Design and validation of high-throughput real-time PCR systems for detection of porcine and bovine respiratory and enteric pathogens using the BioMark HD (Fluidigm) platform

The 4th AVDC-China

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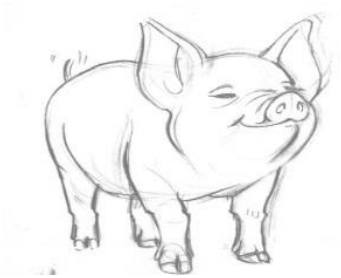
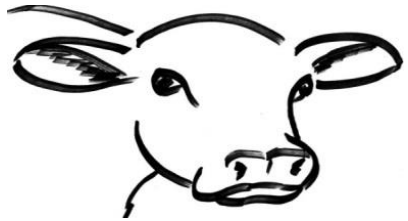
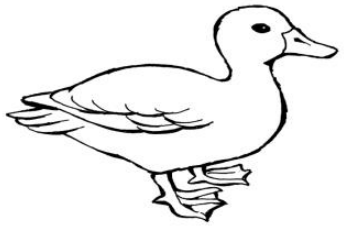
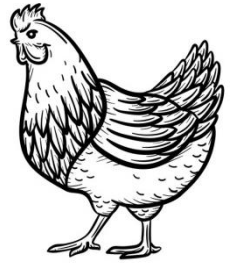
The idea behind the use of high-throughput real-time PCR

- The Danish National Veterinary Institute
  - Large diagnostic unit
    - Main focus on diseases of domestic animals
    - Identification of cause of disease and pathogens
    - Surveillance of avian and swine influenza A viruses









The diagnostic unit – the flow

Diseased animal

Sample submission

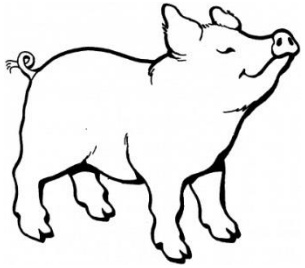
Laboratory

Laboratory analyses

Test result

Veterinarian

Treatment



Healthy animal

Happy farmer

The used low-throughput real-time PCR platform

Low-throughput real-time PCR platform

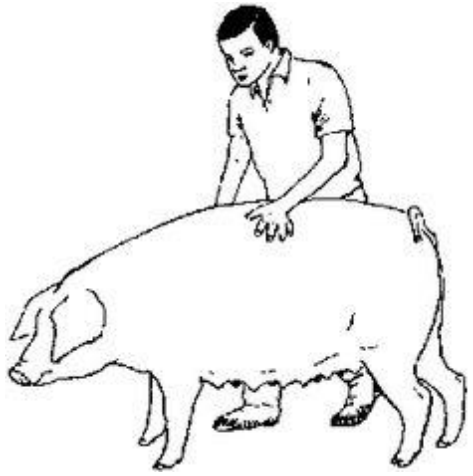
Rotor-Gene Q

- Number of samples: 36, 72, 100
- PCR assay per sample:
  - 1 (singleplex) → most test
  - 6 (multiplex) → few test

Challenges with the low-throughput real-time PCR platform

- Limitation of the platform capacity
- Expensive analyses
  - High analysis cost per sample
    - Many farmers keep the sample submissions to a minimum

- The problem with few sample submissions:
  - Fewer animals get diagnosed
  - Lack of laboratory analysis
    - The disease causing pathogen is unknown
  - Medication and vaccination is based on clinical observation
    - Risk of overuse of antibiotics
    - Risk of suboptimal vaccine programs



The change of real-time PCR platform

- Development of high-throughput real-time PCR systems used for detection and typing of pathogens (viruses and bacteria)
  - Pathogens which have importance to the health and welfare of Danish pigs
- Purpose
  - Reduce the analysis cost per sample and working hours

Low-throughput real-time PCR platform

Rotor-Gene Q

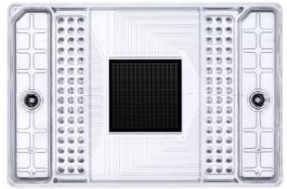
High-throughput real-time PCR platform

BioMark

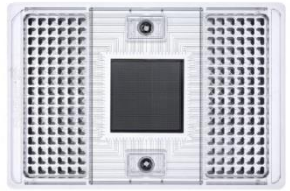
The high-throughput real-time PCR platform

- High-throughput real-time PCR platform – BioMark HD (Fluidigm)
  - Requires less sample and reagents (nL vs. µL)
  - Providing up to 9,216 reactions in one chip (96x96)

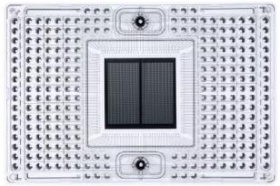
48 samples x 48 assays



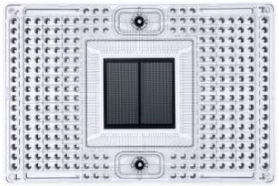
96 samples x 96 assays

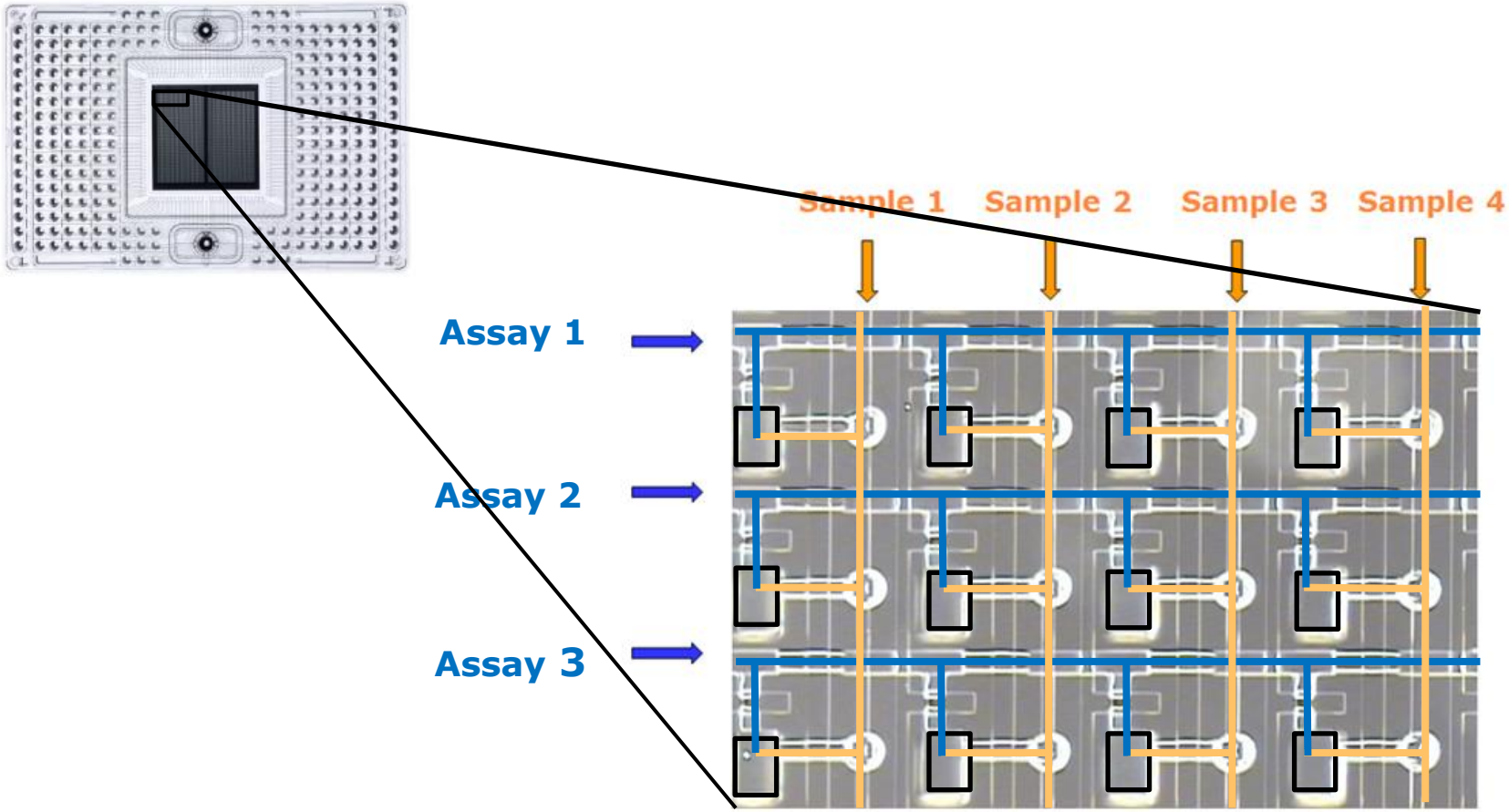


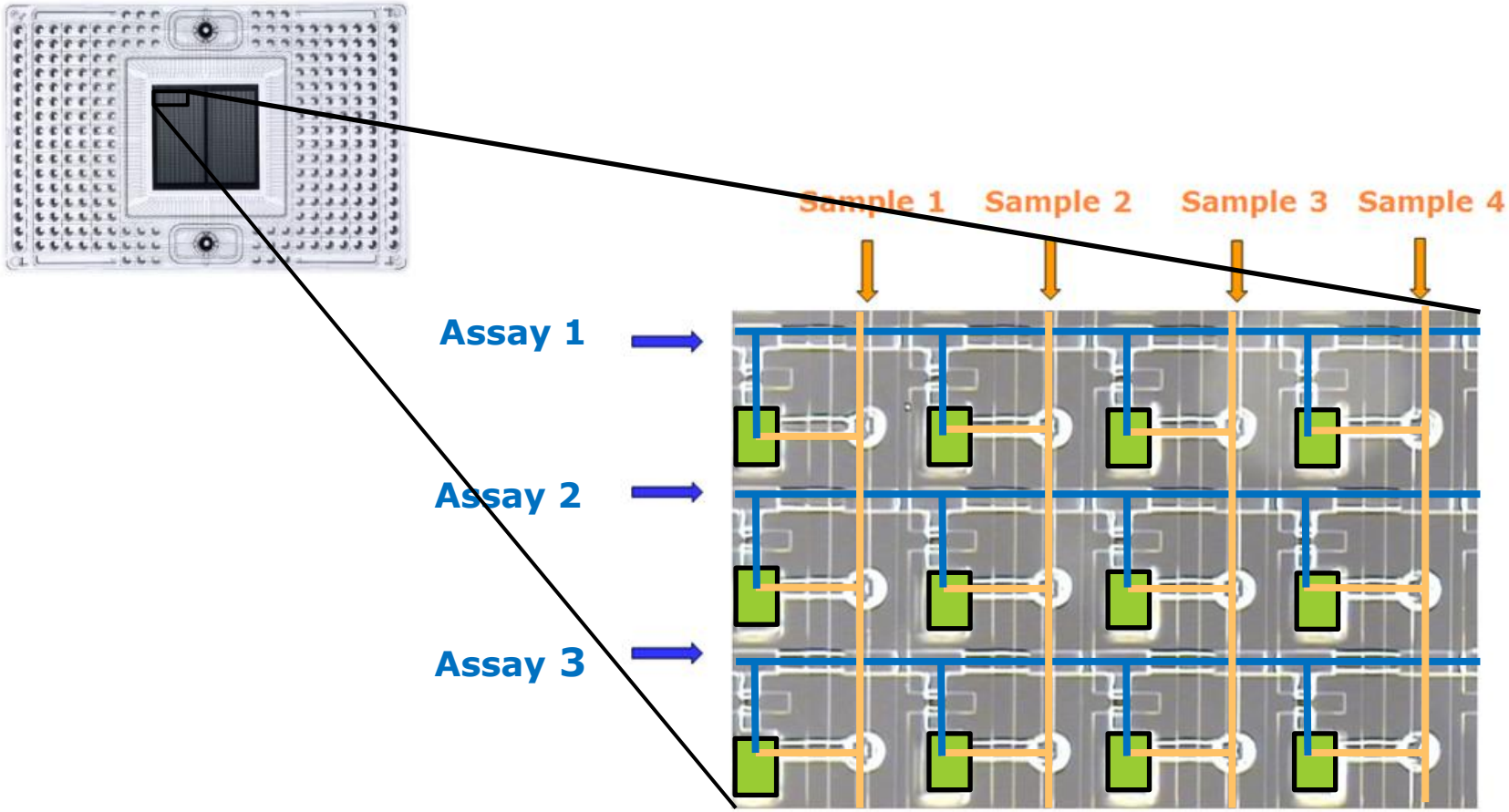
192 samples x 24 assays



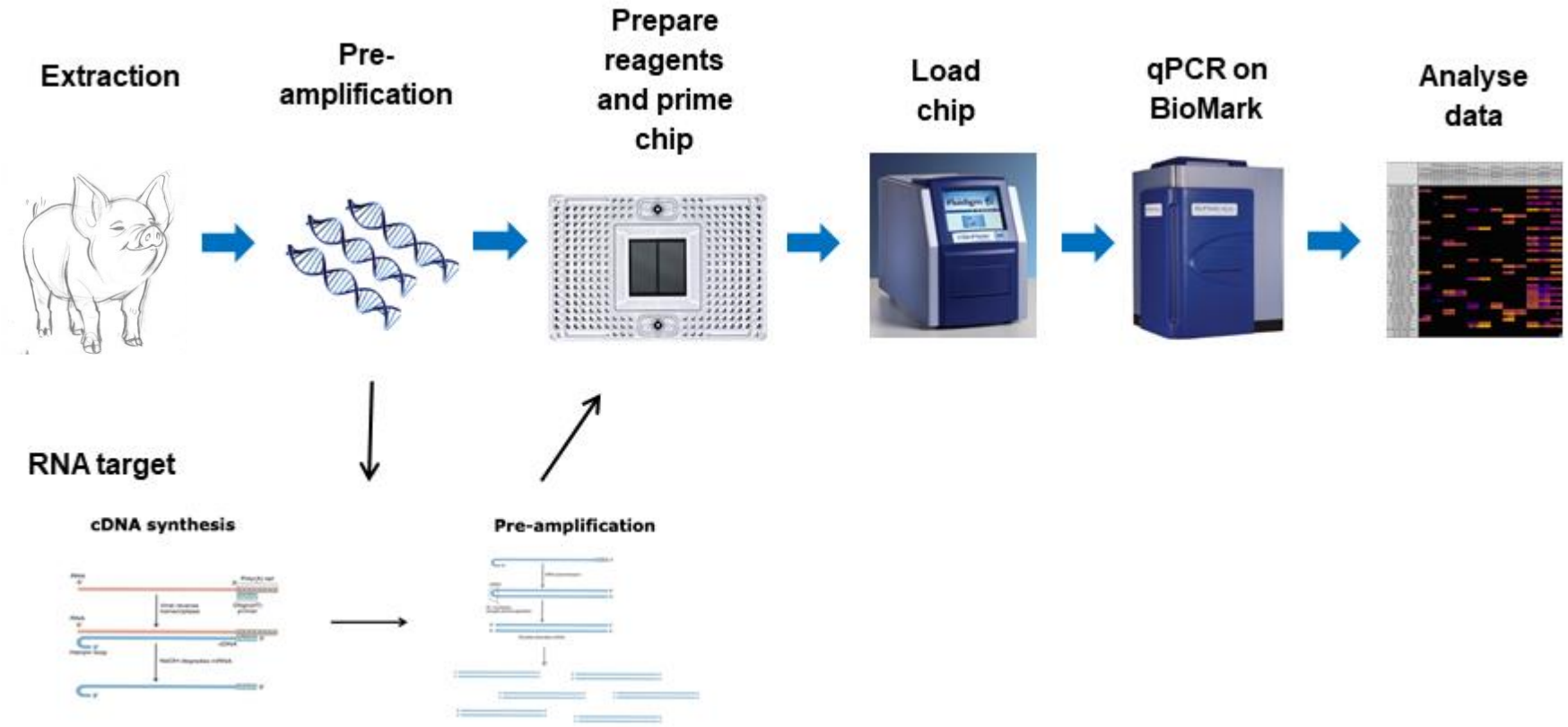
24 samples x 192 assays




Sample 1
Sample 2
Sample 3
Sample 4
Assay 1
Assay 2
Assay 3


Sample 1
Sample 2
Sample 3
Sample 4
Assay 1
Assay 2
Assay 3

The Workflow



The first porcine high-throughput real-time PCR system

Swine influenza A virus (swIAV)

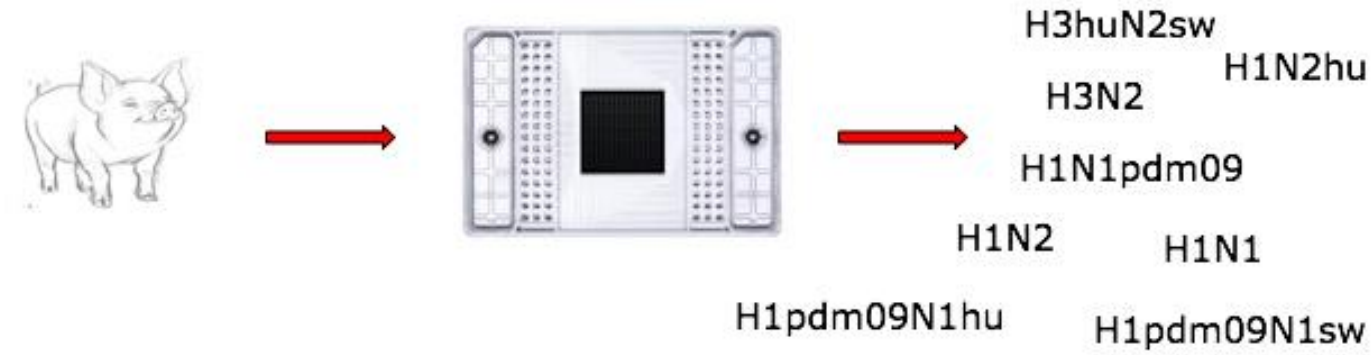
- Establishment of a high-throughput system for detection and subtyping of swIAVs

frontiers in Cellular and Infection Microbiology
ORIGINAL RESEARCH
published: 22 May 2018
doi: 10.3389/fcimb.2018.00165

Subtyping of Swine Influenza Viruses Using a High-Throughput Real-Time PCR Platform

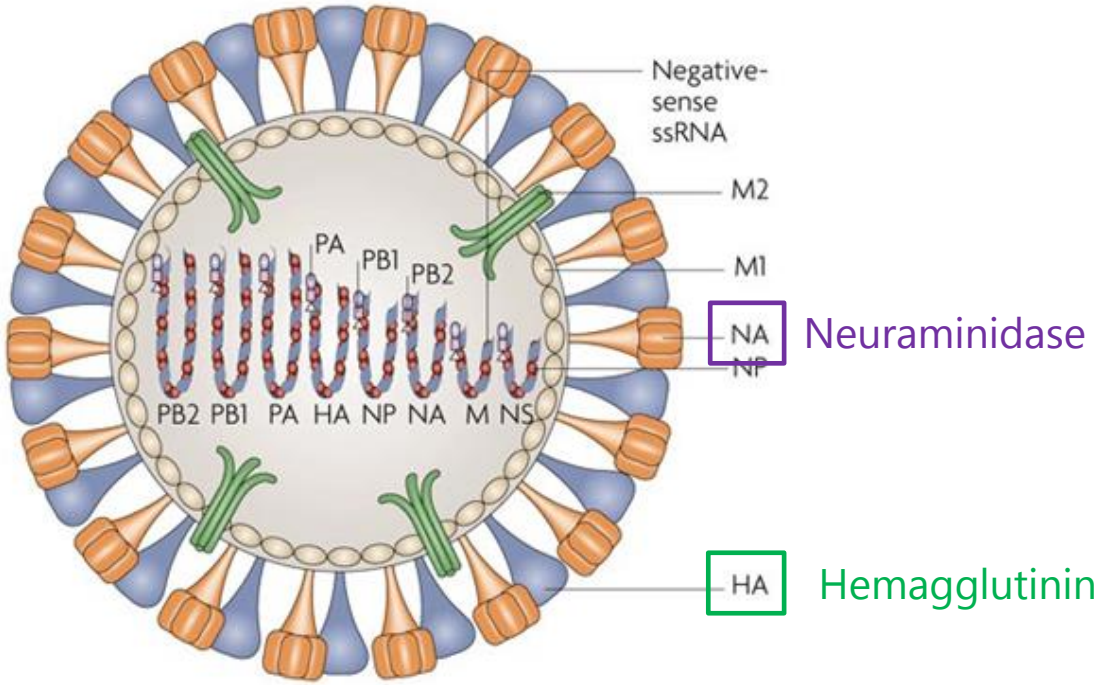
Nicole B. Goecke[1], Jesper S. Krog[1], Charlotte K. Hjulsager[1], Kerstin Skovgaard[1], Timm C. Harder[2], Solvej Ø. Breum[1] and Lars E. Larsen[1]*

[1] Division for Diagnostics & Scientific Advice, National Veterinary Institute, Technical University of Denmark, Kongens Lyngby, Denmark, [2] Institute of Diagnostic Virology, Federal Research Institute for Animal Health, Friedrich-Loeffler Institute, Riems, Germany



Subtyping of swine influenza A viruses

- Currently 18 hemagglutinin (HA) and 11 neuraminidase (NA) genes identified
- Different variants of the HA and NA genes exist
  - H1 lineage
    - H1-human like
    - H1-avian like
    - H1-pandemic 2009
- Subtypes **H1N1**, **H1N2** and **H3N2** are commonly found in pigs



Subtyping of swine influenza A viruses

- A passive surveillance program for swIAVs has been conducted in Denmark since 2011
  - The aim is to identify which subtypes circulate in Danish pigs
    - Be able to act fast on new virus variants

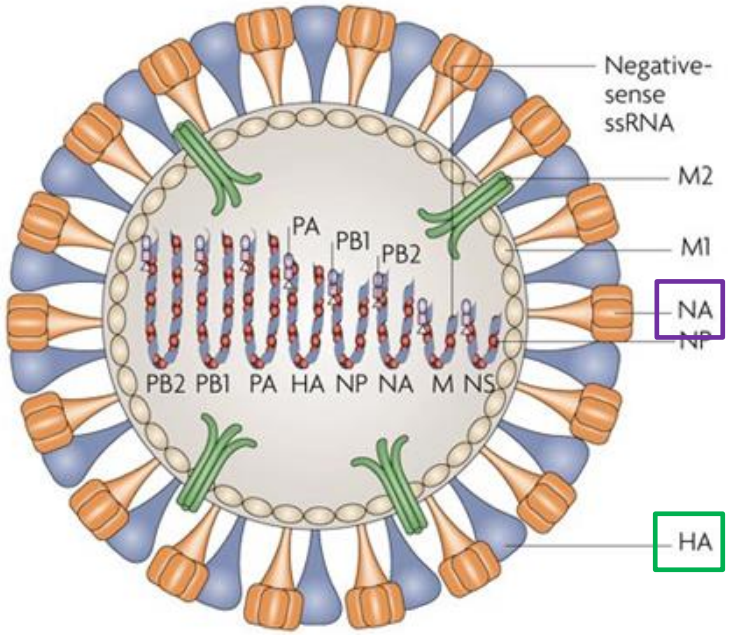
- Subtyping of swIAVs
  - Subtyping is conducted in a multiplex setup
  - Several PCR assays are needed to cover the wide range of circulating subtypes
  - High analysis cost → A limited number of samples can be subtyped

Subtyping of swine influenza A viruses

- Change of real-time PCR platform

→ A more detailed characterization

Internal genes:
- PB2
- PB1
- PA
- NP
- M
- NS



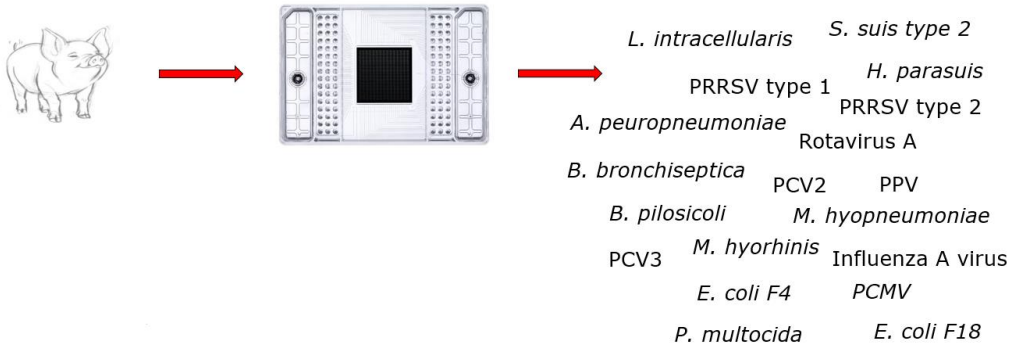
Neuraminidase

Hemagglutinin

The second porcine high-throughput real-time PCR system

Development of a porcine high-throughput system

- Used for detecting of 18 different porcine viruses and bacteria
- All the relevant pathogens causing respiratory and intestinal diseases in pigs are included
  - Reduce the risk of not detecting the relevant pathogen(s)



Full Scientific Report

Development of a high-throughput real-time PCR system for detection of enzootic pathogens in pigs

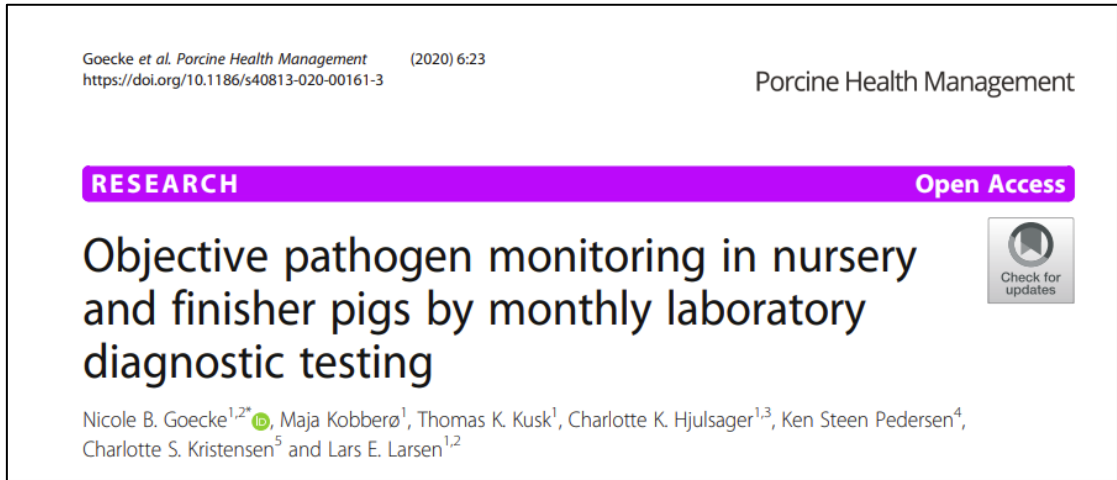
Nicole B. Goecke,[1] Charlotte K. Hjulsager, Jesper S. Krog, Kerstin Skovgaard, Lars E. Larsen

Journal of Veterinary Diagnostic Investigation
2020, Vol. 32(1) 51–64

DOI: 10.1177/1040638719890863
jvdi.sagepub.com

The use of the porcine high-throughput real-time PCR system

- Test the performance of the high-throughput diagnostic system on field samples
  - Samples collected from ten Danish pig production units (6 herds)
- Investigate the connection between prevalence of different pathogens and clinical signs observed
- Test if monthly monitoring of pathogens on herd level could be a supportive tool for the veterinarians
  - Create a more objective basis for intervention



Goecke *et al. Porcine Health Management* (2020) 6:23
https://doi.org/10.1186/s40813-020-00161-3

Porcine Health Management

RESEARCH Open Access

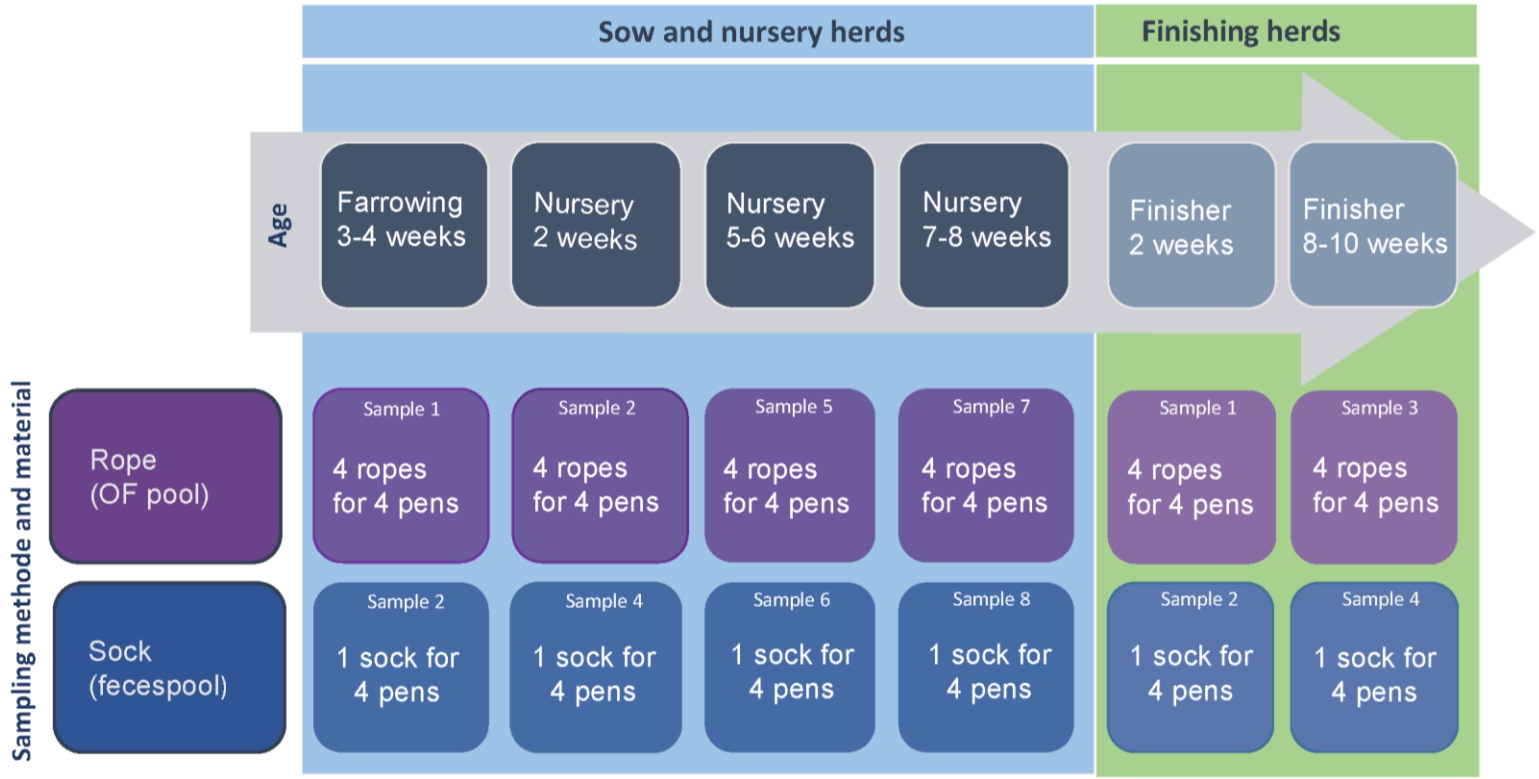
Objective pathogen monitoring in nursery and finisher pigs by monthly laboratory diagnostic testing

Nicole B. Goecke[1,2*], Maja Kobberø[1], Thomas K. Kusk[1], Charlotte K. Hjulsager[1,3], Ken Steen Pedersen[4], Charlotte S. Kristensen[5] and Lars E. Larsen[1,2]

SOS "Swine, Objective Surveillance"

The concept behind SOS:

- Herds are sampled at regular intervals throughout the year
- Common guidelines for all herds
- The farmer is responsible for the sampling



The pathogens in SOS

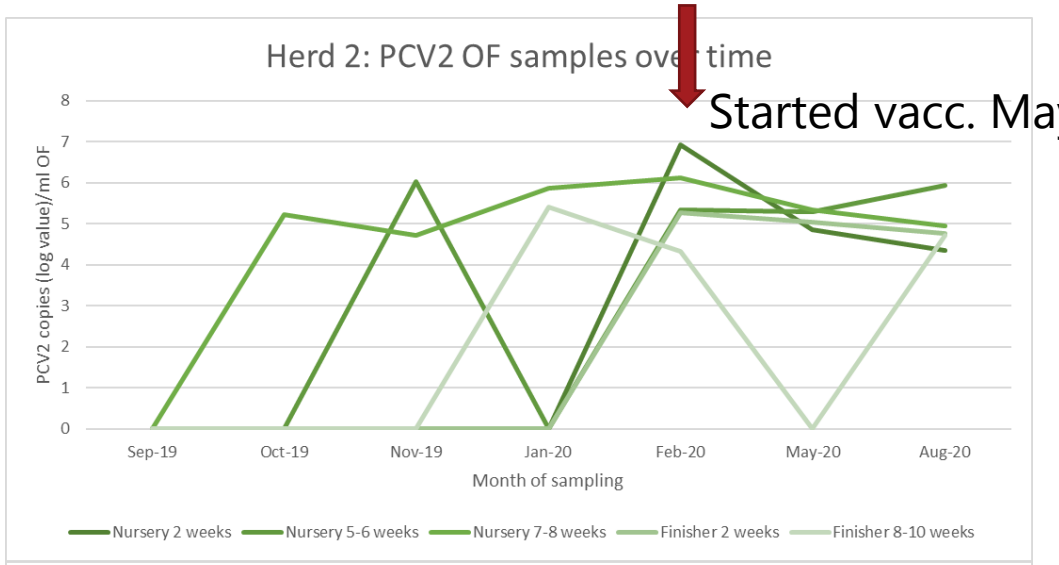
Oral fluid samples:

- Influenza A virus
  - Pandemic H1 (H1pdm)
- Porcine Circovirus Type 2 (PCV2)
- Porcine Circovirus Type 3 (PCV3)
- Porcine Cytomegalovirus (PCMV)
- *Streptococcus suis type 2*
- *Haemophilus parasuis*
- *Pastuerella multocida*
- *Actinobacillus pleuropneumoniae*
- *Mycoplasma hyopneumonia*
- *Mycoplasma hyorhinis*
- *Bordetella bronchiseptica*

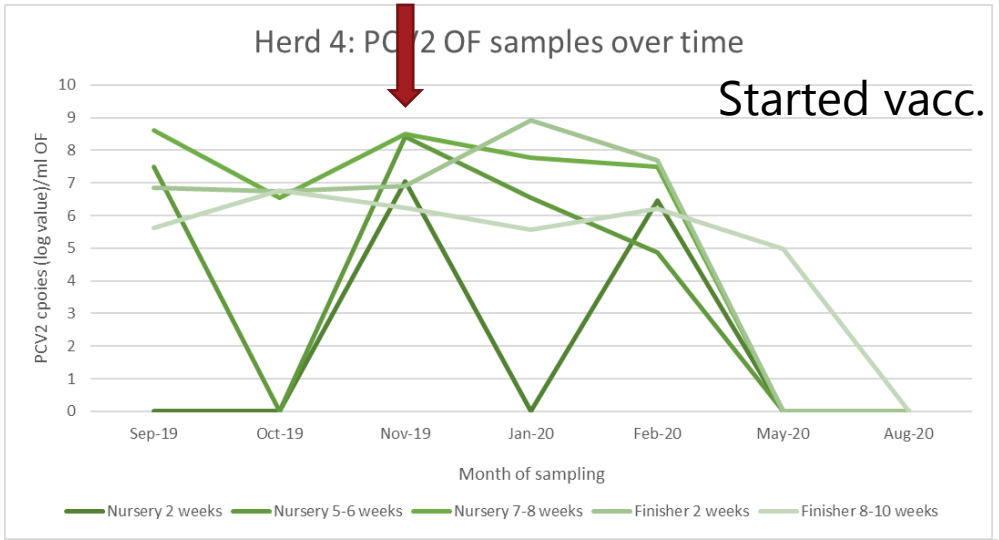
Sock samples:

- *Escherichia coli F4*
- *Escherichia coli F18*
- *Lawsonia intracellularis*
- *Brachyspira pilosicoli*
- Rotavirus A
- Porcine Circovirus Type 2 (PCV2)
- Porcine Circovirus Type 3 (PCV3)

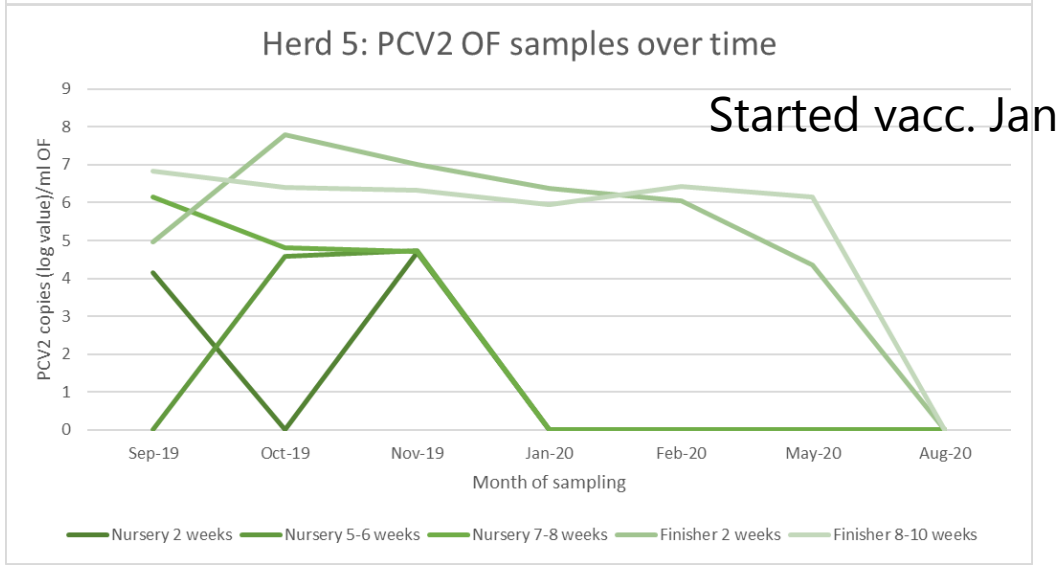
PCV2 – Result example for four herds



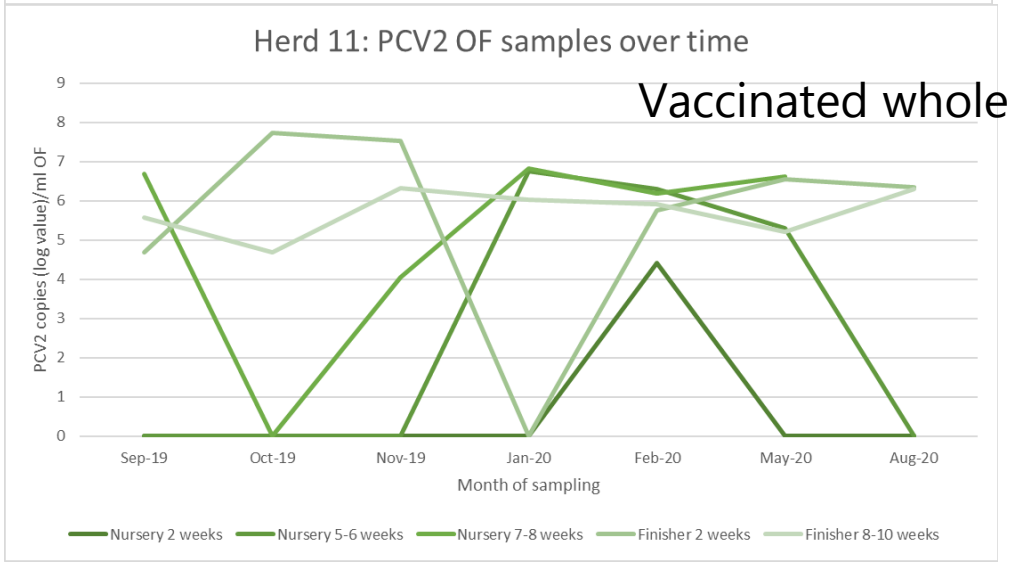
Started vacc. May 20



Started vacc. Nov 19



Started vacc. Jan 20



Vaccinated whole period

The outcome of SOS

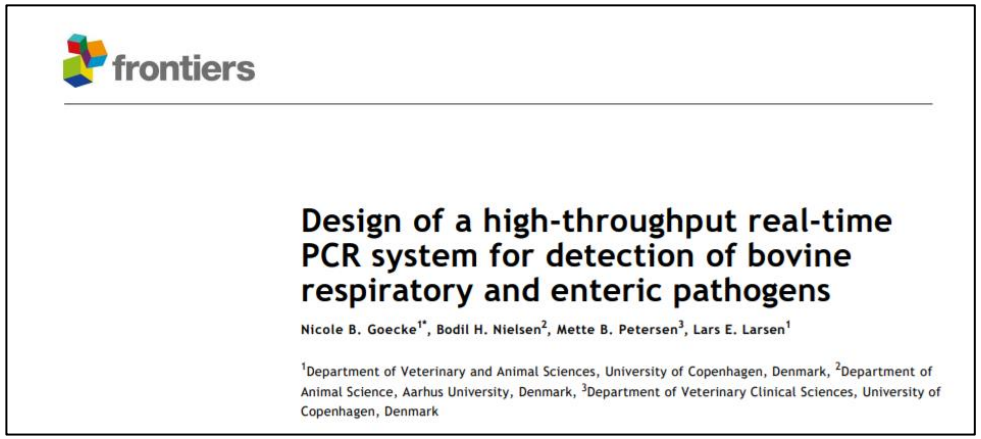
- Overview of the pathogen dynamic in the herd
- Monthly monitoring of pathogens provides information about:
  - The distribution of pathogens in a healthy status
  - An outbreak situation → benchmarking the findings of pathogens
    - → More specific treatment

- Enables follow-up on interventions and change in management

The bovine high-throughput real-time PCR system

Development of a bovine high-throughput system

- Used for detecting of 11 different bovine viruses and bacteria
- All the relevant pathogens causing respiratory and intestinal diseases in calves are included
  - Reduce the risk of not detecting the relevant pathogen(s)


frontiers

Design of a high-throughput real-time PCR system for detection of bovine respiratory and enteric pathogens

Nicole B. Goecke[1*], Bodil H. Nielsen[2], Mette B. Petersen[3], Lars E. Larsen[1]

[1]Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark, [2]Department of Animal Science, Aarhus University, Denmark, [3]Department of Veterinary Clinical Sciences, University of Copenhagen, Denmark

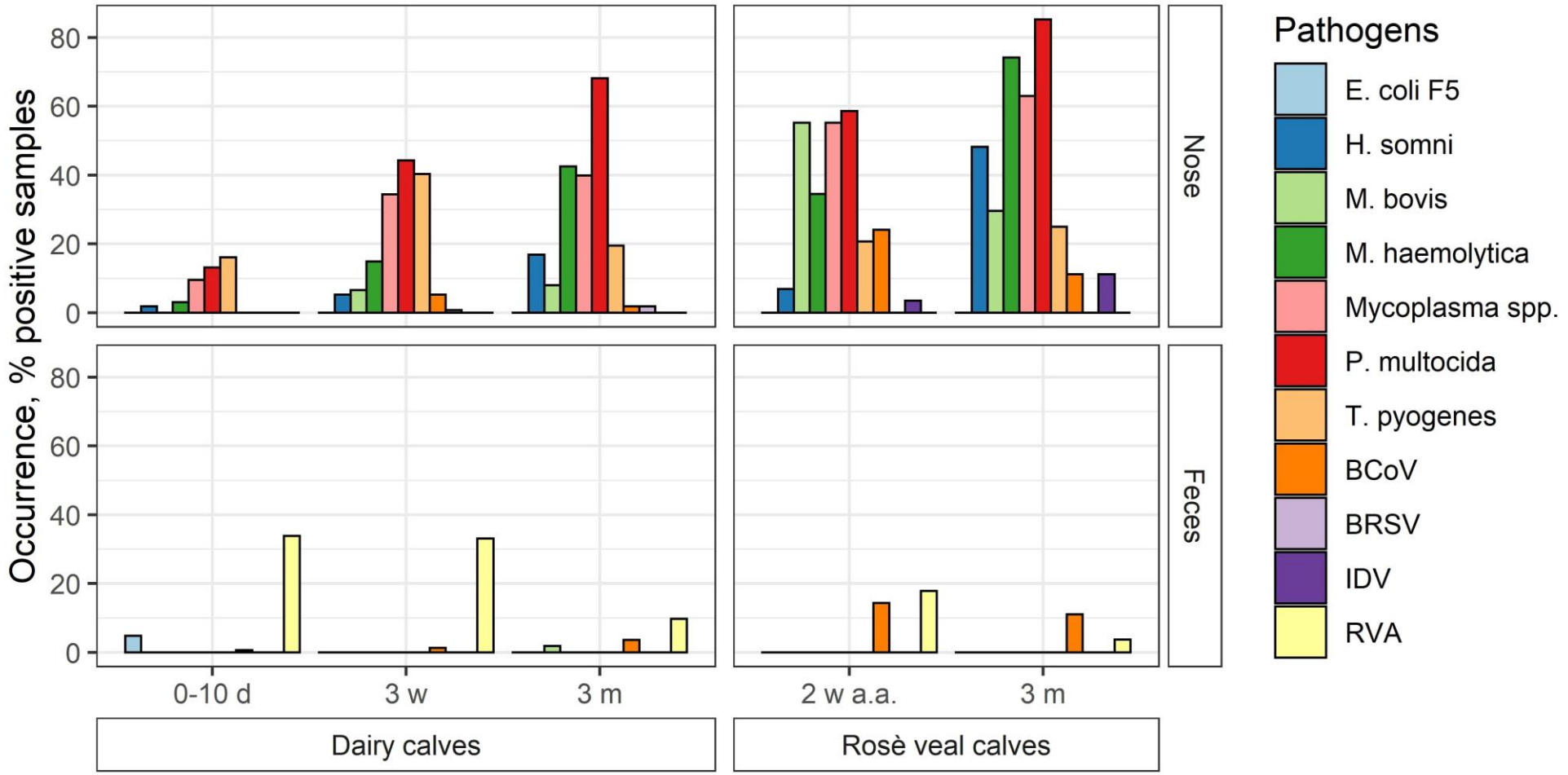
Viruses
Bovine coronavirus (BCoV)
Bovine respiratory syncytial virus (BRSV)
Influenza D virus (IDV)
Rotavirus A (RVA)

Bacteria
Mannheimia haemolytica
Pasteurella multocida
Histophilus somni
Mycoplasma spp.
Mycoplasma bovis
Trueperella pyogenes
Escherichia coli F5

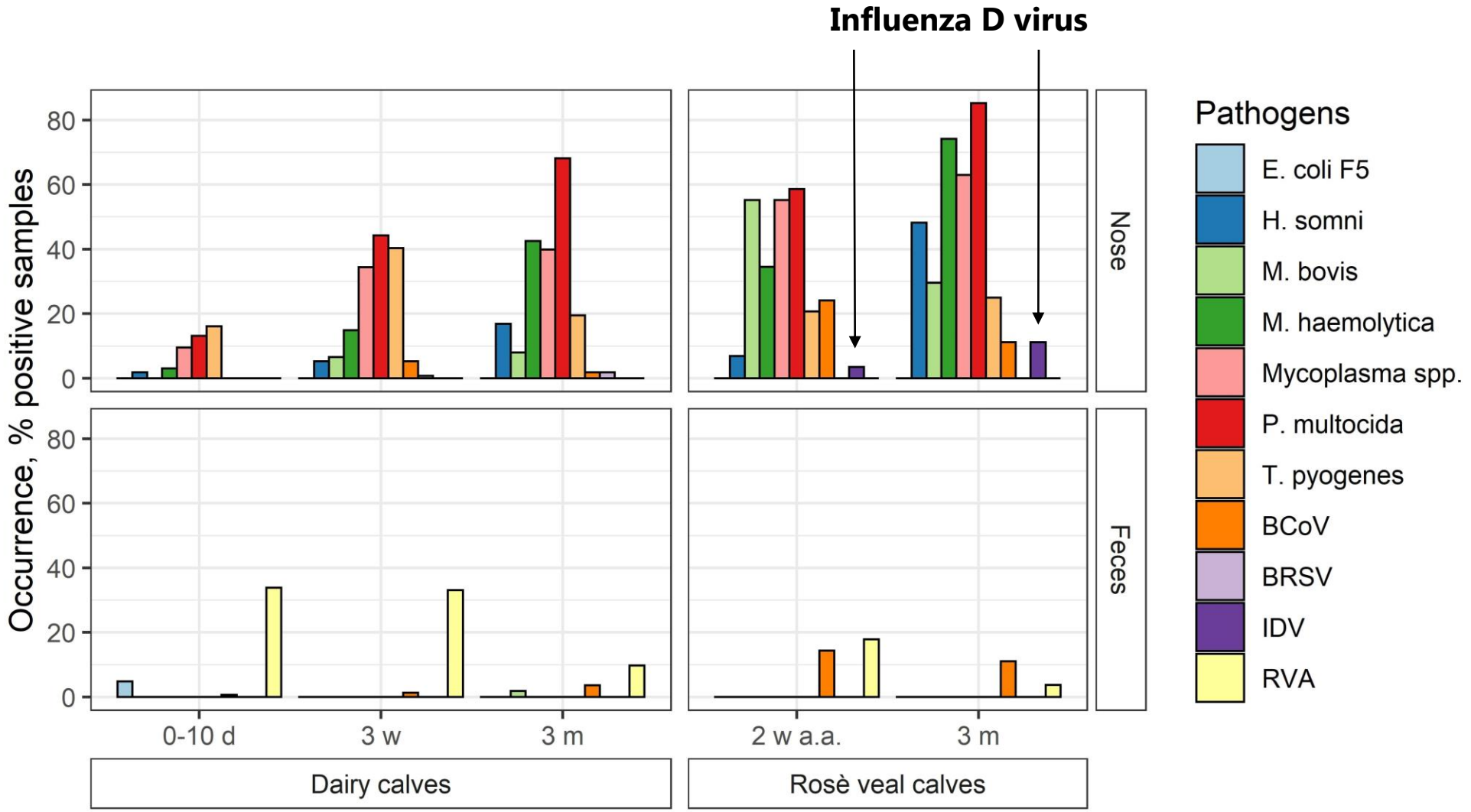
Study design

- Sampling
  - Nasal swab, serum and fecal samples were collected from 100 Danish herds (83 dairy and 17 slaughter herds)
    - Dairy herds: three age groups (0-10 days, 3 weeks and 3 months of age)
    - Slaughter herds: two age groups (2 weeks after arrival and 3 months of age)
  - Sample periods: September-April 2018-2019 and 2019-2020
- Clinical observations
  - Rectal temperature, coughing, nasal and eye discharge, diarrhea, hair coat, joints, body condition

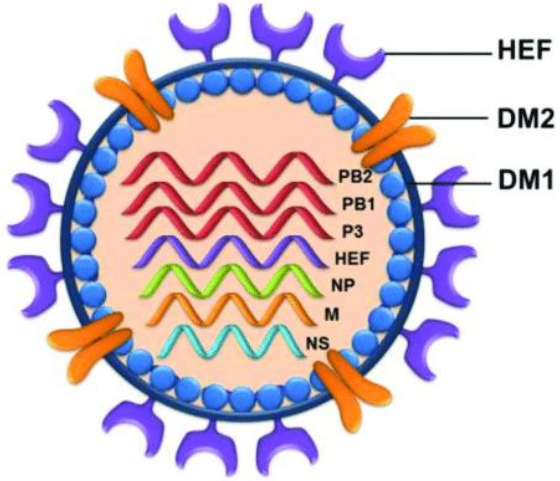
Occurrence of respiratory and enteric pathogens in pools



Occurrence of respiratory and enteric pathogens in pools



Influenza D virus



- The family Orthomyxoviridae
  - Influenza A virus – 8 gene segments
  - Influenza B virus – 8 gene segments
  - Influenza C virus – 7 gene segments
  - Influenza D virus – 7 gene segments - encodes only one glycoprotein (HEF)

- Isolated for the first time in 2011 in the US (Oklahoma)
- Detected in Asia, Europe and USA
- Detected in bovine, pigs, horses, sheep and goats

Influenza D virus – HEF gene

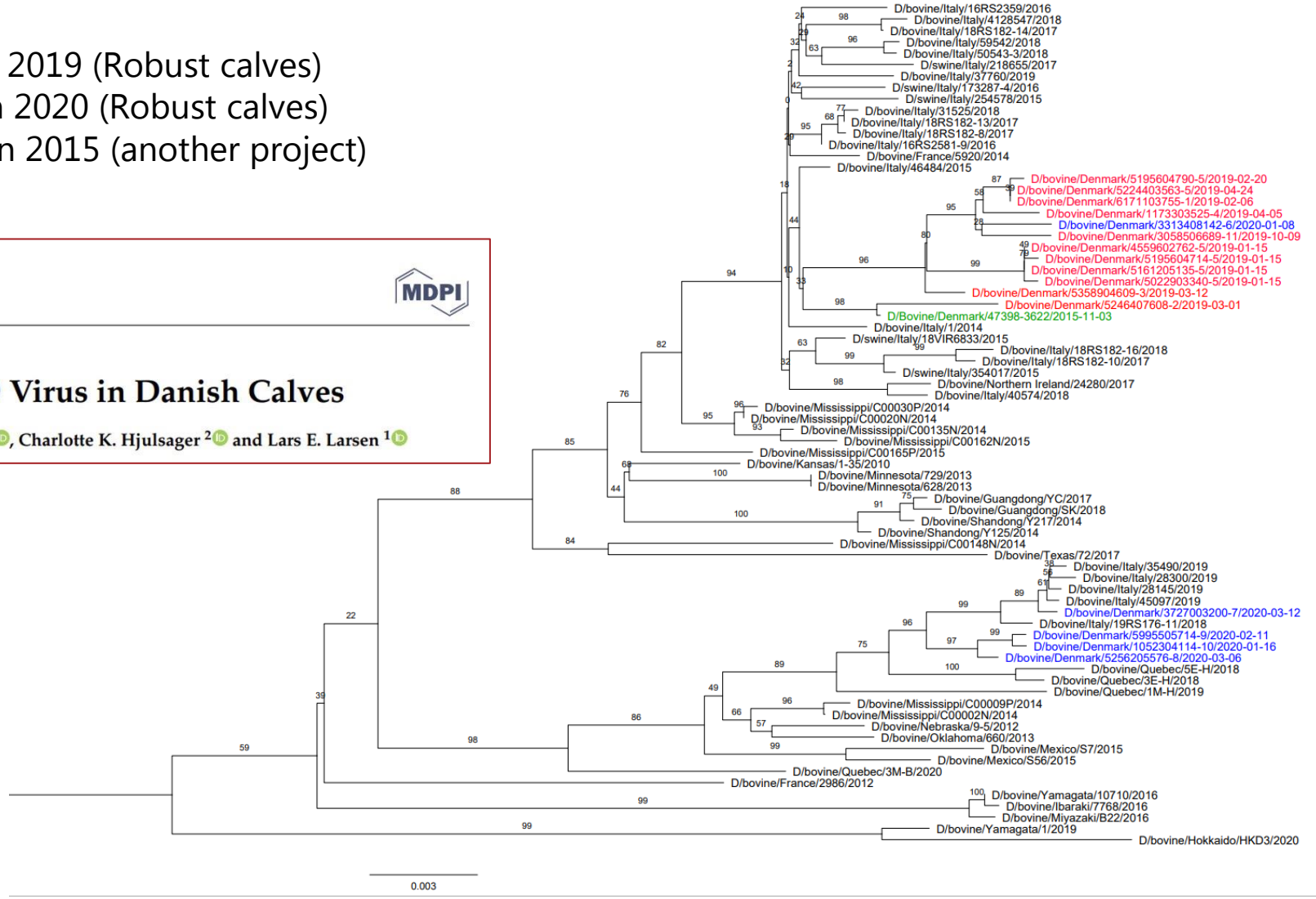
Red: Samples collected in 2019 (Robust calves)
Blue: Samples collected in 2020 (Robust calves)
Green: Sample collected in 2015 (another project)

viruses MDPI

Article

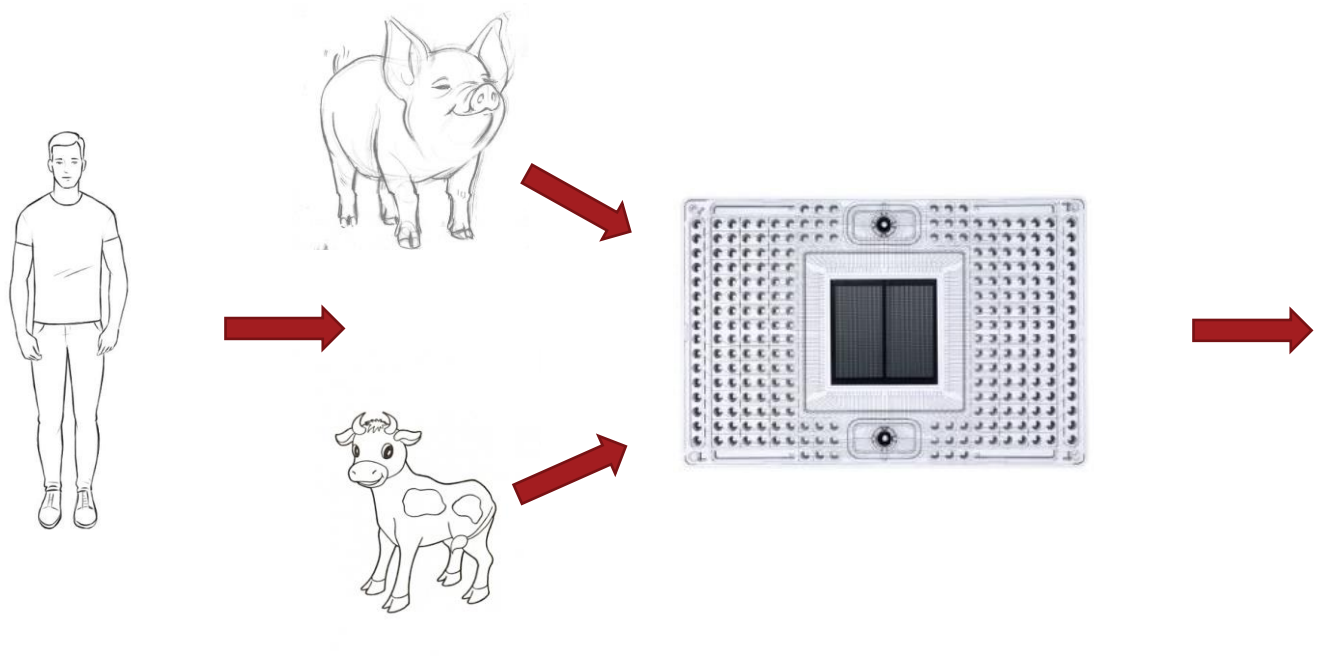
Characterization of Influenza D Virus in Danish Calves

Nicole B. Goecke [1,*,†], Yuan Liang [1,†], Nina D. Otten [1], Charlotte K. Hjulsager [2] and Lars E. Larsen [1]



An AMR high-throughput real-time PCR system

- AMR: Antimicrobial resistance genes



Antimicrobial classes (55 genes)

Aminoglycoside (5)
Macrolide (4)
Phenicol (3)
Sulphonamide (2)
Tetracycline (5)
Trimethoprim (3)
Vancomycin (2)
Beta-Lactamase (4)
Low affinity PBPs (2)
ESBL (7)
Quinolone (6)
Ampc Beta-Lactamase (3)
Carbapenemase (6)
Colistin (2)
Fosfomycin (1)

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Mogens Vestergaard
Henrik L. Martin
Martin Bjerring
Henrik H. Møller
Dorte B. Lastein
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Franziska H. S. Pedersen
Stine Lindgren
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