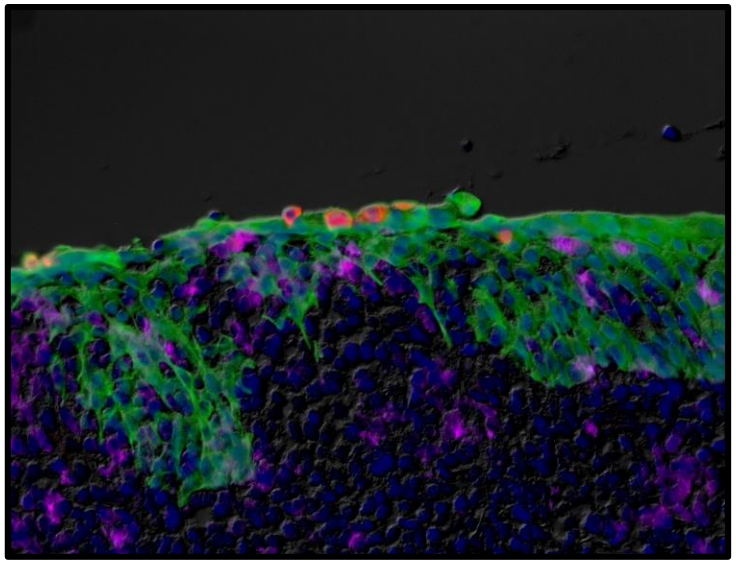
New models of FMDV pathogenesis



Carolina Stenfeldt & Jonathan Arzt
USDA-ARS, Plum Island Animal Disease Center

USDA ARS
United States Department of Agriculture
Agricultural Research Service

University of Minnesota
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New models of FMDV pathogenesis

Outline

- Historical aspects of FMD pathogenesis research
- Old and New models for FMD studies in cattle
- Current state of knowledge of FMDV pathogenesis in cattle
- FMDV pathogenesis in pigs
- A little bit about sheep

Acknowledgements

Jonathan Arzt

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Michael Eschbaumer

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U.S. Department of Homeland Security, Science & Technology Directorate (DHS S&T)

PRIVY COUNCIL

AGRICULTURAL RESEARCH COUNCIL

THE QUANTITATIVE STUDY OF FOOT-AND-MOUTH DISEASE VIRUS

By W. M. HENDERSON, D.Sc., M.R.C.V.S.

Research Institute (Foot-and-Mouth Disease Research Committee)

Pirbright, Surrey

1949

LONDON: HIS MAJESTY'S STATIONERY OFFICE

1949

“Intra-dermal lingual” (IDL) inoculation

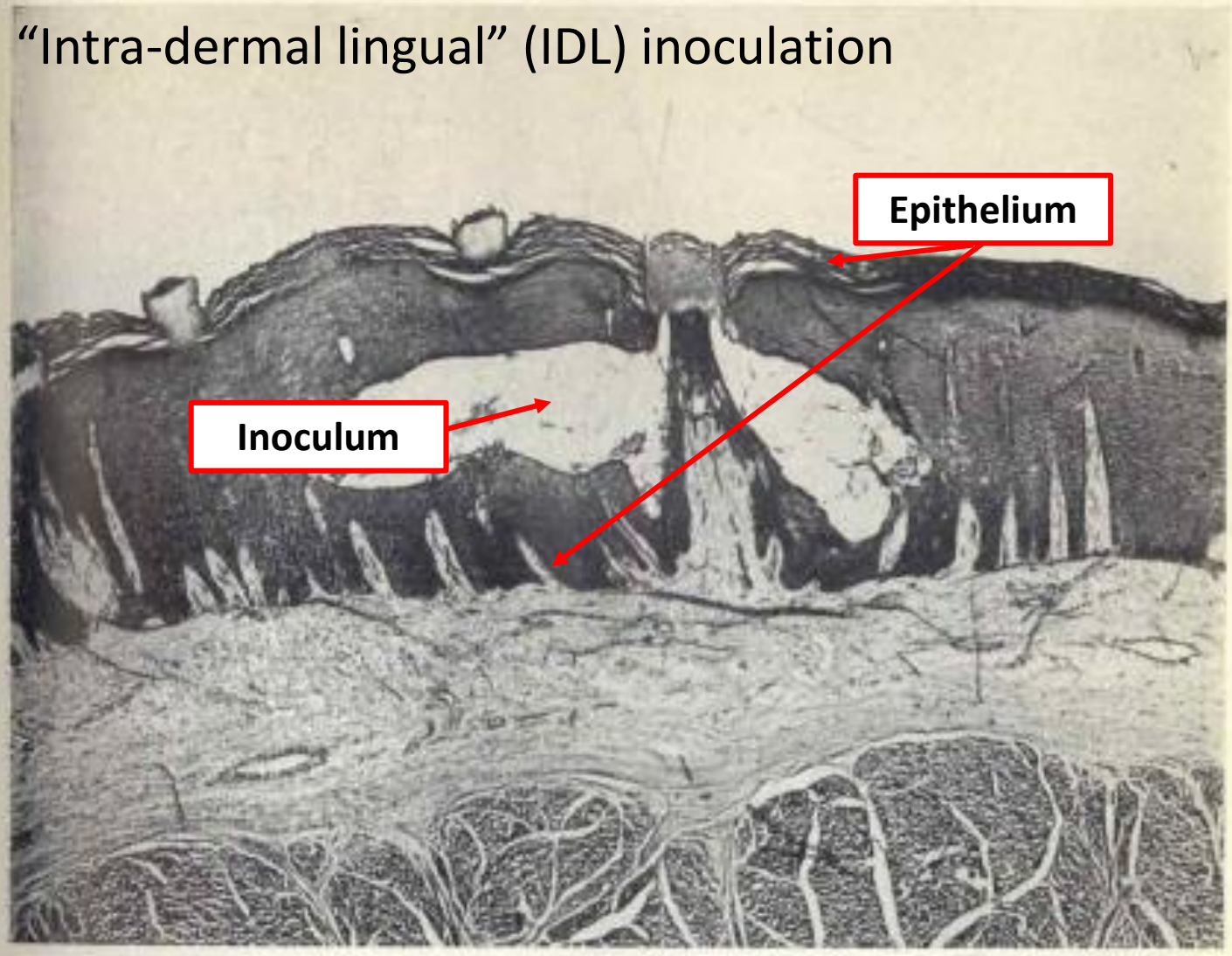


Plate 1

Section of the mucous membrane of a steer's tongue inoculated intradermally with india ink. Animal killed 20 minutes after inoculation. The cavity in the epithelial layer indicates the position of the bleb of inoculum which has been lost during the preparation of the section.

H. & E. x 17.

Early FMDV pathogenesis studies

J. Comp. Path. 1981. Vol. 91.

THE PATHOGENESIS OF NATURAL AND SIMULATED NATURAL FOOT-AND-MOUTH DISEASE INFECTION IN CATTLE

By

R. Burrows, J. A. Mann, A. J. M. Garland, A. Greig and D. Goodridge

The Animal Virus Research Institute, Pirbright, Surrey GU24 0NF, U.K.

INTRODUCTION

For many years it had been thought that the normal method of infection in foot-and-mouth disease (FMD) was by ingestion (Sellers, 1971), virus gaining entry to susceptible cells through minor abrasions in the mouth or other areas of the digestive tract. This view was supported by the known susceptibility of tongue epithelium to inoculation compared with other methods of exposure (Henderson, 1952). This concept of the pathogenesis of the disease was not questioned until Korn (1957) described histopathological changes in the nasal mucosae prior to the development of clinical signs and concluded that the primary sites of virus multiplication were in the nasal passages. Subsequently, Hyslop (1965) confirmed that virus was present in aerosol form in the vicinity of infected cattle and that cattle could be infected by artificially produced aerosols of virus. Some features of the 1967/1968 outbreak of FMD in the

Early FMDV pathogenesis studies

THE PATHOGENESIS OF NATURAL AND SIMULATED NATURAL FOOT-AND-MOUTH DISEASE INFECTION IN CATTLE

By

R. Burrows, J. A. Mann, A. J. M. Garland, A. Greig and D. Goodridge

The Animal Virus Research Institute, Pirbright, Surrey GU24 0NF, U.K.

FMD VIRUS RECOVERY BEFORE AND AFTER SLAUGHTER FROM 6 STEERS KILLED 2 TO 4 DAYS AFTER CONTACT* WITH INFECTED CATTLE (A_5-EYSTRUP VIRUS)

Animal number		*HI* 59	*HI* 58	*HJ* 66	*HG* 60	*HG* 75
Days after exposure	1	+† −‡	− −	− −	− −	− −
	2	+ −	+ −	− −	+ −	− −
	3		+ −	3·2 −	+ −	2·5 −
	4				+ +	1·7 −
Day killed		2	3	3	4	4
Serum		−	−	−	+	−
Retropharyngeal LN		−	−	1·3	+	NT
Dorsal surface of soft palate		+	+	5·2	+	1·5
Pharynx		−	+	6·3	+	4·0
Trachea		−	−	2·2	+	−
Bronchi		−	−	−	−	−
Lung		−	−	−	+	−
Bronchial LNs		−	−	−	+	−
Mediastinal LNs		−	−	−	+	−
Tonsil		−	+	2·7	+	NT

* Housed for 6 to 8 h with an infected animal and then removed to a clean room.

Models for FMDV pathogenesis studies
"simulated natural exposure"

J. Hyg., Camb. (1983), **91**, 319–328
Printed in Great Britain

Aerosol exposure of cattle to foot-and-mouth disease virus

JOHN W. McVICAR AND ROBERT J. EISNER

Plum Island Animal Disease Center, USDA, ARS, S&E, P.O. Box 848, *Greenport, New York* 11944

(*Received* 14 *February* 1983; *accepted* 19 *April* 1983)

SUMMARY

Slight modifications of a small, plastic covered greenhouse provided a chamber for the exposure of cattle of all ages to aerosols of foot-and-mouth disease virus. Particle size distributions of aerosols were 76% < 3 μm, 17% 3–6 μm, and 7% > 6 μm immediately after the deVilbis no. 40 nebulizer used was turned off and 90% < 3 μm, 8% 3–6 μm, and 2% > 6 μm 20–30 min later. Pharyngeal virus growth curves and viremia patterns correlated well with the dose of virus to which test cattle were exposed and were similar to those of cattle inoculated intranasally.

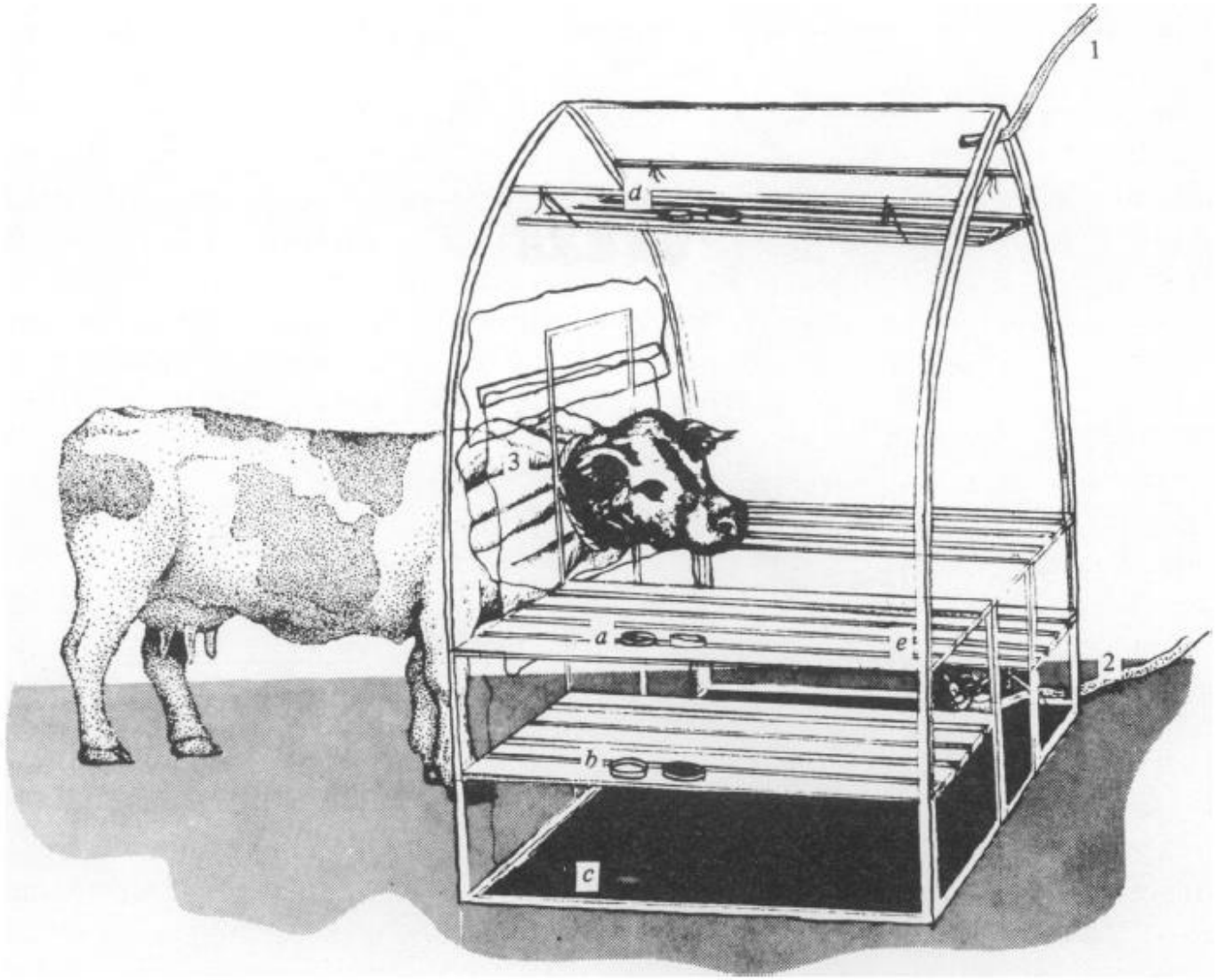
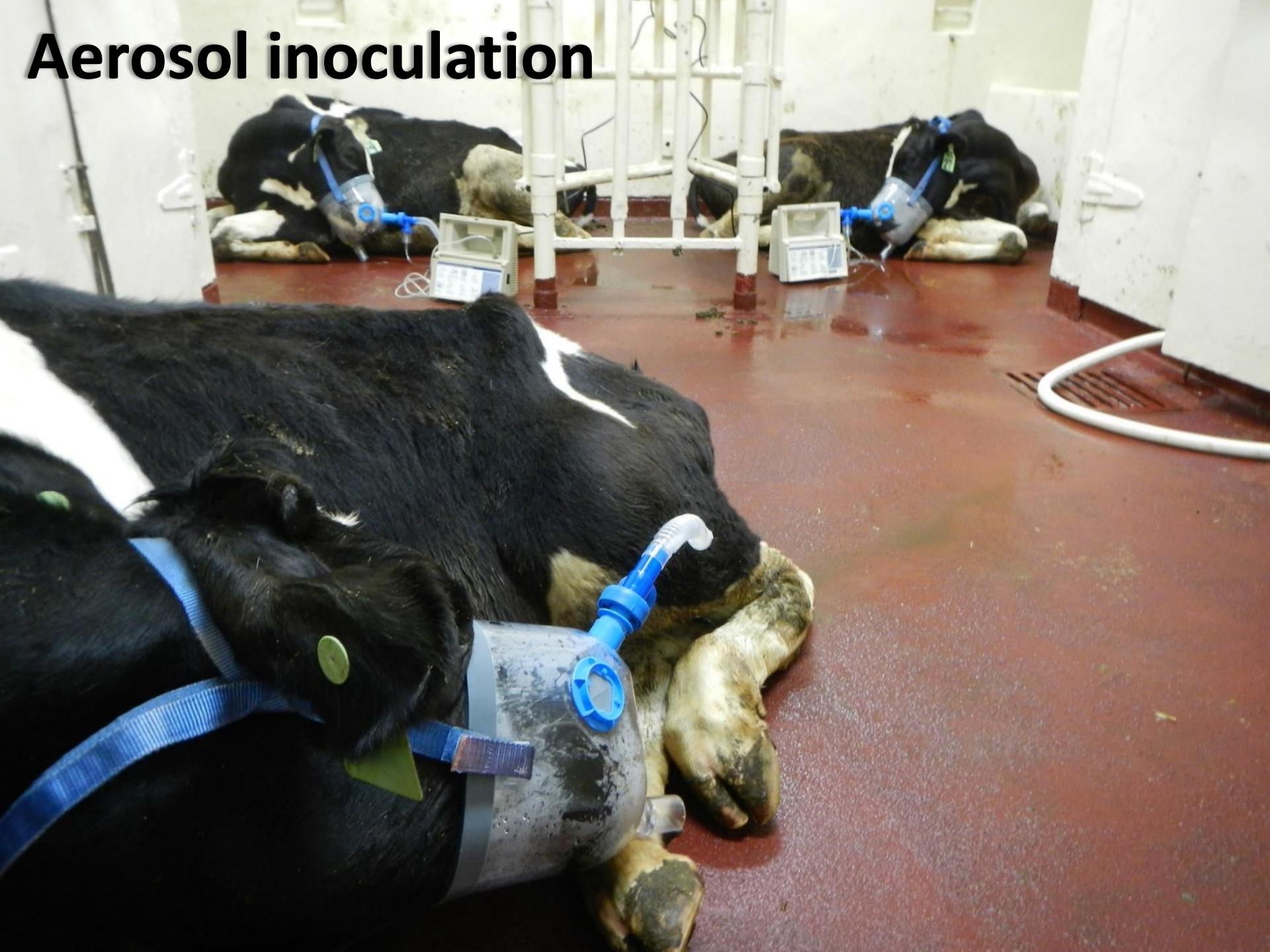


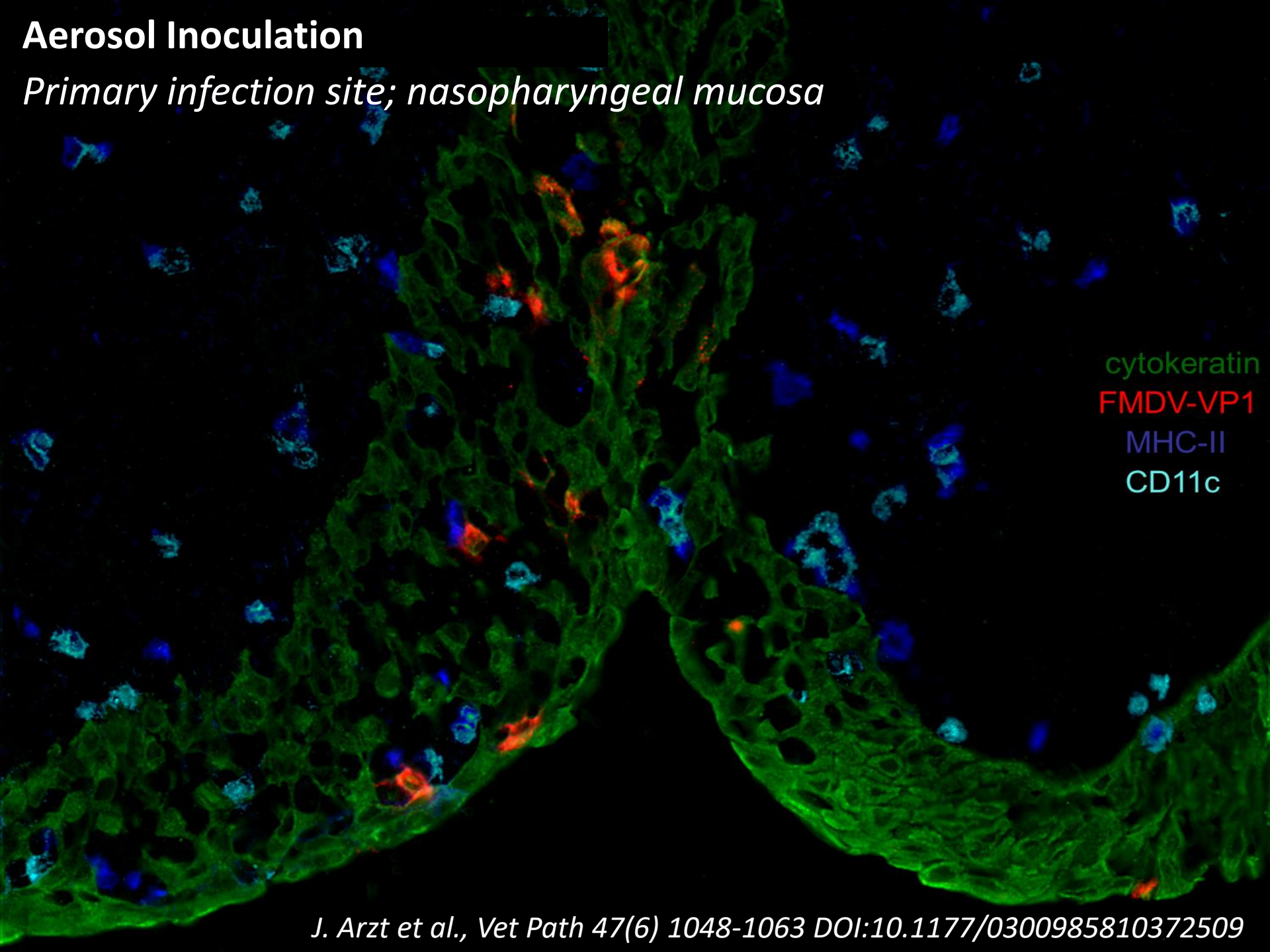
Fig. 1. Aerosol chamber in place for exposure of an adult bovine. 1 = exhaust hose, 2 = air pressure supply hose, 3 = neck sleeve of plastic sheeting, *a*, *b*, *c*, *d* = open Petri dish air samplers, *e* = location of May air sampler if used.

Aerosol inoculation



Aerosol Inoculation

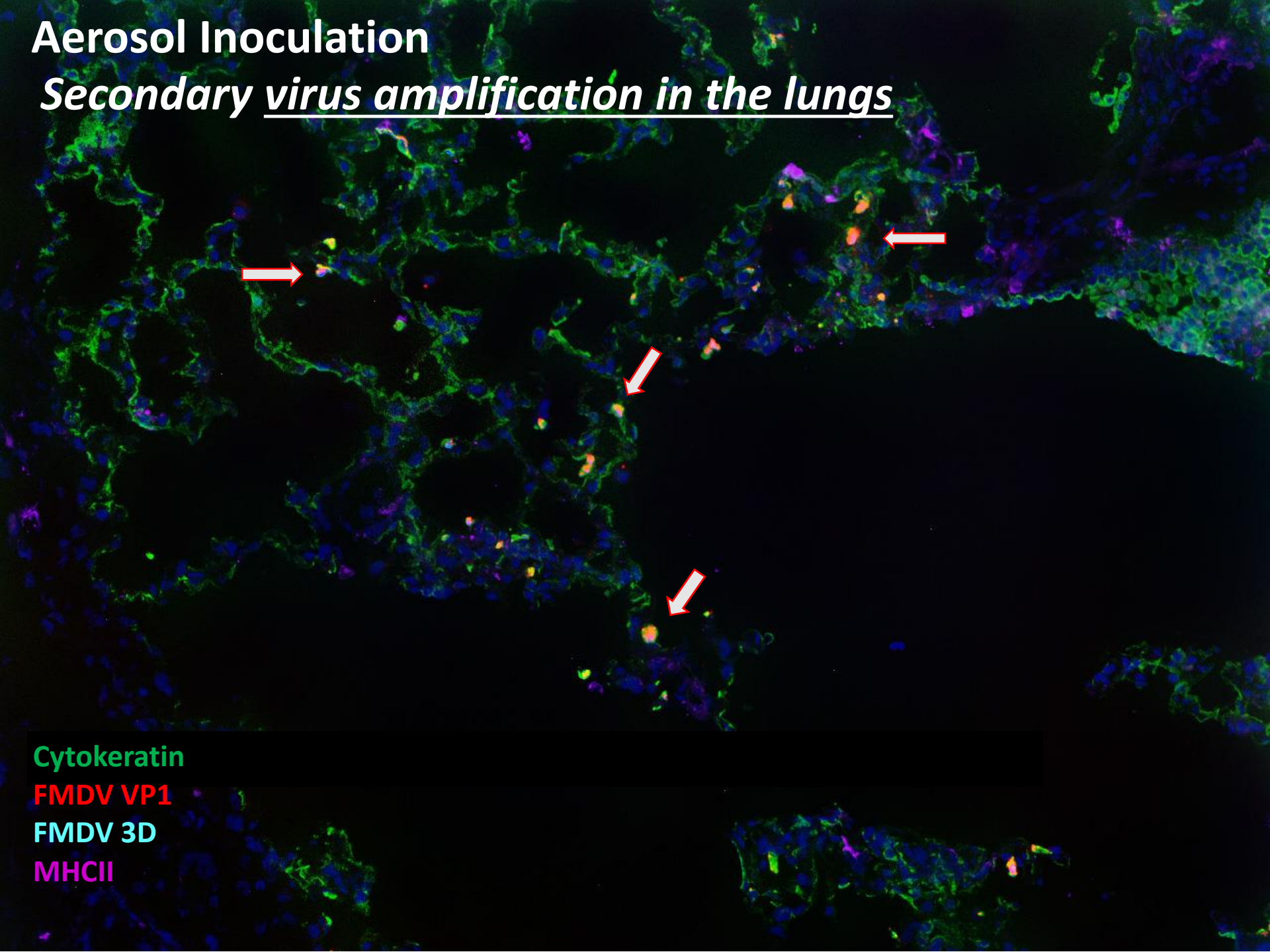
Primary infection site; nasopharyngeal mucosa



J. Arzt et al., Vet Path 47(6) 1048-1063 DOI:10.1177/0300985810372509

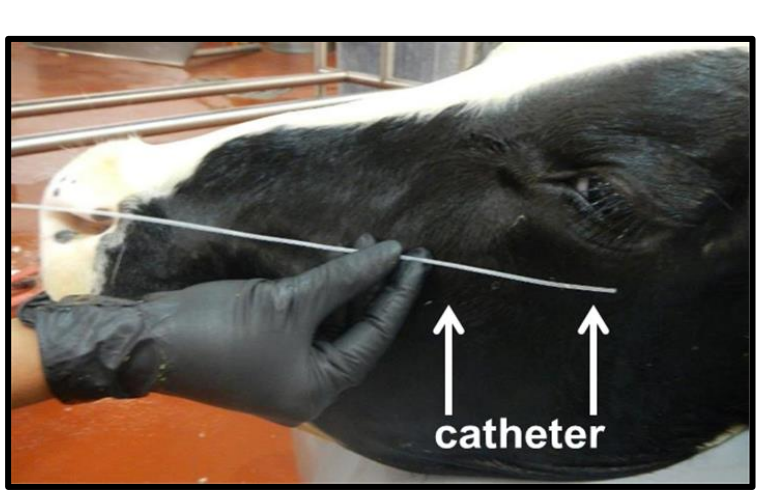
Aerosol Inoculation

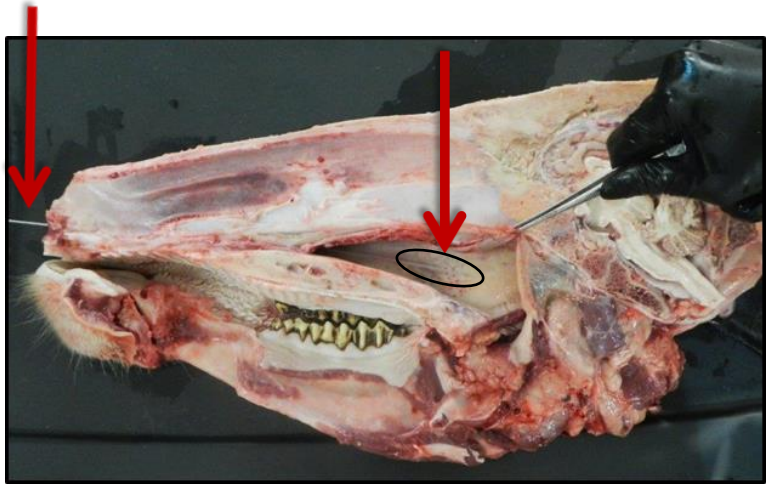
Secondary virus amplification in the lungs



Simulated natural virus exposure; 2nd generation:

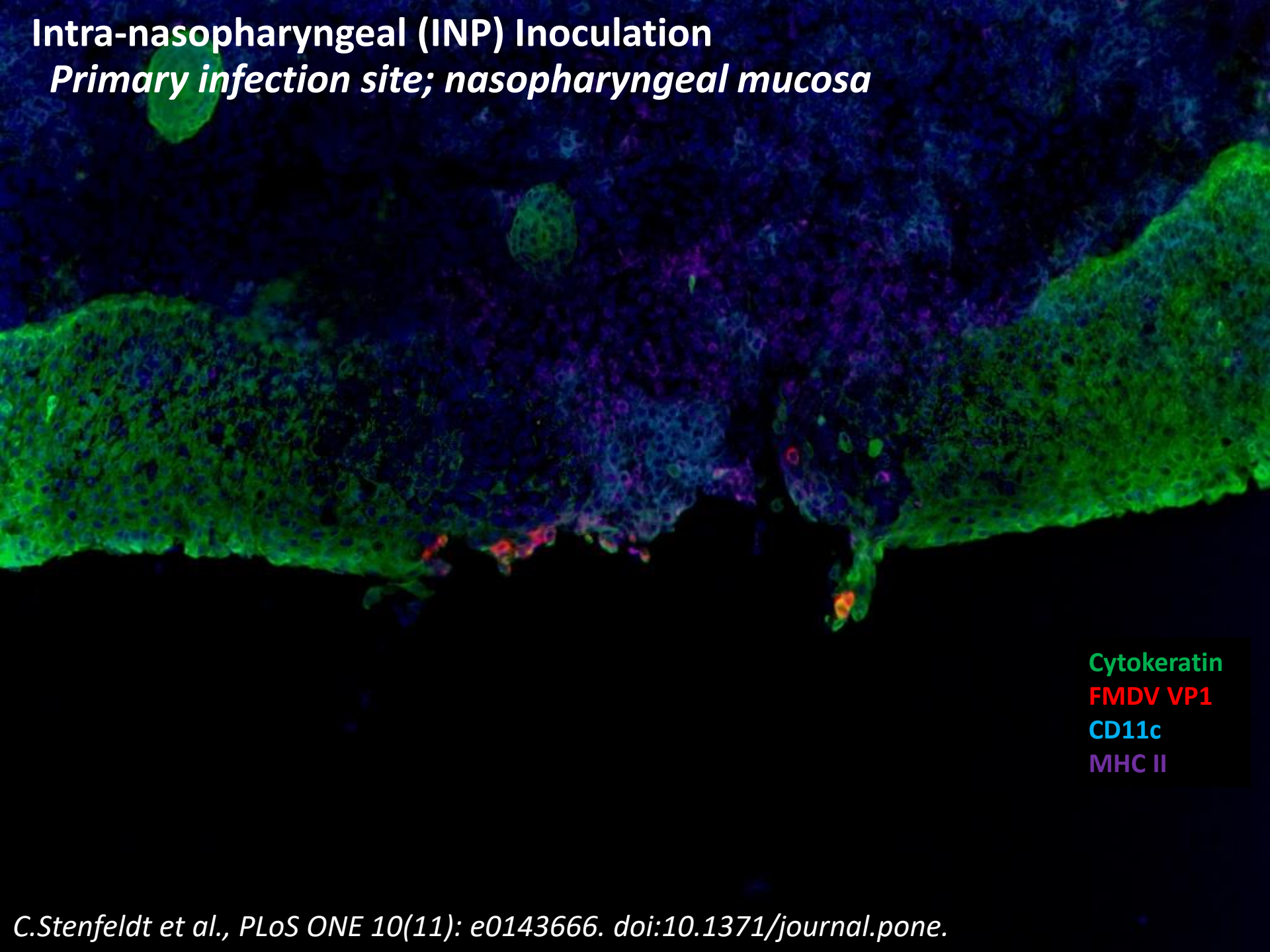
- *Intra-Nasopharyngeal (INP) inoculation*
- **Optimized system based on refined knowledge of FMDV pathogenesis in cattle**
- **Engages natural mucosal defenses**
- **Facilitates control of dose and timing of challenge**
- **User friendly/ time-efficient**



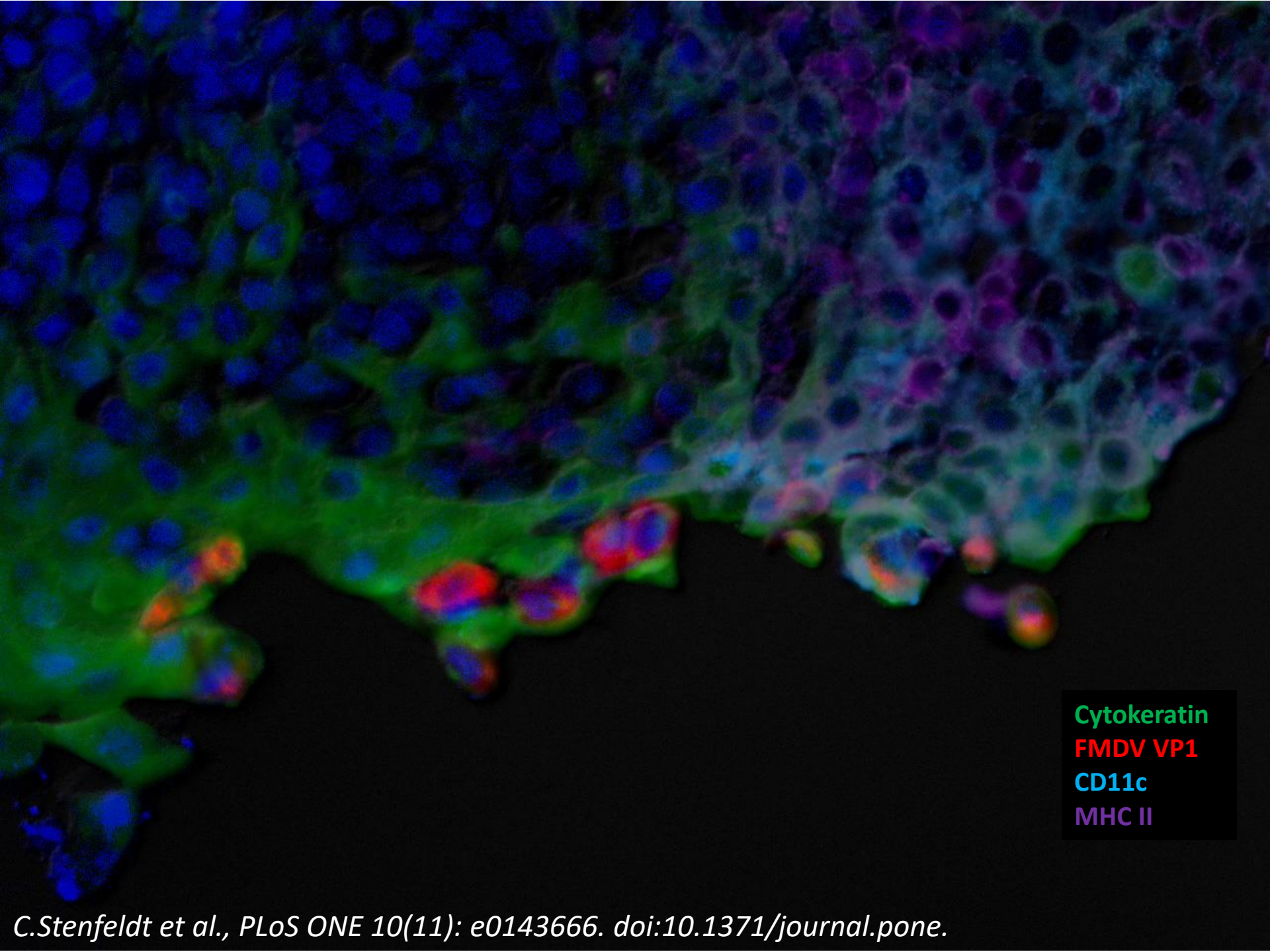


Intra-nasopharyngeal (INP) Inoculation

Primary infection site; nasopharyngeal mucosa



C.Stenfeldt et al., PLoS ONE 10(11): e0143666. doi:10.1371/journal.pone.

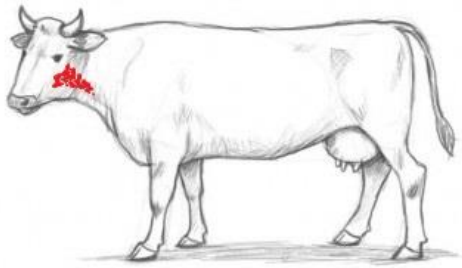


C.Stenfeldt et al., PLoS ONE 10(11): e0143666. doi:10.1371/journal.pone.

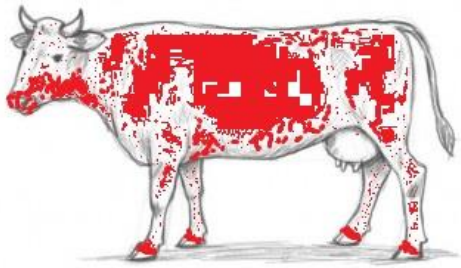
The FMDV carrier state divergence

-Tissue distribution of infectious virus

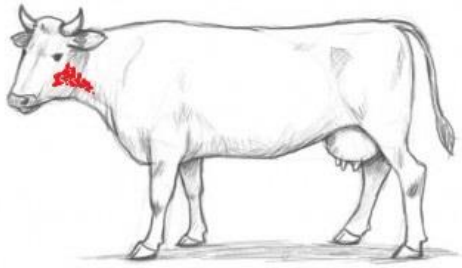
Non-Vaccinated



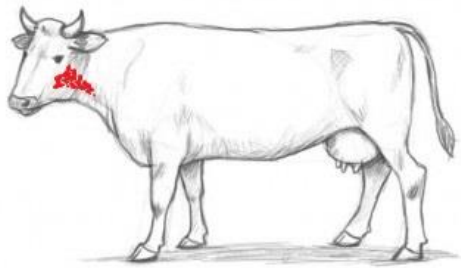
Primary Infection



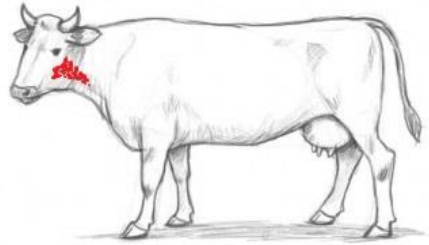
Systemic Generalization



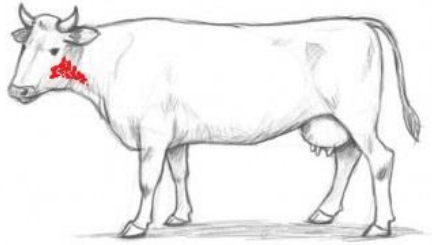
Subclinically infected

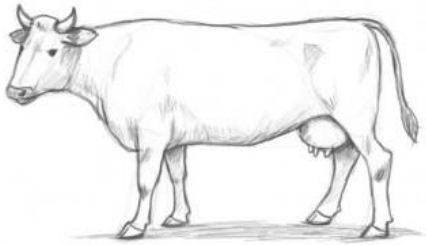


Vaccinated



Persistent Infection

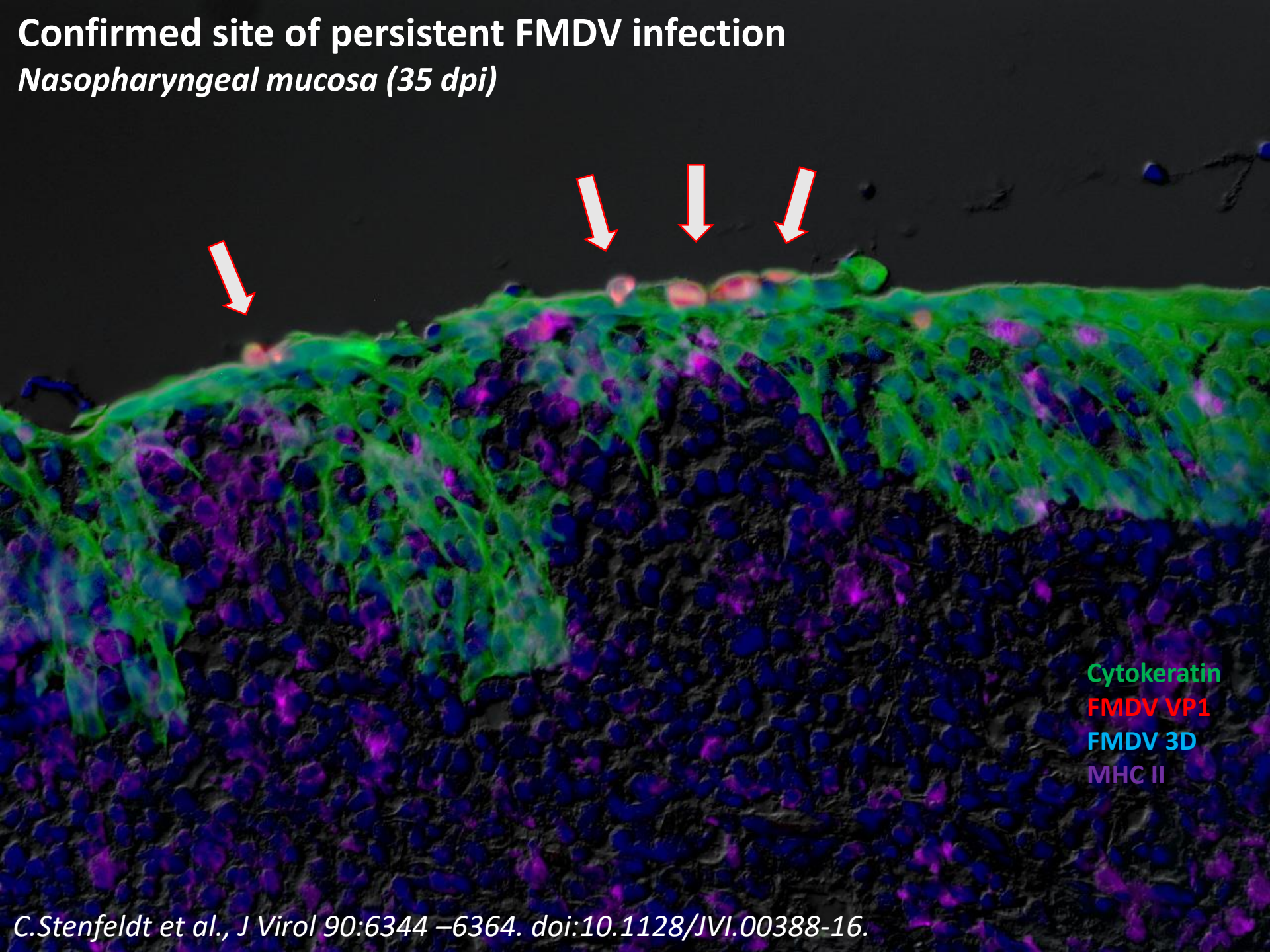




Transitional Phase

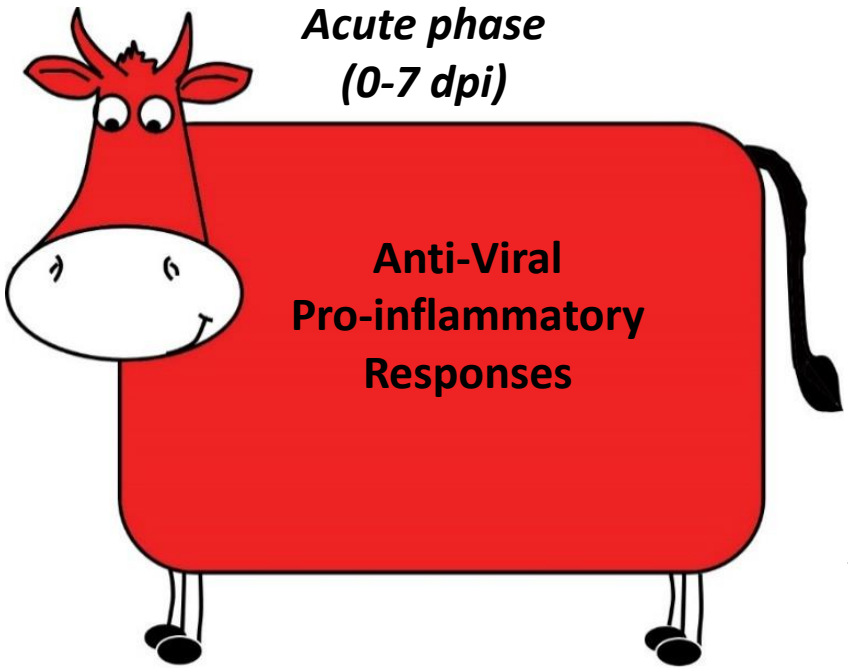
Confirmed site of persistent FMDV infection

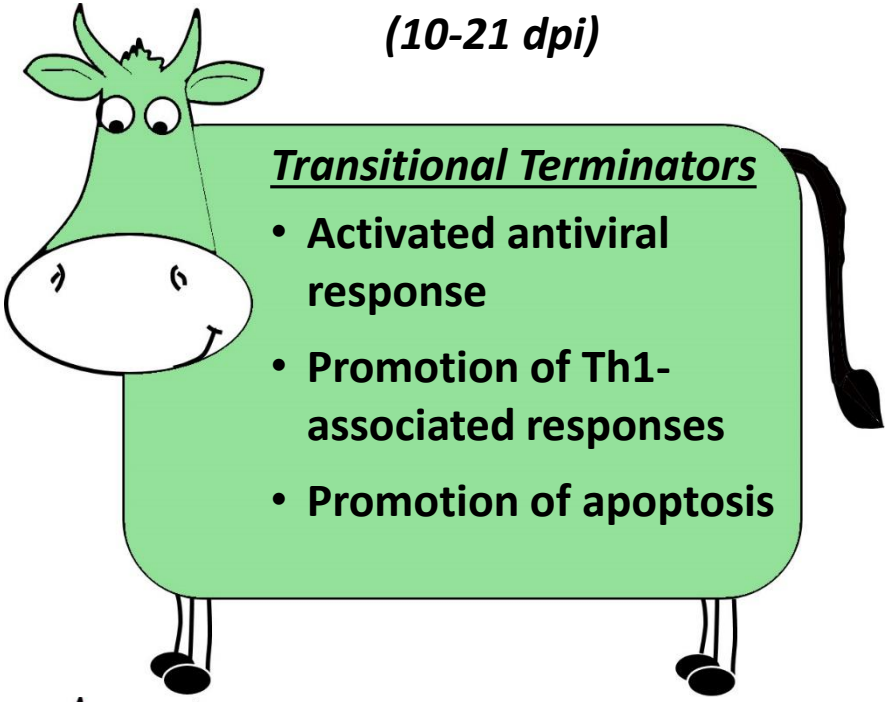
Nasopharyngeal mucosa (35 dpi)

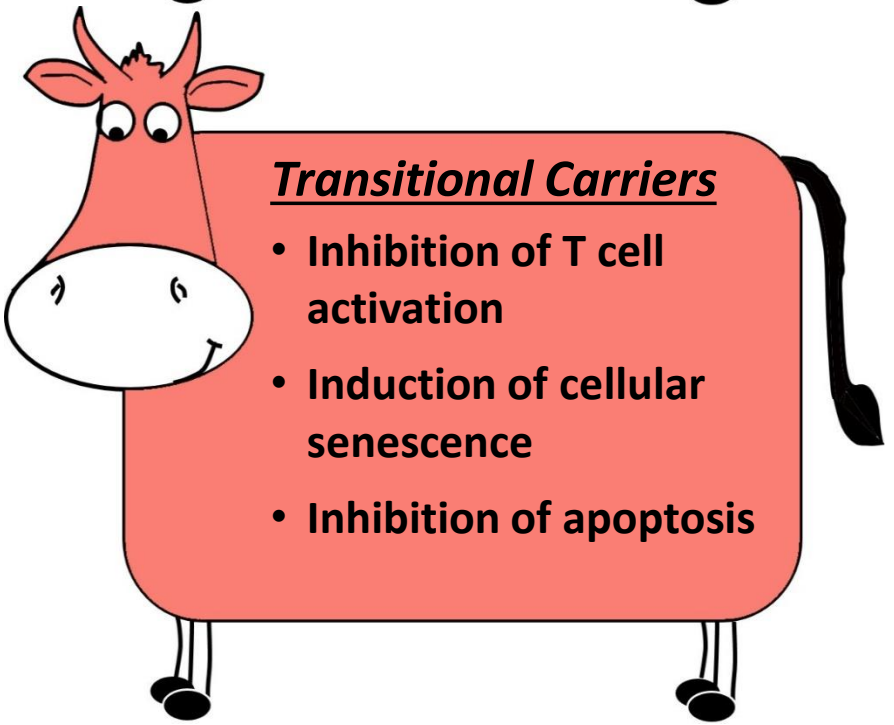


C.Stenfeldt et al., J Virol 90:6344 –6364. doi:10.1128/JVI.00388-16.

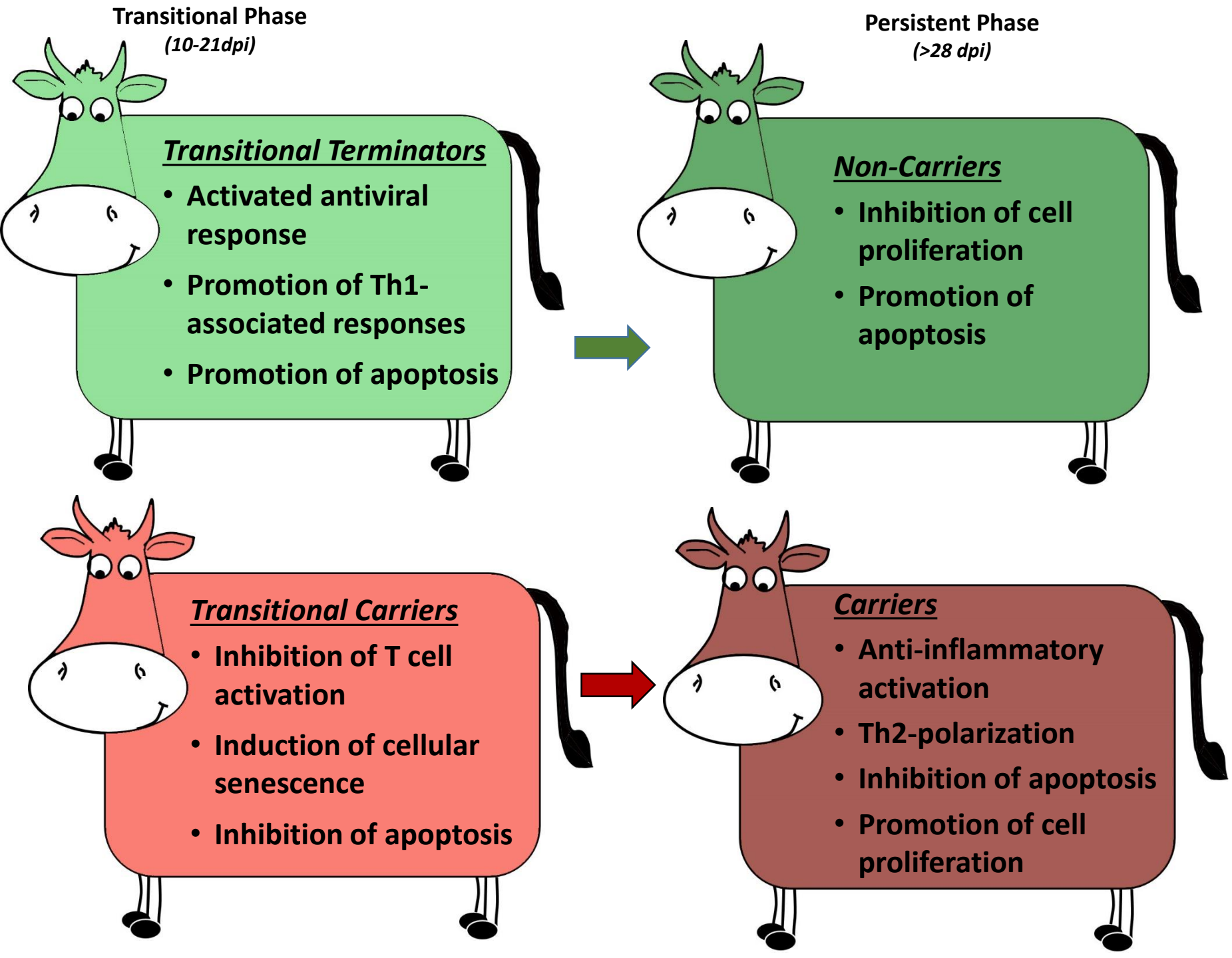
Bovine host responses associated with stages of infection in cattle; transcriptomics







- Zhang et al ’06, ‘07
- Stenfeldt et al ‘15, ’16, ‘17
- Zhu et al ‘12
- Perez-Martin et al ‘12
- Eschbaumer et al ‘16


Transitional Phase
(10-21dpi)
Transitional Terminators
• Activated antiviral response
• Promotion of Th1-associated responses
• Promotion of apoptosis
Persistent Phase
(>28 dpi)
Non-Carriers
• Inhibition of cell proliferation
• Promotion of apoptosis
Transitional Carriers
• Inhibition of T cell activation
• Induction of cellular senescence
• Inhibition of apoptosis
Carriers
• Anti-inflammatory activation
• Th2-polarization
• Inhibition of apoptosis
• Promotion of cell proliferation

Aerosol versus intra-nasopharyngeal inoculation

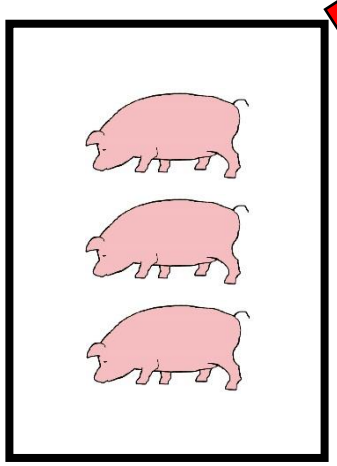
Both systems:

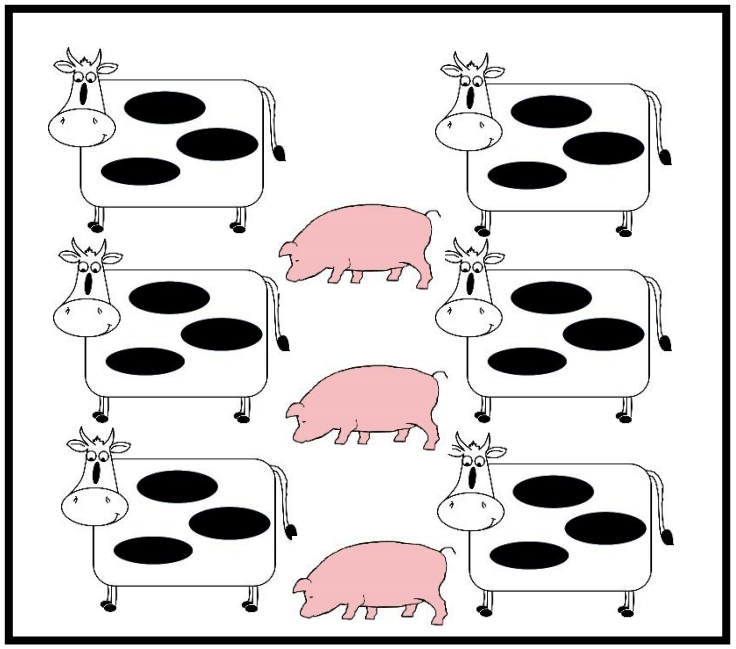
+ **Controlled virus exposure of upper respiratory tract**
+ **Engagement of natural mucosal immunity**
+ **Reproducible**

Aerosol	Intra-nasopharyngeal
+++ Lungs	+/- Lungs

Pig-to-cow contact transmission

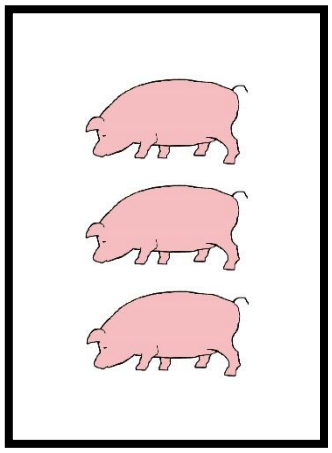
Pigs inoculated with FMDV (0 hpi)

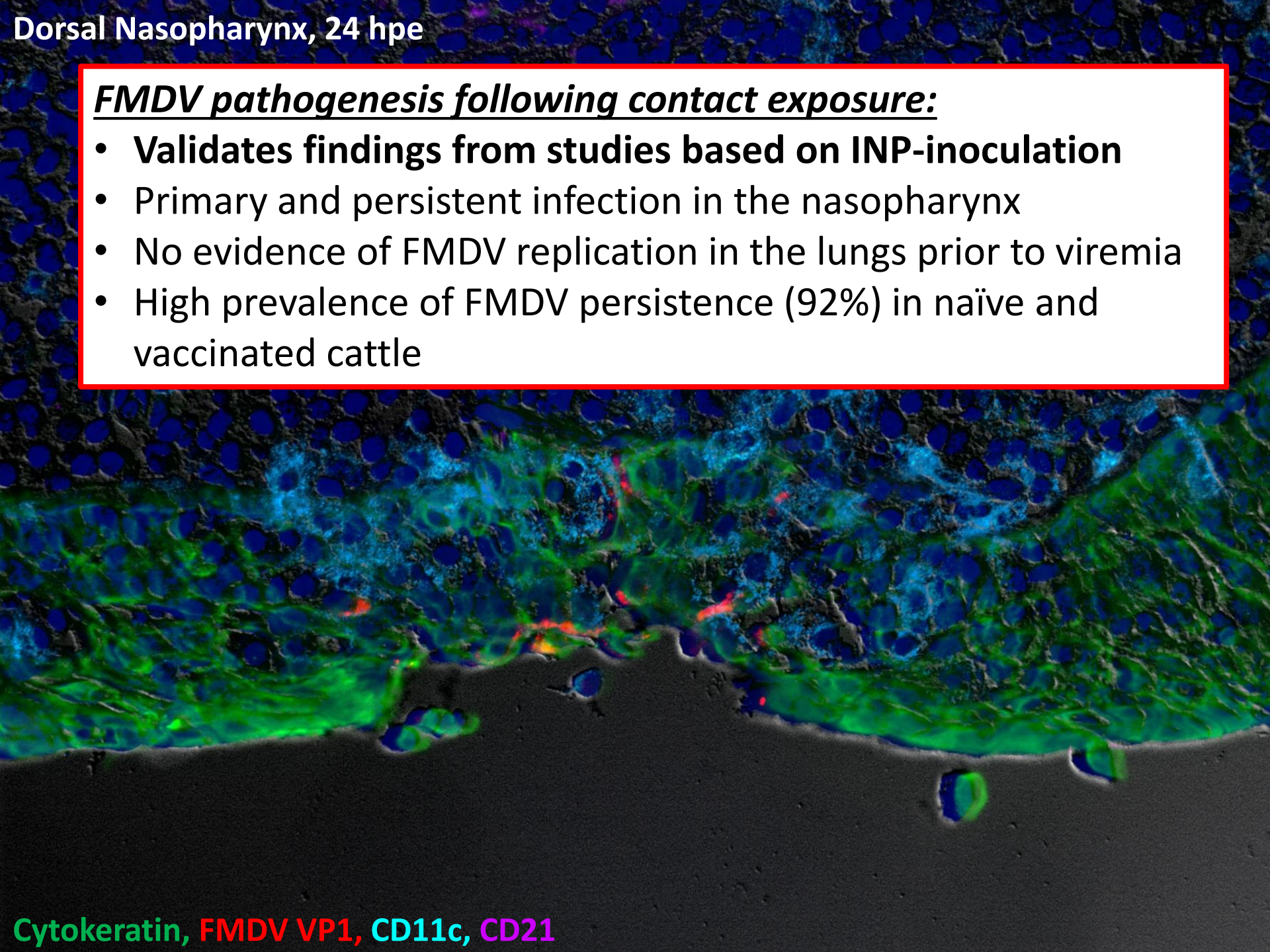




At 48 hpi, pigs relocated for 24 h co-habitation with cattle

Pigs removed from the room at 72 hpi




Dorsal Nasopharynx, 24 hpe
FMDV pathogenesis following contact exposure:
• Validates findings from studies based on INP-inoculation
• Primary and persistent infection in the nasopharynx
• No evidence of FMDV replication in the lungs prior to viremia
• High prevalence of FMDV persistence (92%) in naïve and vaccinated cattle
Cytokeratin, FMDV VP1, CD11c, CD21

FMDV pathogenesis studies in pigs

FMDV pathogenesis in pigs

Bull. Off. int. Epiz., 1972, 77 (5-6), 859-874.

Pathogenesis of Foot-and-Mouth Disease in experimentally infected pigs

by

C. TERPSTRA (*)

SUMMARY

Quantitative studies on the pathogenesis of Foot-and-Mouth Disease in pigs have shown that animals could successfully be infected by inhalation of an aerosol containing maximal 400 mouse LD_{50} of a pig adapted O_1 strain. Initial replication of virus occurred in the lungs and generalization was not observed until 72 hours after exposure. Considerable larger amounts of virus were required for a successful infection by oral route.

Relative resistance of pigs to infection by natural aerosols of FMD virus

A. I. DONALDSON, S. ALEXANDERSEN

FOOT-AND-MOUTH disease (FMD) can be spread by the wind under certain epidemiological and climatic conditions (Henderson 1969, Hugh-Jones and Wright 1970, Tinline 1970, Sellers and Forman 1973). Most commonly, airborne spread is from pigs at source to ruminants downwind. This

Office, Bracknell, collaborated during the 1970s to develop a computer-based model to predict the risk of airborne spread of FMD (Gloster and others 1981, 1982). The model can be used to predict the risk of spread up to 10 km from a source and was used successfully under operational conditions in 1981 (Donaldson and others 1982). Since that time, there have been significant advances in the development of models which can simulate the atmospheric dispersion of particles, including those for predicting the spread of FMD virus. The Pirbright laboratory of the IAH has collaborated with the Danish Meteorological Institute and the Risø National Laboratory, Denmark, to incorporate the aerobiological properties of FMD virus into a model called Rimpuff (Mikkelsen and others 1984, 1997, Sørensen and others 2000, 2001). Rimpuff can be used to predict the risk of airborne spread of FMD to cattle and sheep, but until recently it could not predict

Veterinary Record (2001) **148,** 600-602

In conclusion, on the basis of these results, the probability of pigs being infected as a result of exposure to a plume of airborne FMD virus under field conditions is very low.

FMDV pathogenesis in pigs

Journal of General Virology (2001), **82**, 747–755. Printed in Great Britain

The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time-course study using TaqMan RT–PCR

Soren Alexandersen, Martin B. Oleksiewicz† and Alex I. Donaldson

Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK

Preventive Veterinary Medicine 88 (2009) 158–163

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Foot and mouth disease virus transmission during the incubation period of the disease in piglets, lambs, calves, and dairy cows

K. Orsel [a,*], A. Bouma [a], A. Dekker [b], J.A. Stegeman [a], M.C.M. de Jong [c]

[a] Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht University, Utrecht, The Netherlands
[b] Central Institute for Animal Disease Control Lelystad (CIDC-Lelystad), Wageningen UR, The Netherlands
[c] Quantitative Veterinary Epidemiology, Wageningen University and Research Centre, Wageningen, The Netherlands

J. Comp. Path. 1995 Vol. 113, 51–58

Pathogenesis of Foot-and-Mouth Disease in Swine, Studied by In-situ Hybridization

C. C. Brown, *H. J. Olander and R. F. Meyer

*Foreign Animal Disease Diagnostic Laboratory, NVSL-VS-APHIS-USDA, P.O. Box 848, Greenport, NY 11944 and *Department of Veterinary Pathology, University of California, Davis, CA 95616, USA*

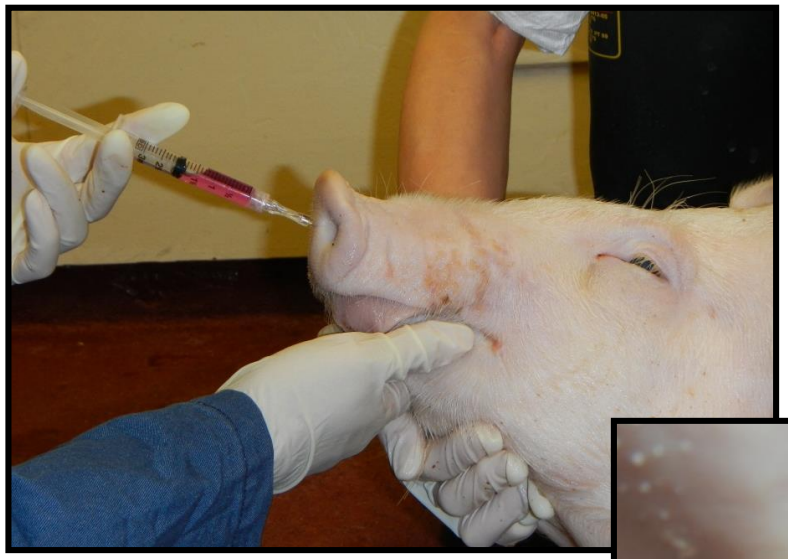
Foot-and-mouth disease viral loads in pigs in the early, acute stage of disease

C. Murphy, J. B. Bashiruddin, M. Quan, Z. Zhang, S. Alexandersen

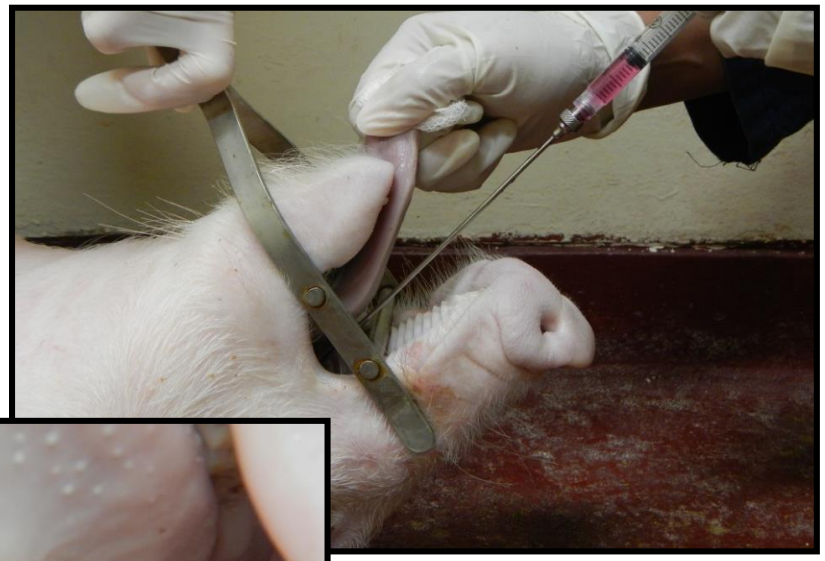
The progress and pathogenesis of foot-and-mouth disease virus (FMDV) was studied in infected pigs by observing the development of clinical signs in two separate experiments. Viral loads were determined by real-time quantitative RT-PCR in the liver, spleen, cervical lymph node, mandibular lymph node, retropharyngeal lymph node, soft palate, pharynx, tonsil, tongue and skin (coronary band area). Tissue samples were collected from both inoculated and contact-infected pigs at several time points during infection, and blood samples were collected to assess viraemia and its relationship to tissue viral load. Virus first appeared in the lymph nodes, followed by viraemia and then clinical signs. The results suggested that FMDV accumulated in lymphoid tissue up to six hours after infection, in the tissues drained by the mandibular lymph node and tonsil and then disseminated throughout the body where epithelial cells were the favoured sites of replication.

Inoculation systems for FMDV studies in pigs

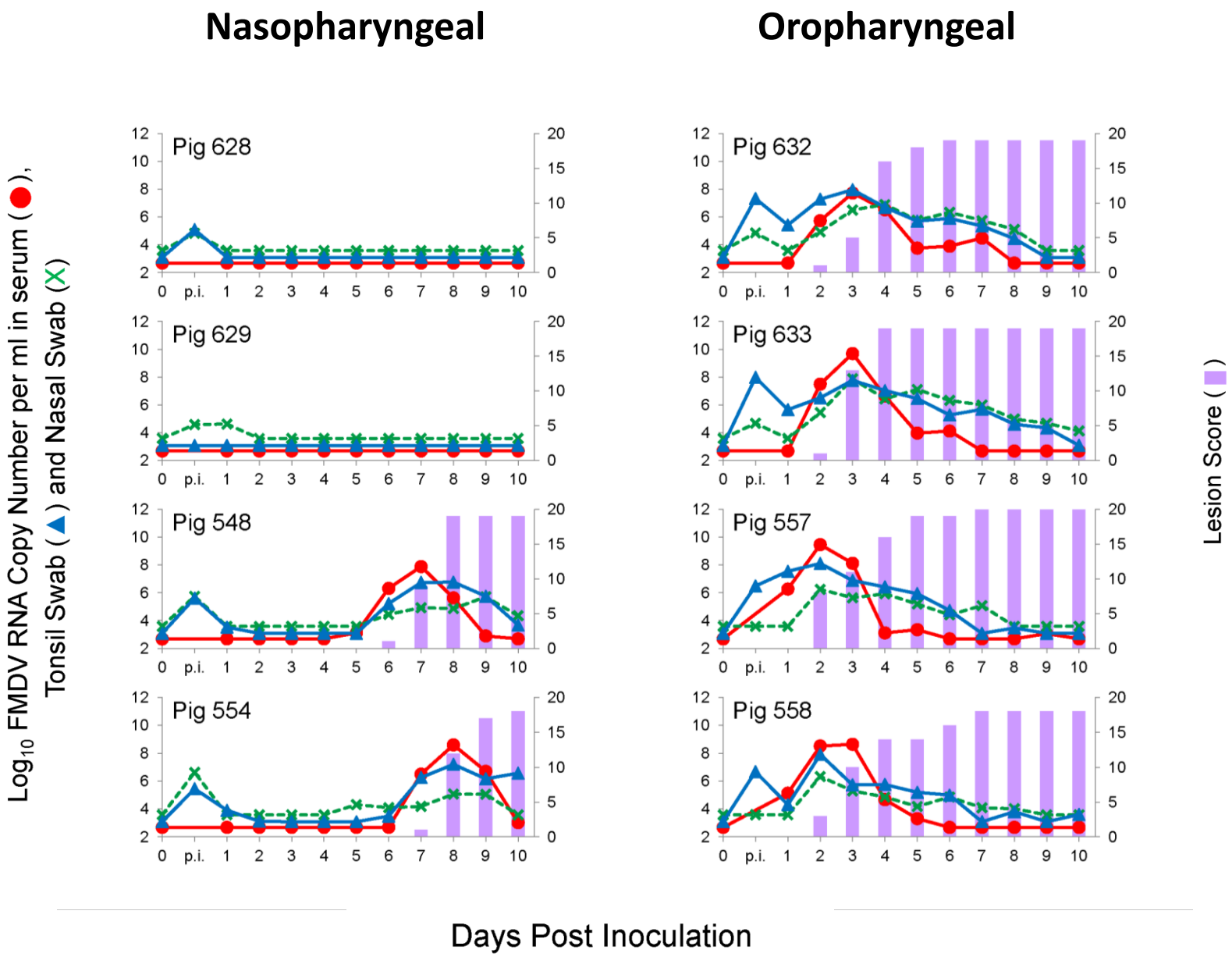
Intra-nasopharyngeal inoculation (INP)



Intra-oropharyngeal inoculation (IOP)



Development of simulated-natural challenge system for FMDV studies in pigs

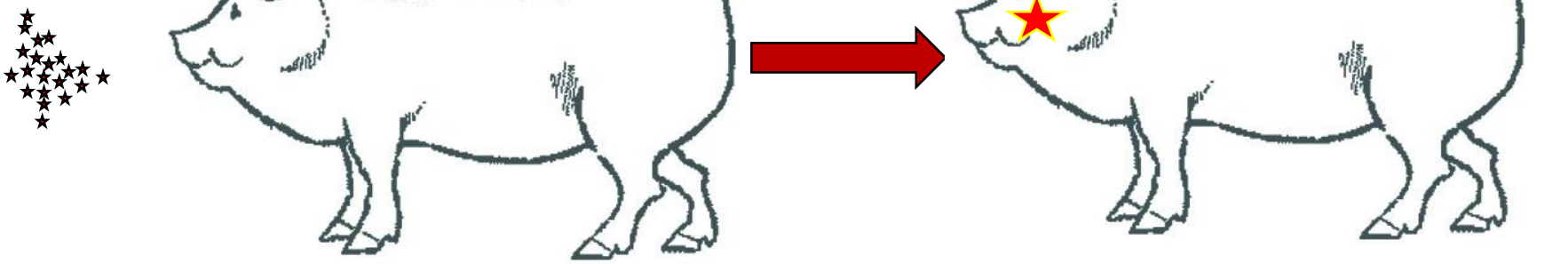


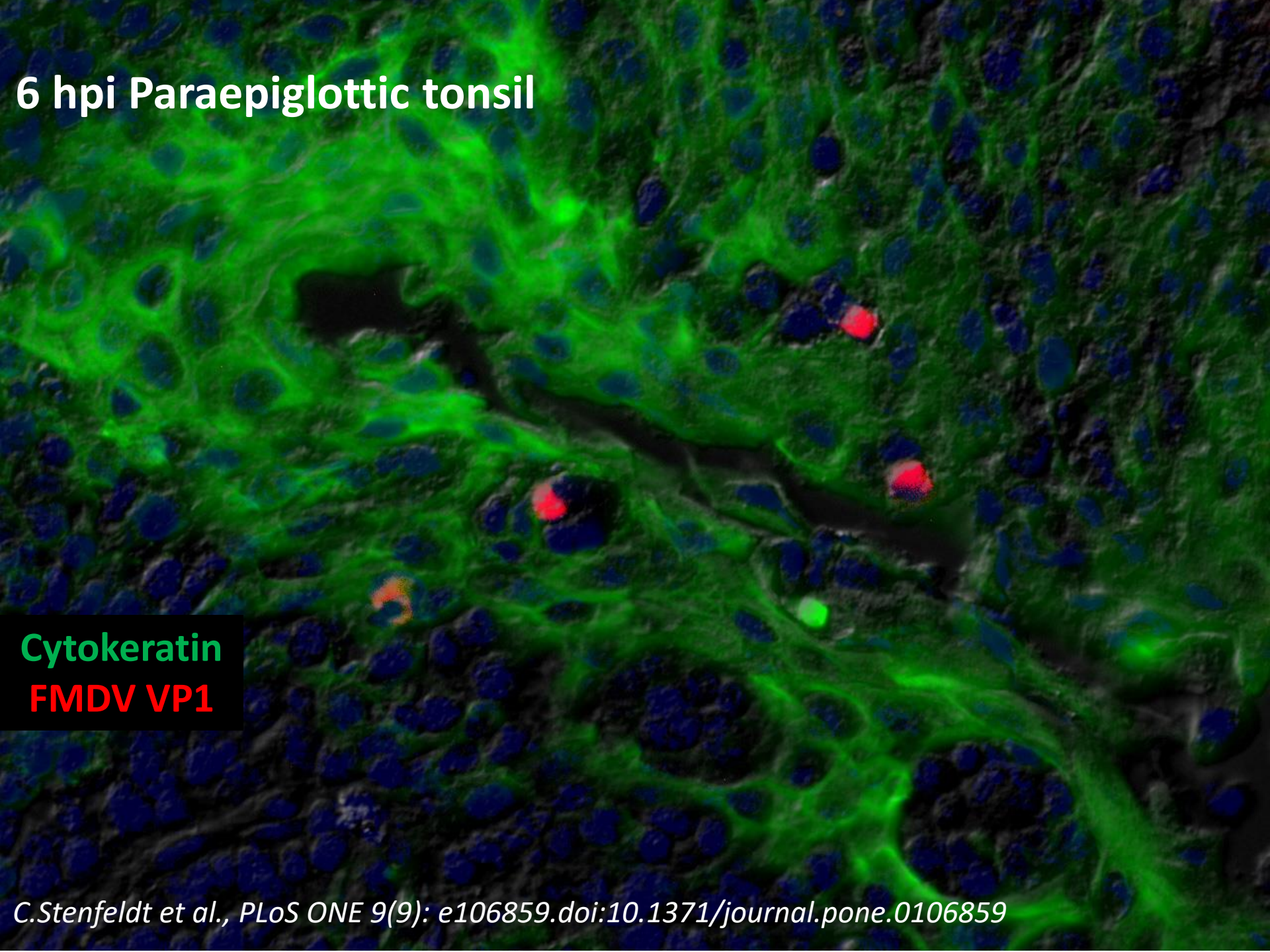
Exposure

0 HPI

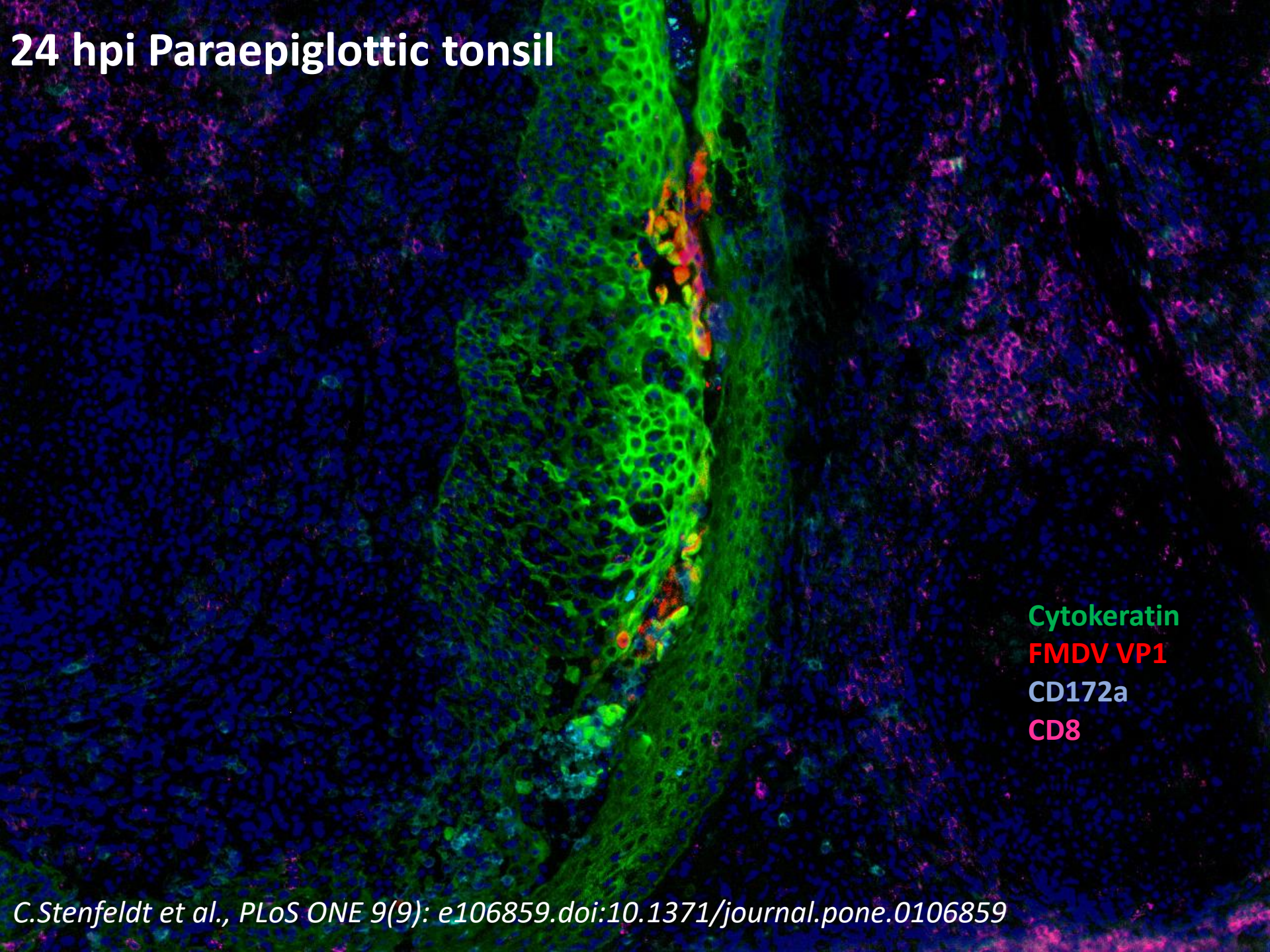
Primary infection

6-12 HPI





C.Stenfeldt et al., PLoS ONE 9(9): e106859.doi:10.1371/journal.pone.0106859

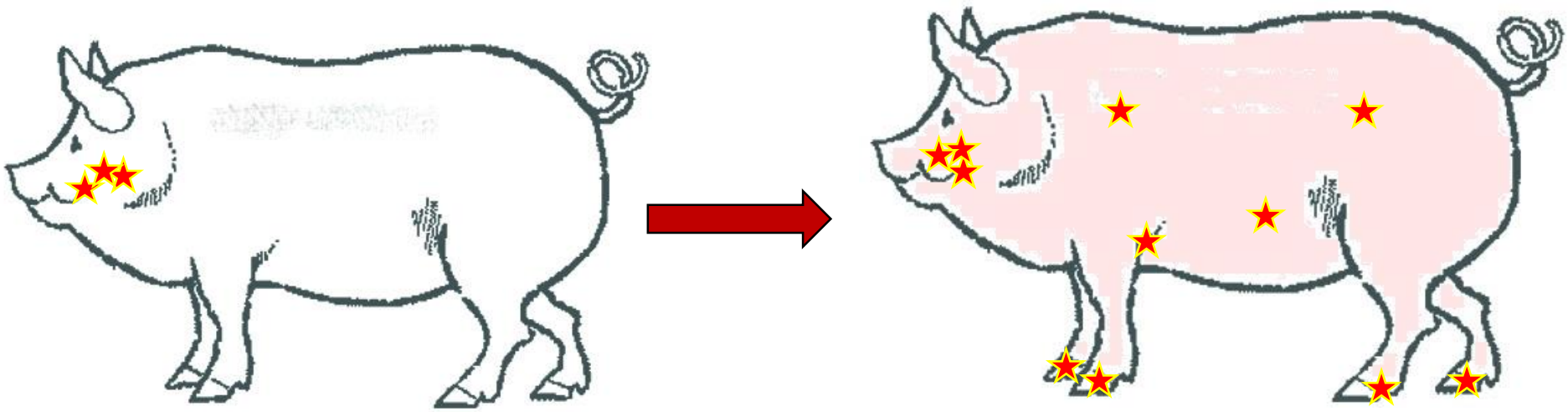

24 hpi Paraepiglottic tonsil
Cytokeratin
FMDV VP1
CD172a
CD8
C.Stenfeldt et al., PLoS ONE 9(9): e106859.doi:10.1371/journal.pone.0106859

Subclinical Infection

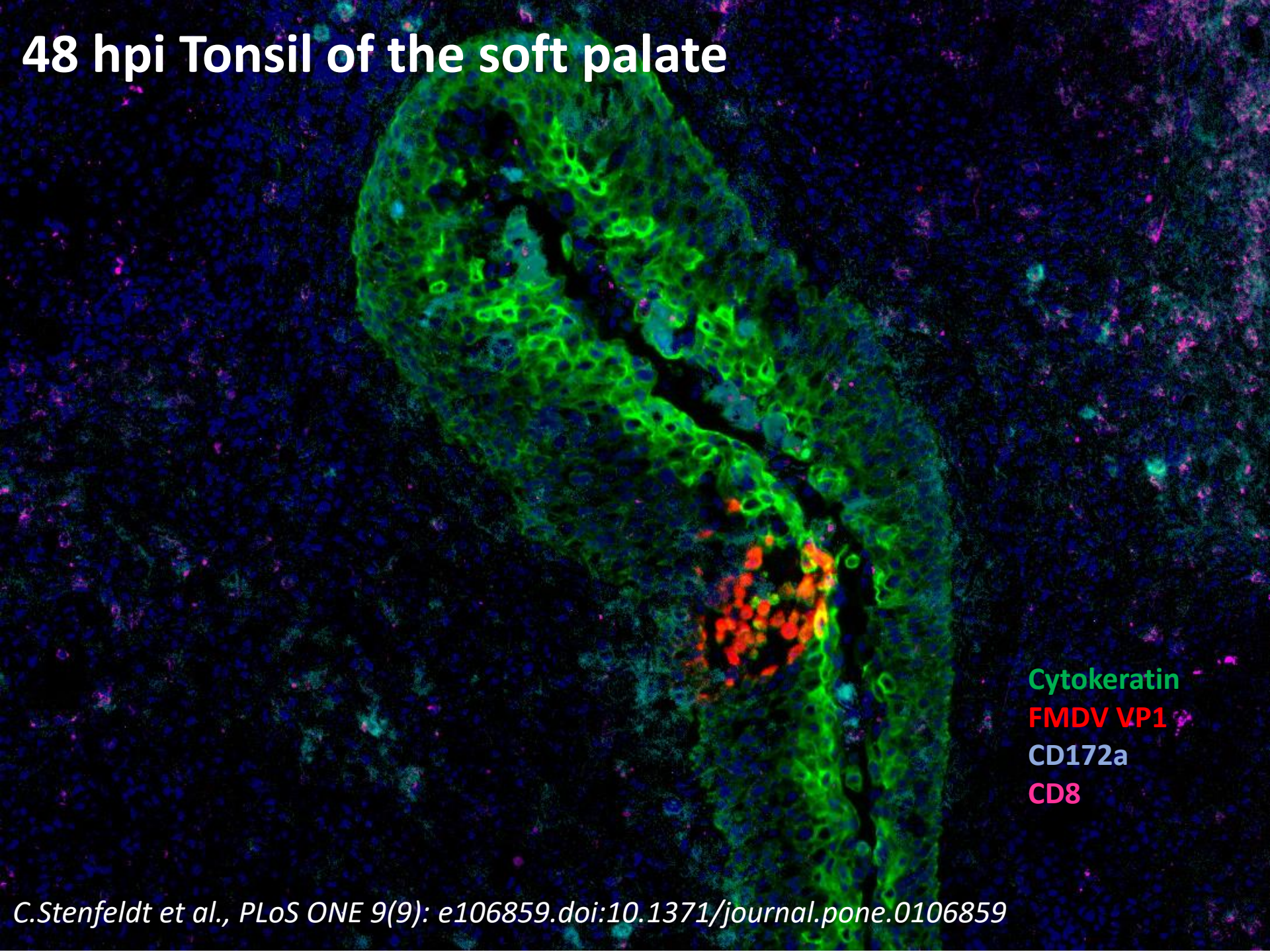
6-24 HPI

Clinical infection

48 HPI

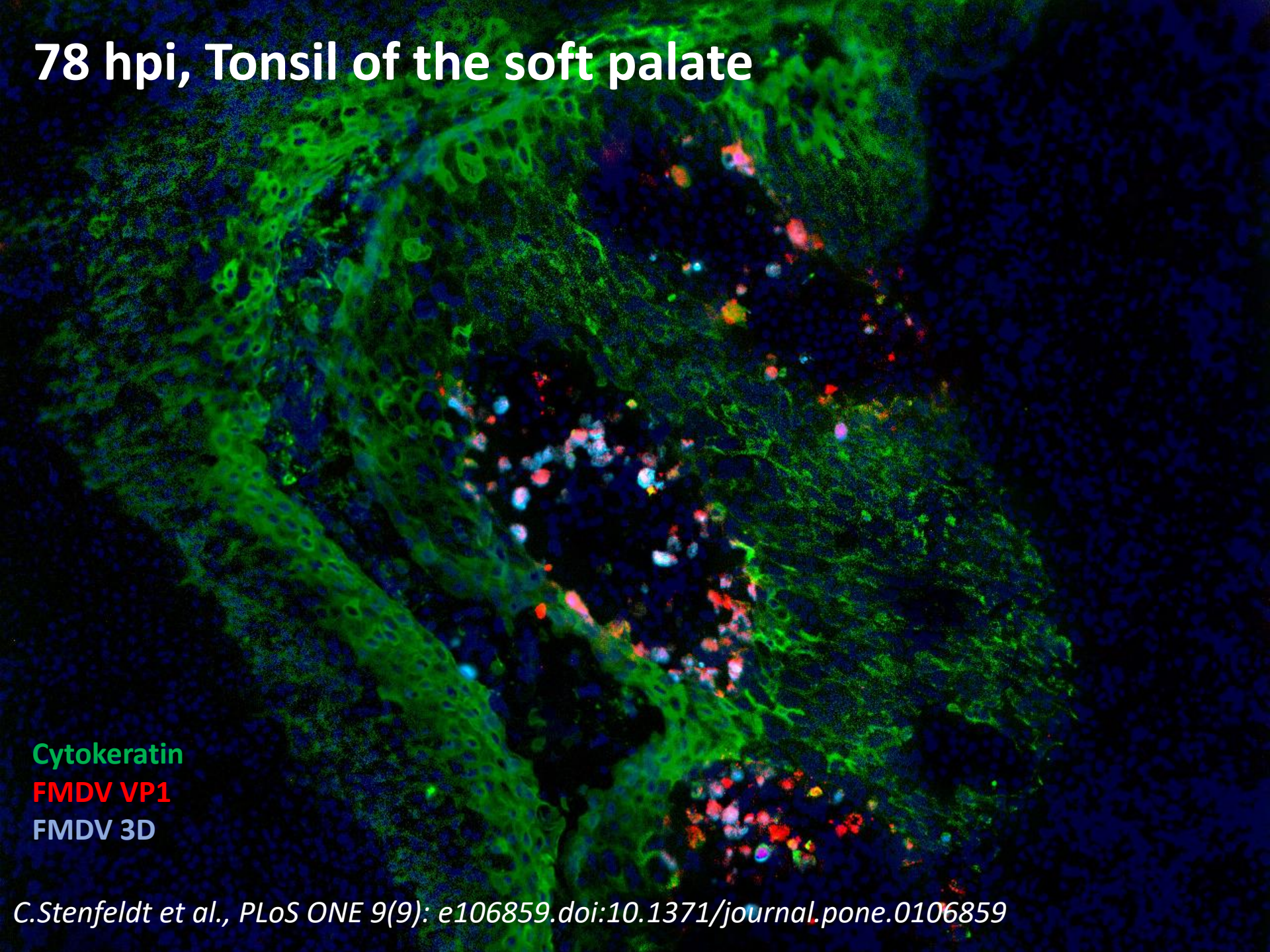


48 hpi Tonsil of the soft palate



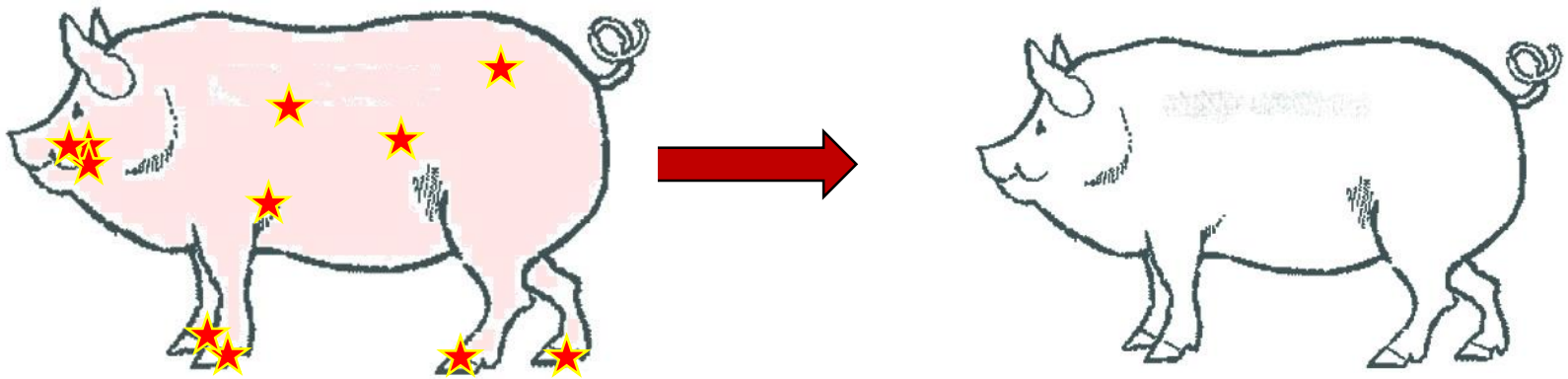
C.Stenfeldt et al., PLoS ONE 9(9): e106859.doi:10.1371/journal.pone.0106859

78 hpi, Tonsil of the soft palate

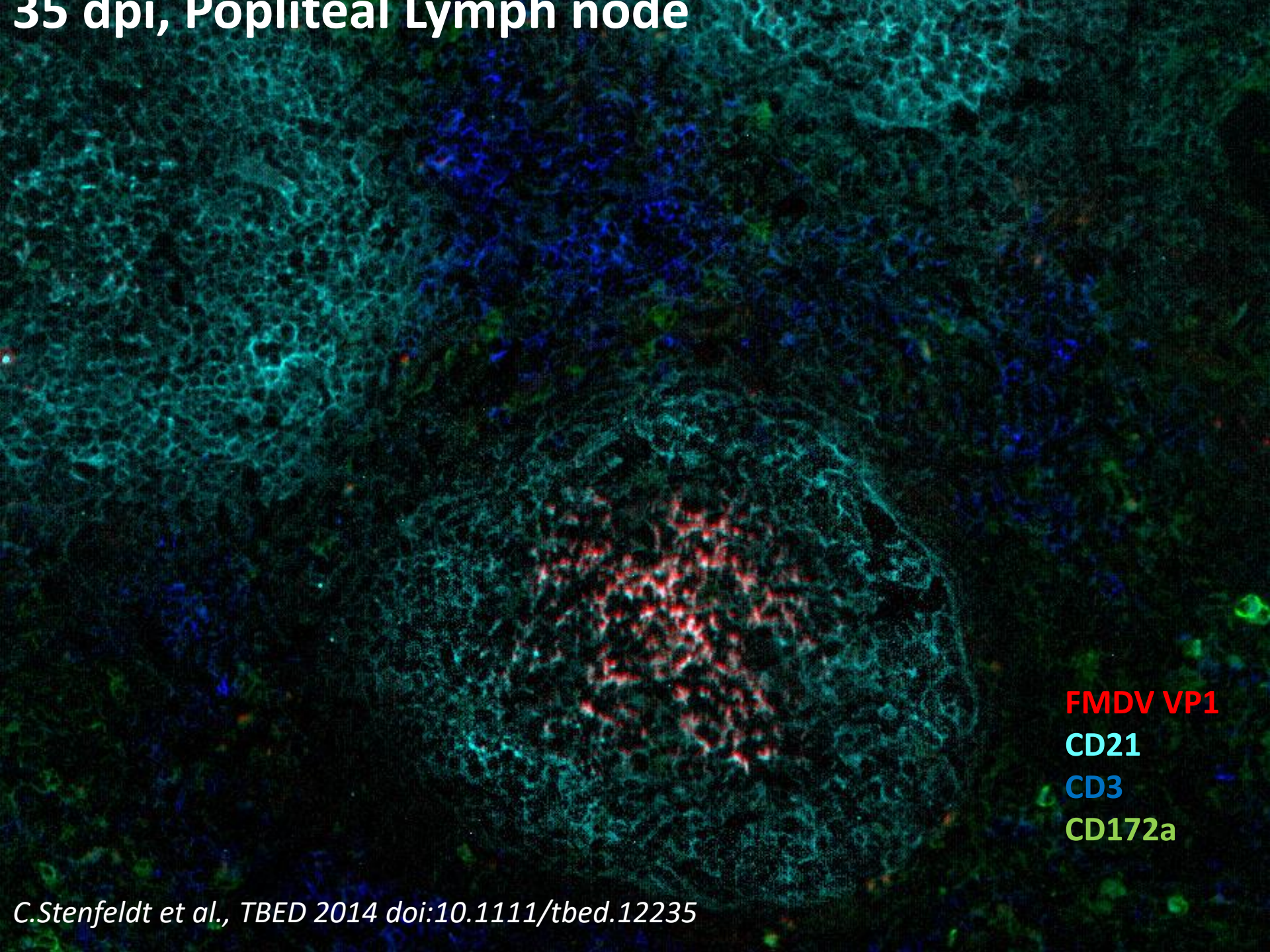


C.Stenfeldt et al., PLoS ONE 9(9): e106859.doi:10.1371/journal.pone.0106859

Clinical infection **FMDV clearance**



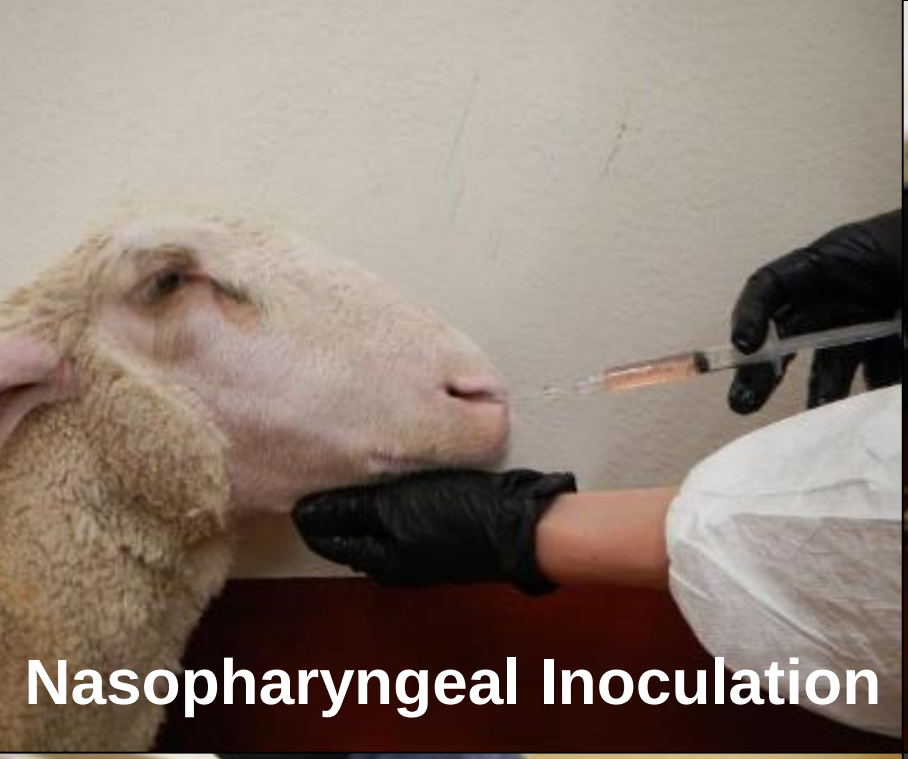
≥28 dpi: <u>NO persistence</u>
of infectious FMDV in porcine tissues

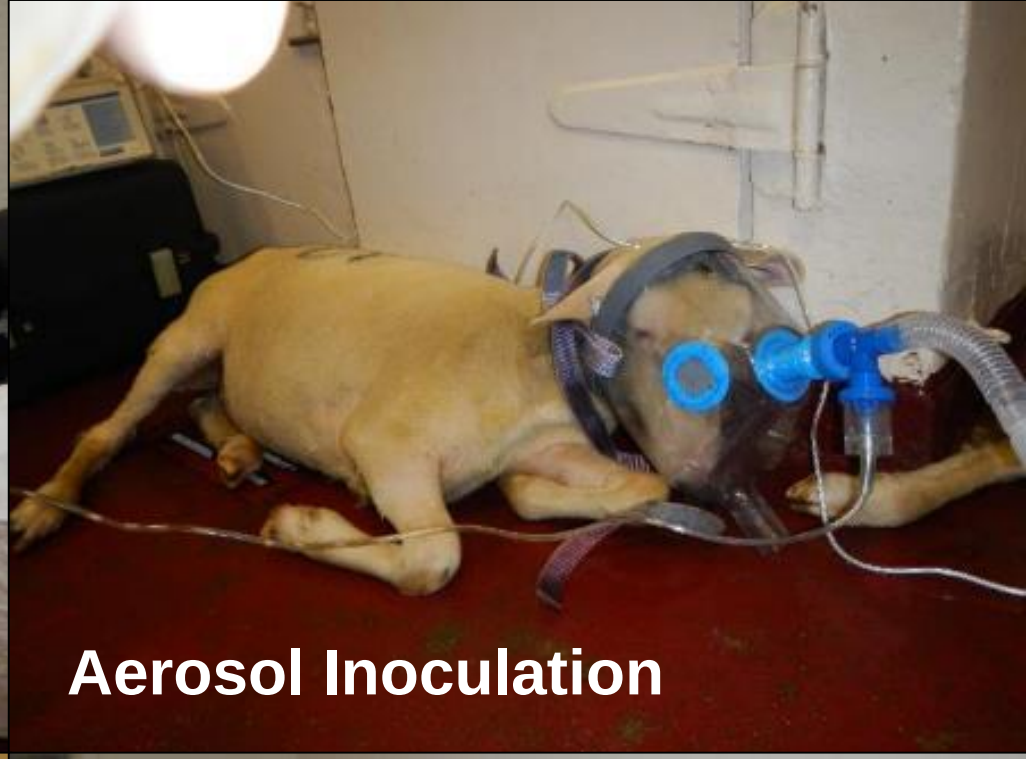


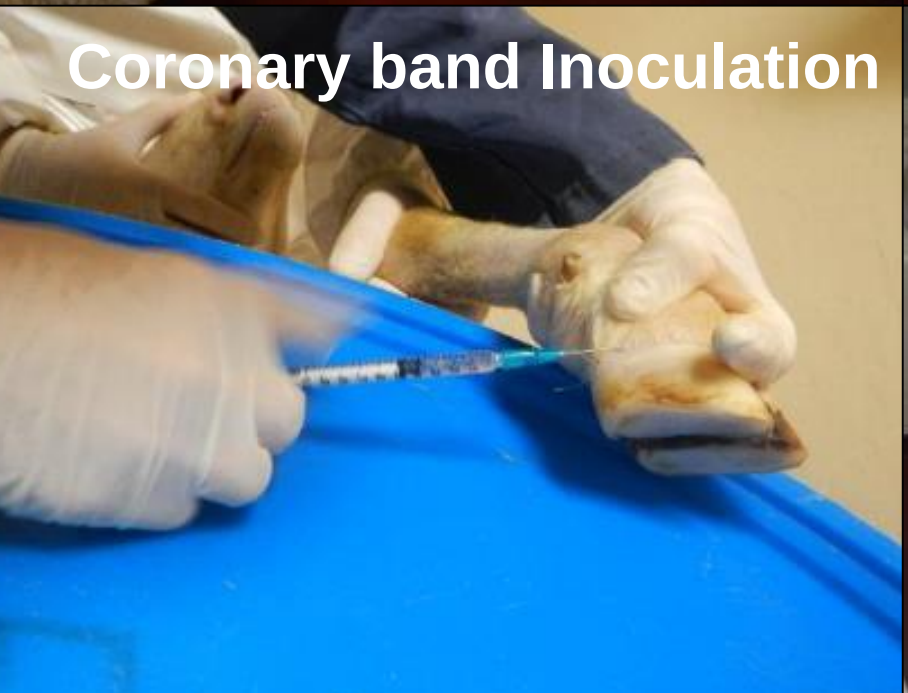
C.Stenfeldt et al., TBED 2014 doi:10.1111/tbed.12235

FMDV pathogenesis studies in sheep

“Little Cows” or “Furry Pigs”?


Nasopharyngeal Inoculation


Aerosol Inoculation


Coronary band Inoculation


Direct contact exposure

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journal homepage: www.elsevier.com/locate/vetmic

Clinical and virological dynamics of a serotype O 2010 South East Asia lineage foot-and-mouth disease virus in sheep using natural and simulated natural inoculation and exposure systems

Carolina Stenfeldt [a,b,1], Juan M. Pacheco [a,1], Nagendrakumar B. Singanallur [c], Helena C. de Carvalho Ferreira [a,b], Wilna Vosloo [c], Luis L. Rodriguez [a], Jonathan Arzt [a,*]

[a] Plum Island Animal Disease Center, Foreign Animal Disease Research Unit, Agricultural Research Service, United States Department of Agriculture, Greenport, NY, USA
[b] Oak Ridge Institute for Science and Education, PIADC Research Participation Program, Oak Ridge, TN, USA
[c] CSIRO-Australian Animal Health Laboratory, Geelong, VIC, Australia

Vaccine 36 (2018) 6095–6102

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Vaccine

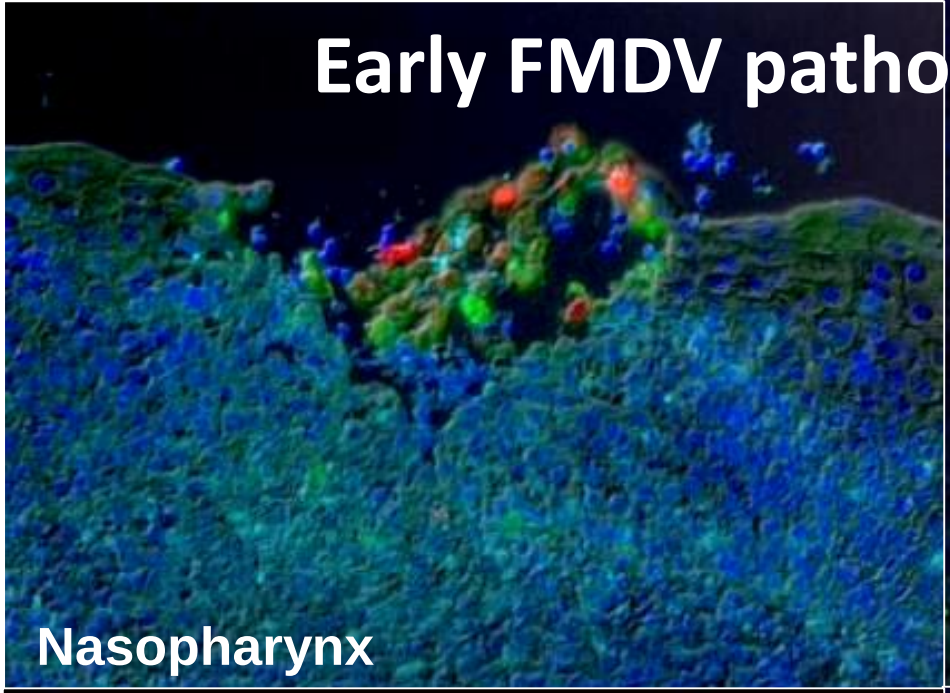
journal homepage: www.elsevier.com/locate/vaccine

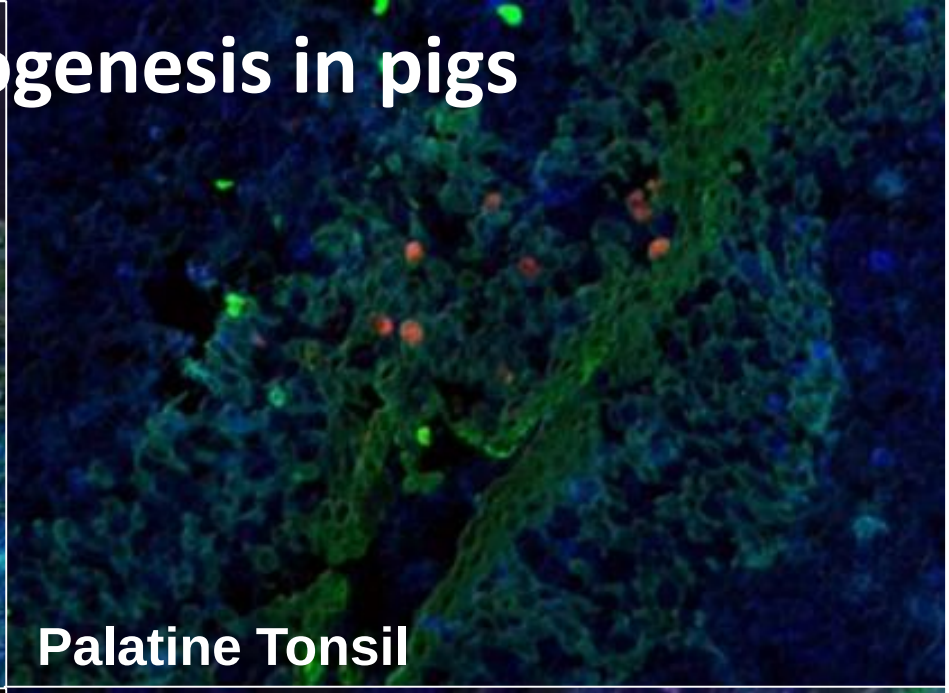
Protection in sheep against heterologous challenge with serotype Asia-1 foot-and-mouth disease virus using high potency vaccine

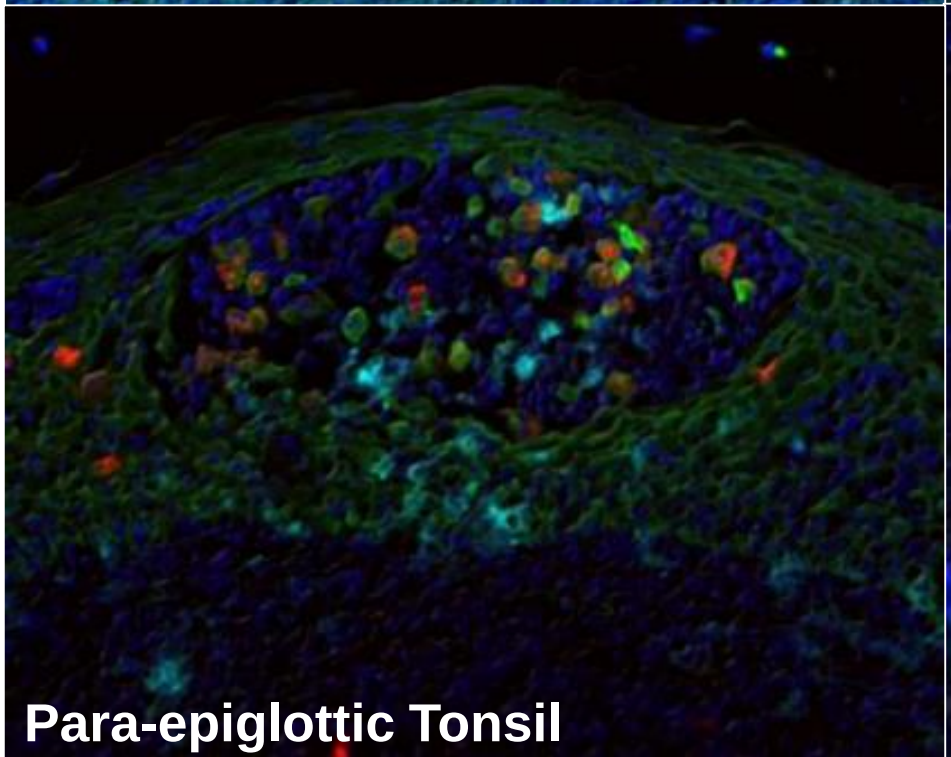
Jacquelyn Horsington [a,*], Charles Nfon [b], Jose L. Gonzales [c], Nagendrakumar Singanallur [a], Hilary Bittner [b], Wilna Vosloo [a]

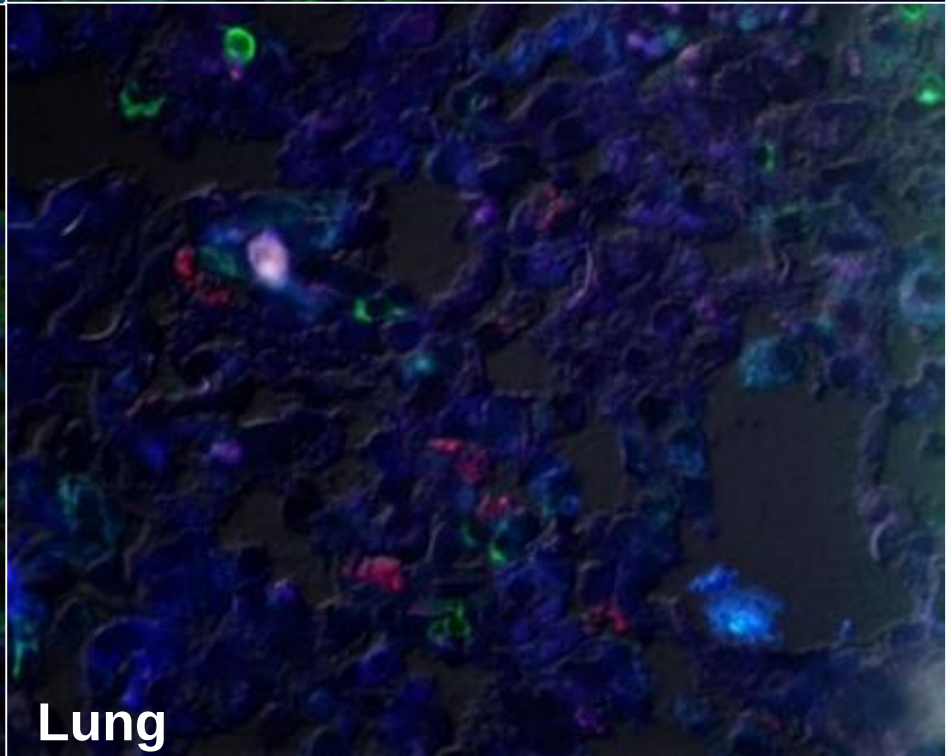
[a] CSIRO-Australian Animal Health Laboratory, 5 Portarlington Rd, Geelong, Australia
[b] Canadian Food Inspection Agency, National Centre for Foreign Animal Diseases, 1015 Arlington St, Winnipeg, Canada
[c] Wageningen Bioveterinary Research, Department of Bacteriology and Epidemiology, Houtribweg 39, 8221RA Lelystad, The Netherlands

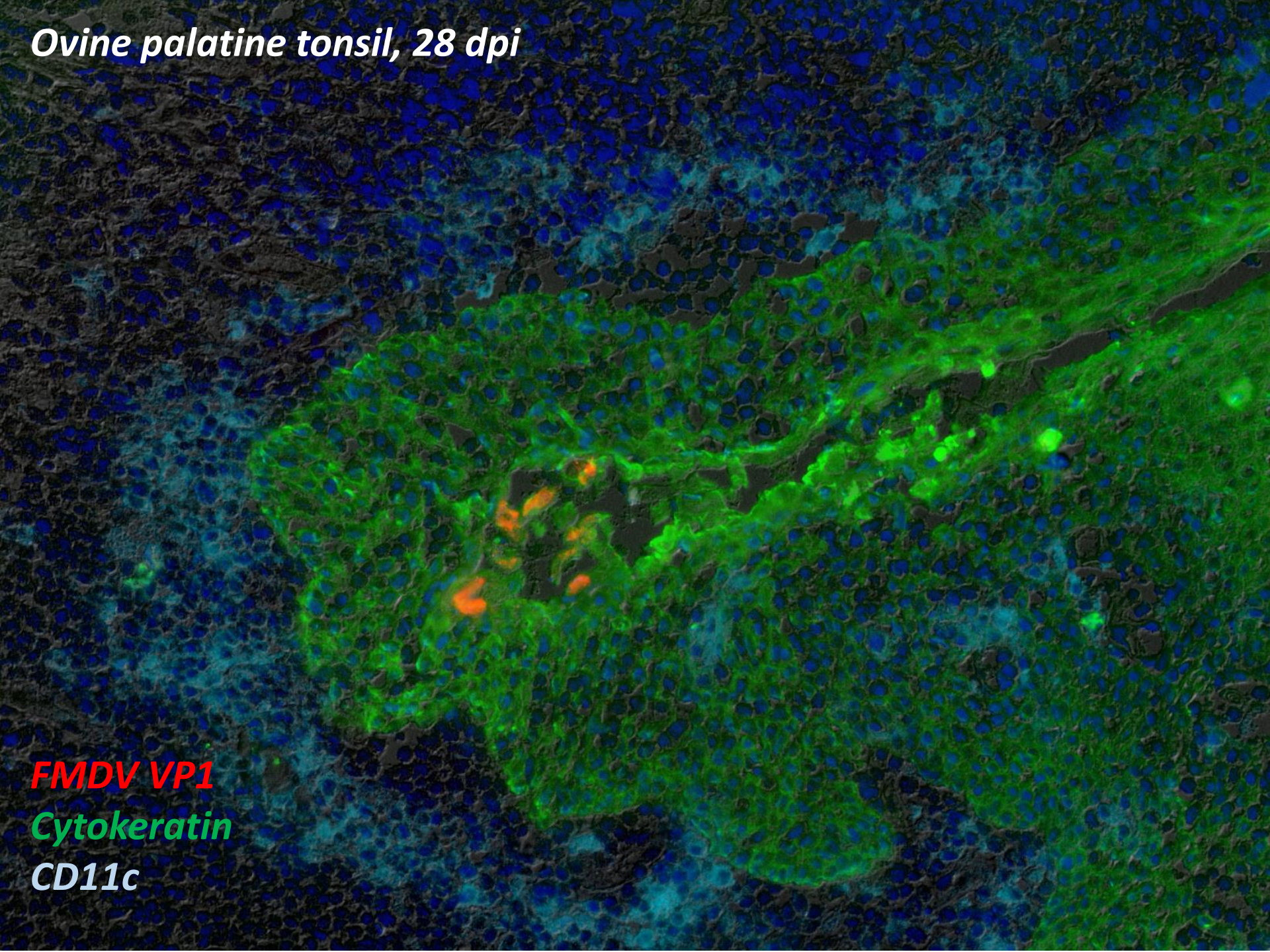
Early FMDV pathogenesis in pigs










Ovine palatine tonsil, 28 dpi
FMDV VP1
Cytokeratin
CD11c


Thank You!