



# DAIRY VETERINARY NEWSLETTER

January 2010

## Some New Information Regarding Handling of Diagnostic Milk Samples for Mycoplasma Mastitis

Dr. Larry Fox from Washington State University was just here at Utah State University to present a seminar, "Epidemiology of Mycoplasma Mastitis in Dairy Cows". Some of the very interesting information from his presentation included:

He recommends that culture methods on milk samples for diagnosis of *Mycoplasma* spp. include use of Modified Hayflick media in a 10% CO<sub>2</sub> incubator (temperature used is usually 37° C); this is in accordance with what most mastitis microbiologists recommend.

### Repeatability of detection of mycoplasma in cows in a given milk sample over time

Dr. Fox cultured milk from a number of cows already diagnosed as positive for mycoplasma infection in the mammary gland for 28 consecutive days. 28% of the milk samples were culture-negative; over 60% were strongly positive at  $> 10^6$  cfu/ml, and 72% were positive in that at least one mycoplasma colony was seen. Very few cows were negative on all of the 28 days cultured (all cows had been confirmed positive at least once earlier).

Previous work done by Dr. Ruben Gonzalez at Cornell University showed that within 21 days of onset of clinical mastitis, or within 21 days after cows had calved, 93% of milk samples from positive cows were positive for *Mycoplasma* spp. Dr. Fox sampled his cows over a one-week longer period, and they were not necessarily sampled only beginning at calving or the onset of clinical mastitis. Taken together, the results suggest that when cows are sampled at longer time intervals after they first become infected with mycoplasma mastitis, some culture results will be false-negative. This is true to some degree for the isolation of all mastitis pathogens.

### Freeze or refrigerate milk samples?

There has been some debate about this for years. Milk samples for mycoplasma diagnosis are often collected in two ways that result in a delay from collection until they are streaked for isolation:

If milk samples are collected following onset of signs of clinical mastitis, and from fresh cows following calving, they are often saved in batches on farms until they are either collected by a veterinarian or sometimes by other sample pick up personnel, or are sent via courier to a laboratory. The interval from collection to laboratory testing is typically at least one week to a month or so, especially if sample collection occurs when a veterinarian makes a herd health visit to the farm. Thus there is considerable delay from collection to culture.

If all or part of a dairy herd is sampled at the same time during a milking, it is quite common to test one milk sample collected from each bulk tank on the farm for *Mycoplasma* spp. before proceeding to test all of the individual cows' milk samples for mycoplasma. This is often done even when all of the individual samples are cultured for other mastitis pathogens, with those results usually available within 48 hours. Mycoplasma culture results typically require 3 to 10 days (usually 7 to 10 days) until all positive growth of mycoplasma can be seen. Therefore there is a delay until the bulk tank results are finished and if mycoplasma-positive, the herd owner makes a final decision on whether to culture the individual milk samples for mycoplasma.

In both of the above scenarios, it is almost universally recommended, and widely done, that the milk samples are stored frozen until they are cultured. Dr. Fox tested milk samples with separate aliquots of the same sample cultured fresh, frozen one week, 2, 3 and 4 weeks. He did not re-freeze and thaw the same milk samples repeatedly which could result in more loss of mycoplasma organisms over time.

Dr. Fox found that fresh milk samples that cultured positive for mycoplasma averaged more than  $10^6$  cfu/ml of mycoplasma, while aliquots frozen for one week averaged 39,000 cfu/ml of mycoplasma. Both numbers are quite high and he does not expect that this would affect diagnostic ability. By 4 weeks, they averaged approximately 100 cfu/ml which would not preclude diagnosis but makes false negatives more likely, and some were. It seems that freezing milk samples for one week is not too bad, but for much longer, and certainly by the time they are frozen for 4 weeks, this is not ideal for mycoplasma diagnosis.

Therefore he also investigated refrigeration times and their effects on milk samples for diagnostic purposes. Apparently a different set of samples was used, because these samples when fresh averaged 25,000 cfu/ml and after 5 days of refrigeration they averaged 12,500 cfu/ml. These are still quite high numbers and should not preclude diagnosis; there was little practical difference in isolation of mycoplasma after 5 days' refrigeration.

### **Practical conclusions**

During a productive discussion, Dr. Fox said that one should ideally refrigerate milk no longer than 5 days or freeze it longer than 7 days before culture for *Mycoplasma* spp. In the two scenarios described above, one could collect the on-farm milk samples and then once each week send them via overnight delivery to a laboratory. An alternative would be to refrigerate samples for 5 days and then freeze them for another week before they are sent for culture.

When the entire herd is cultured but bulk tank results are awaited before proceeding with mycoplasma culture of the individual cow samples, one could refrigerate the cow samples for 5 days. One would check bulk tank results at 3 and 5 days, and if positive, test the cow samples. However, mycoplasma diagnosis often takes 7 to 10 days. Therefore, one could refrigerate the cow samples for 5 days and the freeze them for an additional 7 days. By then the tank results would be complete and the decision whether to test the cows' milk could be made.

## **USDA Announces Changes in the Animal Disease Traceability Program**

The USDA just announced "a new, flexible framework for animal disease traceability in the United States - -with changes that respond directly to the feedback we heard." The USDA recently concluded a listening tour to hear comments about the National Animal Identification System (NAIS).

A new Questions and Answers Fact Sheet from the USDA APHIS can be viewed at [http://www.aphis.usda.gov/publications/animal\\_health/content/printable\\_version/faq\\_traceability.pdf](http://www.aphis.usda.gov/publications/animal_health/content/printable_version/faq_traceability.pdf)  
Some of what is contained in the 7 page document follows:

### **Acknowledged criticism of NAIS efforts**

" - - the vast majority of participants were highly critical of the [NAIS] program. Some of the concerns and criticisms raised included confidentiality, liability, cost, privacy and religion." "[In the past] - - USDA tried to

implement NAIS. USDA spent more than \$120 million, but only 36 percent of producers participated. It is no secret that there are concerns about and opposition to NAIS.”

There are a number of statements regarding flexibility, lower cost, administration by States and Tribal Nations, and overcoming “mistrust caused by NAIS”.

### **Only animals crossing state lines will in the new traceability program**

“The new framework focuses only on animals that move in interstate commerce.” A later statement is, “Animals moving in interstate commerce into normal marketing channels are those that will fall under USDA’s new animal disease traceability approach.”

“The details of the new system will be developed - -“.

### **Discussion of costs and who will pay for the new program**

There is quite a bit of discussion of costs and it is clear that these details remain to be worked out. However, some statements regarding cost include:

“U.S. taxpayers have already made a significant investment in USDA’s past animal disease traceability efforts, and it is vitally important that funding for this initiative not go to waste. - - USDA funds previously allocated to NAIS will be used for the new animal disease traceability approach.”

“The money invested in NAIS will not go to waste. Many elements of the NAIS system can be used in this new animal disease traceability framework, should States and Tribal Nations elect to use them. These elements include:

A strong IT infrastructure; an allocator to provide unique identification identifiers; and 840 tags.”

### **What about those participating in the original NAIS ID program?**

“Producers who registered their locations as part of NAIS and currently use 840 numbers may continue to do so. [If 840 tags were used] retagging will not be necessary.”

### **Confidentiality of producer information**

“USDA understands that the confidentiality of producer information is a foremost concern. USDA believes that producer information gathered through the animal disease traceability efforts is exempt from provisions of the Freedom of Information Act. USDA also intends to continue to work with stakeholders and Congress to thoroughly consider this issue.” This certainly sounds as though confidentiality issues and applicability of FOIL have not been completely decided upon. This would seem to be a very important point to get clarified before all (or most) livestock owners and veterinarians would fully endorse the program.

### **Other issues**

There is discussion of protecting against introduction of foreign animal disease through imported animals, consumer protection, and prevention of food borne illness. It seems that the program is directed toward controlling animal health, and the statement is made, “Animal disease traceability isn’t a food safety program.” It is mentioned that quarantine of diseases dangerous to livestock or zoonotic diseases transmissible to humans can be instituted more rapidly with a traceback program.

### **How soon will the new rules be finalized?**

The fact sheet suggests that they will not be finalized for some time, probably at least a year.

### **How can you participate?**

Input and feedback are welcomed. “You can either contact your USDA area veterinarian in charge (AVIC), your State veterinarian, or Tribal animal health officials with comments.” There is some web site contact information described in the fact sheet.

Again, to see the new Fact Sheet from the USDA APHIS go to:

[http://www.aphis.usda.gov/publications/animal\\_health/content/printable\\_version/faq\\_traceability.pdf](http://www.aphis.usda.gov/publications/animal_health/content/printable_version/faq_traceability.pdf)

As always I urge our readers to contact me, including suggestions for future topics of interest. I can be reached at (435) 797-1899 M-W, (435) 797-7120 Th-F or [David.Wilson@usu.edu](mailto:David.Wilson@usu.edu).



David Wilson, DVM  
Extension Veterinarian

"Utah State University is an affirmative action/equal opportunity institution."
---