Virginia Cooperative Extension
Virginia Tech • Virginia State University
www.ext.vt.edu

Department of Dairy Science
Blacksburg, VA 24061
540/231-6870 Fax: 540/231-5014
www.vtdairy.dasc.vt.edu

DAIRY PIPELINE

Volume 39, No. 4 May 2018

Using PCR for mastitis diagnosis: just because we can, does it mean we should?

—Nicole Steele, Ph.D. Student with Dr. Christina Petersson-Wolfe, milk@vt.edu

"...questions remain around the clinical relevance of detecting dead and low levels of bacteria (i.e., <100 CFU/mL), especially from a quarter that appears otherwise healthy."

New technologies are being brought to the market ever more frequently. While many prove to be useful, others are capable of generating information that can confuse users. One such example is the molecular technology, polymerase chain reaction (PCR), for the rapid identification of mastitis pathogens in milk. The PCR is initiated by an enzyme called polymerase, which takes a piece of DNA and makes millions of copies in a chain reaction. For mastitis, the target is DNA from bacteria commonly implicated in intramammary infection. Identifying the type of bacteria helps to define a herd's mastitis problem and supports management decisions, such as selecting appropriate treatments. The traditional method, bacterial culture, involves the growth of bacteria, taking at least two days for a result. PCR detects DNA in as little as 4 hours, but DNA may be from either live or dead bacteria and only the bacterial species included in the test design will be identified.

One study compared the performance of a commercial PCR test with bacterial culture for detecting *Streptococcus uberis* in quarter milk samples collected in early and mid-late lactation (Steele et al., 2017). Bacterial culture results were used to select samples for PCR testing, so that samples represented quarters with a current or previous *Strep. uberis* infection. The study focussed on *Strep. uberis* as it is the most common cause of mastitis in seasonal-calving pasture-based dairy cows. Performance measurements included relative sensitivity, or the proportion of positive culture samples correctly identified as positive by PCR, and relative specificity, which is the proportion of negative culture samples correctly identified as negative by PCR. The correct identification was assumed to be the culture result, as it is the current industry standard for diagnosing mastitis pathogens.

PCR had a relative sensitivity of 87% (Table 1), as 13% of *Strep. uberis* infections were missed. Sensitivity remained similar throughout lactation (range 85-90%), excluding the 30 days in milk timepoint (57.1%) as few quarters (7/82) were infected at this sampling point. Locating DNA proved difficult, especially in colostrum and clinical mastitis samples. Milk contains many potential PCR inhibitors and without successful removal during DNA extraction, these inhibitors can block the polymerase enzyme's activity. Dilution of the sample after the DNA extraction step improved detection by PCR. Without this adjustment, the overall sensitivity would have been 79%.

The PCR test specificity was 87%, since 12% of quarters without *Strep. uberis* in culture were positive in PCR. Specificity was lower at the first milking post-calving (64%) and higher in mid-late lactation (98%). Either PCR was detecting DNA from dead bacteria and, therefore, not an existing infection, or PCR was detecting lower levels of bacteria than culture. The latter

	N	Relative Se. (%)	Relative Sp. (%)
All samples	315	86.8	87.7
First milking	87	89.0	64.3
Tenth milking	73	85.3	71.8
30 days in milk	82	57.1	94.7
Mid-late lactation	71	89.3	97.7

Table 1. Relative sensitivity (Se) and specificity (Sp) of the PCR test for detecting *Strep. uberis* in bovine milk; for all samples, and split into early (first milking, tenth milking and 30 days in milk) and mid-late lactation (100-200 days in milk) (NB: two clinical mastitis samples not included in early or mid-late lactation).

Upcoming Events

See VTDairy for details.

April 9-June 1, 2018
MPP Sign-up period

May 14, 2018
Hokie Cow Classic

May 19, 2018
District Dairy Judging Workout

May 26, 2018
Food Science Workshop, Weyers Cave

June 2018 – Date TBD
Quickbooks for Producers, Franklin County

June 9, 2018
Franklin County Livestock Show

June 9-10, 2018
Show like a Pro Workshop Rockingham County

June 16, 2018
Virginia State Dairy Judging Contest

June 28, 2018
Franklin County DHIA Banquet

July 2018—Date TBD
Hoof Care Management Franklin County

July 8-13, 2018
Southeast Youth Dairy Retreat, GA

If you are a person with a disability and require any auxiliary aids, services or other accommodations for any Extension event, please discuss your accommodation needs with the Extension staff at your local Extension office at least 1 week prior to the event.

For more information on Dairy Extension or to learn about current programs, visit us at VT Dairy—Home of the Dairy Extension Program on the web at: www.vtdairy.dasc.vt.edu.

Christina Petersson-Wolfe, Ph.D.
Dairy Extension Coordinator & Extension Dairy Scientist, Milk Quality & Milking Management

explanation implies that specificity can be underestimated, a limitation of an imperfect reference test. However, questions remain around the clinical relevance of detecting dead and low levels of bacteria (i.e., <100 CFU/mL), especially from a quarter that appears otherwise healthy. In many cases, this would not warrant treatment, so the information provided by PCR may not add value above that from culture, at a lower cost.

Reported data indicate that PCR should not be used on its own to diagnose *Strep. uberis* infections and make decisions regarding treatment, particularly in early lactation. When using PCR, we may end up with more questions than answers. Looking ahead, technological advances, such as the ability to distinguish DNA from live and dead bacteria, will improve the value of PCR as a mastitis diagnostic tool. But for now, combining PCR results with other cow data will improve the overall confidence about the presence or absence of pathogens and their impact on mammary health.

Heifers—Assets or Liabilities?

—Jeremy Daubert, Extension Agent, Rockingham County; jdaubert@vt.edu

Heifers are the future milking animals of your herd, but until then they are an expense that needs to be managed. There was a time when many herds had a 10% mortality rate for calves, today if that is over 2% it is atrocious. Combine that with the rapid acceptance of sexed semen and many farms are finding themselves overwhelmed with heifers. For a herd that is expanding this is a positive thing and allows expansion without purchase. For herds that are not expanding, this can force a higher cull rate in cows and overflowing heifer pens.

> "Heifers are the future milking animals of your herd, but until then they are an expense that needs to be managed."

How a dairy should manage this depends some on their situation. First, it is important to know how many replacements are needed each year to maintain your herd size. Table 2 below shows the number of heifers needed per 100 lactating cows. As you can see, the cull rate and age at first calving can have a significant impact on how many replacements are needed. The extra needed replacements at a later calving age can have a significant impact on herd profitability.

Are you spending money to raise heifers that are not needed? Perhaps it is time to start culling heifers before you have significant expenses in raising them. There are several ways to manage this. Start by culling heifers that have had a significant health event, pneumonia, scours, or just 'poor doing' heifers. Cull calves out of poorer dams or sires. Genomics may be a tool that can be used to manage the culling of heifers. Why spend $1600 to raise a heifer that you do not need?

Table 2. Heifer herd size for a 100-cow herd and a 10% heifer cull rate

Cull Rate (%)	Age at First Calving 22 months	Age at First Calving 24 months	Age at First Calving 26 months	Age at First Calving 28 months	Age at First Calving 30 months
26	53	58	63	67	72
30	61	66	72	78	83
34	69	76	82	88	94
38	77	84	92	99	106
42	86	93	101	109	117

Table from Heifer Economics; 2017 Penn State Extension