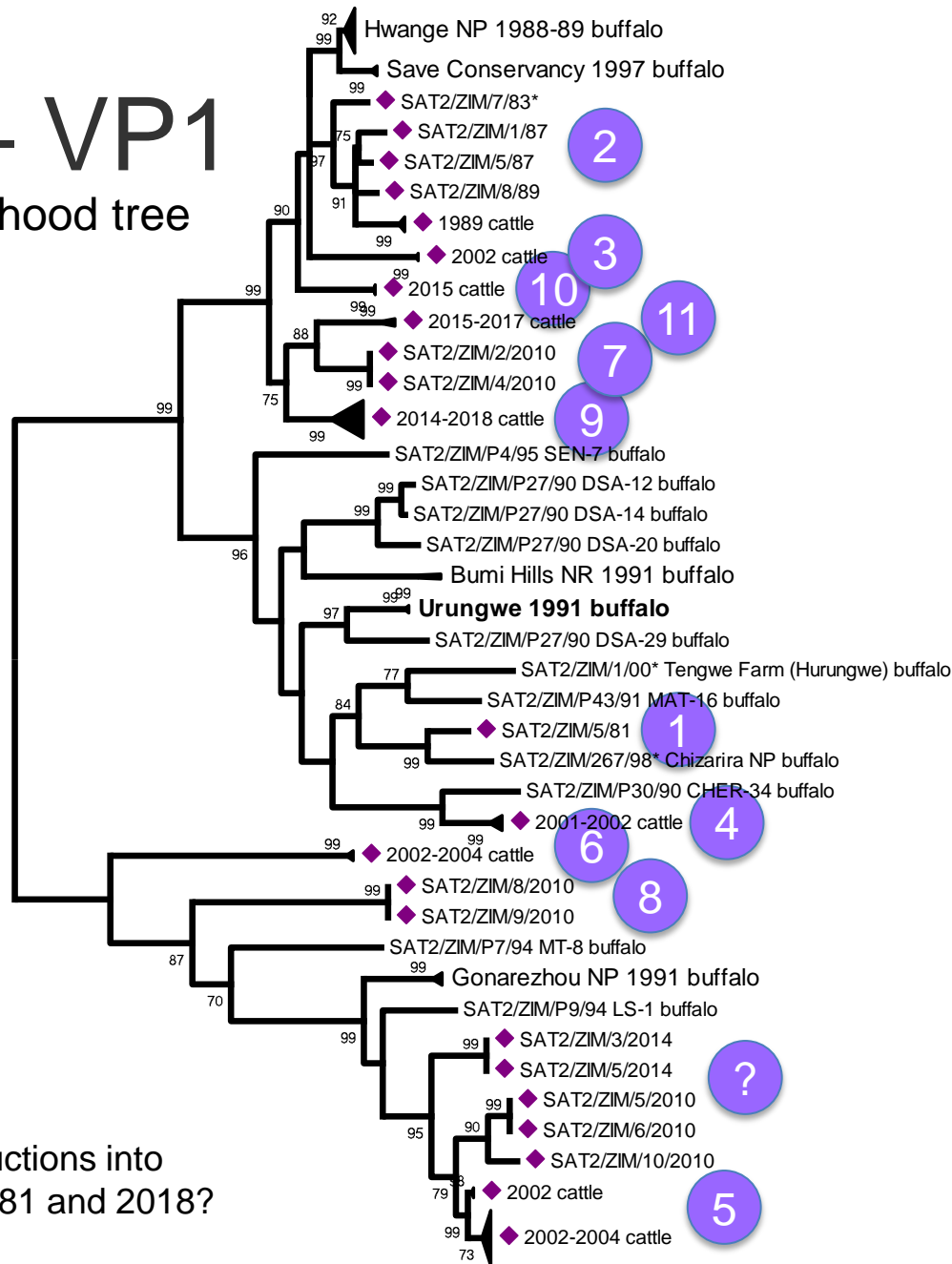
# SAT 2 – VP1

## Maximum Likelihood tree

◆ cattle isolates



Origin of the introductions



Topotype Ila (W Zimbabwe)

Topotype IIb (NW Zimbabwe)

Topotype 1 (SE Zimbabwe)

□ At least 11 introductions into cattle between 1981 and 2018?

0.05



# Conclusions

- Phylogenetic analyses of the different genome regions demonstrated the virus clustering by buffalo herd.
- The lack of clustering by serotype in non-capsid regions suggests that extensive recombination has taken place between the serotypes.
- The close relationship between some within-herd viruses suggested the possibility of acute infection epidemics.
- Comparison of VP1 sequences with those of cattle outbreak isolates revealed a defined number of introductions of SAT 1 and SAT 3 viruses, but a more complex situation with SAT 2 viruses with possible longer-term persistence in the cattle population.

# Acknowledgements

- Funding for the original buffalo study was provided by the Overseas Development Administration (ODA), now Department for International Development (DFID) and was facilitated by the Department of Veterinary Services, Zimbabwe.
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