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Virulent footrot : mild or severe?

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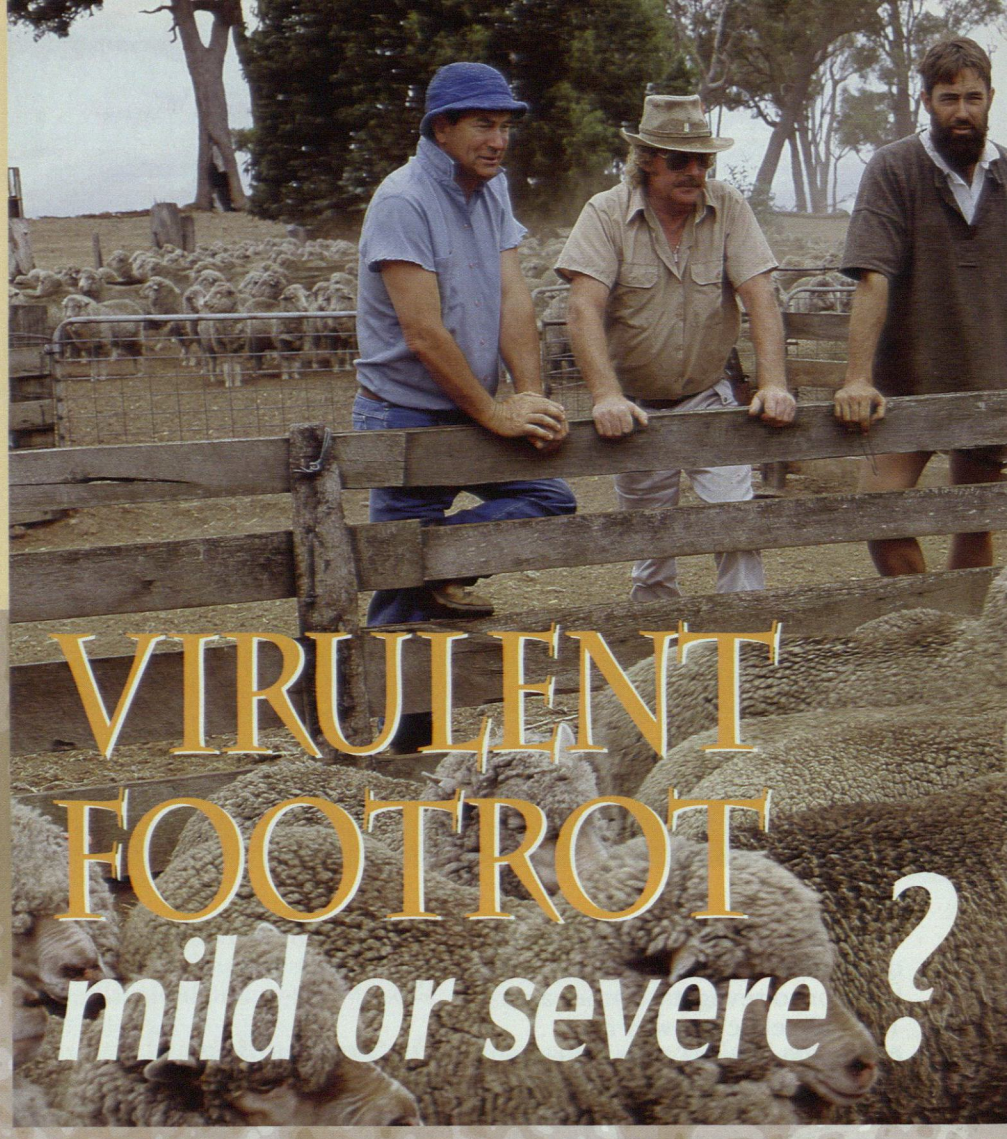


VIRULENT FOOTROT mild or severe?

The footrot eradication scheme in Western Australia has resulted in a low prevalence of severe footrot. To appreciate the achievement, we need to understand what exactly is being eradicated. 'S' strains of Dichelobacter nodosus, *the infectious bacterium of footrot, are the target of footrot eradication. Laurie Depiazzi examines the basis for eradicating 'S' strains.*

Carrier animals

When the eradication scheme began in 1949, footrot was one of the most serious diseases facing the sheep industry. With the scheme well under-way, footrot outbreaks flared up in areas of the south-west previously considered safe from the disease. Carrier animals, that carry the footrot bacterium but show little or no signs of footrot, were held responsible for these and similar outbreaks. Recent research has confirmed that mild symptoms on one farm may flare up into severe footrot when carriers are relocated.

If potentially severe footrot can be carried in mild forms, how is it possible to selectively eradicate the severe, or virulent forms of footrot? The question is important in economic evaluations because lasting benefits of eradication will only come by counteracting the carrier threat.

To detect potential carriers of footrot, we use a test that quantifies virulence independently of clinical observation. However, in developing footrot tests, severity of symptoms and potency of *D. nodosus* must both be quantified to establish a correlation.

Quantifying footrot virulence

The symptoms of footrot are quantified by:

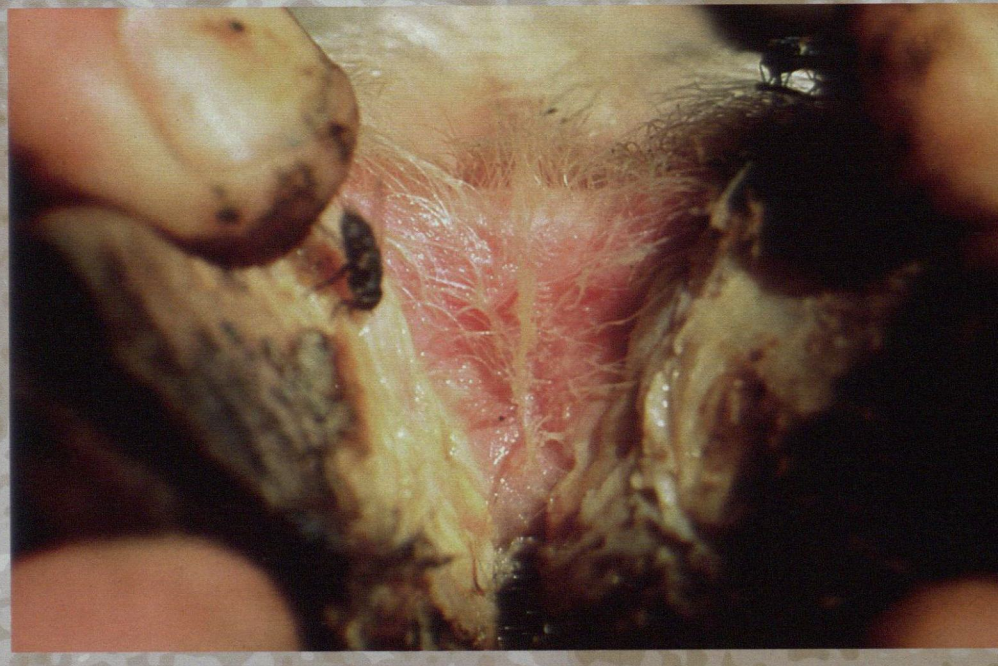
- grading the severity of lesions from 0 to 7. Lesions greater than score 2, in which the horn detaches from the foot to varying degrees, are more likely to lower productivity;
- measuring the spread of disease by recording the percentage of feet with lesions; and
- recording occurrences, or chronicity, of lesions. Generally, the more virulent the disease, the more chronic are the symptoms.

Under favourable conditions virulent footrot is associated with lesions greater than score 2 in a significant number of sheep. Benign footrot is characterised by lesion scores of 1, 2, and 3a and may show a high prevalence in sheep flocks in some environments.

Carriers of virulent footrot often show lesion scores of 0 or 1. When the feet are pared, pockets of infection are sometimes found that vary from obvious rotting

Score 0 lesion.

Score 1 lesion.



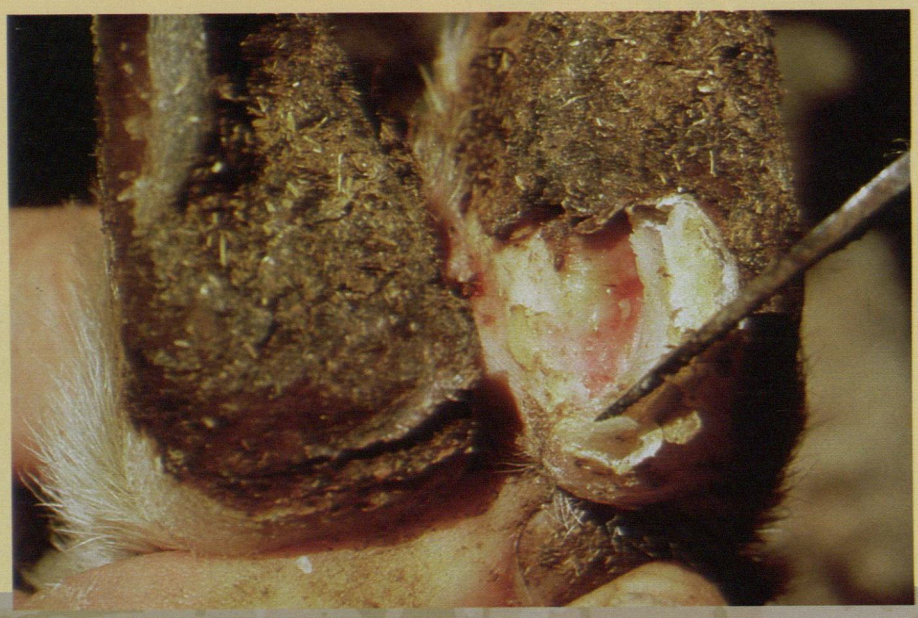
Score 2 lesion.

enclosures to barely visible foci of infection. The disease usually becomes active and easily detectable when the environment becomes warm and wet, particularly where there is lush pasture.

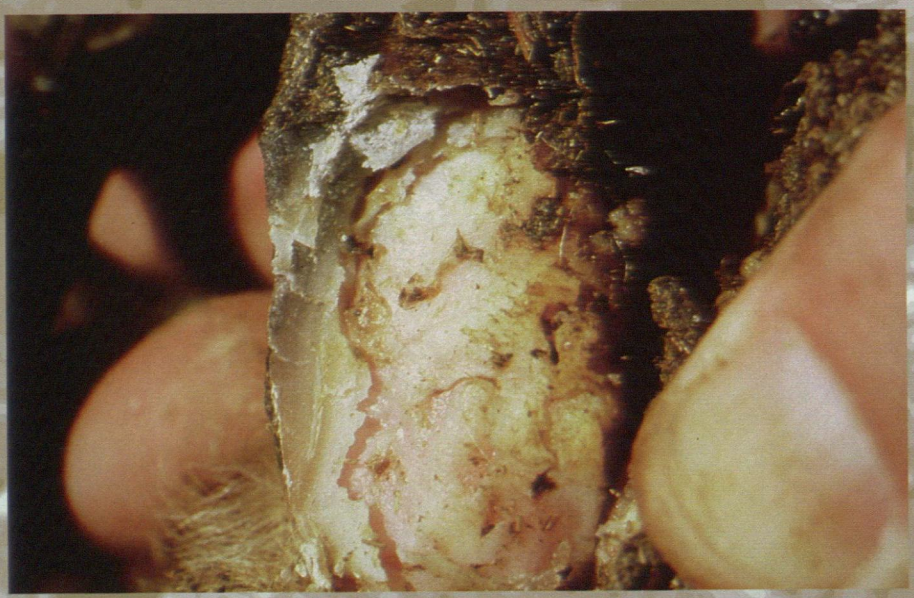
Because footrot symptoms are not always a reliable indicator of the potential for virulent footrot, recent policy in Western Australia has been to eradicate virulent strains of *D. nodosus*, after they have been identified by laboratory tests. How accurate are these tests?

Laboratory tests

Laboratory tests for footrot are tests for bacterial virulence. The characteristics of bacteria that relate to severity of disease are called virulence markers. In *D. nodosus*, different virulence markers combine to produce the overall virulence level. For more than a decade in Western Australia, two virulence markers have been used in the laboratory diagnosis of footrot: protease stability (also called the gelatin gel test) and protease zymogram.



Score 3 lesion.



Score 4 lesion.

Table 1. Percentage distribution of lesions in five sheep flocks at two sites.

	Mount Barker					Wokalup				
	Severe benign	Mild virulent	Farm			Severe benign	Mild virulent	Farm		
Lesion score	U305	S2127	A	B	C	U305	S2127	A	B	C
1	65	47	33	30	77	28	28	29	42	54
2	32	43	59	58	20	53	45	51	50	41
3a	3	6	6	10	2	16	16	15	6	5
>3a	0	4	2	2	1	3	11	5	2	0
Lesions %*	44	26	48	66	24	73	58	71	60	45
Observations**	688	728	720	732	536	760	760	760	756	576

Data were recorded from October to December.
* Feet with lesions as percentage of total.
** Total number of observations.

Table 2. Lesion scores in 3 groups of sheep infected with strain S2127 at Mount Barker.

	1991	1992	1993
Low	6	26	27
Medium	29	32	31
High	65	42	42

Data were recorded from October to December each year. Numbers refer to percentage of lesions greater than score 1.

Protease is an enzyme produced by *D. nodosus* that helps break down the structure of epidermal tissue, causing rotting of interdigital skin and hoof horn. The protease itself gradually loses its activity at warm temperatures and in solutions deficient in metal ions, particularly calcium. *D. nodosus* has two major sub-populations, those producing relatively stable (S strain) and unstable (U strain) protease. Rarely, aberrant strains that do not fit this dichotomy are found, but these are readily identified by the zymogram test.

Protease stability is the best currently available test for diagnosing virulent footrot. Repeated studies have shown that all highly virulent strains of *D. nodosus* have stable protease whereas protease unstable strains have limited virulence. This is not always obvious in the field because environmental, bacterial and host resistance factors combine to influence the severity of symptoms.

In particular, some S strains lack additional virulence markers resulting in reduced potency. After decades of footrot eradication, the proportion of these mild strains is exceptionally high in Western Australia. The limitation of the protease stability test stems from the fact that it does not detect these additional markers, some of which are as yet unknown. Extensive research has been carried out to identify other markers, without much success.

S and U proteases have several molecular components called isoenzymes. Isoenzyme markers are analysed in the zymogram test, which, along with protease stability, is carried out on all *D. nodosus* strains cultured from animals in Western Australia.

Currently, S strains have three and U strains have six zymogram types. Zymogram typing has provided insights into epidemiology and the problem of footrot carriers, but generally gives the same information on virulence as protease stability.

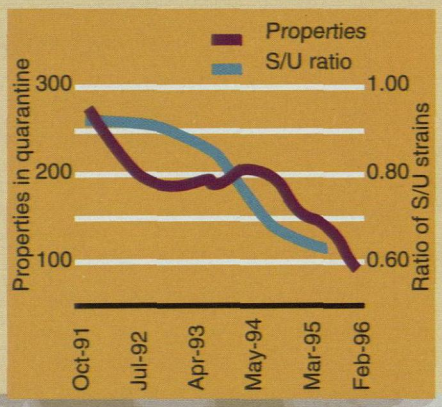


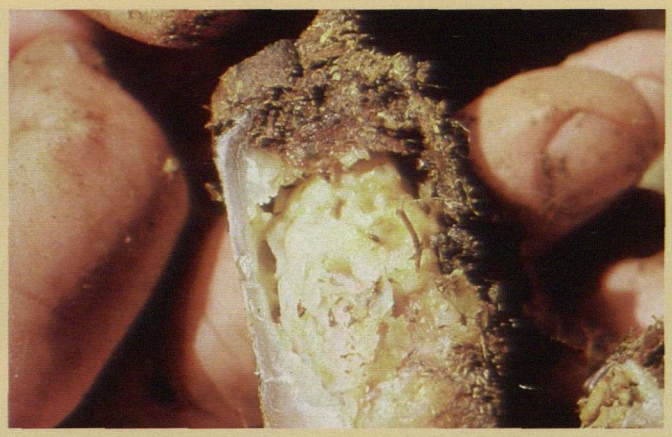
Figure 1. Decline of the S strain of D. nodosus *means lower risk of virulent footrot shown by properties in quarantine and ratio of S and U strains.*

Surface translocation, or the ability to move across a solid surface, is another marker of *D. nodosus*. Virulent strains are more mobile than benign strains. Unfortunately, it is not easy to apply this marker in routine virulence tests. Eventually, DNA tests may detect translocation and other markers, making footrot diagnosis more accurate.

Interpretation

Markers other than protease stability and zymogram may influence the interpretation of laboratory tests. Some S strains may be deficient in markers other than protease, so their overall virulence is relatively mild. Alternatively, some U strains may possess additional markers, making them unusually virulent. Consequently, there may be overlap in the virulence of a few S and U strains.

S and U strains suspected of being in this overlapping range were compared in trials duplicated at Mount Barker and Wokalup Research Stations. Footrot affected sheep were obtained from five sources including farms A and B that had acquired S strains by natural infection. A flock artificially infected with a relatively aggressive U strain (U305) and a flock with a mild S strain (S2127) were used as controls. Sheep from farm C were found to have self cured and were re-infected, after a delay, with an S strain previously isolated from the same farm. The data for farm C are shown but were not included in the comparative analyses.



Score 5 lesion.

At Mount Barker, sheep associated with farms A and B and strain S2127 had more severe footrot than sheep with strain U305. However, at Wokalup, where sheep grazed on irrigated pasture, the severity of S and U strains overlapped. Also, the severity of footrot was higher at Wokalup than at Mount Barker only for artificially infected controls (Table 1).

The trial confirmed that when virulence levels of S and U strains are close, the effects of environment, previous exposure and host resistance may over-ride the relative virulence of each strain.

The effect of environment on footrot was researched in a detailed study from 1991 to 1994. This research also showed how host resistance, or previous footrot exposure, affected the interpretation of virulence levels.

In 1991, for example, sheep at Mount Barker were classified by tagging into low, medium and high virulence groups two months after inoculation with strain S2127. The virulence scores in the high and low groups were different in the next two years, probably resulting from some immunity acquired during initial exposure (Table 2).

The virulence scores of the three groups also had the same ranking over three years, even though all sheep grazed in the same flock. Between groups there were obviously different levels of host resistance to footrot. If the histories of the sheep were unknown, it could be wrongfully concluded that strain S2127 had three levels of potency.

Why eradicate S strains?

There are several reasons for eradicating S strains even though all virulence markers of *D. nodosus* have yet to be identified:

1. The most potent strains of *D. nodosus* have the S marker. Eradicating the S strain most effectively reduces the overall virulence of *D. nodosus* in the field.
2. Some virulence markers may be transferred genetically between strains. Eradicating the S strain excludes the possibility of mild S strains becoming highly virulent by acquiring additional markers through genetic transfer.
3. The remaining U strains are a new benchmark for assessing low virulent strains. For example, some rare U5 strains, while not highly virulent, are more potent than typical U strains. This strain is detected by the zymogram test and could yield significant information on additional virulence markers.

Can S strains be eradicated?

The number of W.A. sheep farms quarantined for footrot has been decreasing since 1991. The ratio of S to U strains has also decreased, showing that specific strains can be successfully targeted (figure 1). Active surveillance and tracebacks have ensured the detection of all forms of virulent footrot. The mild form, similar to that produced by strain S2127, is now predominant in Western Australia and can be eradicated from farms.

Acknowledgements

Woolgrowers who contributed sheep for trials are gratefully acknowledged. Without the work of field officers and colleagues in the footrot research group this work could not have been completed.