Gene editing livestock

Simon Lillico

ISBR 2025

ROSLIN

THE UNIVERSITY of EDINBURGH
Royal (Dick) School of Veterinary Studies

UKRI Biotechnology and Biological Sciences Research Council

Gene edited livestock with positive regulatory decisions

Rumigen

TOWARDS IMPROVEMENT OF **RUMI**NANT BREEDING THROUGH **GEN**OMIC AND EPIGENOMIC APPROACHES

RUMIGEN, coordinated by INRAE, involves 18 partners in 7 Member states plus Norway and United Kingdom

- Start date: 1st June 2021
- End date: 31st May 2026
- EU contribution: 6.998.851 €
- Project coordinator: Eric Pailhoux

UKRI Biotechnology and Biological Sciences Research Council

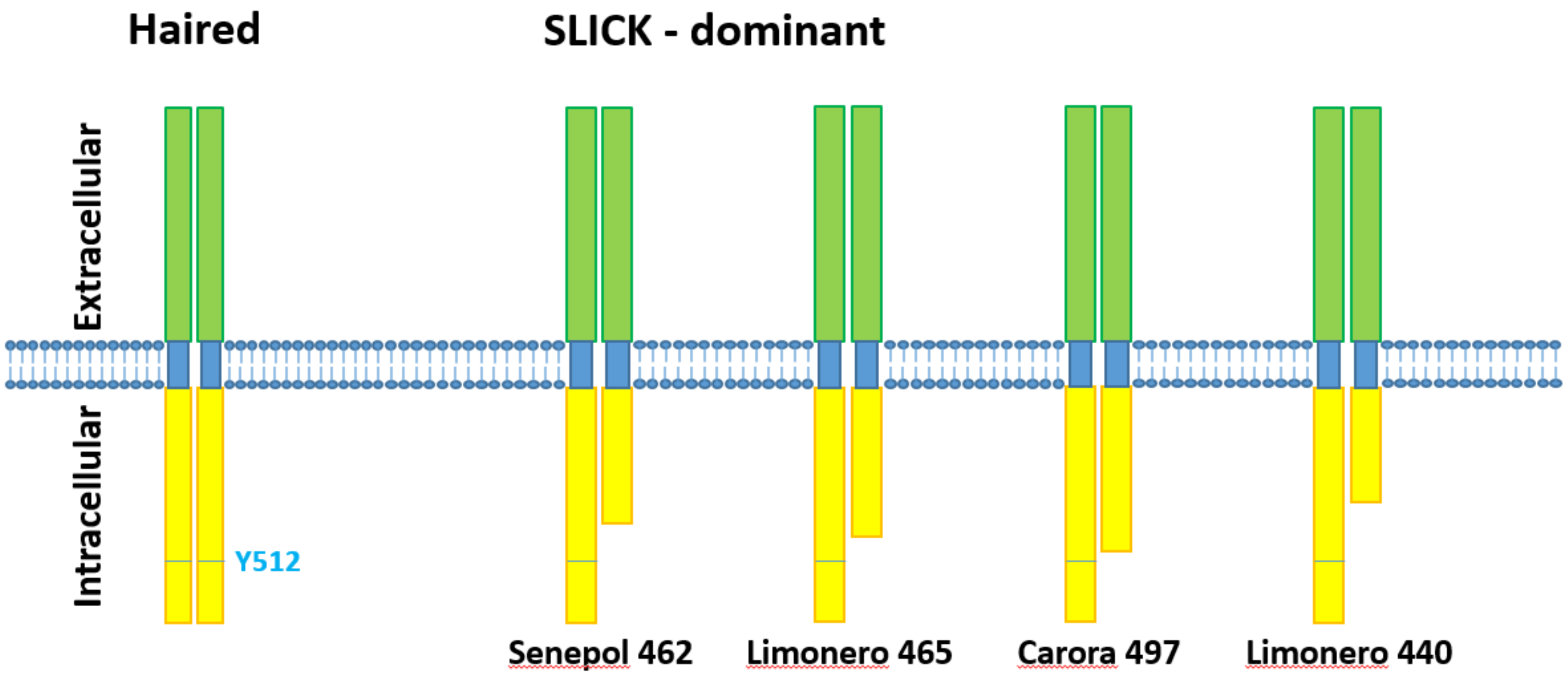
ROSLIN

THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

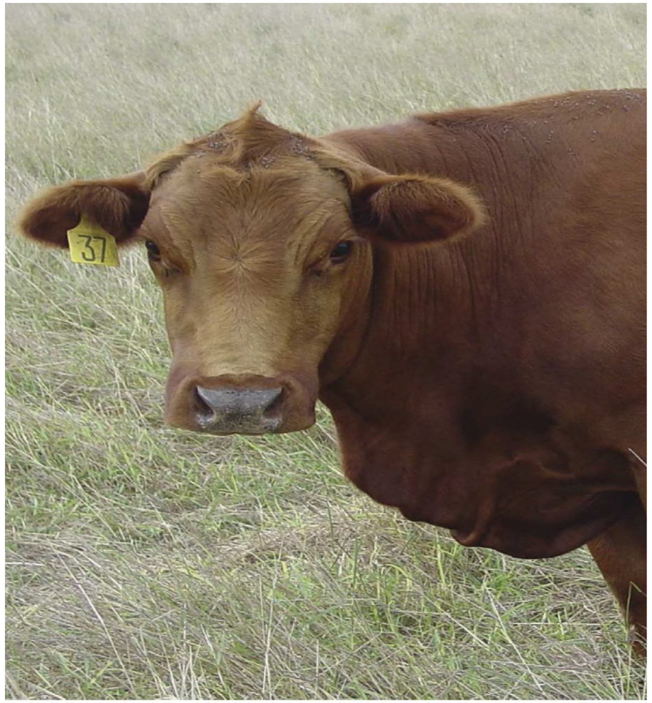
The slick phenotype in cattle

Mutations in the prolactin receptor (PRLR) gene are associated with improved thermal tolerance in cattle



Y512 dimerization required for Jak2/STAT5 activation

(adapted from Porto-Neto et al., 2018)



Biotechnology and Biological Sciences Research Council

ROSLIN

Rumigen

THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Will PRLR mutation in sheep produce a slick phenotype?

Control (RUM02)

Homozygote (RUM03)

UKRI Biotechnology and Biological Sciences Research Council

Rumigen

ROSLIN

THE UNIVERSITY of EDINBURGH
Royal (Dick) School of Veterinary Studies

Will PRLR mutation in sheep produce a slick phenotype?

Control (RUM02)

Homozygote (RUM03)

August
6 months old

Biotechnology and Biological Sciences Research Council

THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

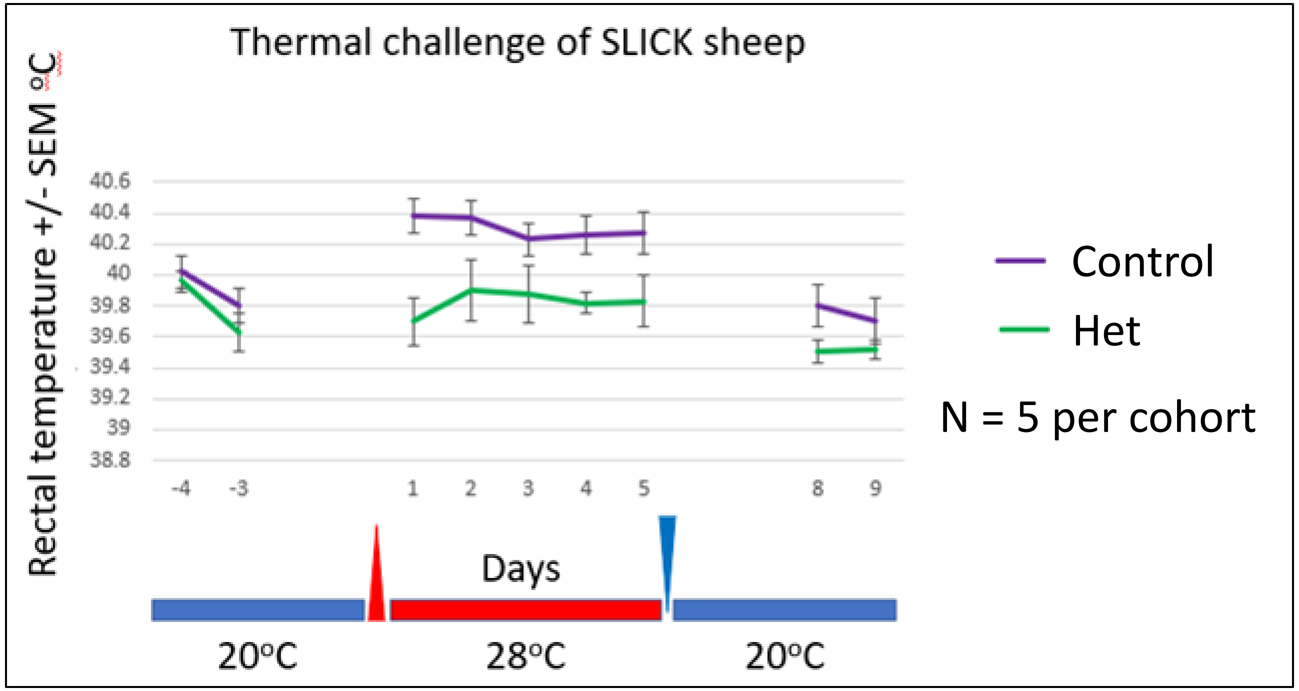
SLICK sheep have improved thermal tolerance

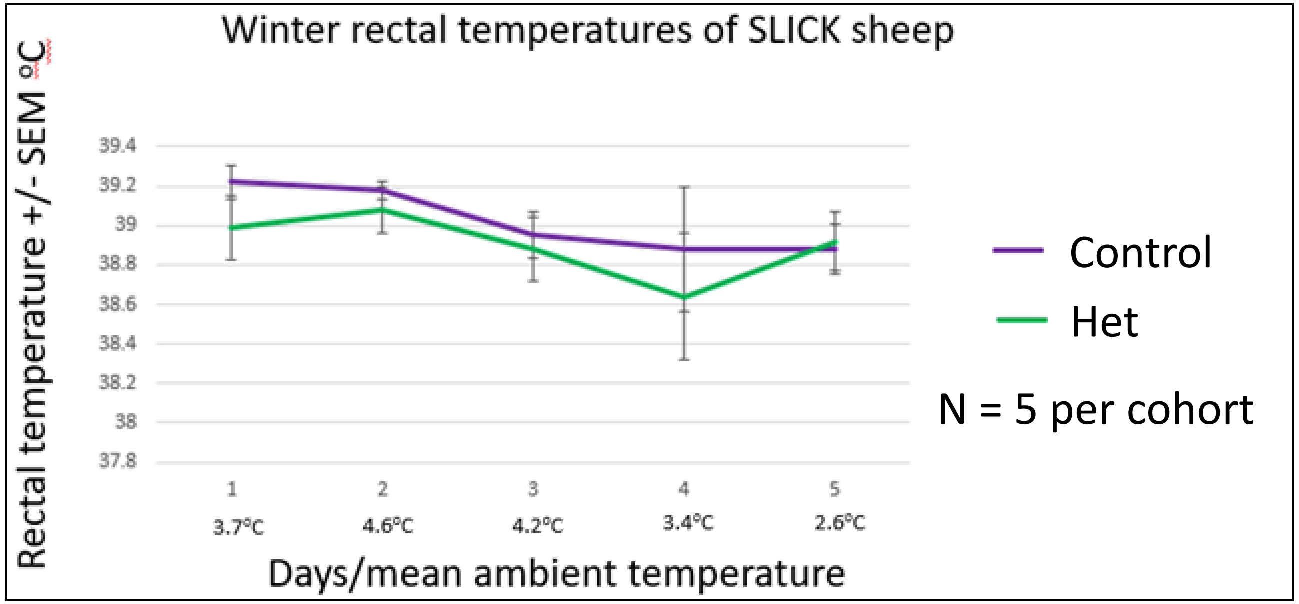
Biotechnology and Biological Sciences Research Council

Rumigen

ROSLIN

THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies



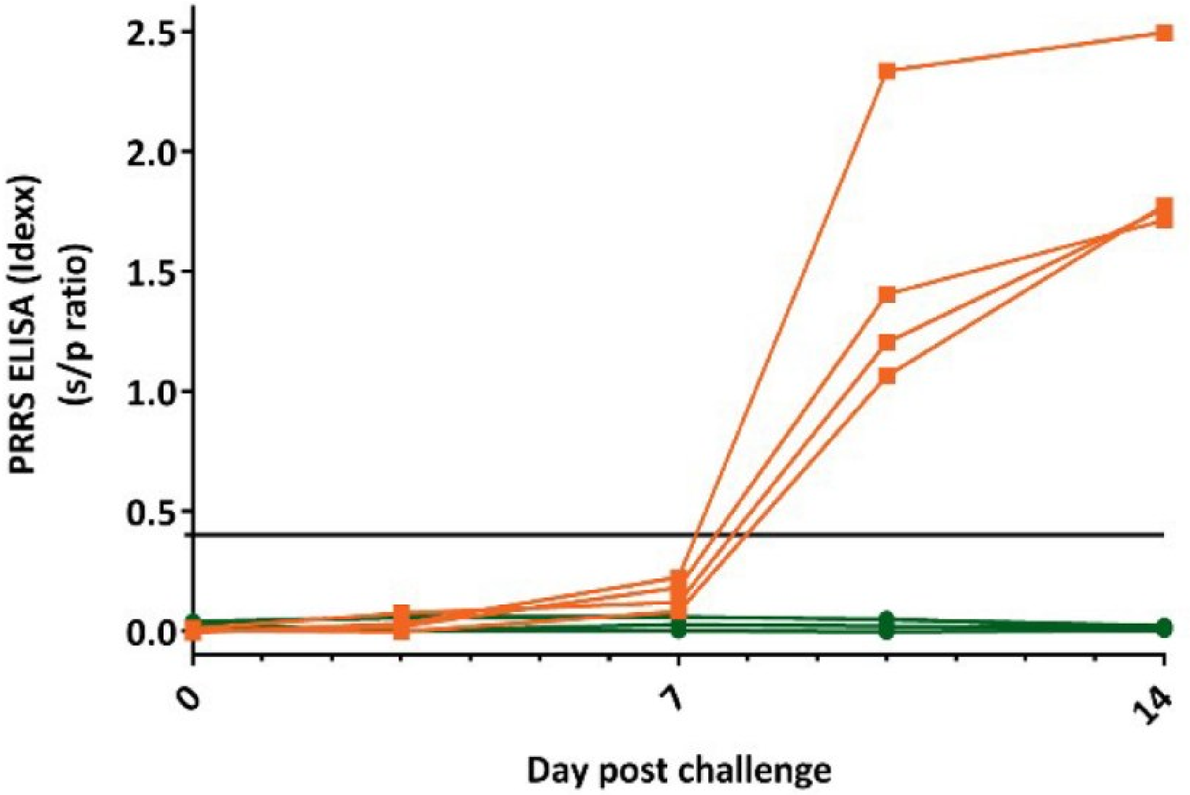


Biotechnology and Biological Sciences Research Council

ROSLIN

Consequences of viral disease

Porcine Reproductive and Respiratory Syndrome



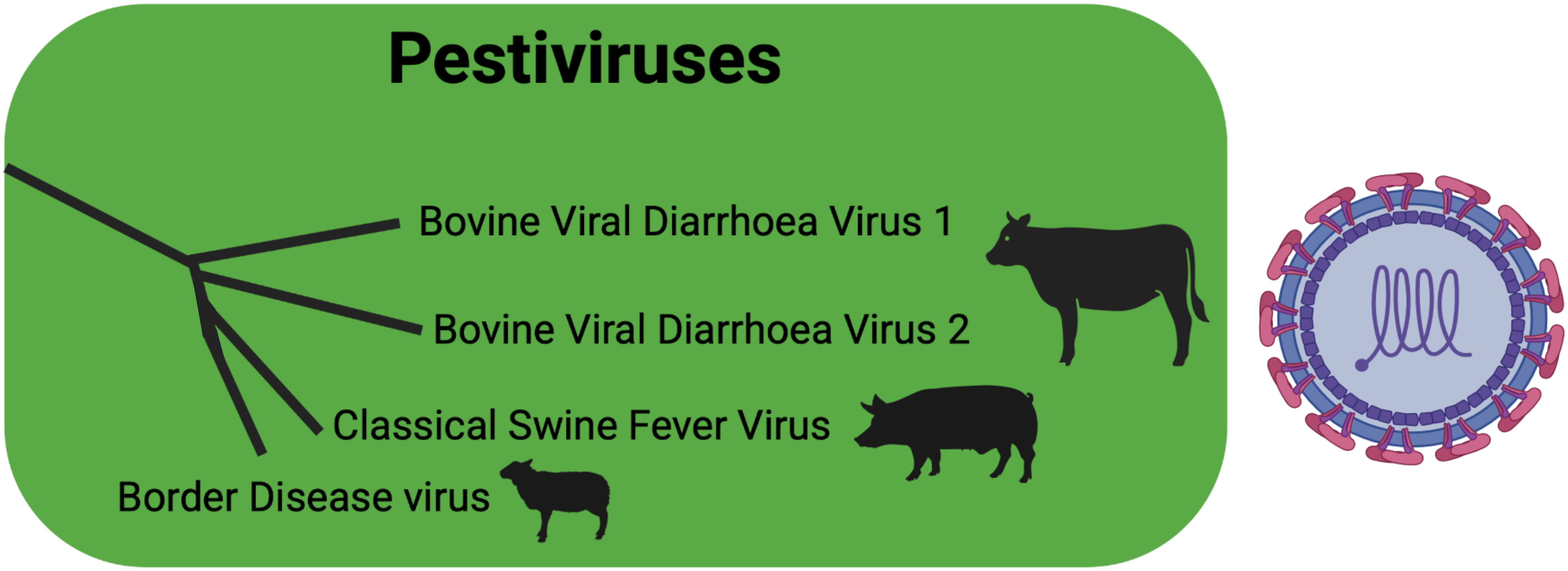
Biotechnology and Biological Sciences Research Council

ROSLIN

Genus

THE UNIVERSITY of EDINBURGH
Royal (Dick) School of Veterinary Studies

Pestiviruses infect livestock

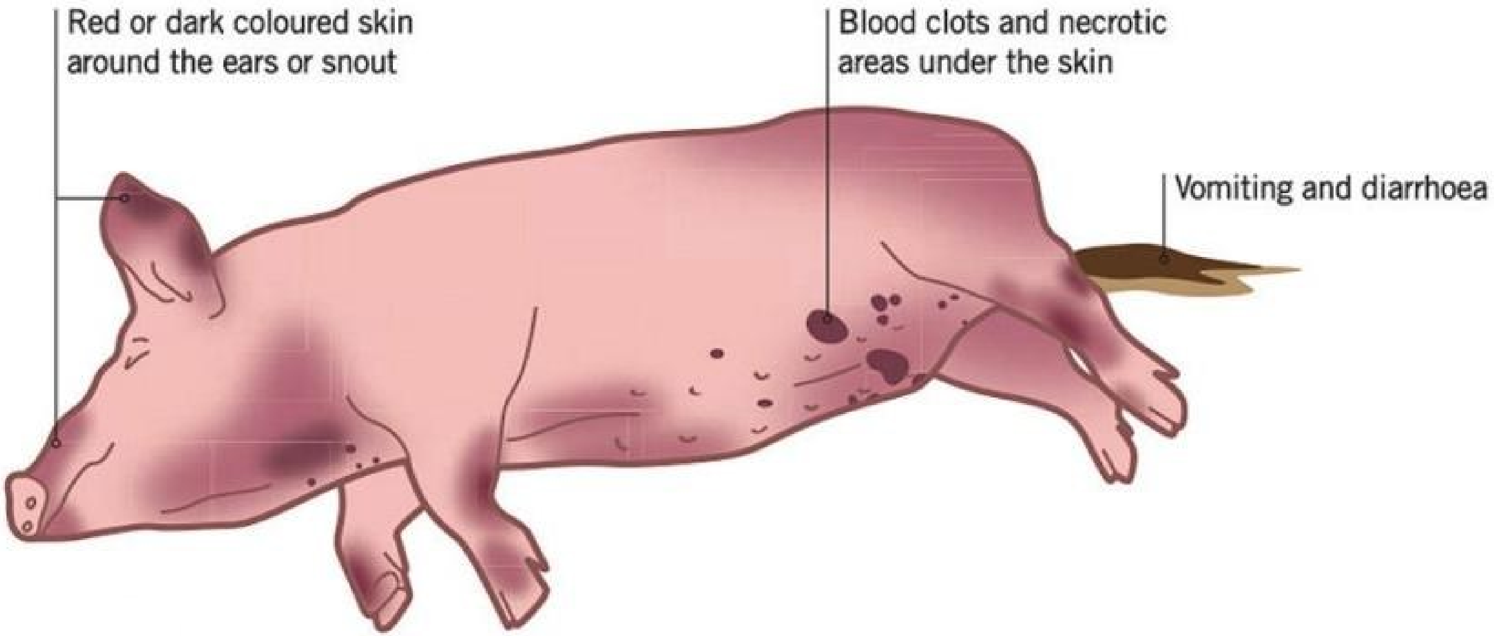


Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Classical swine fever (also known as Hog Cholera)

NOT to be confused with African Swine Fever or swine influenza

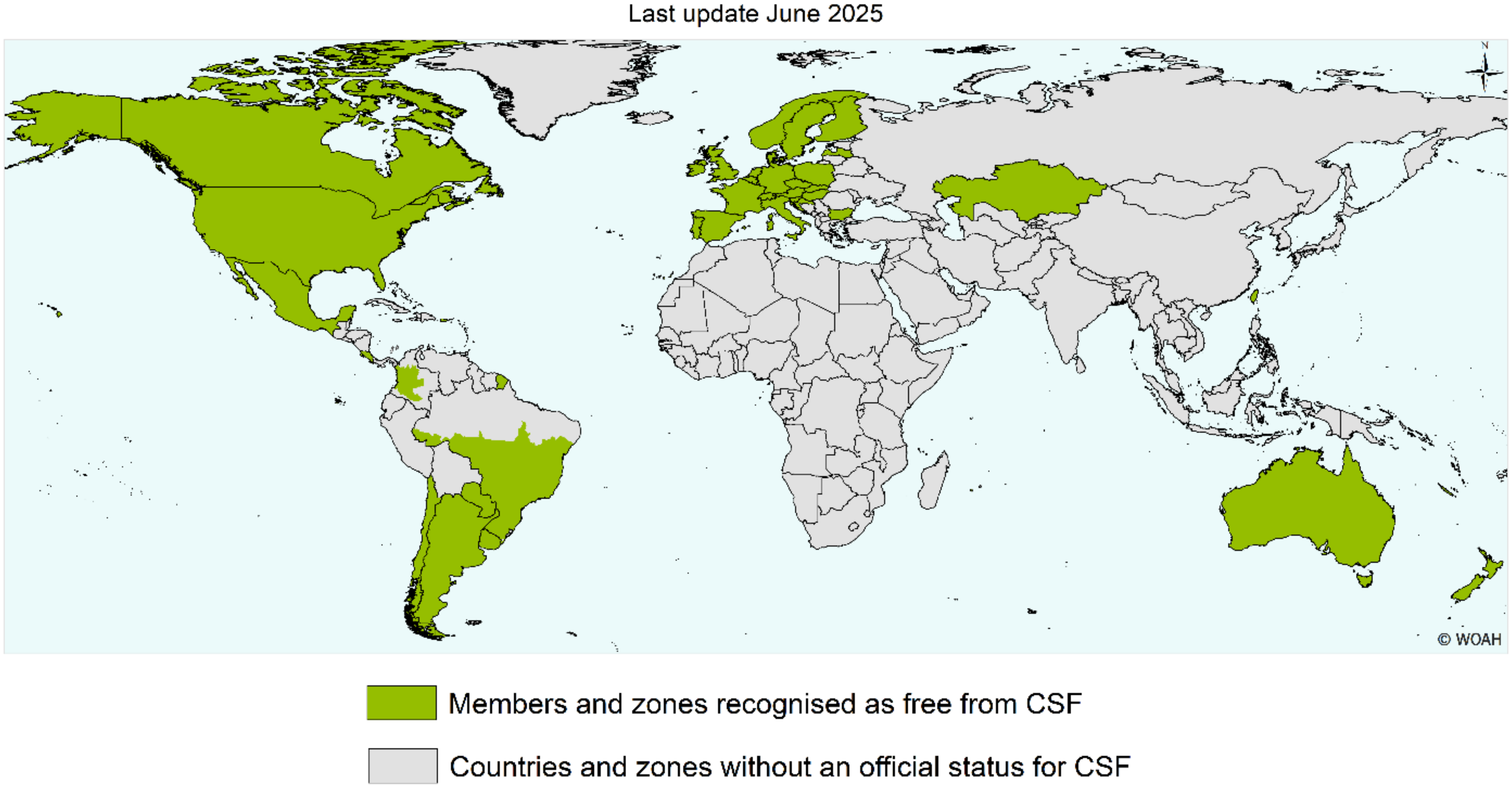
- High Fever
- Decreased Appetite
- Weakness, Incoordination, Paddling
- Red, blotchy skin
- Red ears
- Nasal and Eye Discharge
- Diarrhoea and Vomiting
- Coughing and difficulty Breathing
- Miscarriage, stillbirths, weak litters
- Congenital Tremor
- Death, which may be sudden



Big impact on animal welfare and productivity

Adapted from: Asian Agribiz Team, John Carr

Classical swine fever – a disease of global importance



Eradicated in UK in 1966

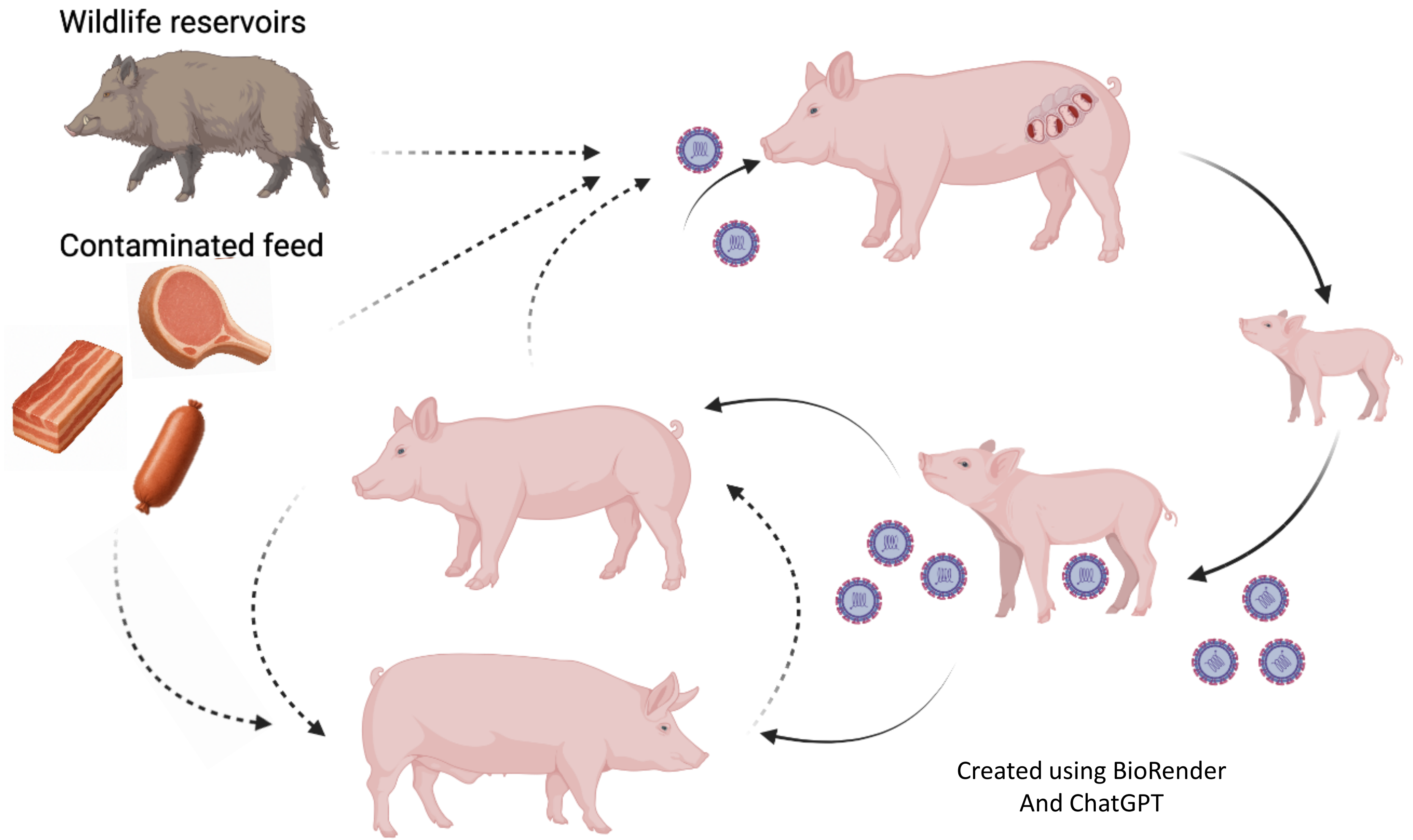
Outbreaks in: 1971
1986
2000

2000 UK outbreak required cull of 75,000 pigs to contain it

Source: World Organisation for Animal Health (previously OIE)

Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Classical swine fever - transmission



Created using BioRender
And ChatGPT

Current management

- Vaccination
- Biosecurity
- Depopulation
- Trade restrictions

Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Pestiviruses interact with many host proteins

viruses MDPI

Review

Current Status of Genetically Modified Pigs That Are Resistant to Virus Infection

Hongming Yuan †, Lin Yang †, Yuanzhu Zhang, Wenyu Xiao, Ziru Wang, Xiaochun Tang, Hongsheng Ouyang * and Daxin Pang *

Viruses **2022**, *14*, 417. https://doi.org/10.3390/v14020417

CD46
Low density lipoprotein receptor
Heparan sulfate
ADAM17

Table 1. Host factors that act in CSFV infection.

Host Factors	Function in CSFV Infection	Promote (+) or Inhibit (−) CSFV Growth in Host Cell
Annexin2	Interacts with CSFV E2 and NS5A, promote CSFV replication	+
IFITM1-3 (interferon-induced trans membrane protein 3)	Modifies the membrane structure or alter endosomal physiology to impair viral membrane fusion	−
ARFGAP1 (ADP-ribosylation factor GTPase-activating protein 1)	Binds to CSFV NS5A and promote CSFV replication	+
β-actin	The amino acids 95-188 of β-actin are responsible for the interaction between β-actin and CSFV E2	+
Caveolin-1	CAV1-mediated endocytosis is necessary for CSFV invasion	+
NDP52 (nuclear dot protein 52)	CSFV inhibits NDP52 expression. Additionally, inhibiting NDP52 promotes interferon and TNF release, acting on the NF-κB pathway	+
GBP1 (guanylate-binding protein 1)	The N-terminal globular GTPase domain of GBP1 interacts with CSFV NS5A. Overexpression of GBP1 inhibits CSFV replication; knocking down GBP1 significantly promotes CSFV replication. Furthermore, the K51 of GBP1 is essential for CSFV replication	−
PSMB10 (proteasome subunit beta 10)	Acts as an NS3-interacting partner in CSFV infection. Overexpression of PSMB10 inhibited CSFV replication	+
POASL (interferon-inducible oligoadenylate synthetase-like protein)	Interacts with MDA5 to enhance MDA5-mediated type I IFN signaling and suppress CSFV replication	−
MERTK (Mer tyrosine kinase)	Interacts with CSFV E2 to facilitate CSFV entry, and down-regulates the expression of IFN-β to enhance CSFV replication	+
MG132	Activates JAK-STAT pathway and up-regulates several interferon-stimulated genes' (ISGs) expression in CSFV infection cells	−
RACK1 (receptor for activated C kinase 1)	RACK1 interacts with NS5A, inhibiting CSFV replication by inhibiting NF-κB activation	−
PRNF114 (porcine RING finger protein 114)	Interacts with NS4B and degrades NS4B through a proteasome-dependent pathway	−
Rab1b, Rab5, Rab7, and Rab11	Regulates CSFV endocytosis	+
Rab18	Interacts with NS5A and mediates virus replication and assembly	+
DCNT6 (dynactin subunit 6)	Interacts with E2, and the DCNT6-E2 interaction is important for CSFV replication and viral virulence	+
Torsin-1A	Interacts with E2, disrupting Torsin-1A-E2 interaction to completely inhibit CSFV replication	+
CCDC115 (coiled-coil domain-containing 115)	CCDC115-E2 interaction is essential for CSFV replication in swine macrophages	+
LamR (laminin receptor)	Acts as an alternative attachment receptor, interacting with E^{rns}	+
Fatty acid synthase (FASN)	FASN participates in the formation of the replication complex. Knocking down FASN in host cells inhibits CSFV replication	+
PCBP1 (poly C-binding protein 1)	Interacts with N^{pro}, down-regulating type I interferon in CSFV infection cells	+

AMERICAN SOCIETY FOR MICROBIOLOGY Journal of Virology®
REPLICATION
1 October 2004 Volume 78 Issue 19
https://doi.org/10.1128/jvi.78.19.10765-10775.2004

Temporal Modulation of an Autoprotease Is Crucial for Replication and Pathogenicity of an RNA Virus

T. Lackner[1], A. Müller[1], A. Pankraz[1], P. Becher[1], H.-J. Thiel[1], A. E. Gorbalenya[2,*], N. Tautz[1,*]

PNAS

Dissection of a viral autoprotease elucidates a function of a cellular chaperone in proteolysis

T. Lackner, H.-J. Thiel, and N. Tautz[†]

Institut für Virologie (Fachbereich Veterinärmedizin), Justus-Liebig-Universität Giessen, Frankfurter Strasse 107, D-35392 Giessen, Germany

Edited by Charles M. Rice, The Rockefeller University, New York, NY, and approved November 18, 2005 (received for review September 21, 2005)

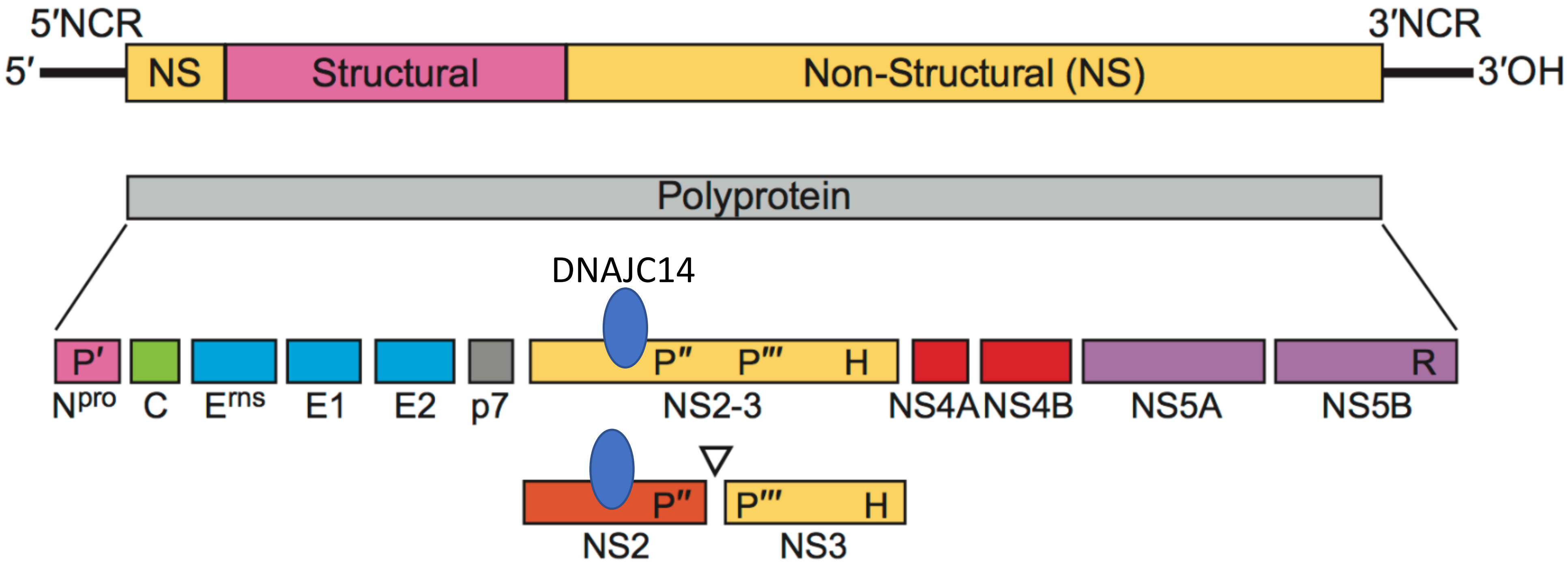
AMERICAN SOCIETY FOR MICROBIOLOGY Journal of Virology®
GENOME REPLICATION AND REGULATION OF VIRAL GENE EXPRESSION
March 2019 Volume 93 Issue 5 10.1128/jvi.01714-18
https://doi.org/10.1128/jvi.01714-18

CRISPR/Cas9-Mediated Knockout of DNAJC14 Verifies This Chaperone as a Pivotal Host Factor for RNA Replication of Pestiviruses

O. Isken[a], A. Postel[b], B. Bruhn[a], E. Lattwein[a,*], P. Becher[b], N. Tautz[a]

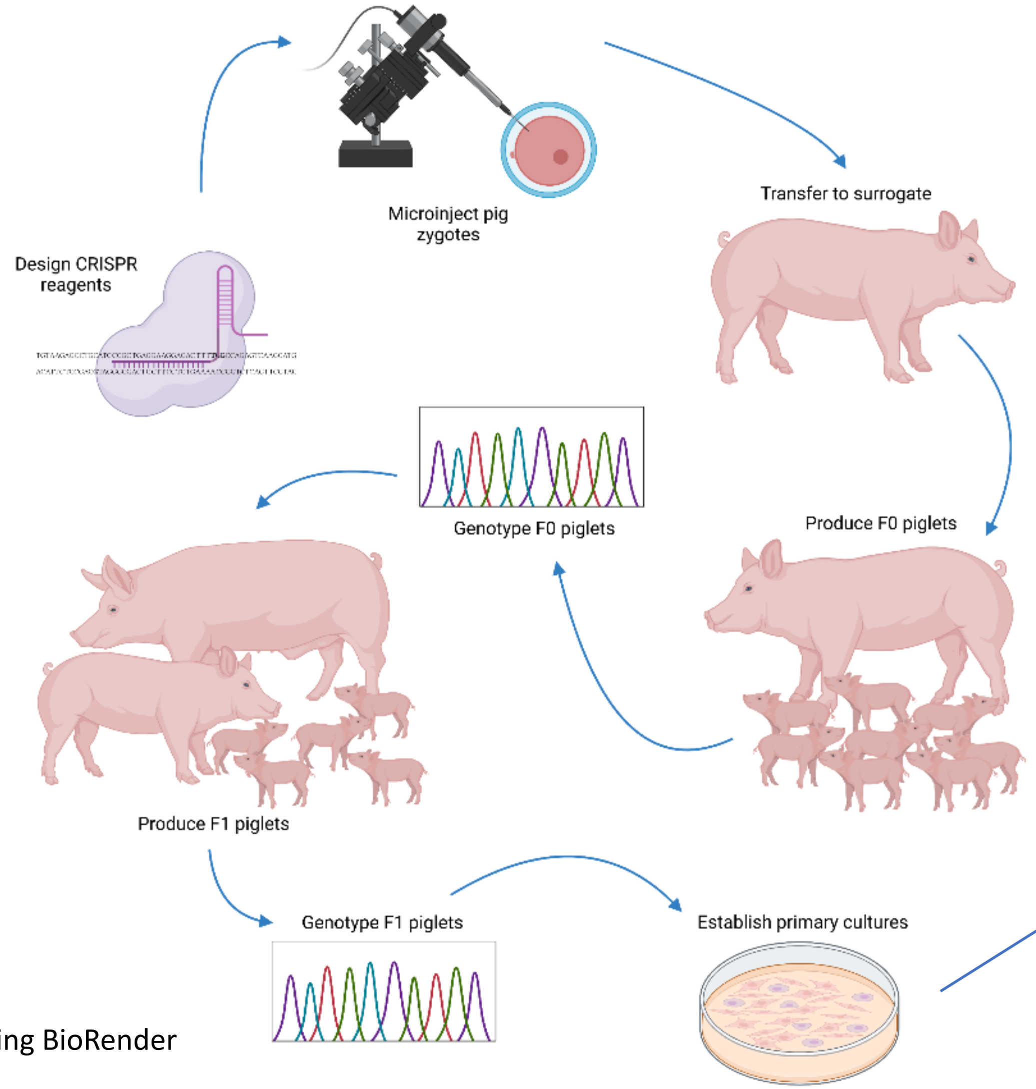
UKRI Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Classical pestiviruses have a compact RNA genome

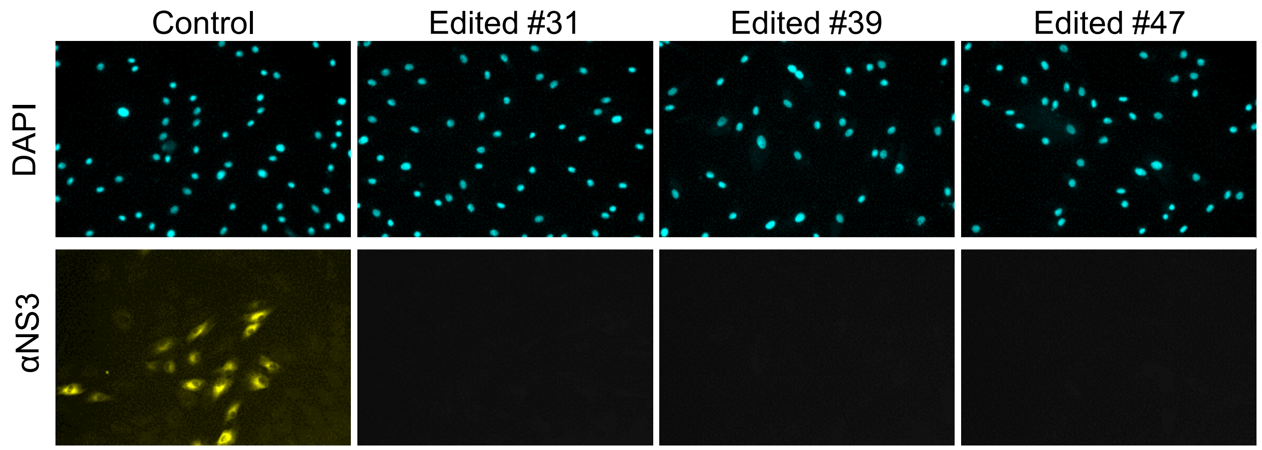


Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

We produced pigs with edited DNAJC14



CSFV challenge



Created using BioRender

Biotechnology and Biological Sciences Research Council

Animal & Plant Health Agency

ROSLIN

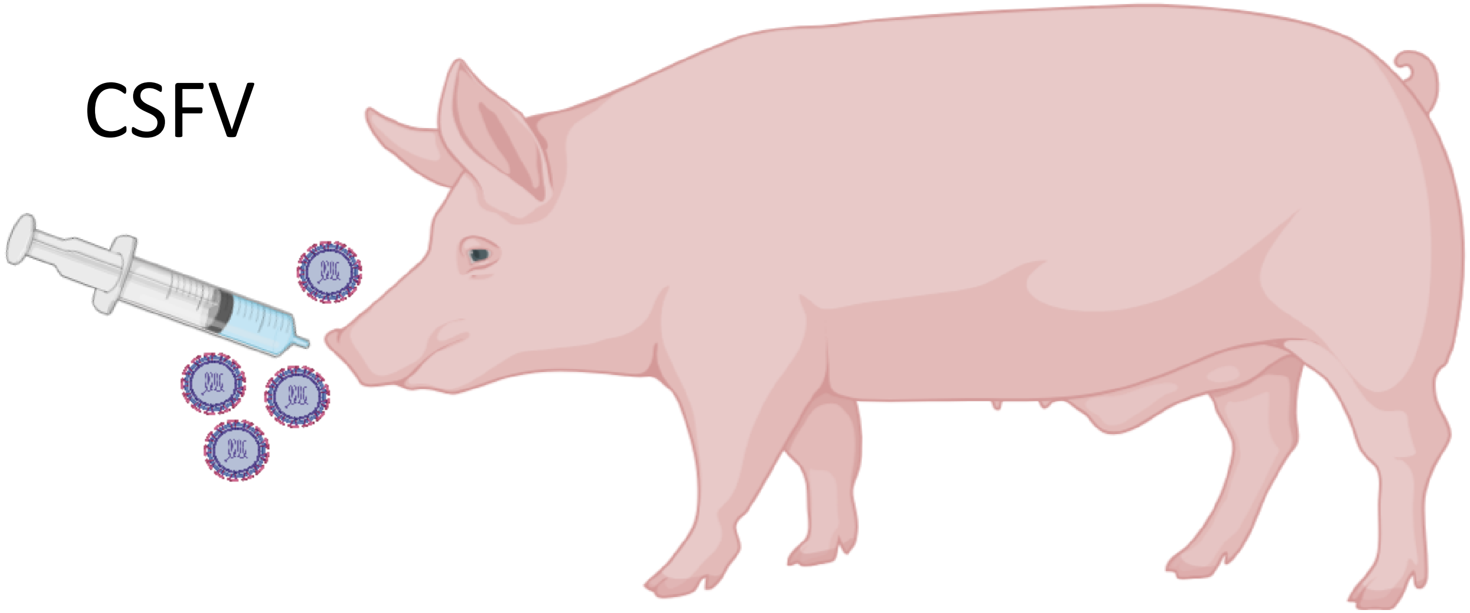
UNIVERSITÄT ZU LÜBECK

Genus

THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Is DNAJC14 essential for viral replication *in vivo*?

CSFV



Created using BioRender

4 x edited **4 x control**

Monitored for 22 days

Clinical signs

Leukopenia

Viraemia

Antibodies

Shedding

Tissue PCR

Biotechnology and Biological Sciences Research Council

Animal & Plant Health Agency

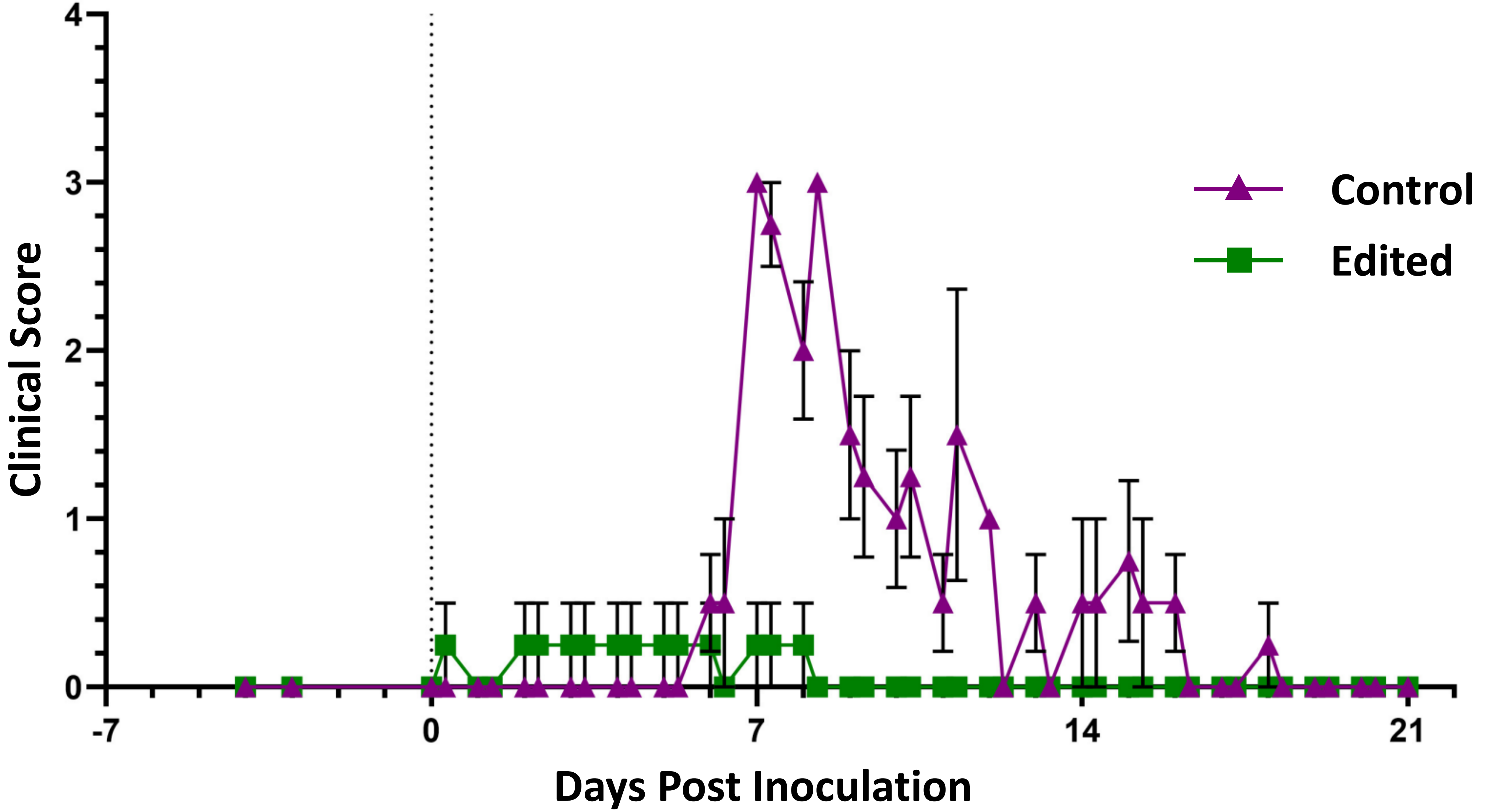
ROSLIN

UNIVERSITÄT ZU LÜBECK

Genus

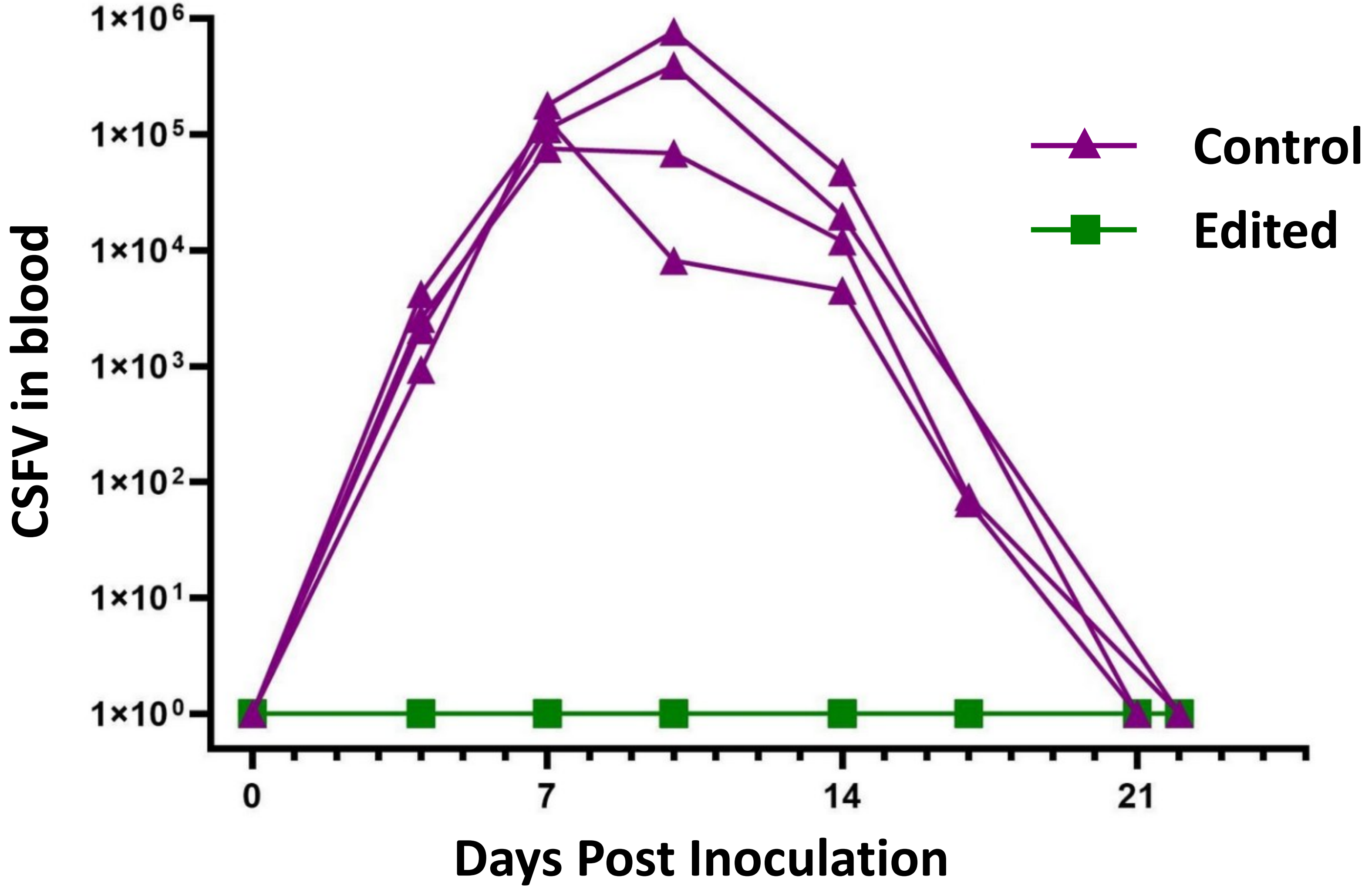
THE UNIVERSITY *of* EDINBURGH Royal (Dick) School of Veterinary Studies

Edited pigs do not develop clinical signs



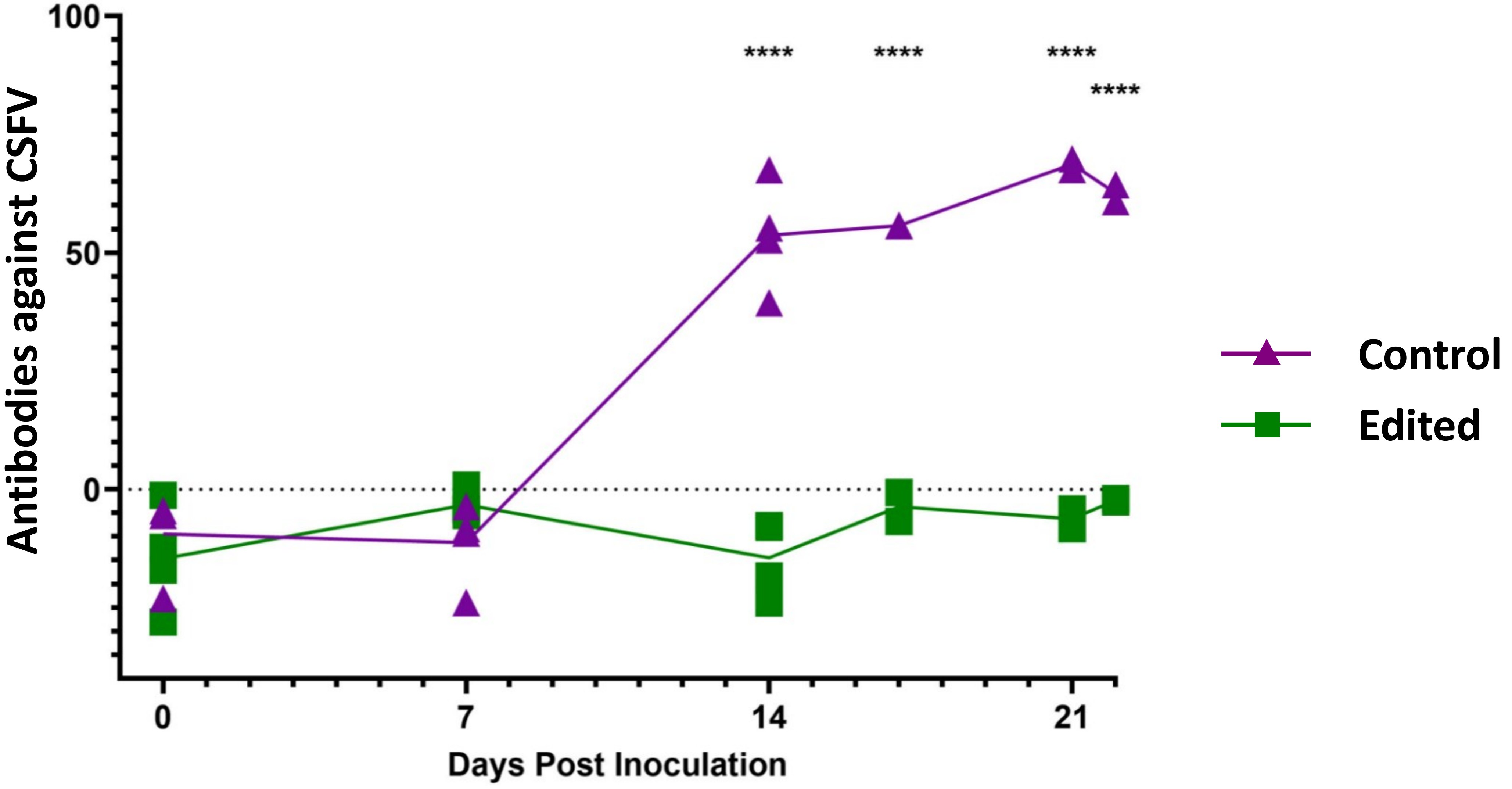
Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Edited pigs have no CSFV in their blood



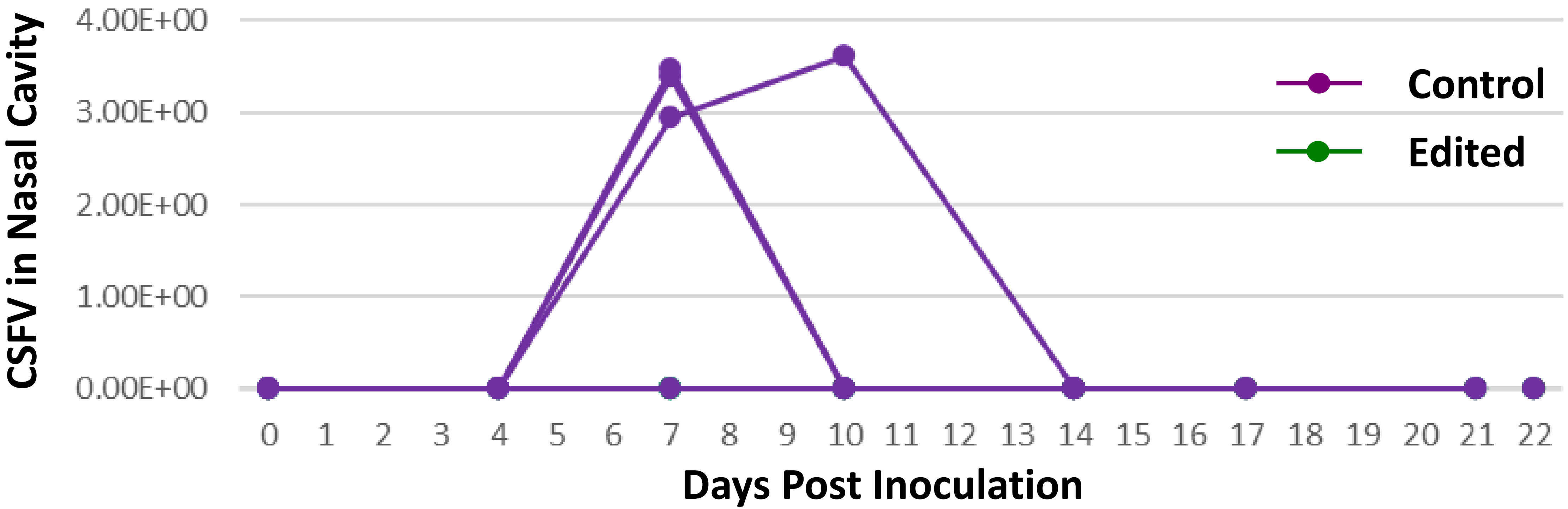
Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Edited pigs do not raise antibodies



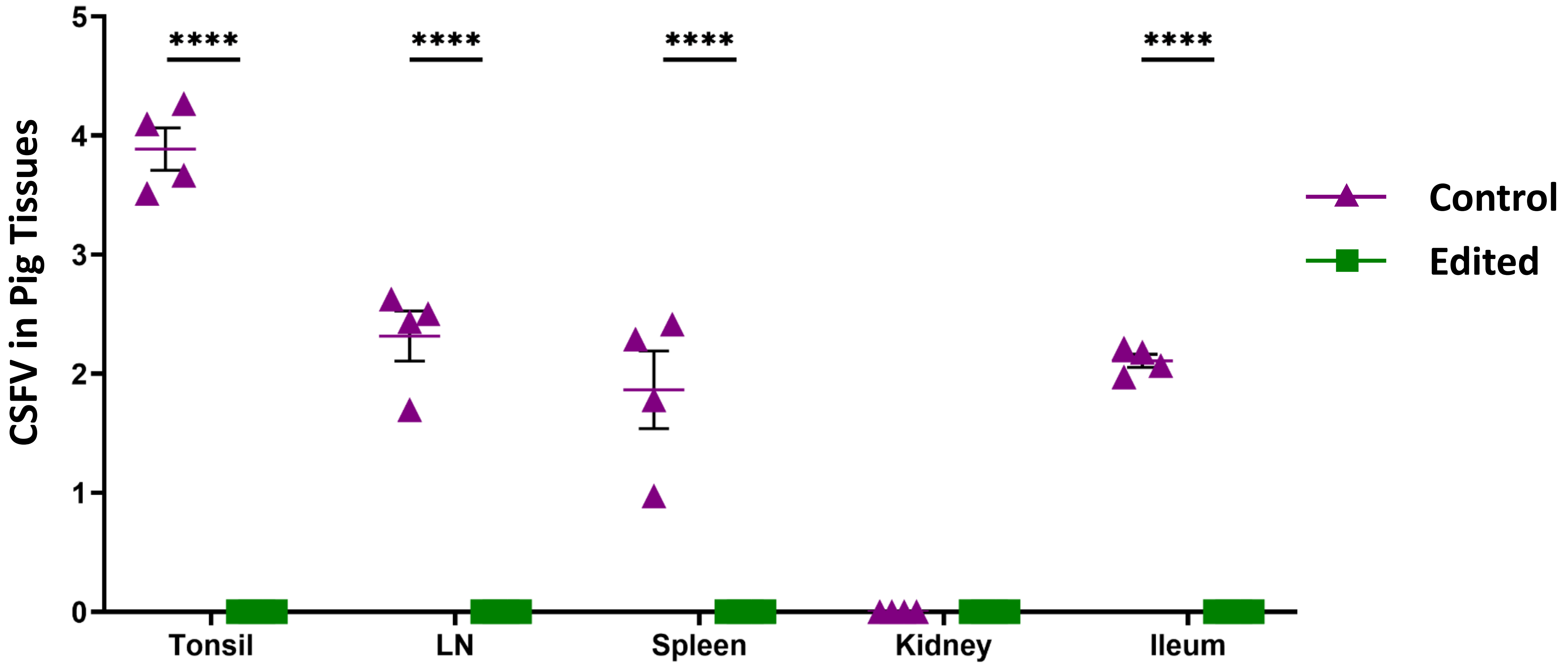
Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Edited pigs do not shed CSFV



Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Edited pigs do not have CSFV in their tissues



Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Trends in Biotechnology

CellPress

Report

DNAJC14 gene-edited pigs are resistant to classical pestiviruses

Helen Crooke[1,4], Stefanie Schwindt[2,4], Sarah L. Fletcher[3], Olaf Isken[2], Sophie Harding[1], Nicholas Berkley[1], Christine Tait-Burkard[3], Claire Warren[3], C. Bruce A. Whitelaw[3], Norbert Tautz[2], and Simon G. Lillico[3,*]

doi: 10.1016/j.tibtech.2025.09.008.

Biotechnology and Biological Sciences Research Council

Animal & Plant Health Agency

ROSLIN

UNIVERSITÄT ZU LÜBECK

Genus

THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

Biotechnology and Biological Sciences Research Council

THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies

- Application of NGTs has significant potential to contribute to the control of animal diseases
- Implementation could:
  - Improve welfare
  - Reduce reliance on antimicrobials
  - Reduce environmental footprint of animal agriculture
  - Support reliable production outcomes

- Failure to implement such technologies could be viewed as a biosafety failure

Thank you

UKRI Biotechnology and Biological Sciences Research Council
Animal & Plant Health Agency
ROSLIN
UNIVERSITÄT ZU LÜBECK
Genus
THE UNIVERSITY of EDINBURGH Royal (Dick) School of Veterinary Studies