Porcine Deltacoronavirus Fulfilling Koch's Postulates

Dick Hesse

K-State Swine Day 20 November 2014





Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 20, No. 9, September 2014 Porcine Coronavirus HKU15 Detected in 9 US States, 2014

Forcine Colonavirus Tiko 13 Detected in 9 03 States, 2014

Leyi Wang, Beverly Byrum and Yan Zhang, Ohio Department of Agriculture, Reynoldsburg, Ohio

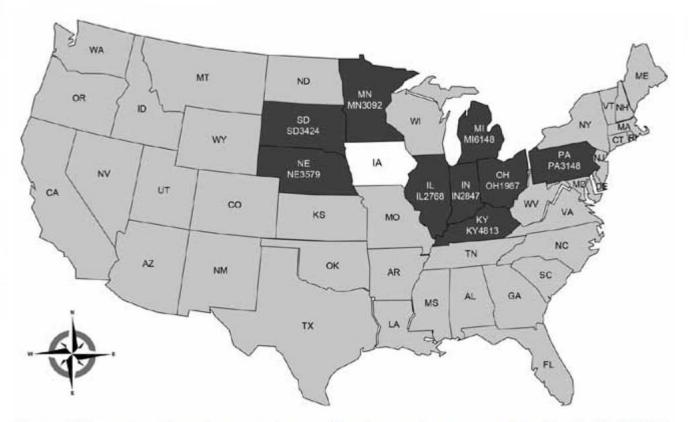
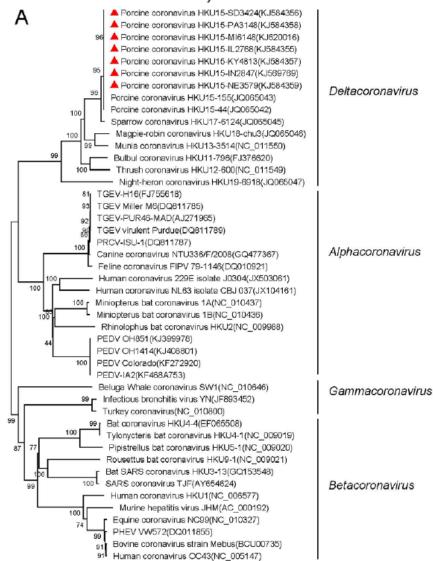


Figure. US states with swine samples positive for porcine coronavirus (PorCoV) HKU15, between February and April 2014. A total of 435 samples from 10 states were selected to be tested for the presence of PorCoV. Of those samples, 109 (25%) from 9 states (dark gray) were positive for PorCoV HKU15. Another recent article reported the presence of PorCoV HKU15 in Iowa (white) (3). Strain names are indicated below state abbreviations.



Porcine Coronavirus HKU15 Detected in 9 US States, 2014









United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Swine Enteric Coronavirus Disease Testing Summary Report

September 3, 2014

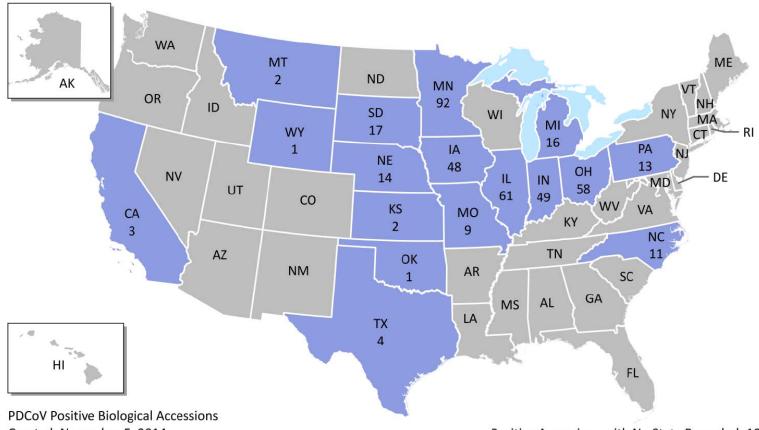
This report summarizes NAHLN laboratory testing for Porcine Epidemic Diarrhea Virus (PEDV) and Porcine Delta Coronavirus (PDCoV) through 8/30/2014. The report was prepared by USDA APHIS VS NVSL National Animal Health Laboratory Network (NAHLN) and VS STAS Center for Epidemiology and Animal Health (CEAH). Critical notes about the data used in this report are provided at the end of this report.





PDCoV Positive 5 Nov 14

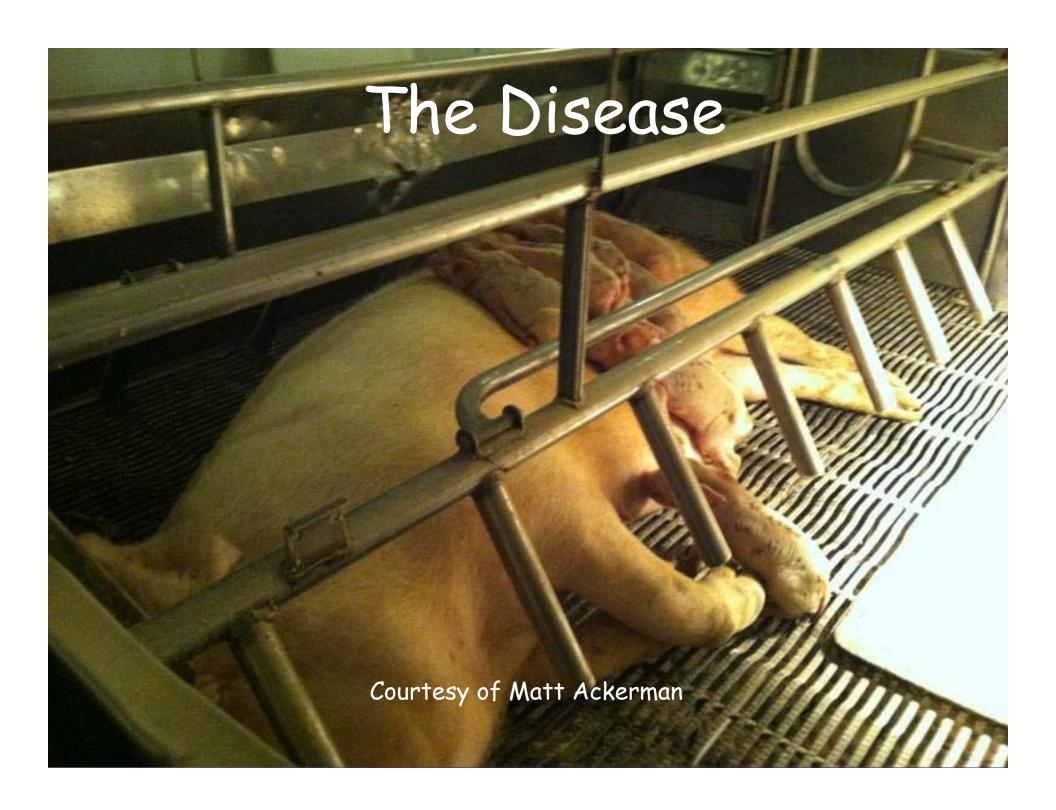
PDCoV Positive Biological Accessions



Created: November 5, 2014

Positive Accessions with No State Recorded: 10











Determination of the sites of tissue localization, routes of viral shedding, duration of virus carriage, kinetics of antibody response, and potential of aerosol transmission of

Porcine Deltacoronavirus (PDCoV) following inoculation of nursing pigs and their dams.

Koch's Postulates Fulfilled Preliminary Results





PDCoV Team Members

KSVDL--Dick Hesse, Barb Breazeale, Alex Fuller, Joe Anderson, Erin Schirtzinger, Elizabeth Poulsen, Jianfa Bai, Gary Anderson.

UNL--Sarah Vitosh-Sillman, Clayton Kelling, Bruce Brodersen, John Loy, Alan Doster, Chris Topliff.

SDSU-Eric Nelson.

Thanks to the National Pork Board for providing Funding





Housed in five BSL2 isolation rooms at the Life Science Annex at the University of Nebraska.

10 PEDV/PDCoV naïve Bred Sows obtained from a high health commercial source.

Group	Treatment	# of Animals
A	PDCoV oronasal inoculated	~48 (6 litters)
В	None—Contact Control	12 (2 from 6 litters)
С	None—aerosol transmission controls	~20 (2 litters)
D	Negative Controls	~20 (2 litters)
A-Dams	PDCoV oronasal inoculated	6
C-Dams	None—aerosol transmission controls	2
D-Dams	Negative Controls	2



Challenge: Pool of fecal derived virus that was passaged in 2-4 day old pigs.

Kindly provided by Eric Nelson

The inocula had a PDCoV nucleic acid "CT titer" of 22 in a real-time PCR assay.

Nursing pigs and their Dams challenged when pigs were ~2 days of age via intranasal and oral routes with 5 ml of inocula per route.



Day Post Challenge	Blood	Nasal Swab	Fecal Swab	Sequential Sacrifice Group # Sac	Oral Fluid Rope Sample
-2	Sows only	Sows only	Sows only		
0	A,B,C,D	A,B,C,D	A,B,C,D		
1	A-3 + sac	A,B,C,D	A,B,C,D	A-3 D-1	
2	A-3 + sac	A,B,C,D	A,B,C,D	A-3 D-1	
3	A-3 + sac	A,B,C,D	A,B,C,D	A-3 D-1	
4	A-3 + sac	A,B,C,D	A,B,C,D	A-3 D-1	
5	A-3 + sac	A,B,C,D	A,B,C,D	A-3 D-1	
6	A-3 + sac	A,B,C,D	A,B,C,D	A-3 D-1	
7	A,B,C,D	A,B,C,D	A,B,C,D	A-3 D-1	
10	A,B,C	A,B,C,D	A,B,C,D	A-3 D-1	4.0.4.6
14	A,B,C,D	A,B,C,D	A,B,C,D	A-3 D-1	A,B,C,D
21	A,B,C,D	A,B,C,D	A,B,C,D	A-3 D-1	A,B,C,D
28	A,B,C,D	A,B,C,D	A,B,C,D	A-3 D-1	A,B,C,D
35	A,B,C,D	A,B,C,D	A,B,C,D	A-3 D-1	A,B,C,D
42	A,B,C,D	A,B,C,D	A,B,C,D	A-12, B-12, C,-12,D-8	A,B,C,D
Research and Extension					Lije

Tissues for Histopathology, qPCR and IHC

turbinates, tonsils, esophagus, stomach, duodenum, jejunum (two segments), ileum, cecum, spiral colon, descending colon, trachea, lungs (representative samples from cranial, middle and caudal lobes), liver, spleen, kidney, thymus and submandibular, tracheobronchial, mesenteric and inguinal lymph nodes.



Clinical Observations Following Challenge

- Sows developed soft feces on day 2 postinoculation and diarrhea on day 3 postinoculation.
- All sows were clinically normal after day 8 postinoculation.
- Aerosol contact sows developed soft to diarrheic feces on day 5 of the study and returned to normal after day 8.
- Non-infected control sow remained clinically normal throughout the study.

04Jun14 PID 2

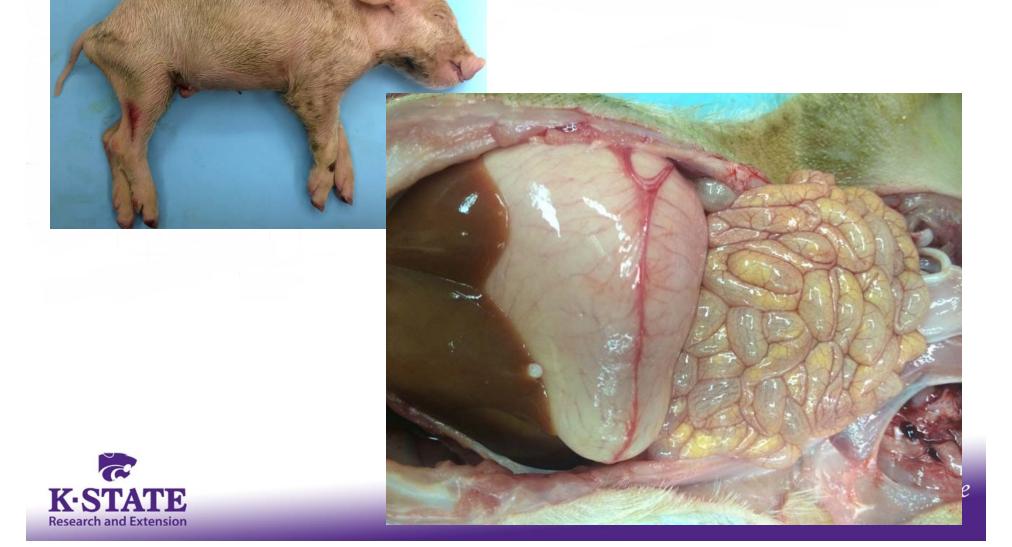


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05Jun14 Pig 4-5 PID 3



Clinical Observations Following Challenge

- Inoculated piglets developed soft to profuse diarrheic feces on day 2 post-inoculation, as did the contact controls.
- Morbidity was 100%.
- Mortality was variable among the litters, but was especially high in two of the six inoculated litters.
- All piglets from those two litters were either euthanized due to severe dehydration or had died by day 8 post-inoculation.

Clinical Observations Following Challenge

- Aerosol contact litters developed diarrhea on days 3 and 4.
- All surviving piglets had returned to normal by day 12.
- Non-infected negative control piglets remained clinically normal



16Jun14 PID 14

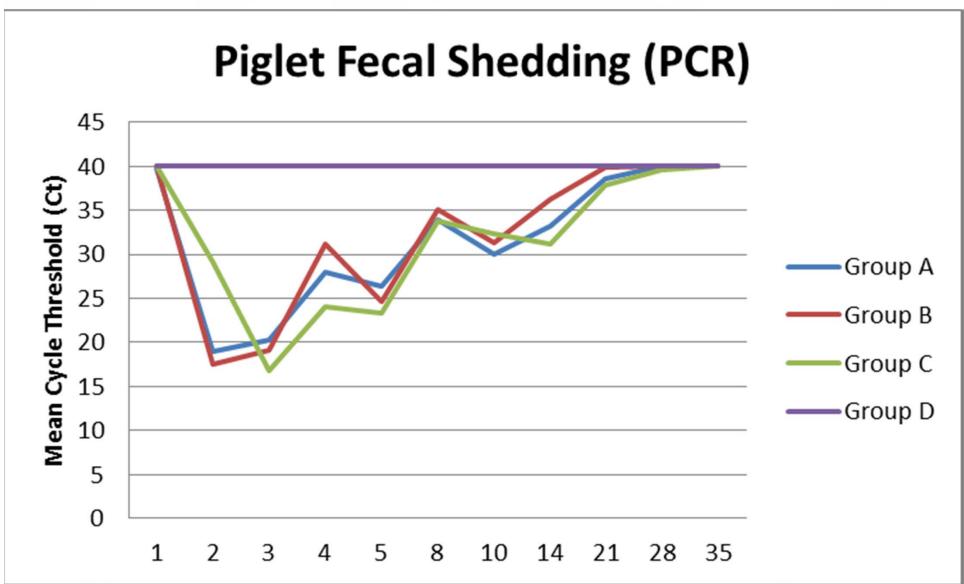


Live Animal Fecal Swabs PCR

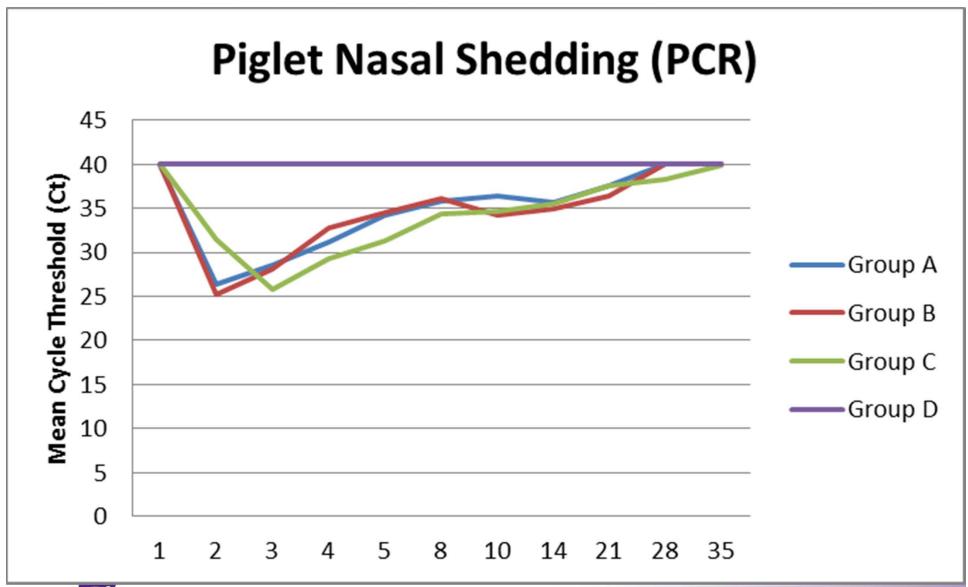
_	Dig ID	Noc Day	Day 1		Day 2	Day 4	Day E	Day 9	Day 10	Day 14	Day 21	Day 20	Day 25	Day 42
-	Pig ID	Nec Day		Day 2	Day 3	Day 4	Day 5	Day 8	Day 10	Day 14	Day 21	Day 28	Day 35	Day 42
-	4-8	1.1	40.0											
ŀ	1-4	1.3	40.0											
F	5-1	1.3	40.0											
ŀ	6-8	1.3	38.5											
L	4-6	2.2	40.0	30.3										
Ļ	1-1	2.3	40.0	18.9										
_	4-2	2.3	40.0	15.1										
	6-7	2.3	40.0	19.2										
	1-2	3.1	40.0	19.1	23.4									
L	6-3	3.1	40.0	18.9										
L	4-5	3.2	40.0	17.5	18.2									
	6-4	3.2	40.0	21.8	16.5									
L	1-7	3.3	40.0	16.6	21.1									
L	3-4	3.3	40.0	18.9	17.5									
L	4-10	3.3	40.0	17.5	18.4									
L	1-3	4.3	39.1	19.1	19.2	29.5								
L	1-8	4.3	40.0	20.9	20.0	17.3								
L	2-6	4.3	37.7	17.8	16.2	20.8								
	3-7	4.3	37.7	15.4	18.1	33.0								
	5-3	4.3	40.0	15.4	28.4	32.2								
L	6-2	5.1	40.0	17.2	19.1	36.7								
	6-9	5.3	38.5	16.2	21.5	26.6								
	1-5	6.3	40.0	17.8	20.1	32.2	20.1							
	3-3	6.3	40.0	17.5	19.7	19.3	22.6							
	3-5	7.1	40.0	15.1	16.0	19.1	17.0							
	3-6	8.1	40.0	25.6	17.0	21.2	16.5							
	1-6	8.3	40.0	17.6	21.7	23.3	31.6	40.0						
	6-5	8.3	40.0	30.6	18.1	27.6	16.2							
	6-6	8.3	40.0	14.3		36.1	19.5	22.1						
	2-3	9.1	40.0	17.0	20.4	23.1	22.7	37.4						
	3-8	9.1	40.0	25.0	20.4	26.2	18.1	39.1						
	2-7	10.3	40.0	30.3	22.2	30.2	31.3	33.4	35.8					
	3-1	10.3	40.0	16.4	19.1	19.4	31.4	33.2	31.7					
	4-1	14.3	40.0	19.6	22.7	37.2	24.1	30.1	23.5	35.3				
	5-7	14.3	40.0	17.2	18.6	31.8	31.0	36.5	23.7	26.0				
	2-9	21.3	40.0	26.9	20.4	33.2	25.1	25.6	26.0	25.1	39.3			
	5-4	21.3	40.0	12.6	22.9	22.2	22.2	30.5	22.4	32.4	40.0			
	5-5	28.3	40.0	16.0	25.3	18.3	20.7	40.0	34.4	35.8	40.0	40.0		
	2-8	35.3	38.7	20.3	17.8	38.3	30.1	32.2	35.5	40.0	40.0	40.0	40.0	
	5-2	35.3	40.0	15.5	20.6	40.0	28.9	38.1	35.1	26.0	36.7	40.0	40.0	
	2-2	42.3	40.0	28.7	18.1	33.3	38.6	26.7	35.4	40.0	39.3	40.0	40.0	40.0
	2-4	42.3	37.6	21.8	30.6	36.1	34.9	32.8	36.1	40.0	40.0	40.0	40.0	40.0
Ī	2-5	42.3	40.0	14.1	18.3	22.0	31.8	34.9	26.3	25.5	36.8	40.0	40.0	40.0
Ī	3-2	42.3	40.0	16.0	19.9	29.5	18.3	39.1	33.6	40.0	38.0	40.0	40.0	38.5
Ī	4-3	42.3	40.0	18.2	26.1	29.6	39.8	38.2	33.4	31.2	31.0	40.0	40.0	40.0
1	4-7	42.3	40.0	17.7	20.3	24.4	36.1	29.3	29.8	24.2	40.0	40.0	40.0	
	5-6	42.3	40.0	15.5	16.0	32.2	33.5	33.7	19.2	40.0	40.0	40.0	40.0	40.0
	5-8	42.3	40.0	14.3	19.1	27.4	23.0	36.5	29.1	37.1	40.0	40.0	40.0	40.0



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Pig ID	Group	Nec Day	Blood	Serum	Trachea	Turbinate	Tonsil	Lung	Liver	Kidney	Spleen	Lymph Node	Thymus	Esophagu s	Stomach	Des. Colon	Spiral Colon	Small Intestine	Cecum
2-1	Стопр	-1.1	5,000	oc. u.i.	Huelleu	, u. o.ii.u.te	. 6.1.5.11	-48			opice		yus		Gtomaen	661611	Colon	comic	CCCami
4-4		-1.1																	
4-9		-1.1																	
5-11		-1.1			40.0	40.0	40.0	40.0	40.0	40.0	40.0		40.0	40.0	40.0	40.0	40.0	40.0	40.0
6-1		-1.1																	
6-10		-1.1																	
4-8	A	1.1	22.10	2.2.															
1-4	A	1.3	32.19 31.13	34.31 31.63	33.5 32.5	35.3	40.0 36.7	32.4	32.7 29.1	36.8	36.4	35.9	37.6	33.6	29.0 32.0		38.8	18.7	23.7
5-1 6-8	A	1.3	32.15	34.38	36.9	36.1 35.6	40.0	32.2 31.2	29.1	26.6 29.5	26.5 37.8	23.1 28.9	33.4 36.3	37.0 36.9			29.0 29.8	16.3 19.6	20.5
1-9	В	2.2	29.59	28.14	23.7	21.6	23.0	21.5	22.4	17.7	25.1	20.0	23.3	20.1	19.9		17.6	13.0	19.0
4-6	A	2.2	23.33	27.35	24.3	24.4	31.2		25.7	22.3	24.8	26.1	25.1	25.8		_	22.9		
5-9	В	2.2	33.71	31.52	24.0	20.6	23.3		26.8	23.3	28.2	19.6	27.7	22.8	21.4		13.9	13.9	
1-1	Α	2.3	29.17	36.7	25.5	23.1	25.8		25.4	21.9	21.0	20.5	23.7	24.1	21.9	20.1	17.6	17.1	
4-2	Α	2.3	40	39.2	24.3	23.6	24.0	21.9	24.5	20.9	21.5	20.4	21.9	25.4	20.5	19.0	15.1	16.3	18.4
6-7	Α	2.3	32.05	27.89	23.2	24.5	25.6	25.0	27.8	20.1	31.6	20.7	31.6	24.2	22.2	19.7	16.5	14.3	16.1
7-10	С	2.3	34.21	32.95	23.8	27.8	25.1	22.2	20.0	18.3	19.5	17.3	22.0	22.7	17.0		12.4	12.4	
9-9	D	2.3	40	40	40.0	40.0	40.0			40.0	40.0	40.0	40.0						
1-2	A	3.1			35.1	34.2	35.0			30.5	33.8	25.2	40.0		26.1		18.9	17.6	
1-10	В	3.1			33.0	32.6	40.0		34.3	27.3	33.0	23.3	40.0	33.2			19.8	20.2	
6-3	A	3.1		34.67	31.2 33.7	32.3	32.6 29.1		32.8 31.3	28.6	32.0	26.8 28.3	31.0	35.2	24.5 30.0		19.1	18.7	
4-5 6-4	A	3.2		35.51	27.1	30.6 26.3	28.8		33.0	31.5 26.9	36.2 34.1	22.5	39.5 33.6	26.5 28.2	24.3		20.4 17.3	17.4 15.9	
7-6	C	3.2		40	35.2	25.4	26.8		31.2	31.0	36.1	23.3	32.4	26.0	22.6		18.4	15.8	
1-7	A	3.3		35.03	37.0	28.9	40.0			27.4	36.3	25.6	37.5	37.2			18.3	18.5	
3-4	Α	3.3		32.14	31.9	24.7	26.9			35.9	30.0	21.6	39.5		23.7		22.5	16.8	
4-10	Α	3.3		36.13	33.3	27.8	32.8		34.2	26.9	34.7	30.4	33.6		30.6		20.1	18.6	
9-5	D	3.3		40	40.0	40.0	40.0	40.0	34.6	40.0	36.2	38.7	40.0	40.0	40.0	40.0	40.0	40.0	40.0
9RA	D	3.3		40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
1-3	Α	4.3	40	32.55	27.9		32.1	28.2	30.7	27.9	30.2	26.3	29.7	29.3	24.1	19.3	24.1	19.4	16.5
1-8	Α	4.3	40	37.7	34.4	31.4	28.8	34.9		31.5	33.2	28.2	37.8	27.8	21.5		20.3	15.0	
2-6	Α	4.3	34.24	36.22	28.6	25.6	35.1	34.3	35.8	27.7	29.5	26.6	32.5	32.5		1	20.2	16.9	
3-7	A	4.3	33.84	29.99	38.8	28.8	36.1		31.8	37.8	31.7	28.6	37.1	36.1	25.3		22.1	19.5	
5-3	A	4.3	34.69	32.74	35.5	35.1	36.3		32.2	40.0	34.0	23.5	38.4	33.2	29.7		24.8	17.6	
9RB 6-2	D A	4.3 5.1	40	40	40.0 37.6	40.0 40.0	40.0 35.0		40.0 38.6	40.0 33.1	40.0 37.5	40.0 24.4	40.0 37.5	40.0 36.0	40.0 39.3		40.0 29.3	40.0 32.5	
6-9	A	5.3	40	33.26	38.6	32.2	34.8			36.5	34.0	23.2	37.3	34.7			29.5	20.1	
1-5	A	6.3	40	40	40.0	35.9	39.2			33.1	35.7	23.5	36.1	37.2			20.6	22.2	
3-3	A	6.3	40	40	34.1	33.1	31.2		37.4	34.6	35.1	25.2	35.0	30.4			20.0	17.1	
8-5	С	6.3	40	40	38.7	35.2	34.2	40.0		40.0	39.5	35.0	40.0	32.8	28.3	25.6	24.7	17.1	
9-8	D	6.3	40	40	40.0	40.0	40.0	39.1	40.0	40.0	40.0	40.0	40.0	39.4	40.0	40.0	40.0	36.9	
3-5	Α	7.1			40.0	35.6	34.9	35.4	38.4	34.6	39.2	25.1	39.4	40.0	40.0	33.4	26.5	21.1	36.8
3-6	Α	8.1			40.0	34.2	40.0			38.5	40.0	29.9	37.0				26.1	28.0	
8-2	С	8.1			40.0	38.0	40.0			35.2	40.0	32.4	40.0	 			32.4	23.0	
8-4	С	8.1			40.0	39.2	40.0			40.0	35.3	28.8	40.0				30.8		
8-7	C	8.1		20.22	36.1	34.0	32.0		33.0	28.3	40.0	31.3	35.6	40.0			21.6	26.3	
1-6	A	8.3 8.3	40	39.03	40.0 35.4	35.8 30.1	39.3 34.5	36.8	20.5	40.0	40.0	28.2 28.9	40.0				38.7 25.2	40.0	
6-5 6-6	A	8.3	40 40	40 40						38.0 34.9	40.0 38.0		40.0 35.8					25.4 25.3	
2-3	A	9.1	40		40.0					40.0		25.9	40.0				37.3		
3-8	A	9.1	40		37.3					38.9		33.1	38.9						
2-7	A	10.3	40	40						40.0		32.7	40.0						
3-1	Α		40	40						38.7		31.8	40.0			1	38.6		
9-2	D	10.3	40	40						40.0		40.0	40.0						
4-1	Α	14.3	40	40	40.0	38.1	36.7	40.0	40.0	40.0	40.0	28.4	40.0	40.0	37.2	35.5	33.5	25.9	35.3
5-7	Α	14.3	40	40	36.8	35.7	35.7	33.3	38.8	32.9	35.8	26.7	37.1	36.0	34.1	29.0	25.8	28.2	26.3
7-5	С	14.3	40								35.0		36.8						
8-3	С	.n <u>and</u>	40												1				
9-6	D	14 3	40	40	40.0	40.0	40.0	40.0	40.0	40 O	40 O	40.0	40.0	40 O	40.0	40.0	40.0	40.0	40.0

Dead/Sacrificed Tissue PCR

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Pig ID	Group	Nec Day	Blood	Serum	Trachea	Turbinate	Tonsil	Lung	Liver	Kidney	Spleen	Lymph Node	Thymus	Esophagu s	Stomach	Des. Colon	Spiral Colon	Small Intestine	Cecum
4-1	Α	14.3	40	40	40.0	38.1	36.7	40.0	40.0	40.0	40.0	28.4	40.0	40.0	37.2	35.5	33.5	25.9	35.3
5-7	Α	14.3	40	40	36.8	35.7	35.7		38.8		35.8	26.7	37.1	36.0	34.1		25.8	28.2	26.3
7-5	С	14.3	40	40	32.0	31.1	31.9	33.2	36.4		35.0	31.0	36.8	32.2	35.8		24.1	28.0	23.5
8-3	С	14.3	40	40	40.0	40.0	38.2		40.0	1	40.0	30.3	40.0	40.0	40.0		40.0	32.7	40.0
9-6	D	14.3	40	40	40.0		40.0				40.0	40.0	40.0	40.0	40.0		40.0	40.0	40.0
2-9	A	21.3	40	40	40.0	38.5	34.5				36.1	31.4	40.0	36.9	35.3		40.0	28.4	35.9
5-4 7-2	A C	21.3 21.3	40 40	40 40	40.0 40.0	40.0 40.0	40.0 36.2		40.0 40.0		40.0 37.9	29.9 27.1	40.0	40.0	40.0 38.7		37.9	30.2 25.0	40.0 32.6
8-1	С	21.3	40	40	40.0	40.0	40.0	40.0 40.0	40.0		40.0	28.0	40.0 40.0	40.0 40.0	40.0		36.6 40.0	26.4	40.0
9-1	D	21.3	40	40	40.0	40.0	40.0		40.0		40.0	40.0		40.0	40.0		40.0	40.0	40.0
5-5	A	28.3	40	40	40.0	40.0	40.0		40.0		40.0	32.7	40.0	40.0	40.0		40.0	39.6	38.9
7-7	C	28.3	40	40	40.0	40.0	39.1				40.0	35.5	40.0	40.0	40.0		40.0	39.0	35.4
8-8	С	28.3	40	40	40.0	40.0	40.0				40.0	32.7	40.0	40.0	40.0	-	40.0	31.0	40.0
9-3	D	28.3	40	40	40.0	40.0	40.0		40.0		40.0	40.0		40.0	40.0		40.0	40.0	40.0
S2	A	35.3	i,	40		.0.0	40.0	1010	40.0	.0.0	40.0	31.6	.0.0	.0.0	40.0		.0.0	35.4	40.0
2-8	A	35.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	39.1	30.8	40.0	40.0	40.0		40.0	32.2	40.0
2-11	В	35.3			40.0	40.0	38.3	40.0	38.9		40.0	31.4	40.0	40.0	40.0		40.0	30.5	40.0
S3	Α	35.3		40			40.0		40.0		40.0	29.7			40.0	40.0	40.0	40.0	40.0
S4	Α	35.3		40			38.5		40.0		40.0	32.4			40.0	40.0	40.0	40.0	40.0
S5	Α	35.3		40			39.0		40.0		37.6	31.3			40.0	40.0	40.0	37.9	40.0
5-2	Α	35.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	32.6	40.0	40.0	40.0	40.0	40.0	31.2	40.0
S6	Α	35.3		40			40.0		40.0		40.0	27.3			40.0	38.9	40.0	37.3	40.0
S7	С	35.3		40			40.0		40.0		40.0	32.6			40.0	40.0	40.0	31.2	40.0
7-3	С	35.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	32.3	40.0	40.0	40.0	40.0	40.0	32.3	40.0
7-8	С	35.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	32.0	40.0	40.0	40.0	39.3	29.2	29.8	40.0
8-10	С	35.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	38.0	40.0	40.0	40.0	40.0	40.0	33.1	39.6
S9	D	35.3		40			40.0		40.0		40.0	40.0			40.0		40.0	40.0	40.0
9-4	D	35.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0		40.0	40.0	40.0
S1	Α	36.3		40			38.6		40.0		40.0	29.1			40.0		34.6	40.0	40.0
S8	С	36.3		40			40.0		40.0		36.8	34.6			40.0		40.0	32.7	38.2
2-2	Α	42.3	40	40	40.0	40.0	38.8		40.0		40.0	34.8	40.0	40.0	40.0		40.0	34.2	40.0
2-4	A	42.3	40	40	40.0	40.0	40.0		40.0		40.0	34.2	40.0	40.0	40.0		40.0	33.8	40.0
2-5	A	42.3	40	40	40.0	40.0	40.0		40.0		40.0	32.6	40.0	40.0	40.0	1	39.4	32.8	40.0
2-10 3-2	B A	42.3 42.3	40 40	40 40	40.0 40.0	40.0 40.0	40.0 40.0		40.0 40.0		40.0 40.0	35.3 35.3	40.0 40.0	40.0 40.0	40.0 40.0		40.0 40.0	32.4 31.2	40.0 40.0
3-2	В	42.3	40	40	40.0	38.5	40.0				40.0	40.0		40.0	40.0		40.0	33.8	40.0
3-10	В	42.3	40	40	40.0	40.0	40.0		40.0		40.0	33.2	40.0	40.0	40.0		40.0	40.0	40.0
4-3	A	42.3	40	40	40.0		40.0		40.0		40.0	34.5	40.0	40.0	38.4		40.0	33.6	40.0
4-3	A	42.3	40	40	40.0	40.0	40.0		40.0	1	40.0	33.2	40.0	40.0	40.0	1	40.0	31.4	40.0
5-6	A	42.3	40	40	40.0	40.0	40.0				40.0	34.4	40.0	40.0	40.0		40.0	32.8	40.0
5-8	A	42.3	40	40	40.0	40.0	40.0		40.0		40.0	32.0	40.0	40.0	40.0		40.0	34.5	38.8
5-10	В	42.3	40	40	40.0	40.0	40.0		40.0		40.0	33.8	40.0	.0.0	40.0		40.0	35.5	40.0
7-1	С	42.3	40	40	40.0	40.0	40.0				40.0	37.6		40.0	40.0	1	40.0	35.5	40.0
7-4	С	42.3	40	40	40.0	40.0	40.0		40.0		40.0	35.9	40.0	40.0	40.0		40.0	34.4	40.0
7-9	С	42.3	40	40	40.0	40.0	40.0		40.0		40.0	33.8	40.0	38.7	40.0		40.0	32.8	40.0
8-6	С	42.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	33.8	40.0	40.0	40.0	40.0	40.0	40.0	40.0
8-9	С	42.3	40	40	40.0	40.0	37.5	40.0	40.0	40.0	40.0	32.7	40.0	40.0	40.0	40.0	40.0	33.3	40.0
9-7	D	42.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
9-10	D	42.3	40	40	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0

Dead/Sacrificed Tissue PCR

Oral Fluid PCR

Oral Fluid	Day 14	Day 21	Day 28	Day 35	Day 39
Litter 1					
Litter 2	30.1	32.1	30.3	40.0	38.5
Litter 3	33.7	34.7	37.0	38.7	40.0
Litter 4	34.5	35.2	40.0	40.0	40.0
Litter 5	31.3	31.8	40.0	40.0	40.0
Litter 6					
Litter 7	28.1	36.9	36.6	40.0	40.0
Litter 8	35.1	35.4	34.5	40.0	37.6
Litter 9	40.0	40.0	40.0	40.0	40.0



Sow Data Following PDCoV Challenge

Serum PCI	R										
Serum PCI Pig ID	Day 8	Day 14	Day 21	Day 28	Day 35						
S1	40.0										
S2	40.0	40.0									
	40.0	40.0	40.0	40.0	40.0						
S3			40.0	40.0	40.0						
S4	40.0	40.0	40.0	40.0	40.0						
S5	40.0	40.0	40.0	40.0	40.0						
S6	40.0	40.0	40.0	40.0	40.0						
S7	40.0	40.0	40.0	40.0	40.0						
S8	40.0	40.0	40.0	40.0	40.0						
S9	40.0	40.0	40.0	40.0	40.0						
Fecal PCR											
Pig ID	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8	Day 10	Day 14	Day 21	Day 28	Day 35
S1	40.0		24.6	22.8	23.2	24.0	27.3		37.2	40.0	
S2	40.0	30.5	23.9	24.0	22.5	23.4	18.9	28.4	36.6	40.0	40.
S3	40.0	29.9	30.0	25.0	27.4	19.8	21.2	33.0	38.4	40.0	40.
S4	40.0	40.0	31.5	33.8	40.0	27.2	24.5	35.6	40.0	40.0	40.
S5	40.0	29.3	21.8	26.5	20.2	27.9	26.9	36.1	40.0	40.0	40.
\$6	40.0	32.0	32.8	23.3	23.9	22.8	25.4	27.2	34.6	40.0	40.
	40.0	25.6	28.4	24.1	27.3	29.9	27.9	35.6			
S7	40.0	37.0	29.1					31.7	36.8	40.0	38.
S8				20.2	19.4	21.4	24.4		35.4	33.4	40.
S9	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.
Nasal PCR											
Pig ID	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8	Day 10	Day 14	Day 21	Day 28	Day 35
S1	40.0	28.0	29.2	29.1	35.9	37.6	40.0	38.9	40.0	40.0	40.
S2	40.0	27.4	29.3	32.2	35.4	35.1	34.7	37.0	40.0		
S 3	40.0						5	37.0	40.0	40.0	40.
S4		31.1	29.3	33.1	33.3	37.4	35.2	40.0	38.6	40.0 40.0	
	40.0	31.1 33.5	29.3 33.2	33.1 36.8	33.3 39.7	37.4 40.0					40. 40. 40.
S5	40.0 40.0						35.2	40.0	38.6	40.0	40. 40.
S5 S6						40.0	35.2	40.0	38.6	40.0 40.0	40.
	40.0	33.5	33.2	36.8	39.7	40.0 32.1	35.2 36.4	40.0 40.0	38.6 40.0	40.0 40.0 40.0	40. 40. 40.
S6	40.0 40.0	33.5 32.6	33.2	36.8	39.7 34.5	40.0 32.1 29.6	35.2 36.4 36.3	40.0 40.0 40.0	38.6 40.0 40.0	40.0 40.0 40.0 40.0	40. 40. 40.
\$6 \$7	40.0 40.0 40.0	33.5 32.6 29.8	33.2 30.2 31.7	36.8 32.1 34.3	39.7 34.5 30.8	40.0 32.1 29.6 40.0	35.2 36.4 36.3 34.0	40.0 40.0 40.0 35.5	38.6 40.0 40.0 40.0	40.0 40.0 40.0 40.0 40.0	40. 40. 40. 40. 40. 39.
\$6 \$7 \$8	40.0 40.0 40.0	33.5 32.6 29.8 36.1	33.2 30.2 31.7 30.1	36.8 32.1 34.3 32.2	39.7 34.5 30.8 33.7	40.0 32.1 29.6 40.0 36.9	35.2 36.4 36.3 34.0 39.3	40.0 40.0 40.0 35.5 40.0	38.6 40.0 40.0 40.0 40.0	40.0 40.0 40.0 40.0 40.0 40.0	40. 40. 40. 40. 40. 39.
\$6 \$7 \$8	40.0 40.0 40.0	33.5 32.6 29.8 36.1	33.2 30.2 31.7 30.1	36.8 32.1 34.3 32.2	39.7 34.5 30.8 33.7	40.0 32.1 29.6 40.0 36.9	35.2 36.4 36.3 34.0 39.3	40.0 40.0 40.0 35.5 40.0	38.6 40.0 40.0 40.0 40.0	40.0 40.0 40.0 40.0 40.0 40.0	40. 40. 40. 40. 40. 39.
\$6 \$7 \$8 \$9	40.0 40.0 40.0	33.5 32.6 29.8 36.1	33.2 30.2 31.7 30.1	36.8 32.1 34.3 32.2	39.7 34.5 30.8 33.7 40.0	40.0 32.1 29.6 40.0 36.9	35.2 36.4 36.3 34.0 39.3 40.0	40.0 40.0 40.0 35.5 40.0 40.0	38.6 40.0 40.0 40.0 40.0 5mall	40.0 40.0 40.0 40.0 40.0 40.0	40. 40. 40. 40. 40. 39.
\$6 \$7 \$8 \$9	40.0 40.0 40.0 40.0	33.5 32.6 29.8 36.1 40.0	33.2 30.2 31.7 30.1 40.0	36.8 32.1 34.3 32.2 40.0	39.7 34.5 30.8 33.7 40.0 Lymph Node	40.0 32.1 29.6 40.0 36.9 40.0	35.2 36.4 36.3 34.0 39.3 40.0	40.0 40.0 35.5 40.0 40.0 Spiral	38.6 40.0 40.0 40.0 40.0 5mall	40.0 40.0 40.0 40.0 40.0 40.0	40. 40. 40. 40. 40.
\$6 \$7 \$8 \$9	40.0 40.0 40.0 40.0	33.5 32.6 29.8 36.1 40.0	33.2 30.2 31.7 30.1 40.0	36.8 32.1 34.3 32.2 40.0 Spleen	39.7 34.5 30.8 33.7 40.0 Lymph Node	40.0 32.1 29.6 40.0 36.9 40.0	35.2 36.4 36.3 34.0 39.3 40.0 Des.	40.0 40.0 35.5 40.0 40.0 Spiral	38.6 40.0 40.0 40.0 40.0 40.0 Small Intestine	40.0 40.0 40.0 40.0 40.0 40.0	40. 40. 40. 40. 39.
\$6 \$7 \$8 \$9 Pig ID \$1	40.0 40.0 40.0 40.0 Serum	33.5 32.6 29.8 36.1 40.0 Tonsil	33.2 30.2 31.7 30.1 40.0 Liver	36.8 32.1 34.3 32.2 40.0 Spleen 40.0	39.7 34.5 30.8 33.7 40.0 Lymph Node	40.0 32.1 29.6 40.0 36.9 40.0 Stomach	35.2 36.4 36.3 34.0 39.3 40.0 Des. Colon	40.0 40.0 35.5 40.0 40.0 Spiral	38.6 40.0 40.0 40.0 40.0 40.0 Small Intestine 40.0	40.0 40.0 40.0 40.0 40.0 40.0 40.0	40. 40. 40. 40. 40. 39.
\$6 \$7 \$8 \$9 Pig ID \$1 \$2	40.0 40.0 40.0 40.0 5erum 40	33.5 32.6 29.8 36.1 40.0 Tonsil 38.6 40.0	33.2 30.2 31.7 30.1 40.0 Liver 40.0 40.0	32.1 34.3 32.2 40.0 Spleen 40.0 40.0	39.7 34.5 30.8 33.7 40.0 Lymph Node 29.1 31.6	40.0 32.1 29.6 40.0 36.9 40.0 Stomach 40.0	35.2 36.4 36.3 34.0 39.3 40.0 Des. Colon 36.1 38.1	40.0 40.0 35.5 40.0 40.0 Spiral Colon	38.6 40.0 40.0 40.0 40.0 5mall Intestine 40.0 35.4	40.0 40.0 40.0 40.0 40.0 40.0 40.0 Cecum 40.0	40. 40. 40. 40. 40. 39.
\$6 \$7 \$8 \$9 Pig ID \$1 \$2 \$3	40.0 40.0 40.0 40.0 Serum 40 40	33.5 32.6 29.8 36.1 40.0 Tonsil 38.6 40.0	33.2 30.2 31.7 30.1 40.0 Liver 40.0 40.0	32.1 34.3 32.2 40.0 Spleen 40.0 40.0	39.7 34.5 30.8 33.7 40.0 Lymph Node 29.1 31.6 29.7	40.0 32.1 29.6 40.0 36.9 40.0 Stomach 40.0 40.0	35.2 36.4 36.3 34.0 39.3 40.0 Des. Colon 36.1 38.1 40.0	40.0 40.0 35.5 40.0 40.0 Spiral Colon 34.6	38.6 40.0 40.0 40.0 40.0 5mall Intestine 40.0 35.4 40.0	40.0 40.0 40.0 40.0 40.0 40.0 40.0 Cecum 40.0 40.0	40. 40. 40. 40. 40. 39.
\$6 \$7 \$8 \$9 Pig ID \$1 \$2 \$3 \$4	40.0 40.0 40.0 40.0 Serum 40 40 40	33.5 32.6 29.8 36.1 40.0 Tonsil 38.6 40.0 40.0 38.5	33.2 30.2 31.7 30.1 40.0 Liver 40.0 40.0 40.0	32.1 34.3 32.2 40.0 Spleen 40.0 40.0 40.0	39.7 34.5 30.8 33.7 40.0 Lymph Node 29.1 31.6 29.7 32.4	40.0 32.1 29.6 40.0 36.9 40.0 Stomach 40.0 40.0 40.0	35.2 36.4 36.3 34.0 39.3 40.0 Des. Colon 36.1 38.1 40.0 40.0	40.0 40.0 35.5 40.0 40.0 Spiral Colon 34.6 40.0	38.6 40.0 40.0 40.0 40.0 5mall Intestine 40.0 35.4 40.0	40.0 40.0 40.0 40.0 40.0 40.0 40.0 Cecum 40.0 40.0 40.0	40. 40. 40. 40. 40. 39.
\$6 \$7 \$8 \$9 Pig ID \$1 \$2 \$3 \$4	40.0 40.0 40.0 40.0 Serum 40 40 40 40	33.5 32.6 29.8 36.1 40.0 Tonsil 38.6 40.0 40.0 38.5 39.0	33.2 30.2 31.7 30.1 40.0 Liver 40.0 40.0 40.0 40.0 40.0	36.8 32.1 34.3 32.2 40.0 Spleen 40.0 40.0 40.0 40.0 37.6	39.7 34.5 30.8 33.7 40.0 Lymph Node 29.1 31.6 29.7 32.4 31.3	40.0 32.1 29.6 40.0 36.9 40.0 Stomach 40.0 40.0 40.0 40.0	35.2 36.4 36.3 34.0 39.3 40.0 Des. Colon 36.1 38.1 40.0 40.0	40.0 40.0 35.5 40.0 40.0 Spiral Colon 34.6 40.0 40.0	38.6 40.0 40.0 40.0 40.0 Small Intestine 40.0 35.4 40.0 40.0	40.0 40.0 40.0 40.0 40.0 40.0 40.0 Cecum 40.0 40.0 40.0	40. 40. 40. 40. 40. 39.
S6 S7 S8 S9 Pig ID S1 S2 S3 S4 S5 S6	40.0 40.0 40.0 40.0 Serum 40 40 40 40 40	33.5 32.6 29.8 36.1 40.0 Tonsil 38.6 40.0 40.0 38.5 39.0 40.0	33.2 30.2 31.7 30.1 40.0 Liver 40.0 40.0 40.0 40.0 40.0 40.0	36.8 32.1 34.3 32.2 40.0 Spleen 40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0	39.7 34.5 30.8 33.7 40.0 Lymph Node 29.1 31.6 29.7 32.4 31.3 27.3	40.0 32.1 29.6 40.0 36.9 40.0 Stomach 40.0 40.0 40.0 40.0 40.0	35.2 36.4 36.3 34.0 39.3 40.0 Des. Colon 36.1 38.1 40.0 40.0 38.9	40.0 40.0 35.5 40.0 40.0 Spiral Colon 34.6 40.0 40.0 40.0	38.6 40.0 40.0 40.0 40.0 5mall Intestine 40.0 35.4 40.0 40.0 37.9	40.0 40.0 40.0 40.0 40.0 40.0 40.0 40.0	40. 40. 40. 40. 40. 39.

Viremia

Fecal Shedding

Nasal Shedding

Day 35
Tissue PCRKnowledge

for Life

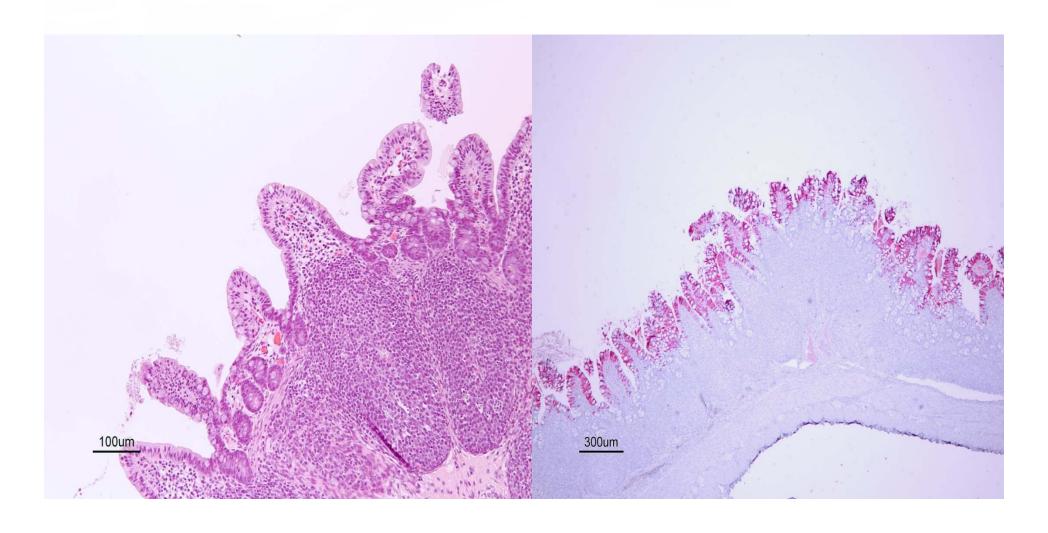
Histopathology Virus Localization

- Histological lesions of the GI tract were extensive, mainly confined to the small intestine.
- PDCoV was demonstrated via IHC in the villi of the small intestine.
- Detection of virus localization in other tissues is currently underway.



Histopathology/ Virus Localization

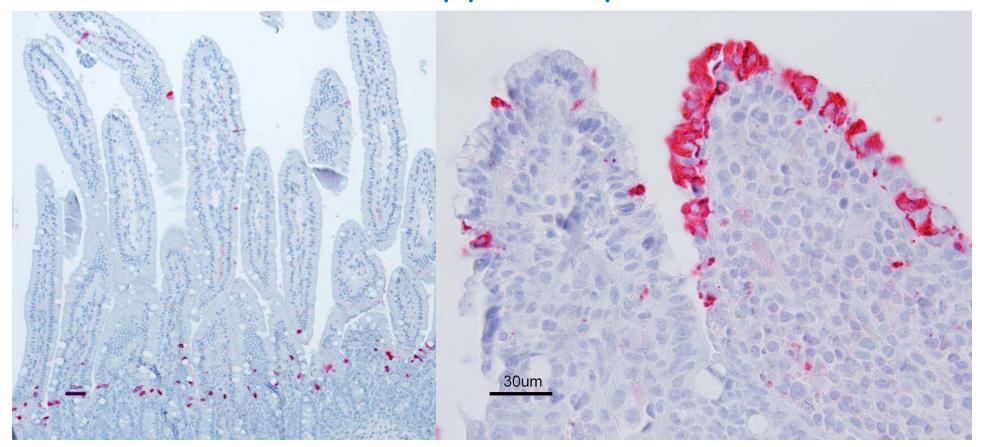
Day 1 post inoculation



Immunohistochemistry Virus Localization

Courtesy of Bruce Brodersen and Sarah Vitosh-Sillman.

Detection Antibody provided by Eric Nelson



IFA and SN Response following PDCoV Inoculation

- All sows seroconverted to PDCoV (IFA & SN) around D-14 post inoculation except the Negative Control group.
- All surviving piglets seroconverted to PDCoV (IFA & SN) around D-14 post inoculation except the Negative Control group.



Thanks To:

 NVSL—Sabrina Swenson & Melinda Jenkins-Moore for providing cell culture adapted PDCoV virus, culturing methods and sharing assay results.



Feed Transmission of Swine Enteric Corona Virus: What have we learned in the last year?

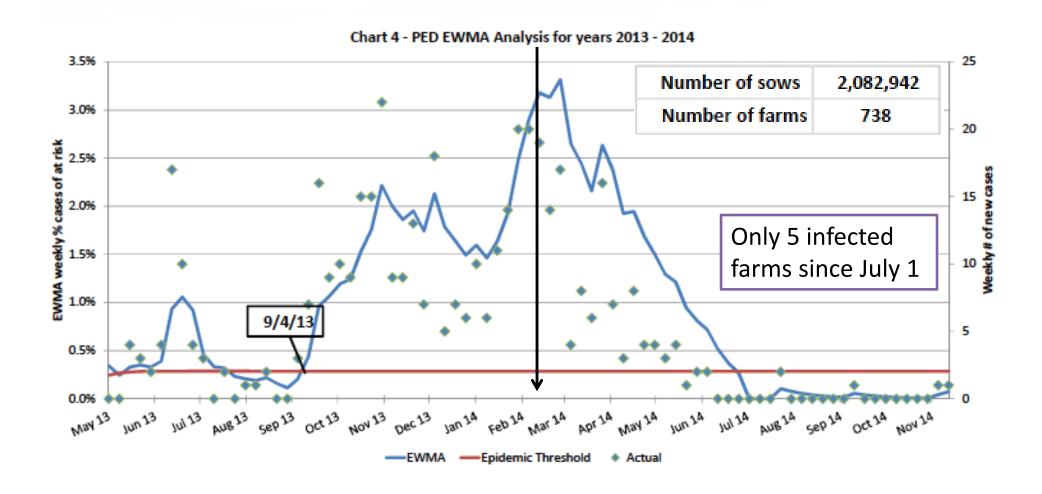
Kansas State University
Applied Swine Nutrition Team







Current US PEDv Status





PEDv is Highly Infectious

- ✓ ONE mL solution of ONE gram of infected baby pig feces in 2,800 liters of water still is infectious
- ✓ Another way of expressing is that 1 ml of diarrhea is capable of infecting almost 3,000,000 pigs.
- ✓ (Goyal & Rovira, 2013)



History of increased suspicion of a feed transmission connection:

- Concern about feed transmission since introduction of the PED virus into the US in May 2013:
 - Vitamin premixes
 - Products from Asia
- Initially, though we did not think feed would be a significant transmission route



History of increased suspicion of a feed transmission connection:

- Early work indicated truck transport to slaughter would be the primary concern
 - Clean trucks were becoming contaminated at the slaughter plant, Lowe et al. 2014
 - Cull sow assembly yards as a point source, Turner et al. 2013



History of increased suspicion of a feed transmission connection:

- Late December 2013 to Early February 2014 our K-State group was experiencing multiple suspected cases with a feed link to PEDv transmission
 - Common theme of clinical signs appearing in early nursery
 - Upstream sow farms remaining negative



Case Study

- 50,000 Sow Midwest USA production system
- 18 Sow Farm Sites
- 2 sow farms PEDv positive
 - 1 in November and 1 in December 2013
- Two actions:
 - Improved biosecurity practices
 - Removed creep feed from all sow farms
- To date no further sow farms have been infected



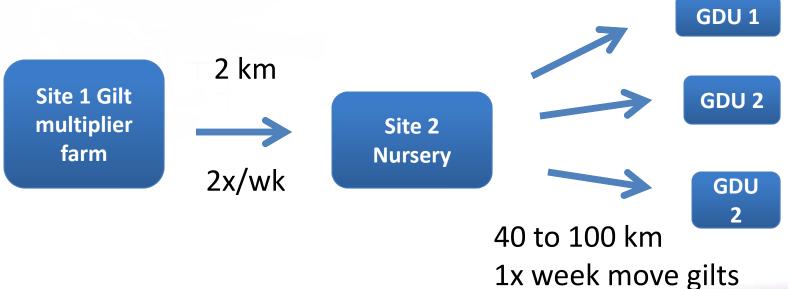
Case Study

- Remaining negative Sow Farms
- 12/21 commercial nurseries filled between
 1/16/2014 and 2/17/2014 were categorized as
 PEDv positive
 - Pigs originating from sow farms showing no evidence of infection
 - Clinical signs were observed shortly after pig arrival



Case Study February 2014

- Same production system Gilt multiplication nursery
 - Isolated site used in gilt multiplication for over 25 years without PRRS infection





Knowledge ^{for}Life

Case Study History – Multiplier Farm Nursery

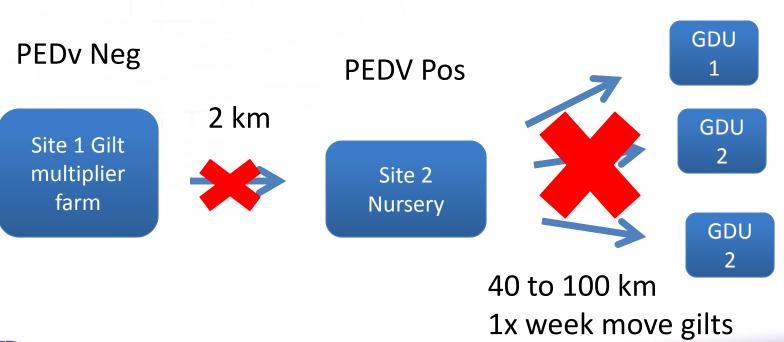
- Diarrhea noted in newly weaned pigs
 - Feed sample PCR PEDv positive,
 - Clinical signs of diarrhea increased over the next 3 days
 - Subsequent testing of fecal swabs confirmed active PEDv infection
 - Clinical signs moved through site from younger to older pigs



Case Study February 2014

 Upstream sow farm and downstream gilt flow has never shown clinical or diagnostic evidence of PEDv

PEDv Neg





Recommendations made:

- January 2014
 - Remove all creep and nursery feed products from sow farms
- February 2014
 - Removed all porcine proteins from creep and nursery diets



Site	Date in	PED	Diet	Site	Date in	PED	Diet
Bentein WF	11/25/2014	POS	Old	Steuer 2 WF	2/10/2014	Neg	Old
Circle A	1/2/2014	Neg	Old	Ward	2/10/2014	Neg	Old
Richter	1/3/2014	Neg	Old	Newry WF	2/11/2014	Neg	Old
Nelson	1/3/2014	Neg	Old	River Valley	2/12/2014	Neg	Old
Pork Ave.	1/6/2014	Neg	Old	Lonetree WF	2/13/2014	POS	Old
Busch WF 1	1/7/2014	Neg	Old	G1	2/13/2014	Neg	Old
Lafayette	1/7/2014	Neg	Old	Coughlin	2/15/2014	POS	Old
Kruckeberg	1/8/2014	Neg	Old	JAT	2/17/2014	Neg	Old
Thor WF	1/13/2014	Neg	Old	AR&J 2 WF	2/17/2014	POS	Old
Wenger WF	1/13/2014	Neg	Old	V1-1		Neg	Old
Friesen WF	1/16/2014	Neg	Old	Die	Diet Change		New
Schatz WF	1/17/2014	POS	Old	Echard WF	2/24/2014	Neg	New \
Willow Creek	1/17/2014	POS	Old	G2	2/25/2014	Neg	Old
Nevada One	1/20/2014	POS	Old	Circle A	2/25/2014	Neg	New
Grove WF #2	1/21/2014	POS	Old	Sanborn	2/26/2014	Neg	New
Hillcrest	1/24/2014	Neg	Old	Lafayette	3/3/2014	Neg	New
BellM WF	1/24/2014	POS	Old	Haler	3/5/2014	Neg	New
Andy WF	1/27/2014	Neg	Old	Busch WF 1	3/6/2014	Neg	New
R&C	1/28/2014	POS	Old	Hillcrest	3/7/2014	Neg	New
Geneva WF	1/31/2014	POS	Old	Concord WF	3/7/2014	Neg	New
Haugen	2/3/2014	POS	Old	Lechtenberg	WF 3/13/2014	Neg	New
Bear Creek	2/3/2014	Neg	Old	Haugen	3/13/2014	Neg	New
JM&TD WF	2/4/2014	POS	Old	Nelson	3/13/2014	Neg	New



Site	Date in	PED	Diet	Site	Date in	PED	Diet	
Bentein WF	11/25/2014	POS	Old	Steuer 2 WF	2/10/2014	Neg	Old	
Circle A	1/2/2014	Neg	Old	Ward	2/10/2014	Neg	Old	
Richter	1/3/2014	Neg	Old	Newry WF	2/11/2014	Neg	Old	
Nelson	1/3/2014	Neg	Old	River Valley	2/12/2014	Neg	Old	
No new sow farm				Lonetree WF	2/13/2014	POS	Old	
Bus INO I	iew so	W Ic	11111	G1	2/13/2014	Neg	Old	
infections to date.			ato	Coughlin	2/15/2014	POS	Old	
Kru IIII C	20113	to a	ate.	JAT	2/17/2014	Neg	Old	
Thor WF	1/13/2014	Neg	Old	AR&J 2 WF	2/17/2014	POS	Old	
Wenger WF	1/13/2014	Neg	Old	N		Neg	Old I	
Friesen WF	1/16/2014	Neg	Old	Diet Ch	ange	Neg	New	
Schatz WF	1/17/2014	POS	Old	Echard WF	2/24/2014	Neg	New \	
Willow Creek	1/17/2014	POS	Old	G2	2/25/2014	Neg	Old	
Nevada One	1/20/2014	POS	Old	Circle A	2/25/2014	Neg	New	
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Andy WF	1/27/2014	Neg	Old	Bus	2/2/22		<u> </u>	
R&C	1/28/2014	POS	Old	Hill As of C	October	31	onlv	
Geneva WF	1/31/2014	POS	Old	I ICorl				
Haugen	2/3/2014	POS	Old	Lec 1 infec	1 infected nursery.			
Bear Creek	2/3/2014	Neg	Old	Наивен	J) 1J) 2U14		INCW	
JM&TD WF	2/4/2014	POS	Old	Nelson	3/13/2014	Neg	New	



Risk of porcine origin products: the Canadian experience

10/11 of the first cases of PED in Canada were associated with a common feed company in Ontario

✓ Geographically diverse farms

✓ Farrowing or nursery only

Cases	Positive
Porcine Plasma in diets	10
No porcine plasma in diets	0

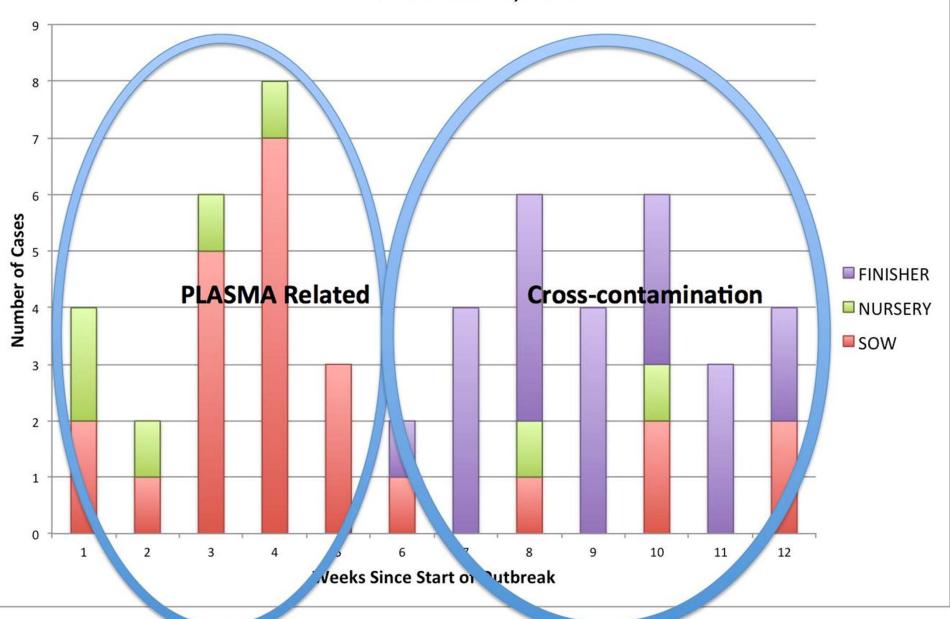


Voluntary recall of feed Subsequent testing of porcine plasma was bioassy PEDv positive Bioassay of the feed was unable to confirm PEDv infective in feed

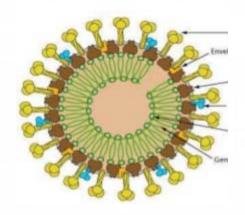
Dr. Martin Misener - Southwest Ontario Veterinary Services - SDEC Symposium 2014

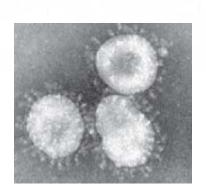


PED Cases per Week by Farm Type in Ontario Since Jan. 22, 2014



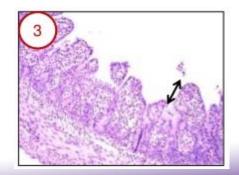
Research Update: K-State studies with PEDv in feed













Partners for our PEDv Research

- K-State Applied Swine Nutrition Team
 - Drs. Nitikanchana, Schumacher, Dritz, Woodworth, Tokach, DeRouchey, Goodband
- K-State Grain Science
 - Drs. Jones, Stark, and Huss; Roger Cochrane, Joel McAtee
- K-State VDL
 - Drs. Hesse, Bai, Haus, Anderson, and their team
- Iowa State University VDL
 - Drs. Main, Zhang, Gauger, and their team
- National Pork Board









Can actions be taken in the feed mill to mitigate the transfer of PED virus?

K-State Research has focused on these areas:

- Establish a minimum infectious dose in feed
- Impact of thermal processing (steam conditioning/pelleting)
- Post manufacture chemical mitigations

All supported by the National Pork Board

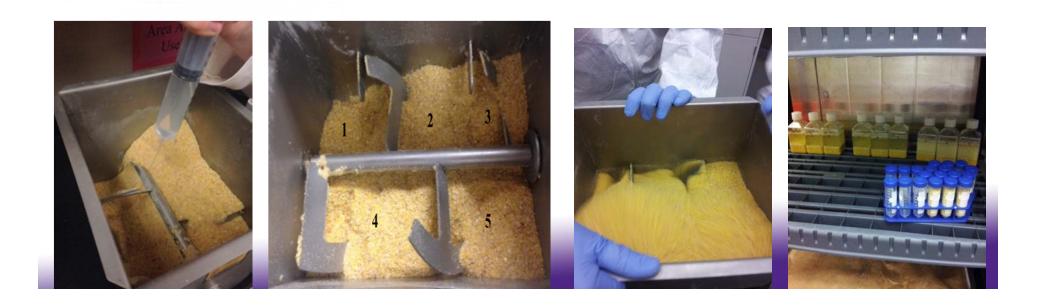




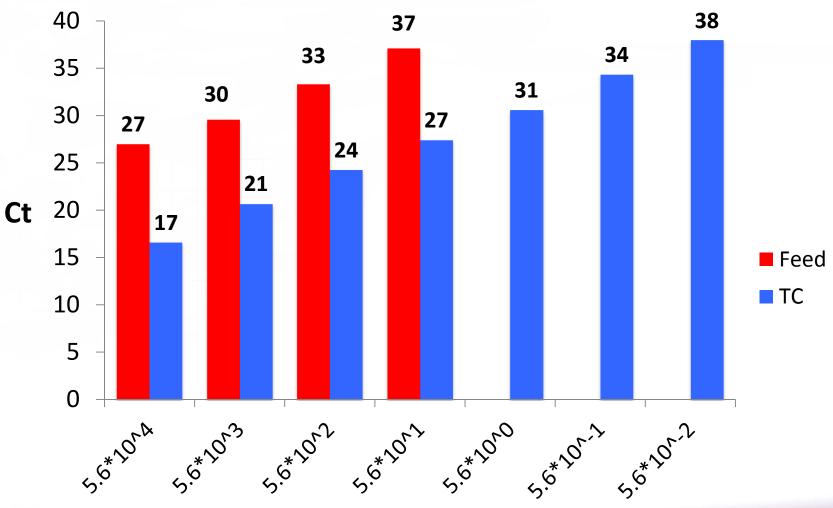


Study 1: Confirm the minimum infectious dose of PEDv in a feed matrix using a 10 d old pig bioassay

- Serial 10-fold dilution of PEDv in tissue culture media
- Dilutions added to 4.5 kg of complete feed and blended for 2.5 minutes to homogenize and distribute virus.
- Blended feed added to PBS and supernatant then collected for bioassay.
- Inoculated into 3 pigs per dose plus a non-inoculated control.



Tissue Culture (TC) vs Feed TCID₅₀ and Ct values







TCID 50/g

-0.0-0.4	PEDV N-gene rRT-PCR						
TCID50/g (spiked feed with theoretical titers)	Inocula Ct	0 DPI Fecal	2 DPI Fecal	4 DPI Fecal	6 DPI Fecal	7 DPI Fecal	7 DPI Cecum
,		swab	swab	swab	swab	swab	content
5.6 x 10^0	>45	>45	>45	>45	>45	>45	>45
	>45	>45	>45	>45	>45	>45	>45
	>45	>45	>45	>45	>45	>45	>45
5.6 x 10^1	37	>45	19	22	19	23	24
	36	>45	35	21	20	33	26
	38	>45	>45	19	20	20	19
5.6 x 10^2	34	>45	22	22	24	25	24
	33	>45	27	24	24	28	31
	33	>45	34	20	16	19	24
5.6 x 10^3	29	>45	37	25	23	27	21
	30	>45	38	25	22	23	25
	30	>45	18	18	18	25	26
5.6 x 10^4	27	>45	21	26	27	30	31
	27	>45	31	18	18	26	26
	27	>45	30	19	21	19	19





A low dose can infect naïve pigs

- ✓ A dose as low as 200 infectious particles in feed has been demonstrated to result in pig infection (Schumacher, Woodworth, Zhang, Gauger et al., 2015)
- ✓ An acutely infected piglet can produce 100,000,000 infectious particles per gram of feces
- ✓ Thus, 1 gram of feces from an acutely infected pig could contaminate up to 500 tonnes of feed with each gram of feed being infectious















K-State Study 2: Determine the impact of conditioning time and temperature of pelleted complete feed

- Treatments organized in a
 2 x 3 x 3 factorial
 - PEDv dose (low and high)
 - Conditioning time (45, 90, and 180 s)
 - Conditioning temperature (155, 175, 195 F)
- PCR and bioassay conducted





Effect of Conditioning Time and Temperature on PEDv PCR

Low Dose PCR Ct Values

		Time, se	С
Temp, F	45	90	180
155	39	38	40
175	37	38	39
195	37	37	36

High Dose PCR Ct Values

	Time, sec			
Temp, F	45	90	180	
155	30	30	30	
175	30	30	30	
195	30	31	30	

Low Dose Feed No processing = 31 Low Dose Tissue Culture = 20 High Dose Feed No processing = 24 High Dose Tissue Culture =13

(Schumacher, Woodworth, Zhang, Gauger et al., 2015)





KSU Study 3: Chemical mitigation strategies to control post-processing PEDV contamination in feed and feed ingredients

- Roger Cochrane and Dr. Cassie Jones as lead investigators
- Sister project to a similar study utilizing salmonella contamination that is funded by FPRF.
- 4 different feed or ingredients to be treated:
 - Complete nursery diet, porcine meat and bone meal (MBM), blood meal, and animal plasma
- 7 chemical treatments:
 - Organic acids, essential oils, medium chain fatty acids, sodium bisulfate, commercial formaldehyde (Termin-8), sodium chlorate, and an untreated control





Procedures: Chemical mitigations for post-processing contamination

Procured nursery diet, blood meal, porcine meat and bone meal, and animal plasma



Applied the 6 chemical mitigations + untreated control to each feed or ingredient



4 feed treatments x 7 chemical treatments = 28 - 1 kg batches



Added PEDv virus to each batch the following day to provide 5.6 x 10⁴ TCID50/g



Segregated 3 replicate samples of each batch for each day of analysis

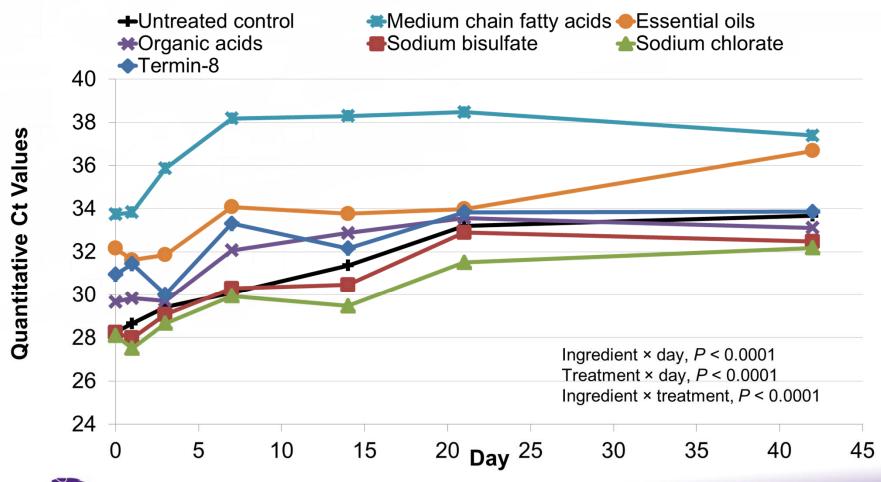


Stored samples at room temperature and analyzed on each day after infection



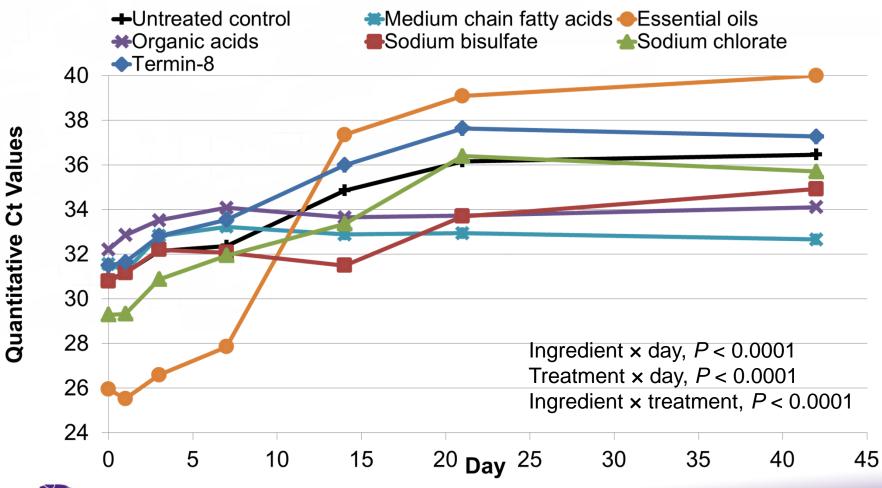


PEDv contamination post-treatment in **swine diet** stored at Room Temperature



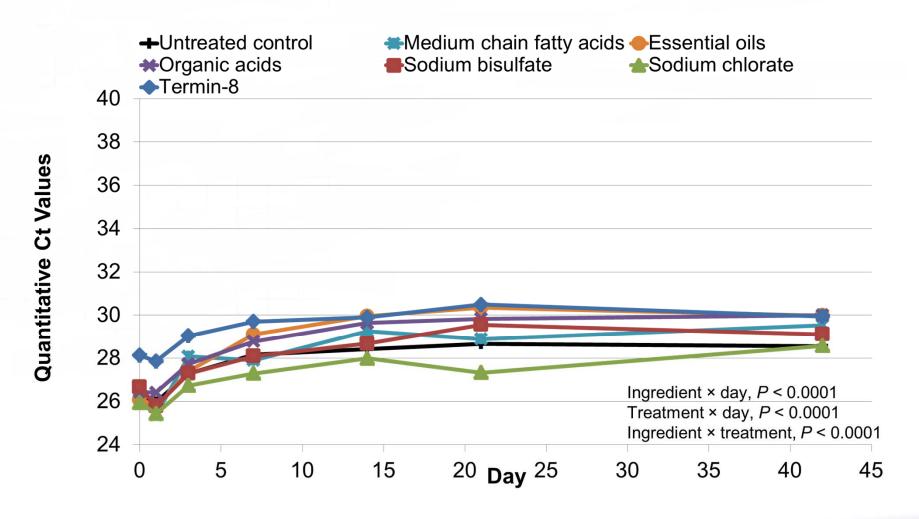


PEDv contamination post-treatment in **blood meal** stored at Room Temperature



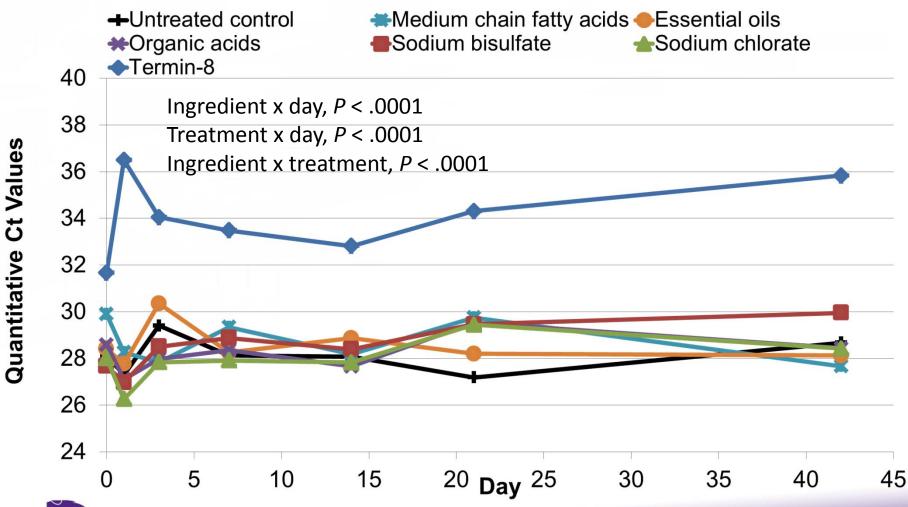


PEDv contamination post-treatment in **porcine meat and bone meal** stored at Room Temperature





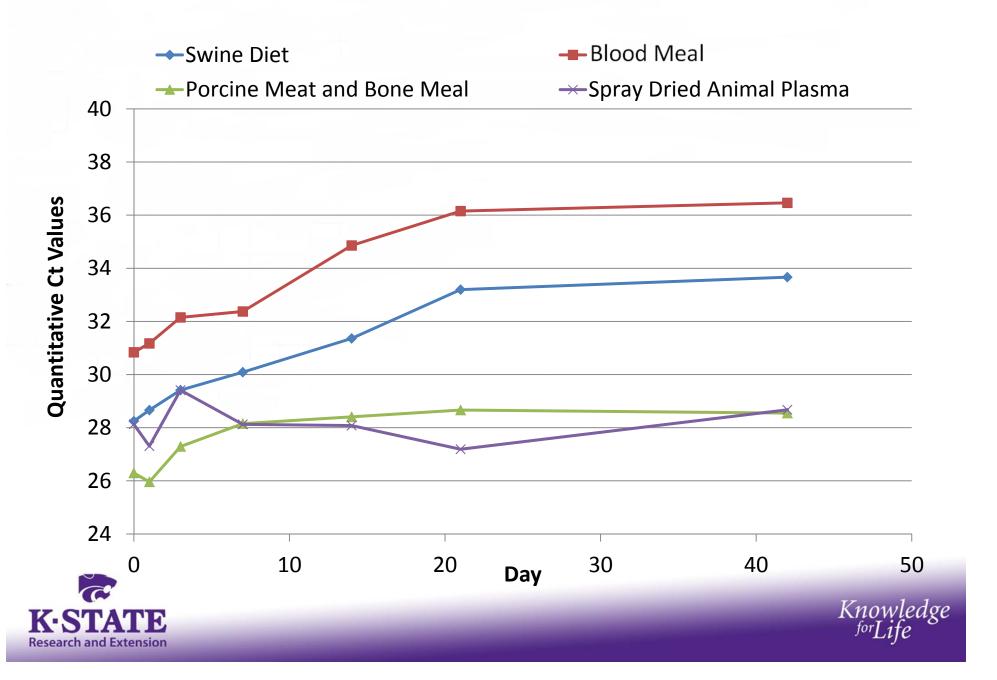
PEDv contamination post-treatment in **spray dried animal plasma** stored at Room Temperature





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Untreated Controls stored at Room Temperature



Summary of what we have learned from our K-State Studies:

- We lose about 10 Ct when going from tissue culture into feed
- Detectable Ct can result in infectivity
- The infective minimum Ct can be above the cutoff points for some labs
- We can detect low amounts PEDv RNA in feed on a consistent and repeatable basis with PCR
 - Although PCR results may not always correlate with infectivity





Summary of what we have learned from our K-State Studies:

- May be some opportunity for chemical mitigations to help minimize post-processing PEDv contamination
 - Appears they may be matrix specific (i.e. formaldehyde in plasma or MCFA in feed)
- There appears to be something about plasma and porcine meat and bone meal that leads to PCR stability



Recommended Focus Areas for Feed Mill Biosecurity

- Ingredients in the feed mill
 - No porcine ingredients used in breeding stock, boar or multiplication diets
 - Prefer using feed mills with no porcine proteins for breeding herd
 - Minimize usage of porcine ingredients in commercial mills
 - Sequencing and flushing
- Ingredient Receiving
- Transport Protocols
 - Biosecurity pyramid
 - Delivery to positive sites last
- Assessment of the feed mill as part of biosecurity



What are some fundamental concepts of Swine Enteric Corona Virus Biology to remember ?

- Viruses need living cells to multiply. Thus, will not multiply outside of the pig.
- Young infected pigs excrete large amounts of virus in their fecal material
 - 1 g can potentially infect millions of pigs
 - Relatively short period of time when peak shedding occurs
- Newly infected farms will have lots of virus
 - May contaminate the environment outside the buildings
 - Large volume of dead pigs lead to potential cross contamination path ways
- Nursery and Growing pig sites
 - May exhibit mild clinical signs
 - Many sites may be infected but unknown status
 - Packing plant and truck transport transmission is a primary route
 - Need to always treat these sites as if they were positive





What are some fundamental concepts of Swine Enteric Corona Virus Biology to remember ?

- On a positive note many farms have been able to keep from getting infected using good biosecurity procedures:
 - Segregated trucking
 - Weaned pigs vs market pigs
 - Washing, disinfecting and drying trucks every time before hauling pigs
 - Managing risk ingredients appears to have an influence on transmission pathway
 - Eliminating creep feeding
 - Eliminating porcine proteins from diets
 - Do not let the virus load become extreme



What are some fundamental concepts of Swine Enteric Corona Virus Biology to remember ?

- Many infected farms have been able to:
 - Eliminate the virus from the sow farm environment
 - Begin producing negative weaned pigs
 - Recover reproductive performance
- The Canadian Swine Industry has had success limiting the spread of the virus



"At the end of the day, we still need to feed the pigs!"

K-State Applied Swine Nutrition Team

