OntarioSheep
FARMERS

How Maedi Visna Testing Works in the Ontario Maedi Visna Flock Status Program[1]

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Summary Points:

- The Ontario Maedi Visna Flock Status programme (OMVFSP) offers producers and their veterinarians a way to remove maedi visna virus (MVV) infection from their flock and to keep the status of the flock at low risk.
- MVV infects an animal for life.
- The virus is transmitted through respiratory droplets, colostrum, milk and less commonly in utero to the fetus, through semen and from blood.
- Most commonly, a sheep or lamb becomes infected through close contact with infected sheep, but the virus can survive on objects for up to one week.
- Despite a strong immune response on the part of the animal, it is unable to clear the virus. The response causes repeated episodes of inflammation in the target tissues (lungs and udder) which damages those tissues causing disease (hard udder and / or chronic pneumonia) by the time animals are 3 to 5 years of age.
- We detect infection through measuring antibodies that develop to different antigens on the virus; it can take from 2 weeks one year for an animal to seroconvert.
- Less commonly, we detect viral DNA using a PCR test; this can be detected in whole blood or tissues at postmortem.
- The ELISA tests used to detect these antibodies are measured using a reader that determines the optical density (OD) and results are compared to standards and then reported as negative or positive.
- Diagnostic tests are never perfect, and we measure their accuracy by determining
    - Their sensitivity (ability to identify a truly infected animal as infected) which should be very high to minimize false negative results and
    - Their specificity (ability to identify a healthy animal as not infected), which should also be very high to minimize false positive results.
    - The Hyphen Elitest used in the OMVFSP has excellent sensitivity and very good specificity.
- There are several reasons why an infected animal may test negative and a healthy animal may test positive; the OMVFSP attempts to minimizes both issues.
- A research project at the AHL, University of Guelph funded by the Ontario Animal Health Network is investigating the use of a whole blood PCR to help with cases of unexpected positive results.

[1] https://www.ontariosheep.org/maedi-visna

Introduction

Maedi visna (MV), also known in some parts of the world as **ovine progressive pneumonia or OPP**, is caused by maedi visna virus or MVV, one of the small ruminant lentivirus (SRLV) group - the other common one being caprine arthritis encephalitis virus or CAEV. SRLVs are RNA viruses which use the DNA of the host cell to replicate and produce new free virus called virions.

This virus infects a sheep for life; a sheep's immune system fights it vigorously but rarely can eliminate it. It causes marked production losses in sheep that are just reaching the age when they should be at the top of their game. The Ontario Maedi Visna Flock Status program (OMVFSP), offers producers and their veterinarians a way to remove this infection from the flock and to keep the flock at a **low risk** of becoming reinfected.

How Maedi Visna Virus Infects Sheep and Causes Disease

How the Sheep Catches the Virus:

The virus is transmitted mostly by secretions (respiratory droplets, colostrum, and milk) that contain virus-infected cells (monocytes and macrophages) or virions, to the mucous membranes (nasal, lung, oral, eye) of the susceptible animal. The virus may also cross the placenta to the unborn fetus or may be shed in the semen of infected rams and infect the ewe vaginally. Blood also contains the virus so that multiuse needles may transmit the virus. While sheep-to-sheep contact is the most common method of infection, the virus can survive on objects for up to one week depending on temperature and humidity.

How the Virus Infects the Body's Cells:

MVV targets specific types of white blood cells, i.e. macrophages and monocytes, the cell type that develops into macrophages. The virus penetrates the cell and incorporates its RNA into the cell's DNA (now called a provirus) and uses that to replicate new virions that are released to infect more monocytes. MVV prefers specific sites in the body, specifically the lungs, udder and less often the brain and joints. Monocytes are born in the bone marrow; they are infected there by MVV and so every time there is a need to fight other disease agents such as bacteria or other viruses, these infected monocytes migrate to those tissues.

How the Immune System Fights the Virus:

The sheep's immune system doesn't give up fighting the infection. Lymphocytes, another type of white blood cell, recognize that the virus has infected the sheep and attack the infected tissues. This causes an inflammatory response resulting in damage to the lungs and udder. Because the virus isn't killed, waves of inflammation occur again and again – so as the sheep ages, the damage become more severe - particularly in flocks where there are a lot of infected sheep and particularly where animals are housed in close proximity to each other. This chronic and reoccurring inflammation results in clinical signs of chronic pneumonia and / or hard udder with scant milk generally by the time the sheep is 3 to 5 years of age.

The immune response to the virus, measured by diagnostic laboratories are the antibodies produced by the lymphocytes. Much less commonly, researchers use a PCR (polymerase chain reaction) to detect the DNA

of the provirus in the macrophages and monocytes, in a blood sample or in the tissues of the animal at postmortem.

Detecting Antibodies Produced to MVV

Antibody levels are measured in the blood sample taken from the sheep. After a sheep is infected, it can take as little as two weeks to as long as one-year post-infection for antibodies to be produced at a measurable level; this is termed seroconversion. Antibodies that are absorbed by the newborn lamb through its gut from the dam's colostrum, disappear from a lamb's circulation by the time it is 3 to 4 months of age. That is why in the OMVFSP, we start to test sheep at 6 months of age, and repeat testing every 3 to 4 months until the entire adult flock tests negative.

For MVV, a good diagnostic test should detect antibodies where:

- Lots of antibodies are produced to the targeted antigen(s) on the virus so are readily detected;
- The antibodies are produced at all stages of infection from early to chronic;
- The antigen does not change or shift, i.e. is stable.

This latter part is a challenge because MVV mutates so that some antigens change over time. So, scientists select antigens located on the part of the virus that does not change. We also want to select antigens that are unique to the virus and not shared by another virus or other disease agent. This is to prevent "cross-reactions". Scientists use all those criteria to develop a diagnostic test that is reliable and highly accurate.

How an ELISA Test Works:

ELISA stands for Enzyme-Linked Immuno-Sorbent Assay (in case you wondered). It is a very common tool used to detect antibodies in a blood sample. When a test result is reported, the laboratory indicates it is either a negative or a positive test. This is determined by a machine which reads the amount of colour present in the sample. The amount of colour measured by this machine (called a reader) is directly related to the amount of antibody in the sample. The value is firstly reported as an optical density (OD) - the more colour in the sample, the higher the OD and likely the more antibody in the sample.

That OD is compared to control samples provided with the test kit - a known positive and a known negative. Those determine the cut-point that determines if the OD of the sample is classified as a negative or positive test. This cut-point may vary from day-to-day and could be affected by a variety of factors. Each unexpected reactor is retested in duplicate to make sure the result is repeatable (the sample OD comes out the same each time).

A Test is a Test and is Never Perfect

When we select a test, we want to know how close to perfect it is in detecting infection when its present in an animal (known as test sensitivity) and not calling a healthy animal infected (known as test specificity). So first about sensitivity…

TEST SENSITIVITY: Can a Blood Test Identify all the Infected Sheep as Positive?

When trying to eradicate a disease from a flock, we want a test that has as close to perfect sensitivity as is possible and a very low false negative rate, so all the infected sheep can be identified and removed. For example, if we had a flock of 200 sheep that were all infected, a good test with a sensitivity of 99.5% would

correctly identify 199 of them but would miss one infected animal. So even with a good test, we can still have the odd escape. The goal is to have as few false negatives as possible. MVV is very infectious, so even missing one sheep can lead to return of a high prevalence of infection in the flock in a few years.

What things may make an infected sheep test negative, even with a perfect test?

- Infection is recent, the animal has not yet seroconverted. Unfortunately, with MVV, sometimes seroconversion can take several months up to one year;
- The animal's immune system is stressed with other illness or poor nutrition and so is unable to properly develop antibodies to the MV virus;
- A ewe in late pregnancy to early lactation may be temporarily negative to MV virus when directing large quantities of her antibodies to the colostrum. The period around lambing is also a high stress time for her and her immune system is "downregulated". This is the same phenomenon that causes parasite egg shedding to rise around lambing.
    - For this reason, we strongly recommend not testing ewes 4 weeks before or 4 weeks after lambing.

The test used by the OMVFSP at the Animal Health Laboratory, University of Guelph (HYPHEN Elitest ELISA) has excellent sensitivity. Research we did a few years ago demonstrated the Elitest compared very well to the test we had used previously (The CFIA ELISA), with a comparative sensitivity of 99.3%. So that is good news!

TEST SPECIFICITY: Can a Blood Test Correctly Identify an Uninfected Flock?

In a disease status program such as the OMVFSP, it is important that if the virus is truly not present in a flock, that no sheep test positive. For example, a test with almost perfect specificity of 99.5%, if used in a flock of 200 where all the sheep are healthy, would call 199 not infected and 1 as infected. This is a false positive. Frustratingly, when we use a test with high sensitivity, we rarely also get high specificity. The HYPHEN Elitest ELISA has an estimated specificity of ~ 95%. OK but not great.

What things may make a healthy sheep test positive, even with a perfect test?

- Vaccination with any vaccine or an unrelated illness has been suspected as potentially causing false positives. When an animal is sick or challenged with another disease agent, the immune system will create antibodies that may not be specific to the disease agent – sort of like a buckshot response. These non-specific antibodies may give a false reaction to the test.
- For this reason, we strongly recommend waiting at least 3 weeks after vaccinating before testing, although vaccination can be done on the same day as sampling.
- If an animal that tests positive in a flock that is believed to be negative, the follow-up test may show the OD has dropped below the cut-point and the animal is now classified as negative.

Why Would an Animal Change from Being Positive to Negative

This is one of the most frustrating issues with disease testing and it common in all disease testing programs. It may be that the original test was wrong because the animal was reacting to another infection as outline

above and not MV virus. It may be that the follow-up test is wrong, the animal is truly infected but for a reason is no longer producing enough antibodies (pregnancy, illness, nutritional stresses).

How Can We Confirm that the ELISA Test Result is Correct?

The AHL, University of Guelph with the support of the Ontario Animal Health Network, is developing a different confirmatory test to help figure out cases when a flock has unexpected positive results. The best way to "prove" that a flock is infected is to identify the presence of the virus in one or more animals. This is usually done by performing a PCR on a whole blood sample. If the virus is detected in even one of the samples, then the virus is circulating in the flock and the ELISA tests are correct. As part of the research project, the Animal Health Laboratory has sequenced some of the viruses detected with the PCR to make sure that the virus is really the MV virus and not a related but different virus. This research is ongoing but early work is promising. The drawback to PCR testing is that it is more expensive than serology and so is usually only used in cases when the ELISA results for a flock are unexpected. Keep tuned for more information as test development continues.

The OMVFSP committee is continuously working to improve the programme including updating protocols and working with the Animal Health Laboratory, University of Guelph to refine diagnostic tools.