TB Testing – What it all means – One person's view.

When the CFIA come to test your cattle for TB, the tests they will use can be divided into 2 categories:

1) Tests which look for evidence that your cattle either have active TB, or have been exposed to TB in the past, and
2) Tests that look for the presence of an active TB bacteria.

Category 1 tests are looking for specific immune system responses (the animal's response to the presence of TB), while category 2 tests are looking for living TB bacteria (*Mycobacterium bovis*).

Category 1: Immune system response tests.

Let us use the common cold as an example of how your body has an immune system response. When you get a cold, your body's Primary Immune Response is to make chemicals called antibodies which circulate in your blood and kill the specific cold virus which has infected you. Because this Primary Immune Response is slow in developing (7 to 14 days from first infection), you suffer from cold symptoms for a few days before your antibodies can wipe out the infection. But a very important side benefit is that this Primary Immune Response also generates a Memory within your immune system for this specific infection. Should you again become infected with this specific cold virus, this Memory triggers a Secondary Immune Response, which is much faster to develop and much stronger than the Primary Response. The upshot is that the Secondary Response kills off the cold virus usually before you even feel symptoms (2 to 5 days from first infection), as long as it is exactly the same cold virus that attacks you a second time (the Secondary Response is only generated for a specific virus, and since the cold virus exists in a large number of varieties, and mutate to form other varieties we continue to suffer from colds).

This long-term protection which develops after exposure to a pathogen was recognized 2400 years ago by Thucydides of Athens, who described how those sick and dying of the plague were cared for by others who had recovered, "for no one was ever attacked a second time."

The slate of Immune System Response tests for Tuberculosis are all based on determining whether your cattle demonstrate this rapid and strong Secondary Immune Response – in other words, whether their immune system contains the Memory of a current or past TB infection. These tests include:

1) Caudal Fold or Tail Test:

   This is the initial test the CFIA do on a cattle herd. A very small amount (1/10 cc) of Tuberculin (a protein extract obtained from Bovine TB cultures, which can trigger a Secondary Immune Response, but **cannot infect** an animal with TB) is injected into

the caudal fold at the base of the tail. The injection site is examined 3 days later, and a Secondary Immune Response is indicated if a large amount of swelling has occurred. But this response can come from exposure to any of the bacteria in the TB family (in 2002/3, during the testing of about 50,000 cattle around the Park, there were 1100 positive reactions to the tail test). In such animals, a second test (the neck test) is done to determine if this Secondary Immune Response is due to the more commonly-occurring Avian TB.

2) The Neck Test:

An area on the neck is shaved, and Tuberculin derived from cultures of both Avian TB and Bovine TB (the same Tuberculin that was used for the tail test) are injected side-by-side. The injection sites are examined 3 days later, and if the Avian site is more swollen and inflamed than the Bovine site, it is concluded that the tail test reaction was due to Avian TB, and that the animal is negative for Bovine TB. However, if the Bovine site is more swollen and inflamed than the Avian site, the herd is quarantined and the animal is slaughtered and further testing is done, looking for signs of active TB.

3) The Bovigam Test:

The acquisition of Memory by the Immune System following a TB infection occurs in 2 forms. The first is in B (Bone Marrow) cells, which produce specific anti-disease antibodies, is tested for using the above-described Tail and Neck Tests.

The second form of Immune System response is found in the T (Thymus) cells, which produce Interferon, a chemical which "interferes" with the spread of disease within an animal. The T Cells also acquires Memory for an infection, and the presence of this form of Memory indicates that the animal has been exposed to bovine TB in the past, and in turn may have an active case of TB. To measure the presence of T cell memory, a blood sample is taken, and the T cells in it are stimulated with a Bovine TB derivative. If the T cells have a Memory for TB, they will release Interferon. The amount of Interferon released is measured and determines a positive result.

The Cervigam Test, which can be used on deer and elk, operates in a similar fashion. The Bovigam and Cervigam tests have performed well in Australia and New Zealand. The Bovigam was first used in the RMEA in the fall of 2003, and produced an unexpectedly high number of positive results. Research on the effectiveness of this test in our area is continuing.

Category 2: Tests for Active TB:

1) Necropsy:

Animals coming up positive on the Neck Test are slaughtered, and a full necropsy by a qualified pathologist is performed. Visible suspicious signs of TB infection include

lumps or lesions in the lungs, other organs, and the body cavity, and swelling and inflammation of the tonsils and lymph nodes. The purpose of the Lymphatic system is to collect the fluids which bathe and nourish the cells of the body, and to return them to the bloodstream. The Lymph Nodes are small filters, concentrated in the neck and chest, but also found throughout the body, which collect debris and disease-causing organisms from these fluids. They are often the site of the battles between diseases and the body's immune system. Because of their role in filtering out disease, they are natural sampling sites for further tests for the presence of TB. Both the Lymph Nodes and Tonsils are collected for further testing, which is done only at the CFIA Laboratory in Nepean, Ontario.

2) Acid-Fast Test:

Part of the Lymph Node sample obtained during Necropsy is preserved and exposed to a stain that can only be absorbed by bacteria from the TB family. The sample is examined by a pathologist using a microscope, who determines the presence/absence of stained bacteria. Since several Lymph Nodes are usually examined, and since several sites on each Lymph Node are checked, the Acid Fast Test can take several hours to come up with a negative result. A combination of a positive Bovigam, lesions observed during Necropsy, and a positive Acid Fast Test will lead to a whole herd quarantine. Producer samples in for Acid-Fast testing, take precedence over wildlife samples at the Nepean Lab.

3) PCR (Polymerase Chain Reaction) Test:

Should stained bacteria be found during the Acid Fast Test, DNA is withdrawn from these bacteria, and multiplied using the PCR method in order to get enough DNA material to test. The DNA is then tested to determine if the bacteria is from Bovine TB or another type of TB. However, positive PCR results, although thought to be a reliable indicator of Bovine TB, will not, by themselves, lead to herd eradication.

4) Culture Test:

The second part of the Lymph Node sample obtained during Necropsy arrives at the Nepean Laboratory in fresh, living condition. The sample is prepared, introduced into a nutritive culture medium, and incubated. Because the Bovine TB bacterium is of weak constitution, and grows very slowly, 3 months are required before a negative or positive test result can be determined. Should apparent TB bacteria appear to be growing in culture after, perhaps, 6 weeks, a PCR Test can be performed on part of this culture to determine if it is Bovine TB.

The culture test is currently the gold standard in TB testing, and is the only way a positive animal can be conclusively identified. Herd eradications can only occur after a positive culture result.