# Methods for inactivating PEDV in hog trailers

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## Overview

- Summary of four studies evaluating trailer decontamination measures with and without washing for ability to inactivate or eliminate PEDV.
  - Not everyone can wash all livestock trailers
  - Washing does not remove 100% of organic material
- Started with PEDV-positive <u>feces</u>
- Evaluated the treatments in conditions similar to a trailer environment
- Recollected the feces
- Evaluated infectivity via pig bioassay
  - PCR+ does not indicate infectious virus is present



## Study 1: Evaluation of Time & Temperature

- Object was to evaluate a range of time and temperature combinations that might represent Thermo-Assisted Drying and Decontamination (TADD) systems or no use of TADD
  - 71°C (160°F), 10 minutes
  - 63°C (145°F), 10 minutes
  - 54°C (130°F), 10 minutes
  - 38°C (100°F), 12 hours
  - 20°C (68°F), 24 hours
  - 20°C (68°F), 7 days





# Study 2: Evaluation of Stalosan<sup>®</sup> F disinfectant powder

- Object was to evaluate a commercially-available, environmentally-applied disinfectant that is efficacious in the presence of feces and at freezing temperatures
  - Product is a fine, red powder
  - Applied to animal environments
  - Causes lysis of cell membranes
- Evaluated efficacy of Stalosan<sup>®</sup> F Disinfectant after 1 hour of contact time
- Three groups
  - Negative Control (n=8)
  - Positive control (n=8)
  - Stalosan<sup>®</sup> F treatment group (n=8)





# Study 3: Evaluation of Accel<sup>®</sup> liquid foaming disinfectant

- Object was to evaluate the efficacy of a commerciallyavailable disinfectant in the presence of fecal material.
- Accel<sup>®</sup> (accelerated hydrogen peroxide) is supposed to have longer residual activity due to enhanced contact.
- Four treatment groups:
  - Light feces (5mL) @ 1:32 concentration for 30m
  - Heavy feces (10mL) @ 1:32 concentration for 30m
  - Light feces (5mL) @ 1:16 concentration for 30m
  - Heavy feces (10mL) @ 1:16 concentration for 30m
  - Transmission Control group





Study 4: Evaluation of various combinations of TADD following a wash and Synergize®

- Object was to evaluate the efficacy of various TADD configurations following a wash and disinfection with Synergize<sup>®</sup> disinfectant @ 1:256.
  - Wash, 10m Synergize<sup>®</sup>, 68°C for 10m
  - Wash, 10m Synergize<sup>®</sup>, 66°C for 10m
  - Wash, 10m Synergize<sup>®</sup>, 60°C for 20m
  - Wash, 10m Synergize<sup>®</sup>, 49°C for 20m
  - Wash, 10m Synergize<sup>®</sup>, 20°C for 12h
  - Wash, 60m Synergize®
  - Wash, 10m Synergize<sup>®</sup>





## Source of virus and feces

- PEDV-positive feces was obtained from 3-week old pigs that were experimentally infected with PEDV
  - Feces from multiple pigs was collected and pooled
  - Pooled feces was analyzed via PCR to ensure presence of virus
  - Frozen at -70C until time of challenge
  - Feces was retested following thawing on day of challenge
- PEDV-negative feces was obtained from the negative control pigs from this same study
  - Negative feces was handled, stored, and evaluated for virus similarly



## Analysis of Results

- Descriptive statistics
  - Outcomes would be described as a percentage or proportion of bioassays in a group that became PEDV-positive
  - Mean PCR CT values of tray swabs before and after treatments
- Fisher's Exact test for comparison of bioassays between groups



## **Tray Preparation**

- Four (4) trays per treatment group
  - Each tray was the experimental unit
  - Eight (8) trays per group in Study 2
- 5 or 10 mL of feces was placed on each tray depending upon the study / treatment group





## **Tray Preparation**

- Feces was spread in a thin layer to represent a trailer floor that had been scraped or swept, but not washed
- A sample of the feces on the tray was taken for PEDV PCR prior to experimental treatment





## **Timed Thermal Application**

 Timed thermal application for the high temperature groups was performed using an incubator capable of reaching and holding temperatures of 75°C





### Room temperature treatment groups

• Replicates from the low temperature groups were placed in an insulated cooler indoors.





## **Collection of Treated Feces**

- Feces was resuspended with 10 mL of sterile saline and drawn up for inoculation of the swine bioassays
- A second sample of the feces was collected following re-suspension and tested via PEDV PCR





## Swine Bioassay

- A 14 Fr rubber catheter was used to pass the challenge material directly into each pig's stomach
- Each pig corresponded to an individual tray





## Individual Housing



1 tub per room, 1 treatment group per tub.

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## **Transmission Control Group**



#### Three negative pigs, one positive pig



## **Clean Sample Collection**

- Pigs were monitored daily for signs of PEDV infection
- Rectal swabs were collected on day 3 and day 7 postchallenge to determine bioassay status





## Results of tray swabs – Time & Temperature

Table 1: PCR results for swabs of trays before and after treatment was applied in Study 1.

Treatment Group	Pre-Treatment RT-PCR: mean CT (± SD)	Post-Treatment RT-PCR: mean CT (± SD)
Negative Control	>40	N/A
Positive Control	15.22 (0.73)	N/A
71C-10M	13.40 (0.30)	24.10 (0.76)
63C-10M	13.16 (0.48)	21.56 (0.71)
54C-10M	13.41 (0.32)	20.83 (0.53)
38C-12H	13.28 (1.21)	20.19 (0.09)
20C-24H	14.45 (0.57)	15.07 (0.18)
20C-7D	12.94 (0.55)	17.71 (0.41)



## Bioassay Results – Time & Temperature

Table 2. Description of treatment groups and bioassay outcomes for Study 1.

Treatment Group	Temperature & Time applied	Percentage of PEDV positives (out of 4)
Negative Control	No heat, no time	0% (0/4) <sup>a</sup>
Positive Control	No heat, no time	100% (4/4) <sup>b</sup>
71C-10M	71°C (160°F), 10 minutes	0% (0/4) <sup>a</sup>
63C-10M	63°C (145°F), 10 minutes	25% (1/4) <sup>a, b</sup>
54C-10M	54°C (130°F), 10 minutes	25% (1/4) <sup>a, b</sup>
38C-12H	38°C (100°F), 12 hours	50% (2/4) <sup>a, b</sup>
20C-24H	20°C (68°F), 24 hours	25% (1/4) <sup>a, b</sup>
20C-7D	20°C (68°F), 7 days	0% (0/4) <sup>a</sup>

Groups with different superscripts indicate statistically significant differences (P ≤0.05)



## Results - Stalosan® F treatment

Table 3. PCR results for swabs of trays before and after treatment was applied in Study 2.

Treatment Group	Pre-Treatment RT-PCR: mean CT (± SD)	Post-Treatment RT-PCR: mean CT (± SD)
Negative Control	>40	N/A
Positive Control	14.4 (0.67)	15.35 (0.73)
Stalosan <sup>®</sup> F Treatment	15.2 (0.42)	18.4 (1.51)

Table 4. Description of treatment groups and bioassay outcomes for Study 2.

Treatment Group	Description of Treatment	Percentage of PEDV positives (out of 8)
Negative Control	No Stalosan® F contact	0% (0/8) ª
Positive Control	No Stalosan <sup>®</sup> F contact	100% (8/8) <sup>b</sup>
Stalosan <sup>®</sup> F Treatment	One hour of Stalosan® F contact time	100% (8/8) <sup>b</sup>
Groups with different superscrip	ots indicate statistically significant differences	(P ≤0.05)



## Results of tray swabs – Accel<sup>®</sup> disinfectant

Table 5: PCR results for swabs of trays before and after treatment was applied in Study 3.

Treatment Group	Pre-Treatment RT-PCR: mean CT (± SD)	Post-Treatment RT-PCR: mean CT (± SD)
Negative Control	>35	N/A
Positive Control	14.15 (0.41)	N/A
5mL-1:16	13.43 (0.57)	>35
10mL-1:16	13.83 (0.46)	>35
5mL-1:32	14.30 (0.22)	>35, (one tray @ 32.2)
10mL-1:32	13.83 (0.27)	>35, (one tray @ 34.3)
Transmission Control	>35 (3 negative animals), 14.4	NA



### Bioassay Results – Accel<sup>®</sup> disinfectant

Treatment group	Description of treatment	Percentage of PEDV positives (out of 4
Negative Control	No treatment, pigs received a gavage of PEDV-negative feces	0% (0/3) ª
Positive Control	No treatment, pigs received a gavage of PEDV-positive feces	100% (4/4) <sup>b</sup>
5mL-1:16	A 1:16 concentration of Accel <sup>®</sup> disinfectant was applied to 5 ml of PEDV- positive feces for 30 minutes	0% (0/4) ª
10mL-1:16	A 1:16 concentration of Accel <sup>®</sup> disinfectant was applied to 10 ml of PEDV- positive feces for 30 minutes	0% (0/4) ª
5mL-1:32	A 1:32 concentration of Accel <sup>®</sup> disinfectant was applied to 5 ml of PEDV- positive feces for 30 minutes	0% (0/4) ª
10mL-1:32	A 1:32 concentration of Accel <sup>®</sup> disinfectant was applied to 10 ml of PEDV- positive feces for 30 minutes	0% (0/4) °
ransmission Control	1/4 pigs in the group was gavaged with PEDV-positive feces, 3/4 were gavaged with PEDV-negative feces	25% (1/4)



## Results of tray swabs – Wash, Synergize<sup>®</sup>, & TADD

PCR results for swabs of trays before and after treatment was applied in Study 4.

Treatment Group	Pre-Treatment RT-PCR: mean CT (± SD)	Post-Treatment RT-PCR: mean CT (± SD)
Negative Control	>35	N/A
Positive Control	20.93 (0.35)	N/A
WD-68C-10	21.85 (0.19)	34.43 (0.32), >35*
WD-66C-10	21.08 (0.69)	33.6 (1.41), >35*
WD-60C-20	21.33 (0.50)	32.83 (0.45), >35*
WD-49C-20	21.55 (0.40)	33.93 (0.81), >35*
WD-20C-12	28.48 (0.74)	>35
WD60	20.68 (0.39)	31.65 (2.83)
WD10	20.43 (0.25	31.6 (0.67)

\* One or more trays in this group had a CT >35, these trays were not included in calculation of the mean CT value.



## Bioassay Results – Wash, Synergize<sup>®</sup>, & TADD

Treatment group	Description of treatment	Percentage of PEDV positives (out of 4
Negative Control	No treatment, pigs received a gavage of PEDV-negative feces	0% (0/4) ª
Positive Control	No treatment, pigs received a gavage of PEDV-positive feces	100% (4/4) <sup>b</sup>
WD-68C-10	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, heated to 68° C (155° F) in an incubator and held at this temperature for 10 minutes	0% (0/4) ª
WD-66C-10	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, heated to 66° C (150° F) in an incubator and held at this temperature for 10 minutes	0% (0/4) ª
WD-60C-20	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, heated to 60° C (140° F) in an incubator and held at this temperature for 20 minutes	0% (0/4) ª
WD-49C-20	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, heated to 49° C (120° F) in an incubator and held at this temperature for 20 minutes	0% (0/4) ª
WD-20C-12	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, left at 20° C (room temperature) for 12 hours	0% (0/4) ª
WD60	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 60 minutes	0% (0/4) ª
WD10	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes	0% (0/4) ª



## Conclusions

- Only two combinations from Study 1 were 100% successful:
  - $-71^{\circ}$  C (160° F) for 10 minutes
    - If you can't wash, heat trailers @ 160° F to inactivate PEDV
  - 20°C (68° F) (room temperature) for 1 week
    - This is not practical for many farms or transporters
    - These samples dried out completely
    - Humidity / moisture would likely play a role in survival



## Conclusions

- Stalosan<sup>®</sup> F is ineffective at inactivating PEDV from contaminated hog trailers
- Accel<sup>®</sup> (accelerated hydrogen peroxide) appears to effective at inactivating PEDV in presence of feces
  - This may have applications in situations where washing isn't possible or practical



## Conclusions

- A wider range of TADD configurations were found to be effective following wash and disinfection with Synergize<sup>®</sup>
  - 20°C for 12h to 68°C for 10m
  - Synergize<sup>®</sup> and wash alone were also effective
  - Underscores the importance and utility of wash and disinfection steps in trailer sanitation
- Environmental swabs could not differentiate infectious from non-infectious PEDV
  - Implications for environmental sampling



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