Seeking Harmonization of Trichomoniasis Regulatory and Testing Standards

Information synthesized from the April 3, 2014, “NIAA/USAHA Joint Forum on Trichomoniasis Standards” in Omaha, Neb. Full presentations are available online at www.animalagriculture.org

DISCLAIMER: The information provided in this White Paper is strictly the perspectives and opinions of individual speakers and results of discussions at the 2014 NIAA/USAHA Joint Forum on Trichomoniasis Standards.
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ABSTRACT

Bovine Trichomoniasis, commonly referred to as “trich,” is a sexually transmitted parasitic infection caused by *Tritrichomonas foetus* and was first discovered in Western beef herds in 1958. Spread primarily by infected bulls that do not exhibit symptoms, trich has resurfaced in the United States and is causing significant monetary losses to the beef industry.

While 26 states have trichomoniasis control/management program regulations in place as of April 1, 2014, there is a wide variation in regulation and testing among the states, making compliance difficult and confusing. Other states are in the process of establishing regulations and testing protocols or are seeking guidance so regulations and protocols can be formulated and the disease can be managed.

The April 3, 2014, “NIAA/USAHA Joint Forum on Trichomoniasis Standards” provided a platform where industry leaders, producers, diagnostic laboratory veterinarians, animal health officials and others involved in the control and management of trichomoniasis obtained a broader understanding regarding best management practices, economics, sample collection and laboratory handling and processing procedures, plus gained insight into various state regulations. The Forum also allowed for an in-depth exchange of science-based information and individual opinions so challenges could be discussed and areas identified where harmonization of regulations and testing protocols would be beneficial to state officials and beef producers.

The National Institute for Animal Agriculture (NIAA) is a non-profit, membership-driven organization that unites and advances animal agriculture: the aquatic livestock, beef, dairy, equine, goat, poultry, sheep, and swine industries. NIAA is dedicated to furthering programs working toward the eradication of diseases that pose risk to the health of animals, wildlife and humans; promote the efficient production of a safe and wholesome food supply for our nation and abroad; and promote best practices in environmental stewardship, animal health and well-being.

U.S. Animal Health Association (USAHA) is a science-based, non-profit, voluntary organization that serves as a forum for communication and coordination among State and Federal governments, universities, industry, and other concerned groups for consideration of issues of animal health and disease control, animal welfare, food safety and public health. It is a clearinghouse for new information and methods, which may be incorporated into laws, regulations, policy, and programs. It develops solutions of animal health-related issues based on science, new information and methods, public policy, risk/benefit analysis, and the ability to develop a consensus for changing laws, regulations, policies, and programs.
HIGHLIGHTS OF PRESENTATIONS

Overview of Trichomoniasis

Trichomoniasis, commonly referred to as “trich,” is a highly contagious venereal disease that has resurfaced in the beef industry. While the “traditional range” of spread of the disease in the past has been limited to the Western states and Florida, trichomoniasis is now considered a nation-wide disease as it has been reported in Nebraska, Georgia, Tennessee and other states beyond the traditional range.

The protozoan organism *Trichomonas foetus* is sexually transmitted, with 80 percent to 90 percent of females exposed to the organism becoming infected. Infected females often conceive but lose the fetus 50 to 80 days into gestation. Late-term abortions are also known to occur. Infected females are often open, have a prolonged calving interval, and are often culled from the herd. Over time, a majority of infected females clear the infection and return to normal fertility in two to five months.

While carrier or chronic females comprise less than 1 percent of infected females, chronic cows can carry the infection for up to 22 months post breeding and 63 to 97 days post calving.

Trich-infected bulls do not exhibit clinical signs. Chronic carrier bulls are typically three years of age or older. While bulls younger than three years of age do not typically become chronically infected, they have the ability to transmit trich to females they service. Chronically infected bulls do not clear the infection, and, because there is no effective, approved treatment, they must be culled.

Economic Loss Estimates Related to Trichomoniasis

A study conducted in Texas looked at the potential economic impact of trichomoniasis in that state based on the estimated number of calves not produced due to the disease. Conclusions were drawn based on several assumptions: 90-day calving interval, 20 percent of the state’s 150,000 beef herds affected by trich, and using a weighted average of weaning rates (85 percent on 80 percent of the cattle and 73 percent on 20 percent of the cattle).

Analysis showed the economic impact of the disease would mean 2.5 percent fewer calves born—96,000 head—that would have resulted in $95 million in missed revenue to cow-calf producers. Using these figures, an individual ranch with 355 cows, and trich leading to the weaning of 73 percent of calves instead of its normal 85 percent weaning rate, would have its revenue reduced by almost $37,000 a year, with its net farm income decreased by almost $44,000. The cumulative effect of trich on a single ranch would be significant, and lessen its borrowing power and ability to remain a viable business.

When the economic impact was extended to the feedlot/finishing level, total revenue lost increased to $156 million. The economic impact attributed to direct, secondary and indirect losses jumps the figure to approximately $300 million.

Additional work is needed regarding trich’s impact on economic losses, with further work involving alternative calving intervals and the impact on the number of calves and calf weights. More analysis is also needed regarding the benefits and costs of testing vs. return on investment and the potential economic impact of trichomoniasis nationwide.
Sample Handling and Collection
Sample handling and collection has three phases where something can go wrong: 6
• Pre-analytical Phase—Error sources include wrong sample, mishandled sample, improper sample collection, etc.
• Analytical Phase (equipment and technician)—Error sources include mechanical wear and tear, bad sensors, inherent errors, software errors and improper cutoff values.
• Post-analytical Phase—Errors include misread or misreported values, transposition of figures, etc.

With sample handling, collection and testing critical to the management of trich, it is important that field veterinarians and laboratories work as partners. 7

Sample Handling and Collection –Veterinarian Perspective
While states agree that veterinarians should collect and handle samples, diagnostic laboratories across the country have different recommended protocols in place regarding the collection and handling of samples. A survey of 10 diagnostic laboratories showed 10 different recommended procedures to follow when collecting and handling samples.

Differences among laboratories include:
• Preferred collection medium: Tubes, saline or InPouch™
• The specific window from time of collection to having the sample at the laboratory. Some laboratories have a 24-hour window requirement while others have windows of 48 and 72 hours.
• Approved temperature range for the shipping of samples: 60°F to 98°F vs. a more narrow window.
• Whether samples should be frozen prior to shipment, must be put on ice, are OK at room temperature, etc.
• If samples need to be incubated.
• If samples can be pooled in the laboratory. If pooling is allowed, then how many samples can be pooled?

Collection of samples in the field can be a challenge to veterinarians. In addition to bulls not being the most cooperative patient, bulls can come into the chute with mud on them, necessitating the removal of mud to prevent contamination of the sample collected. When a sample becomes contaminated from mud/dirt, the identification of infected animals decreases since a “dirty sample rarely returns positive results.” One study showed that 20 out of 20 samples without bacteria had at least one positive while no samples out of 20 contaminated samples had a positive.

A challenge among veterinarians is to have proper collection media and current collection pouches on hand. Many clients give only a one-day notice to the veterinarian which means the veterinarian must have a sufficient supply of pouches or tubes with current dating to hold each sample.
Recommended collection protocols:

- Before undertaking sample collection, know the tending diagnostic lab’s sample collection protocols.
- Always change gloves between animals. At a minimum, gloves should be cleaned between animals.
- Avoid contamination by wiping the animal’s clipped hair from the scissors before placing the scissors into cleaning solution.
- Be aggressive but not overly aggressive when collecting samples. Blood and fecal matter in the collection pouch is unacceptable.
- Properly record bull identification on the pouch sent to the lab before adding the sample to the pouch.
- Protect the sample from extreme weather or exposure to sunlight while at chute side.

An additional challenge is that veterinarians in some rural areas do not have timely, reliable transportation for shipping samples to the laboratory. Thus, the question arises “How long can a sample be in transit and at what temperature and still produce an accurate result?” It is known that the longer the time between sample collection and work in the diagnostic laboratory, the less reliable the test results.

Sample Handling and Collection – Laboratory Perspective

A *Tritrichomonas foetus* (*T. foetus*) sample contains ~0.5 to 1.5 mL preputial smegma and ~4mL collection media, with the most important component being the *T. foetus* organism. Culture diagnosis depends on the visual observation of live, motile *T. foetus* while real-time polymerase chain reaction (PCR) testing depends on the preservation of *T. foetus* DNA integrity. Factors that can negatively influence DNA integrity and PCR results are bacteria or yeast overgrowth, excess blood or urine in the sample and/or tissue factor necrosis.9

Most laboratories follow good laboratory practice on sample intake and may reject or issue a disclaimer against “regulatory” use if a sample is out of temperature range, did not arrive within the specific window of time between collection and arrival at the laboratory, or is visibly contaminated.10 In some states, where samples are received outside the designated window of acceptance, tests are still run and information is provided to the veterinarian and to the producer—but the information provided is not viewed as “official.”11

Concerns voiced by laboratory diagnostician panelists include:12

- Professional literature has conflicting information about various aspects of collection, shipping, pooling, etc.
- Collection and transport of samples significantly impacts diagnostic accuracy.
- Animal owners sometimes pressure laboratories, requesting leniency in procedures.
- AAVLD accreditation does not mean all laboratories are doing the same thing.
- Not all laboratories are ISO accredited.
- Veterinarians should not use or submit outdated pouches.
• Some laboratories are more strict on sample acceptance; relaxed protocols can lead to missed identification of trich-infected animals/herds and can be taken advantage of by some individuals.

An informal survey of veterinary diagnostic laboratories was conducted February and March 2014 by BioMed Diagnostics, White City, Ore., and addressed sample collection, submission times, handling, accepted material and protocols. Results of 32 university and state departments of agriculture veterinary diagnostic laboratories included:

• States conducting the highest volume of testing for trichomoniasis include Utah and Texas, with near or more than 20,000 samples a year, followed by Wyoming, Kansas, Oregon and California that conduct more than 5,000 tests per year. Colorado also conducts more than 15,000 tests a year.
• Six laboratories responding use culture only; three use PCR only; and 23 out of 32 offer a choice of either culture or PCR.
• PCR sample submission format: 15 out of 32 laboratories use InPouch only; nine InPouch or TF-Tube; five InPouch, TF-Tube or sterile saline; two InPouch, TF-Tube or lactated ringers; and one InPouch or Diamond’s modified medium.
• Submission window: two laboratories had a 24-hour submission window; 21 out of 32, a 48-hour window; three, 72-hour window; two, 120-hour window; and four did not specify submission window.
• All states recommend or specify preputial scraping as the recommended site for bulls. Only two states have a recommendation for cow samples: aspirate cervical mucus.
• Some states will issue technical recommendations by telephone but do not have written sample handling and submission rules.

The same survey identified two sample handling trends for high-test-volume states:

• 15 states incubated the sample at the practitioner’s lab for 24 to 48 hours at 37°F. Other states did not specify incubation period.
• 15 states allow a sample to be frozen and shipped on ice but thawed samples may be rejected.

PCR technology is considered the best technology available today as it is more sensitive and specific than the culture method. Peer-reviewed publications and the experience of various laboratories indicate pooling up to five properly handled samples is an effective method for reducing the costs of diagnostic testing for *Tritrichomonas foetus* while obtaining accurate results. Pooling five samples is shown to deliver 96 percent accuracy. Currently 11 state programs have validated pooling of samples in the laboratory.

A 2010 study, proposed by the American Association of Veterinary Laboratory Diagnosticians parasitology committee and led by Dr. Lee Effinger of Oregon State University, was conducted to determine if *T. foetus* samples could be pooled in order to reduce the costs of testing for trichomoniasis while maintaining a high-degree of accuracy. The study findings:

• 1:5 pooling of positive samples with a cycle time (CT) of 35 and below were all detected.
• Three of nine positive samples with CTs between 36 and 39.9 were detected in 1:5 pools.
• Eight of 15 positive samples with CTs between 36 and 39.9 were detected in 1:3 pools.
Pooling at 1:5 missed 4 percent (7 out of 176) of *T. foetus*-positive samples.  
Pooling at 1:3 missed 3.5 percent (6 out of 176) of *T. foetus*-positive samples.

While diagnostics use of PCR for testing of an individual animal sample is more accurate, pooling of samples makes PCR testing more affordable while delivering a 96 percent confidence in the results.\textsuperscript{17} It must be noted that no test delivers 100 percent accuracy.\textsuperscript{18} Pooling of samples can benefit producers through cost reduction and benefit laboratories in numerous areas.\textsuperscript{19}

Diagnostic laboratory panelists agreed that:\textsuperscript{20}

- Quality control must be a priority from start to finish.
- Regulations and communication should be in place so states and producers feel confident in testing results.
- Establishing a concrete definition for pooling would be beneficial.
- Laboratories should collaborate to determine the range of acceptable criteria to obtain good quality diagnostic results as this would assist both field and regulatory personnel.
- Producer and veterinarian educational programs are important and should be ongoing and high quality.

**Trich Testing—What Has Worked in Colorado**\textsuperscript{21}

While Colorado has been testing for trichomoniasis since the 1970s, testing increased in 2000. Starting with offering culture with InPouch and PCR confirmation, the state moved to allowing pool PCR in 2009. The state approved use of the Biomed Transit Tube in 2013 and is now evaluating XCR from Fluoresentric Inc.

Submission guidelines include:

- 48-hour submission for InPouch.
- 24-hour submission for Lactated Ringers Solution (LRS).
- 72-hour submission for PCR and pooled PCR.
- Same-day submission for transit tubes that will be incubated 24 hours at the lab with pre-incubation not encouraged.

Pooled PCR is increasing, moving from 19 percent in 2009 to 51 percent in 2012 and 68 percent in 2013. In 2013, PCR and pooled PCR accounted for 82 percent of total tests. Some individuals prefer using the Transit Tube because it is easier to use in the field and in the lab. A late 2013 tube change resulted in minimal difference in CP/CT between InPouch and the Transit Tube.

XCR shows promise. Even with the tubes being transported 72 hours in cold conditions, the laboratory has found it can still detect <1 cell in only 25 minutes.

Testing is evolving, with detection becoming more efficient, sensitive and faster. While culturing is a five- to six-day process, conventional PCR reduces the time to three hours. Real-time PCR lowers the time to one hour and 15 minutes, and XCR is a 25-minute process.
Snapshot of Four State Control/Management Programs

Four state program leaders of the 26 states that have a trichomoniasis control/management program in place shared information about their specific programs.

Texas.\textsuperscript{22}

The control program in place in Texas has been industry-driven from its start in January 2010. Borrowing components from programs in other states, Texas Animal Health Commission (TAHC) officials agreed with producer organizations and veterinarians that the program should be a voluntary program and that it should be a control program rather than an elimination program, with risk management rather than risk avoidance driving decisions. Veterinarians collecting samples were mandated to be trained; only official labs would run regulatory tests; and trich was a reportable disease.

A working group comprised of representatives from the Texas & Southwestern Cattle Raisers Association, the Texas Independent Cattlemen’s Association, Texas Farm Bureau and Texas Veterinary Medical Association met with TAHC staff in May 2010 for the first time and changes to the original program that was launched five months earlier were recommended.

The Texas control program has evolved through the years—and continues to evolve. Today’s regulations include, but are not limited to:

- A trichomoniasis test is valid for only 60 days.
- Neighbors should be notified when a ranch finds a positive animal but neighbors are not required to test for trich.
- “Cutter” bulls can move to feedlot for castration at the feedlot.
- Buyers of untested bulls have seven days to decide whether to test, slaughter, feed or resell said bulls.
- Virgin bulls moving interstate into Texas more than 12 months of age must be tested, while those under 12 months of age do not need to be tested.
- Breeding bulls from Canada or Mexico entering directly into Texas may move under an entry permit and remain under hold order at designated premises, away from female cattle, until they test negative.
- Out-of-state untested bulls can go to feedyards.
- Infected bulls can be sold at markets and moved under permit to slaughter.
- Large ranches identified with trich have three years to manage it and must at least test once a year. Bulls testing positive must go to slaughter. The ranch must enter into a herd plan with TAHC and their veterinarian to effectively remove the disease from the herd during the three years.
- The maximum time for retesting bulls in infected herds is 60 days.

Four factors contributing to the success of the Texas control program include:

- Annual scheduled stakeholder meetings that allow for valuable dialogue so differences can be worked out.
- Having an adequate infrastructure at markets, feedlots, special sales and roadstops.
• A strong working relationship with official laboratories.
• Being flexible and industry-driven.

**Kansas.**

Elements of the Kansas program include:

• Recognize only PCR testing.
• Seed stock producers can go from 18 months to 24 months before testing. A herd management plan is submitted that details their animal movement and biosecurity plans.
• Whenever a positive trich herd is identified, a producer and herd veterinarian survey focused on the bull, female and management practices are conducted. The data obtained helps identify trends that then influence changes in the state’s trich regulations.
• A female component to the regulations addresses requirements to move animals interstate into Kansas.
• A trich working group tackles proposed changes to the current trich regulations. The state’s program continues to evolve with industry support critical to formulating any changes.

Input from the beef industry in Kansas led to regulation changes in 2013 involving two key components:

1. Testing of the non-virgin bull whenever there is a change in ownership.
2. Veterinarians in Kansas are certified to test and submit samples. The Kansas State Veterinary Diagnostic Laboratory assisted with the development of the certification program.

A component under consideration in Kansas is neighbor notification.

**Wyoming.**

When Wyoming’s original trichomoniasis regulations were taken to the public for comment, backlash occurred. Over the next 18 months, rules were developed that were more practical and garnered significant buy-in from the industry.

Wyoming’s regulations focus on common grazing, as turning out one trich-infected bull can result in significant problems.

Among the state’s current regulations:

• When a report is received about an incidence of trichomoniasis, the infected herd is quarantined and any exposed herd is quarantined. At this point in time, Wyoming is addressing only the bull issue and not the female issue.
• If a bull trespasses onto another’s property, the state veterinarian can require that the animal be tested.
• With certain areas of the state being “high pocket areas,” these special focus areas have enhanced requirements.
• A producer with a trich-infected herd must notify his/her neighbor in writing, and the state must be given a copy of the letter sent to the neighbor. The state also sends a letter to all neighbors of trich-infected herds.

Colorado.\textsuperscript{25}

Colorado has interstate and intrastate regulations and is in the process of rewriting certain rules because the state is currently more heavy-handed on out-of-state owners than in-state owners.

Elements of the Colorado trich management program include:

• Testing is required before cattle go on Bureau of Land Management, U.S. Forest Service land and public grazing land.
• Brand inspectors check that an animal is negative for trich when there is a change of ownership.
• If a herd is quarantined, two negative PCRS are required to release them. Test-positive open cows and test-positive bulls must go to slaughter. Cows under 120-days pregnant must go to slaughter or be held and rechecked after being away from the bull for 120 days.

Some cattle producers in Colorado are taking responsibility for the spread of trichomoniasis very seriously, with one producer communicating to fellow producers by posting “My herd has trich” at the local livestock auction.

POINTS OF AGREEMENT, AREAS WARRANTING FURTHER DISCUSSION

Consensus was voiced regarding numerous points, including but not limited to:

• Trichomoniasis is a nation-wide disease and can have significant negative economic implications.
• Differences in facilities, staffing and other factors can lead to variations between laboratory protocols and policies, and these variations are understood and accepted.
• No test is 100 percent accurate.
• To be successful, trich control/management programs must have industry buy-in, be flexible and evolve to meet needs.
• Outreach and education are key elements that can increase industry compliance.

Areas were identified where further exploration of possible harmonization of trichomoniasis regulations and testing standards across states could help reduce confusion and increase understanding, cooperation and compliance.

Points Regarding Interstate Movement

• Designate 60 days as the time frame for which a trich test is valid.
  – Tested bull should have no exposure to females during this 60-day timeframe.
  – Require an owner to sign an affidavit or statement on CVI.
– PCR will be the defining test, knowing newer technology might change this defining test.
• Pooling is an accepted method.
• Accept virgin status, and have 24 months of age as a starting point for discussions.
• Trichomoniasis is a reportable disease, and laboratories must report all trich-positive animals.

Sample Collection, Shipping, Handling
• Shipping of samples.
  – Use an enriched/selective medium.
  – 48 hours is acceptable if a sample is unincubated and protected at 15° to 37° C (60° to 98° F); 120 hours is acceptable if a sample is incubated and frozen.
• Collecting veterinarians must be certified.

Recommendations on Follow up of Infected Herds
• Neighbor notification.
• Other effective avenues/opportunities for herd follow up.

Additional Topics for Consideration
• Focus on how to address females.
  – Open cow movement.
  – Communication with markets/impacts.
• Laboratory (AAVLD) increased collaboration.
  – Should a scientific advisory committee be established?
  – Does trichomoniasis merit a full committee within USAHA?
FOOTNOTES


2. Ondrak, Jeff.


6. Ondrak, Jeff. Referencing work performed by Jim Kennedy, DVM, Colorado State University.


15. Smith, Anthony.

16. “Harmonization—Sample Collection & Handling” panel.


FORUM SPEAKERS AND TOPICS  (Listed in the order the presentations were given at the Forum)

“Trichomoniasis Overview—The Disease, History & Management” — Jeff Ondrak, DVM, Beef Cattle Clinical Veterinarian and Assistant Professor, University of Nebraska, Great Plains Veterinary Educational Center

“Economic Aspects of Trichomoniasis: Effects on U.S. Cattle Herd” — David Anderson, PhD, Professor and Extension Economist, Livestock and Food Products Marketing, Department of Agricultural Economics, Texas A&M University

“Field Perspective—Sample Handling & Collection” — Jeremy Van Boening, DVM, Republican Valley Animal Center P.C., Alma, Neb., and Anthony Smith, PhD, BioMed Diagnostics

“Trich Laboratory Testing Standardization” — Jeff Baxter, Senior Product Manager, Ruminant-Animal Health, Life Technologies, and Ivan Leyva-Baca, DVM, Animal Health and Food Safety Group, Life Technologies

“Harmonization—Sample Collection & Handling” — Panel members: Gary Anderson, DVM, Director of the Kansas State Veterinary Diagnostic Laboratory, Kansas State University; Tom Hairgrove, DVM, Program Coordinator for Livestock and Food Animal Systems, Texas Veterinary Medical Diagnostic Laboratory and Texas A&M AgriLife Extension; and Tiffany Brigner, DVM, Laboratory Director, Rocky Mountain Regional Animal Health Laboratory, Colorado Department of Agriculture. Panel moderator: Chuck Massengill, DVM, Consulting Epidemiologist and President of the Missouri Cattlemen’s Association

“Harmonization Panel State Regulations” — Panel members: Bill Brown, DVM, State Animal Health Commissioner, Kansas Department of Agriculture; Dee Ellis, DVM, State Veterinarian and Executive Director of the Texas Animal Health Commission; Jim Logan, DVM, State Veterinarian, Wyoming Livestock Board; and Carl Heckendorf, DVM, Livestock Disease Veterinarian, Colorado Department of Agriculture. Panel moderator: Chuck Massengill, DVM, Consulting Epidemiologist and President of the Missouri Cattlemen’s Association

“Trich Testing—What Has Worked in Colorado” — Tiffany Brigner, DVM, Laboratory Director, Rocky Mountain Regional Animal Health Laboratory, Colorado Department of Agriculture

“Open Forum Discussion” — Moderator: Wes Ishmael, BEEF magazine
NIAA/USAHA JOINT FORUM ON TRICHOMONIASIS STANDARDS PLANNING COMMITTEE

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