# Using Aggregated Diagnostic Data in Swine Disease Surveillance and Monitoring

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## Surveillance vs Monitoring

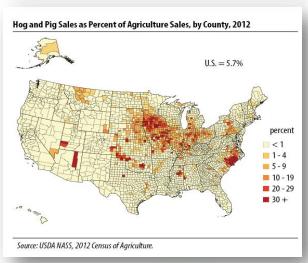
- While often used interchangeably, there is a difference in intent:
  - Surveillance intends to detect a pathogen if present
    - Often attempts to prove a negative
    - May be used to assess geographic distribution
    - Can be done periodically
    - Is generally 'actionable' if positive
      - Examples:
        - Sick pig surveillance for ASF/CSF
        - Processing fluids for PRRSV in a negative herd
  - Monitoring aims to detect temporal changes or trends
    - Can be pathogen detection or disease expression
    - Typically a process evaluation
    - Is generally performed continually
      - Examples:
        - M. hyopneumoniae ELISA in a negative herd
        - Processing fluids for PRRSV in unstable herd





- What is your specific goal?
  - Confidence can be considered at various levels:
    - Animal
      - Confidence relates solely to test characteristics (DSe, DSp)
    - Round
      - Where multiple animals are tested around the same time by the same method
      - Expected prevalence impacts sampling and confidence
    - Herd
      - One related unit of animals with a geographical area
      - Rounds of testing over time add to confidence
    - Zone
      - All herds within a geographical area (county, state, region, etc.)
  - Type & amount of testing is dependent upon use case









- How Many Samples Do I Need?
  - Classic Statistical Tables (Cannon and Roe, 1982)
    - Simple random sampling
      - Assumes perfect tests, homogeneous distribution, binomial distribution
      - Truly random, not just easiest pigs to catch
    - Best for point in time analysis (confirming regional status)
    - May require many samples to achieve desired confidence in a negative
  - Accumulated Temporal Data (Rotolo et al. 2017)
    - Fixed spatial sampling
      - Collect the same location repeatedly [same pen(s) every time]
    - Best for individual herd surveillance
    - Repeated sampling increases confidence with fewer samples





- Chase and Polson, 2000
  - Based upon Cannon and Roe
    - Extrapolated for large herd sizes
  - This is where the 30 serum samples per month for PRRSV surveillance is derived
    - If PRRSV enters a negative herd, 95% confident that more than 10% will be positive

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			Popu	ılation Si	ze (Dete	cting One	e or More	Positive	s)			
Prevalence Estimate % Positive	Confidence Level	100	200	400	600	800	1000	2000	4000	6000	8000	10000
>1%	70%	71	92	105	110	113	114	118	120	120	120	121
	80%	81	112	133	142	147	149	155	158	160	160	160
	90%	91	138	176	192	201	206	218	224	226	227	228
	95%	96	156	211	236	250	259	278	289	292	294	295
	99%	100	181	274	321	350	369	411	434	443	447	449
>2%	70%	46	53	57	58	59	59	60	61	61	61	61
	80%	56	67	74	76	77	78	80	80	81	81	81
	90%	69	88	101	105	108	109	112	114	114	115	115
	95%	78	106	125	133	137	139	144	147	148	148	149
	99%	91	137	175	191	200	205	217	223	225	226	227
>5%	70%	22	24	24	25	25	25	25	25	25	25	25
	80%	28	30	32	32	32	32	33	33	33	33	33
	90%	37	42	44	45	45	45	46	46	46	46	46
	95%	45	52	56	57	58	58	59	59	60	60	60
	99%	60	73	82	85	86	87	89	90	91	91	91
>10%	70%	12	13	13	13	13	13	13	13	13	13	13
	80%	16	16	16	17	17	17	17	17	17	17	17
	90%	21	22	23	23	23	23	23	23	23	23	23
	95%	26	28	29	29	29	30	30	30	30	30	30
	99%	37	41	43	44	44	44	45	45	45	45	45





- Chase and Polson, 2000
  - Each month is a new "round" of testing on the herd
    - Can combine the confidence of successive rounds
    - Probability that two independent rounds fail to detect a disease is the product of the probabilities from each

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	95%	26	28	29	29	29	30	30	30	30	30	30
	99%	37	41	43	44	44	44	45	45	45	45	45





### • Per Cannon (2002):

 Combining confidence levels for rounds of random testing follows the equation:

• 
$$\gamma = 1 - [(1 - \gamma_1)(1 - \gamma_2)]$$

 Thus, combined confidence (γ) of 2 rounds of 30 serum samples for PRRSV surveillance would yield:

• 
$$\gamma = 1 - [(1-0.95)(1-0.95)]$$

• 
$$\gamma = 0.9975$$

• Four rounds (months):

• 
$$\gamma = 1 - [(0.05)(0.05)(0.05)(0.05)]$$

• 
$$\gamma = 0.99999375$$

• One positive detection resets.

#### Table 1

			Popu	ulation Si	ze (Dete	cting One	or More	Positive	s)			
Prevalence Estimate % Positive	Confidence Level	100	200	400	600	800	1000	2000	4000	6000	8000	10000
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- How Many Samples Do I Need?
  - Fixed Spatial Sampling (Rotolo et al. 2017)
    - Technically less complex
      - Collect the same location repeatedly
        - same pen(s) every time
      - Amenable to composite samples
    - Similar sensitivity as random sampling

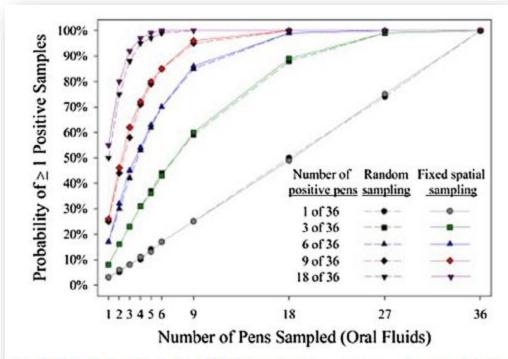


Fig. 1. Probability of detecting PRRSV in a single barn using pen-based oral fluids tested by RT-rtPCR as a function of sample allocation (simple random sampling vs. fixed spatial sampling), sample size, and prevalence.





- How Many Samples Do I Need?
  - Fixed Spatial Sampling (Rotolo et al. 2017)
    - In 4 weeks, 95% confidence to detect one positive with 4 or 6 oral fluids / wk
      - 4 x 4 weeks = 16 samples
        - versus 30 sera in one round randomly
    - Number of samples needed would depend upon the expected pathogen dynamics
      - Upper and lower graphs are slower vs faster spread

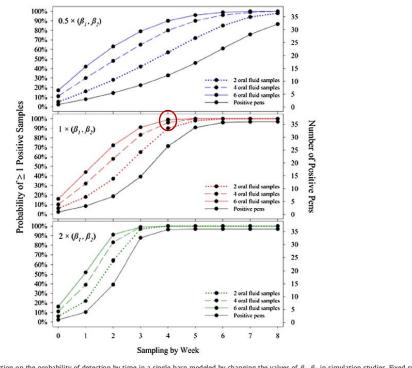


Fig. 2. Effect of spread of infection on the probability of detection by time in a single barn modeled by changing the values of  $\beta_b$ ,  $\beta_2$  in simulation studies. Fixed spatial sampling was used with sample sizes 2, 4, and 6 while allowing prevalence to change over time. For simplicity, diagnostic sensitivity and specificity were assumed to be 100%.





- How Many Samples Do I Need?
  - Herd surveillance does not need to be rigid and inflexible (Cannon 2002)
    - Not "one size fits all"
      - Generally, a binary approach (presence/absence)
    - Should be customized to the needs of the user (cost, convenience, speed, etc.)
      - Point in time information or ongoing assessment?
    - Must meet the needs of the customer (replacement stock versus grower/finisher)
    - Can combine different strategies:
      - Random sampling and statistical tables to establish herd or regional status initially
      - Fixed spatial sampling of fewer samples for ongoing surveillance
        - Cumulative rounds of negative testing raise confidence level over time





- Potential Application for Dysentery Surveillance on a Sow Farm
  - Subclinical breeding herds likely have low prevalence of B. hyodysenteriae < 2% (Duff et al. 2014)
    - Traditional surveillance methods require many samples to reach 95% confidence in negative
      - ~145 samples for 2,500 hd
      - Reflects one timepoint
    - Sampling specific subsets can increase detection (lactating animals, just weaned pigs, etc.)

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- Potential Application for Dysentery Surveillance on a Sow Farm
  - How could fixed spatial sampling be used?
    - Select a subset of crates in farrowing
    - Sample the same crates monthly
      - Individual Brachyspira culture
      - ± Pooled PCR

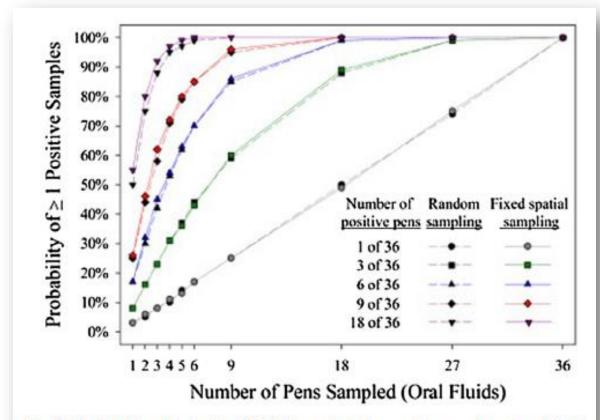
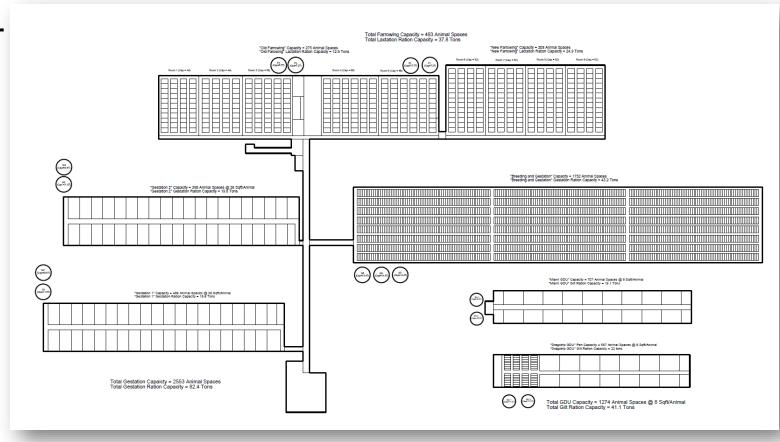


Fig. 1. Probability of detecting PRRSV in a single barn using pen-based oral fluids tested by RT-rtPCR as a function of sample allocation (simple random sampling vs. fixed spatial sampling), sample size, and prevalence.





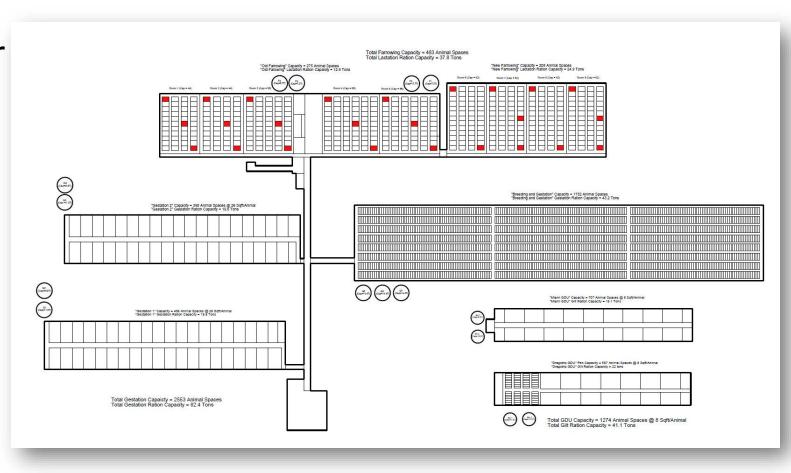
- Potential Application for Dysentery Surveillance on a Sow Farm
  - How could fixed spatial sampling be used?
    - Select a subset of crates in farrowing (ex. 25)
    - Sample the same crates monthly
      - Individual *Brachyspira* culture
      - ± Pooled PCR







- Potential Application for Dysentery Surveillance on a Sow Farm
  - How could fixed spatial sampling be used?
    - Select a subset of crates in farrowing (ex. 25)
    - Sample the same crates monthly
      - Individual *Brachyspira* culture
      - ± Pooled PCR
    - So, what is the expected confidence of 25 samples?







- Potential Application for Dysentery Surveillance on a Sow Farm
  - According to Cannon and Roe (1982):
    - Sampling 25 animals at 2% prev is 60.3% likely to fail to detect (39.7% DSe)
    - Recall the combined confidence eq:

• 
$$\gamma = 1 - [(1 - \gamma_1)(1 - \gamma_2)]$$

• Modify for n = # of repeated rounds

• 
$$\gamma = 1 - (1 - \gamma_1)^n$$

• 
$$0.95 = 1 - (1 - 0.397)^n$$

• 
$$0.95 = 1 - (0.603)^n$$

• 
$$n = \sim 6$$
 months

• After 6 months, you may assume with >95% confidence the herd is and remains negative with continued testing

#### Table 3: Probability of Failure to Detect Diseased Animals

The table gives the probability of failure to detect diseased animals from an 'infinite' population with the specified proportion of positives in the population.

prevalence	5	10	number 25	of anim 50	als in 75	sample 100	tested 200	250	500	1000
18	0.951	0.904	0.778	0.605	0.471	0.366	0.134	0.081	0.007	0.00
2%	0.904	0.817	0.603	0.364	0.220	0.133	0.018	0.006	0.000	
3%	0.859	0.737	0.467	0.218	0.102	0.048	0.002	0.000		
4%	0.815	0.665	0.360	0.130	0.047	0.017	0.000			
5%	0.774	0.599	0.277	0.077	0.021	0.006	0.000			
6%	0.734	0.539	0.213	0.045	0.010	0.002	0.000			
7%	0.696	0.484	0.163	0.027	0.004	0.001	0.000			
88	0.659	0.434	0.124	0.015	0.002	0.000				
9%	0.624	0.389	Ó.095	0.009	0.001	0.000				
10%	0.590	0.349	0.072	0.005	0.000					
12%	0.528	0.279	0.041	0.002	0.000					
14%	0.470	0.221	0.023	0.001	0.000					
16%	0.418	0.175	0.013	0.000						
18%	0.371	0.137	0.007	0.000						
20%	0.328	0.107	0.004	0.000						
24%	0.254	0.064	0.001	0.000						
28%	0.193	0.037	0.000							
32%	0.145	0.021	0.000							
36%	0.107	0.012	0.000							
40%	0.078	0.006	0.000							
50%	0.031	0.001	0.000							
60%	0.010	0.000								





- Potential Application for Dysentery Surveillance on a Sow Farm
  - According to Cannon and Roe (1982):
    - Sampling 25 animals at 2% prev is 60.3% likely to fail to detect (39.7% DSe)
    - Recall the combined confidence eq:

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$$0.95 = 1 - (1 - 0.397)^n$$

• 
$$0.95 = 1 - (0.603)^n$$

- $n = \sim 6$  months
- After 6 months, you may assume with >95% confidence the herd is and remains negative with continued testing

### **Total tests required:**

- Traditional (point in time) = 145
- Fixed spatial
  - 25/month \* 6months = 150



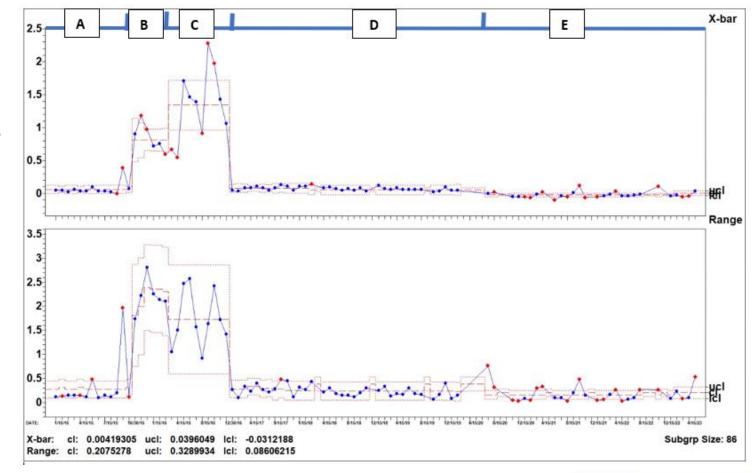


- If a herd is known to be positive for a given pathogen, or vaccinated, it may be desirable to temporally monitor quantitative data
  - ELISA or PCR results
- Specific Process Control (SPC) Charts
  - Well-suited for aggregated quantitative diagnostic data of individual pathogens
    - Looking for variation, loss of stability
  - Several commercial software packages:
    - Northwest Analytics Quality Analyst
      - <a href="https://www.nwasoft.com/products/nwa-quality-analyst">https://www.nwasoft.com/products/nwa-quality-analyst</a>
    - Microsoft Excel





- SPC Chart for Mhp ELISA
  - Results are reported as S/P
    - positive/negative cutoff is 0.5
  - The charts' three-sigma limits recalculate when a new method is introduced or if a change in output average is detected
  - A = Mhp negative
  - B = Acute Mhp infection
  - C = Herd closure + vaccination
  - D = Depop / repop
  - E = Unstable variation
    - Needs investigating

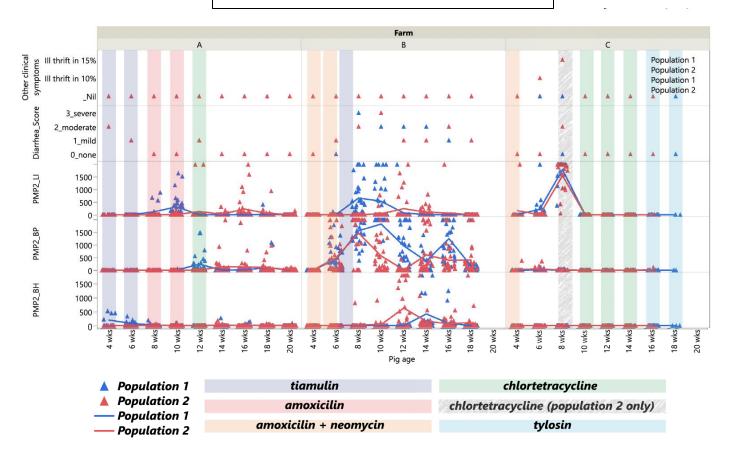






#### Pork MultiPath™ Enteric panel (PMP2) results

- PCR Panels for Endemic Pathogens
  - Copy number or Ct values can be used to estimate pop pathogen burden
  - Useful for composite samples over time
    - Oral fluids, feces
  - Consistency of sampling is important
  - Indirect assessment of mitigation effectiveness
    - Unexpected spikes warrant investigation



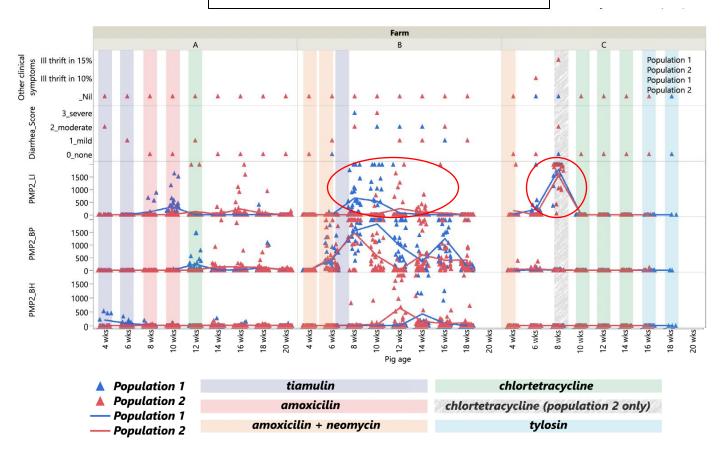
Gerszon J, et al. 2024. The use of oral fluids and sock samples for monitoring key pathogens in pig populations for surveillance purposes. *Prev Vet Med* 228:106237.





### Pork MultiPath™ Enteric panel (PMP2) results

- PCR Panels for Endemic Pathogens
  - Will generate more questions than answers
    - Requires knowledge of the herds behind the data
    - What do these Lawsonia detections reflect?
      - Is live vaccine used?



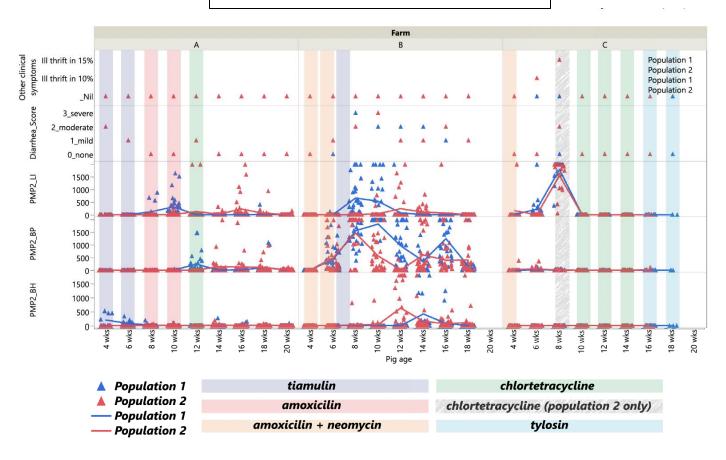
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### Pork MultiPath™ Enteric panel (PMP2) results

- PCR Panels for Endemic Pathogens
  - Will generate more questions than answers
    - Requires knowledge of the herds behind the data
    - What do these Lawsonia detections reflect?
      - Is live vaccine used?
    - B. pilosicoli appears tiamulin resistant
      - Is this spreading to B. hyo or are these later lateral introductions?
      - Need current MIC and genetic information of both organisms.



Gerszon J, et al. 2024. The use of oral fluids and sock samples for monitoring key pathogens in pig populations for surveillance purposes. *Prev Vet Med* 228:106237.





## Large Scale Data Aggregation

Combining test data from multiple streams for zone-level monitoring





















## Swine Disease Reporting System (SDRS)

sdrs@iastate.edu



### **Project Coordinator**



Dr. Guilherme Cezar

### **Principal Investigators**



Dr. Giovani Trevisan



Dr. Daniel Linhares



### Communications



Dr. Edison Magalhães

### **Graduate Students**



Srijita Chandra



Dr. Elisa De Conti



Jai Tatinani



Alan Moore

### Software Developer



Kinath Rupasinghe

### **Objective**

 GOAL: to share information on the detection of endemic and emerging pathogens affecting the US swine population, thereby assisting veterinarians and producers in making informed decisions on disease prevention, detection, and management.











### A collaborative project across US Vet. Diagnostic Labs

### **Participant Labs**













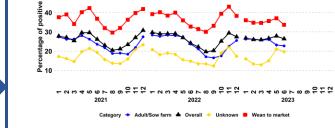


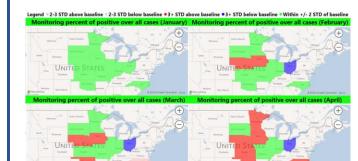
**Data retrieving** 





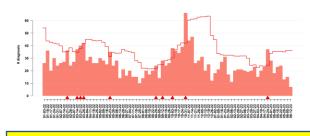






**SDRS team compilation** 

PRRSV percentage of positive submissions by age category



**Numbers + Field Specialists** comments

### **Distribution**















### **Final Report**

PRRSV PCR+/- Lineage/RFLP	MHP PCR+/-
PCV2/3 PCR+/- Ct values	IAV PCR+/- subtyping
PDCoV PCR+/-	PEDV PCR+/-
TGEV	PCR+/-

**Confirmed porcine tissue** diagnosis (ISU VDL only)

### **SDRS** timeline

**Participant** 











**PCV3 PCR** 

**Nov 2023** 

PRRSV, PEDV, PEDV, TGEV PCR; PRRSV ORF5 sequence (ISU)

Tissue-based diagnoses (ISU)

Mar 2019

**IAV IAV PCR** subtype **Apr 2022** PCV2 PCR Oct 2022 May 2022

May 2023

Swine Health Information Center

Funding

















## Information available through monthly PDF reports

















#### Topic 1 – Detection of PRRSV RNA over time by RT-qPCR.

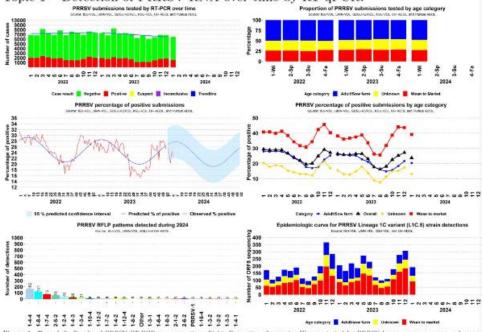


Figure 1. Top: Left: Results of PRRSV RT-PCR cases over time; Right: Proportion of accession ID cases tested for PRRSV by age group per year and season Middle: Left Expected percentage of positive results for PRRSV RNA by RT-qPCR, with 95% confidence interval band for predicted results based on weekly data observed in the previous 3 years; Right: Percentage of PRRSV PCR-positive results, by age category, over time. Wean to market corresponds to nursery and grow-finish. Adult/Sow correspond to Adult, bear stud, breeding berd, replacement, and suckling piglets. Unknown corresponds to not informed site type or farm category. Bottom Left: The 25 most frequently detected RFLP patterns during 2024; Right: Epidemiological curve of detection for PRRSV Lineage IC variant

#### SDRS Advisory Group highlights:

- Overall, 23.76% of 6,486 cases tested PRRSV-positive in January, a moderate decrease from 27.22% of 6,878 in December;
- Positivity in the adult/sow category in January was 20.39% (629 of 3,085), similar to 21.67% (681 of 3,143) in December;
- Positivity in the wean-to-market category in January was 39.1% (701 of 1,793), a moderate decrease from 43.63% (898 of 2,058) in December;
- · PRRSV had a decrease in positivity in both wean-to-market and adult/sow farms in January, which is unusual for this month, according to our historical database. However, this is the fourth consecutive month (since November of 2023) of low average Ct value in the PRRSV submissions (average varies between 25-26).;
- The predominant PRRSV wildtype ORF5 sequences detected since November 2023 are the Lineages L1C.5 (variant) (558), Lineage 1A 1-7-4 (215), L1H 1-8-4 (123), L1C.2 1-2-4 (122), and L1C.5 (variant) 1-4-3 (77);
- Different regional PRRSV wild-type ORF5 sequences were detected in 2023. Within states, the major wild-type dominant strain and percentual of state detections were: a) Lineage 1A: NC 83%, IN 55%, OH 53%, IL 34%; b) Lineage 1C.5 9 (L1C variant) MO 75%, SD 67%, MN 64%, NE 45%, IA 43%; c) Lineage 1H; KS 76%, OK 51%;
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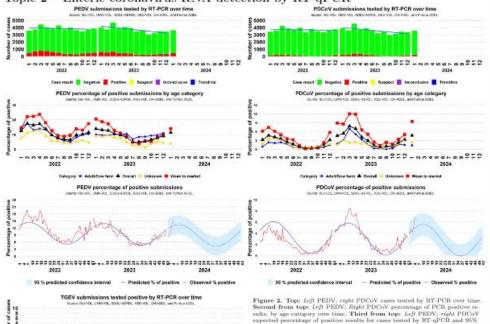




confidence interval for 2024 predicted value. Bottom: Number of TGEV posi-



Topic 2 – Enteric coronavirus RNA detection by RT-qPCR



#### SDRS Advisory Group highlights:

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2021

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#### Topic 1 – Detection of PRRSV RNA over time by RT-qPCR.

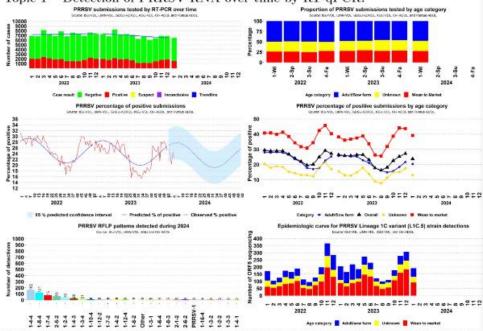


Figure 1. Top: Left: Results of PRRSV RT-PCR cases over time; Right: Proportion of accession ID cases tested for PRRSV by age group per year and season Middle: Left Expected percentage of positive results for PRRSV RNA by RT-qPCR, with 95% confidence interval band for predicted results based on weekly data observed in the previous 3 years; Right: Percentage of PRRSV PCR-positive results, by age category, over time. Wean to market corresponds to nursery and grow-finish. Adult/Sow correspond to Adult, bear stud, breeding berd, replacement, and suckling piglets. Unknown corresponds to not informed site type or farm category. Bottom Left: The 25 most frequently detected RFLP patterns during 2024; Right: Epidemiological curve of detection for PRRSV Lineage IC variant

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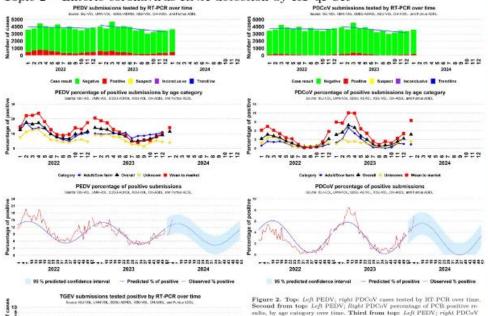


expected percentage of positive results for cases tested by RT-qPCR and 95%

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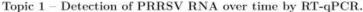












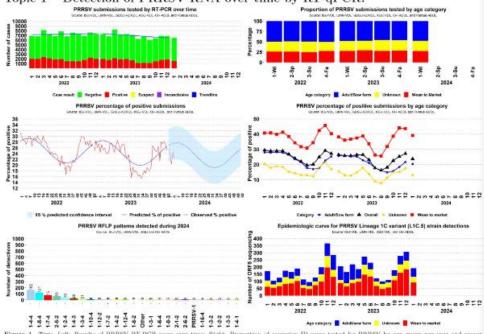


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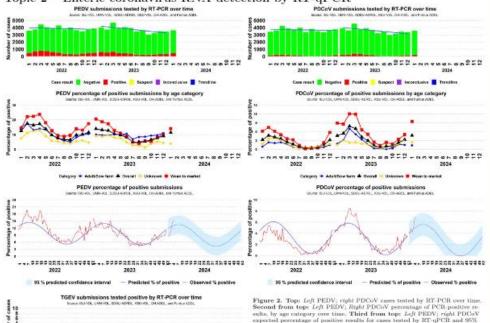


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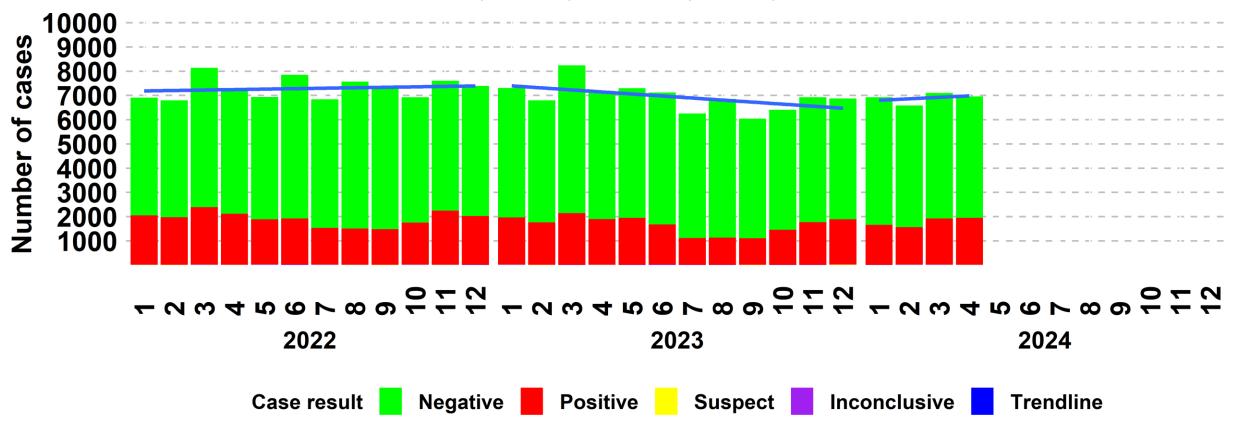
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Communications and information conot to be construed as recommending

Interpret the decrease in positivity with caution because some production systems are not sampling the animals downstream once the sow farm is positive for PRRSV

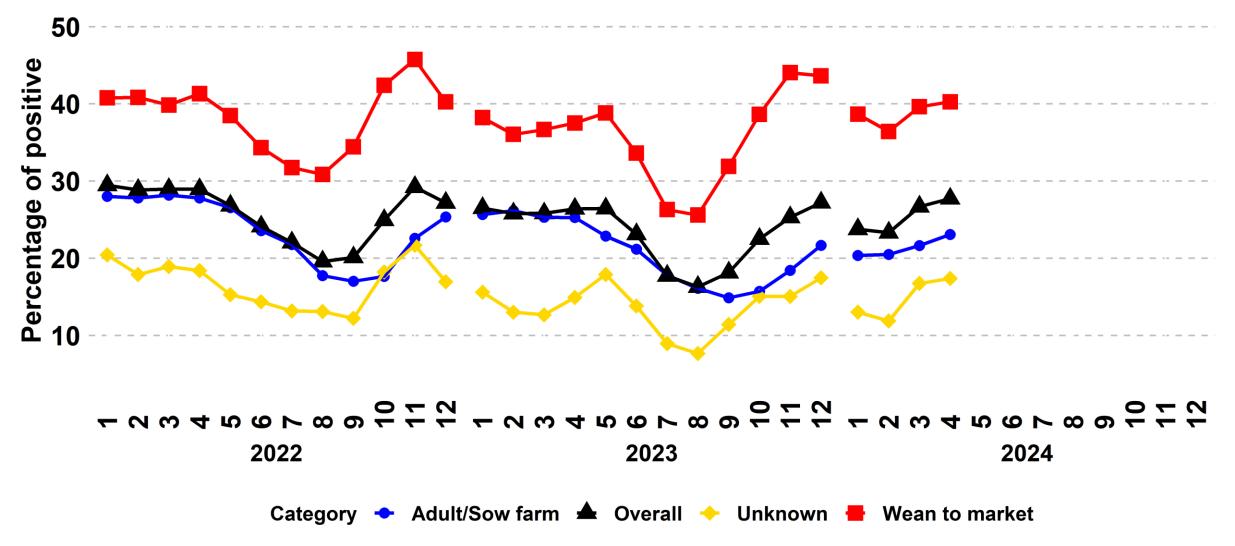
### PRRSV submissions tested by RT-PCR over time

Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, OH-ADDL and Purdue ADDL.



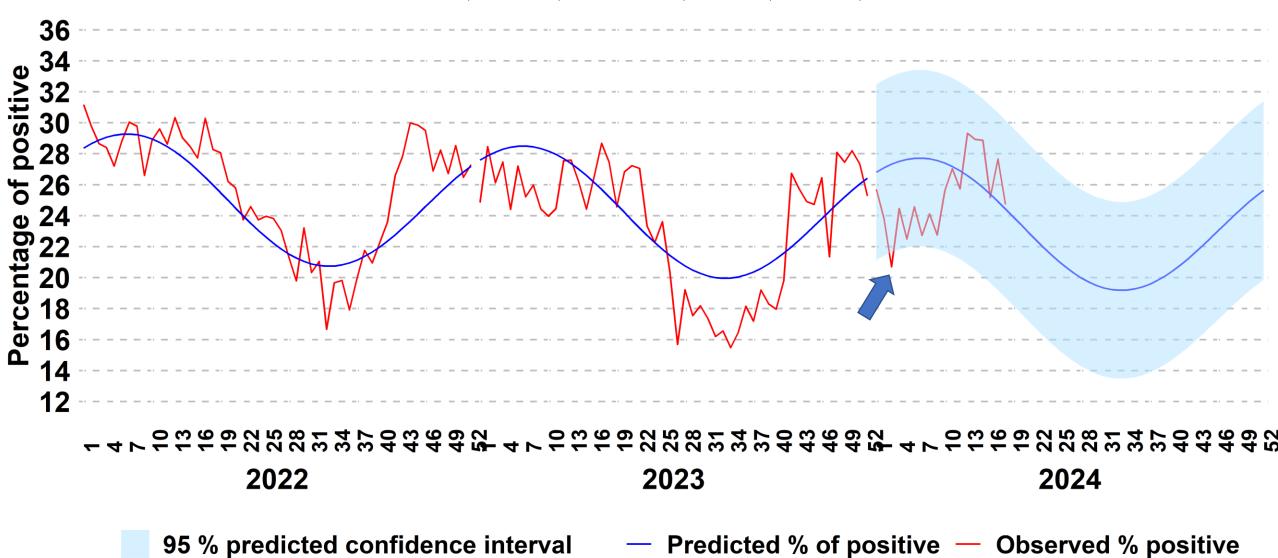
### PRRSV percentage of positive submissions by age category

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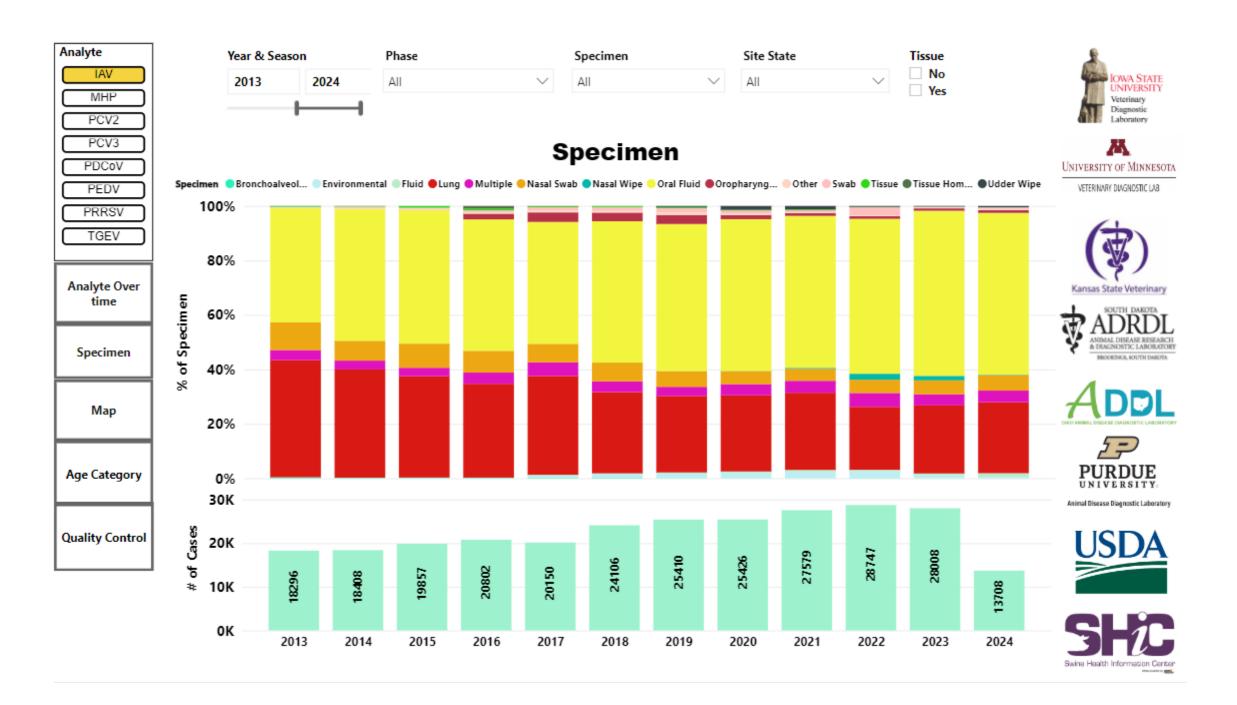


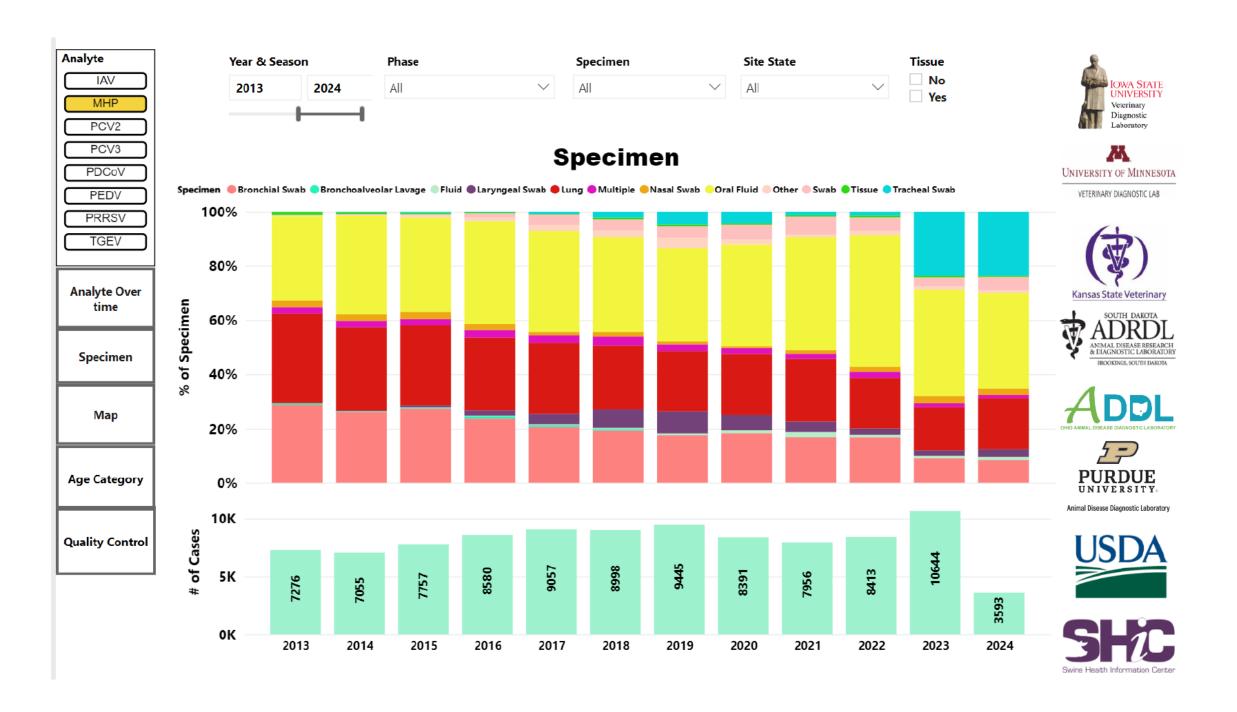




# Information available through online dashboards







- Relatively easy to do for pathogen detection data.
- What about disease diagnosis?
  - For endemic agents, disease diagnosis requires detection + evidence
    - To be of value, there should be standardization of what is accepted an disease confirmation
  - Pathology (gross and microscopic) is the gold standard
    - The narrative nature of traditional pathology reporting makes data aggregation challenging
    - Currently, there is no uniform method for disease reporting in veterinary medicine
  - Can disease be coded in a uniform way?
    - Disease diagnostic codes can be messaged and aggregated similar to other test data





- Disease Diagnostic Codes (Dx Codes)
  - Have been used at the ISU VDL since 2003
    - Early codes were not standardized, no clear hierarchy
      - Examples:
        - MHD = mulberry heart disease
        - ABOR PPV = parvovirus abortion
        - ENTE SERP HYOD = swine dysentery
  - In 2017, we decided a new hierarchal system was needed
    - Each disease code contains 4 components:
      - **SYSTEM** (respiratory, digestive, nervous, urogenital, etc.)
      - INSULT (bacterial, viral, parasitic, toxicity, etc.)
      - LESION (pneumonia, enteritis, arthritis, etc.)
      - ETIOLOGY (PRRSV, Salmonella, E. coli, etc.)



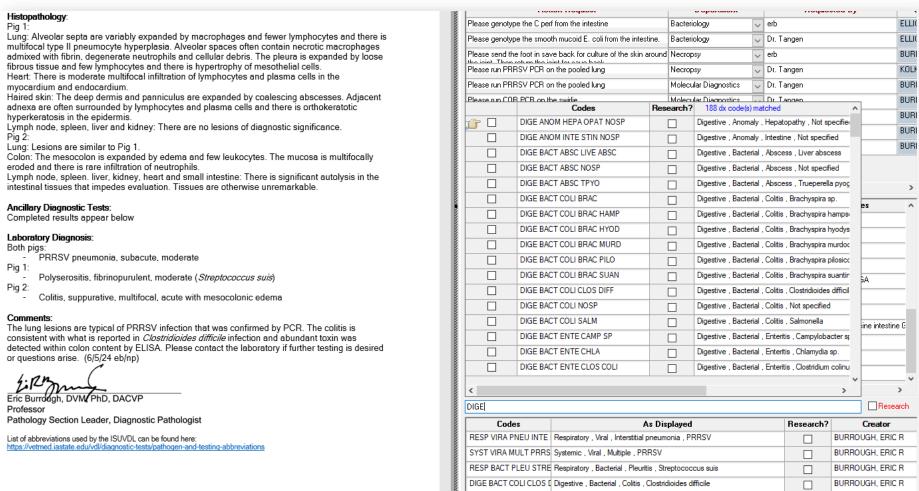


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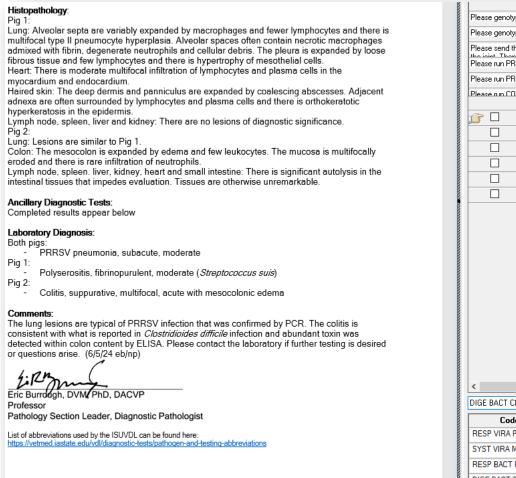
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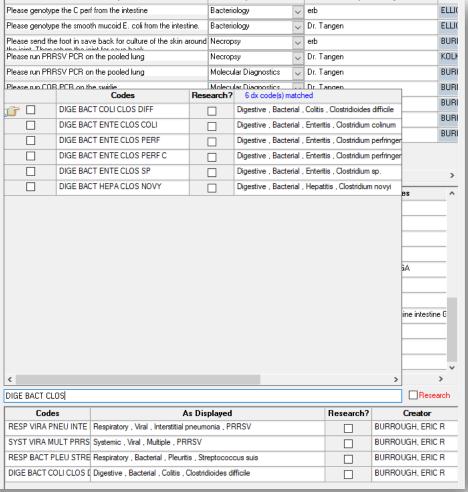






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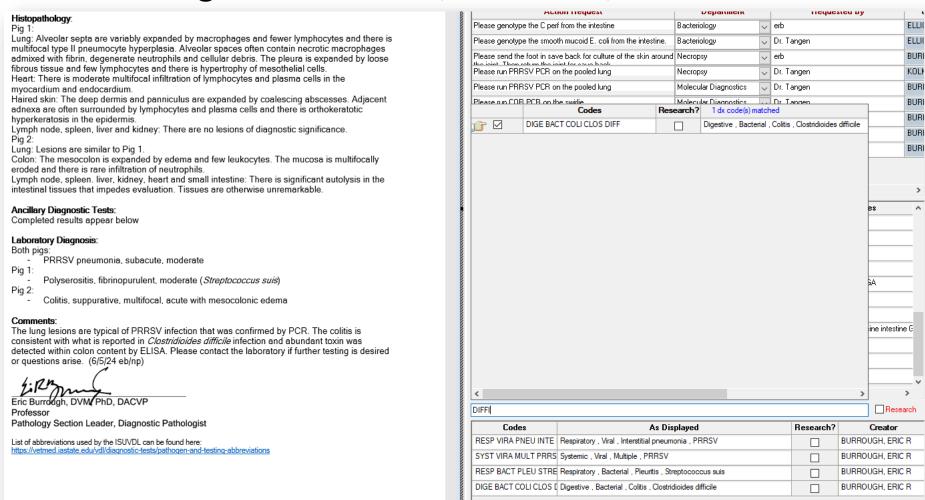
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Disease Diagnostic Codes (Dx Codes)



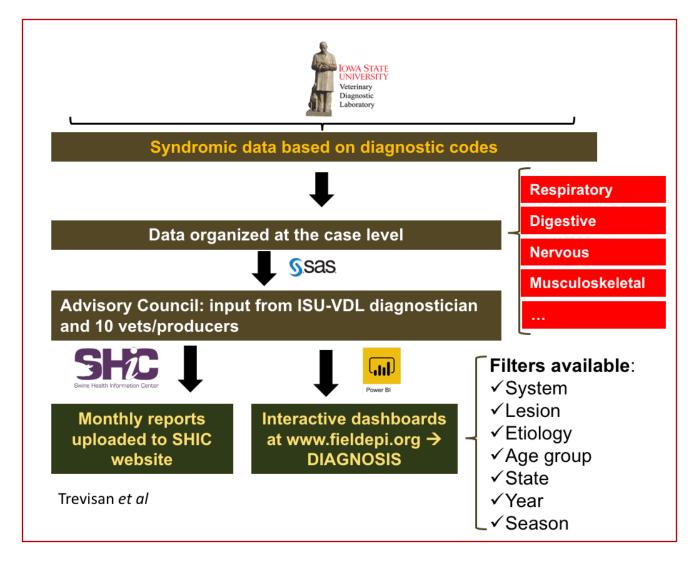




- Disease Diagnostic Codes (Dx Codes)
  - Challenges
    - Requires pathologist training/retraining
    - Codes from previous system must be mapped to new system to avoid
      - This is essential to avoid loss of historical data
  - Opportunities
    - Disease data is now filterable and can be aggregated
      - A completely new stream of data is available
        - Disease data versus test result data (both useful but different)
    - Improved denominators:
      - e.g., number of IAV diagnoses in a period over:
        - Total respiratory cases received
        - Total cases with respiratory viral disease
        - Total cases with bronchitis



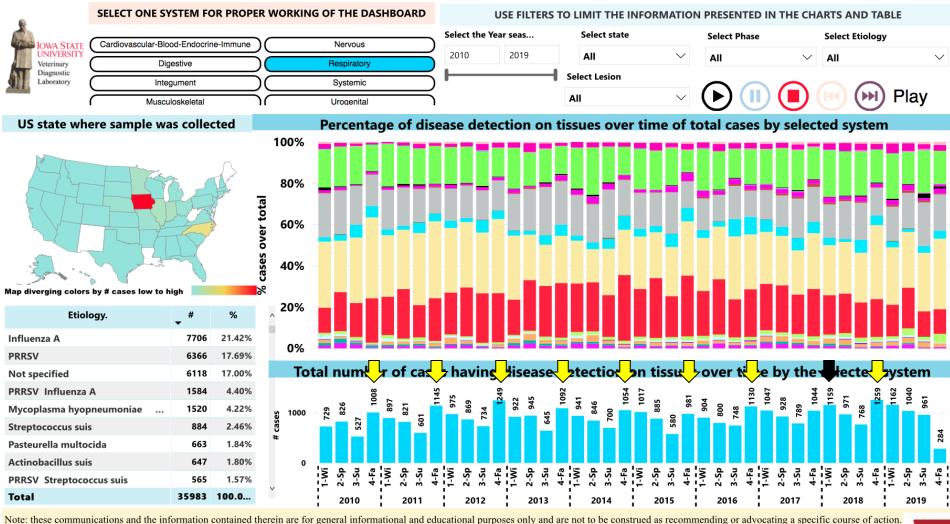








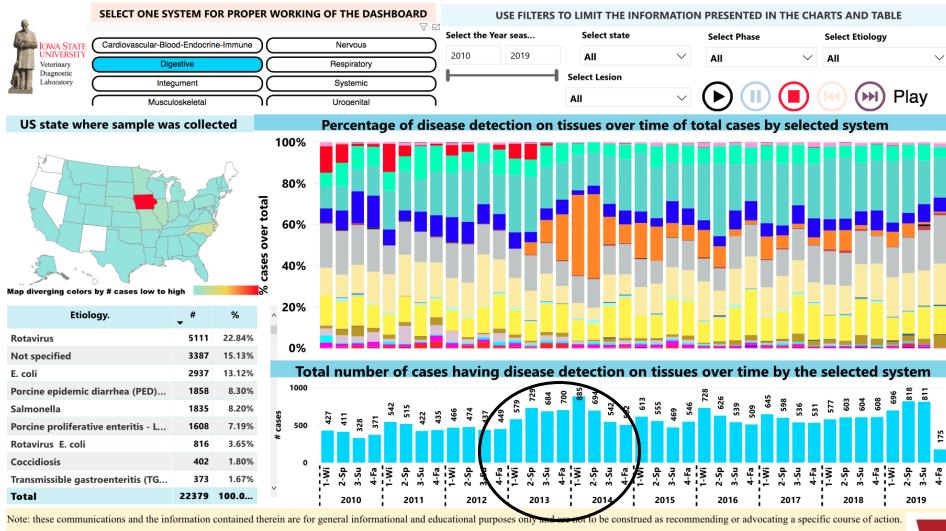
# Fall / winter months have the highest number of respiratory diagnosis





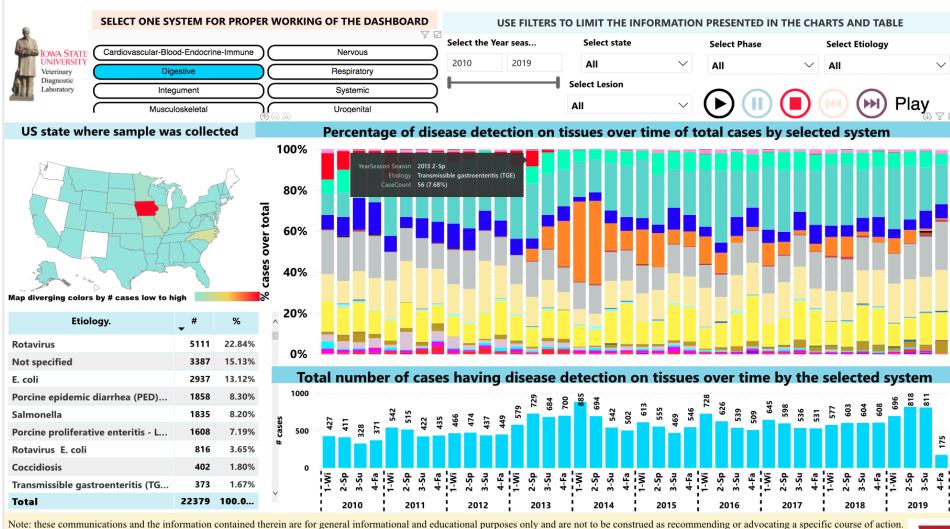
Veterinary
Diagnostic
Laboratory

# Increased number of digestive diagnosis in 2013/2014





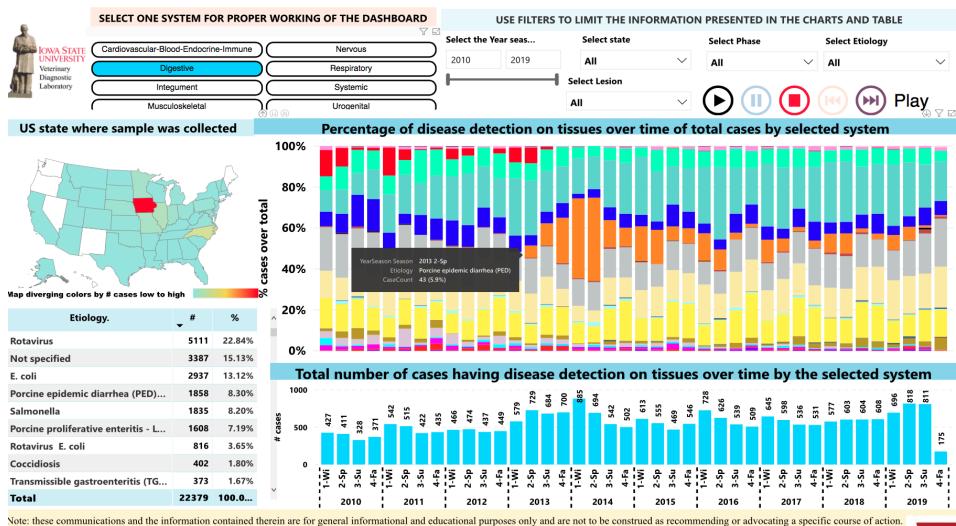
## Low frequency of TGEV diagnosis after 2013







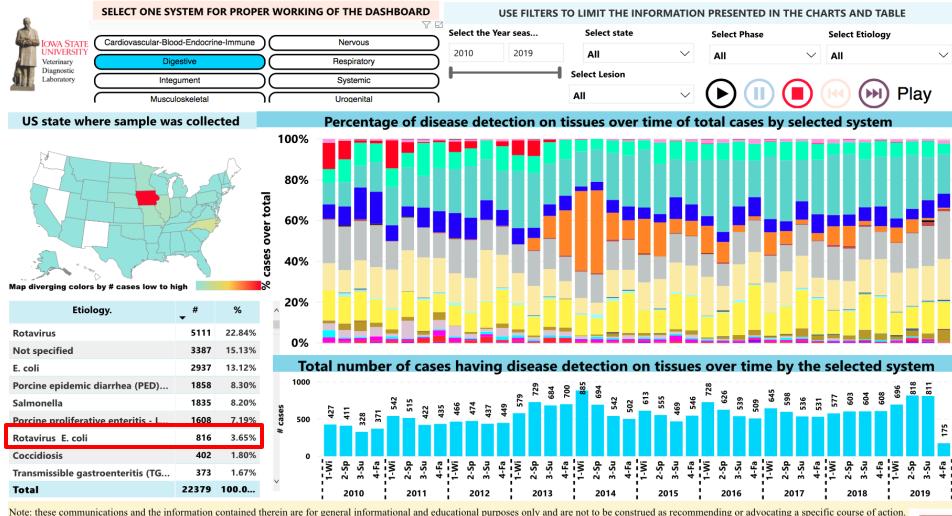
## High frequency of PEDV diagnosis in 2013/2014





Veterinary
Diagnostic
Laboratory

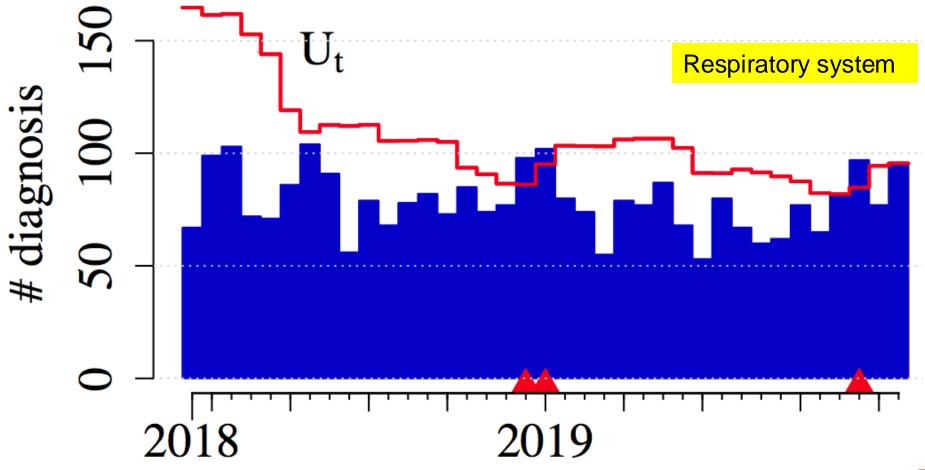
## Sorted by system the tool has ability to inform multiple agents detected in a case





Veterinary Diagnostic Laboratory

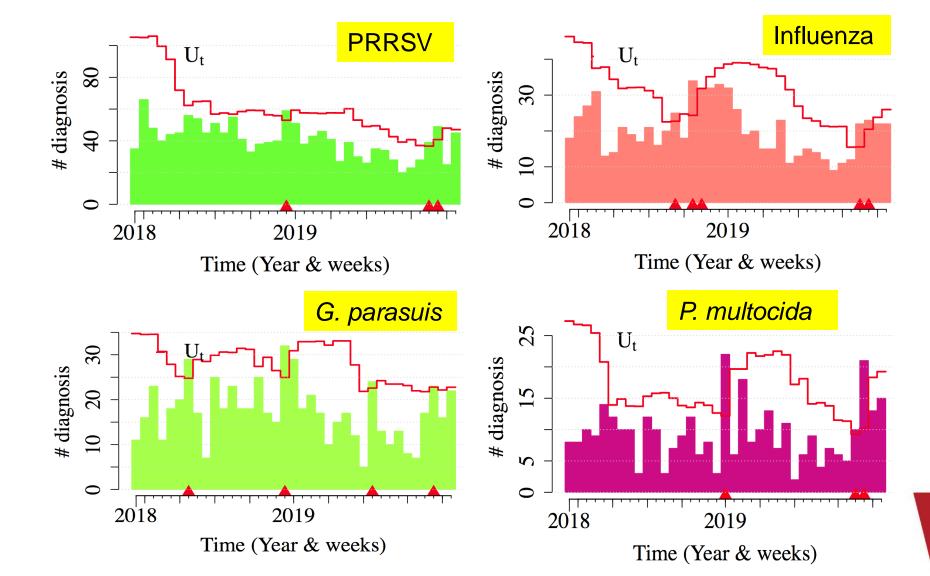
# Weekly monitoring of disease diagnosis by system can create alert signals for increased diagnoses







# At the agent level, 2 signals in a 4 week interval are suggestive of a potential outbreak



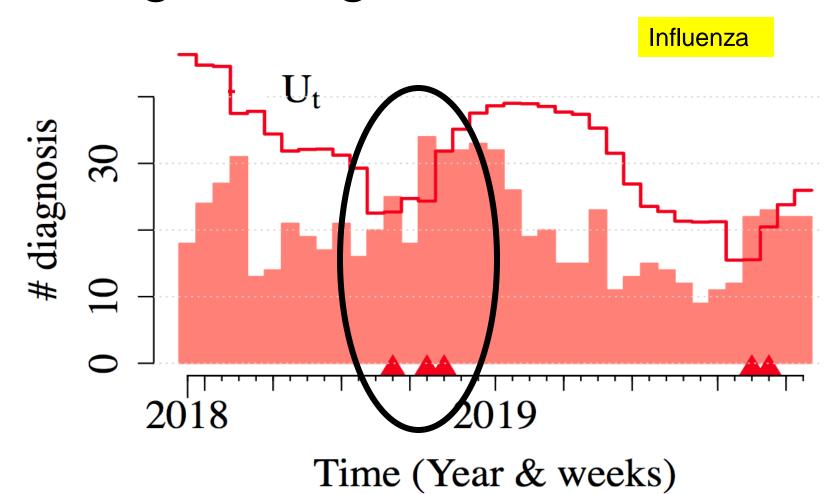
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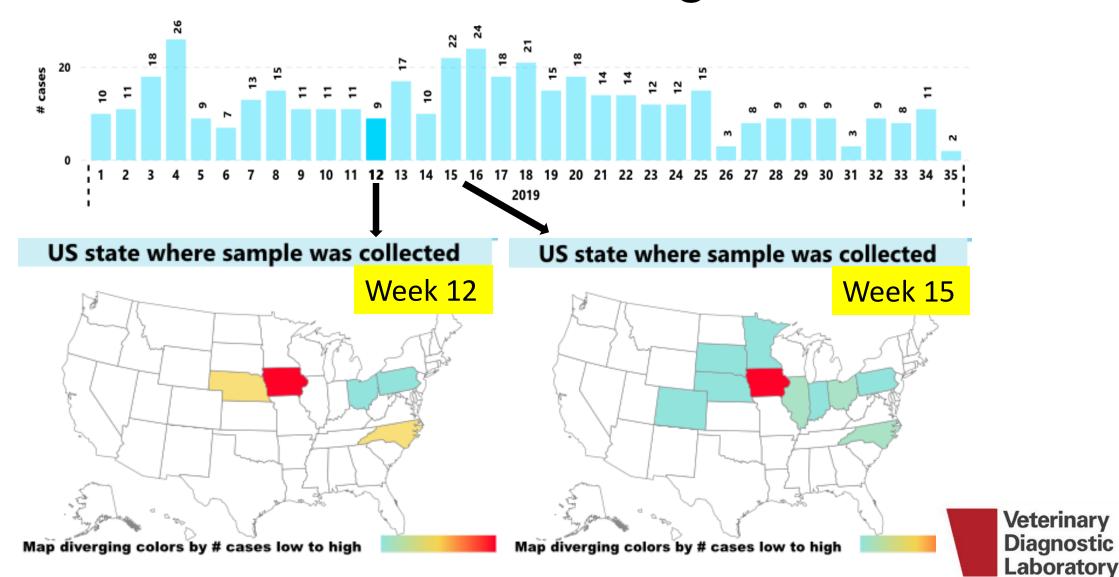
# Triggers investigation of geographical distribution of diagnosis signal in week 15







# Dashboards are then used to investigate weekly cases of influenza diagnosis



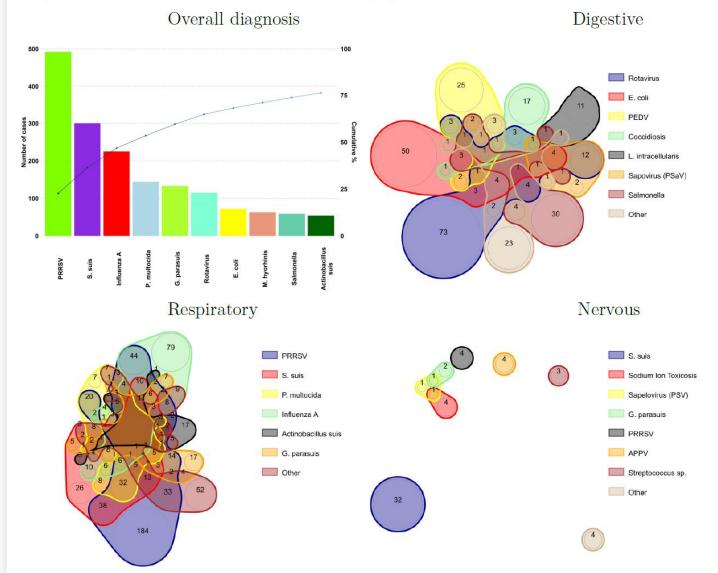


# Dx Code Data from ISU VDL is Summarized Monthly in the SDRS Report





Topic 6 – Confirmed tissue cases etiologic/disease diagnosis at the ISU-VDL.





### Large Scale Data Aggregation – Take Home

- Use of DX code information to monitor swine disease diagnosis:
  - Can keep swine industry informed on endemic disease trends
  - Large scale monitoring of endemic disease diagnosis can help scientists, producers, and veterinarians:
    - Better understand the pattern of disease occurrence
    - Develop better disease control strategies

#### Next steps:

- Explore additional statistical tools to monitor disease diagnosis trends
- Collaborate with other VDLs in US and globally to aggregate information?
  - Will require standardization of coding for useful messaging





## Summary

- Different tools are needed for Surveillance versus Monitoring
  - Surveillance often used to prove freedom from disease
    - Is well suited for binary data (presence/absence)
    - Fixed spatial sampling and combined confidence from successive rounds of testing can reduce sample numbers per round
  - Monitoring is used to observed patterns or changes in endemic disease
    - Poorly suited for binary data (finding it is not unexpected)
    - SPC charts can be used for quantitative data
    - Disease diagnosis data is best, but harder to aggregate
- Aggregated anonymized data is useful for the swine industry
  - Requires purposeful collaboration (and funding)
  - Data access via dashboards help generate new questions





## Questions?





