Potentiating Effect of Plasma on Physical and Chemical Inactivation of PEDV
PEDV: Introduction

- PEDV: faeco-orally transmitted enveloped ssRNA alphacoronavirus

- Presence of viral RNA in multiple body tissues and fluids of infected pigs implies risk of product contamination

- Prevention of further geographical spread of PEDV is important

- Adequate biosafety measures should be in place, including those for feed and feed ingredients

- **Viral genome** can be detected by RT-qPCR, **Viral protein** can be detected using antibody binding assays

- **Infectious virus** can only be detected by showing virus replication: quantification of newly made viral RNA or protein, or visualization of virus infection in cell culture, *in vitro*, or bioassay, *in vivo.*

- **Virus inactivation is by processing** raw material. The viruses remain in the final product in non-infective form.

- Non-exhaustive list of virus inactivation methods used in food industry, blood plasma industry, water purification, vaccine production, and air purification is shown. **Combination of inactivation methods is best.**
PEDV Life Cycle and Pathogenesis

1. Ingestion

2. Virus replication
   - a. Attachment
   - b. Entry
   - c. Replication
   - d. Spread
   - e. Egress

3. Fecal shedding

Feco-Oral infection

PEDV receptor

Mature enterocytes

Epithelial lining of the intestine

Cell culture

Syncytium (plaque)

Vero-Ba cells

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### Analysis for Presence of PEDV

#### Viral genome
- target of RT-qPCR - small RNA fragment
- unit: Ct value or genomic equivalents [ge/ml or g]
- Result: presence of PEDV genetic material, implies risk, but not infection

#### Viral Proteins – Spike protein
- binds to a cellular receptor (APN)
- target of neutralizing antibodies & specific immunity

#### Virus Infectivity
1. **In vitro culture**
   - result: presence of infectious virus replication in a monolayer of susceptible cells
   - ideal for lab virus strains
   - less suitable for clinical samples
2. **In vivo culture – Bioassay (animal trial)**
   - result: presence of infectious virus replication in susceptible pigs
   - ideal for clinical samples

**Size:** 60-160 nm
PEDV RNA can be present in acute phase serum

Liver, spleen, kidneys

Fecal Shedding
Highest reported loads

Nasal/Oral
10x less ge/ml than fecal

Milk

Acute Phase Serum
Infectivity not assessed or non-infectious by bioassay
Processing of raw material

Viral removal
- removal of virus by precipitation, nanofiltration or chromatography
- nanofiltration: no denaturation of proteins, function of pore size (<35nm), flow pressure and rate

Viral inactivation
- viruses remain in the final product in non-infective form
- inactivation of virus lipid coat, protein coat or genome by chemical or physical methods
  - Pasteurization, spray drying, vapor heat
  - Dessication (storage at low water activity)
  - Acidity (Low pH +/- temperature)
  - Solvent/Detergent (TritonX-100)
  - UVC (254nm) +/- riboflavin, UVA + psoralen
  - Methylene blue photosensitization
  - Gamma-irradiation
  - Oxidizing with iodine
- Must be allowed for processing materials to be used as feed ingredients
- Chosen in function of deleterious effects on functionality of the product
- Combination of methods is best
PEDV: Present Study Assay

• **Heat treatment and desiccation damage lipid envelope**
  – inactivation assays that simulate industrial spray-dry practices (>4.25 log10 TCID50 / ml plasma) and storage in dry conditions (>2.8 log10 TCID50 / g SDBP: 21d at 4°C, 14d at 12°C, or 7d at 21°C) have been shown elsewhere (overall reduction of 7 log10 TCID50)
  Gerber et al., 2014; Pujols and Segalés, 2014, Univ of Minnesota

• **Acidity is not effective**
  – Orally transmitted intestinal virus is inherently protected from acidic environment of stomach

**Specific Aims**

To investigate overall sensitivity of PEDV in liquid media

– **Chemical**: alkaline pH
– **Physical**: mild heat treatment (up to 48°C)
– **Matrix**: presence of non-immune porcine plasma
– **INTERACTIONS**

To measure infectivity

– Visualization and quantification of virus plaques and syncytia
In vitro PEDV Culture

- **Vero-Ba cells**
- **PEDV Replication**
- **PEDV Syncytium**

- **Actin filaments in cell cytoplasm**
- **Cell nucleus**
- **PEDV**
**In Vitro PEDV Spike-Inactivation Assay**

1. **Temp, pH**
   - Product (plasma or media) at 4°C, pH 7.2
   - Virus stock (PEDV at highest possible titer) at 4°C, pH 7.2

2. **Titration** (TCID$_{50}$/ml)
   - Spike (PEDV) + Test samples (648 of 100µL)
   - Titration (TCID$_{50}$/ml)
   - Survival Curve (pH 7.2, 40°C)

3. **Spike (PEDV) + Product (plasma or media) at 40°C, pH 7.2**
   - 9:1 ratio

4. **Treatment**
   - Conditions + Duration
   - 40°C, pH 7.2
   - 5 min
   - 10 min
   - 15 min

5. **Titer SURVIVAL**
   - Images of virus samples at different time points after treatment
In Vitro PEDV Inactivation Assay

Surviving PEDV titer (log_{10} TCID_{50}/ml) vs Incubation time (min)

- **4°C**
- **40°C**
- **44°C**
- **48°C**
- LDL
- below LDL

Plasma

MEM

**Treatments (648)**

- **Temperature**
  - 4 – 40 - 44 - 48 (°C)

- **pH value**
  - 7.2 - 9.2 - 10.2

- **Medium**
  - MEM or Plasma (porcine)

- **Time (duration, min)**
  - 0.25 - 1 - 3 - 5 - 10 - 30 - 60 - 90 - 120

**Test sample**

- 10% virus-spike (10µL)
- 90% test medium (at pH & temp)

# Replicate assays

N = 3 (mean ± SD)

Submitted to Veterinary Microbiology

Quist-Rybachuk, Nauwynck and Kalmar, 2015
Porcine Epidemic Diarrhea Virus: Results

• **PEDV infectivity**
  - refrigerated plasma
    → no effect
  - pH 10.2 without mild heat treatment
    → no substantial effect in media or plasma
  - neutral-pH culture medium
    → little sensitivity to heat treatment of 40°C
  - incubation in porcine plasma at body temperature = inactivation
    → PEDV would not remain infectious for more than a few hours in the blood of live pigs
  - pH 10.2 at 48°C: 8log10 reduction in virus titer
    within 4.6 min in plasma or 15.2 min in culture medium
    → alkaline pH strongly potentiated thermal sensitivity of PEDV

• plasma has potentiating effect on physical (heat) and chemical (alkaline pH) inactivation of PEDV
Porcine Epidemic Diarrhea Virus: Results

• Reliability of the viral assay
  – Multiple reruns, appropriate statistics
  – Low detection limit as entire sample was analyzed
  – Titered 648 test samples, thus assay was in 96-well plates with small test-volumes (100μl/sample) and only 6log10 reduction as highest titer
  – Calculated D-value should be confirmed within +/- 0,5log10 of 95% confidence interval

• Confirmation of results
  – Larger test-volume & higher virus-spike
  – Measured D value was < UCL95 of expected D value
  – D value was not dependent on test volume or magnitude of virus spike
  – 31 million infectious particles were reduced to 25 infectious particles in plasma kept for 2.5 min at $H_{48^\circ C}A_{pH10.2}$ and to 0 infectious particles in plasma kept for 5 min at $H_{48^\circ C}A_{pH10.2}$

  – $H_{48^\circ C}A_{pH10.2}T_{10\ min}$ would result in $17.4 \ log_{10} \ TCID_{50} / \ ml$ plasma

  – Even in the absence of anti-PEDV IgG, infective PEDV was inactivated in porcine plasma at in vivo conditions at normal pig body temperatures of 37.8-40°C
Conclusions

• Presence of viral RNA in multiple body tissues and fluids of infected pigs implies risk of contamination of raw products of porcine origin

• **Infectious virus** can only be detected by showing virus replication (quantification of newly made viral RNA or protein or visualization of virus infection in cell culture, *in vitro*, or bioassay, *in vivo*).

• **PEDV** is not a resistant virus and is easily inactivated by processing. The viruses remain in the final product in non-infective form.

• PEDV would not remain infectious for more than a few hours in the blood of live pigs

• pH 10.2 at 48°C - 8 log10 reduction in virus titer within 4.6 min in plasma or 15.2 min in culture medium: **alkaline pH strongly potentiates thermal sensitivity of PEDV**

• **Plasma potentiates physical** (heat) and chemical (alkaline pH) PEDV inactivation, and thus is a hostile environment for PEDV (irrespective of presence of specific anti-PEDV antibodies in plasma)