

OPP Eradication Trial: 3rd Year Report, January 2017

Four producers remain in the trial (beginning flock size + baseline % OPP infection noted for each):

- Dan Hammond (30 ewes: 96% positive) Has had two whole-flock 100% negative tests after all positive animals were culled; needs one more whole-flock negative test to confirm status.
- Karen Stormo (55 ewes: 66% positive) Has had two whole-flock 100% negative tests after all positive animals were culled; needs one more whole-flock negative test to confirm status.
- Eric Froehlich (80 ewes: 35% positive) is making excellent progress in spite of the loss of many animals due to copper toxicity following a feed mill mixing error. Test-negative replacements have been retained from all three trial years; several older ewes were segregated and now confirmed test-negative; all positives have been culled.
- Rob Goerger (400 ewes: 65% positive) is eradicating OPP from one group at a time while lambing 4x year. Also making excellent progress with many young test-negative replacements retained from all three trial years, and several older ewes now confirmed test-negative. Positive adults are culled as their production warrants.



1) Genetics

The Minnesota Lamb & Wool Producers (MLWP) requested TMEM154 susceptibility testing of rams as part of the trial and paid all costs (\$12/head) for genotyping one ram per 25 ewes in each flock. However, after the first two years we decided to discontinue this as some rams with desirable genotypes had seroconverted. At about the same time, USDA researchers reported that some strains of the OPP virus had adapted to infect animals regardless of their TMEM154 genotype.

Therefore, much more research needs to be done on genetic susceptibility/resistance before we can depend on it to help eradicate or breed the virus out of a flock. We've been left with more questions than answers, such as: Do the desirable genotypes allow the animal to live with the virus without it being detrimental to their health? And if true, do those animals then still shed the virus to others in the flock?

Though we've put ram genotyping on hold, a few ewes have been TMEM154 tested as we remain curious about the genetics of older ewes, those that are still productive even though test-positive as well as the ones that have managed to fend off the virus in spite of years of exposure in highly infected flocks. A small number of these ewes have already been sampled (we've asked that producers just hold onto those blood cards for now) and there are several more excellent candidates among the trial flocks. We've recently approached USDA researchers to see if these samples would be of use in their work and will keep you posted as more is learned.

2) 'Elitest' MVV/CAEV/OPPV ELISA

The MN VDL has been an invaluable partner in the trial and is the first and only VDL in the country to offer the 'Elitest' ELISA. We can't thank Dr Patnayak and his team enough for all their assistance and patience. While not USDA-licensed, 'Elitest' has long been used in test and control programs worldwide and is the only ELISA for OPP that's been validated to World Organization for Animal Health (OIE) standards.

Some may recall our related March 2013 report of many false positive and inconclusive results due to vaccine cross-reactivity while using the two USDA-licensed tests for OPPV, a cELISA from VMRD and the AGID. Retesting of those same serum sets with 'Elitest' at the University of Guelph found all animals to be negative, further confirmed at various labs by PCR, necropsy, and Western blot. Following additional comparison of both ELISAs, MN VDL imported 'Elitest' and it remains the designated test for both of Minnesota's OPP programs.

Due to the higher sensitivity of 'Elitest' compared to the older AGID, we've been able to reduce testing intervals from one year down to several months. This has the potential to greatly accelerate eradication; what used to take years may now be accomplished by some flocks in one year or less.

As a result of the trial, we've learned much more about how to use 'Elitest' and interpret the results. We continue to work closely with Devi and will be writing Elitest-specific guidelines for use by producers and their veterinarians. After this is published, the lab will be able to report not only positive/negative results but also the actual Elitest OD (optical density) reading for each animal tested.

3) Accuracy of the 'Elitest' ELISA

When the trial began we had no idea how fascinating this part would become. 'Elitest' arrived at the MN VDL in 2013, and at first we received results only as positive or negative. Soon afterward we learned about the OD readings, which are reported by a machine as either above or below a set cutoff (positive or negative), and about the S/N (signal/noise) ratios.

Since the cutoff varies for each 'Elitest' run, an S/N ratio is calculated for each animal by dividing its OD by the cutoff. Once the S/N ratios are known it's possible to track sequential results over time when using this highly sensitive test, and this is when the fun begins. We can now distinguish not only positive from negative, but also follow trends by tracking the S/Ns for each flock, often pinpointing when a breach of the protocol has occurred.

Occasional false positives are a feature of all sensitive ELISAs, including 'Elitest,' and there are strict protocols for retesting, including collection of new samples where necessary. The kicker with all ELISAs is that stress and hormones can skew results, as can infection with other organisms, and this may sometimes result in false positives. These factors are eliminated for human diagnostics but sheep producers would never pay the higher costs for that level of specificity.

While we're not yet sure where all of this will lead, we've learned to pay close attention to the high negatives as well as those testing just above cutoff. These cases need to be assessed carefully while taking the historical status of the entire flock into consideration. At the very least, we'll be writing a protocol to guide producers and their DVMs through the eradication process.

Other challenges:

- Can you show sheep and remain negative? One of our flocks shows heavily and we are helping them to continue this important part of their marketing without bringing new infections back to the flock
- Can a very large flock with multiple lambing groups eradicate using the protocol? Three years in, we've learned that developing a cost-effective protocol for several hundred ewes is far more difficult than dealing with <100. We're working closely with the Goergers to track not only what works but, just as important, where the pitfalls lie so that others may avoid unnecessary expense.

4) Collaborations

And finally, a brief mention of another exciting project: Late last year we shipped nearly 800 serum samples from many different flocks, including some from the eradication trial, for testing in Ireland with a new 'Multiplex' ELISA that looks for Johnes's, CLA, Chlamydia and OPP, all from a single sample. About 200 of these were tested at no charge by MV Diagnostics, the developers of 'Elitest,' and results were shared with producers. The 'Multiplex' is reported to be even more sensitive for OPP than 'Elitest' so we're hopeful that it may someday be available here.

Producers and veterinarians throughout the country are watching the trial's progress. Early results and progress has been reported in lay magazines.

We've just received a grant from *Minnesota Grown* to help finish the trial and write a report due in October 2017.

With MLWP's final donation we are planning to purchase clinical and nonclinical positive-testing older sheep to present in a necropsy demonstration at the annual MLWP Spring Workshop. This will be done to illustrate USDA researchers' findings that >90% of animals showing infection at necropsy exhibit no obvious symptoms of OPP.