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New technique joins the fight against footrot

By Laurie Depiazzi1, Research Officer, Bunbury, Mike Palmer1, Microbiologist and Dave Pitman1, Medical Technologist, Albany

The diagnosis of footrot in sheep and goats is not an easy task.

Two main techniques are used for diagnosis - inspection of diseased feet on a farm and laboratory testing of bacteria isolated from foot scrapings.

The interpretation of the results obtained by these methods requires a good understanding of the various forms of footrot.

On-farm inspection and laboratory testing will allow a diagnosis when interpreted in the correct manner. Diagnosis is crucial because we want to eradicate virulent footrot from Western Australia.

A new laboratory technique has halved the time taken to detect those strains of the bacterium, Bacteroides (Dichelobacter) nodosus, that cause each form of the disease. The new technique combines rapid growth of B. nodosus in the laboratory with increased efficiency in the enzyme (protease) stability test to differentiate the strains.

Forms of footrot

There are essentially only two forms of footrot, virulent and benign. The benign form is often called 'footscald' in Western Australia, but since the term 'footscald' means something else in other states of Australia, benign footrot is the preferred name.

Benign footrot is a mild disease of no economic importance. Virulent footrot is generally a more severe disease and can produce pain, suffering and economic loss. The milder form of virulent footrot is sometimes called 'intermediate' footrot.

The lesions of footrot are determined by environment, breed of sheep, and the bacterium responsible for the disease. Laboratory tests give information about the bacterial factors only. Consequently, the laboratory result may not always reflect what is seen in the field. Some animals, for example, may carry the footrot bacterium without showing obvious signs.

A laboratory test is often the only way to differentiate between the early stages of virulent footrot and well developed benign footrot.

1 Division of Animal Health

1 This foot shows mild changes consistent with either benign footrot (protease unstable) or early virulent footrot (protease stable).

The laboratory test can help distinguish which form of footrot is causing the changes.

Virulent footrot. The forceps are peeling back a section of degenerated tissue underrunning the sole of the hoof.
About the bacterium

The bacterium is an anaerobe, which means it will die if exposed to oxygen in the air, but several conditions help it to survive.

Footrot lesions contain at least 10 other species of bacteria, some of which soak up oxygen. B. nodosus associates with these bacteria, thereby reducing this toxic threat to its survival.

Bacteroides nodosus also needs moisture to survive, and warmth to thrive and spread. This is why the disease is mostly seen in spring, when the weather is warm and paddocks are moist.

As paddocks dry out, the hoof horn may quickly grow over the lesion, forming a protective pocket that excludes air. This pocket of infection lies hidden in the hoof over summer. It is only when the hoof is pared, that the disease can be seen.

It is also possible for B. nodosus to survive over the hot dry summer when moisture exudes from the skin between the toes. This moisture stimulates the oxygen-using organisms, thus protecting the footrot bacterium. Unlike hidden pockets of infection, this form of the disease can be detected without paring.

Detecting the difference

The different strains of B. nodosus can be distinguished in the laboratory by tests that identify protein-digesting enzymes (proteases) produced by the bacteria. The nature of these enzymes is related to the virulence of each strain of B. nodosus.

Strains that cause benign footrot produce enzymes that are unstable (lose their activity) under controlled laboratory conditions. Strains that cause virulent footrot produce stable enzymes under the same conditions (even strains that cause ‘intermediate’ footrot).

By identifying the enzymes associated with each strain it is therefore possible to differentiate benign footrot and early virulent footrot - a task impossible by simply inspecting the foot lesion.

Virulence also influences the survival of B. nodosus and the spread of footrot in animals.

Highly virulent strains, for example, cause under-running (separation of the skin and horn between the toes) and deep lesions which may develop into protected pockets of infection in the hoof during summer. The feet must be heavily pared to locate and expose these infections to prevent the bacteria from surviving over summer. These strains also spread rapidly between sheep.

Diagnosis on the farm

The signs of virulent footrot include lameness and loss of body condition. Close examination of affected feet will show damage which can vary from redness, hair loss, moisture and minor tissue decay of the skin between the toes, to separation of the skin and horn between the toes with extensive decay of the sole of the hoof.

Severe footrot lesions, with their characteristic putrid odour, confirm that the disease is indeed virulent (not benign) footrot.

The difficulty of identification arises because, in the early stages of development, the lesion is confined to the skin between the toes in both virulent and benign footrot. In these cases a sample of the creamy, soft, decaying tissue between the toes is collected and sent to the laboratory for isolation of B. nodosus and testing for enzyme stability. The laboratory test may thus differentiate the two diseases.

Without a confirmed test result, diagnosis must then be based on clinical signs of the disease.

The usual procedure is to wait for some weeks to see if the lesions become more severe. The problem with this method is that the environmental conditions may be unfavourable for further development of the lesion and therefore may camouflage an otherwise virulent form of footrot.
Increased moisture and wrinkling of the soft horn on the inside of each claw (toe) is evidence of virulent footrot. This stage is sometimes called intermediate footrot. The photo shows the space between two toes.

**Laboratory tests for virulence**

Foot scrapings are sent to the laboratory in special (oxygen-reduced) transport media and *B. nodosus* is grown and isolated in airtight (anaerobic) containers. The protease stability test is then conducted to confirm the presence of virulent (stable protease) or benign (unstable protease) strains of *B. nodosus*.

Each test takes about two weeks to complete. The proteases produced by *B. nodosus* actually help to break down the, largely protein, material that makes up the hoof.

The Footrot Reference Laboratory at the Department of Agriculture in Albany has tested more than 3,000 samples of *B. nodosus* from sheep, cattle and goats for protease stability since the early 1980s. The proteases from all samples tested were clearly differentiated as either stable or unstable (Table 1).

### Table 1. Some differences between virulent and benign footrot lesions

<table>
<thead>
<tr>
<th>Foot lesions</th>
<th>Virulent</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over summer survival</td>
<td>mild to severe</td>
<td>mild</td>
</tr>
<tr>
<td>Protease type</td>
<td>good</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>stable</td>
<td>unstable</td>
</tr>
</tbody>
</table>

**Interpretation of laboratory test results**

The overall severity of a footrot outbreak is influenced by several factors, including the proportion of bacteria that are highly virulent. Both protease-stable and protease-unstable strains of *B. nodosus* have been identified in single flocks, in the same animal and even the same foot.

However, it is important to remember that the presence of a stable-protease strain of *B. nodosus* (regardless of the company it keeps) means the potential for virulent footrot is also present. It is these strains that must be eradicated.

If footrot has been present for some time in a flock, there will probably be a high proportion of highly virulent strains of *B. nodosus*. These bacteria survive in hidden pockets of infection in the hoof during dry summer conditions, but the feet may not show obvious signs of footrot. These animals are termed ‘carriers’ and act as a source of infection in the following spring when environmental conditions favour the development of lesions.

**Why we test for stable and unstable strains**

Detection and elimination of the stable-protease strains of *B. nodosus* is important, particularly towards the end of a footrot eradication programme. This is because:

- The stable-protease strains of *B. nodosus* are the more virulent ones; they cause more severe outbreaks and they survive longer than unstable strains.
- The progress of eradication can be monitored by finding out the prevalence of the stable strain on farms.
- The protease stability test is the best test available to differentiate virulent and benign footrot.

**DNA-fingerprinting - a new technology**

DNA-fingerprinting is a technique where the genetic material of a cell (or bacterium) is examined and identified. It is called fingerprinting because it can identify highly specific features of individual bacteria. This very accurate technique is being developed to study the ecology of *B. nodosus*. Information gained will be used in tracing infections, studying the pattern of disease development and geographical spread.

DNA-fingerprinting may also help differentiate new outbreaks of footrot from those that have occurred previously. The habitat of *B. nodosus* may also be investigated using this method. Improved knowledge of the environmental requirements of *B. nodosus* will make it easier to progressively eradicate virulent strains from Western Australia.