TVMDL Texas A&M Veterinary Medical Diagnostic Laboratory

TVMDL Bovine Syndromic Approach to Testing and Testing Trends

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Diagnostic Testing & Methodology

What is the purpose of diagnostic testing?

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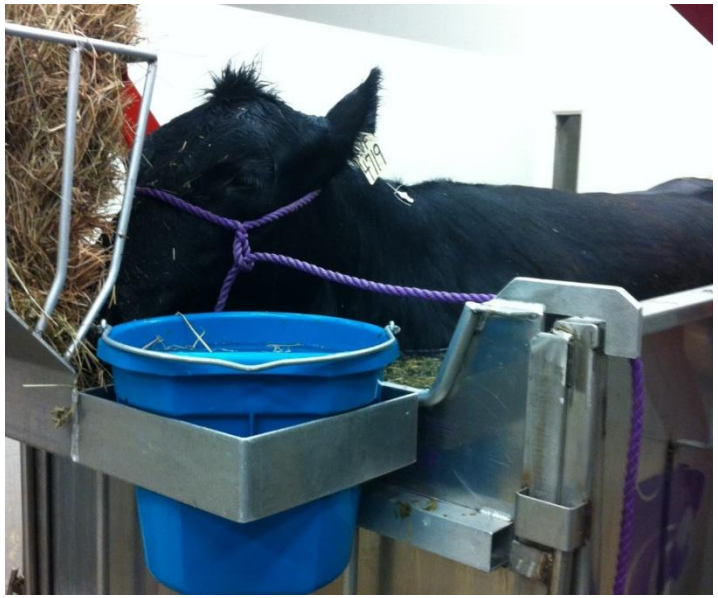
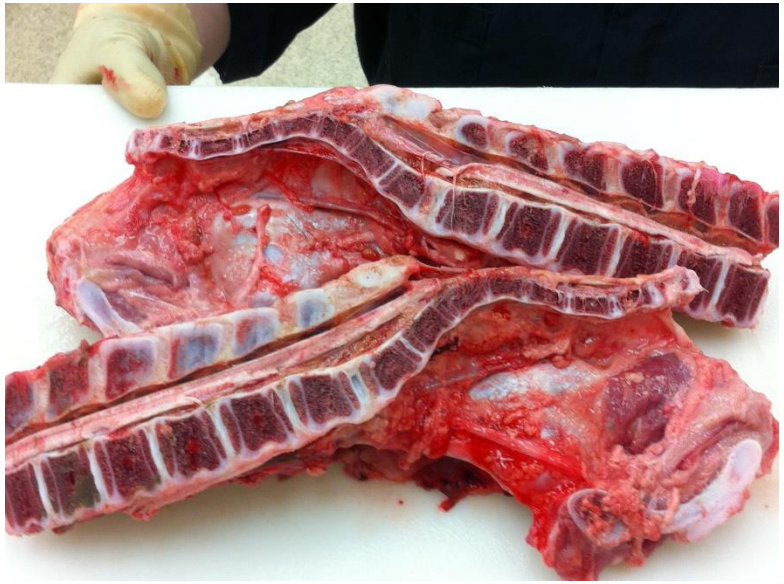
- ~~To find out what is wrong with our patients~~
- Diagnostic tests are tools of prediction, not explanation





What is the purpose of diagnostic testing?

- Diagnosis: to rule in or rule out a specific disease based on pathogen presence, exposure, or physiological effect
- Monitoring: to check response to therapy or the efficacy of preventative, vaccination, or biosecurity programs
- Screening: for genetic diseases, infectious disease carriers, or persistently infected animals
- Research: to understand the pathophysiology of a particular disease process

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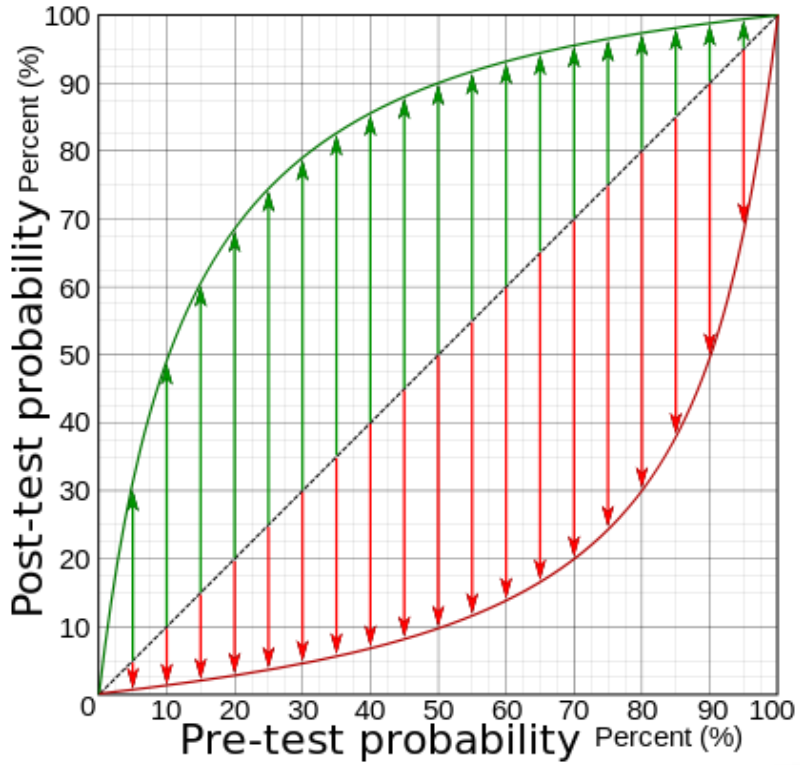
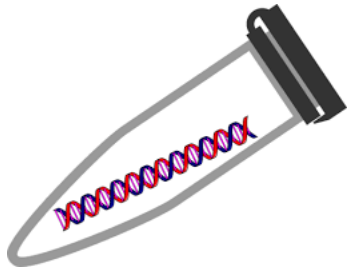
What is the purpose of diagnostic testing

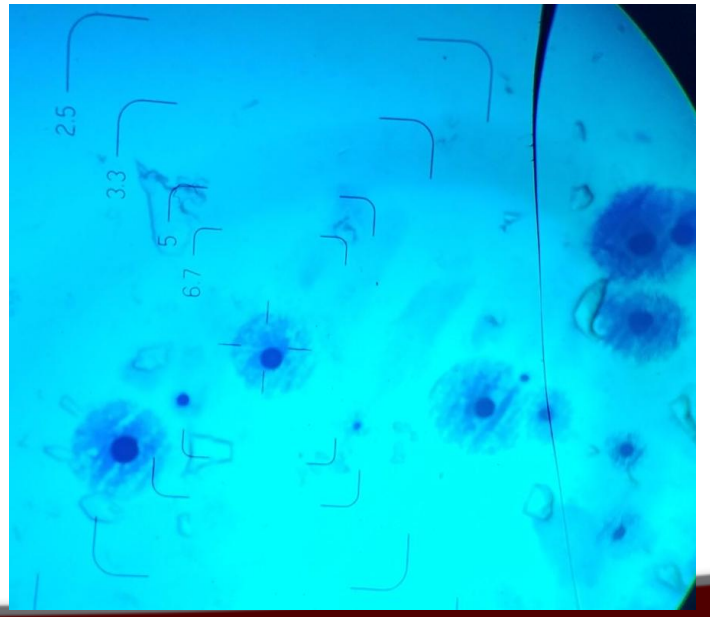
- To gather specific information that closes the gap (amount of uncertainty) between pre-test clinical suspicion and post-test probability of disease
- To inform next steps
  - Treatment & Prognosis
  - Management Changes
  - Prevention Strategies



Testing Methods – How to select tests

- Depends on the diagnostic question
- What information is needed to minimize uncertainty and allow action
  - Anatomic, histologic, or clinical pathology correlated with certain disease processes or pathophysiology
  - Exposure or antibody response to pathogen
  - Presence of pathogen





Testing Methods – Diagnostic Question

- What information is needed for the next step?
- Histologic lesions
- Body system function (dysfunction)
- Pathogen detection
- Pathogen isolation

Testing Methods – Pathogen Detection

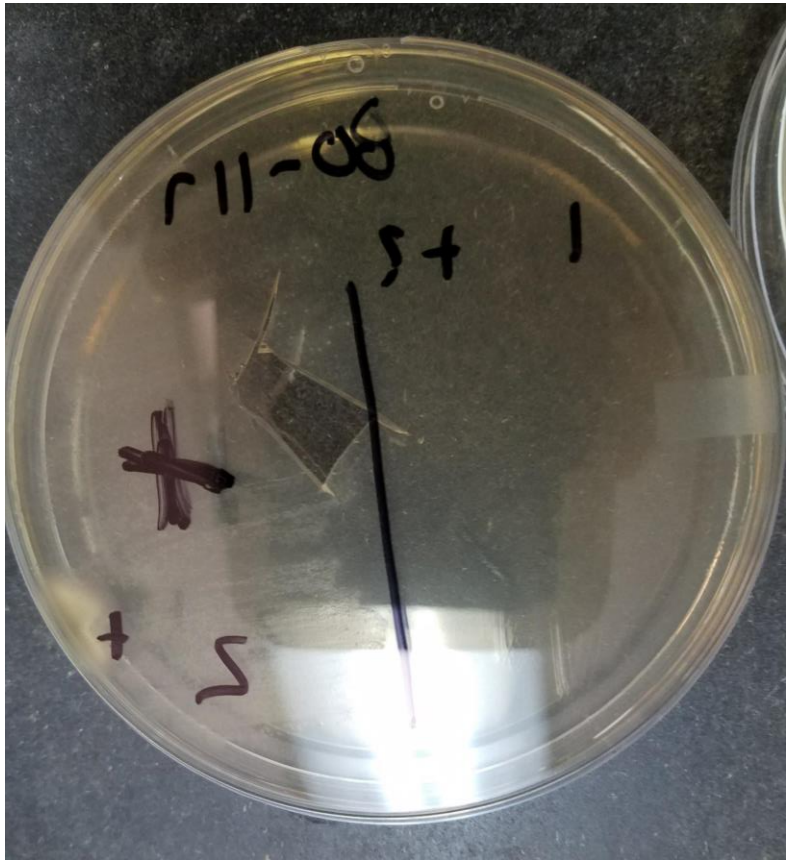
- Molecular Diagnostics
  - PCR
  - qPCR
- Very sensitive and specific
- Quick answer
- Recent MLV can be detected

Testing Methods – Pathogen Isolation

- Virus Isolation
  - Takes longer
  - Dependent on viable virus in the sample
  - Less sensitive than molecular methods
  - Not all viruses can be isolated - BRSV
  - Isolates can be sequenced
  - BRD viral isolates can be from recent MLV

Testing Methods – Pathogen Isolation

- Bacterial Culture
  - +/- Takes longer (depends on culture target)
  - Dependent on viable bacteria in the sample
  - Affected by antibiotic administration
  - Antimicrobial susceptibility testing can be performed on isolates



What test do I want?

- I want to better define the syndromic problem by investigating any pathologic changes in the samples that can be attributed to the syndrome I am investigating
  - Histopathology
- I want to evaluate the organ/body system function (malfunction) attributed to the syndrome in my patients
  - Chemistry, CBC, Tissue (liver) mineral/heavy metal panel
- I want to look for antibody response to pathogens associated with the current syndrome
  - ELISA, Agglutination tests, Virus Neutralization

What test do I want?

- I want to know if a pathogen is present in the sample (yes/no)
  - qPCR
- I want to know what bacteria are present in the sample and want to know the antimicrobial susceptibility +/- send isolates for vaccine development
  - Culture – check what media is required for transport
- I need to be able to differentiate wild type virus from possible MLV vaccine strains
  - VI (sometimes qPCR) followed by sequencing

Sample Submission Tips

- Histopathology
  - Tissue sections should be ¼" thick and submitted in 10% neutral buffered formalin
  - To assure adequate fixation NBF to tissue ratio should be a least 10:1
  - FIX GASTROINTESTINAL TISSUE AND BRAIN TISSUE ASAP!
  - Protect other samples from formalin fumes during shipment
- Clinical Pathology
  - Serum needs to be separated from RBC and placed in secondary vial ASAP
  - Prepare slides from whole blood and send with EDTA tube for CBC
  - Protect whole blood from temperature extremes
  - Urine should be sent with cold pack to decrease cell deterioration and bacterial overgrowth

Sample Submission Tips

- Molecular Diagnostics (PCR/qPCR)
  - Label your swabs!
  - Do not submit swabs in bacteriology media (gel)
  - Do not submit charcoal swabs, cotton swabs, or wood handle swabs
  - Preferred swab type is Dacron or polyester on a plastic handle
  - Keep samples chilled
  - Autolysis negatively affects qPCR sensitivity (nucleic acid degradation)

Sample Submission Tips

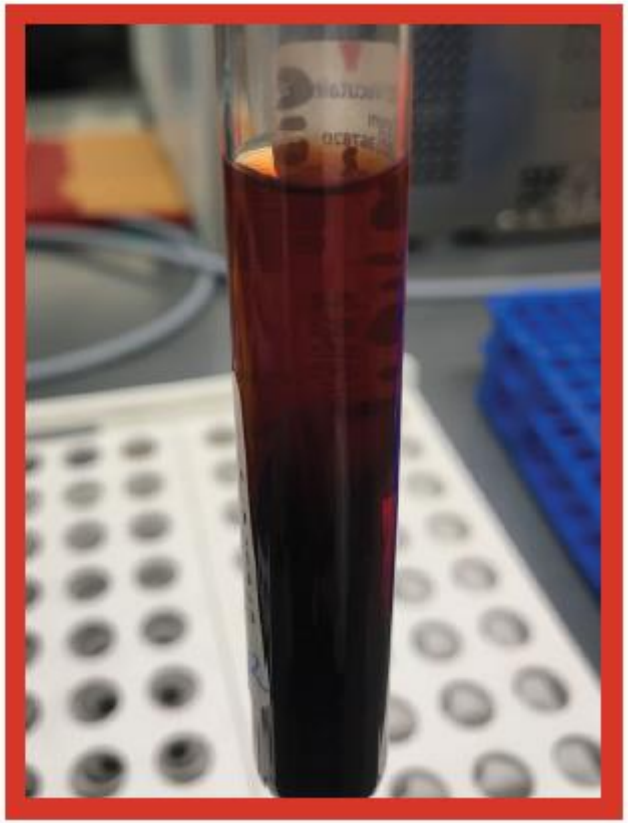
- Bacteriology
  - Label your swabs!
  - Provide a history, including animal age
  - Fresh tissues should be kept chilled
  - Protect samples from formalin fumes
- Virology
  - Swabs must be Dacron or polyester and moist on arrival
    - Viral transport media or 0.25 mL PBS/Sterile Saline
  - Fresh tissues should be kept chilled and shipped overnight
  - Autolysis negatively impacts ability to recover virus from sample

Sample Submission Tips

- Drug testing
  - Call the Drug Lab before collecting samples
- Vitamin Testing
  - Protect serum or tissue from light (wrap in foil)
- Tissue ICP/MS Panels
  - Liver for most mineral and metal targets
  - Kidney required for confirmation of copper toxicity
- Serum Mineral Panels
  - Need royal blue top tubes (not red top tubes) for serum
  - Remove serum from RBC before shipment

Serum Sample Submission in General

- Tests may not be run on samples that are hemolyzed or lipemic



Syndromic Testing Plans

TVMDL Bovine Syndromic Plans

- Tests clustered by syndrome
- Easier test selection
- Entire plan will increase result interpretation potential
- Can add serology panels to most diagnostic plans
- Can customize plans based on pre-test clinical suspicion

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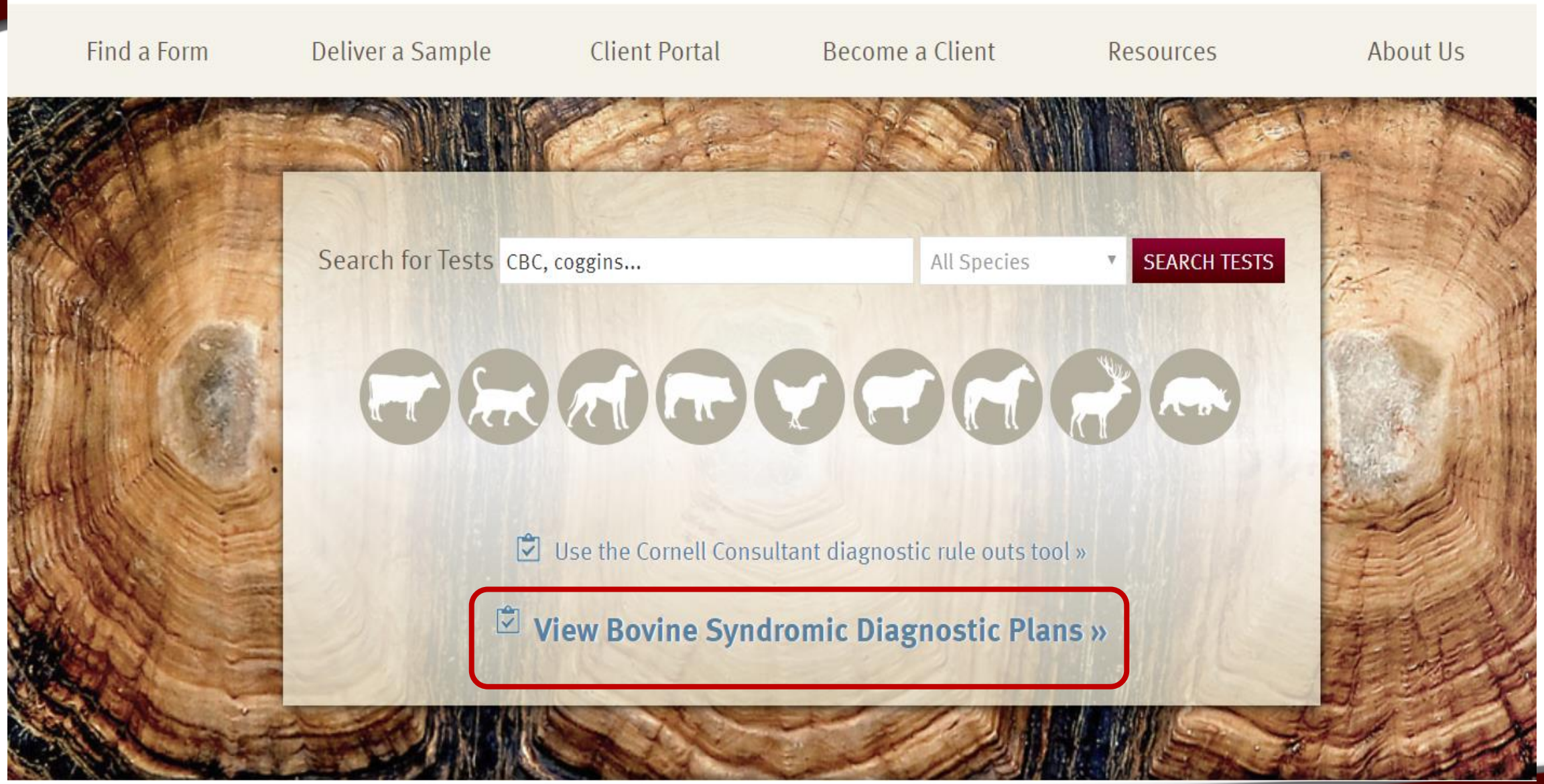

Click below to view each plan:

Bovine BRD Diagnostic Plan	Bovine Sudden Death Diagnostic Plan
Bovine Abortion Diagnostic Plan	Bovine Neurological (CNS) Disease Diagnostic Plan
Bovine Calf Diarrhea Diagnostic Plan	Bovine Pinkeye (IBK) Diagnostic Plan
Bovine Adult Diarrhea/Weight Loss Diagnostic Plan	Bovine Biosecurity Diagnostic Plan

Bovine BRD Diagnostic Plan

This plan was created to assist with the investigation of the pathophysiology and etiologic agents involved the death of cattle with clinical signs or post mortem findings consistent with respiratory disease. Follow the links attached to each test name to see more detailed information from the TVMDL test catalog.

Recommended initial testing:

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Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology (up to 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AM	MTWRF
				CS	MTWRF
Aerobic & Anaerobic Culture – Livestock	tissues or swabs in Amies media	2-7 days	Bacteriology	AM	MTWRFSa
				CS	MTWRFSa
Susceptibility Test-Food Animal(please indicate MIC or KB preference)	pure isolate	1 day	Bacteriology	AM	MTWRF
				CS	MTWRF
Bovine Basic BRD Bacterial PCR Panel	lung, TTW, BAL, nasal/pharyngeal swab	1-3 days	Molecular Diagnostics	AM	TWRF
				CS	TWRF
Bovine Basic BRD Viral PCR Panel	lung, trachea, TTW, BAL, nasal/pharyngeal swab	1-4 days	Molecular Diagnostics	AM	MTWRF
				CS	TWRF

Schedule: M=Monday, T=Tuesday, W=Wednesday, R=Thursday, F=Friday, Sa=Saturday

View secondary or additional tests

TVMDL

View secondary or additional tests

Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology (> 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AMA CS	MTWRF MTWRF
Mycoplasma culture – Livestock	tissues or charcoal swabs in Amies media	14 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Bovine Basic BRD Serology Panel	serum	3-5 days	Serology & Virology	AMA CS	TF TF
Bovine Comprehensive BRD Serology Panel	serum	3-5 days	Serology & Virology	AMA CS	TF TF
Bovine Coronavirus IHC	TVMDL tissue blocks	-	Referral	Michigan	-
Bovine Coronavirus qPCR	lung, TTW, BAL, nasal/pharyngeal swab	-	Referral	KSVDL	-
Bovine Influenza Virus qPCR	lung, TTW, BAL, nasal/pharyngeal swab	-	Referral	KSVDL	-

BRD Case Submission Pointers

- Do not submit swabs for PCR in gel
- PCR has enhanced sensitivity for viruses and *M. bovis*
- Send second swab if you need culture for AST (susceptibility)
  - 3 swabs if you want Mycoplasma culture
- PCR and VI can detect MLV for up to 4 weeks after vaccination (IBR, BVD)
- BRSV detection LRT samples > URT samples (BAL, TTW)
- Coronavirus detection URT samples > LRT samples (NS, NPS)
  - Be familiar with population benchmarks for shedding vs. clinical syndrome
- Send at least 5 mL serum if asking for numerous BRD antibody tests
  - Comprehensive BRD Serology Panel

BRD Testing Plan Modification – Recent MLV

- qPCR and VI can possibly detect virus from MLV
- If qPCR panel is Positive for virus of interest and has a low ct value
  - Send for sequencing to compare vaccine vs wild type
- If qPCR panel is Positive for virus of interest with a ct value > 30
  - VI sample and if isolate is recovered send for sequencing
- Not 100% validated for BRSV, yet

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Histopathology (up to 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AM CS	MTWRF MTWRF
Abortion Culture - Livestock	fetal tissues, fetal stomach contents, placenta	10 days	Bacteriology	AM CS	MTWRFSa MTWRFSa
IBR (BHV-1) qPCR	lung, trachea, nasopharyngeal swab	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
BVD qPCR	lung, trachea, nasopharyngeal swab, ear notch	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
Leptospira spp. qPCR	kidney, liver, placenta	1-4 days	Molecular Diagnostics	CS	TWRF
Neospora caninum qPCR	brain, placenta, liver, lung, heart	2-3 days	Molecular Diagnostics	CS	TWRF
Liver Vitamin A quantification	10 g liver	1-7 days	Toxicology	CS	R
Liver Tissue Mineral Panel	10 g liver	1-4 days	Toxicology	CS	TR

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View secondary or additional tests

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Histopathology (up to 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AM CS	MTWRF MTWRF
B4 qPCR Panel (IBR, BVD, BLV, BTV)	spleen, liver, lung, lymph node	1-4 days	Molecular Diagnostics	CS	TWRF
Bovine Basic BRD Viral Panel (IBR, BVD, BRSV, PI3)	lung, respiratory swabs	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
Anaplasma marginale PCR	spleen, lung, liver, kidney	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
Liver Vitamin Panel (Vitamin A & E)	10 g liver	1-7 days	Toxicology	CS	R
Liver Single Mineral quantification	10 g liver	1-4 days	Toxicology	CS	TR
Nitrates Qualitative	fetal ocular fluid or eyeball	1-2 days	Toxicology	AM CS	MTWRF MTWRF
Fungal Culture/Identification	tissues, placenta, swabs in transport media	21 days	Bacteriology	AM CS	MTWRFSa MTWRFSa

Bovine Basic Abortion Serology	2 mL serum or fetal effusions	2-3 days 2-4 days	Serology	AM CS	MTWRF MR
Bovine Comprehensive Abortion Serology	2 mL serum or fetal effusions	3-5 days	Serology & Virology	AM	MTWRF
Tirtrichomonas foetus qPCR	1-2 mL fetal abomasal contents or cow cervical wash in trich pouch	2-4 days	Molecular Diagnostics	AM CS	MTWRF MTWRF
Campylobacter spp. qPCR	placenta, lung, 1-2 mL abomasal contents or cervical/uterine wash	1-3 days	Molecular Diagnostics	AM CS	TWRF TWRF
Campylobacter fetus differentiation qPCR	placenta, lung, 1-2 mL abomasal contents or cervical/uterine wash	1-3 days	Molecular Diagnostics	AM CS	TWRF TWRF
Listeria monocytogenes PCR	brain, liver, spleen	-	Referral	KSVDL	-

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Abortion Case Submission Pointers

- Basic Abortion Serology Panel is for herd screening
- Comprehensive Abortion Serology Panel is for investigations
  - Need at least 3 mls of serum
- History helps the case coordinators
- Brain is preferred sample type for *Neospora* testing
- Vitamin and mineral abnormalities have been very common this year – might consider testing if infectious disease testing was unrewarding
- Make sure the organs are in the fetus before submitting to necropsy
- If you submit an entire fetus, there will be a necropsy fee
- Send ear notches for BVD Ag ELISA

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Recommended initial testing:

Test	Samples	Turnaround Time	Section	Lab	Schedule
Basic Calf Diarrhea Panel (Bovine Coronavirus, rotavirus, cryptosporidium)	feces, GI contents, intestines	1-4 days	Molecular Diagnostics	CS	TWRF
Aerobic & Anaerobic Culture – Livestock	feces, fresh tissue	2-7 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Salmonella genus qPCR	1 g feces, intestine, fecal swabs	1-4 days	Molecular Diagnostics	CS	TWRF
Salmonella serotyping	salmonella isolate	-	Referral	NVSL	-
E. coli PCR	E. coli isolate	4-6 days	Molecular Diagnostics	AMA	R

Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology (> 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AMA CS	MTWRF MTWRF
Susceptibility Test-Food Animal (please indicate MIC or KB preference)	pure isolate	1 day	Bacteriology	AMA CS	MTWRF MTWRF
Clostridium perfringens typing PCR	pure isolate	1-4 days	Molecular Diagnostics	AMA	R
Ruminant Chemistry Profile	0.5 mL serum	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
CBC – Livestock	1 mL EDTA blood + blood film	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Fecal Flotation Qualitative	3-5 g fresh feces	1-2 days	Parasitology	AMA CS	MTWRF MTWRF
Fecal McMaster EPG (Quantitative)	3-5 g feces	1-2 days	Parasitology	AMA CS	MTWRF MTWRF
Electron Microscopy	feces, GI contents, intestines	5-7 days	Virology	CS	Varies
BVD Antigen Capture ELISA	ear notch, 1 mL serum	1-2 days	Virology	AMA CS	MTWRF TF

Calf Diarrhea Case Submission Pointers

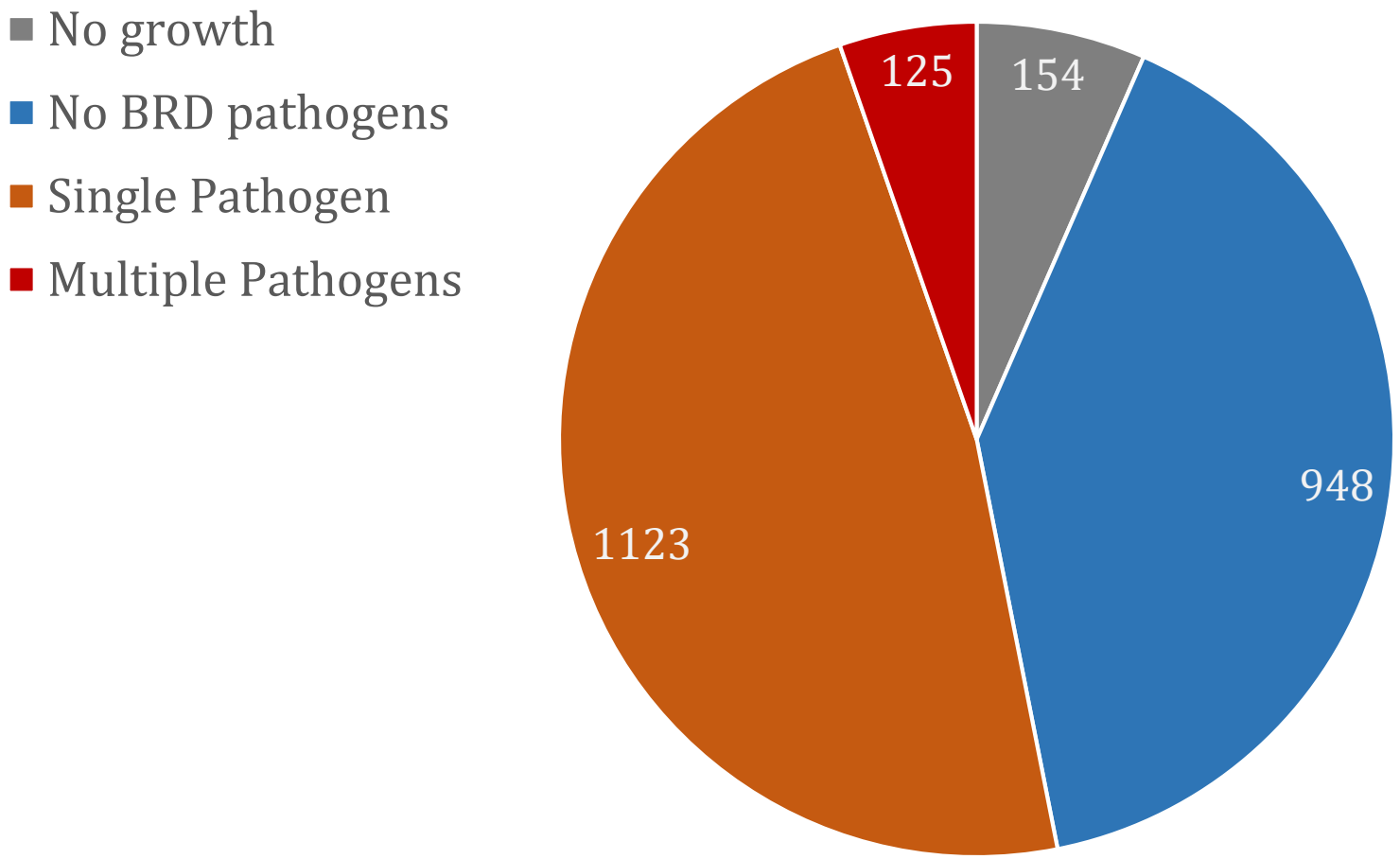
- PCR (Basic Calf Diarrhea PCR Panel) is preferred test for rotavirus, coronavirus, and cryptosporidium
- VI will not isolate (find) coronavirus
- EM will detect rotavirus but it is not as sensitive (or quick) as PCR
- E. coli PCR will type isolates by presence or absence of virulence and toxin genes
  - Will help correlate clinical findings with ETEC, invasive, or other E. coli
- Salmonella PCR + culture with enrichment will increase sensitivity of Salmonella detection in high suspicion cases
- Older calves may need McMaster's EPG and/or additional Clostridium perfringens testing

BRD Testing Results

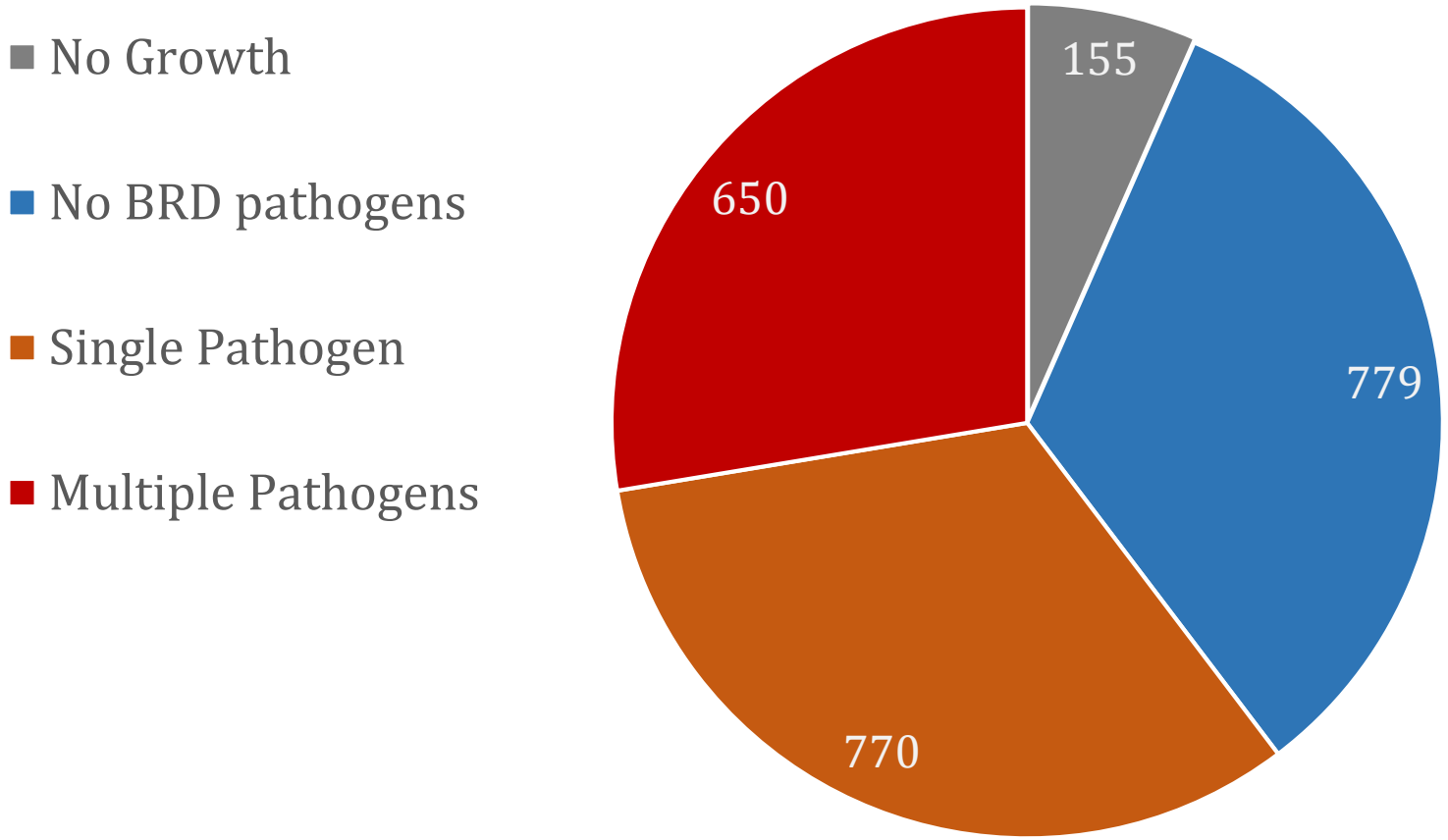
Test results

- January 1, 2014 – December 31, 2017
- TVMDL CS + TVMDL Amarillo
- All submissions – not just TX, OK, NM
- Domesticated cattle (no buffalo/bison)
- Lower Respiratory Tract Samples (Lung, Trachea, Thoracic Cavity)
- Removed all University or Research cases
- Removed all abortion cases or cases that could not be attributed to BRD d/t lack of provided history

LRT Routine Culture Results (n=2350)



LRT Mycoplasma + Routine Culture Results (n=2146)

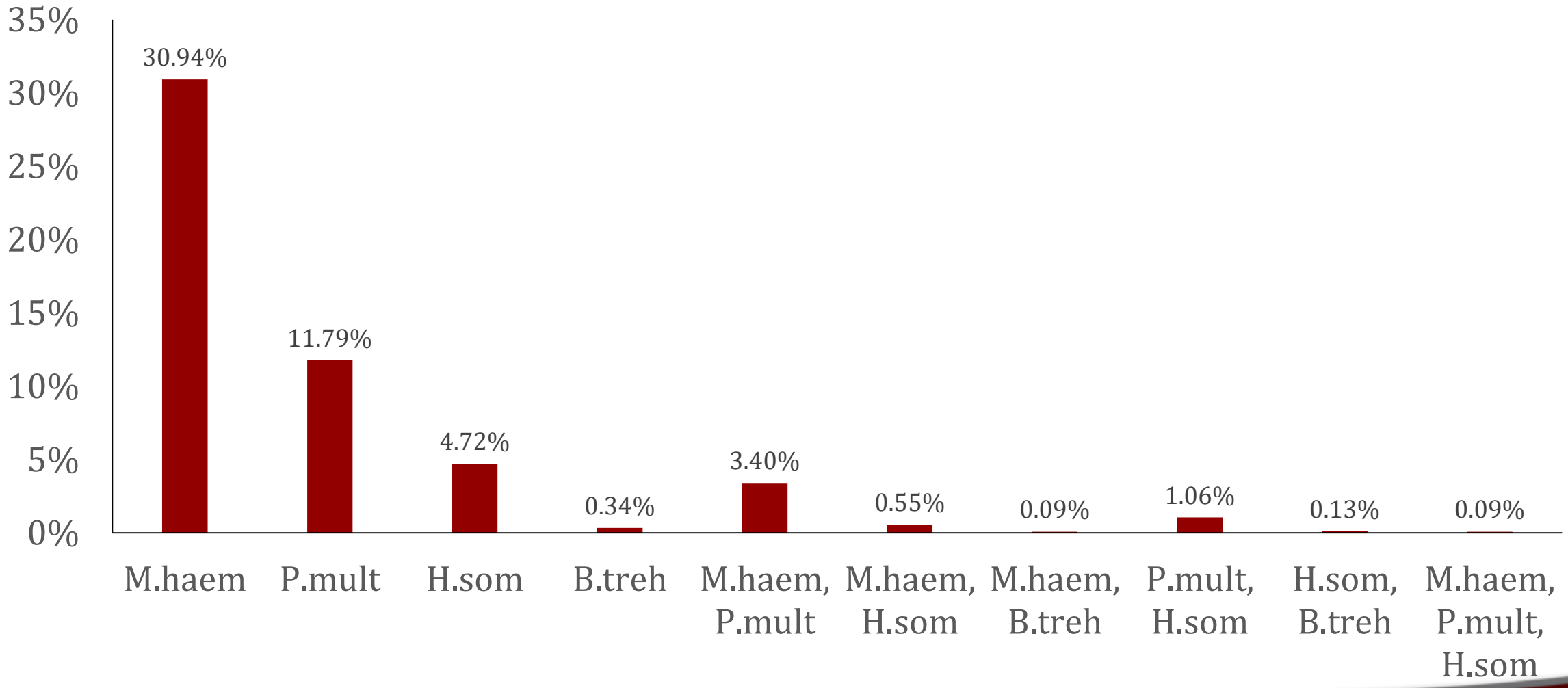


TVMDL BRD LRT Culture 2014 - 2017

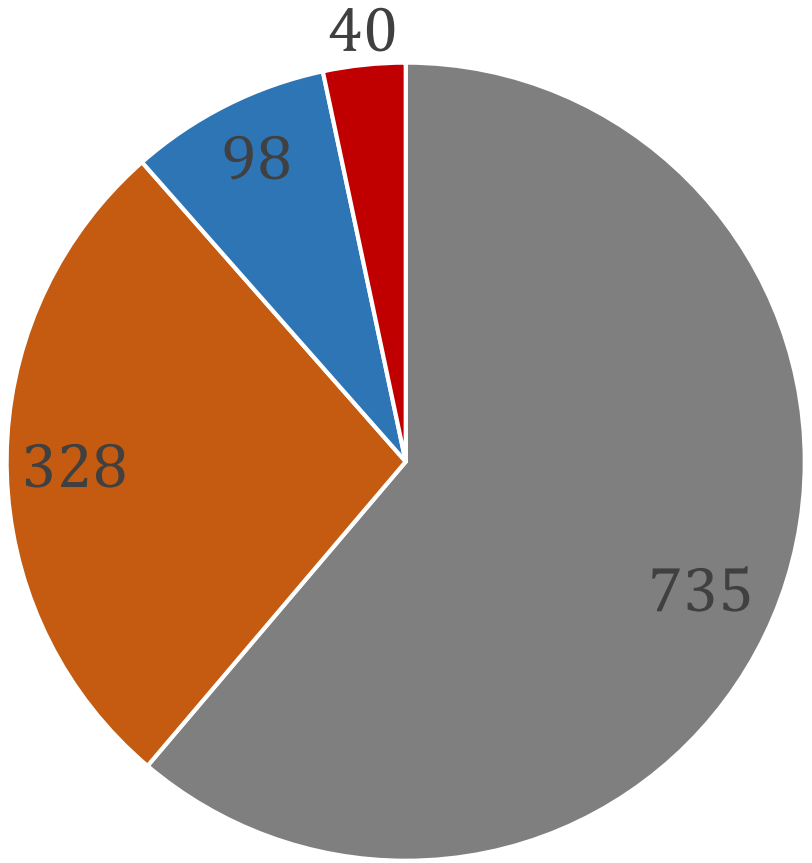
	LRT		
Pathogen	Total #	# POS	Probability of Detection
Mycoplasma	2150	761	35.40%
Mannheimia haemolytica	2350	824	35.06%
Pasteurella multocida	2350	384	16.34%
Histophilus somni	2350	154	6.55%

Total raw prevalence – does not account for pathogens isolated in combination

LRT Pathogen Recovery
Routine Culture



LRT Viral PCR Panel Results (n=1201)



	LRT		
Pathogen PCR	Total #	# POS	Probability
Mycoplasma bovis	118	77	65.25%
Mycoplasma spp.	21	14	66.67%
BRSV	1221	300	24.57%
BVD	1259	240	19.06%
PI3	1202	79	6.57%
BHV1	1247	45	3.61%

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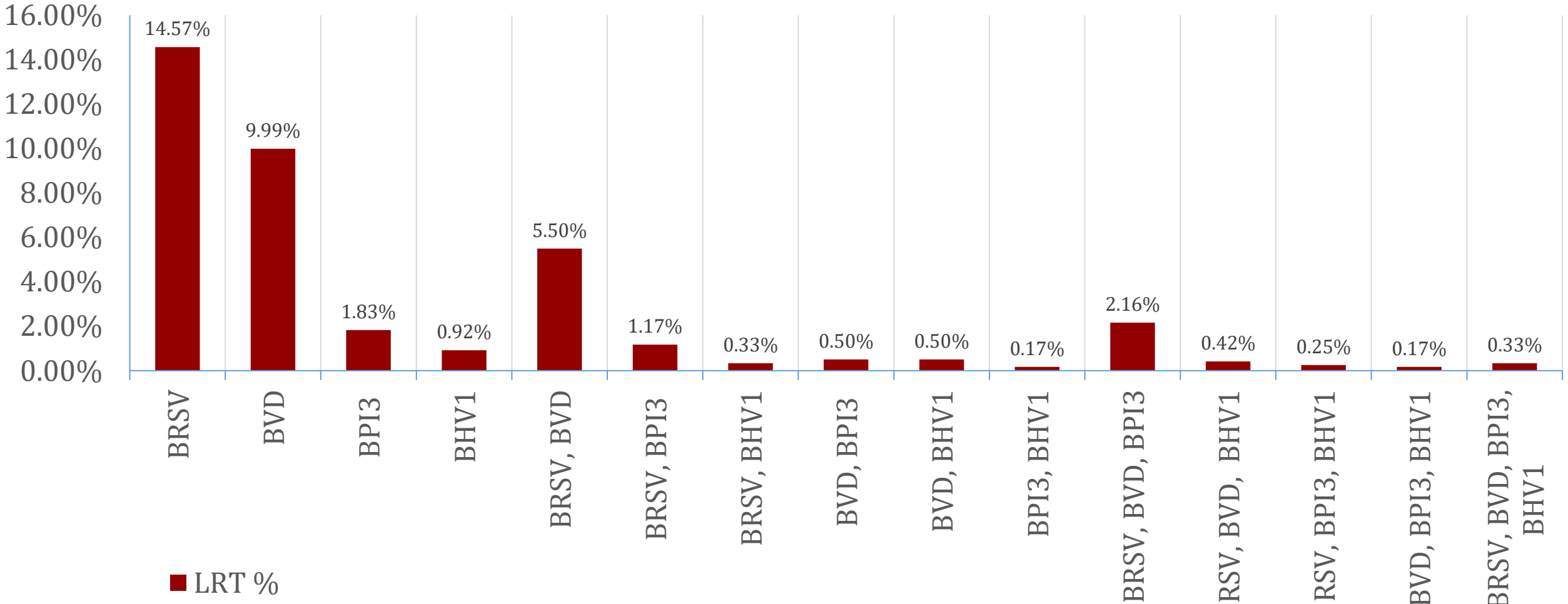
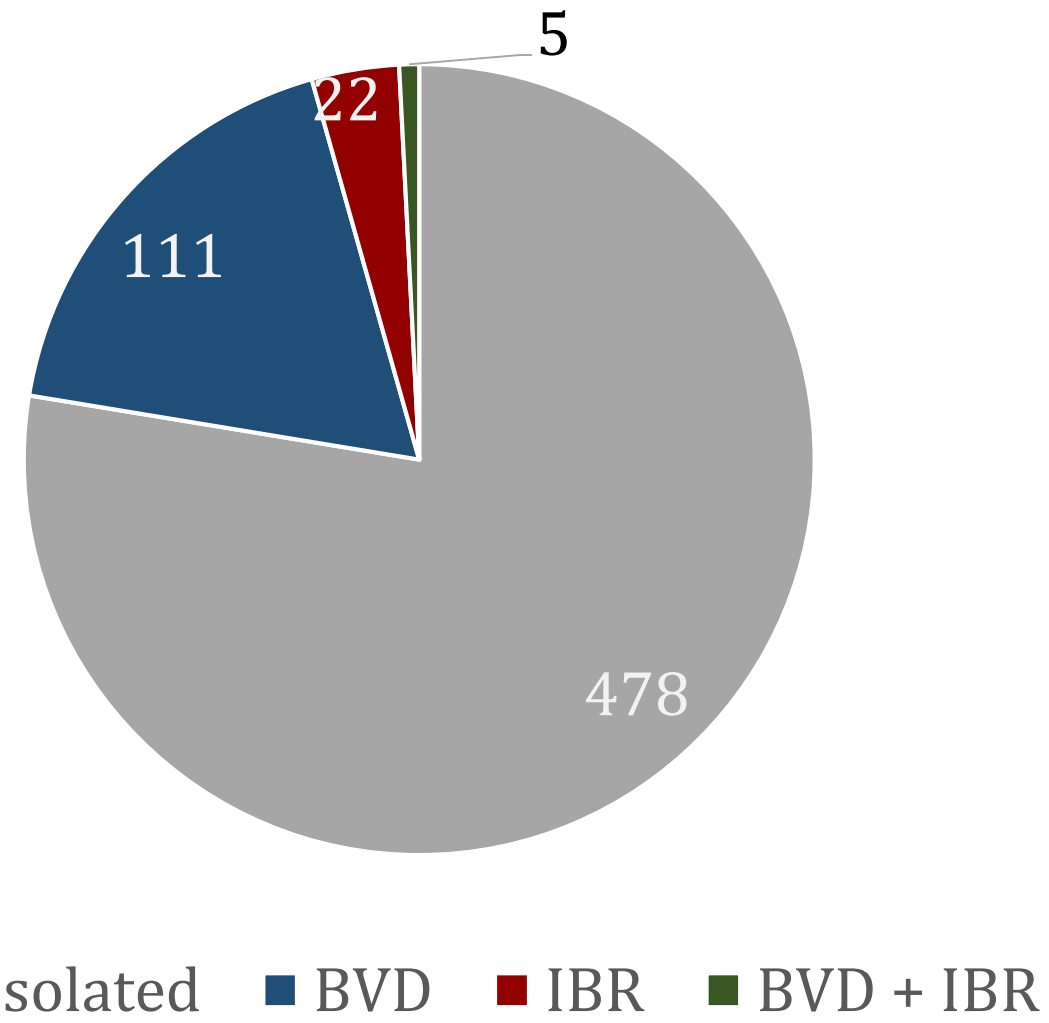
BRD LRT Viral PCR Pathogen Recovery



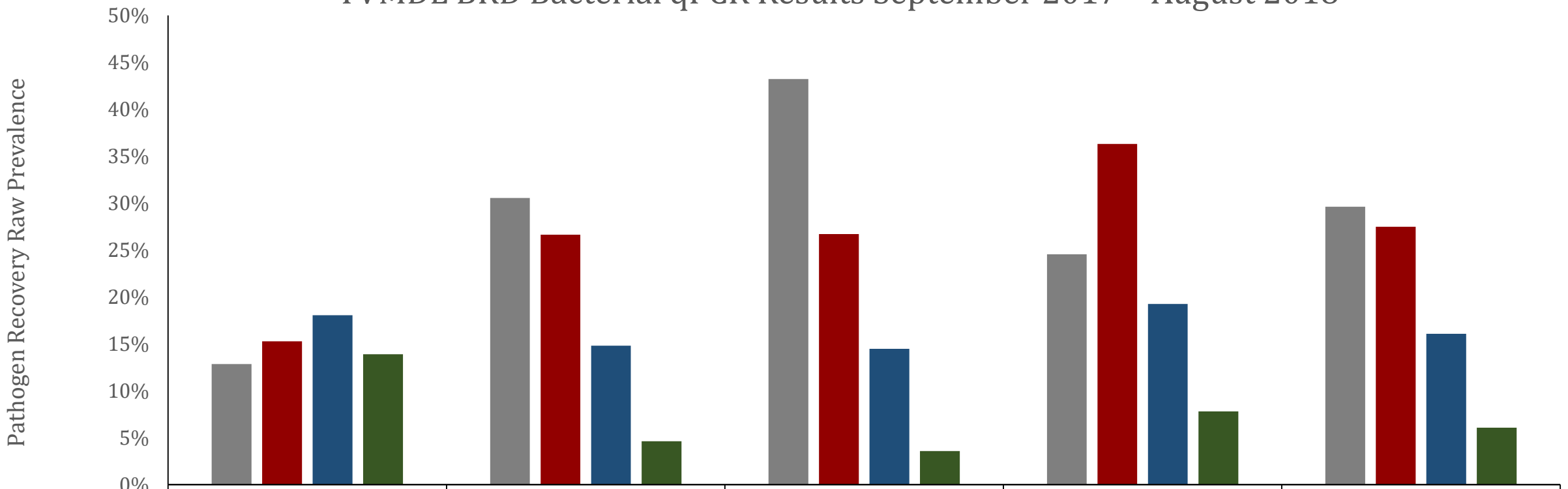
LRT Virus Isolation (n=616)



More Recent Test results

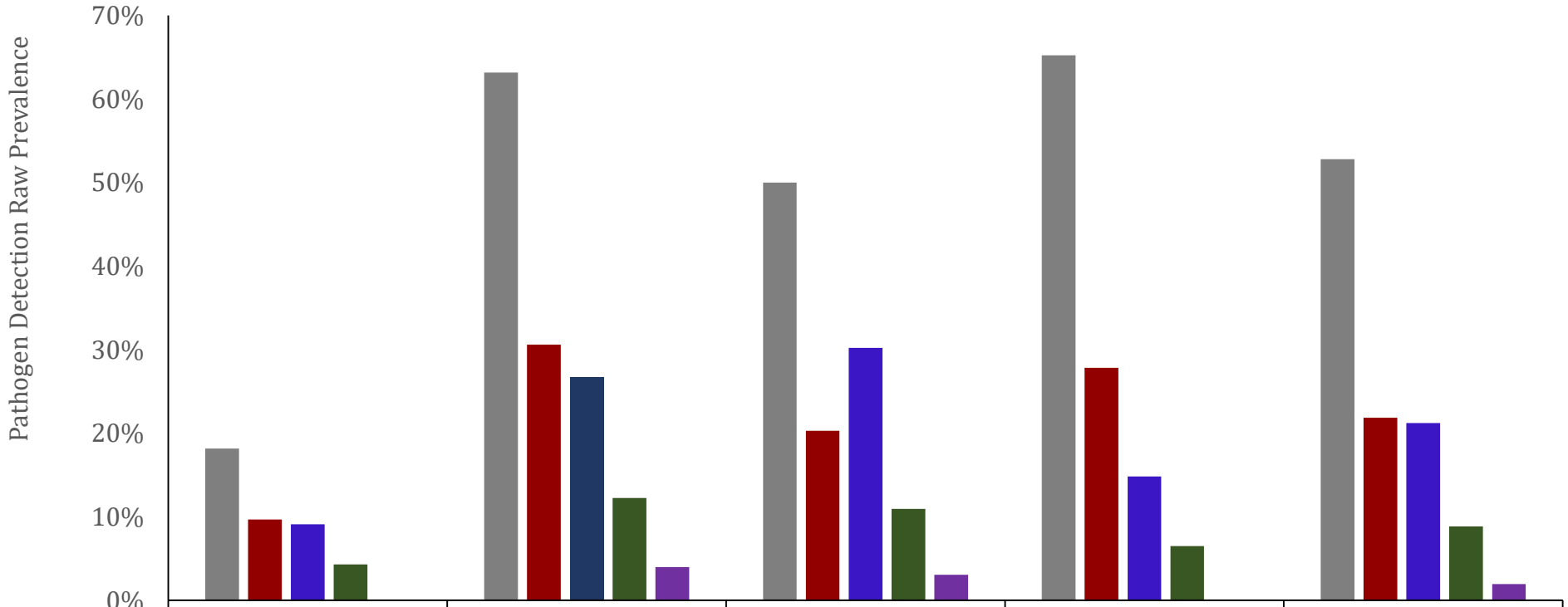
- September 2017 – August 2018
- TVMDL CS + TVMDL Amarillo
- BRD qPCR Bacterial and Viral Panels
- Submissions from TX, OK, NM
- Domesticated cattle (no buffalo/bison)
- All Respiratory Tract Samples
- Removed all University or Research cases
- Removed all abortion cases or cases that could not be attributed to BRD d/t lack of provided history

TVMDL BRD Bacterial qPCR Results September 2017 – August 2018



	Spring	Summer	Fall	Winter	Total
Mycoplasma	12.9%	30.5%	43.2%	24.5%	29.6%
M. haemolytica	15.3%	26.6%	26.7%	36.3%	27.5%
P. multocida	18.1%	14.8%	14.5%	19.3%	16.1%
H. somni	13.9%	4.6%	3.6%	7.8%	6.1%

September 2017 – August 2018 BRD qPCR Viral Detection



	Spring	Summer	Fall	Winter	Total
Mycoplasma	18.2%	63.2%	50.0%	65.2%	52.8%
BRSV	9.7%	30.6%	20.3%	27.8%	21.9%
BVDV	9.1%	26.7%	30.2%	14.8%	21.2%
PI-3	4.3%	12.2%	10.9%	6.5%	8.8%
BHV-1	0.0%	4.0%	3.1%	0.0%	2.0%

Bonus:
TVMDL Syndromic Surveillance

Syndromic Surveillance

- Originally developed for early detection of a large scale release of a biologic agent
- Has been modified for many uses so now it is a blanket term with many definitions (public health's "colic")
- For us: surveillance of test result positive detection % to identify unusual disease clusters or sentinel cases
  - Can also be used retrospectively to determine size, spread, and tempo of an outbreak after detection or to reassure stakeholders/public that a large scale outbreak is not occurring

Syndromic Surveillance

- Categorizing clinical signs and test results into syndromes is fundamental to syndromic surveillance from clinical datasets
- Clean and precise information requires monitoring the frequency of detection (test positive) in animals with specific clinical features
  - Cannot do if history is not reported
  - Nonclinical data sources must be removed if possible (research, healthy animal testing)
  - Surveillance can be modified for biosecurity testing

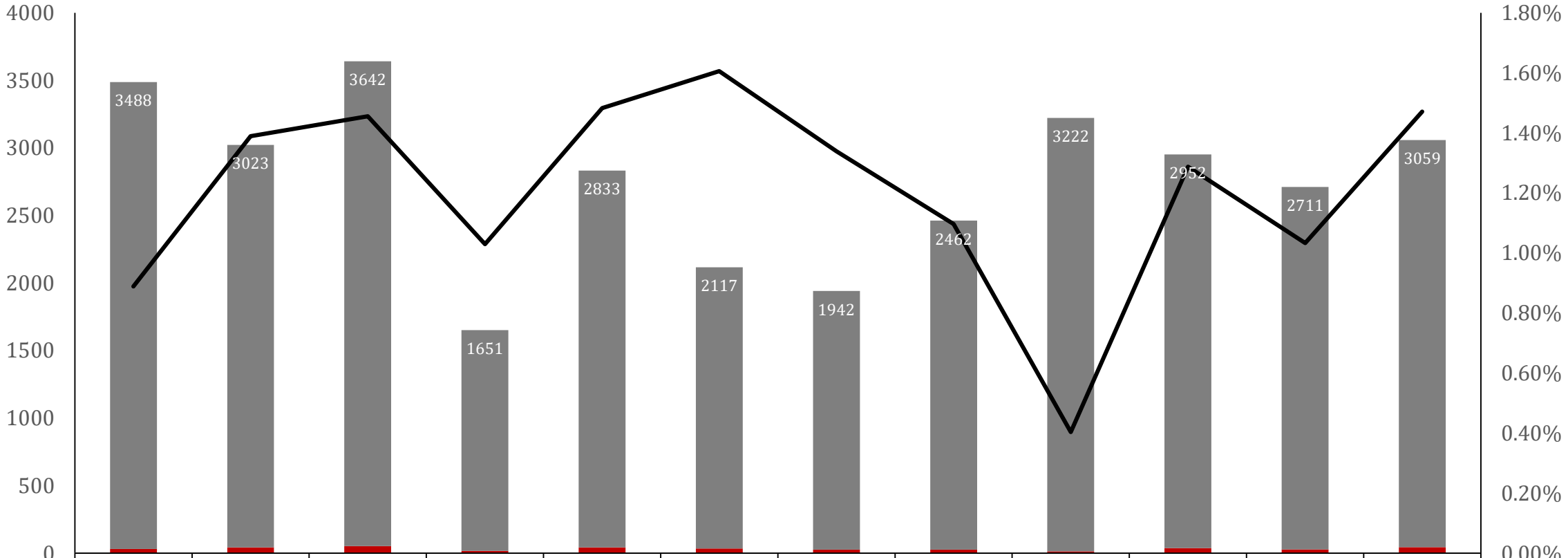
Laboratory result data limitations

- Information is delayed compared to surveillance systems that use data at the DVM/initial symptom level
- Information is biased by nature
  - Only certain clients/DVMs will select/pay for certain tests under certain conditions
    - Data is biased
  - Samples are not always the ideal sample or in the ideal condition to detect certain pathogens/conditions
    - Impacts test sensitivity, specificity, and likelihood ratios
  - Test requests may increase for certain conditions based on factors unrelated to disease presence
    - Anaplasmosis – Drovers article

Interpretation – Danger!

- **<u>Diagnostic Lab data is very messy!</u>**
  - Biased toward disease or health
  - Information that would inform external validation is inconsistently available
  - Need to understand the population of cattle that the samples submitted are representing
- Cannot determine disease prevalence from the following graphs
- Provide an idea of what the lab is seeing every month
- After cleaning they can provide limited benchmark of expectation

BVD Ag ELISA September 2017 – August 2018



	1	2	3	4	5	6	7	8	9	10	11	12
Negative	3457	2981	3589	1634	2791	2083	1916	2435	3209	2914	2683	3014
Positive	31	42	53	17	42	34	26	27	13	38	28	45
% Test Positive	0.89%	1.39%	1.46%	1.03%	1.48%	1.61%	1.34%	1.10%	0.40%	1.29%	1.03%	1.47%

Syndromes: Biosecurity (mostly)

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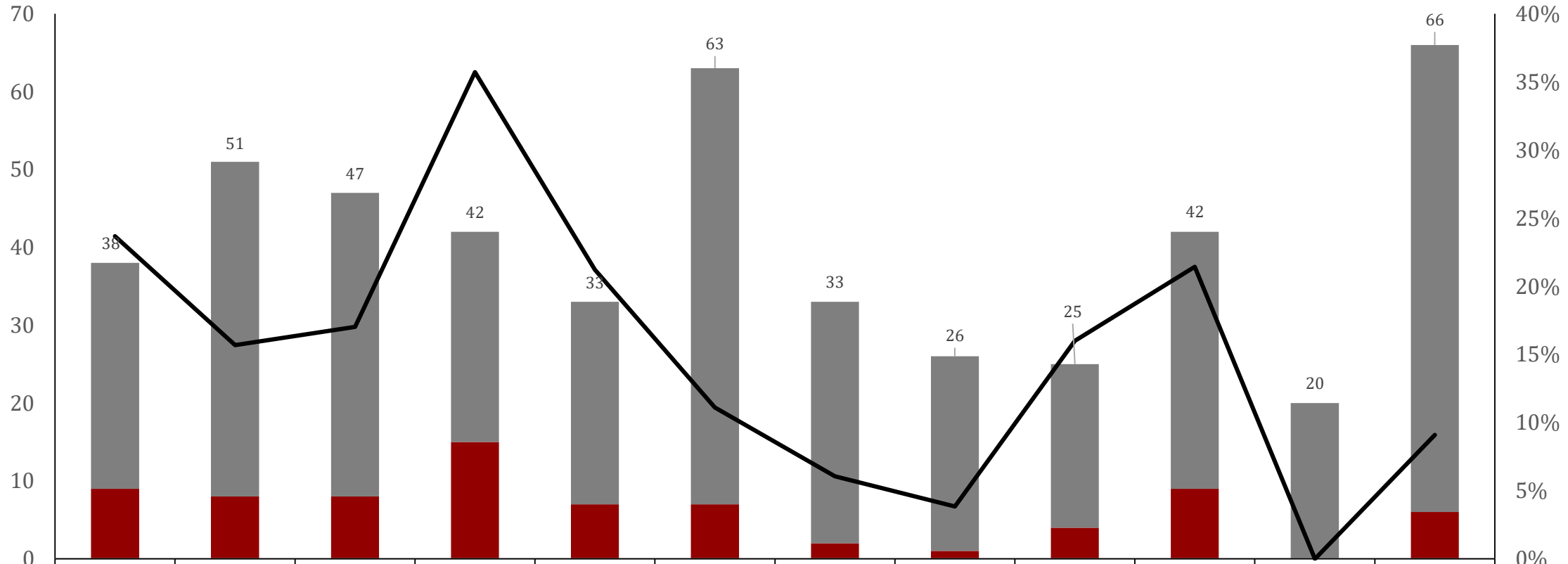
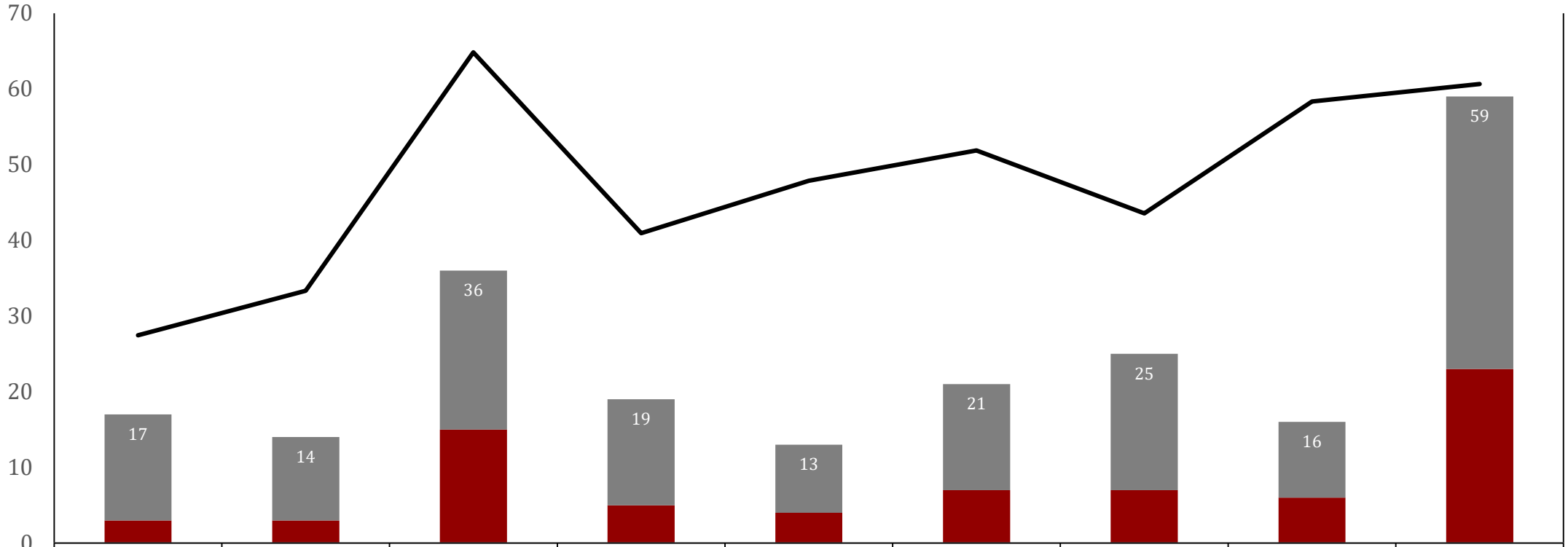
BRSV qPCR September 2017 – August 2018



	1	2	3	4	5	6	7	8	9	10	11	12
Negative	29	43	39	27	26	56	31	25	21	33	20	60
Positive	9	8	8	15	7	7	2	1	4	9	0	6
% Test Positive	23.68%	15.69%	17.02%	35.71%	21.21%	11.11%	6.06%	3.85%	16.00%	21.43%	0.00%	9.09%

Syndrome: Respiratory Disease

P. multocida qPCR September 2017 – August 2018



	1	2	3	4	5	6	7	8	9
Negative	14	11	21	14	9	14	18	10	36
Positive	3	3	15	5	4	7	7	6	23
% Test Positive	17.65%	21.43%	41.67%	26.32%	30.77%	33.33%	28.00%	37.50%	38.98%

Syndromes: Respiratory Disease

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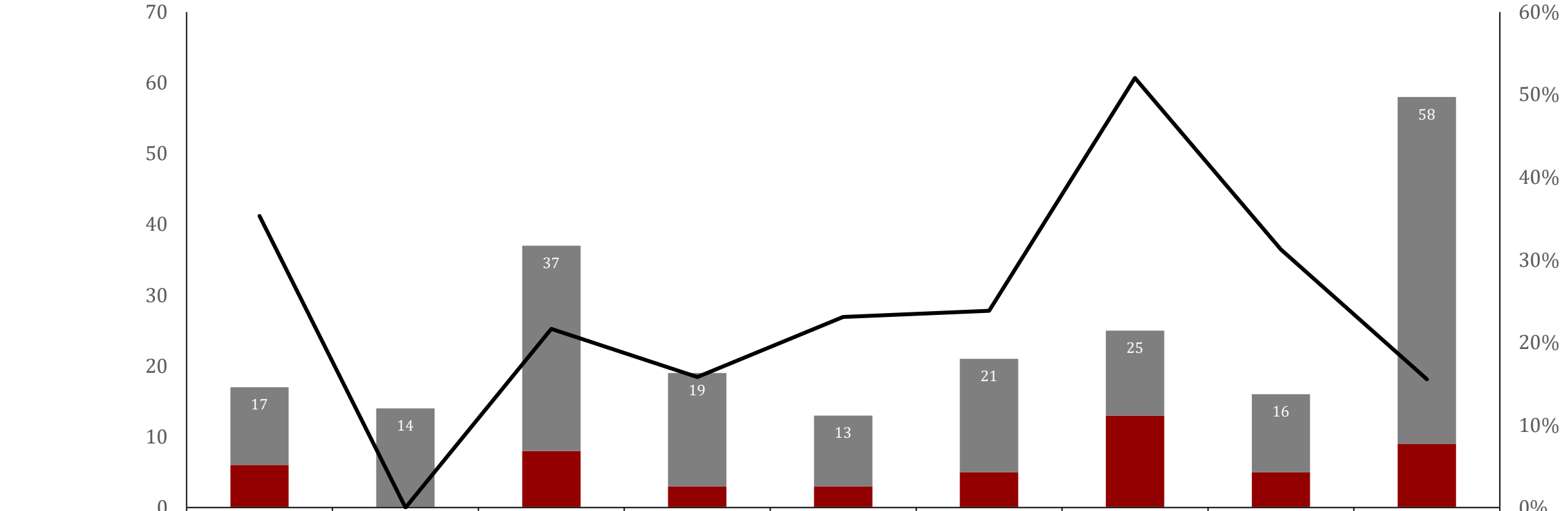
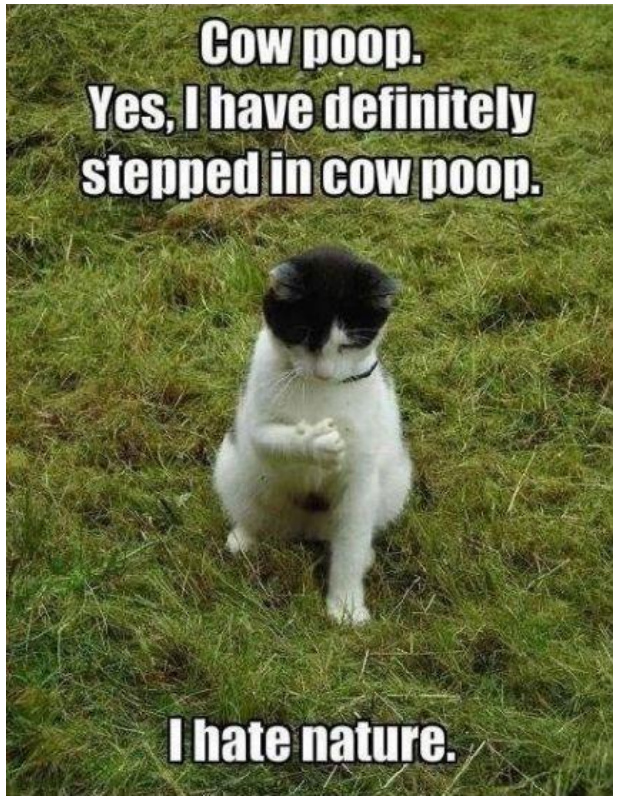
H. somni qPCR September 2017 – August 2018



	1	2	3	4	5	6	7	8	9
Negative	11	14	29	16	10	16	12	11	49
Positive	6	0	8	3	3	5	13	5	9
% Test Positive	35.29%	0.00%	21.62%	15.79%	23.08%	23.81%	52.00%	31.25%	15.52%

Syndromes: Respiratory Disease



Questions?

Feel free to contact me with any questions or feedback:

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