Inactivation of porcine epidemic diarrhea virus (PEDV) by heat-alkalinity-time (HAT) pasteurization

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Porcine Epidemic Diarrhea Virus (PEDV)

- swine alfa-corona virus
- faecal-oral transmission
- highly enteropathogenic
  - villus atrophy
  - acute, watery diarrhea

- Clinical outcome depends on age at infection (virus strain, lactogenic immunity, co-infections, ...)
  - neonatal & suckling: up to 100% mortality
  - weaners to adults: (usually mild) self-limiting
  - gestating sows: reproductive performance ↓
Transmission of PEDV by feed

- **US Field cases** with **entirely vegetal diets** (Dee et al., 2014)
  - Feed-borne (corn, SBM and Vit & Min diets)

- **Ontario cases** (USA → Eastern Canada)
  - Epidemiology: feed-borne transmission
  - **Infectious PEDV in SDPP** sampled at a feedmill (Pasick et al., 2014)
  - **Non-infectious PEDV in SDPP** at the production plant (US FDA, 2014)

- PEDV is sensitive to spray-drying and dessication
- SDPP was produced 10 wks prior to Ontario cases

**Ingredient specific sensitivity** (Dee et al., 2015)
- Outdoor storage (-25 to +20 °C): \(10^{4.2} \text{TIC}_{50}/g\)
- Inactivation: SDPP < 7 d vs SBM < 210 d (>180 d)
Extremely high Fecal shedding

sow milk (RNA)

lung macroph. (replication)

nasal/oral secretions (RNA)

acute phase serum (RNA)

equipment

clothing

lorries

feed

Adequate biosafety measures should also be in place for feed and its ingredients
## Present Trial

### Sensitivity of PEDV to HAT-pasteurisation

<table>
<thead>
<tr>
<th>Heat</th>
<th>Alkalinity</th>
<th>Time</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product temp ([T_{IN}; T_{OUT}])</td>
<td>Product pH ([pH_{IN}; pH_{OUT}])</td>
<td>Holding time ([\text{flow}])</td>
<td>product ([\text{plasma}])</td>
</tr>
</tbody>
</table>

- **Completely characterised process**
- Enables
- Laboratory replication of industrial conditions

**Determination of sensitivity of PEDV**

\[D\text{-value} = \text{time needed to inactivate 90\% of initial infectivity (1 log)}\]
Materials & Methods: Spike Inactivation Assays

1. **Test-samples:** matrix + virus (9:1-ratio)
   - Matrix: ● Minimum Essential Medium (MEM)
     ● porcine plasma

   sterile filtered heat inactivated seronegative for PEDV

   LDL↑
### Materials & Methods: Spike Inactivation Assays

**Inactivation (treatment)**

**Condition**
- pH 7.2, 9.2 or 10.2
- Temperature 4, 40, 44 or 48°C

**Duration**
- 8 time-points up to 120 min
  - (0.25, 1, 3, 5, 10, 30, 60 or 120 min)

**Matrix**
- MEM or porcine plasma
Materials & Methods: Spike Inactivation Assays

Test Matrix 90% 10% PEDV Spike Test Sample INACTIVATION ASSAY

Virus titration
- residual infectivity
- whole test-sample
- end-point dilution assay
- 96-well plates

Survival Curve
- Titer (log_{10})
- Time
- spike
- D-value

Confirmation assays
- D-value, PEDV sterility
Results and Discussion: Survival curves

**Spike-Inactivation Assay**

- **Surviving PEDv titer (log<sub>10</sub> TCID<sub>50</sub>/ml)**
- **Incubation time (min)**
- **4°C, 40°C, 44°C, 48°C**
- **LDL below LDL**

**MEM pH 7.2**

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 15 30 45 60 75 90 105 120</td>
</tr>
<tr>
<td>Surviving PEDv titer (log&lt;sub&gt;10&lt;/sub&gt; TCID&lt;sub&gt;50&lt;/sub&gt;/ml)</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>

**Plasma pH 7.2**

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 15 30 45 60 75 90 105 120</td>
</tr>
<tr>
<td>Surviving PEDv titer (log&lt;sub&gt;10&lt;/sub&gt; TCID&lt;sub&gt;50&lt;/sub&gt;/ml)</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>

**Sensitivity of PEDV**

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

**Results and Discussion**: Survival curves

- **Spike-Inactivation Assay**

  - **4 °C**
  - **Surviving PEDv titer (log<sub>10</sub> TCID<sub>50</sub>/ml) 6**
  - **Incubation time (min)** 0 15 30 45 60 75 90 105 120

**MEM pH 7.2**

- **Surviving PEDv titer (log<sub>10</sub> TCID<sub>50</sub>/ml) 6**

**Plasma pH 7.2**

- **Surviving PEDv titer (log<sub>10</sub> TCID<sub>50</sub>/ml) 6**
Results and Discussion: Survival curves

### Spike-Inactivation Assay

**Surviving PEDv titer**

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>4°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**MEM pH 7.2**

**Plasma pH 7.2**

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### 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)
Results and Discussion: Survival curves

Spike-Inactivation Assay

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

MEM pH 7.2

- 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)

Plasma pH 7.2

- Temp ↑ ⇒ Sensitivity ↑
- Sensitivity_{plasma} ↑↑
- Tailing_{plasma} ↓
Results and Discussion: Survival curves

**Spike-Inactivation Assay**

- **Surviving PEDv titer** (log$_{10}$ TCID$_{50}$/ml)
- **Incubation time (min)**
- **Temperature (°C)**: 4°C, 40°C, 44°C, 48°C

**MEM pH 10.2**

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

**Plasma pH 10.2**

- Sensitive to 40°C in plasma

**Sensitivity of PEDV**

- Temp ↑ ≡ Sensitivity ↑
- Sensitivity$_{\text{plasma}}$ ↑↑
- Tailing$_{\text{plasma}}$ ↓
- pH 10.2 ≡ Not sensitive
- pH ↑ ≡ Sensitivity$_{\text{Temp}}$ ↑↑

**D$_{48^\circ C, \text{pH} 10.2}$** = 35 s in plasma (UCL$_{95}$) 114 s in MEM
**Results and Discussion: Confirmation assays**

**Spike-inactivation assay in tissue culture flasks**

HAT determinants: H = 48°C; A = pH 10.2; $T = 2.5$ min and $T = 5$ min

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### Confirmation of D value

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Spike $(\log_{10} \text{TCID}_{50})$</th>
<th>Vol</th>
<th>pH</th>
<th>Temp $(°C)$</th>
<th>Time (min)</th>
<th>Surviving PEDV</th>
<th>Measured D value (sec or min)</th>
<th>Expected D value mean [UCL95]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>7.37</td>
<td>10 ml</td>
<td>10.2</td>
<td>48</td>
<td>2.5</td>
<td>4</td>
<td>23 sec</td>
<td>20 [35] sec</td>
</tr>
<tr>
<td>Plasma</td>
<td>7.65</td>
<td>1 ml</td>
<td>10.2</td>
<td>48</td>
<td>2.5</td>
<td>25</td>
<td>25 sec</td>
<td>20 [35] sec</td>
</tr>
</tbody>
</table>

- Measured D value (23-25 sec) $< UCL_{95}$ of expected D value (35 sec)
- D value is not dependent on test volume or magnitude of virus spike
- Similar results in confirmation assays of other HAT determinants

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**In other words**

start (spike) **over 31 million** infectious particles is reduced to **25 infectious particles** in **2.5 min HAT-pasteurisation**
Results and Discussion: Confirmation assays

Spike-inactivation assay in tissue culture flasks
HAT determinants: H = 48°C; A = pH 10.2; \( T = 2.5 \text{ min} \) and \( T = 5 \text{ min} \)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Spike</th>
<th>Vol</th>
<th>pH</th>
<th>Temp</th>
<th>Time</th>
<th>Surviving PEDV</th>
<th>Sterility obtained?</th>
<th>Expected time to sterility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>7.37</td>
<td>10 ml</td>
<td>10.2</td>
<td>48</td>
<td>5</td>
<td>0</td>
<td>YES</td>
<td>2.4 [4.2] min</td>
</tr>
<tr>
<td>Plasma</td>
<td>7.65</td>
<td>1 ml</td>
<td>10.2</td>
<td>48</td>
<td>5</td>
<td>0</td>
<td>YES</td>
<td>2.5 [4.4] min</td>
</tr>
</tbody>
</table>

HAT-treatment at 48°C and pH 10.2 during 5 min resulted in PEDV sterility of plasma spiked to 7.65 log\(_{10}\) TCID\(_{50}\) per ml

In other words

start (spike) over 31 million infectious particles

is reduced to 25 infectious particles in 2.5 min HAT-pasteurisation

and is reduced to 0 infectious particles in 5 min HAT-pasteurisation
Spike-inactivation assay in tissue culture flasks

HAT determinants: \( H = 48^\circ C; \ A = \text{pH} \ 10.2; \ T = 2.5 \text{ min} \) and \( T = 5 \text{ min} \)

<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</th>
<th>Sterility obtained?</th>
<th>Expected time to sterility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>7.37</td>
<td>10 ml</td>
<td>10.2</td>
<td>48</td>
<td>5</td>
<td>0</td>
<td>YES</td>
<td>2.4 [4.2] min</td>
</tr>
<tr>
<td>Plasma</td>
<td>7.65</td>
<td>1 ml</td>
<td>10.2</td>
<td>48</td>
<td>5</td>
<td>0</td>
<td>YES</td>
<td>2.5 [4.4] min</td>
</tr>
</tbody>
</table>

Additional assay

<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</th>
<th>Sterility obtained?</th>
<th>Expected time to sterility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>5.80</td>
<td>1 ml</td>
<td>10.2</td>
<td>48</td>
<td>4</td>
<td>0</td>
<td>YES</td>
<td>1.9 [3.4] min</td>
</tr>
<tr>
<td>Plasma</td>
<td>5.80</td>
<td>1 ml</td>
<td>10.2</td>
<td>48</td>
<td>3</td>
<td>0</td>
<td>YES</td>
<td>1.9 [3.4] min</td>
</tr>
</tbody>
</table>

Time to sterility occurred within the expected time
**Summary**

**Spike-Inactivation Assay - survival curves**

### Results:
- **MEM**
  - **pH 7.2**: 
    - $D_{48^\circ C} = 81$ sec ($UCL_{95} = 114$ sec)
  - **pH 10.2**: 
    - $D_{48^\circ C} = 20$ sec ($UCL_{95} = 35$ sec)

- **Plasma**
  - **pH 7.2**: 
    - 
  - **pH 10.2**: 
    - 

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**Note:**
- The survival curves show the decay of PEDV titer over time at different temperatures and pH levels.
- The graphs compare survival curves for 4°C, 40°C, 44°C, and 48°C conditions, with LDL and below LDL thresholds indicated.
Conclusions

1. Inactivation of PEDV is facilitated in plasma

2. Inactivation assays should take matrix into account

3. HAT-pasteurisation at $H_{48^\circ C}A_{pH10.2}T_{10\text{min}}$
   \[ \Rightarrow \text{Inactivates 17.4 log}_{10} TCID_{50} / \text{ml plasma} \]
   (Quist-Rybachuk et al., submitted 2015)

4. PEDV is highly sensitive to HAT-pasteurisation, a redundant additional safety-step?

Standard processing of SDPP

- **Spray-Drying** (Gerber et al., 2014; Pujols and segalés, 2014)
  \[ \Rightarrow \text{Inactivates min 4.2 log}_{10} TCID_{50} / \text{ml plasma} \]

- **Storage at low Aw** (Pujols and segalés, 2014)
  \[ \Rightarrow \text{Inactivates min 2.8 log}_{10} TCID_{50}/g \text{ SDBP} \]
  in 3 w-4°C, 2 w at 12°C, 1 wk at 21°C
Further Take Home Messages

1. ALL ingredient types can be vectors of PEDV (vegetal (Dee et al., 2014), animal (Pasick et al., 2014), micro-ingredients)
   
   Risks of feedborne transmission of PEDV are NOT limited to animal-based ingredients.

2. Inactivation of event. infectious agents is anticipated in the processing of animal-based ingredients.
   
   Processing implies a safety-guarantee, not a safety-risk.

3. Securing feed-safety necessitates proper biosecurity at all points of the distribution chain.

4. PCR-tests do not inform on virus infectivity, they inform on standard necessity of processing.
Acknowledgments

PEDV syncytium

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