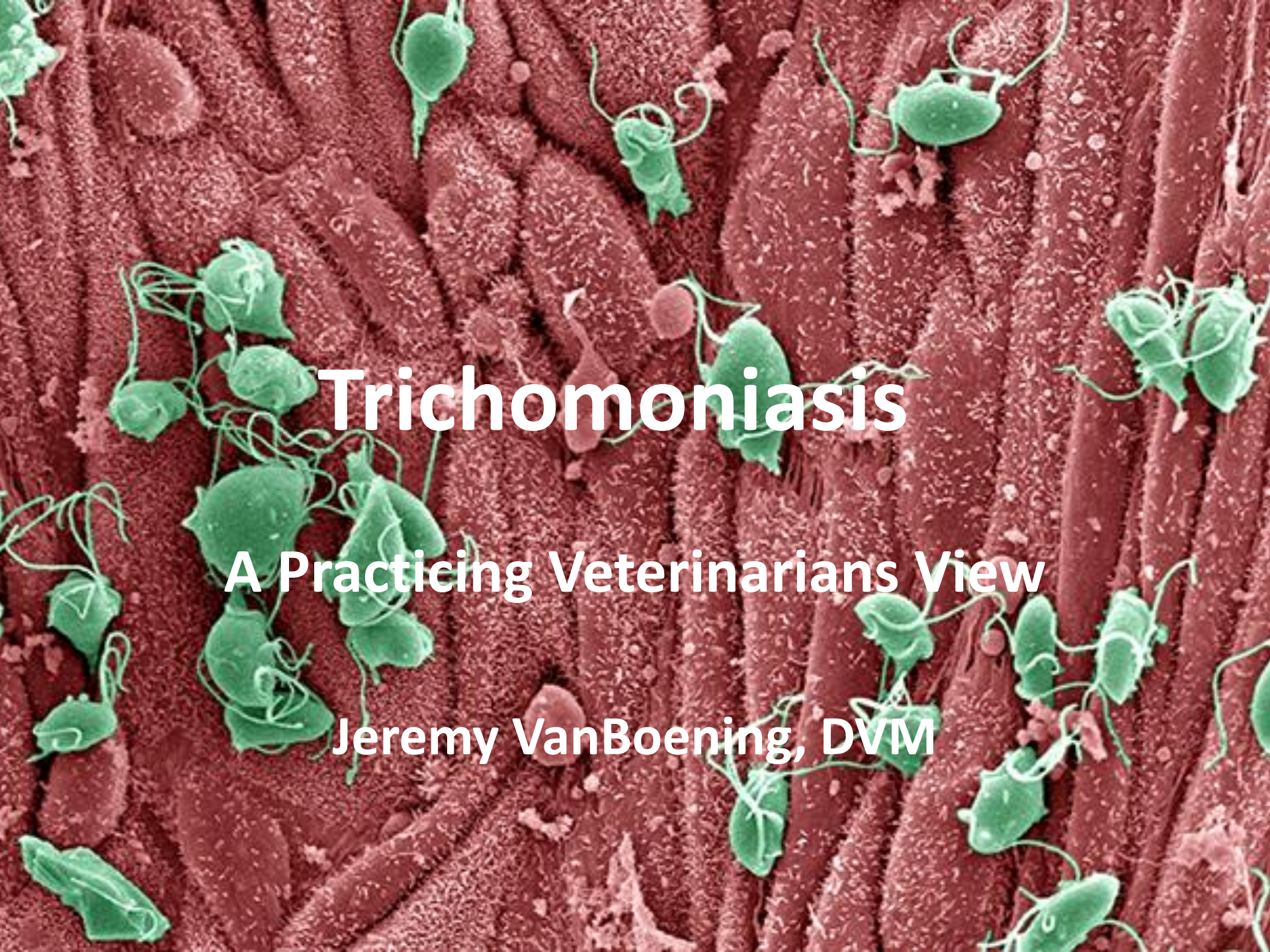

Trichomoniasis

A Practicing Veterinarians View

Jeremy VanBoening, DVM

Republican Valley Genetics

REPUBLICAN VALLEY ANIMAL CENTER

Modern care for animals large & small


Sioux City
Thul
Norfolk
Woodland
Iowa
Nebraska
Omaha
Des Moine
Cheyenne
North Platte
Grand
Island
Lincoln
Kearney
A
Fort Collins
United States
St Joseph
Centennial
Kansas City
Topeka
Hays
Salina
Lee's
Summit
Olathe
Colorado
Springs
Kansas
Pueblo
Wichita
Springfi
Enid

Trichomoniasis Involvement

- Nebraska Cattlemen
  - Animal Health Vice Chair or Chair 2008 to Present
  - Legislation for Notification of Neighbors
  - Took 5 years (2008 to 2013) for an Agreement
  - **Complicated Issue for Producers**
    - ✓ Commercial Cow-Calf Producers
    - ✓ Seedstock Producers
    - ✓ Embryo Recipients
    - ✓ Cull Cow Buyers/Feeders (Commerce Effects)
    - ✓ Livestock Marketing Association Concerns

Nebraska Veterinary Medical Association

- Ad Hoc Committee
  - **Complicated Issue for Veterinarians**
    - ✓ Diagnosis (What's the best test?)
    - ✓ Neighboring Herds (Can we tell them?)
    - ✓ Regulatory Issues (What test is accepted?)
    - ✓ Should There Be Veterinary Certification Programs
      (Training Programs for Veterinarians)

Survey of 10 Diagnostic Labs 10-14-2013

Lab ID	Do the samples need to be incubated?	Do the samples need to be put on ice when shipped?	How many samples can be pooled?	Preferred collection medium?			
A	Yes 24hrs	Yes	Up to 5	tubes			
B	No	No, dark and warm packaging	Up to 5	InPouch			
C	No, room temp	No	Up to 5, **but not recommended**	InPouch			
D	You can if you want, just state on submission form if you incubated samples or not. Have to receive samples within 72hrs of collection.	If cold outside put a thawed ice pack. If hot outside put ice pack but not directly on samples	Up to 5	InPouch or tubes			
E	No, room temp, ship same day	No	No pooling	InPouch			
F	Yes or No, state on form if you did. Has to arrive at lab within 120hrs after collection.	No	Up to 5	InPouch			
G	No	cold pack	Up to 5	Scraping in 5ml of Saline placed in tube.			
H	No	No	Up to 5	InPouch			
I	No	No, room temp	No pooling	InPouch			
J	No, ship overnight	Yes	Up to 3	wash in red top tube with saline			

Summary of PCR Related Questions

Incubation	Yes= 2	No=6	Your Choice=2
Ship on Ice	Yes=2	Room Temp=6	Other=2
Pooling	Up to 5=7	No Pooling=2	Up to 3=1
Preferred Collection Media	In Pouch=6	Saline=2	Tubes=2

Diagnostic Challenges

1. Collections
   - Proper Sampling
     - Preputial Wash or Scrape
     - Culture (3 times every 7 days)
     - Trich-It Device
     - Reading Cultures

   - Bull Restraint (Personal Safety)
     - 2nd and 3rd scraping
   - Collection Media/Containers (Are they all ok?)

Dirty Samples

- Rarely if Ever Return Positive

- Recent Study Huston, et al MSU
  - 20 Samples w/ bacteria injected at increasing #'s
  - 20 Samples clean (Only T. *Foetus* Added)

  - **0 of 20 with bacteria** ever recorded as positive
  - 20 of 20 w/out bacteria had at least one positive recording

Sample Preparation

- Depends on the Diagnostic Lab
- Incubation Time and Time of Incubation
  - Sensitivity and Specificity go down for both culture and PCR as time to incubation increases

	Day 0	Day 2	Day 4	Average
Sensitivity	0.86	0.77	0.55	0.74
Specificity	0.98	0.91	0.87	0.92

Huston et al MSU

Transportation Issues

- Rural Access to Timely **Reliable** Transportation
- How should the sample be packaged?
  - On Ice
  - Room Temperature
  - Depends on the Weather
  - One Ice but NOT Touching Ice Packs

Diagnostic Lab Issues

- Early On: PCR Qualified Personnel (Quality Controls)
- Sample Prep Workflows
- **Disagreements in Results!**
  - **Lead to Loss of Confidence by Vets/Producers**

Quantitative PCR Study

Accepted for Publication in January JVDI 2014

1. Compared Sample Prep Workflows and qPCR
2. Assessed the Accuracy of Pooling
3. Assessed the Specificity of the Primers and Probes Currently Used
4. 5 Labs Participated with the Study Lab
   - ✓ 803 Samples from Across the USA.

Work Flow Issues From Study

- DNA Extraction
  - Boiling (Heat Lysis) -vs- Chemical Extraction
  - 86% of samples that did not agree with study laboratory were from work flows utilizing boiling

Work Flow Issues From Study

- Internal Amplification Control
  - Detecting if something was inhibiting the amplification of DNA
  - 6.2 % of the samples in the study were identified as inhibited and needed more testing

Overall Sensitivity

True Positives

- Heat-Lysis 84.4%
- Chemical Extraction 95.7%

Overall Specificity

	True Negatives
Heat-Lysis	97.5%
Chemical-Lysis	100%

More Findings

- False Negatives and False Positive
  - ✓ 22 False Negatives
    - 91% of those were from the labs utilizing heat-lysis and no internal control
  - ✓ 9 False Positives
- What are the consequences????
- Loss of Customer Confidence
  - Both Veterinarians and Producers
  - Economic Implications for Producers

False Positive Example

- Dual Ownership of Bull (Valued at $50,000)
- Bull goes to owner in southern region for breeding season
- Tested for Trich for return to co-owner in northern region
- Positive result (PCR) from Lab A
- 3 subsequent tests from Lab A and Lab B,C show negative results

How Do We Get Better????

Standardization

1. Adopt the Best Diagnostic Testing Available (qPCR) Using Chemical-Lysis and IAC
2. Promote SOP's for Sampling, Sample Prep, and Shipping
3. Increased Veterinarian/Producer Awareness
4. Standardized Regulations