Brucella ovis serology update

A Johnson, J McGiven, and D Knowles

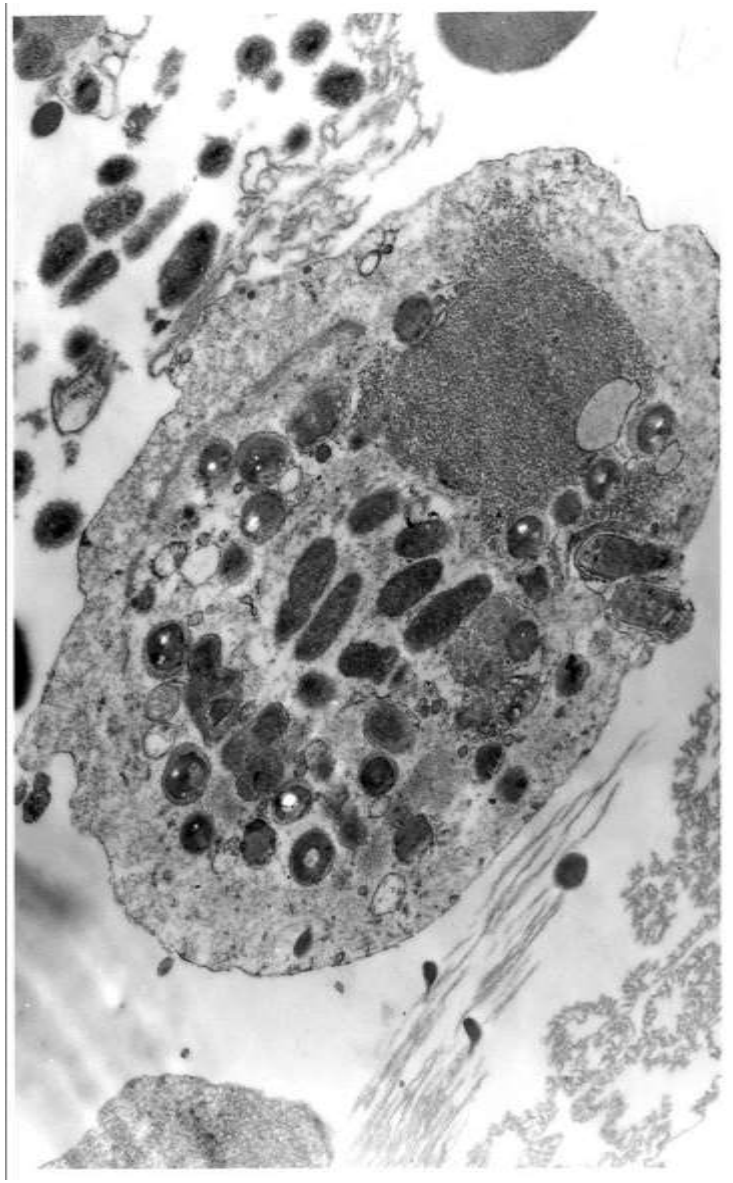
Collaborators:
Colorado Department of Agriculture – Tiffany Brigner (AHL), Jennifer Glenn (AHL), Ed Kline, Dan Love
CSU Western Slope Veterinary Diagnostic Lab – Raye Walck, Katherine Wadsworth
Idaho State Department of Agriculture Animal Health Lab – Keri Banner
Montana Veterinary Diagnostic Lab – Antonio Fuentes-Sanchez
Wyoming Livestock Board - Jim Logan
Optimal Livestock Services - Geri Parsons

Presented by Andrew Johnson, PhD

vmrd Veterinary Medical Research & Development

Brucella ovis – Background

- Discovered in the 1950s in Australia and New Zealand
- Gram-negative intracellular pathogen of the genus *Brucella*
- The genus includes six classical species (*B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis* & *B. neotomae*)
- Limited host range compared to other *Brucella spp.*
    - Natural infection of sheep (primary) and farmed red deer.
    - Infection of other species demonstrated experimentally (e.g. white-tailed deer, bighorn sheep)
    - No evidence of human infection (non-zoonotic)



Brucella ovis – Transmission and Disease

- Venereal transmission (primary)
  - Direct contact between rams
  - Indirect via ewes (mechanical vector)
- Clinical and sub-clinical disease
- Rams primarily affected ➔ epididymitis
  - Infertility
  - Reduction in lamb yield
- Ewes are more resistant to infection but may become transiently infected.
  - Abortion and placentitis (rare)

Picture courtesy of Dan Love

Brucella ovis - Diagnosis

- **Breeding soundness exam (BSE) ➔** Palpable lesions in clinical cases (confirmatory test needed)
- **Serology (indirect detection) ➔** ELISA, AGID, CFT
  - Pro: High sensitivity, high throughput, low cost
  - Con: Exposure vs shedding, cross-reactivity with related organisms
- **Semen PCR (direct detection)**
  - Pro: Identify active shedding
  - Con: Sample collection, cost, intermittent shedding (false negative)
- **Culture (direct detection) ➔** Gold standard

Brucella ovis - Management

- *Brucella ovis* is managed at the state level.
  - 14 states require negative *B. ovis* test for entry (AK, AZ, CA, CO, ID, MT, NV, NC, ND, SD, TX, UT, WA, WY)
  - USDA developed ELISA (NVSL ELISA)
- Cull positive rams
  - No cost-effective antibiotic treatment available
  - Vaccination (*B. melitensis* rev.1) not widely practiced

Brucella ovis – Serology Challenges

- Single serological assay (many tests available for *B. abortus serology*)

- No standardized commercial ELISA reagents available in the U.S.
  - NVSL ELISA made in-house by diagnostic labs
  - Discrepant results between labs
  - Suspect zone complicates management of individual animals

Role of Serology in *B. ovis* management

What does a weak positive result for *B. ovis* antibody mean in terms of infection/disease control? ➔ Depends on clinical context

In the presence of clinical disease and strong seropositive rams:

1. Infection with the potential of disease

In the absence of clinical disease and strong seropositive rams:

2. Could mean recent infection that could lead to clinical disease in some rams
3. Could be cross reactive antibody, i.e. reacting to an organism not yet identified (esp. at cutoff)
4. Could be baseline antibody titer in rams from prior, controlled infection (yes, the expectation is in the absence of reinfection antibody would drop to undetectable levels, but not always the case)

Monitoring change, or lack of change, in antibody titer over time can be helpful in differentiating these cases, but clinical context or diagnostic tools like semen PCR may be needed.

VMRD *B. ovis* ELISA Development

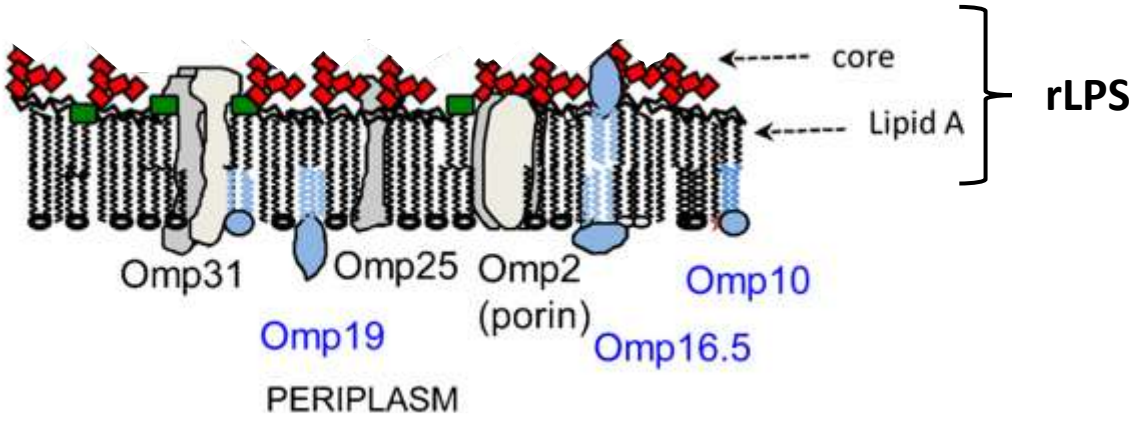
Improve consistency of results between diagnostic labs:

1. Standardization of antigen used to make assay
2. Implementation of commercial manufacturing controls

VMRD *B. ovis* ELISA Development – Antigen

- **Current ELISAs** use hot saline extracted soluble surface antigens:
  - Mix of *Brucella* specific and non-specific antigens
- **VMRD *B. ovis* Screening ELISA** – Defined, highly purified extract of **glycolipid and phospholipid antigens (for stability) ➔ High sensitivity**
- **VMRD in-house recombinant protein (BP26) confirmatory MI-ELISA ➔ High specificity (less sensitive, delayed antibody response)**

Rough *Brucella spp.*



VMRD *B. ovis* ELISA – Manufacturing Controls

- Large-scale batch manufacturing ensures components are optimized to work together and produce consistent results.
- Consistent calibrated equipment (machine vs hand coated plates)
- Qualification of raw materials
- In-process quality control tests e.g. plate uniformity (variation within and between plates)
- Run standardized samples to ensure lot-to-lot consistency
- Stability testing (real time and accelerated)

VMRD *B. ovis* ELISA – Plate Uniformity

Bulk Plate QC – To check plate uniformity, full plates from each bin of coated plates are tested using positive and negative control

Bin 2 positives	0.814	0.838	0.828	0.813	0.797	0.798	0.795	0.782	0.772	0.705	0.722	0.706	0.781
	0.804	0.824	0.784	0.764	0.772	0.794	0.760	0.744	0.737	0.726	0.737	0.753	0.767
	0.780	0.772	0.775	0.780	0.758	0.772	0.741	0.725	0.749	0.742	0.718	0.744	0.755
	0.803	0.803	0.779	0.786	0.798	0.784	0.781	0.771	0.754	0.722	0.748	0.755	0.774
	0.816	0.850	0.768	0.778	0.798	0.793	0.755	0.721	0.715	0.727	0.718	0.745	0.765
	0.825	0.824	0.810	0.776	0.809	0.802	0.787	0.742	0.740	0.738	0.750	0.747	0.779
	0.827	0.811	0.804	0.819	0.769	0.809	0.784	0.739	0.748	0.759	0.756	0.755	0.782
	0.878	0.878	0.837	0.825	0.838	0.877	0.809	0.778	0.730	0.805	0.737	0.694	0.807
	0.818	0.825	0.798	0.793	0.792	0.804	0.777	0.750	0.743	0.741	0.736	0.737	

Avg	SD	CV	Low	High
0.776	0.0399	5.1	0.694	0.878

Intra-assay:

Plate #	Control Sample	Mean (OD)	SD	%CV
Plate 1	Negative	0.169	0.0100	**5.9**
Plate 2	Negative	0.165	0.0091	**5.5**
Plate 3	Postive	0.765	0.0483	**6.3**
Plate 4	Postive	0.776	0.0399	**5.1**
Inter-assay:				
2 Plates	Negative	0.167	0.0028	1.7
2 Plates	Positive	0.770	0.0081	1.1
				1.4

VMRD *B. ovis* ELISA - External Validation

Evaluation completed by CSU WSVDL, CDA, ISDA, MVDL

- Past NVSL proficiency panels ➔ 100% agreement
- Internal banked samples (agreement with NVSL ELISA results)

Field validation by CDA (Compare NVSL and VMRD ELISA results)

- 633 samples from infected flocks tested ➔ likelihood of weak seropositive shedders of *B. ovis*
- 27 known negative and high background negative samples

Results:

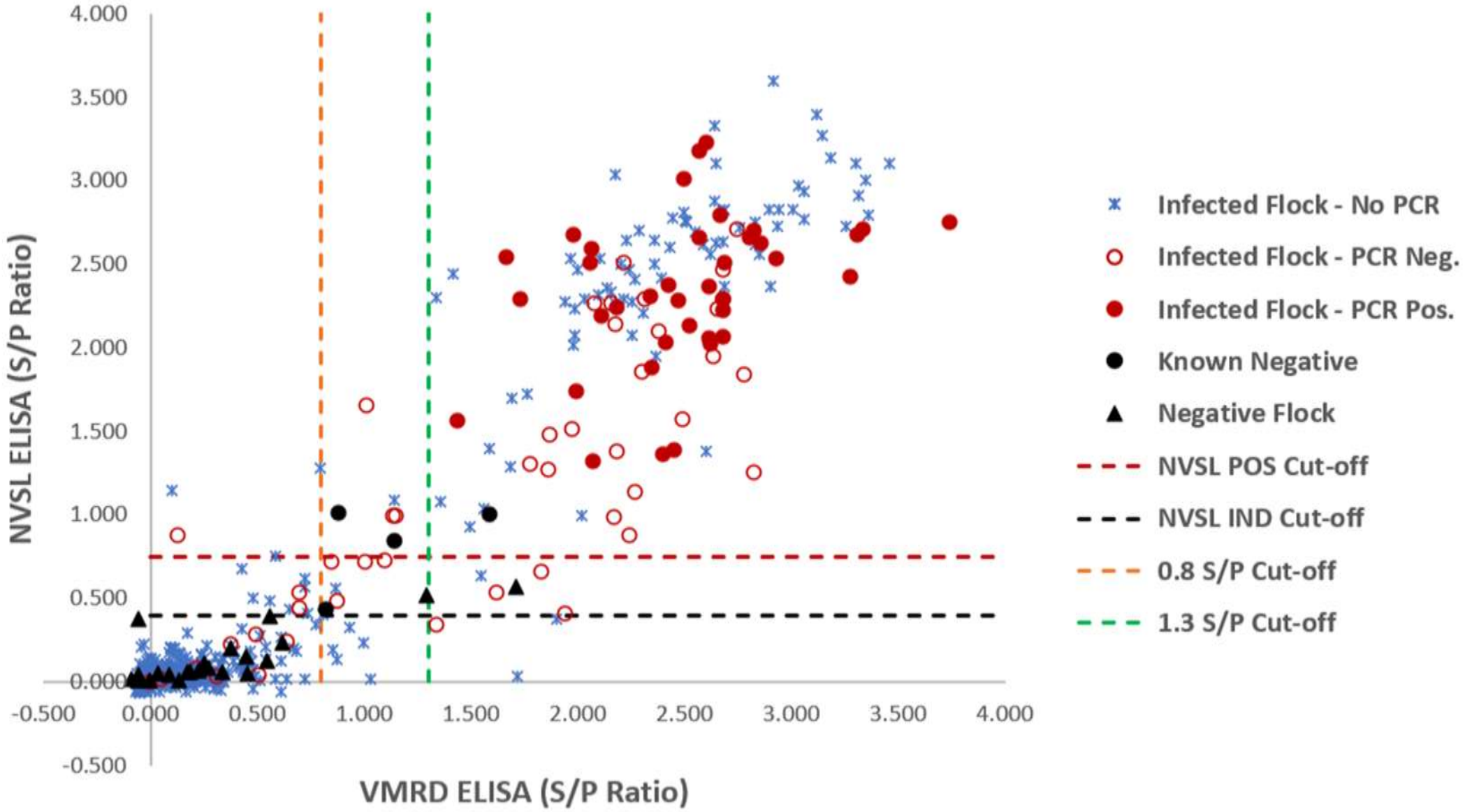
- PCR positive animals were all strongly seropositive (>1.3 S/P)
- State diagnostic lab representatives reviewed data and agreed on an initial 0.8 S/P cut-off. Very good agreement with NVSL ELISA (Kappa = 0.91).
- Cut-off may be increased in the future to further enhance specificity.
    - Evaluate after proficiency testing and periodically throughout the year.

Conclusion

- Standardized antigen and applied manufacturing controls to *B. ovis* ELISA reagent manufacturing.
- Validation data supports this as a sensitive assay with repeatable results in both internal and external testing.
- Diagnostic labs (MVDL, CSU WSVDL, CDA, ISDA) either have, or are in the process of converting to using these reagents.
- 2021 USAHA Western District Meeting ➔ Western district state veterinarians agreed to accept results from VMRD ELISA on entry into their state.
- Complementary in-house ELISA based on recombinant protein (BP26) available.
- External validation data available for *B. canis.*

Extra Slides

VMRD *B. ovis* – CDA Field Validation



VMRD *B. ovis* ELISA - External Validation

NVSL Proficiency Test Panel

Lab	PT Panel	Samples	Agreement with NVSL
WSVDL	PT 2019, 3	15/15	100%*
ISDA AHL	PT 2018, #1	15/15	100%
	PT 2019, Panel #2	15/15	100%
MVDL	PT 2019	15/15	100%
	PT 2018	15/15	100%
	PT 2013	15/15	100%

* Panel included an NVSL ELISA indeterminant sample that was classified as positive in VMRD ELISA

CSU Western Slope Repeatability

Sample #	S/P 1	S/P 2	S/P 3	AVG S/P	SD	%CV
1	1.420	1.361	1.312	1.364	0.054	4.0
2	1.070	1.055	1.023	1.049	0.024	2.3
3	1.601	1.626	1.633	1.620	0.017	1.0
4	0.486	0.529	0.554	0.523	0.034	6.6
5	0.272	0.028	0.350	0.217	0.168	77.5
6	0.877	0.932	0.938	0.916	0.034	3.7
7	1.533	1.539	1.486	1.519	0.029	1.9
8	1.144	1.191	1.176	1.170	0.024	2.1
9	0.633	0.590	0.645	0.623	0.029	4.6
				Intra-assay %CV		**11.5 (4.9)**

B. ovis Infected Flock Case Study*

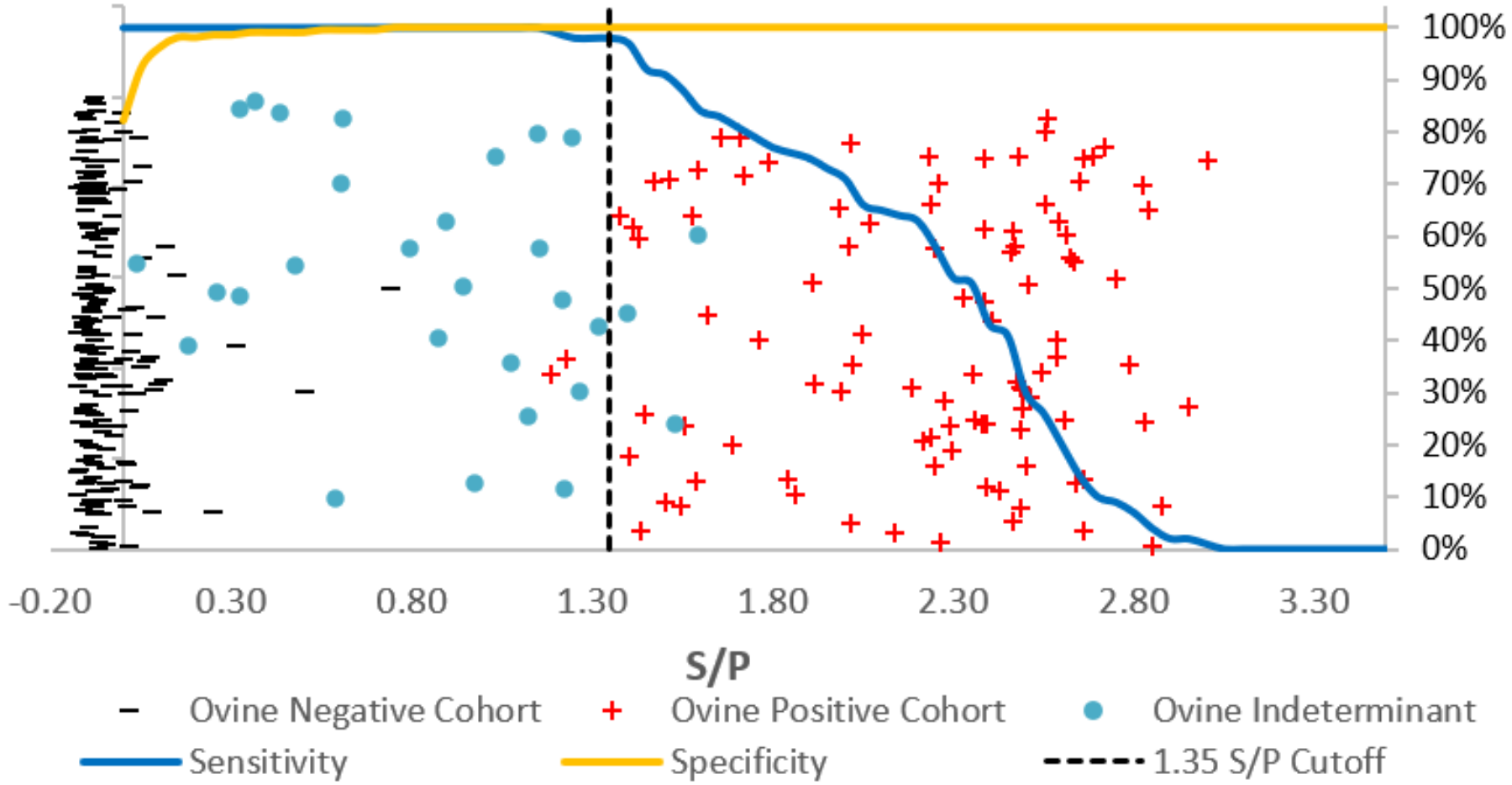
- 31 NVSL ELISA positive rams were culled over two time points from an isolated infected flock of 71 rams.
- 8 additional ELISA positive rams from the flock were monitored for exposure as well as shedding of *B. ovis* by semen PCR.

	10/17/2017	11/29/2017		12/6/2017			1/10/2018 (Necropsy)				VMRD BP26 MI-ELISA		VMRD Rough Ag. ELISA	
ID	NVSL ELISA	NVSL ELISA	PCR	NVSL ELISA	PCR	Culture	NVSL ELISA	Semen PCR	Tissue PCR	Histo.	S/N	+/-	S/P	+/-
393	0.05	1.15		2.07	Pos	Neg	2.44	Pos	Pos	Pos	20.9	+	2.63	+
398	0.14	0.74		0.96	Neg	Neg	2.44	Neg	Neg	Neg	1.7	-	2.41	+
502	0.39	0.65	Neg	1.03	Neg	Neg	0.73	Neg	Neg	Neg	1.0	-	0.84	+
504	0.48	1.89	Neg	2.10	Neg	Neg	2.41	Neg	Neg	Neg	1.1	-	2.28	+
514	0.07	1.69		2.56	Pos	Neg	2.87	Pos	Pos	Pos	27.8	+	2.53	+
516	-0.02	1.70		2.28	Pos	POS	3.25	Pos	Pos	Pos	39.0	+	2.90	+
561	0.06	2.24		2.61	Pos	POS	3.1	Pos	Pos	Pos	27.1	+	2.88	+
584	0.05	0.72		2.26	Neg	Neg	2.63	Neg	Neg	Neg	1.0	-	2.88	+

***Data in gray produced by Dan Love, Tiffany Brigner and Ed Kline.**

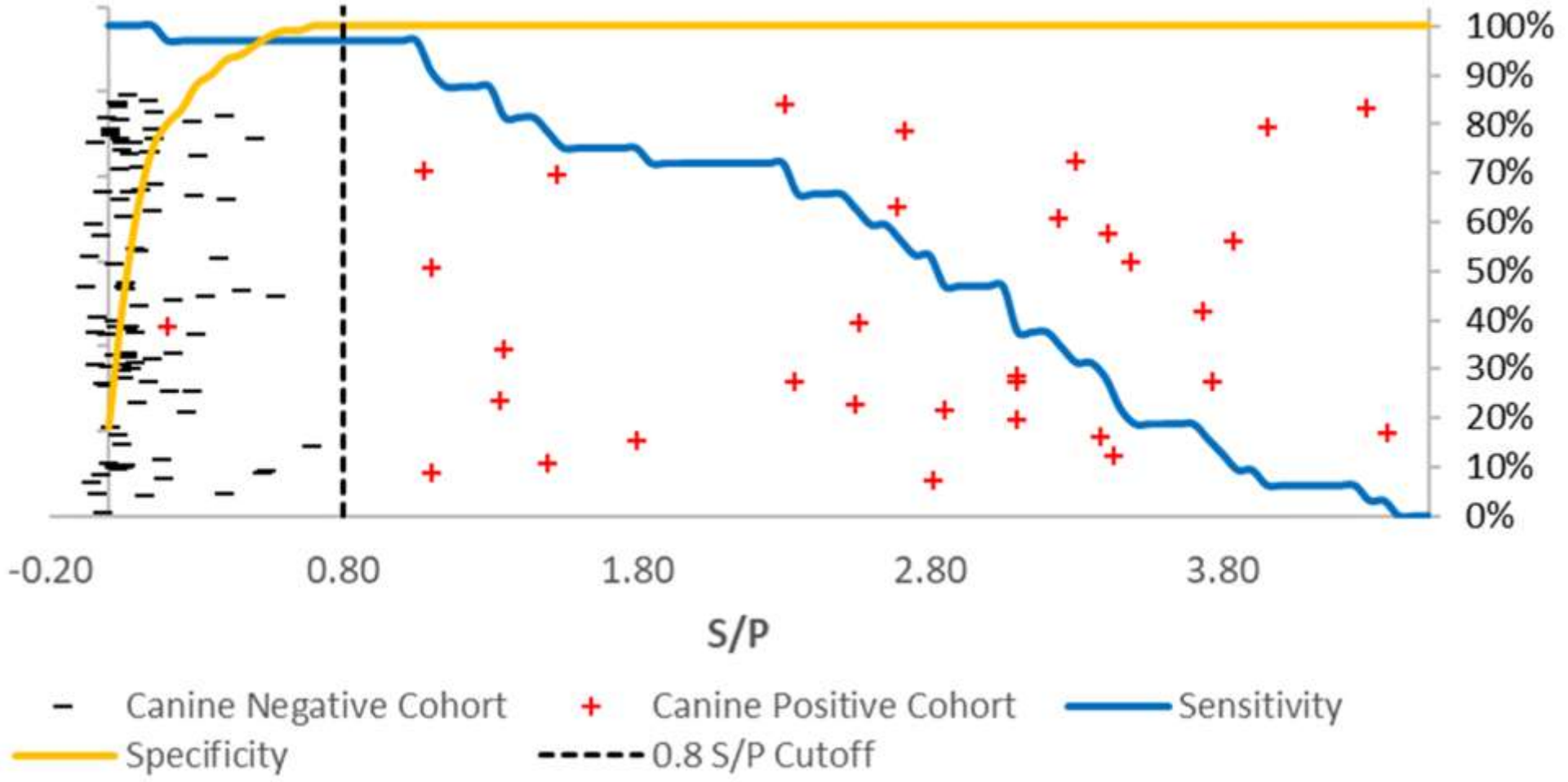
- *B. ovis* quickly spreads in a flock -> Screen with high sensitivity ELISA to isolate/cull exposed rams.
- Not all infected animals develop disease or shed *B. ovis* -> Monitor for disease development and shedding with Semen PCR

VMRD *B. ovis* ELISA - Internal Validation Data



- **98.0% Se (n = 100)/98.7% Sp (n = 208) ➔ indeterminants (n = 29) in negative cohort**
  - **[1.35 S/P Cutoff, YIMax = 0.967]**
- **97.7% Se (n = 100) /98.5% Sp (n = 208) ➔ indeterminants (n = 29) in positive cohort**
  - **[0.3 S/P Cutoff]**

VMRD *B. ovis* ELISA – *B. canis* Internal Validation Data



96.9% Se (n = 32)/100% Sp (n = 100)