Making the most out of BRD diagnostic testing

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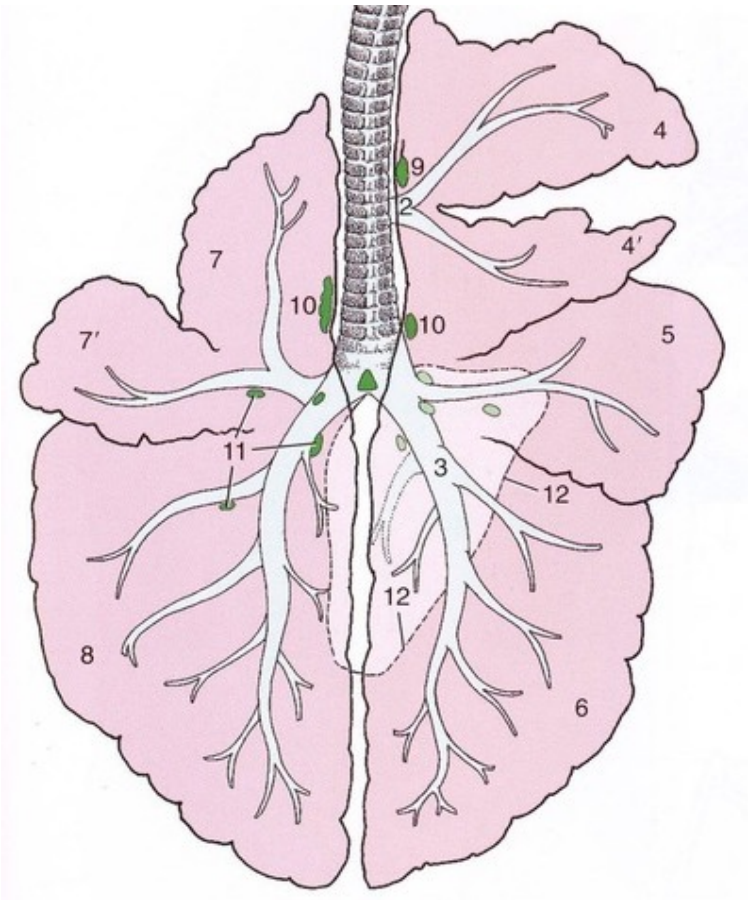
Outline

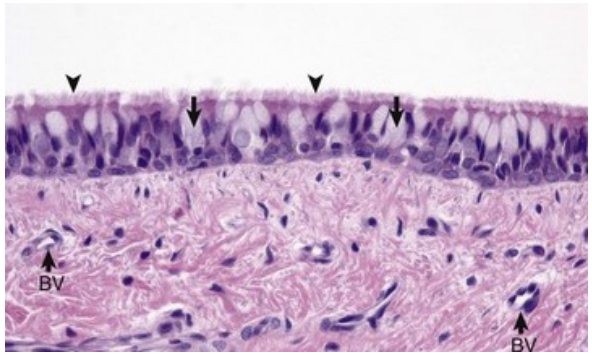
- The Bovine Lung → Pathologic changes
- Typical gross findings attributable to major pathogens
- Considerations for BRD diagnostic testing
  - Sampling
  - Result interpretation
- BVDV testing options

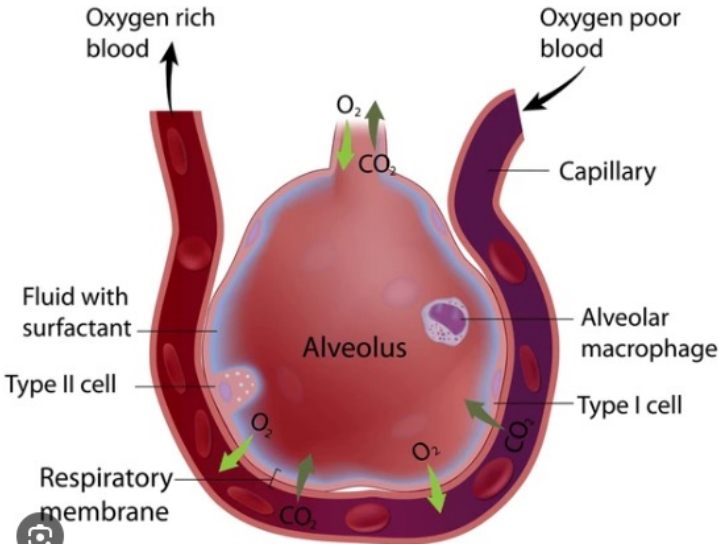
The Bovine Lung



What parts of the lung can be injured?

- Conducting airways
  - Respiratory epithelium/mucociliary escalator
  - Lumen
- Alveolar space
- Alveolar septa/Type I and Type II pneumocytes
- Interlobular septa
- Pleura
- Blood vessels





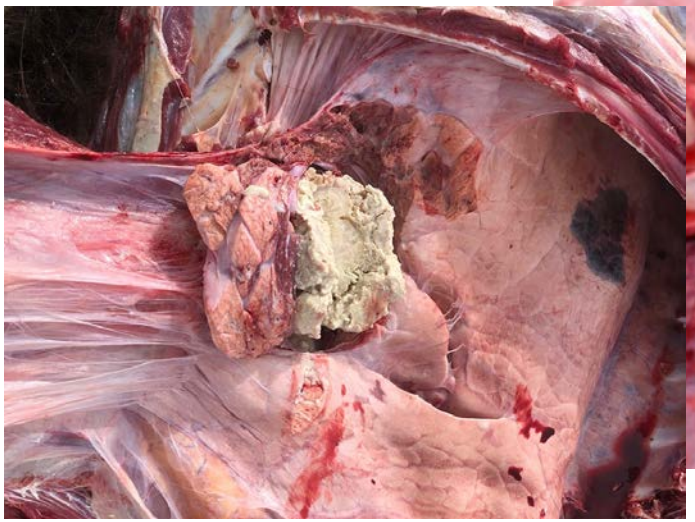
Acute lung lesions

- Edema
- Neutrophils/Pus
- Necrosis
- Fibrin: Interlobular, Pleura
- Hemorrhage

- Emphysema

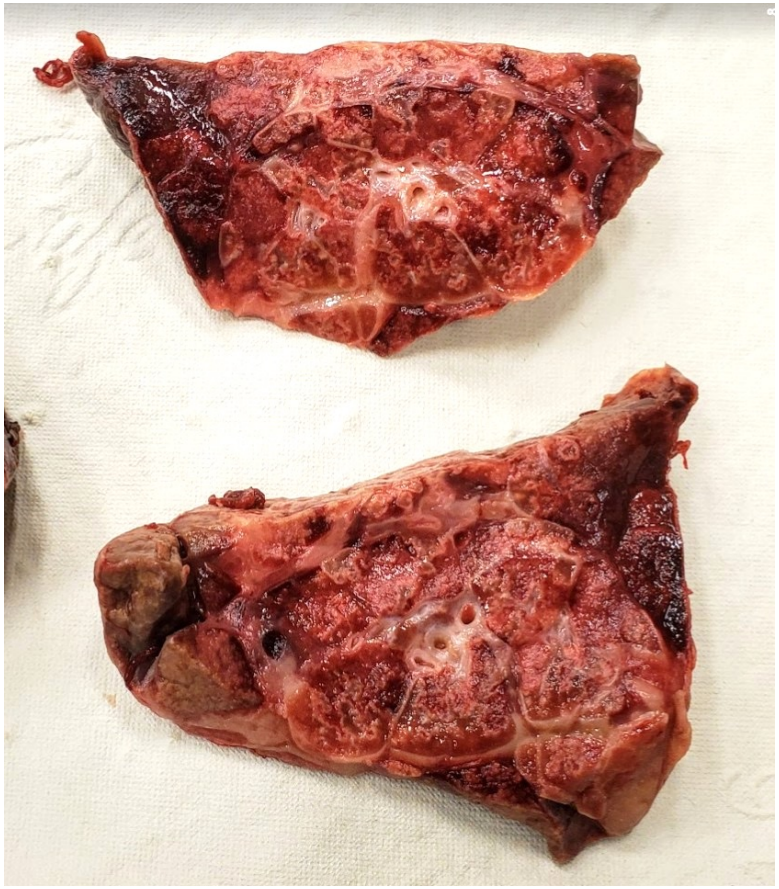
Chronic lung lesions

- Bronchiolitis obliterans
- Bronchiectasis
- Peribronchiolar fibrosis
- Pleural adhesions
- Caseation
- Abscess formation

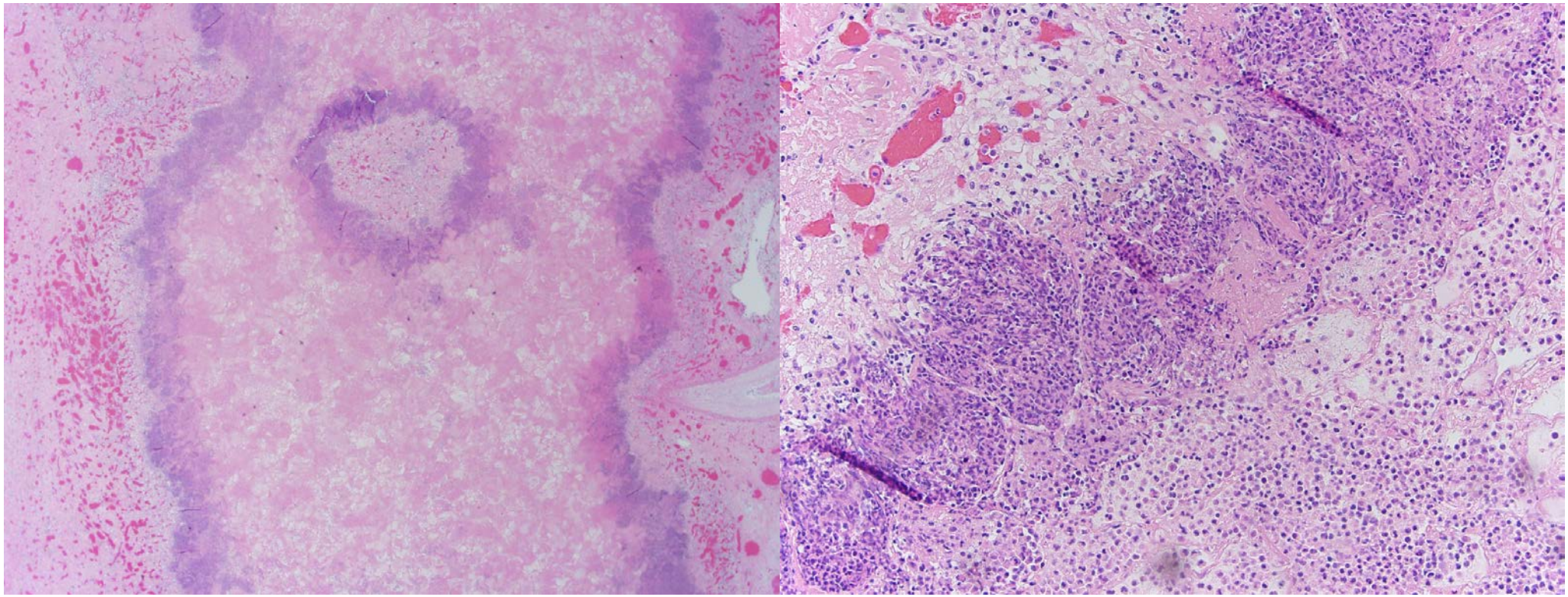


Mannheimia haemolytica

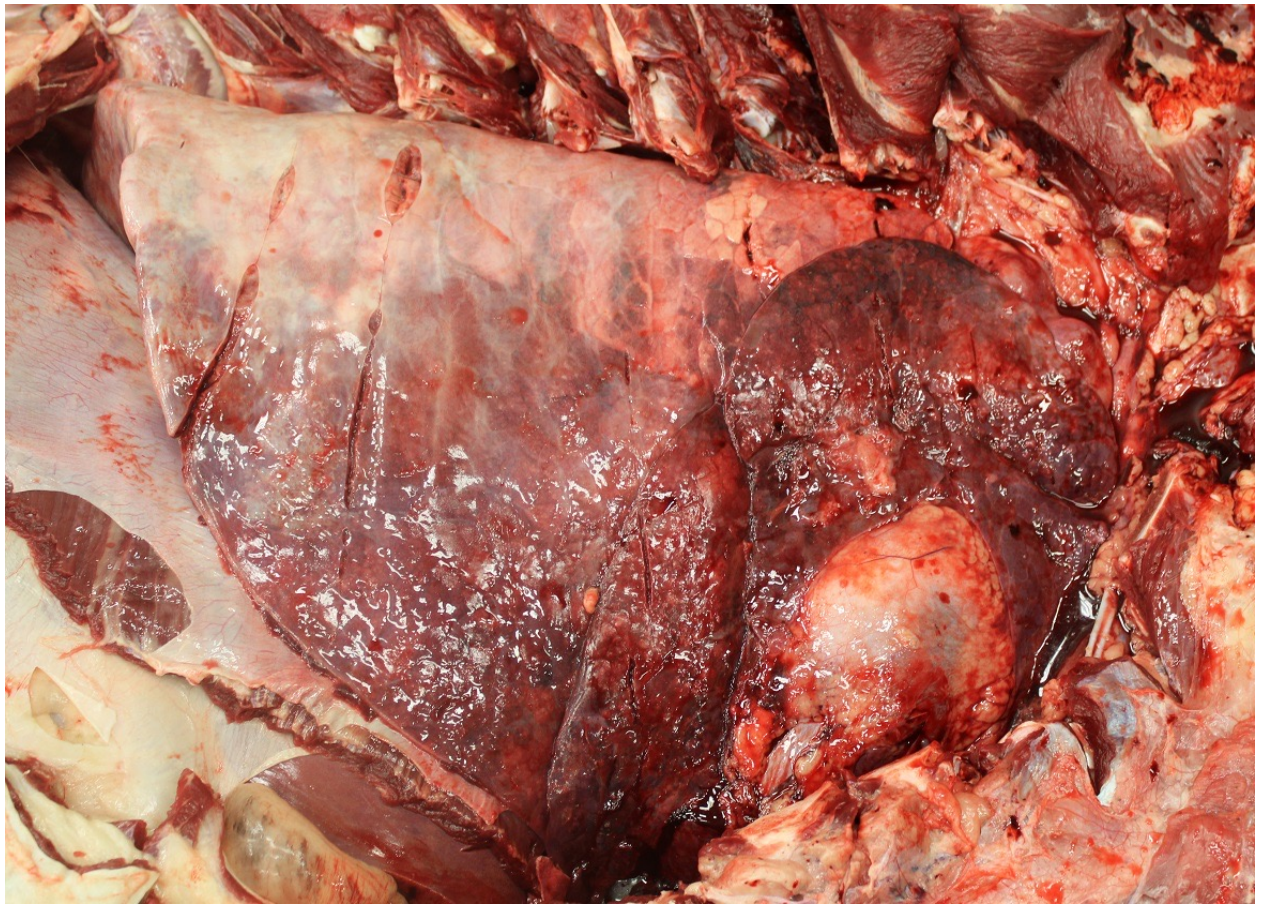
Mannheimia haemolytica



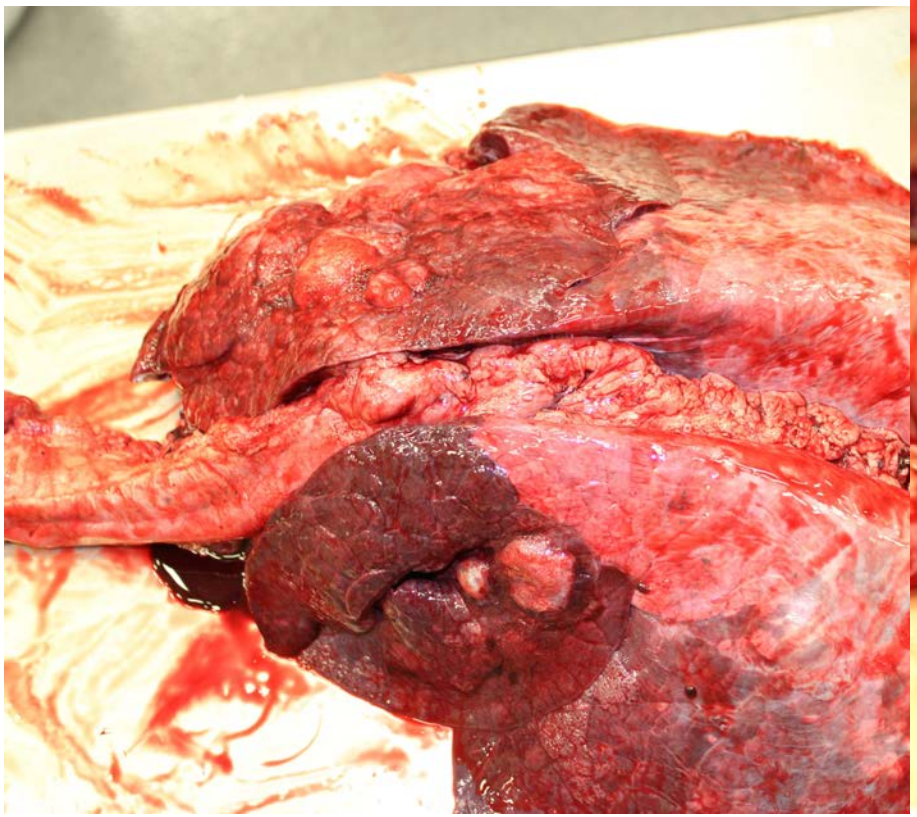
Mannheimia haemolytica



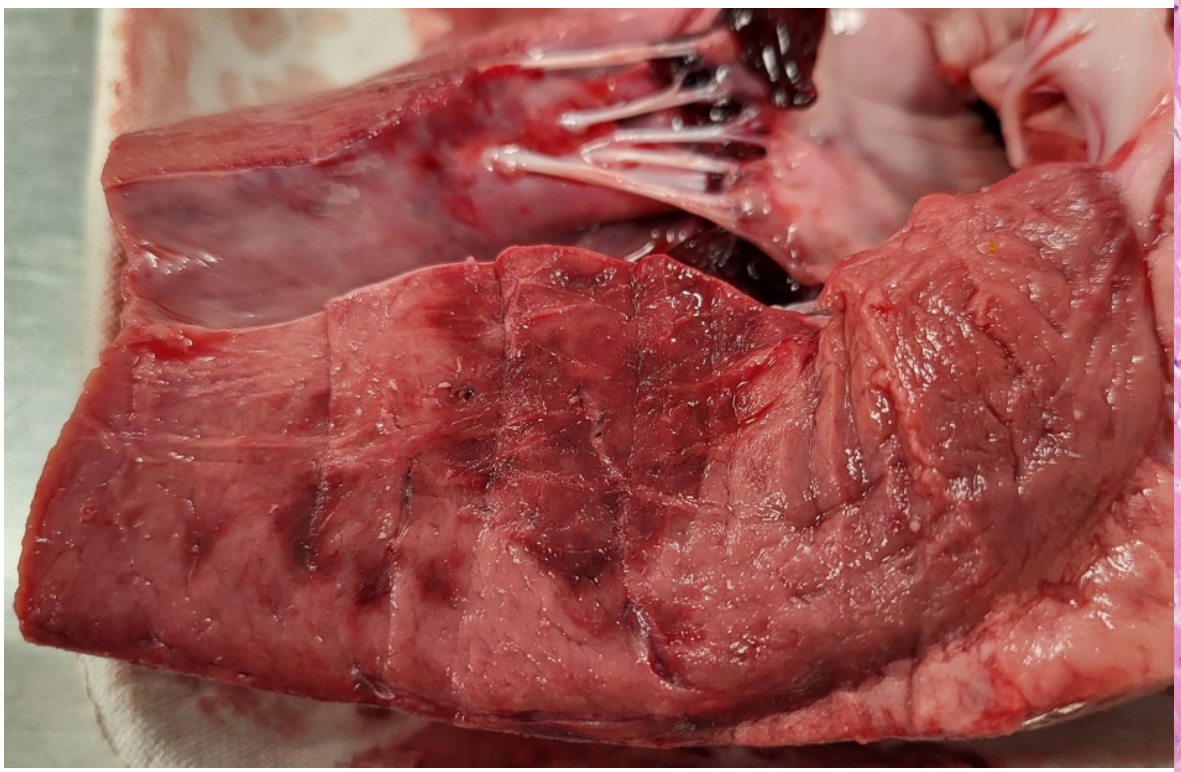
Histophilus somni

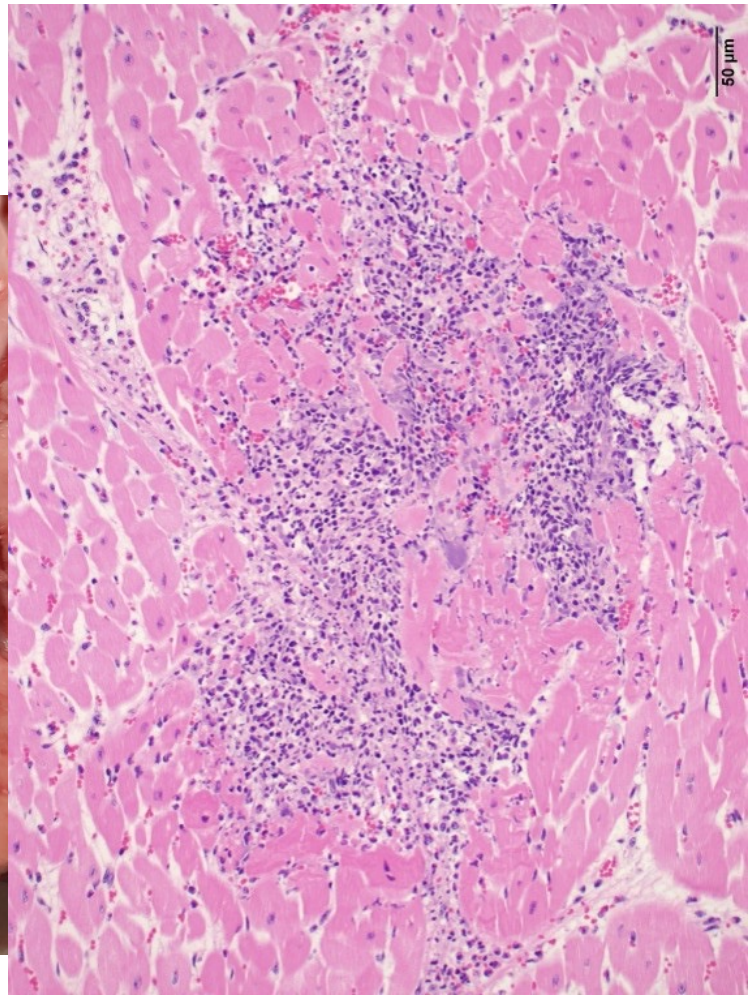


Histophilus somni

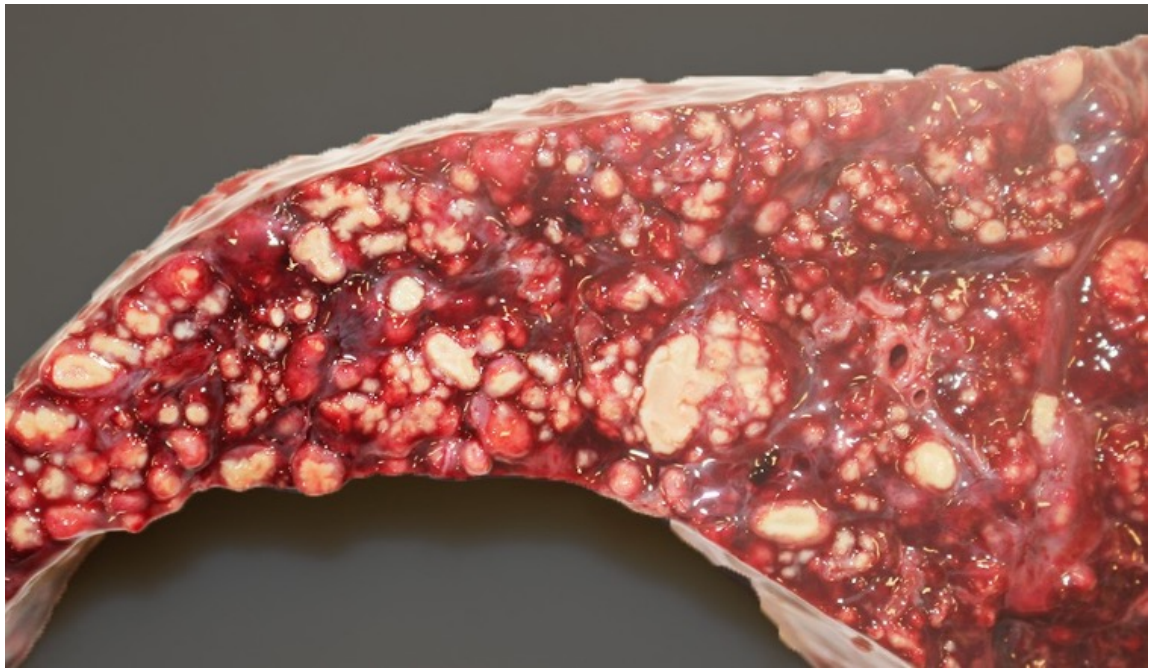


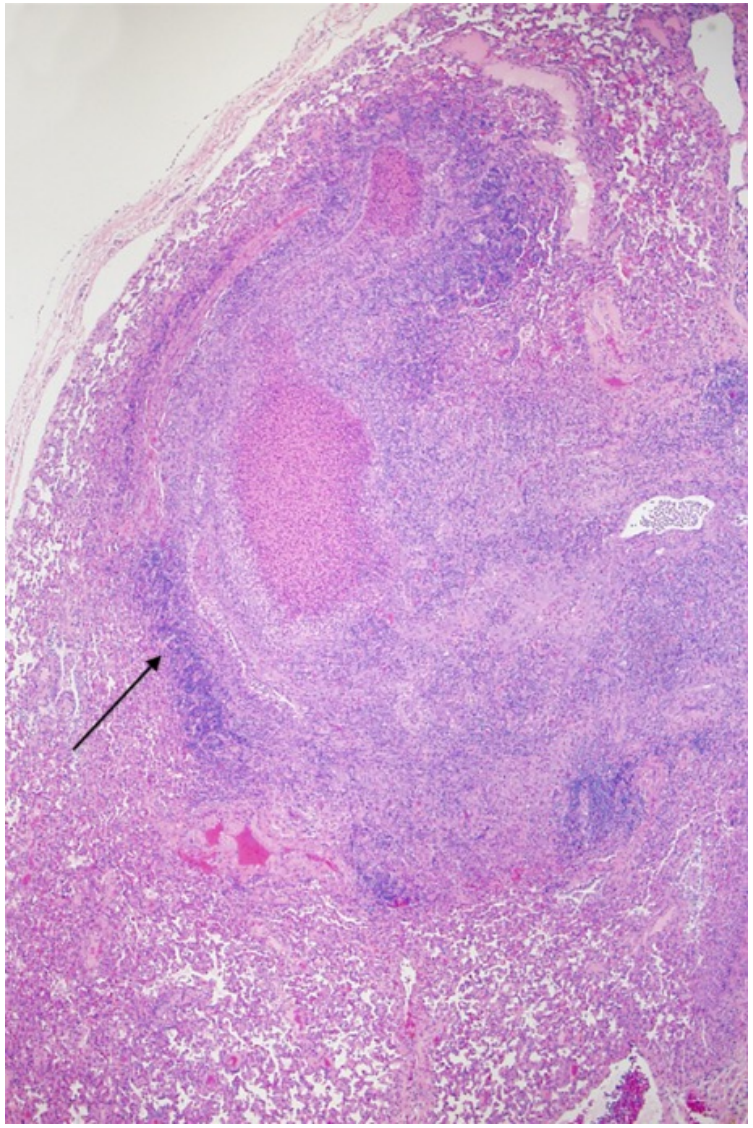
Histophilus somni

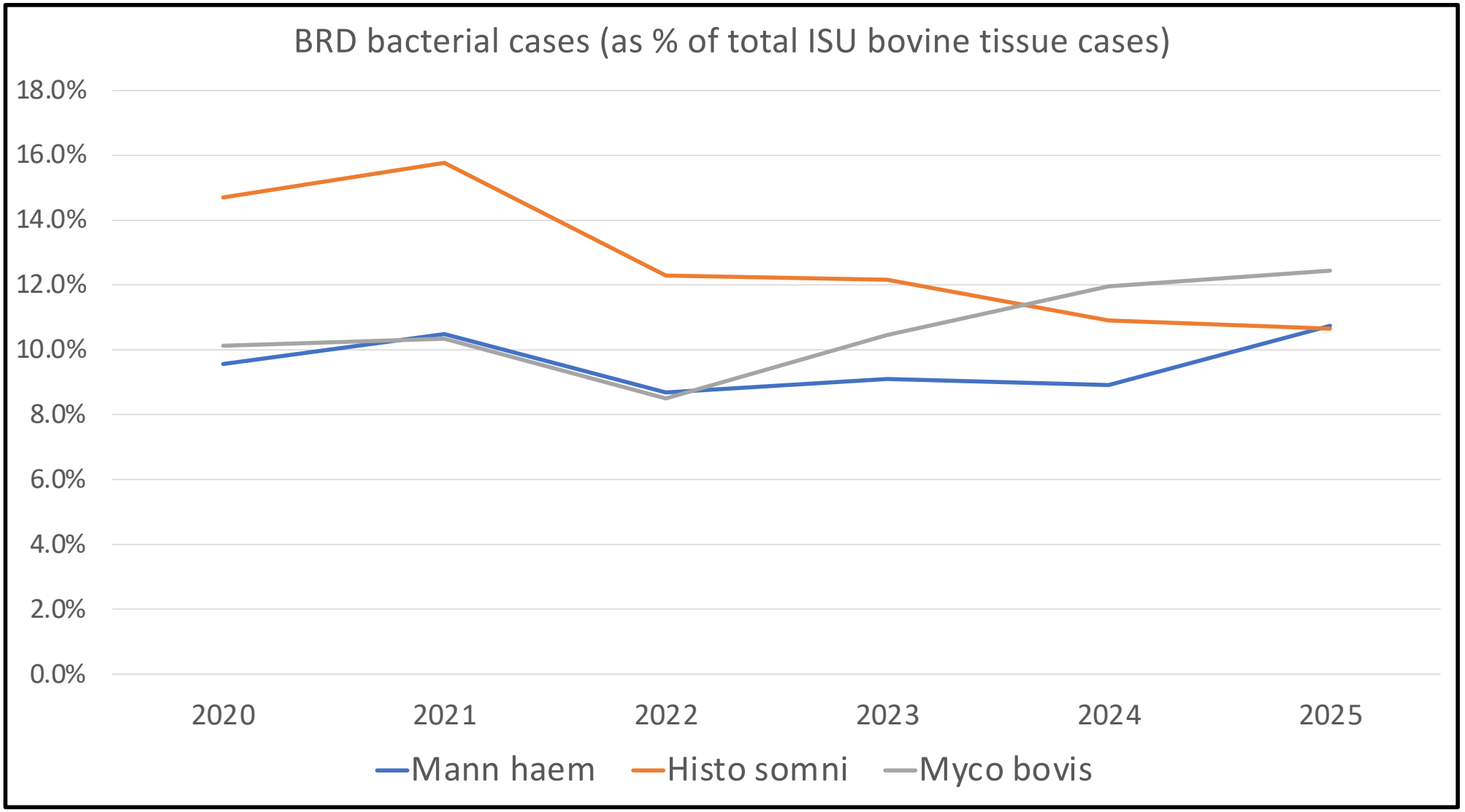




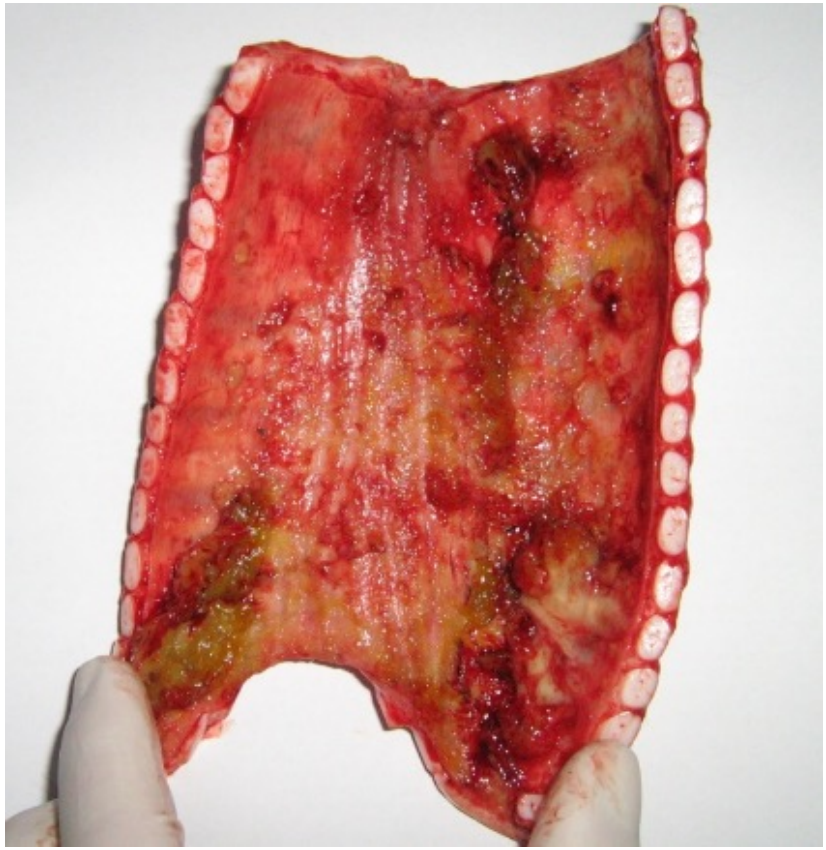
Mycoplasma bovis

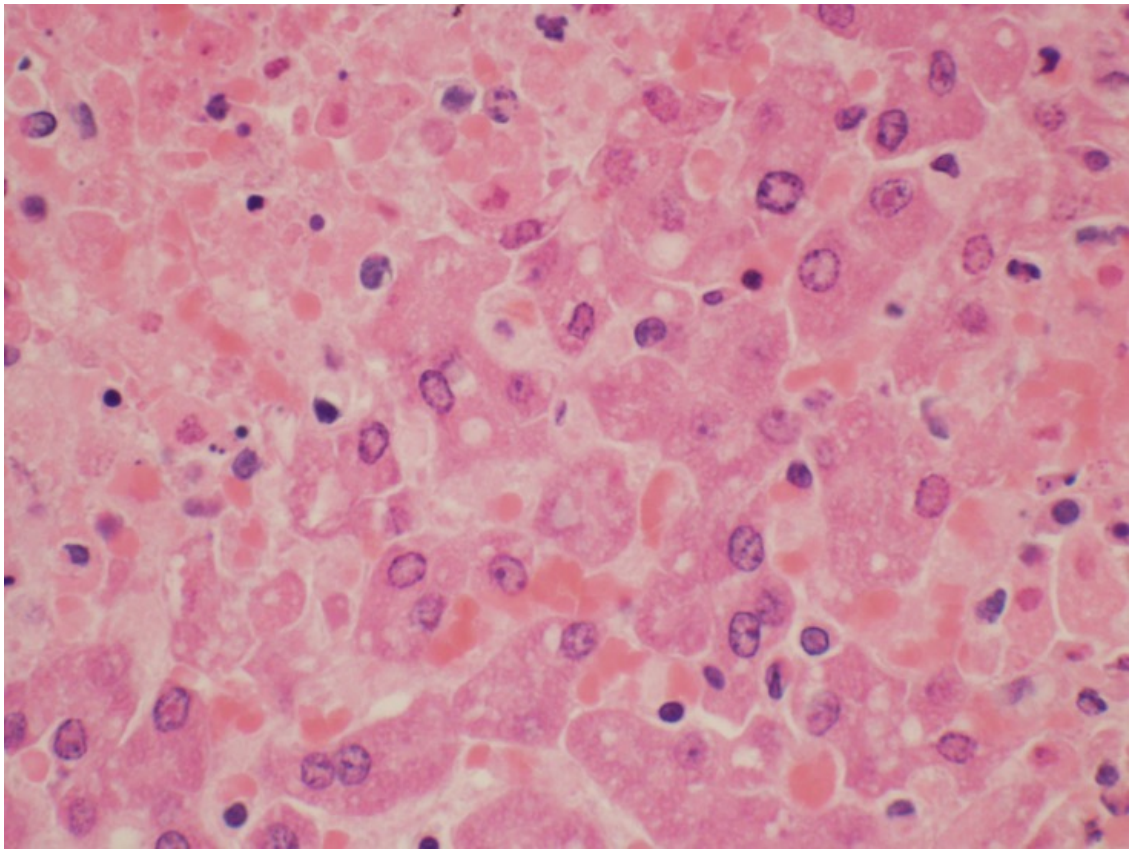




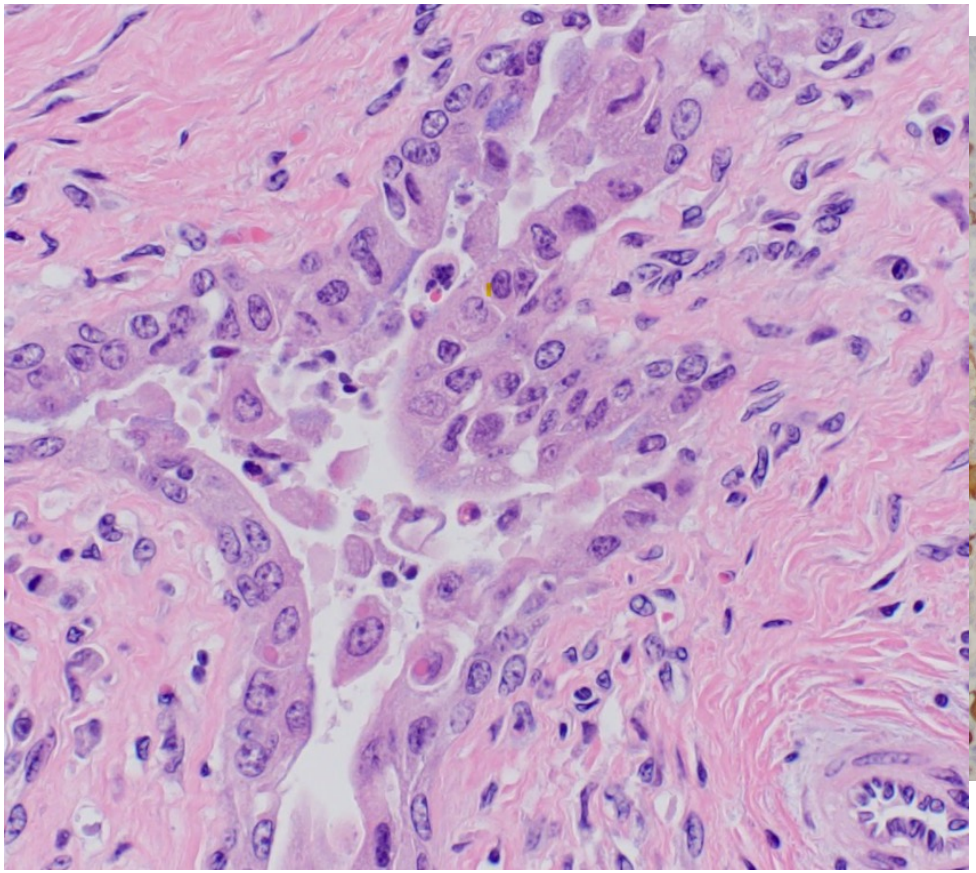

BRD bacterial cases (as % of total ISU bovine tissue cases)
18.0%
16.0%
14.0%
12.0%
10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
2020
2021
2022
2023
2024
2025
Mann haem
Histo somni
Myco bovis

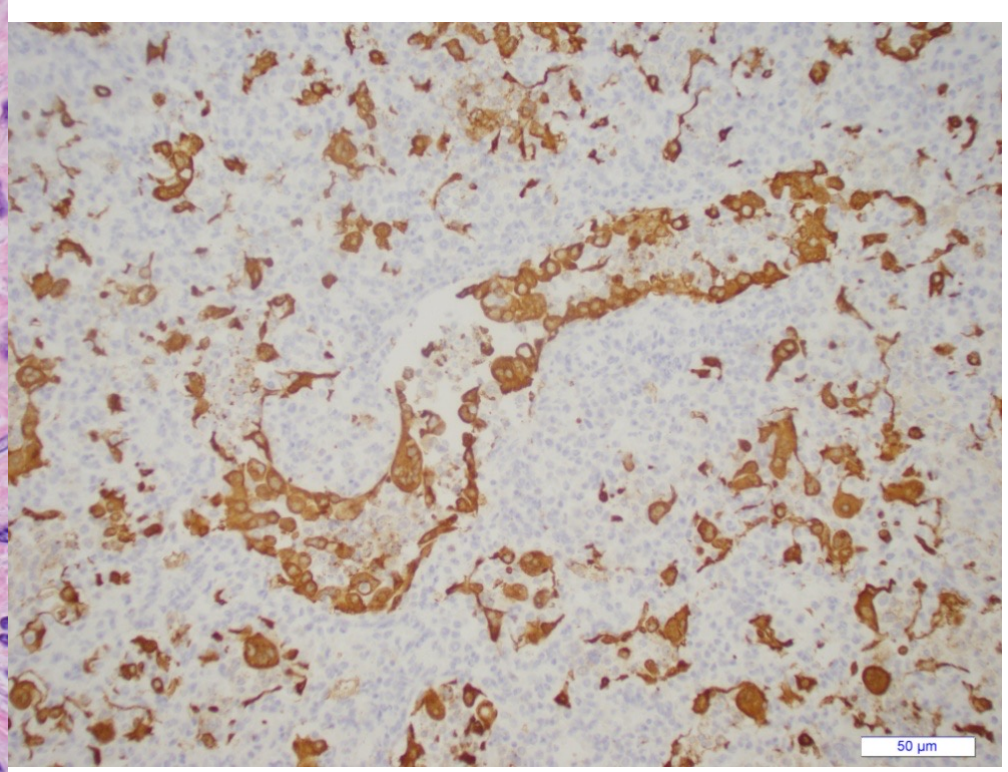
BHV-1 (Infectious bovine rhinotracheitis)





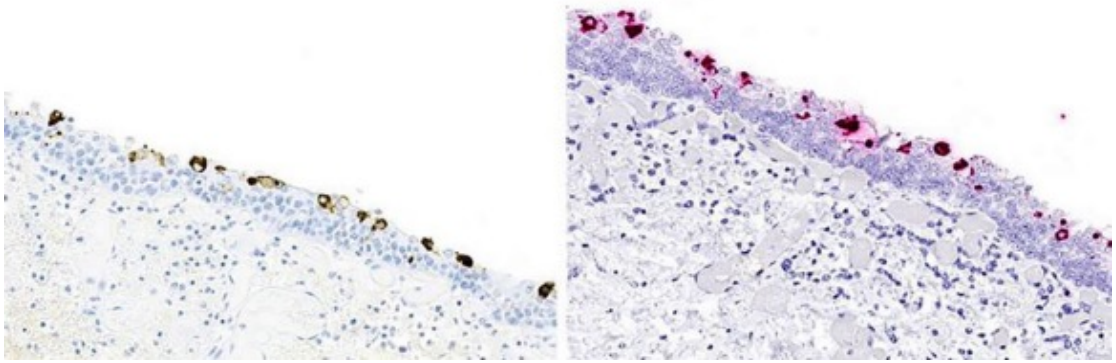
Bovine respiratory syncytial virus





Bovine coronavirus

- Upper respiratory infection: nasal cavity, trachea, primary bronchi
- Generally mild cough in suckling calves
- PCR detection is very common at low levels



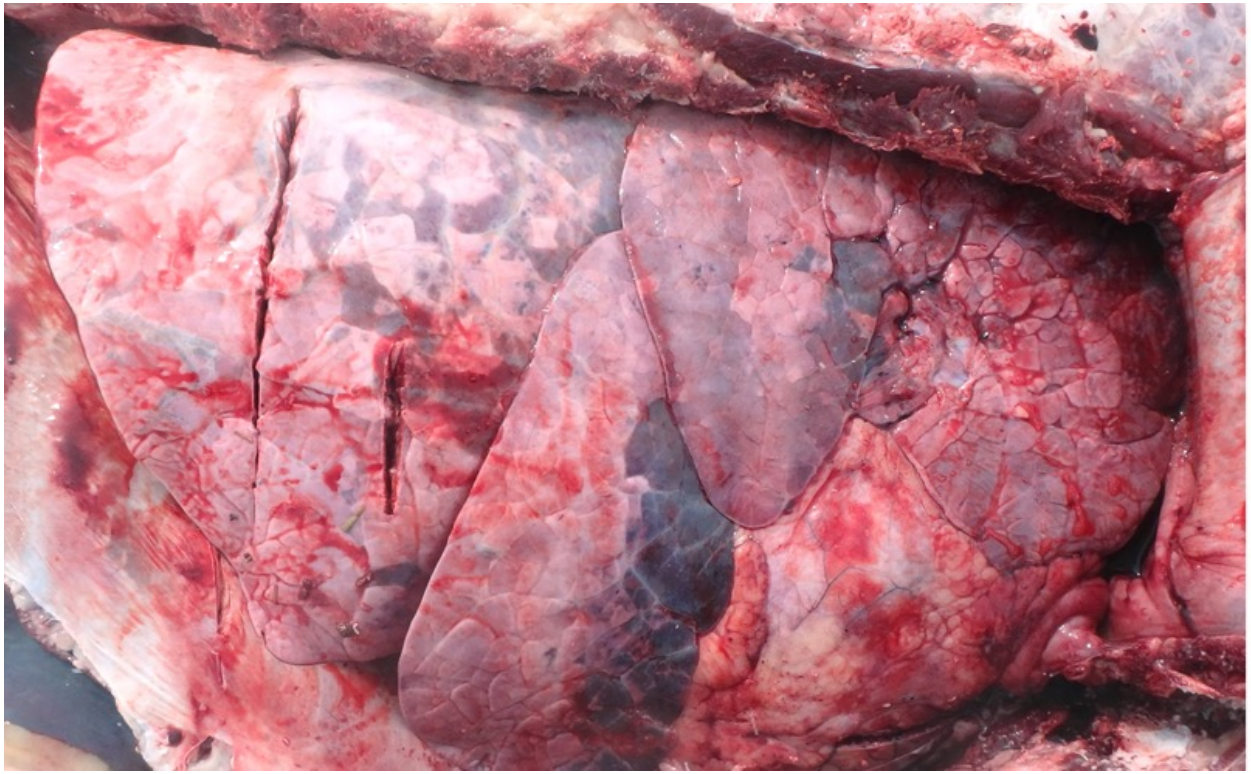
Parainfluenza-3 and Influenza D

- Rarely detected in lung or implicated in disease at ISU-VDL
  - Less than 10 cases combined in past 20 years
- Primarily mild respiratory disease in young suckling calves
- Impacts the respiratory epithelium similar to BCoV
  - Predispose to secondary bacterial pneumonia
- Potentially worth screening pooled nasal swabs via PCR in some cases

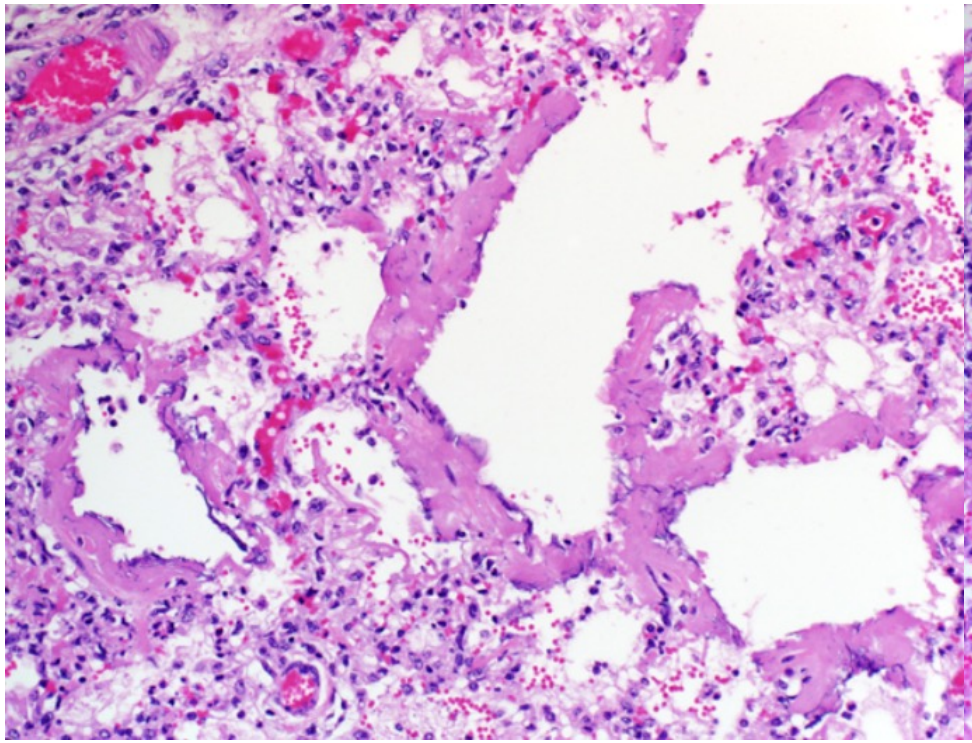
Bovine viral diarrhea virus

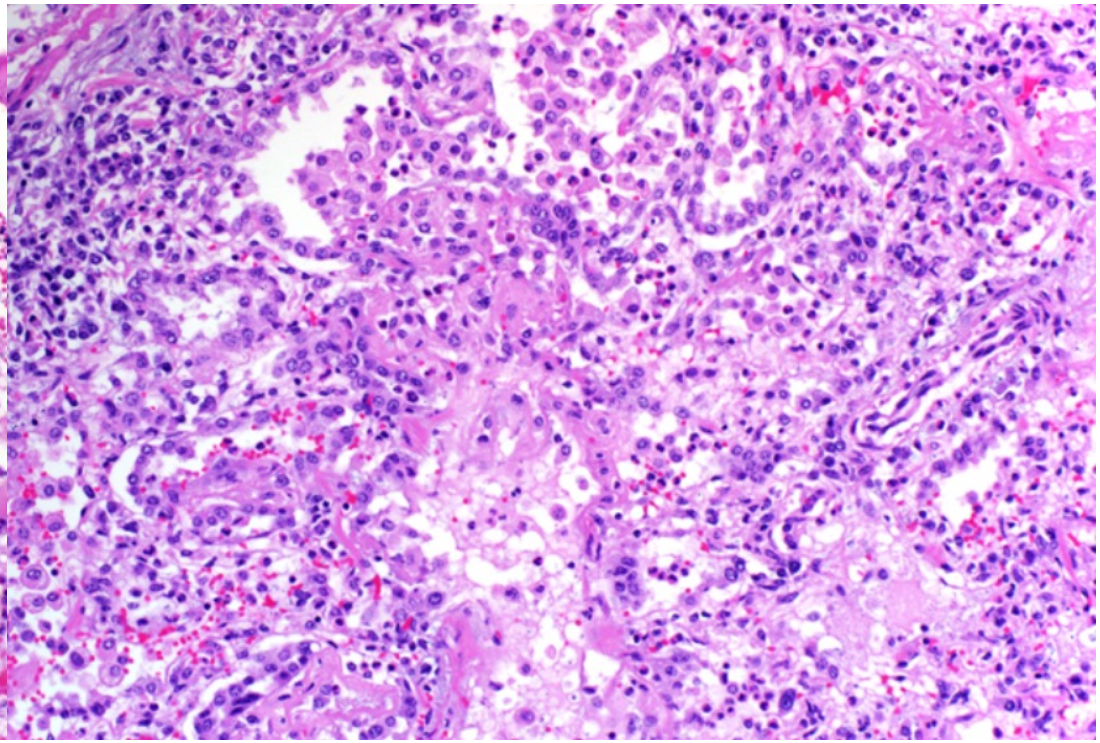
- No specific lung lesions in acute or persistent infection
  - Mucosal disease → Erosions in mouth, nose, and GI tract
- Involvement in respiratory disease stems from immunosuppression
- Persistent infections → strongly positive PCR results on many samples
- Many different ways to screen for BVDV….stay tuned!

Atypical Interstitial Pneumonia



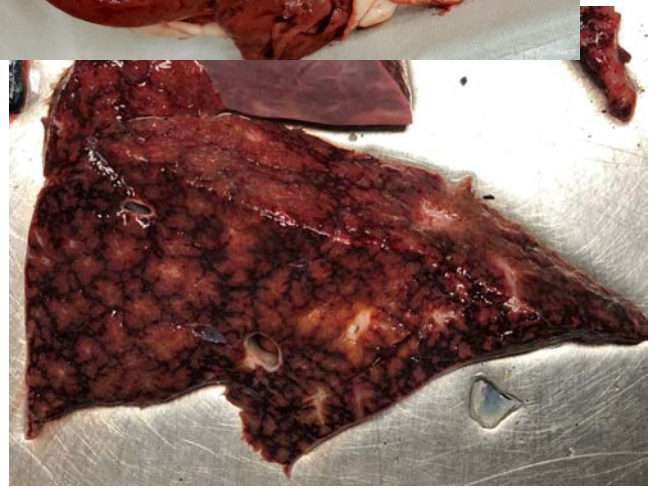
Diffuse alveolar damage/AIP





Lung changes due to other processes

- Sepsis → interstitial pneumonia w/ edema
- Cardiac failure → edema, congestion
- Diphtheria → small foci throughout lung
- Honker syndrome → emphysema
- Anaphylaxis → interstitial pneumonia w/ edema



Important non-infectious factors

- Poor air quality
- Failure of passive transfer
- Stress → weaning, comingling, shipping, environmental
- Nutritional deficiencies/lack of calorie intake
- Dehydration

The Diagnostic Process

- Define your diagnostic question
- What testing options are available to answer the question?
  - Which is the best option?
- What is the best sample for that test?
- What are the best/most appropriate animals to sample?
  - The answer isn't always "the dead one"…..
  - Acutely affected, untreated, and representative of clinical disease

Every single diagnostic test has limitations

- What does a PCR positive result mean?
  - Target nucleic acid sequence is present in the sample at the time of testing
  - Real-time PCR Ct values give us some idea of the amount of target present
- What does a bacterial culture result mean?
  - ID'd organism is present and live in the sample at the time of testing
  - Attempt to provide subjective level/purity of growth (heavy, low, single, etc.)
- What does a positive serum antibody result mean?
  - Target antibody present in serum at the time of testing
  - Attempt to provide subjective level of antibody (titers, % inhibition, S/P ratio)

Interpretation mistakes: Molecular testing

- Low level detection of commensal pathogens
- Effects of recent MLV vaccination on detection
- Sampling after primary cause of disease has resolved
- Pooling: Some dilution factor → slight loss in sensitivity
  - Tip: As long as you sample the right animals at the right time, you can probably pool fairly aggressively to reduce cost.

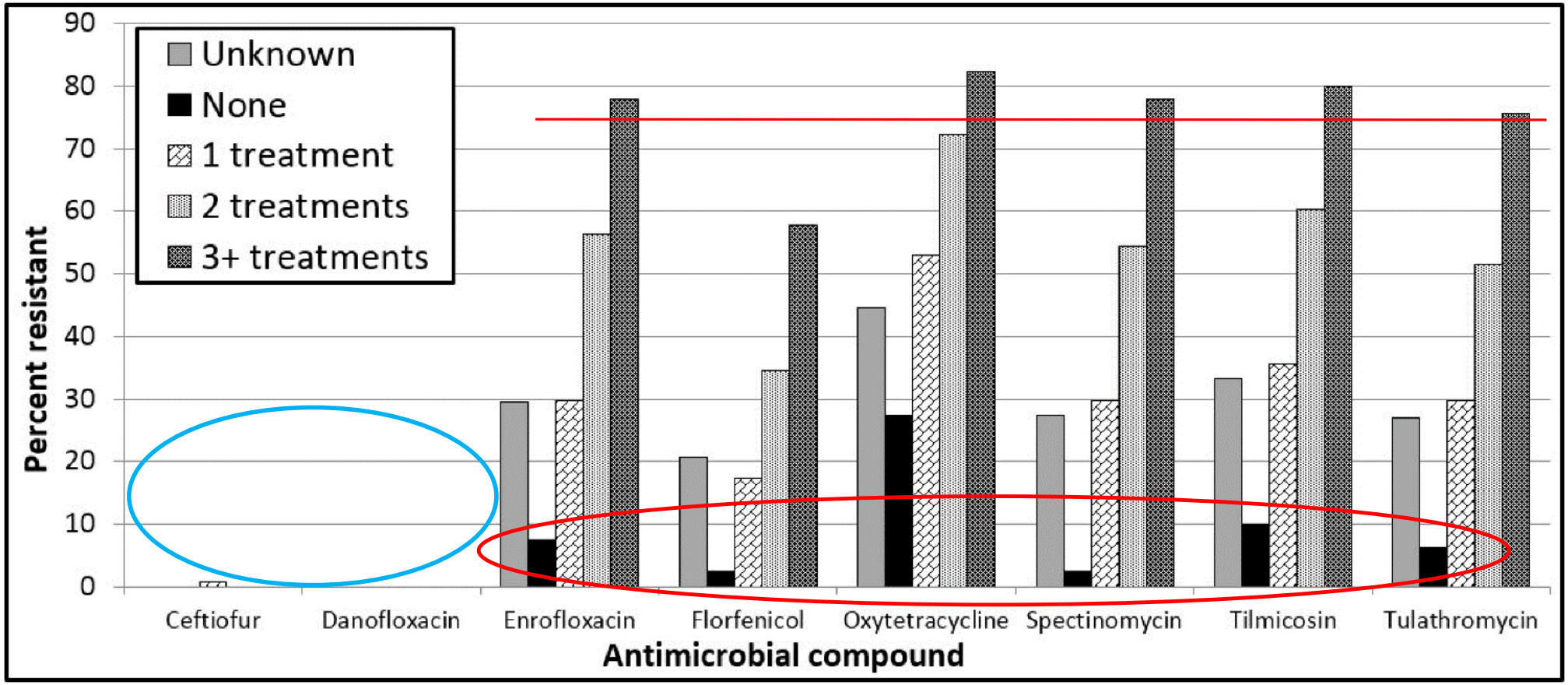
Interpretation mistakes: Bacterial culture

- Overinterpreting culture of commensal pathogens from nasal cavity

- Sample quality
  - Bacterial culture → Need representative sample
  - Tip: Submit larger portions of fresh tissue…..baseball/softball size minimum.

- Recent treatment history
  - Impact on both bacterial growth and antimicrobial sensitivity results

Mannheimia haemolytica – 540 isolates



BRD → Ante mortem sampling

- Nasal swabs
- Deep nasopharyngeal swabs
  - Kansas State: https://www.youtube.com/watch?v=WB3luk1nQjY
- Transtracheal wash
  - Cornell: https://www.youtube.com/watch?v=7xtS0w_-6Uo
- Bronchoalveolar lavage
- Perfect sample collection cannot make up for picking the wrong calves!

BRD diagnostics: Fresh samples (lung/swabs)

- Lung: Large enough to represent the lesion, small enough to chill quickly
  - When in doubt → send bigger pieces than you think we need
  - Several baseball-to-softball sized pieces of affected lung near unaffected tissue
  - Focus on the center of the lung where large airways are located

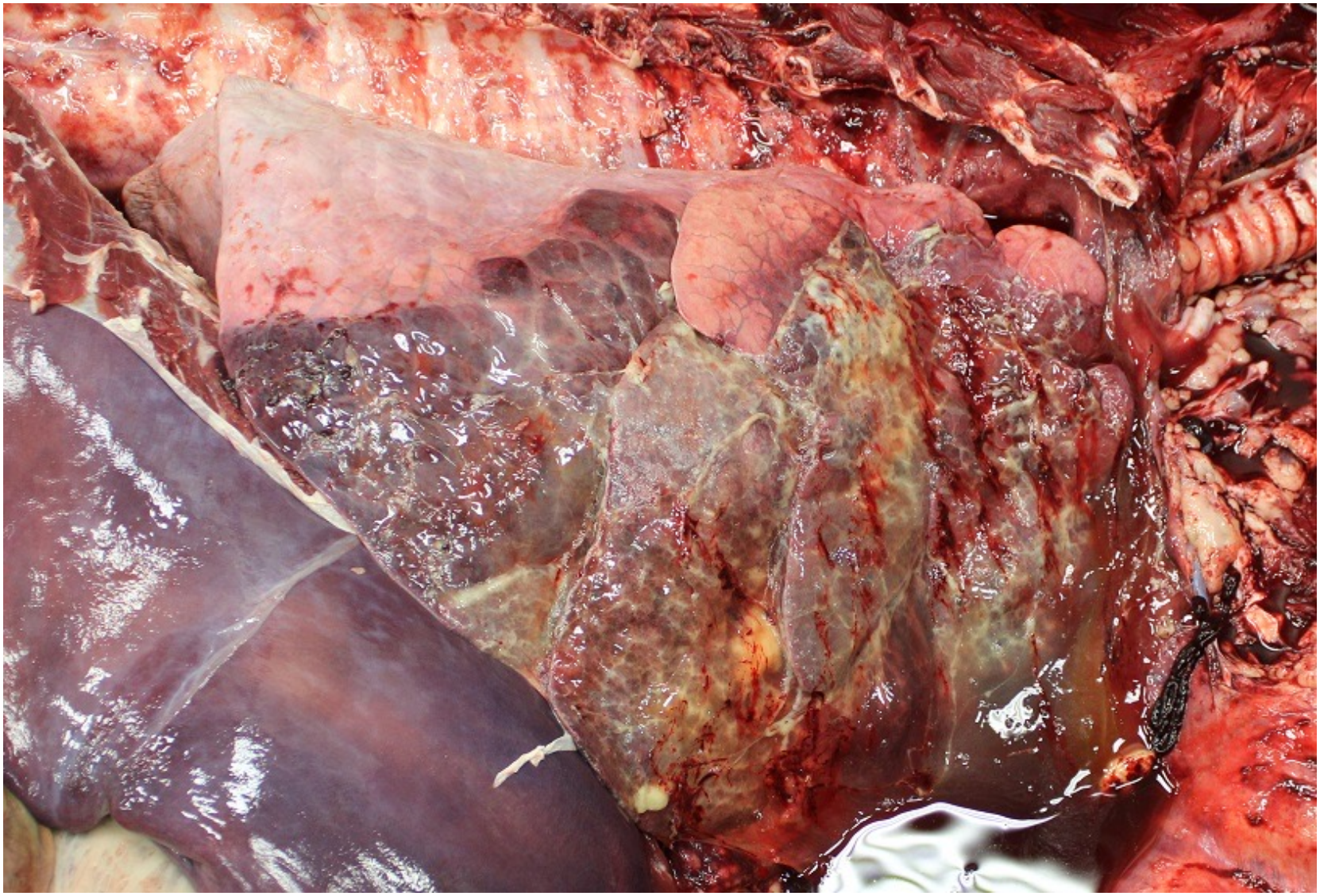
- Swabs for PCR → 100% synthetic material is best (dacron, rayon)
  - Avoid cotton tips, avoid wood shafts, avoid gel media

- Swabs for routine bacterial culture → Amies or Stuart's media

- Swabs for virus isolation/*Mycoplasma* culture → Transport media (UVT)

BRD diagnostics: Fixed tissues

- Formalin fixation stops autolysis and preserves tissue architecture
- 10% neutral buffered formalin
  - Purchased
  - Made from 37% formaldehyde
- Tissue should be no thicker than 1cm in one plane and represent the lesion
- Formalin:tissue ratio → 10:1
- Should happen ASAP after death

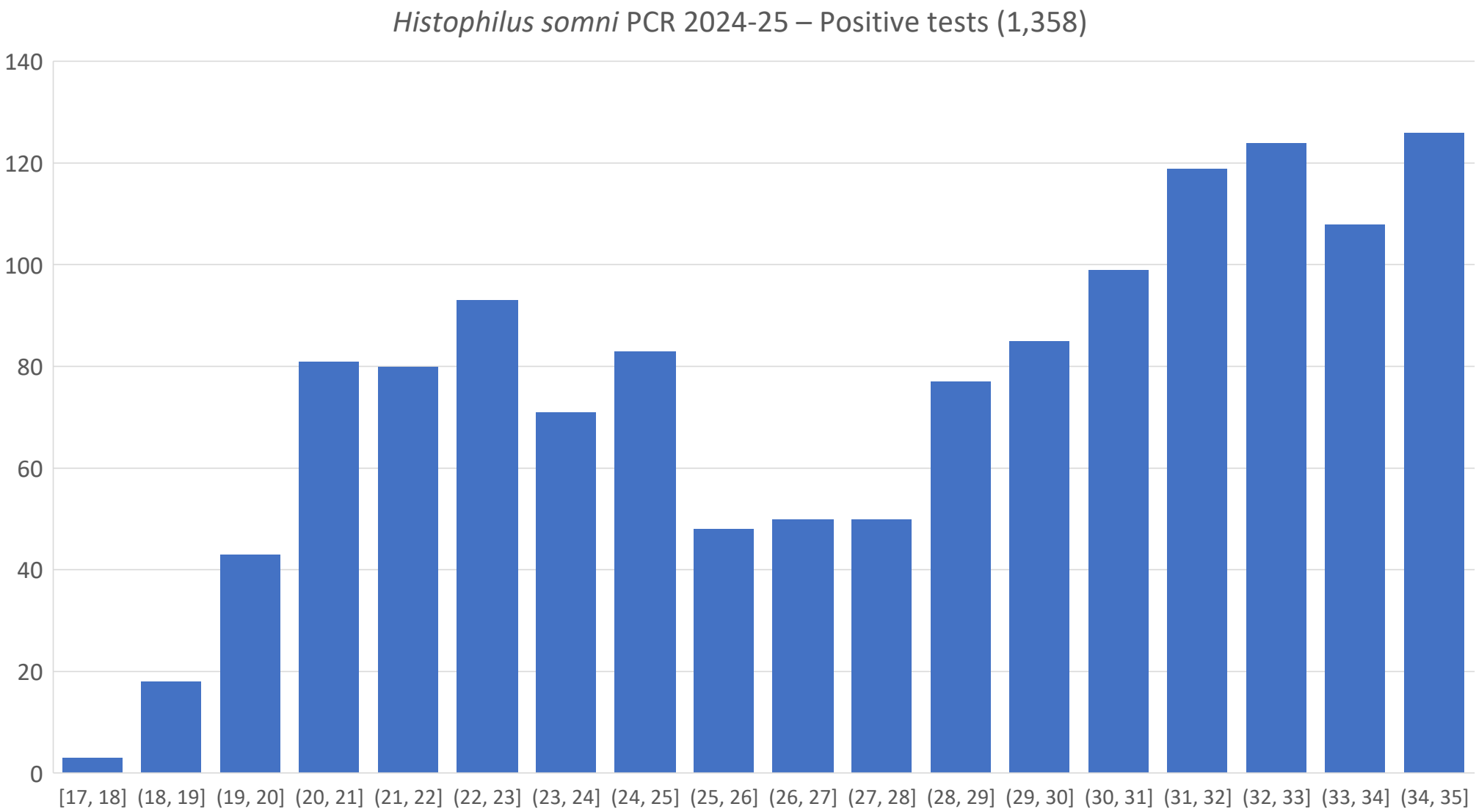


Diagnostic Results and Interpretation

- Incredibly important to sample the best calves for your question
  - Acute clinical disease, untreated, no recent vaccinations (if possible)
  - We want to have confidence in the result….whatever that result is

- Bacterial culture
  - Level of growth/purity of growth

- PCR detection
  - Recent vaccinations/commensal organisms
  - What constitutes a result typical of clinical disease?


Histophilus somni PCR 2024-25 – Positive tests (1,358)
140
120
100
80
60
40
20
0
[17, 18]
(18, 19]
(19, 20]
(20, 21]
(21, 22]
(22, 23]
(23, 24]
(24, 25]
(25, 26]
(26, 27]
(27, 28]
(28, 29]
(29, 30]
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(31, 32]
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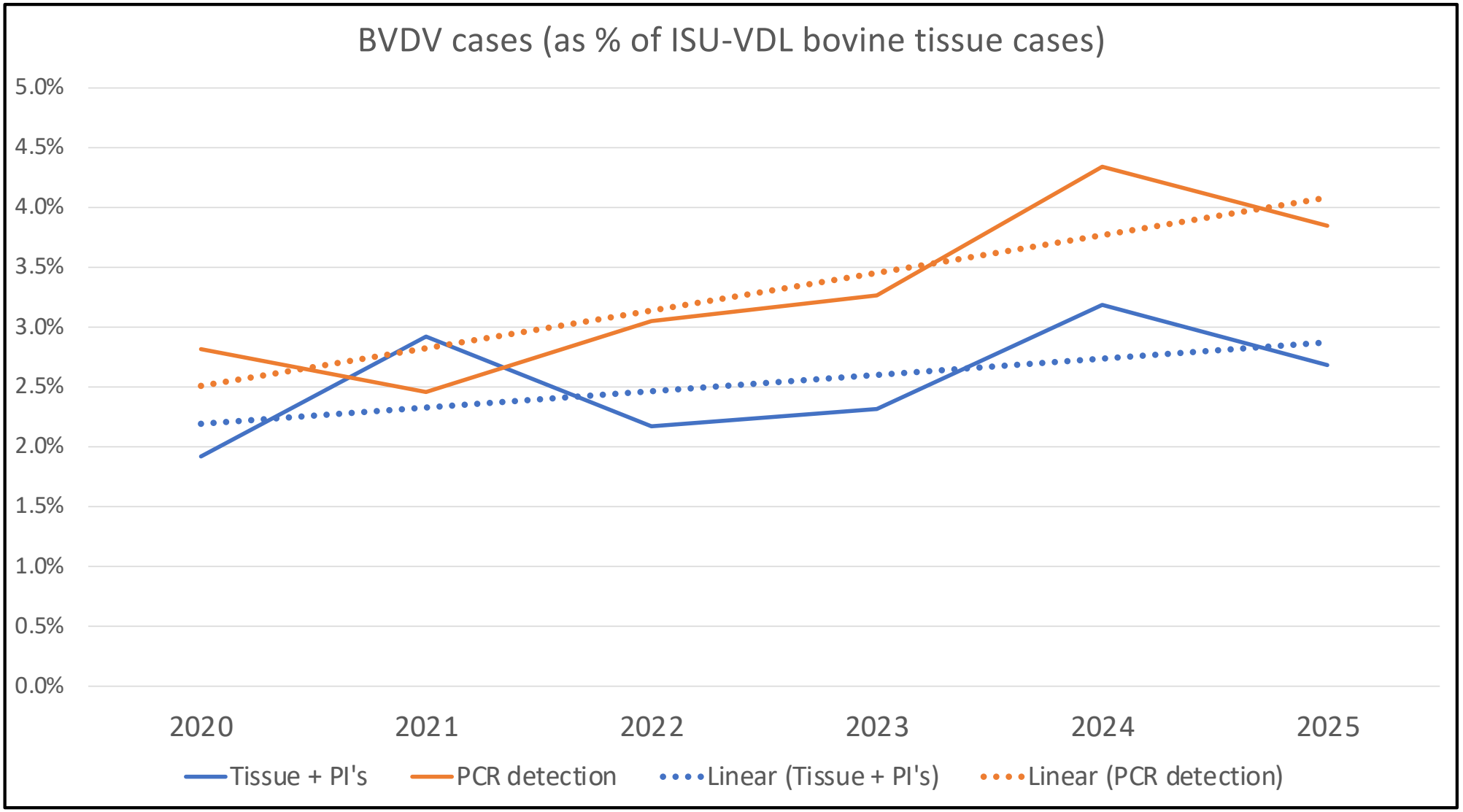
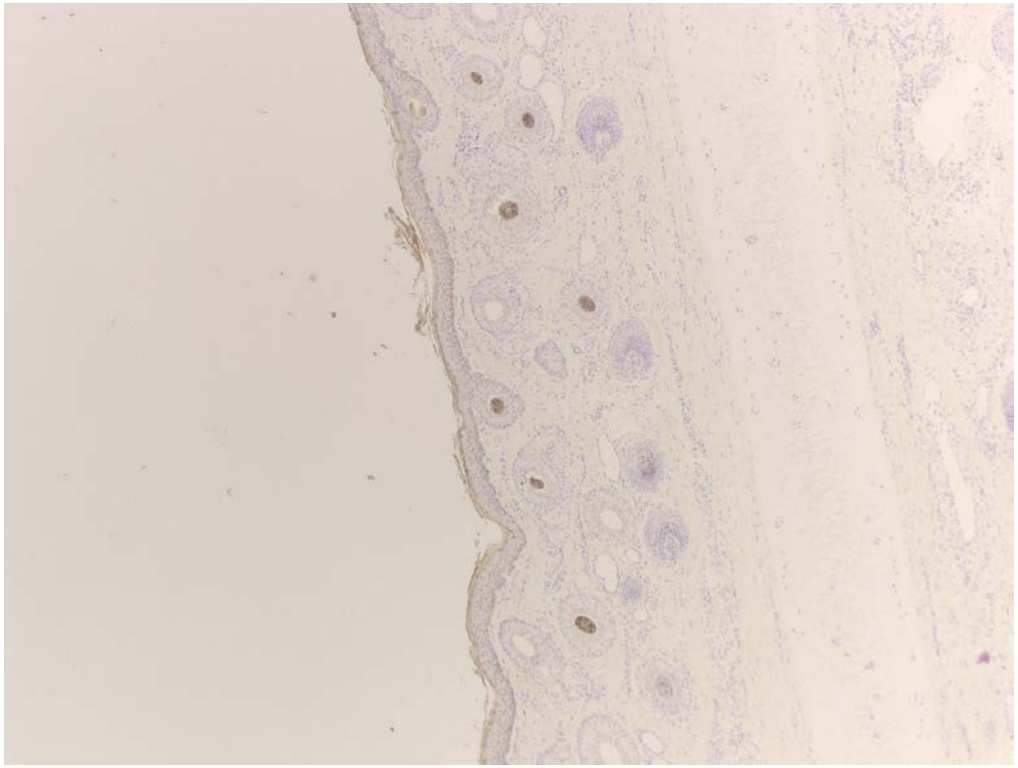
(33, 34]
(34, 35]

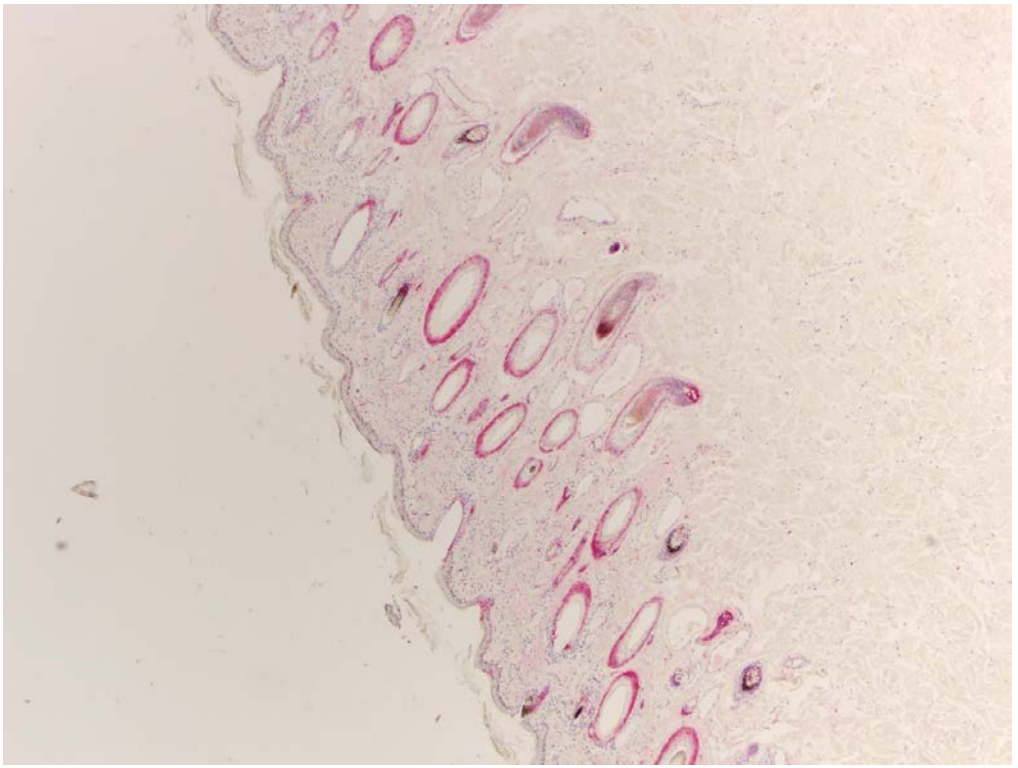

BVDV cases (as % of ISU-VDL bovine tissue cases)
5.0%
4.5%
4.0%
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
2020
2021
2022
2023
2024
2025
Tissue + PI's
PCR detection
Linear (Tissue + PI's)
Linear (PCR detection)

BVDV PI testing options

- Virus detection
  - Antigen-capture ELISA: Serum, ear notch (fresh)
    - Serum: Not for young calves (less than 4 months, interference of passive immunity)
    - Generally an individual animal test
  - PCR: Serum, EDTA buffy coat, ear notch (fresh), milk
    - Rapid and sensitive
    - Can be pooled aggressively to reduce cost
    - Generally used to screen multiple animals
  - Immunohistochemistry: Ear notch (formalin fixed)

BVDV PI testing options: IHC





BVDV PCR testing on fresh ear notches

- Collect individually in empty tube
- Chilled (never frozen) and shipped ASAP

- At the lab: placed in PBS on arrival
- Next day → PBS is pooled for PCR testing
  - Sample placed in formalin

- If any pools are positive, we perform IHC to check for persistent infection

PCR - Bovine viral diarrhea virus

Animal ID	Specimen	ct / Result
GA [1 - 25]	Ear notch	>=35 / Negative
GB [26 - 50]	Ear notch	>=35 / Negative
GC [51 - 75]	Ear notch	>=35 / Negative
GD [76 - 100]	Ear notch	>=35 / Negative
GE [101 - 125]	Ear notch	31.3 / Positive
GF [126 - 150]	Ear notch	>=35 / Negative
GG [151 - 175]	Ear notch	>=35 / Negative
GH [176 - 200]	Ear notch	>=35 / Negative
GI [201 - 203]	Ear notch	>=35 / Negative

BVD PI Biopsy > 3 IHC

Animal ID	Tube #	Result
	SID #101	Negative
	SID #102	Negative
	SID #103	Negative
	SID #104	Negative
	SID #105	Negative
	SID #106	Negative
	SID #107	Negative
	SID #108	Negative
	SID #109	Negative
	SID #110	Negative
	SID #111	Negative
	SID #112	Negative
	SID #113	Positive
	SID #114	Negative
	SID #115	Negative
	SID #116	Negative
	SID #117	Negative
	SID #118	Negative
	SID #119	Negative
	SID #120	Negative
	SID #121	Negative
	SID #122	Negative
	SID #123	Negative
	SID #124	Negative
	SID #125	Negative

BVDV PI testing options: Milk samples

Molecular Diagnostic

PCR - Bovine viral diarrhea virus

Animal ID	Specimen	ct / Result
SID #1	Milk	>=35 / Negative
SID #2	Milk	>=35 / Negative
SID #3	Milk	>=35 / Negative
SID #4	Milk	>=35 / Negative
SID #5	Milk	>=35 / Negative
SID #6	Milk	>=35 / Negative
SID #7	Milk	>=35 / Negative
SID #8	Milk	>=35 / Negative
SID #9	Milk	>=35 / Negative
SID #10	Milk	>=35 / Negative
SID #11	Milk	>=35 / Negative
SID #12	Milk	>=35 / Negative
SID #13	Milk	>=35 / Negative
SID #14	Milk	>=35 / Negative
SID #15	Milk	>=35 / Negative
SID #16	Milk	>=35 / Negative
SID #17	Milk	>=35 / Negative
SID #18	Milk	>=35 / Negative
SID #19	Milk	>=35 / Negative
SID #20	Milk	>=35 / Negative
SID #21	Milk	>=35 / Negative
SID #22	Milk	>=35 / Negative
SID #23	Milk	34.5 / Positive
SID #24	Milk	>=35 / Negative
SID #25	Milk	>=35 / Negative
SID #26	Milk	>=35 / Negative
SID #27	Milk	>=35 / Negative
SID #28	Milk	>=35 / Negative
SID #29	Milk	>=35 / Negative
SID #30	Milk	>=35 / Negative
SID #31	Milk	>=35 / Negative
SID #32	Milk	>=35 / Negative
SID #33	Milk	>=35 / Negative
SID #34	Milk	>=35 / Negative
SID #35	Milk	>=35 / Negative
SID #36	Milk	>=35 / Negative
SID #37	Milk	>=35 / Negative
SID #38	Milk	22.3 / Positive
SID #39	Milk	>=35 / Negative
SID #40	Milk	>=35 / Negative
SID #41	Milk	>=35 / Negative
SID #42	Milk	>=35 / Negative
SID #43	Milk	>=35 / Negative
SID #44	Milk	>=35 / Negative
SID #45	Milk	34.3 / Positive
SID #46	Milk	>=35 / Negative
SID #47	Milk	>=35 / Negative
SID #48	Milk	>=35 / Negative
SID #49	Milk	>=35 / Negative
SID #50	Milk	>=35 / Negative
SID #51	Milk	>=35 / Negative
SID #52	Milk	>=35 / Negative
SID #53	Milk	>=35 / Negative
SID #54	Milk	>=35 / Negative
SID #55	Milk	>=35 / Negative
SID #56	Milk	>=35 / Negative
SID #57	Milk	>=35 / Negative
SID #58	Milk	>=35 / Negative
SID #59	Milk	>=35 / Negative
SID #60	Milk	>=35 / Negative
SID #61	Milk	>=35 / Negative
SID #62	Milk	>=35 / Negative
SID #63	Milk	>=35 / Negative
SID #64	Milk	>=35 / Negative
SID #65	Milk	>=35 / Negative

BVD PI Biopsy > 3 IHC

Animal ID	SID #	Specimen	Result
17945	SID #1	Ear notch	Negative
17723	SID #2	Ear notch	Negative
17131	SID #3	Ear notch	Negative
17865	SID #4	Ear notch	Negative
16206	SID #5	Ear notch	Negative
17946	SID #6	Ear notch	Negative
17742	SID #7	Ear notch	Negative
17964	SID #8	Ear notch	Negative
17466	SID #9	Ear notch	Negative
17800	SID #10	Ear notch	Negative
18087	SID #11	Ear notch	Negative
16683	SID #12	Ear notch	Negative
17890	SID #13	Ear notch	Negative
17599	SID #14	Ear notch	Negative
17399	SID #15	Ear notch	Negative
15108	SID #16	Ear notch	Negative
18144	SID #17	Ear notch	Negative
17862	SID #18	Ear notch	Negative
16014	SID #19	Ear notch	Negative
10911	SID #20	Ear notch	Negative
13652	SID #21	Ear notch	Negative
13684	SID #22	Ear notch	Negative
16143	SID #23	Ear notch	Negative
11493	SID #24	Ear notch	Negative
13025	SID #25	Ear notch	Negative
15987	SID #26	Ear notch	Negative
14384	SID #27	Ear notch	Negative
11713	SID #28	Ear notch	Negative
12001	SID #29	Ear notch	Negative
15156	SID #30	Ear notch	Negative
11178	SID #31	Ear notch	Negative
15072	SID #32	Ear notch	Negative
10946	SID #33	Ear notch	Negative
12456	SID #34	Ear notch	Negative
13901	SID #35	Ear notch	Negative
15064	SID #36	Ear notch	Negative
16047	SID #37	Ear notch	Negative
16295	SID #38	Ear notch	Negative
13918	SID #39	Ear notch	Negative
12057	SID #40	Ear notch	Negative
16626	SID #41	Ear notch	Negative
15884	SID #42	Ear notch	Negative
11593	SID #43	Ear notch	Negative
10185	SID #44	Ear notch	Negative
14556	SID #45	Ear notch	Negative
11705	SID #46	Ear notch	Negative
11706	SID #47	Ear notch	Negative
15622	SID #48	Ear notch	Negative
15750	SID #49	Ear notch	Negative
11189	SID #50	Ear notch	Negative
15011	SID #51	Ear notch	Negative
14414	SID #52	Ear notch	Negative
12899	SID #53	Ear notch	Negative
10992	SID #54	Ear notch	Negative
17798	SID #55	Ear notch	Negative
11493	SID #56	Ear notch	Negative
15320	SID #57	Ear notch	Negative
14453	SID #58	Ear notch	Negative
16083	SID #59	Ear notch	Negative
12220	SID #60	Ear notch	Negative
10150	SID #61	Ear notch	Negative
16697	SID #62	Ear notch	Negative
15321	SID #63	Ear notch	Negative
12863	SID #64	Ear notch	Negative
13718	SID #65	Ear notch	Negative
15911	SID #66	Ear notch	Negative
16642	SID #67	Ear notch	Negative
14781	SID #68	Ear notch	Negative
11454	SID #69	Ear notch	Negative
15124	SID #70	Ear notch	Negative
12303	SID #71	Ear notch	Negative
15172	SID #72	Ear notch	Negative
13944	SID #73	Ear notch	Negative
4791	SID #74	Ear notch	Negative
14695	SID #75	Ear notch	Negative
16521	SID #76	Ear notch	Negative
13017	SID #77	Ear notch	Negative
14413	SID #78	Ear notch	Negative
13075	SID #79	Ear notch	Negative
15429	SID #80	Ear notch	Negative
15670	SID #81	Ear notch	Negative
16576	SID #82	Ear notch	Negative
10193	SID #83	Ear notch	Negative
16625	SID #84	Ear notch	Negative
15378	SID #85	Ear notch	Negative
12223	SID #86	Ear notch	Negative
4012	SID #87	Ear notch	Negative
15116	SID #88	Ear notch	Negative
14074	SID #89	Ear notch	Negative
14639	SID #90	Ear notch	Negative
4955	SID #91	Ear notch	Negative
4279	SID #92	Ear notch	Negative
13354	SID #93	Ear notch	Negative
13893	SID #94	Ear notch	Negative
13887	SID #95	Ear notch	Negative
14681	SID #96	Ear notch	Negative
4097	SID #97	Ear notch	Negative
16251	SID #98	Ear notch	Negative
15932	SID #99	Ear notch	Negative
15933	SID #100	Ear notch	Negative
17780	SID #101	Ear notch	Negative
17913	SID #102	Ear notch	Negative
17478	SID #103	Ear notch	Negative
17939	SID #104	Ear notch	Negative
17915	SID #105	Ear notch	Negative
18022	SID #106	Ear notch	Negative
17825	SID #107	Ear notch	Negative
18114	SID #108	Ear notch	Negative
15769	SID #109	Ear notch	Negative
16274	SID #110	Ear notch	Negative
17887	SID #111	Ear notch	Negative
17267	SID #112	Ear notch	Negative
17774	SID #113	Ear notch	Negative
18073	SID #114	Ear notch	Negative
17241	SID #115	Ear notch	Negative
17965	SID #116	Ear notch	Negative
17601	SID #117	Ear notch	Negative
17838	SID #118	Ear notch	Positive
18006	SID #119	Ear notch	Negative
17512	SID #120	Ear notch	Negative

Take home message

- Bovine respiratory disease
  - Fairly well understood pathogens with many testing options
  - Need to sample calves early in disease
  - Need to sample the correct areas of the respiratory tract
  - Try to sample untreated calves with no recent vaccinations

- When utilizing diagnostic testing…..
  - Understand the limitations of various tests
  - Have a diagnostic question with each submission
  - Have a plan to utilize testing results

Questions???

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