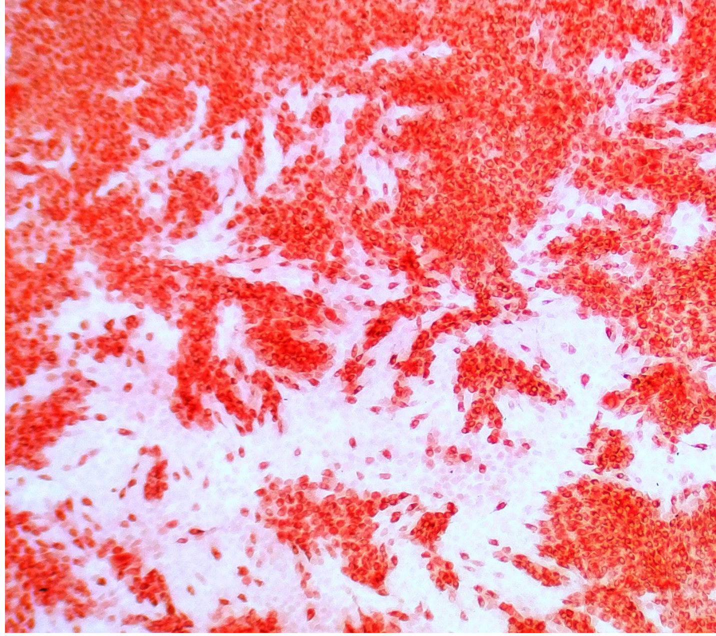
VETERINARY MEDICINE

BVDV in Heterologous Species

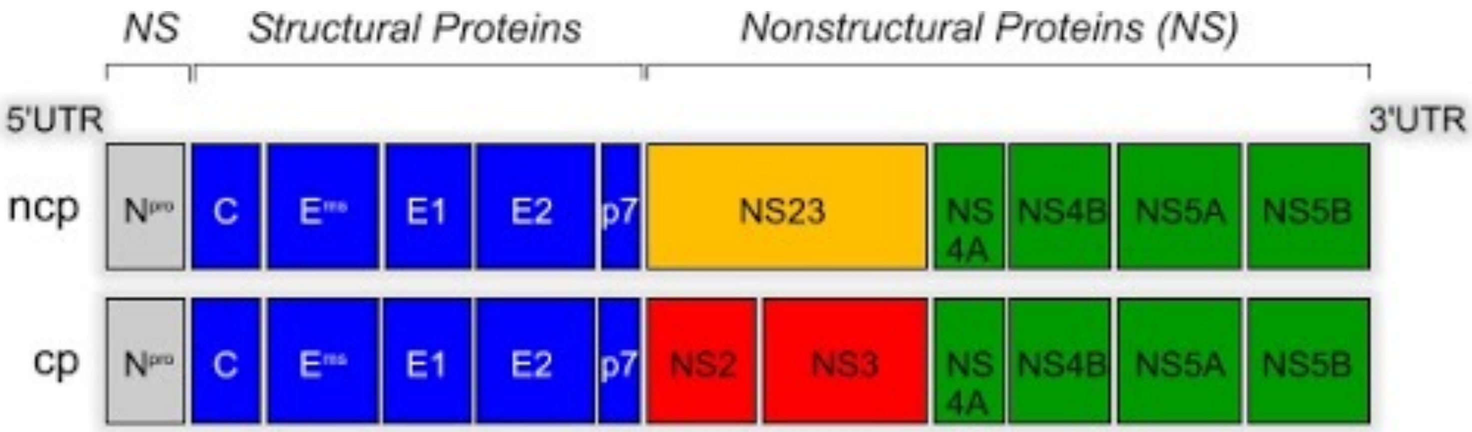
Shari M Kennedy, DVM, PhD. Candidate, DACVIM-LAIM
Assistant Professor, Food Animal Medicine and Surgery

Bovine Viral Diarrhea Virus



Viral Basics

- Genus *Pestivirus*, family *Flaviviridae*
- Positive-sense, single-stranded RNA genome
- Single open reading frame (ORF)- large polyprotein
  - Four structural: C, E^{rns}, E1, E2
  - Eight non-structural: N^{pro}, NS2, NS3, NS4A, NS4B, NS5A, NS5B
- Order Artiodactyla
  - Acute/Transient Infections
  - Congenital



http://www.bvd-info.ch/static/veterinarians/genome.html

Worldwide Antigen prevalence 2010-2021

(Su, Wang et al. 2022)

TABLE 2 Antigen prevalence of Bovine viral diarrhea virus of cattle in the word.

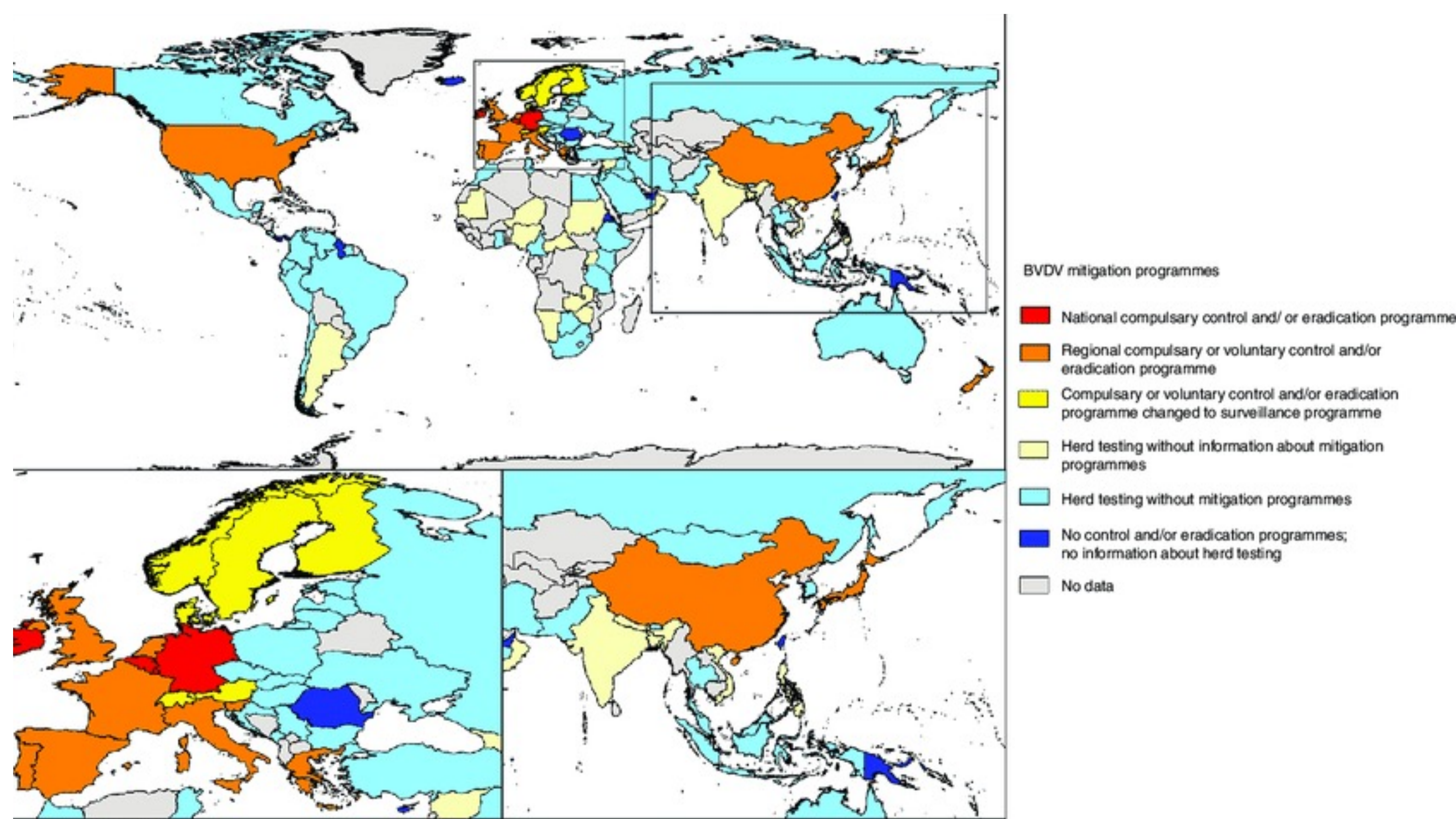
	No. studies	No. tested	No. positive	% (95% CI*)
Area*				
Asia	43	27,333	2,957	16.75% (11.27–23.04)
Europe	2	1,138	96	23.27% (0.00–89.41)
North America	1	7,544	24	0.32% (0.20–0.46)
South America	5	10,196	411	10.55% (2.63–22.82)
Sampling years				
Before 2017	30	26,608	1,625	17.18% (11.08–24.27)
After 2017	21	9,513	1,156	17.91% (10.33–26.99)

Worldwide Antibody prevalence 2010-2021
(Su, Wang et al. 2022)

TABLE 4 Antibody prevalence of Bovine viral diarrhea virus of cattle in the word.

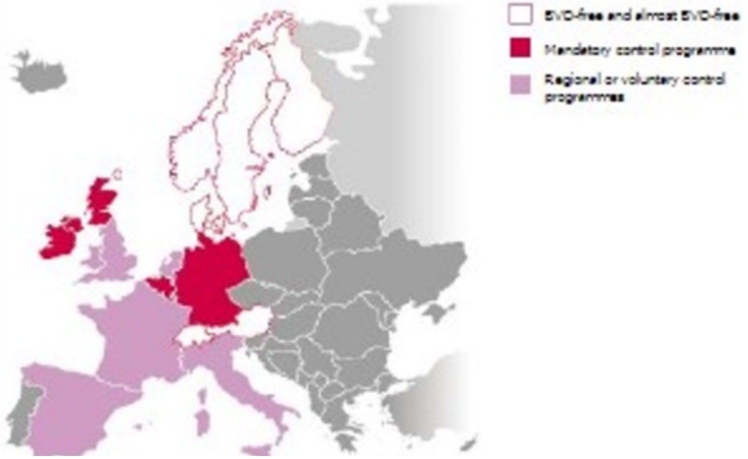
	No. studies	No. tested	No. positive	% (95% CI*)
Area				
Asia	71	53,457	23,597	42.03% (35.99–48.18)
Europe	1	180	82	45.56% (38.32–52.88)
North America	1	385	184	47.79% (42.81–52.79)
South America	1	390	298	76.41% (72.06–80.50)
Africa	3	937	424	46.13% (26.82–66.06)
Sampling years				
Before 2017	55	33,177	15,345	43.63% (37.25–50.13)
After 2017	19	11,619	6,319	41.11% (27.05–55.95)

(Su, Wang et al. 2022)


BVDV mitigation programmes
National compulsary control and/ or eradication programme
Regional compulsary or voluntary control and/or eradication programme
Compulsary or voluntary control and/or eradication programme changed to surveillance programme
Herd testing without information about mitigation programmes
Herd testing without mitigation programmes
No control and/or eradication programmes; no information about herd testing
No data

Implications of a resourceful virus

- The economic impact can be evaluated for BVDV by assessing differences in performance in the presence or absence of a PI animal
  - The average direct losses per:
    - naïve dairy cow were **$199.50**
    - beef cow **$174.60**
    - animal in the feedlot **$93.52**
- Cost of testing
- Exposure of heterologous hosts
  - sheep, goats, deer, camels, alpaca, pigs, and a wide array of ungulate wildlife
  - Seroprevalence varied from 0-45% among herding districts for Reindeer in Norway
  - Feral swine seroprevalence ~6%
    - Individual state range 0-16.7%
    - CA, OK, TX highest seroprevalence
- Fetal bovine serum (500 ml bottles)
  - Super value - $446
  - Value FBS - $676
  - Premium - $800 US, $1656 New Zealand, $1608 Australia
  - Specialty - FBS call for pricing



BVDV Heterologous hosts

DOMESTIC SWINE

Genetic Changes PI vs CI

FERAL SWINE/WILD PIGS

Seroprevalence in US

WHITE-TAILED DEER

Commercial Test Comparisons

CATTLE

Swine vs Bovine BVDV PI Genetic

BVDV Genetic evaluation: congenitally infected piglets

- Hypothesis:
  - Viral change→ clearance of congenital infection
- Specific objectives:
  - Quantify/Characterize nucleotide changes
  - Compare over time, between CI and PI

Chronic Infection Considerations

- CI vs PI
  - Eventual clearance of the virus
  - Serum neutralizing antibodies
- Timing of clearance?
  - Terpstra et al 1997
    - One month of age
    - 6-8 months of age
- Mechanism of clearance?
- Inciting cause of viral clearance?

TIMELINE- Genetic Evaluation Pigs

DAY 26/27 GESTATION	GESTATION	FARROWING	VIREMIA/ SEROCONVERT	GENOME SEQUENCING
IN and IV Inoculation BVDV-1b NCP AU-526	**Gilts** Monthly Trans-abd US and virus neutralization	**Piglets** Ear notch- ACE Virus isolation, whole genome sequencing, virus neutralization	**Piglets** Every 2 weeks: Virus solation and virus neutralization	**PI vs CI piglets V Inoculum** Next-gen and Sanger

Results

- 4 Litters- 2019
  - 26 live-born
  - 9 antigen positive at birth
    - Antigen Capture ELISA
    - PCR
  - 4D- chronically infected
    - Seroconverted ~5.5-6 months of age

Results: Repeat

- 3 Litters- 2020
  - 34 live-born piglets
  - 7 antigen positive at birth
    - Antigen Capture ELISA
  - 6 virus positive at birth
    - Virus isolation
  - 8H Chronic Infection
    - Seroconverted- ~6-8 weeks

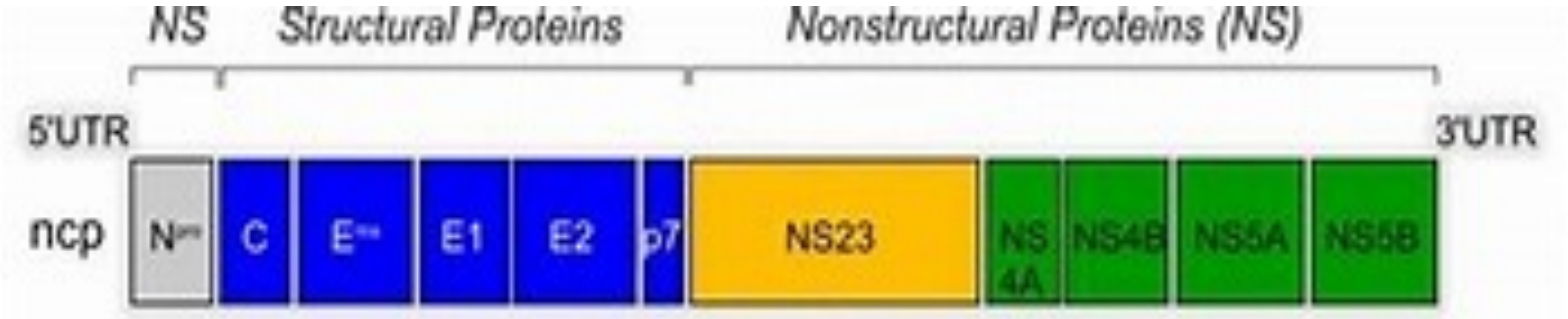
Results- BVDV sequencing

WHOLE-GENOME/NEXT-GEN

- 98.8% Identical to AU526 460F6 inoculum

SANGER

- 15 congenitally infected piglets



Nucleotide difference between Inoculum and Piglets

	Total	Transition	Transversion	Npro	C	Erns	E1	E2
460F6:4A	3	1	2	0	0	0	0	3
460F6:4C	3	2	1	1	0	0	0	2
460F6:4D	6	5	1	1	0	1	0	4
460F6:4F	3	2	1	0	0	0	0	3
460F6:4G	75	52	23	NA	10	6	18	41
460F6:4H	6	5	1	1	0	1	1	3
Median	4.5	3.5	1	1	0	0.5	0	3
Mean	14.4	10.1	4.3	0.7	1.4	1.2	2.7	8.4
	Total	Transition	Transversion	Npro	C	Erns	E1	E2
460F6:8A	2	1	1	0	0	0	0	2
460F6:8B	2	1	1	0	0	0	0	2
460F6:8E	2	1	1	0	0	0	0	2
460F6:8H	2	2	0	0	0	0	0	2
460F6:8L	3	1	2	0	0	0	0	3
Median	2	1	1	0	0	0	0	2
Mean	2.2	1.2	1	0	0	0	0	2.2

Amino Acid difference between Inoculum and Piglets

	Total	**Npro**	**C**	**Erns**	**E1**	**E2**
460F6:4A	13	0	1	2	0	10
460F6:4C	16	0	2	1	0	13
460F6:4D	15	1	1	3	0	10
460F6:4F	11	0	1	1	0	9
460F6:4G	46	10	2	3	1	30
460F6:4H	14	2	1	2	1	8
Median	14.5	0.5	1	2	0	10
Mean	18.5	1.9	1.3	2.0	0.3	12.9
	Total	Npro	C	Erns	E1	E2
460F6:8A	12	0	1	1	0	10
460F6:8B	11	0	1	1	0	9
460F6:8E	11	0	1	1	0	9
460F6:8H	11	0	1	1	0	9
460F6:8L	11	0	1	1	0	9
Median	11	0	1	1	0	9
Mean	11.2	0.0	1.0	1.0	0.0	9.2

Conserved Amino Acids in Piglets

Viral Protein	E2				
AA Position	43	60	103	291	365
460-f6	X	E	L	E	M
460F6:4A	T	E	L	K	L
460F6:4C	T	X	X	K	L
460F6:4D	I	A	L	K	L
460F6:4F	T	E	L	K	L
460F6:4G	T	E	L	K	L
460F6:4H	X	K	L	X	M
460F6:8A	I	K	I	X	M
460F6:8B	I	K	I	X	M
460F6:8E	I	K	I	X	M
460F6:8H	I	K	I	E	M
460F6:8L	I	K	I	X	M

Phylogenic analysis

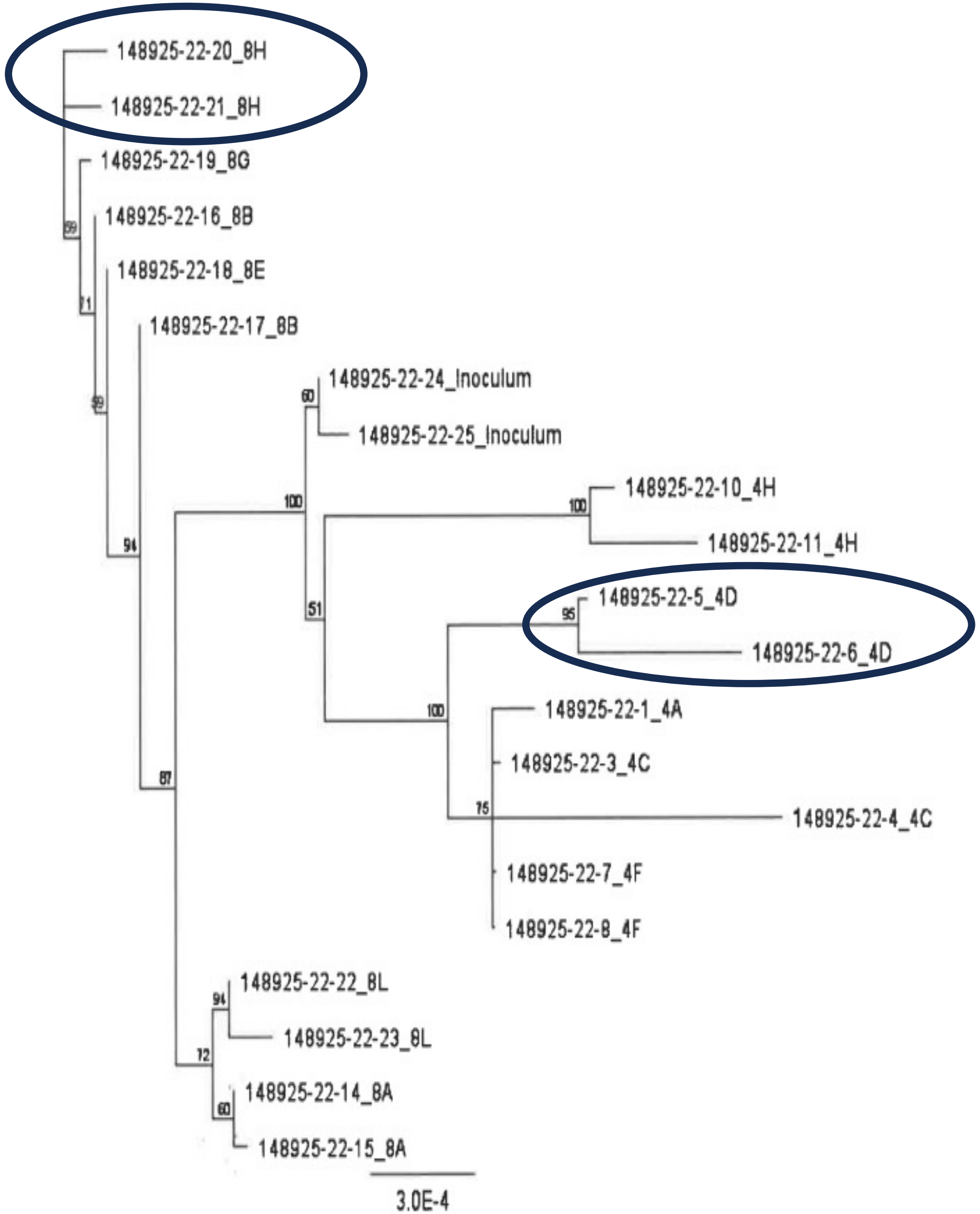


Figure 27: Phylogenetic tree of the whole genome consensus alignments, excludes 148922-22-2, 9, 12, 13 due to having less than 95% coverage.

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Seroprevalence of Bovine Viral Diarrhea Virus in Wild Pigs (*Sus scrofa*) in 17 States in the USA

Shari M. Kennedy,[1,5,6] Thomas Passler,[1] Stephen S. Ditchkoff,[2] Vienna R. Brown,[3] Gage W. Raithel,[4] Manuel F. Chamorro,[1] Paul H. Walz,[4] Constantinos S. Kyriakis,[4] and Shollie M. Falkenberg[4]

USDA Wildlife Services

- Current US Feral Swine/Wild Pig BVDV knowledge:
  - 0% Great Smokey Mountains National Park
  - doi: 10.7589/0090-3558-30.1.103.
- Seroprevalence rate for states with higher feral swine/wild pig or cattle population states

Feral Swine Seroprevalence

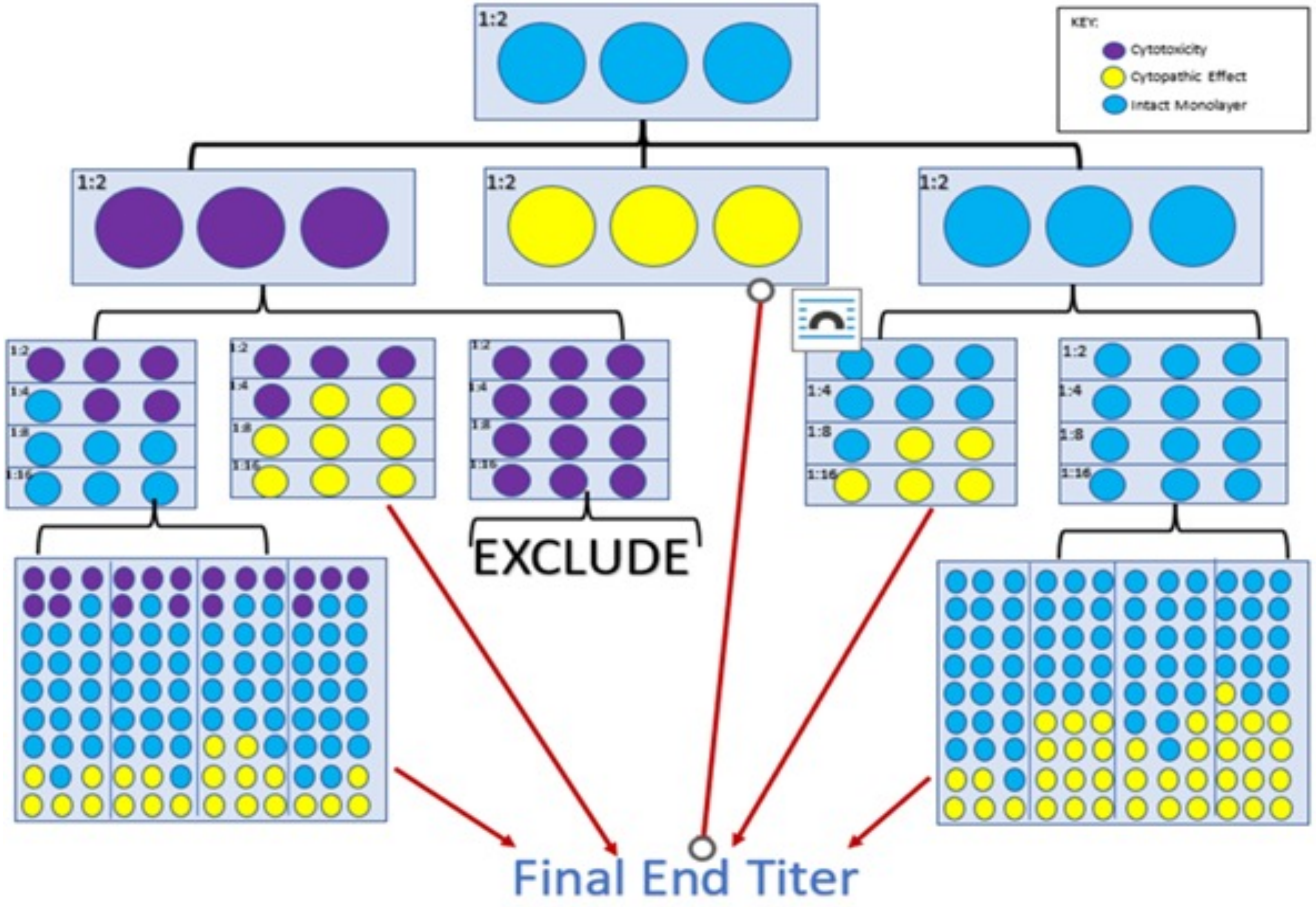
- Objective:
  - Determine BVDV seroprevalence in wild pigs in US
  - Determine if age, sex, or location associated with positive antibody titer

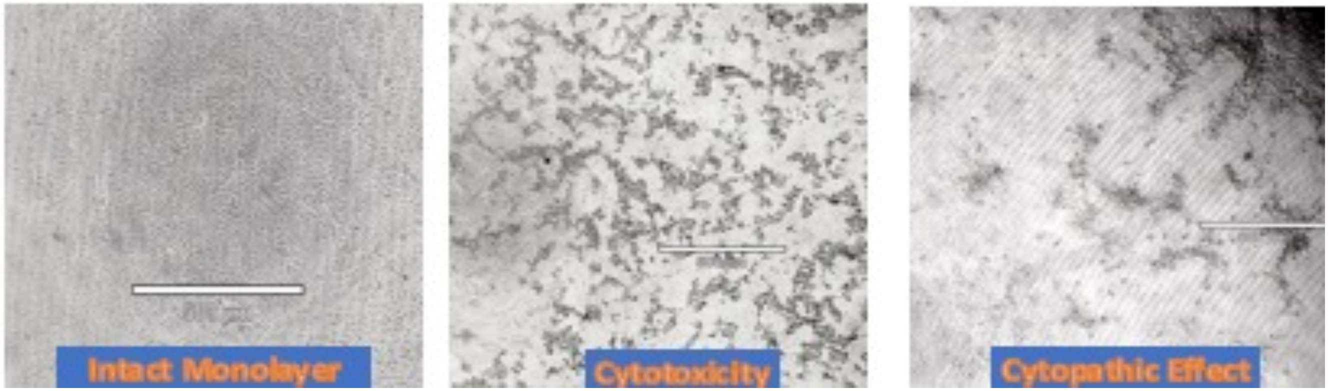

USDA Wildlife Services

Methods

Approach:

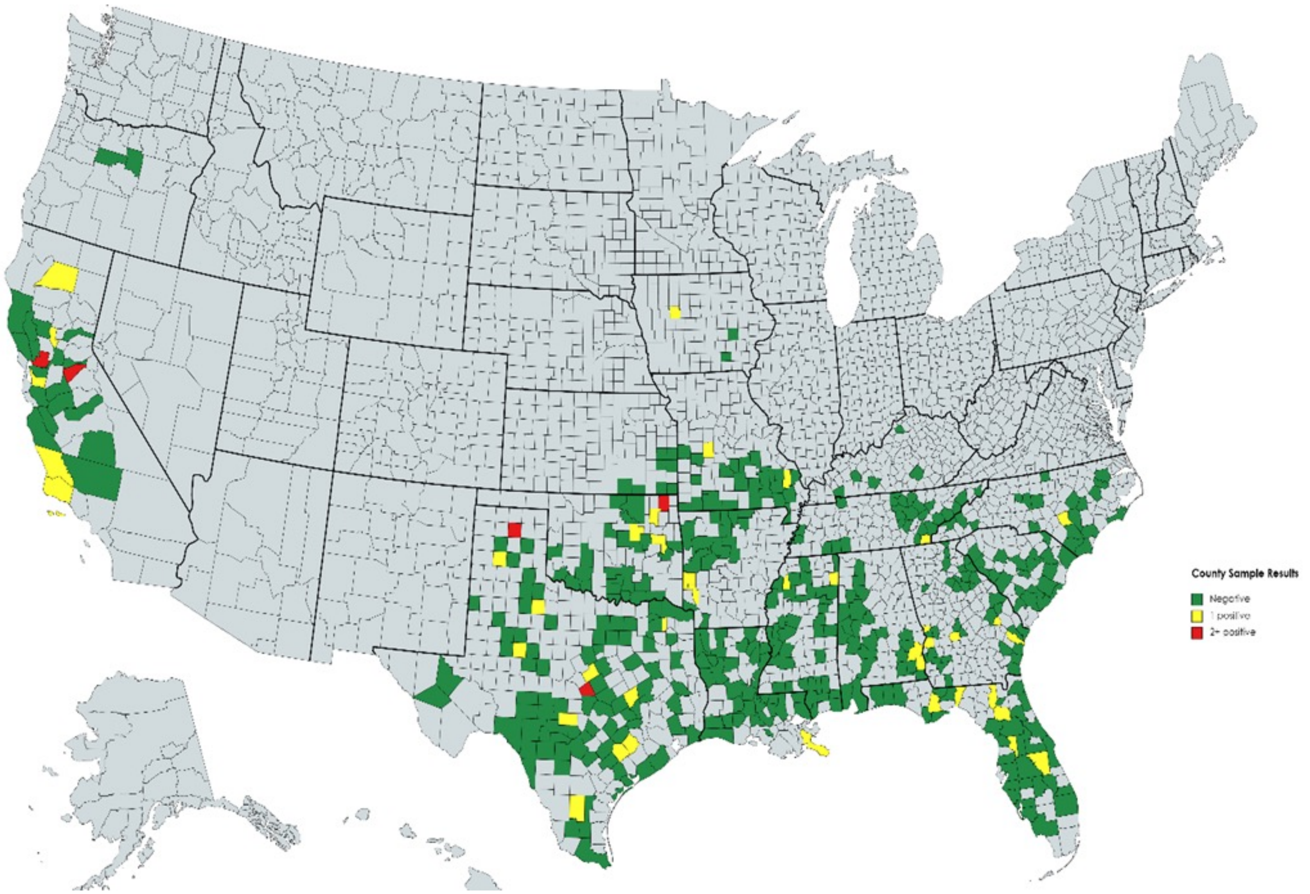
- 945 serum convenience samples from 17 states collected by USDA-ARS NFSDM program and Auburn University College of Forestry, Wildlife and Environment
- Virus neutralization- 1:2 serial dilutions until final titer





Feral swine BVDV seroprevalence: Results

- 67 samples excluded from cytotoxicity
- 878 samples analyzed
- Total BVDV seroprevalence= **5.8%**
- BVDV-1b seroprevalence= **4.4%**
- BVDV-2a seroprevalence= **3.6%**
- Individual states 0-16.7%
- No difference in mean antibody titer BVDV-1b/2a by sex or age
- **CA, OK, TX**= greatest seroprevalence
    - Also, largest cattle and most established wild pig population states



Comparative BVDV COMMERCIAL TESTS

Commercially available and validated for Cattle

- RealPCR BVDV RNA Mix
- IDEXX SNAP BVDV Antigen Test ★
- IDEXX BVDV PI X2 Test
- IDEXX BVDV Total Ab Test
- IDEXX BVDV Ag/Serum Plus Test
- IDEXX BVDV p80 Ab Test
- IDEXX BVDV Ag Point-of-Care Test ★

Hypothesis:

- Heterologous hosts would have an elevated false-positive ACE results

★ Could easily be used by producers and veterinarians

https://www.idexx.com/en/livestock/livestock-tests/ruminant-tests/idexx-bvdv-pi-x2-test/

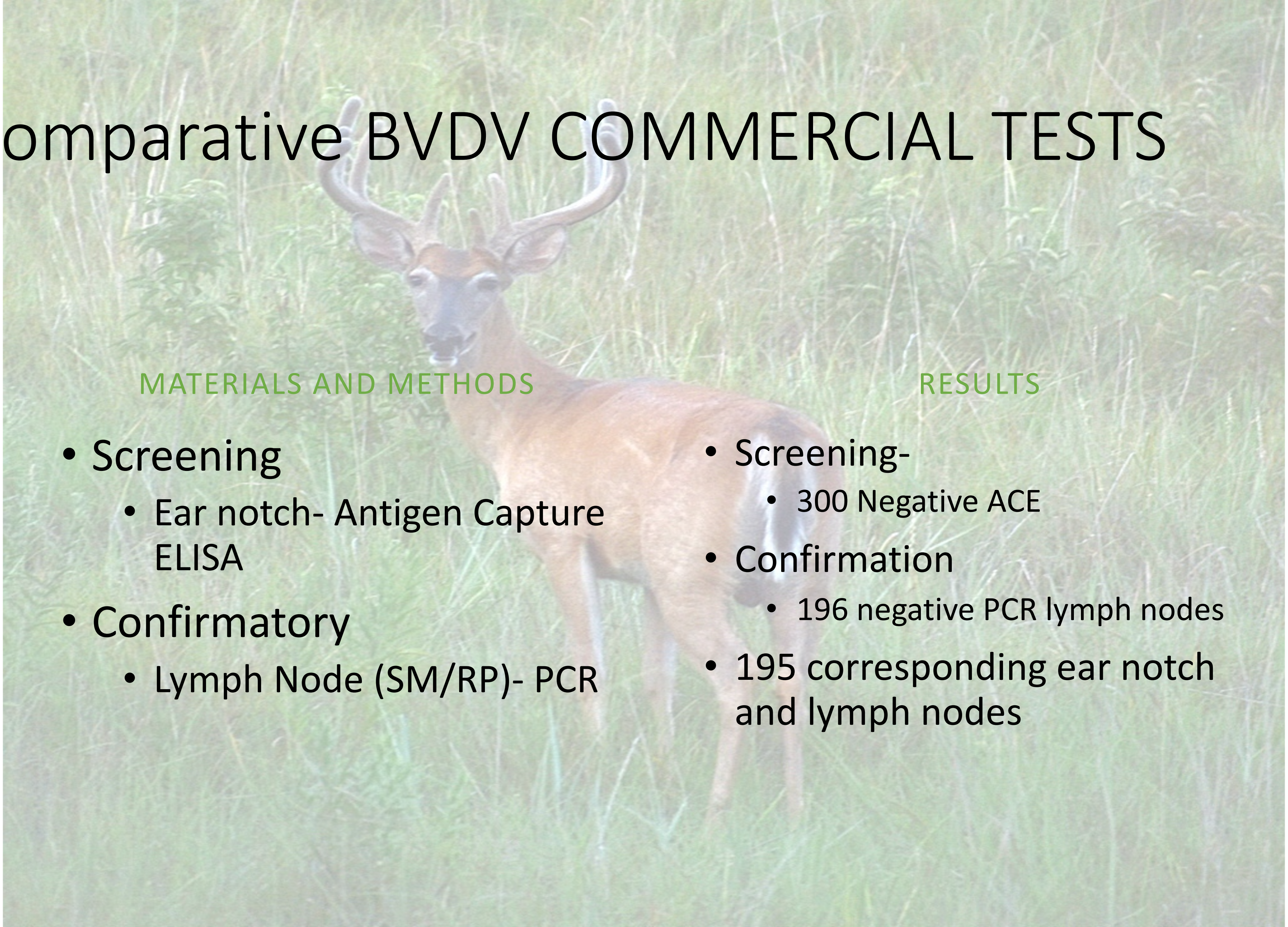
Appropriate testing?- DR. PASSLER

Year	acELISA*	Immunohistochemistry	RT-nPCR
2006/07 440 deer	22 Positive		
2007/08 577 deer	7 Positive, 13 Suspect	5 Positive, 4 Suspect	2 Positive
2008/09 590 deer	4 Suspect, 3 Positive	Pending	0 Positive

*S/P ratios : Suspect 0.2 – 0.39, Positive: > 0.39

Species	Sample	Test	Results: no. (%)	Confirmation?	Reference
1. Rocky Mountain Bighorn Sheep 2. Mountain goats 3. Mule Deer	Serum	BVDV ACE[a]	Antigen positive 1: 0/10 (0) 2: 0/30 (0) 3: 6/84 (7.1); 0/19 (0)	No	Wolff et al 2016
Algerian Dromedary Camel	Serum	BVDV antibody* ELISA and BVDV antigen[t] ELISA	Antibody positive • 10/111 (9.0) Antigen positive • 46/111 (41.4)	No	Saidi et al 2018
White-tailed deer	Ear notch/ Lymph node	BVDV ACE[a]	Antigen positive • Ear notch 1/311 (0.32) • Lymph node 1/434 (0.23)	Yes • PCR, VI, Sequence analysis- positive samples	Pogranichniy et al 2008
White-tailed deer	Ear Notch	BVDV ACE[b]	Antigen suspect • 3/367 (0.82)	Yes • Immunohistochemistry, VI, and PCR- suspect samples Screening subset • 89/367 PCR negative	Ilha et al 2012
Austrian Red Deer	Ear Notch	BVDV ACE[a]	Antigen positive • 0/764 (0)	Yes • PCR- all samples	Glawischnig et al 2010
Bactrian camels	Serum	BVDV ACE[a]	Antigen positive • 17/56 (30.4)	Yes • PCR- positive samples	Gao et al 2013
Sheep	Serum	BVDV[c,d]	Antibody positive • 27/42 (64.3) inoculated sheep	Yes • Agarose gel immunodiffusion (AGID) • All samples	Evans et al 2017

Comparative BVDV COMMERCIAL TESTS



MATERIALS AND METHODS

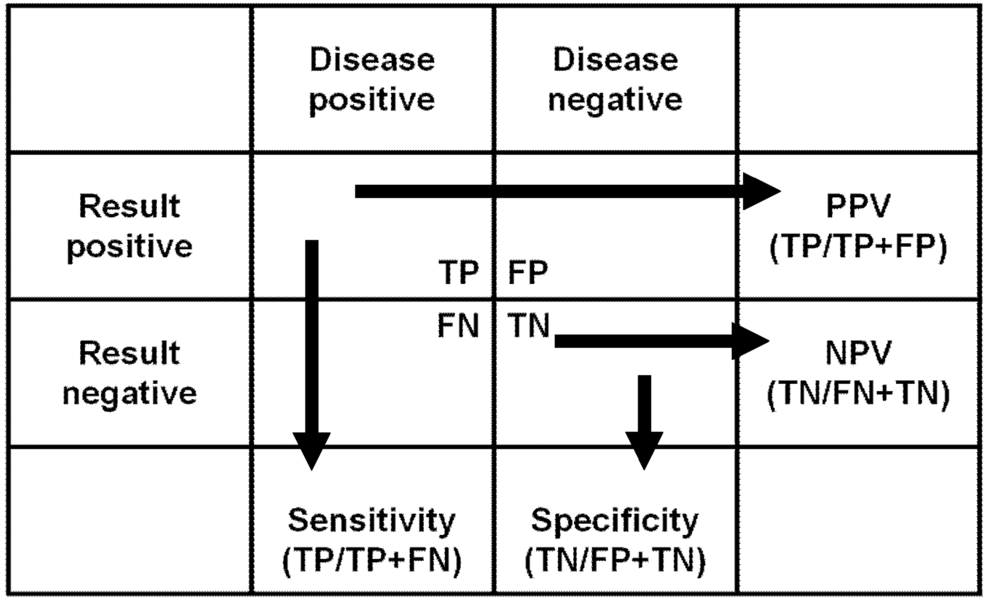
- Screening
  - Ear notch- Antigen Capture ELISA
- Confirmatory
  - Lymph Node (SM/RP)- PCR

RESULTS

- Screening-
  - 300 Negative ACE
- Confirmation
  - 196 negative PCR lymph nodes
- 195 corresponding ear notch and lymph nodes

Comparative test conclusion

- Reliably detect negative or non-viremic animals.
- High correlation (sensitive) between IDEXX BVDV PI X2 Antigen-capture ELISA with PCR
- High negative predictive value
- Unknown specificity- likely high false positives in previous studies?



https://www.google.com/url?sa=i&url=https%3A%2F%2Fundefined.photos%2Fphoto-gallery%2Fnegative-predictive-value-vs-specificity&psig=AOvVaw0HiFSp4ukg3i3ddAKlwdEr&ust=1717518195553000&source=images&cd=vfe&opi=89978449&ved=0CBIQjhxqFwoTCLCiiYHsv4YDFQAAAAAdAAAAABAI

Swine vs Bovine BVDV isolate PI infection

- Evaluate antigenic changes during immune recognition of pregnant cattle and creation of PI
  - BVDV propagated in heterologous hosts
  - BVDV propagated in cattle hosts

Swine Isolate

460f6

Pre-colostrum viremia PI calf

Acute Infection Heifer

Inoculum

VS

Bovine Isolate

AU-526

Pre-colostrum viremia PI calf

Acute Infection Heifer

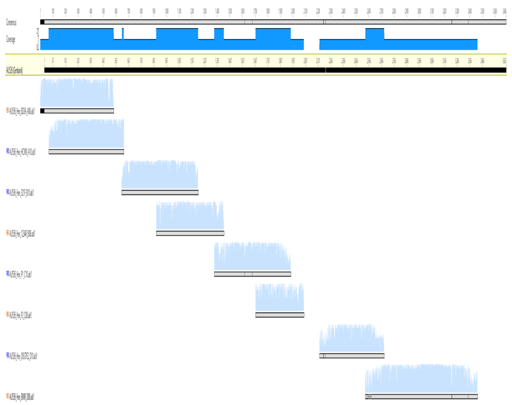
Inoculum

Research timeline- swine vs cattle

DAY 75 GESTATION	ACUTE INFECTION	GESTATION	PARTURITION	SEQUENCING







Nucleotide difference between Inoculum and PI Calves

	Total	Transition	Transversion	Npro	C	Erns	E1	E2
01L	41	21	10	2	5	11	4	19
02L	9	3	0	0	0	1	4	4
03L	27	22	5	NA	3	9	4	11
04L	7	4	3	1	1	1	2	2
05L	35	29	6	5	3	10	5	12
06L	32	26	6	0	4	12	4	12
07L	38	31	7	3	4	11	5	15
08L	6	3	3	1	1	1	1	2
09L	34	25	9	4	3	9	5	13
10L	4	2	2	1	1	0	1	1
Median	29.5	21.5	5.5	1	3	9	4	11.5
Mean	23.9	17.0	5.1	1.8	2.5	6.7	3.5	9.3

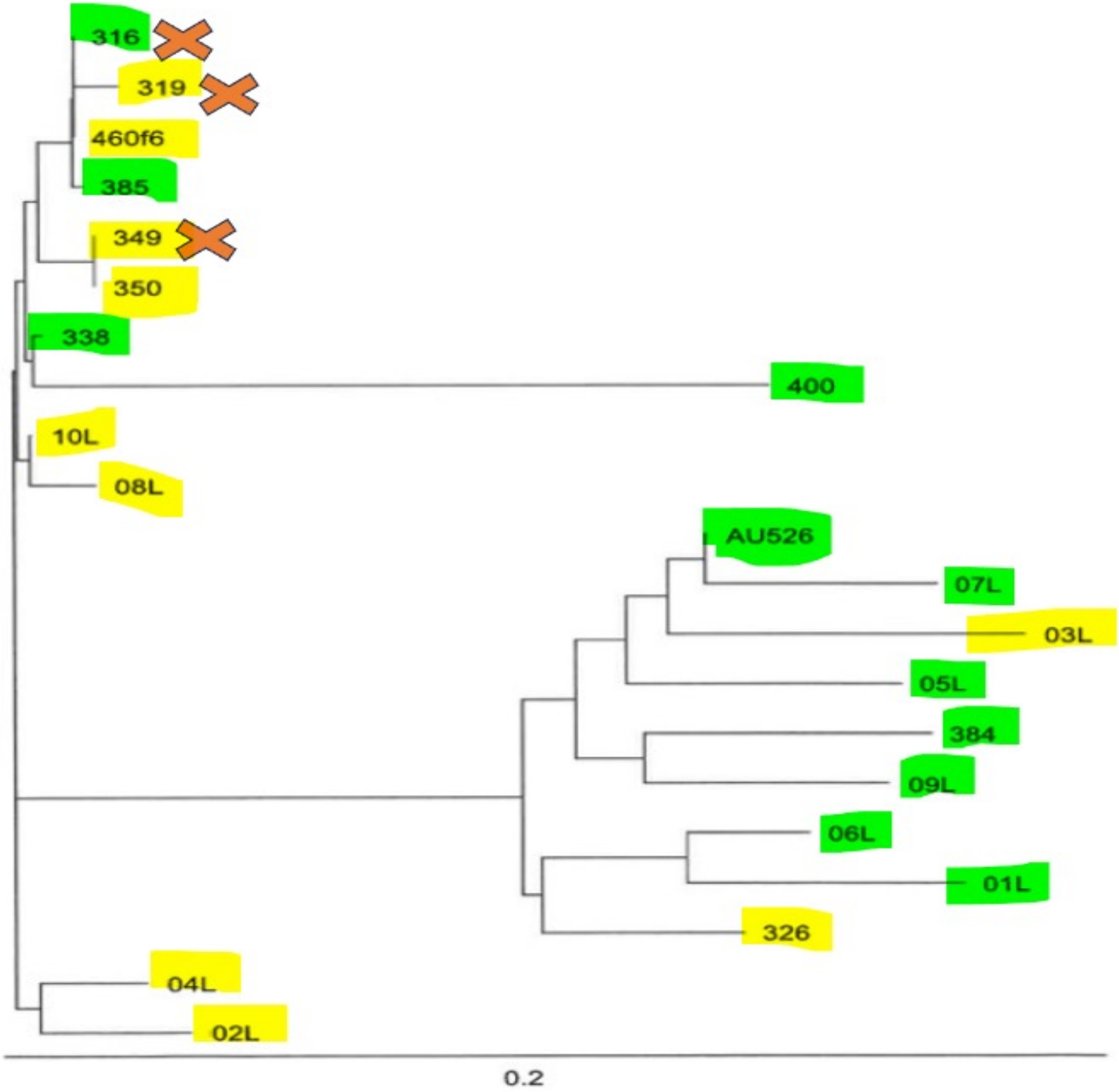
Amino Acid difference between Inoculum and PI Calves

	Total	Npro	C	Erns	E1	E2
01L	19	1	2	7	2	7
02L	8	0	0	1	4	3
03L	11	NA	1	1	2	7
04L	6	0	1	1	2	2
05L	11	0	1	1	2	7
06L	16	0	1	8	2	5
07L	11	0	2	1	2	6
08L	3	0	1	0	1	1
09L	15	1	1	3	3	7
10L	3	0	1	0	1	1
Median	11	0	1	1	2	5.5
Mean	10.4	0.2	1.1	2.2	2.1	4.7

Conserved Amino Acids in PI calves

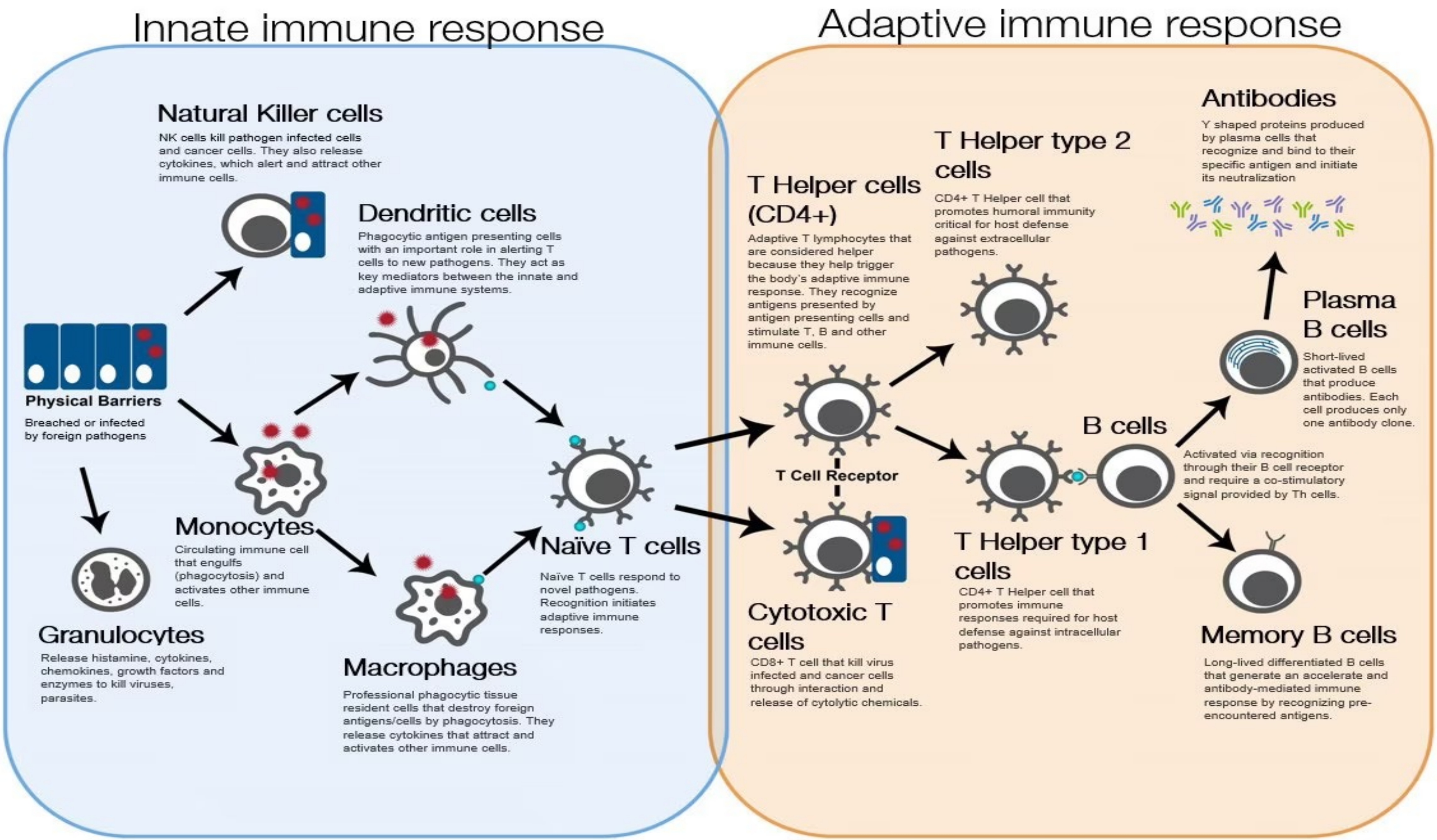
Viral Protein	C	Erns	E1				E2							
AA Position	6	36	49	169	178	209	8	36	57	213	254	281	301	373
460-f6	N	K	T	D	A	R	Y	R	T	S	R	H	K	L
01L	D	R	I	H	T	K	Y	G	T	F	R	Y	T	S
02L	N	K	T	D	A	G	Y	R	T	S	Q	H	K	L
03L	D	K	T	H	T	K	Y	G	I	F	R	Y	S	S
04L	D	K	T	D	A	G	Y	R	T	S	Q	H	K	L
05L	D	K	T	H	T	K	F	G	I	F	R	Y	R	S
06L	D	R	I	H	T	R	Y	G	T	F	R	Y	T	S
07L	D	K	T	H	T	K	F	G	I	F	R	Y	S	S
08L	D	K	T	D	A	R	Y	R	T	S	R	H	K	L
09L	D	K	T	H	T	G	F	G	T	F	L	H	R	S
10L	D	K	T	D	A	R	Y	R	T	S	R	H	K	L

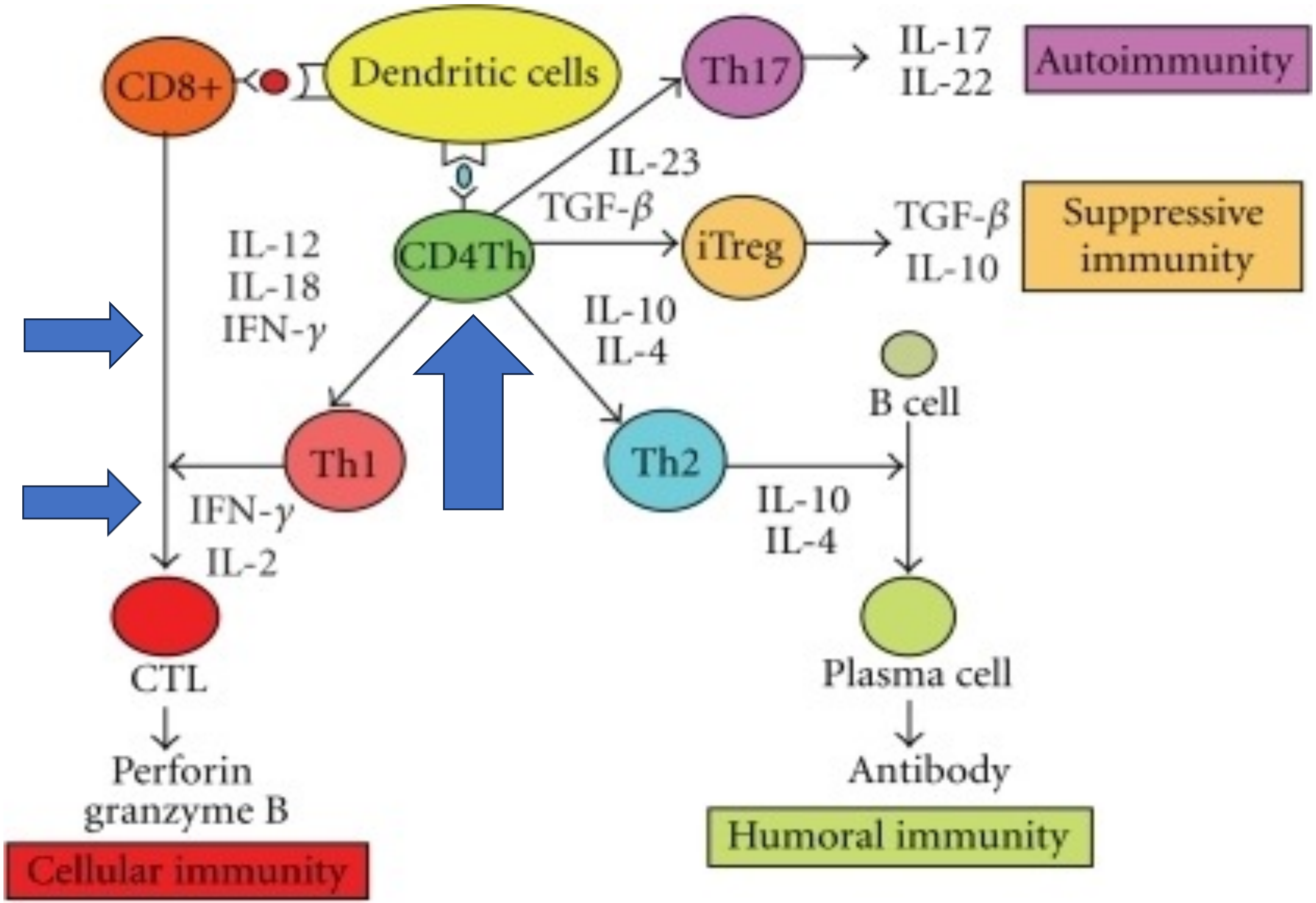
Swine vs Cattle Genetics Results



Cell-mediated immunity during pregnancy recognition with a PI fetus

- Does pregnancy with a PI fetus alter the cell mediated immunity and the ability for the adaptive immune system to respond to both specific and non-specific challenges?


Innate immune response
Adaptive immune response
Natural Killer cells
NK cells kill pathogen infected cells and cancer cells. They also release cytokines, which alert and attract other immune cells.
Dendritic cells
Phagocytic antigen presenting cells with an important role in alerting T cells to new pathogens. They act as key mediators between the innate and adaptive immune systems.
Physical Barriers
Breached or infected by foreign pathogens
Monocytes
Circulating immune cell that engulfs (phagocytosis) and activates other immune cells.
Granulocytes
Release histamine, cytokines, chemokines, growth factors and enzymes to kill viruses, parasites.
Macrophages
Professional phagocytic tissue resident cells that destroy foreign antigens/cells by phagocytosis. They release cytokines that attract and activates other immune cells.
Naïve T cells
Naïve T cells respond to novel pathogens. Recognition initiates adaptive immune responses.
T Helper cells (CD4+)
Adaptive T lymphocytes that are considered helper because they help trigger the body's adaptive immune response. They recognize antigens presented by antigen presenting cells and stimulate T, B and other immune cells.
T Cell Receptor
Cytotoxic T cells
CD8+ T cell that kill virus infected and cancer cells through interaction and release of cytolytic chemicals.
T Helper type 2 cells
CD4+ T Helper cell that promotes humoral immunity critical for host defense against extracellular pathogens.
T Helper type 1 cells
CD4+ T Helper cell that promotes immune responses required for host defense against intracellular pathogens.
Antibodies
Y shaped proteins produced by plasma cells that recognize and bind to their specific antigen and initiate its neutralization
Plasma B cells
Short-lived activated B cells that produce antibodies. Each cell produces only one antibody clone.
B cells
Activated via recognition through their B cell receptor and require a co-stimulatory signal provided by Th cells.
Memory B cells
Long-lived differentiated B cells that generate an accelerate and antibody-mediated immune response by recognizing pre-encountered antigens.


CD8+
Dendritic cells
Th17
IL-17
IL-22
Autoimmunity
IL-23
TGF-β
iTreg
TGF-β
IL-10
Suppressive immunity
IL-12
IL-18
IFN-γ
CD4Th
IL-10
IL-4
B cell
Th1
Th2
IFN-γ
IL-2
IL-10
IL-4
CTL
Plasma cell
Perforin
granzyme B
Antibody
Cellular immunity
Humoral immunity

CMI Assay Heifers

- Materials and Methods

Measuring CMI responses using the PrimeFlow RNA assay: A new method of evaluating BVDV vaccination response in cattle

Shollie M. Falkenberg[a,*], Rohana P. Dassanayake[a], John D. Neill[a], Paul H. Walz[b], Eduardo Casas[a], Julia F. Ridpath[a], James Roth[c]

[a] Ruminant Disease and Immunology Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, IA, 50010, United States
[b] Department of Pathobiology, College of Veterinary Medicine, 129 Sugg Laboratory, Auburn University, AL, 36849, United States
[c] Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, IA, United States

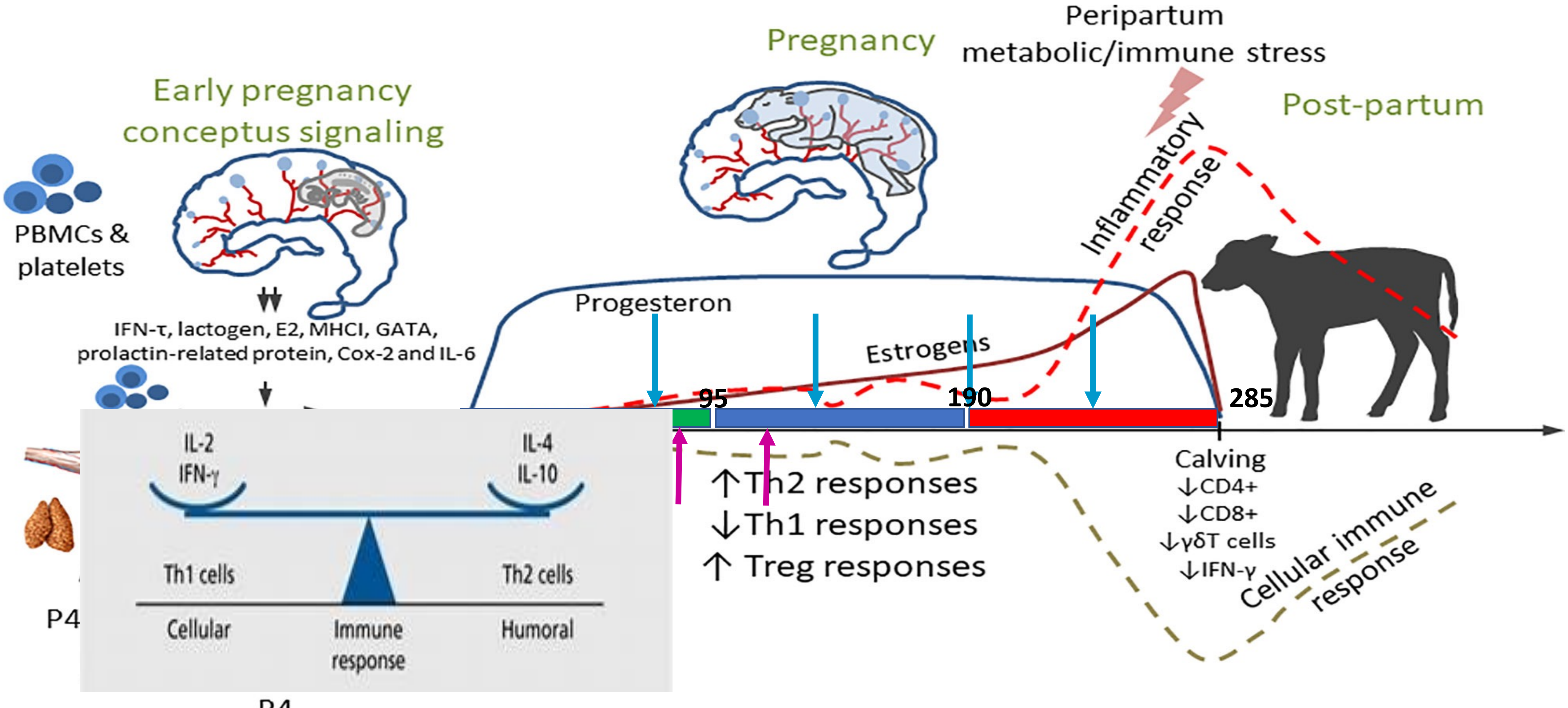
76
- Prior to Challenge
- Early gestation

130
- Early gestation
- 50 days post challenge

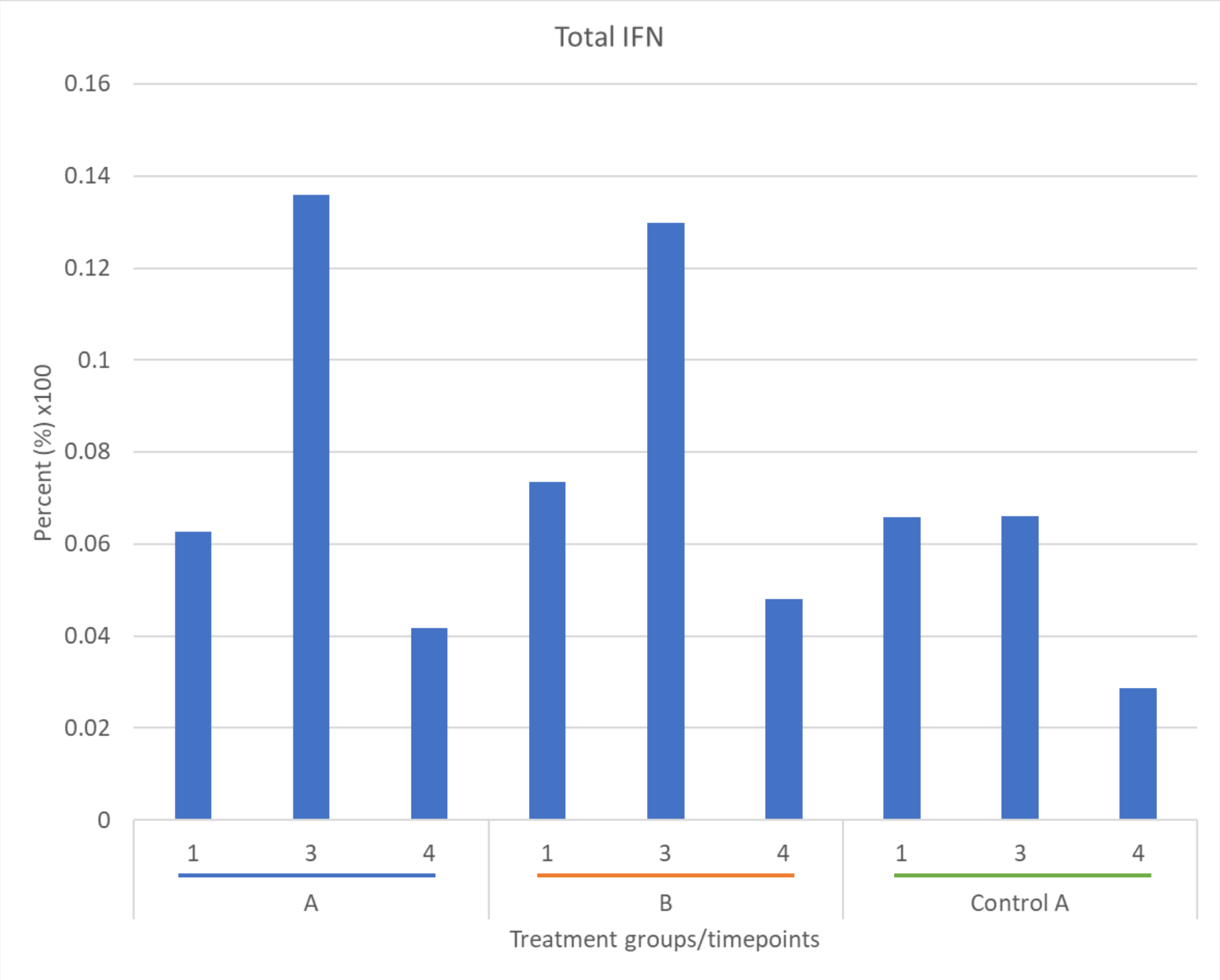
190
- Mid-gestation
- 110 days post challenge

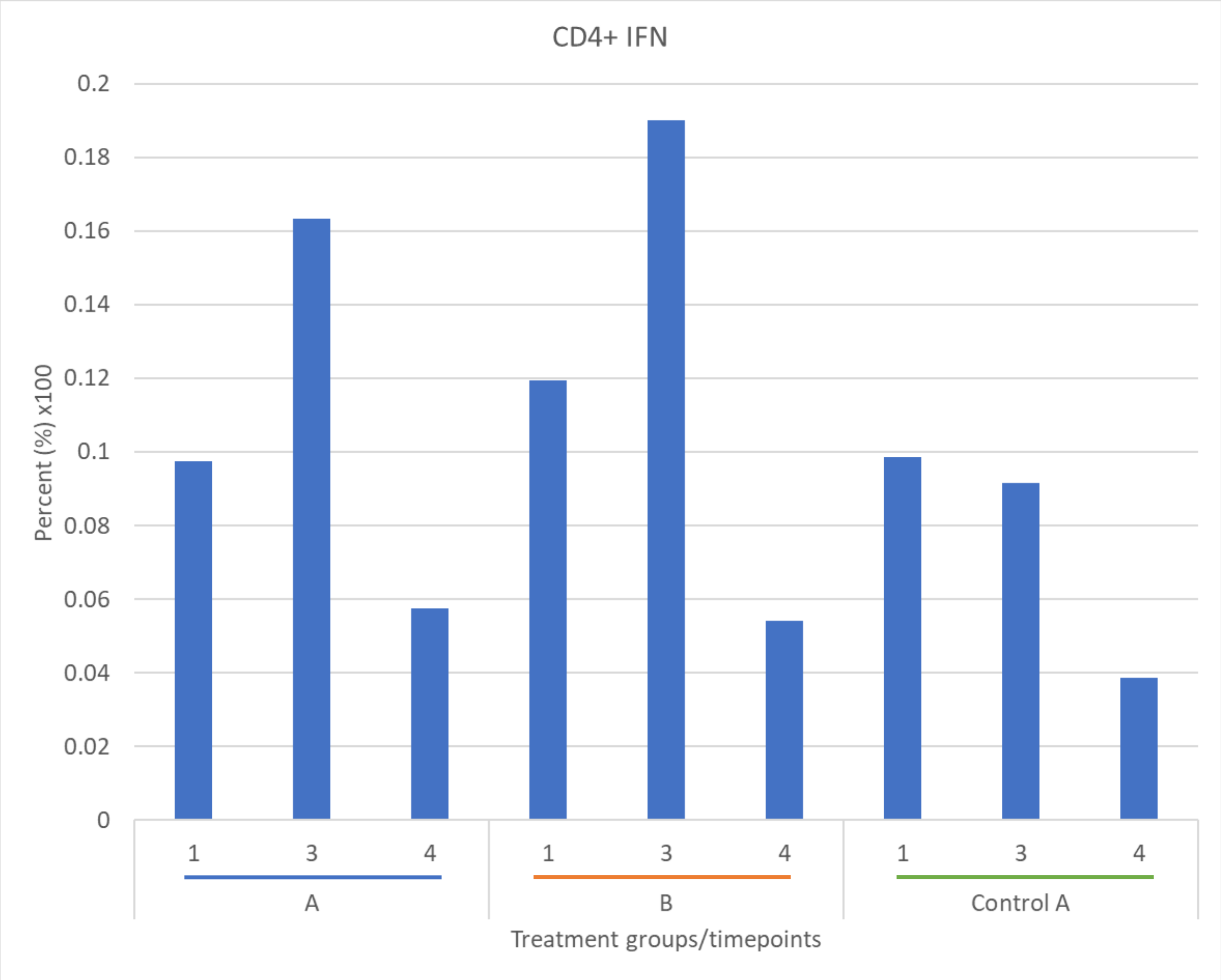
240
- Late-gestation
- 160 days post challenge

Immune response during pregnancy

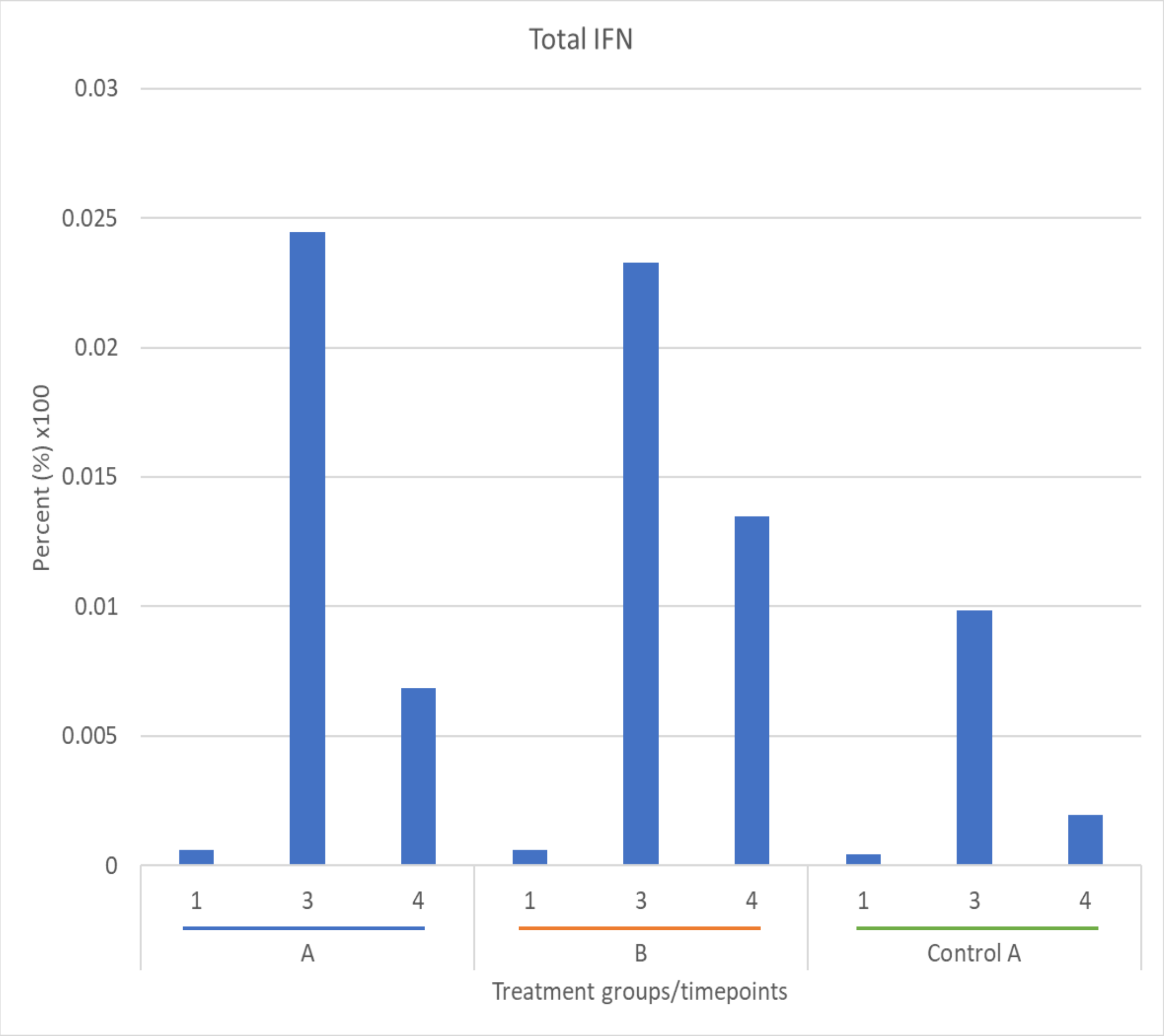


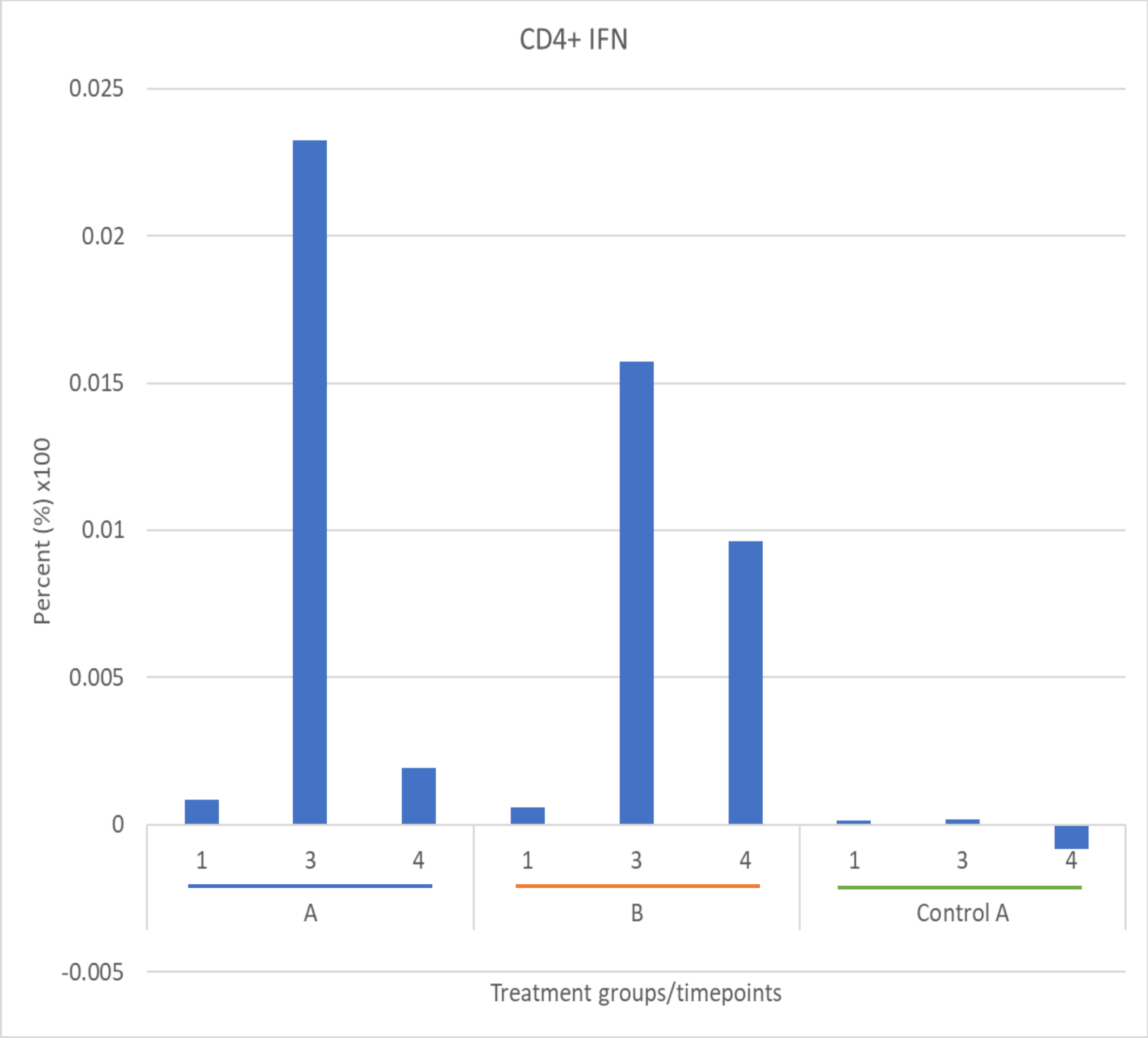
Heifers with PI and non-PI fetuses Mitogen stim- Total IFN and CD4s





Heifers with PI and non-PI fetuses BVDV stim – Total and CD4





Thank you

Shari Kennedy, DVM, PhD Candidate, DACVIM-LAIM

Department of Veterinary Clinical Sciences

O | 405.744.7000
C | 405.744.7000
E | shari.kennedy@okstate.edu
2065 W. Farm Road

vetmed.okstate.edu