Non-specific antiviral components in plasma can contribute to the safety of SDPP towards PEDV

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• swine alfa-corona virus
• major infection route: Faecal-Oral
• highly enteropathogenic
  → small intestinal villus atrophy
  → acute, watery diarrhea
  (malabsorptive & maldigestive)

• Severity strongly depends on age at infection
  (also on virus strain, lactogenic immunity, co-infections, ...)

<table>
<thead>
<tr>
<th>Neonatal &amp; Suckling</th>
<th>Weanling to Adult</th>
<th>Gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>➤ up to 80-100 M%</td>
<td>➤ self-limiting (mild)</td>
<td>➤ Reproduction ↓</td>
</tr>
<tr>
<td>o fetid, watery diarrhea</td>
<td>o diarrhea</td>
<td>o FR ↓ - RR ↑ - AR ↑</td>
</tr>
<tr>
<td>o anorexia</td>
<td>o depression</td>
<td>o TB ↓ - BA ↓ - Mu ↑</td>
</tr>
<tr>
<td>o vomiting</td>
<td>o loss of apetite</td>
<td>o especially gilts, d1-d30</td>
</tr>
<tr>
<td></td>
<td>o weight loss</td>
<td>(Orlanratmanee et al., 2010)</td>
</tr>
</tbody>
</table>
Background - Transmission of PEDV

- **Faecal-Oral**
  - **Direct** Infected pigs
  - **Indirect** Faecally contaminated fomites (equipment, clothing, lorries,...)

- **Live pig collection points** (USA July ‘14)
  - 6.6% of trailers: RNA-pos at arrival
  - 5.2% of RNA neg trailers: left RNA-pos
  (Lowe et al., 2014)

- **Milk-borne** lactating sows
  - RNA detected in sow milk
  - Infectivity not tested
  (Sun et al, 2011; Li et al, 2011; Chen et al, 2013)

- **Aerosolised**
  - faeces
  - vomit
  - saliva
  - RNA in air samples 16 km downwind infected farms
  - Field samples: Not infectious
  - Exp. samples: Infectious
  (Alonso et al., 2014)
Background - Transmission of PEDV

- Faecal-Oral

  **Direct** Infected pigs

  **Indirect** Faecally contaminated fomites equipement, clothing, lorries,

Proven field cases (USA Jan ‘14)
- 3 breeding herds: “emergency feed”
- Origin diets: different mills
- clinical sings 2 d post-delivery

- Ingredients: corn - soybean meal - Vit&Min

\[ \text{Ct feed} = 19.5-22.2 \]

- Bioassay: infectious PEDV

(Dee et al., 2014)

Adequate biosafety measures should be in place for feed and its ingredients.
**Background - What about SDPP?**

- **Ontario-cases, 2014**
  - Feed: common factor (Ct 37-40)
  - SDPP: Ct 36-37 (Ct 37-40)
  - bioassay CFIA: POS (sampled at feedmill) *(Pasick et al., 2014)*
  - bioassay US FDA: NEG (sampled at production plant) *(US FDA, 2014)*
  - Ontario-cases occurred > 10 weeks post-production of SDPP

**Epidemiology**  
Brazil & West-Canada remained neg *(Crenshaw et al., 2014)*
Despite large imports of PCR-pos US-SDPP
(2.5-3.5 M Brazilian pigs/3.5-4.0 M West-Canadian pigs)

**Timing**  
PEDV is sensitive to dessication *(Pujols & Segalés, 2014)*
Inactivation of $2.8 \log_{10} \text{TCID}_{50}/g$ (Ct 22)
3 wk at 4°C (Ct 31.5); 2 wk at 12°C (Ct 24); 1 wk at 21°C (Ct 21)

**Processing**  
PEDV is sensitive to spray-drying *(Gerber et al., 2014; Pujols & S, 2014)*
Inactivation of $4.2 \log_{10} \text{TCID}_{50}/ml$ (Ct 13.9)
Spraydrying at 80 °C or 70 °C outlet temp (Ct 23.3-23.8)
Present Trial

Why?
- Prevention of further geographical spread of PEDV is critical
- Adequate biosafety measures should be in place for feed and feed ingredients, including SDPP

Extremely High Fecal Shedding

- RNA detected in sow milk
- Replication in lung macrophages
- RNA detected in nasal/oral secretions (? faecal contamination)
- RNA detected in acute phase serum

BIOSecurity

SDPP (US)
PIGS
Air
Lorries
Clothing
Equipm.
Present Trial

Research Questions

1. Does porcine plasma contain non-specific anti-PEDV activity?
   - 3-5 dpi: 2.3-3.2 log PEDV ge/ml serum (Opriessnig et al. 2014)
   - Bio-assay: 3-5 dpi, seronegative serum (body temp): non-infectious (Gerber et al., 2014)

2. Are anti-PEDV effects in plasma temperature dependent?
   - [refrigerated plasma (4°C) vs living pig (37.8-40°C)]
   - Bio-assay: PEDV spiked, seronegative serum (4°C): infectious (but high spike)

3. Is PEDV inactivation facilitated in porcine plasma?
   - ingredient-specific PEDV survival at outdoor storage (-25 to 20 °C)
     - SDPP, VTM, soy oil, corn: ≤ 7 d
     - DDGS, M&B, Lys, stock-virus: > 30 d
     - Soybean meal: > 180 d (≤ 210 d)
     - non-infectious (in vitro and in bio-assay)
       (Dee et al., 2015)
Materials & Methods - Spike Inactivation Assays

1. 90:10 matrix (MEM or porcine plasma):virus

   sterile filtered heat inactivated seronegative for PEDV
Materials & Methods - Spike Inactivation Assays

1. **Test Matrix**
   - 90%
   - 10%

2. **PEDV Spike**

3. **Test Sample**

   ![Diagram](image)

**Inactivation (treatment)**

- **Condition**
  - **pH**: neutral (pH 7.2)
  - **temperature**: 4, 37 or 40 °C (body temp: 37.8 - 40 °C)

- **Time**
  - up to 180 min

- **Matrix**
  - MEM or porcine plasma

**ASSAY**

In vitro infectivity
Materials & Methods - Spike Inactivation Assays

1. Test Matrix
2. PEDV Spike
3. Test Sample
4. SPIKE
5. INACTIVATION
6. ASSAY
7. In vitro infectivity

**virus titration of whole test sample**
- 100 ul test samples
  - TCID\textsubscript{50} in 96-well plates
  (survival curves, n=3)
- 1 ml test samples
  - PFU count
  - 1:10 in 175 cm\textsuperscript{2} tissue culture flasks
Spike-Inactivation Assay

Surviving PEDv titer (log_{10} TCID_{50}/ml) vs Incubation time (min) at different temperatures:
- **4 °C**

**MEM** pH 7.2

Stable in MEM at 4°C
Stable in plasma at 4°C

**Sensitivity of PEDV**

**Results & Discussion - Survival Curves**
Results & Discussion - Survival Curves

Spike-Inactivation Assay

**Surviving PEDv titer (log\(_{10}\) TCID\(_{50}\)/ml)**

- **4 °C**
- **40°C**

**Incubation time (min)**

**MEM** p\(\text{H} 7.2\)

**Stability**

- **Stable in MEM at 4°C**
- **Stable in plasma at 4°C**

- **Stable in MEM at 40°C**
- **Sensitive to 40°C in plasma (tailing effect in plasma)**

**Sensitivity**

- **D\(_{40°C,\text{MEM}}\) = 120 ± 17 min**
- **D\(_{40°C,\text{plasma}}\) = 16 ± 5 min**
- **7.5 x faster “in vivo” conditions**
Results & Discussion - Survival Curves

**Spike-Inactivation Assay**

**Surviving PEDv titer**

- **MEM, pH 7.2**

- **Temperature Impact**:
  - **4 °C**
  - **40 °C**

**Sensitivity of PEDV**

- **Stable in MEM at 4°C**
- **Stable in plasma at 4°C**
- **Stable in MEM at 40°C**
- **Sensitive to 40°C in plasma (tailing effect in plasma)**

**Incubation Time** (min)

**D<sub>40°C,MEM</sub> = 120 ± 17 min**

**D<sub>40°C,plasma</sub> = 16 ± 5 min**

**7.5 x faster “in vivo” conditions**

**HAT-pasteurisation plasma**

- **[48°C - pH 10.2 - 10 min]**
- **D-plasma<sub>UCL95</sub> : 35 sec**
- **17.4 log PEDV TCID<sub>50</sub>/ml**

(in MEM: **D<sub>UCL95</sub> = 114 sec → 5.3 log**)

Quist-Rybachuck, Nauwynck, Kalmar, subm
Acute phase serum has been reported to contain PEDV
Does PEDV remain infectious in plasma at *in vivo* conditions?

⇒ Spike-inactivation assay in tissue culture flasks
⇒ HAT determinants: H = 37°C; A = pH 7.2; \( T = 120 \) or \( T = 180 \) min

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Spike ( (\log_{10} \text{TCID}_{50}) )</th>
<th>Vol</th>
<th>pH</th>
<th>Temp ( (^\circ C) )</th>
<th>Time ( (\text{min}) )</th>
<th>Surviving PEDV</th>
<th>Measured D value ( (\text{sec or min}) )</th>
<th>Expected D value</th>
<th>Measured mean</th>
<th>UCL95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>5.80</td>
<td>1 ml</td>
<td>7.2</td>
<td>37</td>
<td>120</td>
<td>3</td>
<td>23.2 min</td>
<td>30.5 [55.8] min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Flask assay at *in vivo* conditions: \( D_{37^\circ C, \text{pH} 7.2} = 23.2 \) min
Even in absence of anti-PEDV IgG, infective PEDV is inactivated in porcine plasma at *in vivo* conditions
(normal pig body temperature = 37.8-40°C)
Acute phase serum has been reported to contain PEDV Does PEDV remain infectious in plasma at *in vivo* conditions?

- Spike-inactivation assay in tissue culture flasks
- HAT determinants: $H = 37^\circ C$; $A = pH 7.2$; $T = 120$ or $T = 180$ min

### Obtainment of PEDV sterility at *in vivo* conditions

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Spike ($\log_{10}$ TCID$_{50}$)</th>
<th>Vol</th>
<th>pH</th>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>Surviving PEDV # PFU</th>
<th>Sterility obtained? (yes/no)</th>
<th>Expected time to sterility (mean, [UCL95])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>5.80</td>
<td>1 ml</td>
<td>7.2</td>
<td>37</td>
<td>180</td>
<td>0</td>
<td>YES</td>
<td>177 [324] min</td>
</tr>
</tbody>
</table>

**Flask assay at *in vivo* conditions:** $D_{37^\circ C, pH 7.2} = 23.2$ min

Even in absence of anti-PEDV IgG, infective PEDV is inactivated in porcine plasma at *in vivo* conditions (normal pig body temperature = 37.8-40°C)

*In vivo* conditions result in PEDV sterility of a 5.8 $\log_{10}$ TCID$_{50}$ per ml spike within 180 min
1. Porcine plasma at body temperature shows anti-PEDV activity
   □ but refrigerated plasma does not
   □ tailing effect when inactivation at 37 - 40 °C
   □ no tailing effect at 48 °C / important dilution effect (further trials)

   PEDV inactivation in seronegative porcine plasma at 37°C
   ➡ ± 0.44 million infectious particles /ml plasma (5.8 log TCID$_{50}$/ml)
   ➡ reduced to 3 (0) infectious particles in 2 h (3 h)
   ➡ viraemic PEDV would not remain infectious for prolonged times

2. Inactivation of PEDV is facilitated in plasma
   non-specific anti-viral activity of plasma increases safety of SDPP

3. Viral inactivation assays should take matrix-effects into account

4. Biosafety measures: needed at all points of the distribution chain
PEDV syncytium

- cell nuclei
- PEDV virions

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