Atlantic Healthy Herds

Research Summary for Recently completed projects for Johnes Disease, BVD and BLV.

With the winding up of the Atlantic Healthy Herds projects on Johnes Disease (AJDI2), BVDv and BLV, some noted success came from all three projects. Take-aways for producers include:

Atlantic Johnes Disease Initiative:
The critical take-away for producers is that the continued completion of Risk Assessments and Management Plans (RAMPs) and the modifying or practices around them has proven to work for farms as they are slowly starting to see a decrease in infection levels. While Johnes cannot be eliminated overnight, the application of the RAMPs on farms is showing that progress can be made over time.

BVDv:
The critical take-aways for producers are:

1. There are still herds that are not vaccinating.
2. Even though most herds do vaccinate, there are still herds that demonstrate active BVDv on the farm.
3. The finding of persistently infected (PI) animals (even though it is a low number of animals) is vital to stop the effects of BVD.
4. Feedbunk swabs may be an easy way for producers to collect screening samples themselves for locating PI animals on their farm.

BLV:
The take-aways for producers are:

1. With the introductions of RAMPs with BLV, over the past 3 years we saw a decrease in the percent of herds in the highest infection levels.
2. Based on the results from the106 farms that completed the RAMPs, the practices most associated with having a higher number of BLV-infected cows in the herd were a combination of factors associated with reproductive practices. These included practices around changing rectal sleeves and cleaning ultrasound/AI equipment, sequence of cows when performing pregnancy checks, use of estrus synchronization protocols, and use of bulls for live breeding on the farm.
3. By determining when calves or heifers become infected with BLV, producers can focus on preventing new infections in certain age groups rather than having to implement a wider control program.

More details on these projects are available in the attached project summaries.

Graduate students and faculty members involved with the projects are available to present the project results at producer meetings across the region.

Atlantic Johne's Disease Initiative 2 Update

There has been continued success with the Atlantic Johne's Disease Initiative (AJDI) and participation from producers in the program continues to be strong end and we hope will continue going forward. The continued support of the changes we have made to the program, from both dairy producers and their veterinarians, shows we have a very sustainable program.

In light of keeping this program financially sustainable, there were some fundamental changes made to the program as part of AJDI 2. One of the first changes made was simplifying the risk assessment form while focusing on the major areas of risk and eliminating areas that did not produce actions points for farms typical of our area. There was also an option for "self-assessments" that the producer could fill out on their own or with the AJDI sampling staff a the time of environmental sample collection.

Table 1 Summary of herds tested and the number of environmental positive herds and percent positive of herds tested from end of 2011 up until end of 2017.

Province	Test Year	#Herds Enrolled	Herds exited	Positive herds
New Brunswick	2011-12	129		27 (21% pos)
	2017-current	115	14	16 (14% pos)
Nova Scotia	2011-12	169		10 (6% pos)
	2017-current	142	27	12 (8.5% pos)
Prince Edward Island	2011-12	140		36 (26% pos)
	2017-current	108	32	24 (22% pos)
Newfoundland and Labrador	2011-12	25		16 (67% pos)
	2017-current	4	N/A	N/A

The number of herds being tested each year fluctuates because only positive herds are tested yearly, but herds that test negative two years consecutively skip a year. Of the herds in Atlantic Canada that are no longer participating, 40 of those herds sold and the remaining 33 chose to no longer participate. The percent positive is the number of herds with positive samples out of the herds enrolled during that period. Overall, we have evidence for slight decrease in herd prevalence, although the statistical power is low.

The other major advancement in the program was moving to direct environmental PCR testing instead of cultures. The primary reason was to minimize cost and decrease testing time. We verified this by comparing 2220 environmental samples simultaneously with culture and PCR. The overall test performance of direct PCR as compared to culture showed that the sensitivity and specificity was quite good, 95.5 and 96.1% respectively, with a negative predictive value of 99.6%. This means that when a herd was called negative by PCR, you could be 99.6% certain you would get the same negative answer on culture.

In combination, the revised risk assessment and "self-assessment" along with the changes in laboratory testing make the continuation of the AJDI very feasible. These changes have been well accepted by the producers and their continued support and desire for participation continues.

BVD Screening in Atlantic Dairy Herds

Bovine Viral Diarrhea virus (BVDv) is a significant production limiting disease of cattle that can remain endemic within herds due to persistently infected (PI) individuals. When a cow comes in contact with BVDv between 35-120 days gestation, the fetus has no functioning immune system and the virus remains "un-detected" in that fetus. When that calf is born it becomes a life-long shedder of BVDv. It is these individuals that must be identified in order to achieve control and eventual eradication of the disease.

There are many ways of testing for BVDv, but most often it is laborious and cost-prohibitive. Developing methods of screening groups of cattle for BVDv, especially PI animals, would allow a reduction in the number of tests performed as well as reduced animal handling requirements.

This project had two main objectives: the first was to screen dairy herds for BVDv infection and the second was to evaluate the ability of herd-level screening methods to find BVDv PI animals. We compared bulk tank milk (BTM) samples, the use of sentinel (unvaccinated) animals, and a consumption surface swab assay (CSSA). We hypothesized that if a PI animal was present within the herd, or group of animals tested, then these screening methods would indicate the presence of the virus.

A suspect positive herd is identified as a herd having a positive BTM PCR, a BTM ELISA >1.0 in an unvaccinated herd, or a BTM ELISA >1.0 on both dates, because vaccine titres should go down. There were 9 farms which were PCR-positive on BTM, while 35 additional farms had antibody ELISAs greater than 1.0 on both sampling occasions. Therefore, a total of 44 herds (7.1%) were considered suspect positive for BVDv. Some herds declined further testing, so 33 herds underwent whole herd testing of individual cows and all young-stock, which can be a rather larger undertaking. Milking animals were tested by milk antigen ELISA and dry cows, bulls, and young-stock greater than 6 months were tested by blood testing. All young-stock under 6 months were tested by taking an ear notch skin PCR test. Additional testing done to look for evidence of a PI animal was sentinel testing and consumption surface swab testing.

Table 1: Bulk Tank Milk test results for 619 dairy herds in Atlantic Canada in 2016

	PEI	NS	NB	NL	Total
Herds tested	170	217	199	33	619
BTM PCR +	4	3	2	0	9
BTM ELISA +	9	15	8	3	35
Herds Suspect +	13	18	10	3	44

A total of 5109 individual animals were tested for BVDv among 32 initial suspect positive herds from BTM testing, and from an additional 2 herds that were suspect negative based on BTM testing but were positive on screening tests.

After whole herd testing was performed, out of 5109 animals tested, a total of 12 PI animals were identified in 9 herds. Therefore, in our study population, the prevalence of PI individuals was 0.23%, while 25.7% (9/35) of herds receiving whole herd testing had at least one PI animal. There were 3, 4, and 2 herds with PI animals (27%, 29%, and 20%) among the 11, 14, and 10 herds tested in PEI, NS and NB, respectively.

Of the 45 herds that underwent sentinel animal screening, 13 herds had at least one positive serum sample on antibody ELISA, giving an apparent prevalence from sentinel testing of 28.9% (Table 2). However, based on the whole herd testing, 7 of these 13 herds did not have a PI on the farm, and therefore were classified as false positives on the sentinel test, producing a positive predictive value (PPV) of 46.2% (6/13).

Of the 58 herds that were tested by the consumption surface swab assay, only 3 tested positive for BVDv, for an apparent prevalence of 5.2%. With no false positives on the swab assay, the predictive value of a positive swab assay was 100%.

The prevalence of herds in Atlantic Canada with BVDv PI animals was estimated to be low at 1.5%. However, the prevalence of transient infections with BVDv is likely to be higher, since the prevalence of suspect positive herds on BTM was 7.1 %.

The two screening tests examined performed quite differently. The sentinel animal test had a 100% sensitivity and negative predictive value, meaning that it detected all tested herds with PI animals. However, it also called some herds positive when no positive PI was found, and these are difficult samples to collect and will not work on herds that vaccinate calves early in life.

The CSSA test had a 100% specificity and positive predictive value, meaning that it classified all PI-free herds correctly, and when a herd tests positive on CSSA, there is likely a PI individual on the farm. However, the CSSA test only detected 3 of 7 tested herds with PI animals, for a sensitivity of 46%, therefore it is not a very sensitive test. On the other hand, the CSSA also had the advantage of locating the PI animal to a particular feed bunk and also can be used to examine all animals located in different locations of a farm. It is also a very easy sample to collect and could be done routinely by producers to monitor for PI animals. More research needs to be done into the utility of the swab assay.

Atlantic Healthy Herds: Surveillance and Control of Enzootic Bovine Leukosis in Atlantic Canada

Regional BLV Surveillance

Over the past 3 years, we collected bulk tank milk samples from all farms shipping milk to determine the number of BLV-infected farms in Atlantic Canada. We were also able to classify these herds into infection tiers to give an estimate of how many cows within each herd are infected with BLV. Overall, the number of BLV-infected herds has stayed constant from 2016 to 2018. In Atlantic Canada, 88-89% of dairy herds were classified as BLV-positive, meaning that they had at least one BLV-positive cow in the herd. All four provinces were about the same in terms of the percentage of BLV-positive herds.

A table showing the percent of dairy herds classified into each infection tier is included below. The percent of BLV-negative or very low prevalence herds has stayed constant over the past 3 years, and the percent of herds in infection tier 2 has increased. Another encouraging result is the decrease in the percent of herds in the highest infection tier over the past 3 years.

Percent of herds in Atlantic Canada classified into each infection tier.

Infection Tier	**2016**	**2017**	**2018**
1 (<5% infected cows)	14	13	13
2 (5-25% infected cows)	9	15	16
3 (15-60% infected cows)	47	61	56
4 (>50% infected cows)	30	11	15

Risk Assessment and Management Program Questionnaire

Based on risk assessment documents used in other locations across Canada and the USA, we developed a risk assessment and management program (RAMP) document for use in Atlantic Canada to help producers identify management practices that may increase the risk of transmitting BLV between animals. In total, 106 farms completed a RAMP and sent the results in, from all 4 provinces. Based on the results from these 106 farms, the practices most associated with having a higher number of BLV-infected cows in the herd were a combination of factors associated with reproductive practices. These included practices around changing rectal sleeves and cleaning ultrasound/AI equipment, sequence of cows when performing pregnancy checks, use of estrus synchronization protocols, and use of bulls for live breeding on the farm.

Timing of New BLV Infections

We were also interested in determining when calves or heifers become infected with BLV, so that producers can focus on preventing new infections in certain age groups rather than having to implement a wider control program. We had 55 herds participate in this part of the project, who submitted blood samples from preweaned calves, weaned heifer calves, and breeding-age heifers.

Of these herds, 19/55 had no BLV-positive youngstock in the blood samples collected. Of these 19 herds, 18 had BLV-positive adult herds, which suggests that heifers become infected with BLV when they enter the milking herd.

There were also 13/55 herds where the preweaned and weaned calves were BLV-negative but the breeding-age heifers were BLV-positive. In this group of herds, the critical control point for BLV seems to be management practices associated with entering the breeding group.

The other 23 herds had a variety of different age groups infected, but 5 of these herds had BLV-positive preweaned calves and 6 herds had BLV-positive samples in all 3 age groups. These herds would need to think about preweaned calf management in terms of preventing new infections.

Future Directions

We are currently recruiting herds for a final segment of the BLV study, where we are looking at cost-effective ways to determine the number of BLV virus particles that each cow is carrying and shedding. Cows that shed higher numbers of virus particles are more infectious than cows shedding low number of virus particles, so identifying these high shedders will hopefully help with preventing new BLV infections in cows not already infected.

If you would like to participate in this study, we are looking for herds who have:

1) Completed individual cow BLV testing, and
2) Have at least 20 confirmed BLV-positive cows currently lactating

If you meet these criteria and would like to participate, or if you would like more information about this part of the project, please contact Emily John at 902-393-2500 or ejohn@upei.ca.