Swine Disease Reporting System
Report # 55 (September 06, 2022)

What is the Swine Disease Reporting System (SDRS)? SDRS includes multiple projects that aggregate data from participating veterinary diagnostic laboratories (VDLs) in the United States of America (USA), and reports the major findings to the swine industry. Our goal is to share information on endemic and emerging diseases affecting the swine population in the USA, assisting veterinarians and producers in making informed decisions on disease prevention, detection, and management.

After aggregating information from participating VDLs and summarizing the data, we ask for the input of our advisory group, which consists of veterinarians and producers across the US swine industry. The intent is to provide an interpretation of the observed data, and summarize the implications to the industry. Major findings are also discussed in monthly podcasts. All SDRS reports and podcasts are available at www.fieldepi.org/SDRS. The SDRS projects are:

Swine Health Information Center (SHIC)-funded Domestic Swine Disease Surveillance Program: collaborative project among multiple VDLs, with the goal to aggregate swine diagnostic data and report it in an intuitive format (web dashboards and monthly PDF report), describing dynamics of pathogen detection by PCR-based assays over time, specimen, age group, and geographical area. Data is from the Iowa State University VDL, South Dakota State University ADRDL, University of Minnesota VDL, Kansas State University VDL, and Ohio Animal Disease and Diagnostic Lab.

> **Collaborators:**
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> *Swine Disease Reporting System office*: Principal investigators: Daniel Linhares & Giovani Trevisan; Project coordinator: Guilherme Cezar, Communications: Edison Magalhães.
>
> *Iowa State University*: Gustavo Silva, Marcelo Almeida, Bret Crim, Eric Burrough, Phillip Gauger, Christopher Siepker, Alyona Michael, Panchan Sitthicharoenchai, Rodger Main.
>
> *University of Minnesota*: Mary Thurn, Paulo Lages, Cesar Corzo, Albert Rovira.
>
> *Kansas State University*: Rob McGaughey, Franco Matias-Ferreyra, Jamie Retallick.
>
> *South Dakota State University*: Jon Greseth, Darren Kersey, Travis Clement, Angela Pillatzki, Jane Christopher-Hennings.
>
> *Ohio Animal Disease and Diag. Lab.*: Melanie Prarat, William Hennessy, Ashley Sawyer, Dennis Summers.
>
> *The Ohio State University*: Andreia Arruda.

Disease Diagnosis System: A pilot program with the ISU-VDL consisting of reporting disease detection (not just pathogen detection by PCR), based on diagnostic codes assigned by veterinary diagnosticians.

FLUture: Aggregates influenza A virus (IAV) diagnostic data from the ISU-VDL and reports results, metadata, and sequences.

PRRS virus RFLP and Lineage report: Benchmarks patterns of PRRSV RFLP pattern and Lineages detected at the ISU-VDL, UMN-VDL, KSU-VDL, and OH-ADDL over time by specimen, age group, and US State.

Audio and video reports: Key findings from SDRS projects are summarized monthly in a conversation between investigators and available in the form of an "audio report" and "video report" through SwineCast, YouTube, LinkedIn, and the SDRS webpage.

Advisory Group: Reviews and discusses the data, providing their comments and perspectives monthly: Mark Schwartz, Paul Sundberg, Paul Yeske, Tara Donovan, Deborah Murray, Scott Dee, Brigitte Mason, Peter Schneider, Sam Copeland, Luc Dufresne, and Daniel Boykin.

In addition to this report, interactive dashboards with aggregated test results are available at www.fieldepi.org/SDRS.

Note: This report contains data up to August 31, 2022.

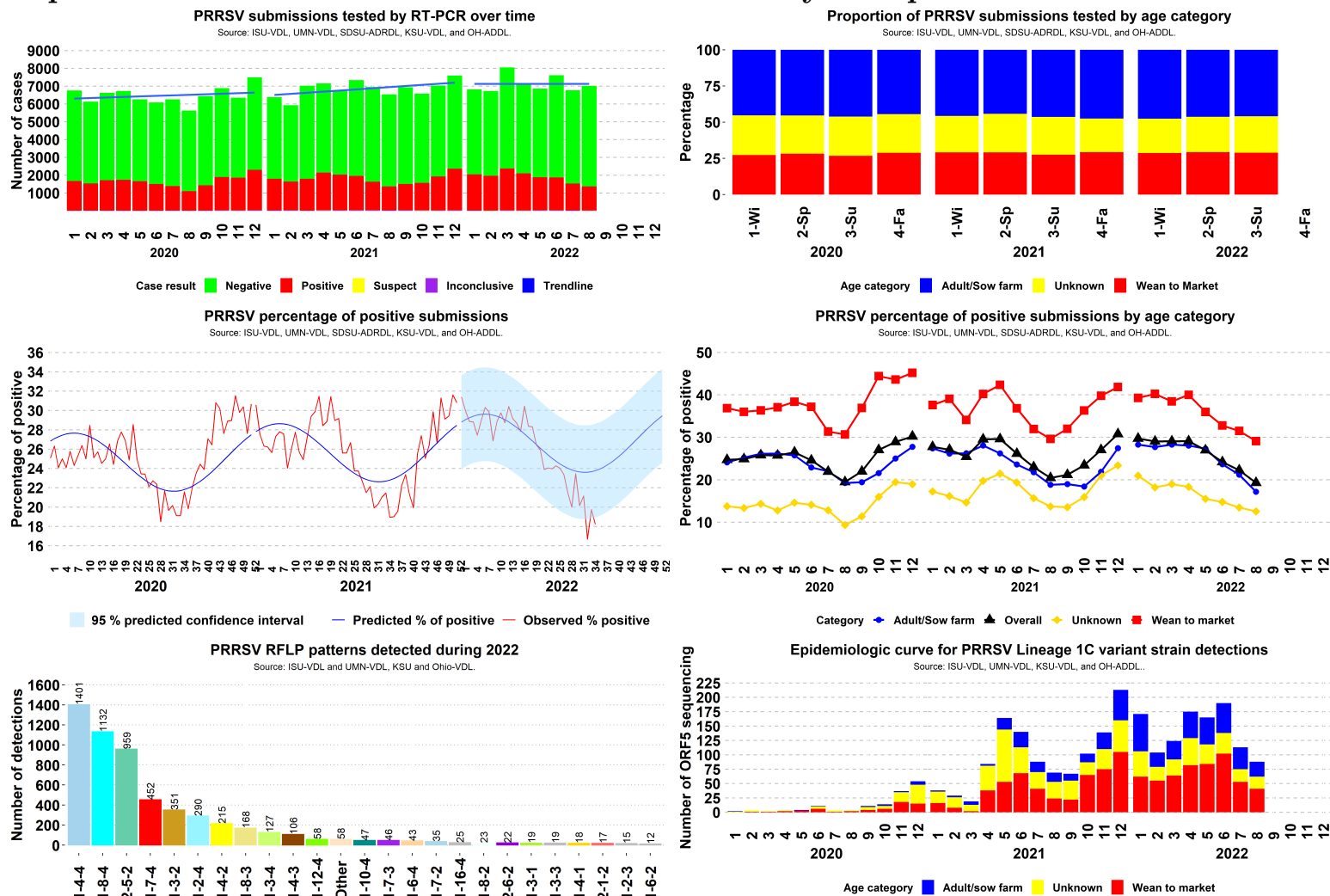# Topic 1 – Detection of PRRSV RNA over time by RT-qPCR.

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, boar stud, breeding herd, replacement, and suckling piglets. Unknown corresponds to not informed site type or farm category. **Bottom** *Left*: The 25 most frequently detected RFLP patterns during 2022; *Right*: Epidemiological curve of detection for PRRSV Lineage 1C variant strain.

SDRS Advisory Group highlights:

- Overall, 19.3% of 7,020 cases tested PRRSV-positive in August, a moderate decrease from 22.26% of 6,771 in July;
- Positivity in the adult/sow category in August was 17.18% (560 of 3,260), a moderate decrease from 21.21% (652 of 3,074) in July;
- Positivity in the wean-to-market category in August was 29.11% (568 of 1,951), a moderate decrease from 31.5% (624 of 1,981) in July;
- Overall PRRSV-percentage of positive cases was 3 standard deviations from state-specific baselines in NE, MO, and IN;
- SDRS has recently detected activity of a PRRSV strain classified as Lineage 1C RFLP 1-2-4 across 5 major pork producer states. This strain shares 8-10% nucleotide divergence with the Lineage 1C variant strain that emerged in 2020 in MN. SDRS is still working with the Advisory Group to understand this strain's clinical relevance and importance.

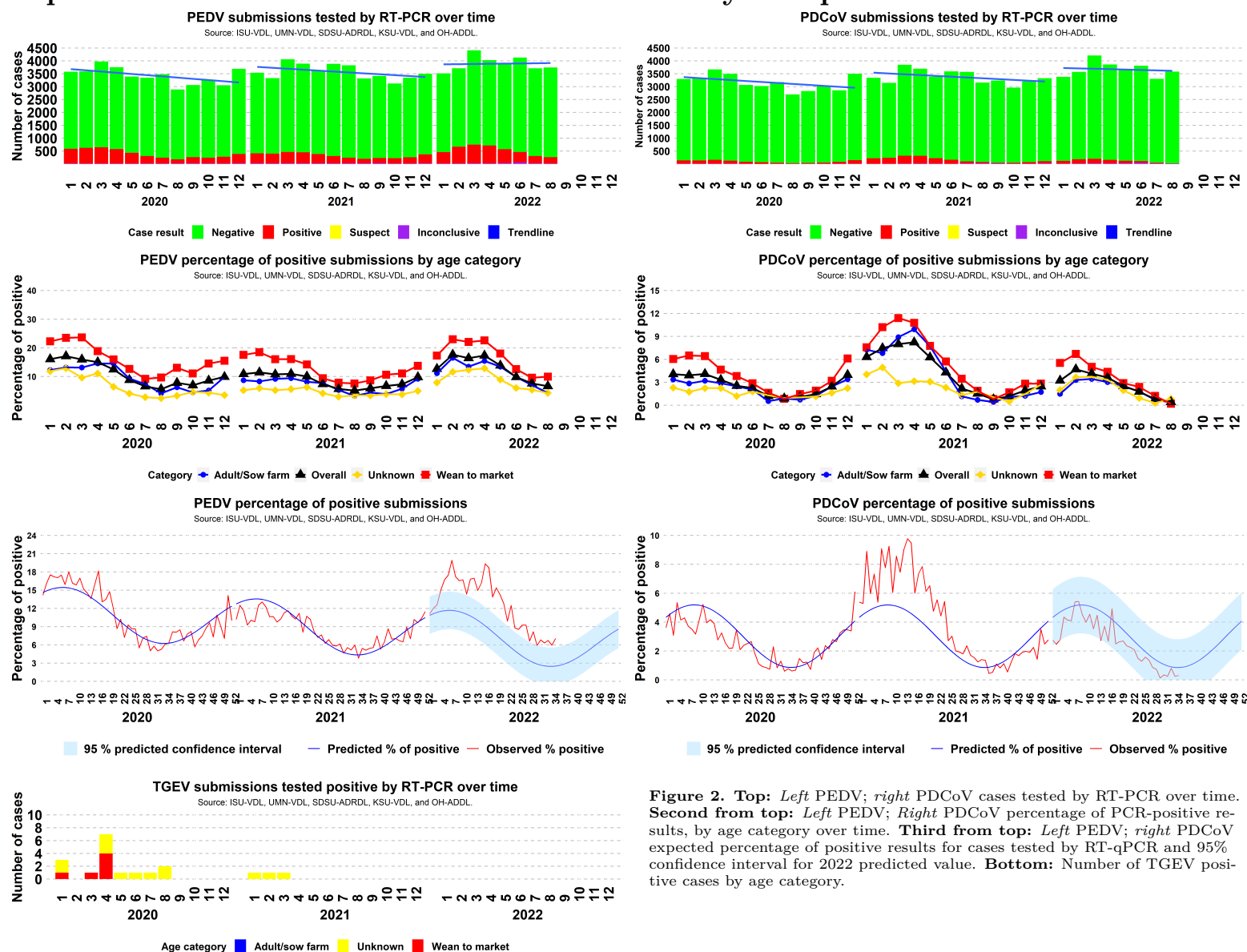# Topic 2 – Enteric coronavirus RNA detection by RT-qPCR

PEDV submissions tested by RT-PCR over time
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

PDCoV submissions tested by RT-PCR over time
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

Case result: Negative | Positive | Suspect | Inconclusive | Trendline

PEDV percentage of positive submissions by age category
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

PDCoV percentage of positive submissions by age category
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

Category: Adult/Sow farm | Overall | Unknown | Wean to market

PEDV percentage of positive submissions
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

PDCoV percentage of positive submissions
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

95 % predicted confidence interval | Predicted % of positive | Observed % positive

TGEV submissions tested positive by RT-PCR over time
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

Age category: Adult/sow farm | Unknown | Wean to market

Figure 2. Top: *Left* PEDV; *right* PDCoV cases tested by RT-PCR over time. **Second from top:** *Left* PEDV; *Right* PDCoV percentage of PCR-positive results, by age category over time. **Third from top:** *Left* PEDV; *right* PDCoV expected percentage of positive results for cases tested by RT-qPCR and 95% confidence interval for 2022 predicted value. **Bottom:** Number of TGEV positive cases by age category.

SDRS Advisory Group highlights:

- Overall, 6.65% of 3,745 cases tested PEDV-positive in August, similar to 7.59% of 3,714 in July;
  - Positivity in the adult/sow category in August was 4.27% (50 of 1,172), a moderate decrease from 7.02% (76 of 1,082) in July;
  - Positivity in the wean-to-market category in August was 9.97% (156 of 1,565), similar to 9.59% (146 of 1,523) in July;
  - The overall PEDV-percentage of positive cases was 3 standard deviations from state-specific baselines in MN, IA, KS, and NC;
- Overall, 0.42% of 3,583 cases tested PDCoV-positive in August, similar to 0.79% of 3,305 in July;
  - Positivity in the adult/sow category in August was 0.36% (4 of 1,124), similar to 0.72% (7 of 979) in July;
  - Positivity in the wean-to-market category in August was 0.2% (3 of 1,507), similar to 1.21% (16 of 1,318) in July;
  - Overall PDCoV-percentage of positive cases was within state-specific baselines in all 11 monitored states;
- There was 0 positive case for TGEV RNA in August, 2022 over a total of 3,470 cases tested;
- PDCoV has achieved the overall lowest percentage of positive cases by RNA PCR detection (0.42%) since 2018;
- The advisory group recommends the interpretation of PDCoV detection levels with some caution. Even though more than 3,000 cases are tested per month for PDCoV, there is not enough testing performed in wean-to-market for production system prevalence estimation purposes. Since PDCoV clinical signs in grow-finish pigs are usually mild, many sites do not perform routine surveillance testing for this agent;
- The advisory group highlighted that the US swine industry has invested in and considerably improved biosecurity measures and practices during recent years, which has also contributed to pathogen outbreaks biocontainment, e.g., the 2022 observed decrease in detection of PDCoV.

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Topic 3 – Detection of *M. hyopneumoniae* and Porcine Circovirus-2 DNA by PCR.
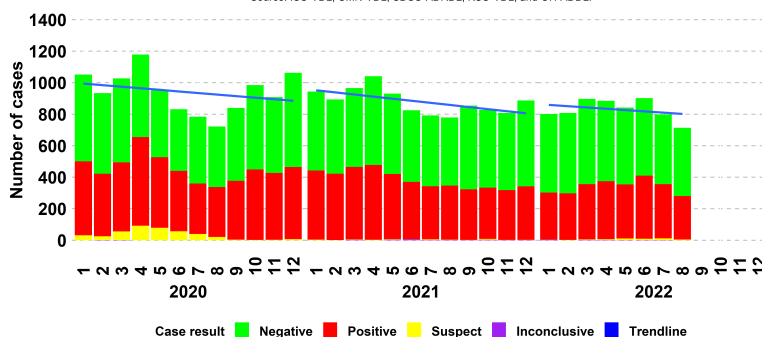

Mycoplama hyopneumoniae submissions tested by RT-PCR over time
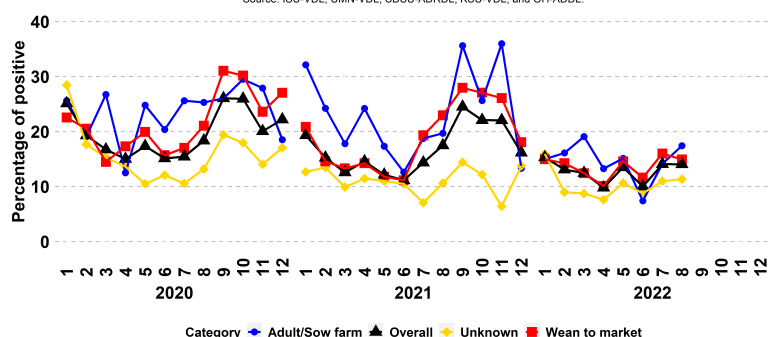Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.
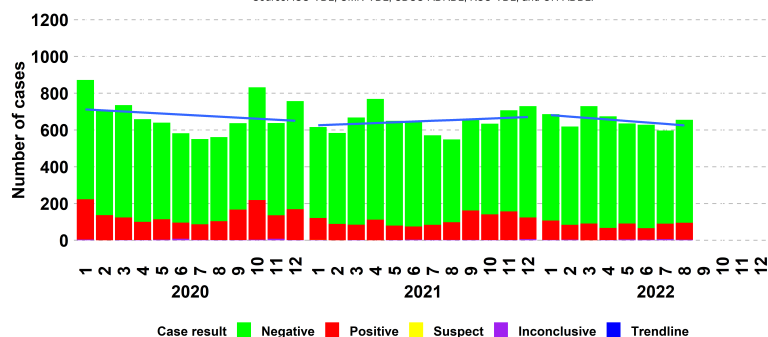
Porcine Circovirus 2 submissions tested by PCR over time
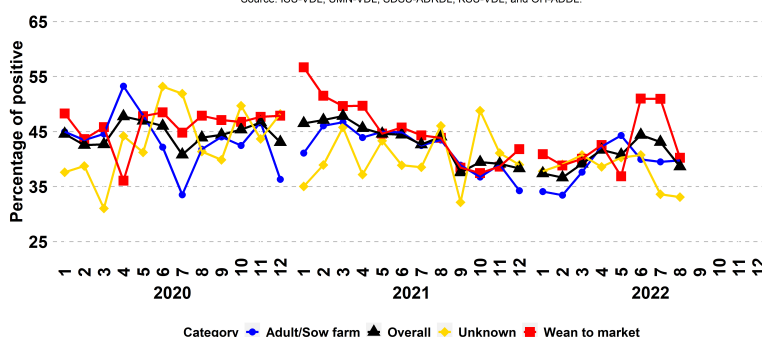Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

Mycoplasma hyopneumoniae percentage of positive submissions by age category
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

Porcine Circovirus 2 percentage of positive submissions by age category
Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

Mycoplasma hyopneumoniae percentage of positive submissions
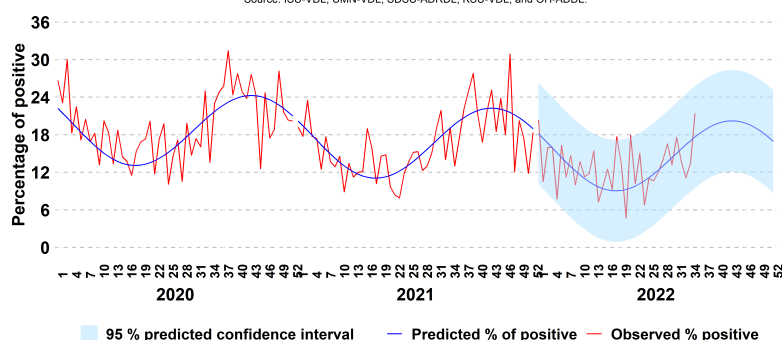Source: ISU-VDL, UMN-VDL, SDSU-ADRDL, KSU-VDL, and OH-ADDL.

Figure 3. Top: Case results tested by PCR over time. *Left* MHP; *Right* PCV2. **Middle:** percentage of PCR-positive results, by category over time. **Bottom:** expected percentage of positive results for MHP by PCR and 95% confidence interval for 2022 predicted value, based on weekly data observed in the previous 3 years.

SDRS Advisory Group highlights:

- Overall, 14.05% of 655 cases tested *M. hyopneumoniae*-positive cases in August, similar to 14.07% of 597 in July;
  - Positivity in the adult/sow category in August was 17.43% (19 of 109), a moderate increase from 14.15% (15 of 106) in July;
  - Positivity in the wean-to-market category in August was 14.94% (46 of 308), similar to 16% (48 of 300) in July;
  - Overall MHP-percentage of positive was within state-specific baselines in all 11 monitored states;
- Overall, 38.66% of 714 cases tested PCV2-positive in August, a moderate decrease from 43.11% of 798 in July;
  - Positivity in the adult/sow category in August was 39.75% (128 of 322),similar to 39.49% (139 of 352) in July;
  - Positivity in the wean-to-market category in August was 40.23% (103 of 256), a marked decrease from 50.94% (162 of 318) in July.

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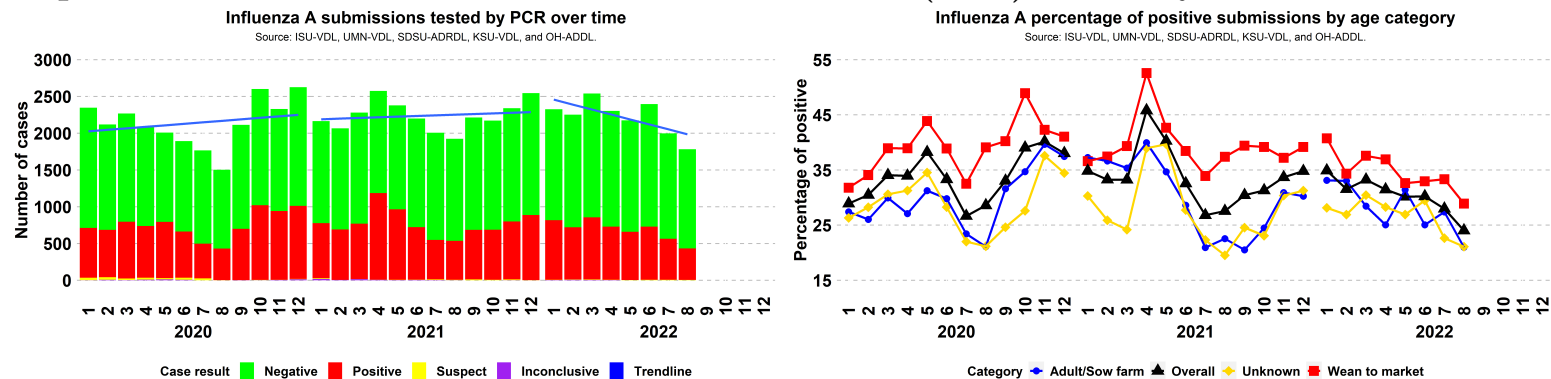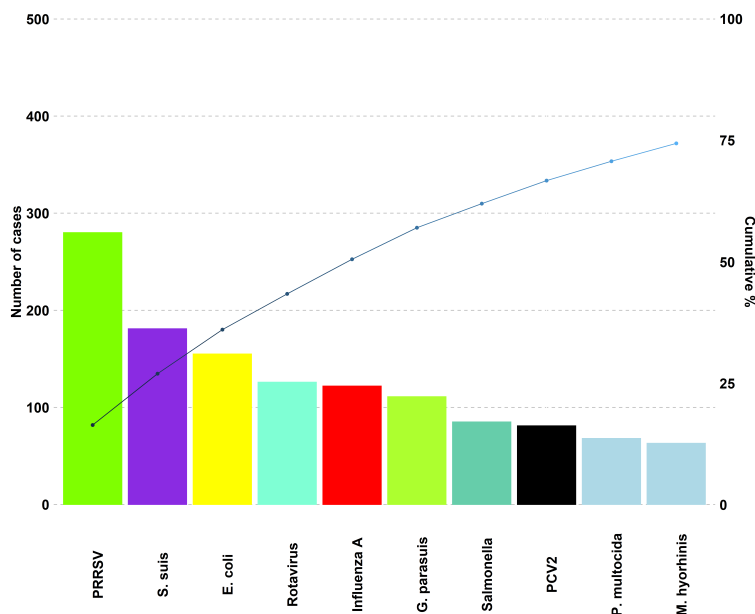# Topic 4 – Detection of Swine Influenza A Virus (IAV) RNA by RT-PCR.

Figure 3. *Left*: Results of IAV PCR cases over time. *Right*: Percentage of IAV PCR-positive results, by category over time.

SDRS Advisory Group highlights:

- Overall, 24.02% of 1,782 cases tested IAV-positive cases in August, a moderate decrease from 27.98% of 1,998 in July;
  - Positivity in the adult/sow category in August was 20.98% (73 of 348), a substantial decrease from 27.38% (112 of 409) in July;
  - Positivity in the wean-to-market category in August was 28.91% (194 of 671), a moderate decrease from 33.33% (272 of 816) in July.

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Topic 5 – Confirmed tissue cases etiologic/disease diagnosis at the ISU-VDL.
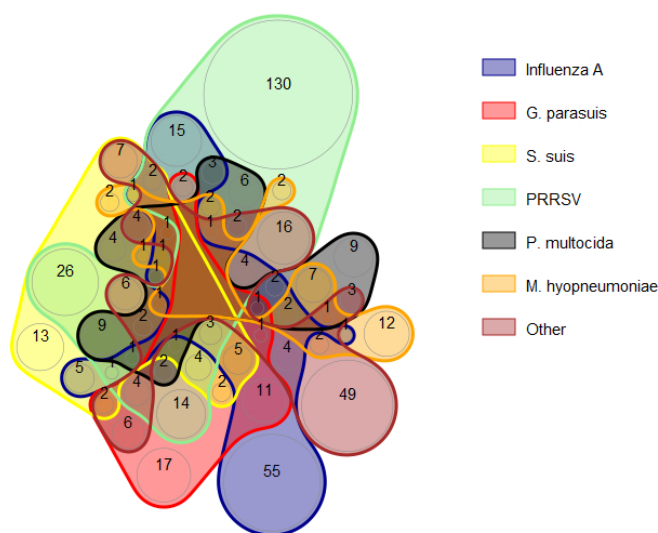
Overall diagnosis

Digestive
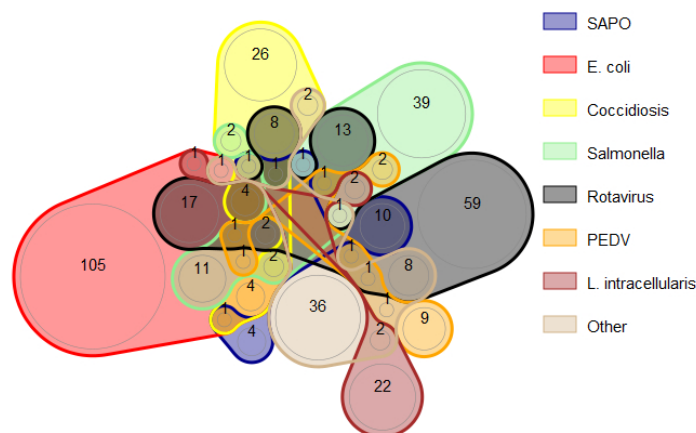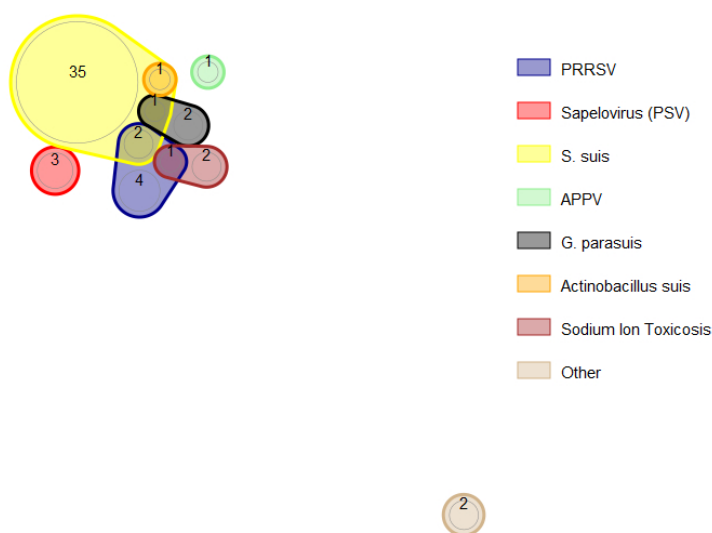

Respiratory

Nervous

Figure 4. ISU-VDL most frequent overall confirmed tissue disease diagnosis. The presented system is described in the title of the chart. Colors represent one agent; line intersections present diagnosis of 2 or more agents within a submission. Only the most frequent etiology/disease are presented. Less frequent etiology/disease are grouped as "other". Non-confirmed diagnoses are not presented.

This work is made possible due to the commitment and teamwork from the ISU-VDL diagnosticians who assign standardized diagnostic codes to each case submitted for histopathology: Drs. Almeida, Burrough, Derscheid, Gauger, Harm, Magstadt, Mainenti, Michael, Piñeyro, Rahe, Schumacher, Siepker, Sitthicharoenchai, and previous VDL diagnosticians who have contributed to this process.

Note: Disease diagnosis takes 1 to 2 weeks to be performed. The graphs and analysis contain data from July. 1 to August. 19, 2022.

SDRS Advisory Group highlights:
• PRRSV (280) led cases with confirmed etiology, followed by *S. suis* (181), and *E. coli* (155). PRRSV (260 of 796) led the number of confirmed respiratory diagnoses, *E. coli* (150 of 519) lead the number of confirmed digestive diagnoses, and *S. suis* (39 of 59) led the number of confirmed neurological diagnoses;
• The advisory group highlighted that across measures for control of *E. Coli*, there are successful cases that relied on the usage of vaccination, medication, or oral inoculation. Oral inoculations have been utilized with non-toxigenic *E.coli*. There is a perception that the disease associated with *E. coli* has been related to diet transitions and environmental stress in post-weaning piglets.

Note: *The SDRS is a collaborative project among multiple VDLs in the US swine industry. The VDL collaborators and industry partners are all invited to submit content to share on this bonus page related to disease prevention, control, and management.Stay tuned for more content in future editions.*

What new information have we learned about PRRSV isolation?

Wannarat Yim-im[1] and Jianqiang Zhang.[1]

1 - Iowa State University, Ames, Iowa, USA.

The full-text reference articles: Improving PRRSV isolation from clinical samples and Characterization of PRRSV in clinical samples

Introduction

Isolation of porcine reproductive and respiratory syndrome virus (PRRSV) in cell culture is a primary means of obtaining virus isolates for autogenous vaccine production and other applications. This report summarizes the major findings from two studies. Study 1: Compare PRRSV virus isolation (VI) from clinical samples in MARC-145 and ZMAC cells. Study 2: Determine whether the virus isolate obtained in cell culture and the virus in the clinical sample are sequence equivalent or not.

Materials and Methods

Study 1. 375 clinical samples representing different specimen types, CT ranges, and genetic lineages were used for PRRSV VI attempts in ZMAC and MARC-145 cells for head-to-head comparisons. These included 305 PRRSV-2 PCR-positive clinical samples (109 serum samples with CT 13.2-36.2, 96 lung samples with CT 13-30.2, 59 oral fluid samples with CT 22.0-34.0, and 41 processing fluid samples with CT 15.6-30.9), and 70 PRRSV-1 PCR-positive clinical samples (31 serum samples with CT 18.4-36.8, 8 lung samples with CT 21.6-35.4, and 31 oral fluid samples with CT 28.0-36.8).

Study 2. 1024 PRRSV PCR-positive (995 PRRSV-2, 26 PRRSV-1, and 3 co-infected PRRSV-1 and PRRSV-2 PCR-positive) clinical samples and their isolates obtained in MARC-145 and/or ZMAC cells during 2010-2020 were included in this study. ORF5 sequences of 1024 clinical samples, 837 MARC-145 isolates (passage 0 or P1), and 270 ZMAC isolates (P0 or P1) were determined and compared for RFLP patterns, genetic lineages, and nucleotide identities. For those cases with non-matching PRRSV between clinical sample and cell culture isolate, next-generation sequencing (NGS) and vaccine-specific PCR were conducted to elucidate the differences.

Major findings and implications

1. It is a challenge to isolate PRRSV from oral fluid and processing fluid samples regardless of whether ZMAC cells or MARC-145 cells are used. The preferred specimen types for PRRSV VI are lung > serum > processing fluid and oral fluid.

2. PRRSV VI success rate was significantly higher in ZMAC than in MARC-145 cells for serum and lung samples containing PRRSV-1, PRRSV-2, or PRRSV-1 & PRRSV-2 co-infection. When PRRSV VI results are negative in MARC-145 cells, PRRSV VI attempts in ZMAC cells are recommended.

3. For cost-effectiveness, it is recommended to conduct PRRSV VI from clinical samples with CT values of < 30.

4. For samples containing lineage 1 and lineage 8 PRRSV-2, VI success rates were significantly higher in ZMAC than in MARC-145 cells. For samples containing lineage 5 Ingelvac PRRS MLV, the VI success rates were similar in two cell lines. If the PRRSV-2 genetic lineage information is unknown in a clinical sample, the VI attempts will result in a better overall outcome in ZMAC cells than in MARC-145 cells.

5. When PRRS virus isolates obtained in ZMAC cells were adapted to grow in MARC-145 cells, 57.3% (47/82) grew and 42.7% (35/82) did not grow. In contrast, all of the evaluated 45 PRRSV-2 isolates obtained in MARC-145 cells grew in ZMAC cells. For those autogenous vaccine production companies using MARC-145 cells in their system, they may sometimes fail to propagate the virus in MARC-145 cells to produce autogenous vaccines when a ZMAC virus isolate is forwarded to them.

6. For clinical samples evaluated in Study 2 (3 positive for both PRRSV-1 and PRRSV-2, 26 PRRSV-1, and 96.2% [957/995] of PRRSV-2), the predominant ORF5 sequences of PRRSV in the clinical samples and the respective cell culture isolates were matching in regards to RFLP patterns, genetic lineages, and nucleotide identities.

7. Small percentage of PRRSV-2 PCR-positive clinical samples (24/995; 2.4%) and their MARC-145 and/or ZMAC isolates had 98.6-99.8% ORF5 nucleotide identity and the same genetic lineages but different RFLP patterns due to point mutation(s) located at the HincII or SacII restriction site, but they were considered as the same virus strains.

8. For small percentage of PRRSV-2 cases (14/995; 1.4%), the predominant ORF5 sequences derived directly from clinical samples were different from those from their corresponding MARC-145 isolates but were similar to their corresponding ZMAC isolates. In those cases, most isolates obtained in MARC-145 cells contained Ingelvac PRRS MLV vaccine-like virus while the predominant viral sequences detected in clinical samples and ZMAC isolates were wild-type strains. This is concerning because autogenous vaccines produced from MARC-145 isolates may not contain the desired wild-type virus strain found on the farm. Additional investigations (vaccine-specific PCR and NGS) confirmed presence of >2 PRRSV-2 strains (mixed infection) in such clinical samples. In co-infected samples, while Sanger sequencing determines the predominant strain ORF5 sequence from the clinical sample (i.e. wild-type A), if wild-type strain A has lower growth adaptability or kinetics compared to the other strain (i.e. vaccine-like strain B) in MARC-145 cells, vaccine-like strain B could be isolated in MARC-145 cells although wild-type strain A is isolated in ZMAC cells.

9. Characterizing PRRSV sequences from clinical samples and cell culture isolates should be conducted before using isolates for producing autogenous vaccines or other applications.