Testing for BLV: Control, reduction, and elimination?

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When COVID-19 achieved pandemic status and began to change the way we all lived, testing was the initial approach to try to control spread. Initial tests were PCR based, detecting the genetic material of the virus on swabs. The PCR test was rapidly developed and effective, but it required specialized equipment for analysis and trained personnel. Time accumulated from sample collection, transport, analysis, and eventual return of results. Further testing developments led to antigen based “dip-stick” methods that are the at-home, swab-your-own-nose, and get-results-in-minutes tests that became the norm and replaced the PCR approach. Eventually, the COVID-19 vaccine reduced the amount of testing. Testing to control COVID-19 spread while developing a vaccine was a key to gaining control of transmission. In dairy herds, Bovine Leukemia Virus (**BLV**) has become endemic in the U.S., and taking a similar test-to-control-spread approach might allow us to eventually eradicate BLV.

It is estimated that almost all dairy herds in the United States have BLV infected cows with prevalence levels around 30-40%. BLV infection can cause a significant economic loss due to immunosuppression, trade restrictions, reduced milk yield and replacement costs. In the US, an economic loss of ~$283 per cow annually resulted in a $2.7 billion national deficit due to BLV infection. BLV was eradicated in western Europe through culling when only single digit percentages of cows were infected. With 40% of dairy cows likely infected with BLV, eradication through culling is economically untenable, but eliminating all infected cows might not be necessary. Although many cows are infected, only a portion are infectious - able to transmit infection to other cows. Eliminating infectious cows might be enough to take control of transmission. At this time, no vaccine is available and the nature of BLV infection limits the effectiveness of developing a vaccine although it is not impossible. If and until a vaccine is developed, testing to prevent new infections with hope for eventual elimination of all infected animals is the option available to producers and this relies on diagnostic tools to detect infected cows.

A number of sophisticated diagnostic tests have been developed to detect BLV infection in cattle. Each tests for a different aspect of the disease. Tests include enzyme-linked immunosorbent assay or **ELISA**, agar gel immunodiffusion, luminescence syncytium induction assay (**LuSIA**), and PCR. The agar diffusion and ELISA tests both detect the antibodies cows produce against BLV when they become infected. Both tests are laboratory-based measurements requiring time for sample collection, shipment, analysis, and return of results along with their associated costs. The LuSIA test measures cell-to-cell infectivity due to BLV infection and is also lab-based. This test requires a facility with a complex optical system and labeling agents that are more commonly used for research purposes that restrict its development of a farm-side testing approach. PCR testing is highly sensitive for detecting BLV infection and measures incorporation of the virus into blood immune cells. It too requires time for sample collection, shipment, lab-based analysis with sophisticated equipment, and associated costs. An accurate test with fewer requirements would be the ideal.

It is possible to use currently available testing approaches to control BLV in a dairy herd. Researchers at Michigan State University used funding from USDA and worked managers at a 3,000 cow dairy in northeast Wisconsin to demonstrate the application of a three test strategy to progressively reduce and eliminate infectious cows in the herd. In the first year, cows were blood sampled at calving and any cows with high immune cell counts that indicated active infections were not rebred and culled. Over the next three years, they tested blood or milk samples with the ELISA test to identify positive cows. Then PCR testing of positive cows was used to determine infectious status. Subsequently, positive cows were monitored yearly by PCR to determine infectious status had changed. Cows with high PCR results were eventually culled but with an eye on maintaining necessary stocking levels, availability of replacements, and market influences as well as other factors that influence managers' culling decisions. Over the four year timespan of the project, the herd reduced its prevalence from over 40% BLV ELISA positive cows to less than 1%. The authors point out that the herd preferred the ease of milk sampling for ELISA testing, and VDACS doesn't currently offer this approach. ELISA sample costs range from $4.40 to $8.75, depending on sample number, and PCR testing costs $12 at the lab used in this study. These prices don't include sample collection or shipping costs.

As faculty researchers at Virginia Tech, we're investigating newer testing approaches that might enable more rapid results and hope that cost will be lower. We're taking two approaches. One is an antigen-based approach analogous to the nasal swab tests that people use at home to determine if they have COVID. The second approach is a biosensor to detect the viral genes present in blood cells. From the perspective of on-farm measurements, biosensors offer many advantages, but they are still not a mature technology for BLV monitoring. Combined with advanced manufacturing (*i.e.* 3D printing), biosensor technology may allow cow-side measurements of BLV prevalence in cattle herds with fast detection (less than a minute), cost-effectiveness, ultra-sensitivity, high selectivity and user-friendly. Both approaches could speed the determination of a cow's infectious status with cow-side determination. With support from VSDA through the Setaside program to the department, we're conducting pilot studies to identify BLV negative and positive cows in the Virginia Tech dairy herd that can be used to conduct the preliminary work to develop these tests. With hope for some initial success, we will build our research efforts further with additional grant funding from other sources such as USDA.

With the possibility that a vaccine might never become available, on-site BLV monitoring is our best option if there's commitment to progressively reduce and eventually eliminate BLV in dairy herds. Tests that are easy, cheap, and effective would lead to wider adoption and faster future progress on BLV eradication.