

***The Cicerone Project Inc.***

**Results**

of the

**FOOTROT TRIAL**

**Funded by**

**Producer Initiated Research  
and Development**

and

**WoolMark**

# THE CICERONE PROJECT'S FOOTROT TRIAL

Funded by PIRD and WoolMark

To study some strains of footrot which are giving stable gel test results but which act as benign in the field (Phase 1, Uralla)

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## SUMMARY, Phase 1

The Cicerone Project developed a trial to compare field expression of various gel stable strains of footrot under similar conditions during the period when footrot expression was likely to occur under New England conditions. The same strains were compared using DNA analysis by Dr Brian Cheetham of the Department of Molecular and Cellular Biology at the University of New England (UNE). The trial was conducted for 20 weeks at the Cicerone Farm near Uralla on the NSW Northern Tablelands.

### Field results indicate:

- Sheep in a paddock with a higher clover content are more susceptible to footrot than those in a lower clover paddock. This occurred across all the strains under test.
- Over the time sheep with field high- grade virulent, gel test stable (ie virulent) [white ear tags] became progressively worse to score 5 and infected clean sheep in the plot.
- Sheep with field low- grade virulence: gel test stable (ie virulent) [green ear tags] became worse but not as bad as the above group, indicating that there is a graduated scale of virulence
- Sheep with field benign; gel test unstable (benign) [red ear tags] did not develop worse symptoms of the disease, they improved and did not infect clean sheep.
- Sheep with a type of field benign: gel test stable (ie virulent)[blue eartags] did not show any indication of virulence in the field, they did not affect clean sheep and the foot scores remained on average below 3a. This indicates the field behaviour is not acting in the same way as we'd expect from the gel test result.
- The sheep [purple ear tags] which were field low grade virulence at home and gel test stable also behaved in the same way as the field benign sheep when brought to the Cicerone plots
- Sheep which had been field benign; gel test stable up to the trial [pink eartags] tested gel test unstable for the first time and behaved in the same way as the benign in the trial. Stable strains were indicated at the end of the trial.

Thus we have shown that in this area, some gel stable strains act in a benign manner in the field.

**DNA fingerprinting** The bacterial strains which acted as virulent in the field showed 'bands' in the DNA test and those strains which acted as benign in the field were 'non-banded' in the DNA test. This suggests that in the future it may be possible to develop a test based on DNA which is more accurate than the current elastase and gelatin gel tests, both of which look at just proteases. There are additional factors at work, not just the protease, which influence virulence or non- virulence being expressed.

## Introduction

Armidale Pastures Protection Board (now Rural Land Protection Board [RLPB]) was a declared Footrot Protected Area in 1969. This Protected Area was extended to cover the majority of the New England Tablelands with the introduction of the NSW Footrot Strategic Plan in 1988. This Strategic Plan was introduced to eradicate virulent footrot as opposed to benign footrot, the former being a quarantinable disease. In Western Australia the gelatin gel test is used to determine a cut off point for decisions of virulence, with 'stable' isolates indicating 'virulence' and 'unstable' isolates indicating 'benign' strains of the disease. However in NSW the use of the "laboratory test must not be used on its own to establish a diagnosis of virulence in a flock of sheep." A recent footrot survey in New England, organised by NSW Agriculture and carried out by an independent contractor, served as a catalyst in reviewing this situation.

Following a number of district meetings it was apparent that footrot was looming as a major issue for local graziers. In July 1998 a meeting held by The Cicerone Project was attended by over 100 wool producers. At meetings held in January 1999 in Armidale, Glen Innes, Guyra and Walcha there was obvious anger, heartache and frustration shown by local producers as they tried to come to terms with recognising and treating footrot and the quarantine restrictions imposed on them. Many stories related how the disease was causing no ill thrift in the sheep, production remained good and the foot problems seemed to come and go without treatment. This was all anecdotal but obviously was believed by many producers who were therefore very suspicious of the results of the footrot survey.

The Cicerone Project developed a trial to compare field expression of various gel stable strains of footrot under similar conditions during the period when footrot expression was likely to occur under New England conditions. The same strains were compared using DNA analysis by Dr Brian Cheetham of the Department of Molecular and Cellular Biology at the University of New England (UNE).

## METHOD

Fourteen (14) one hectare plots previously used in pasture trials were slashed, seeded and fertilised in the early spring of 1999. The plots were assessed for clover content and the plots were then paired to contain one with a higher and one with a lower clover content.

Sheep infected with different strains of *Dichelobacter nodosus* came from seven district properties. They carried the following strains of footrot:

The three controls:

- Field high grade virulent: lab stable (ie virulent) *Elastase* + at 7 days      White eartags
- Field benign: lab unstable (ie benign)      Red eartags
- Clean control sheep with no footrot      No eartags

The four problem flocks:

- Field low grade virulent : lab stable (ie virulent) *Elastase* + at 10 days      Green eartags
- Field benign: lab stable (ie virulent) *Elastase* + at 7 - 10 days      Purple eartags
- Field benign: lab stable (ie virulent) *Elastase* + at 7-10 days      Blue eartags
- Field benign: lab unstable (ie benign) *No elastase*      Pink eartags  
*(this result for the first time, previous tests had all been + and is now confirmed as containing stable isolates))*

At the beginning of November 1999, four or five infected sheep were placed in each plot, the strains being kept separate, for example the green eartags were placed in plots 3 and 7, the white eartags in plots 6 and 11. Thus each strain was placed in both a plot of higher clover and one of lower clover content.

Five clean sheep were also placed in each plot to see if clean sheep became infected. These sheep were all 4 tooth wethers from the same bloodline.

Thus each plot had a total of nine or ten sheep.

Every sheep had each claw of each hoof scored for footrot by RLPB inspectors every week for 20 weeks commencing 1 November 1999.

Care was taken to ensure there was no cross contamination between plots. Each person who went into the plots wore "Gubba" rubber over-boots and went via a footbath of Hibitane or Stericide into and out of the plot. Even the dog went through the footbath on his way in and out of the plots. The inspectors washed their hands and foot clippers in the disinfectant between each plot.

Sheep from all farms were inspected and 10 swabs collected prior to the trial. These were sent to NSW Agriculture Regional Veterinary Laboratory, Orange where *Dichelobacter nodosus* was isolated and cultures forwarded to UNE for DNA analysis.

After 20 weeks swabs were taken again and sent for analysis to see if the strains had changed at all. In addition a blood sample from each sheep was collected at the request of John Seaman, Program Leader (Flock Health) NSW Agriculture, for the possible development of an anamnestic test by NSW Agriculture.

## FIELD RESULTS

The weekly foot scores have been placed on computer. These results have then been analysed into graphs. As computers cannot cope with the scores 3A, 3B and 3C, the scoring system found on the graphs is:-

Footrot score 1	=	Graph score 1
Footrot score 2	=	Graph score 2
Footrot score 3A	=	Graph score 3
Footrot score 3B	=	Graph score 4
Footrot score 3C	=	Graph score 5
Footrot score 4	=	Graph score 6
Footrot score 5	=	Graph score 7
Removed from trial on welfare grounds	=	Graph score 8

The graphs indicate the field results for each strain of footrot.

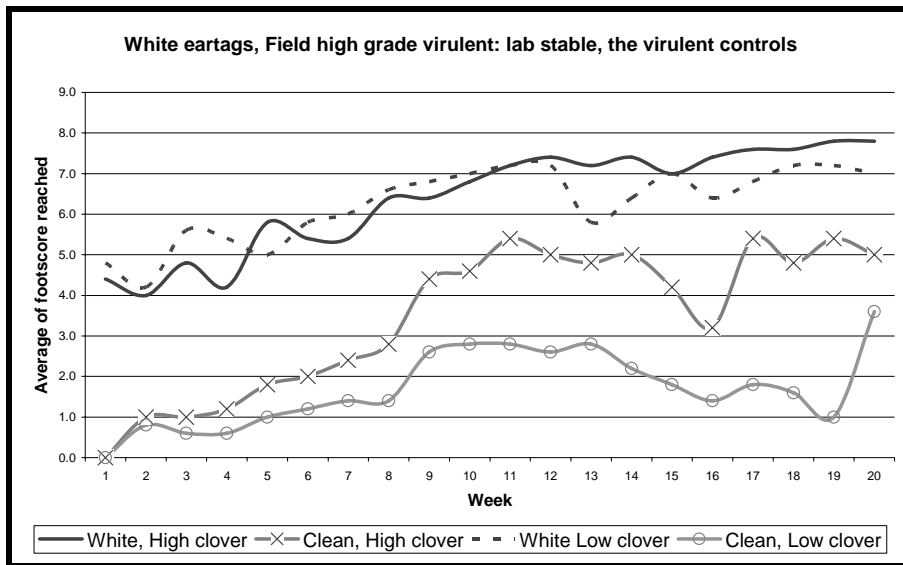
## RESULTS

# 1. The effect of clover content in the plot on the various strains of footrot.

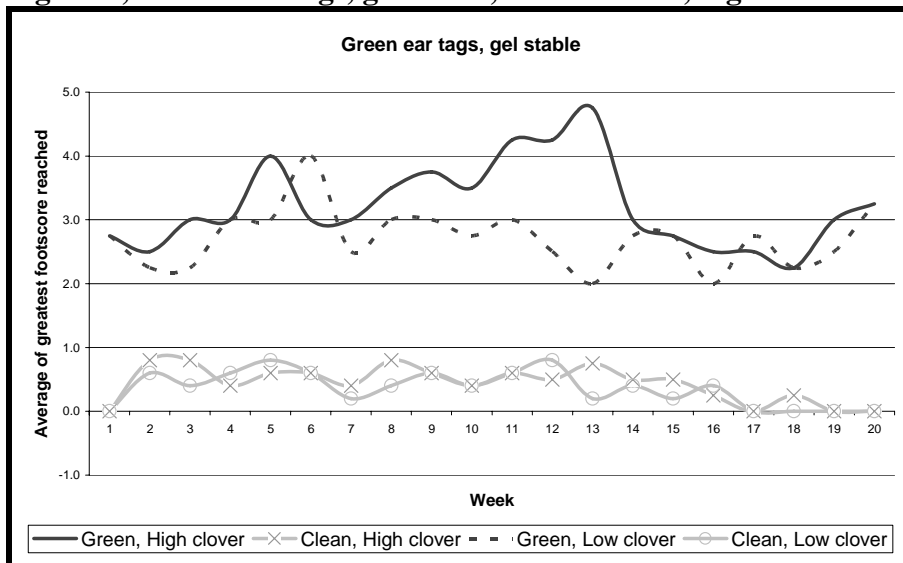
See Figures 1 to 7  
 For each strain the lines are

<b>Solid line</b>	<b>infected</b> sheep in <b>higher</b> clover
<b>Dotted line</b>	<b>infected</b> sheep in <b>lower</b> clover
<b>X on line</b>	<b>clean</b> sheep in <b>higher</b> clover
<b>O on line</b>	<b>clean</b> sheep in <b>lower</b> clover

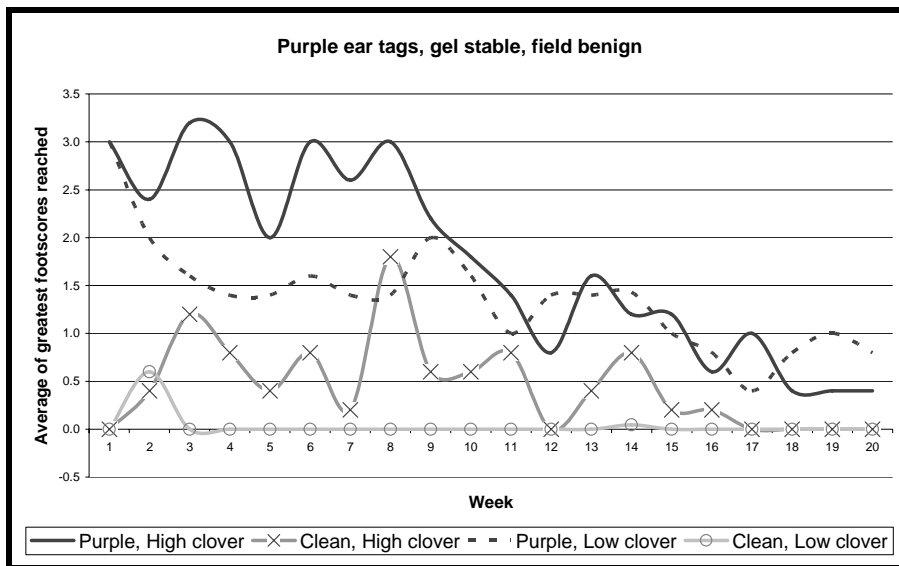
**Figure 1, White ear tags, gel stable, field high grade virulent, virulent controls in high and lower clover**



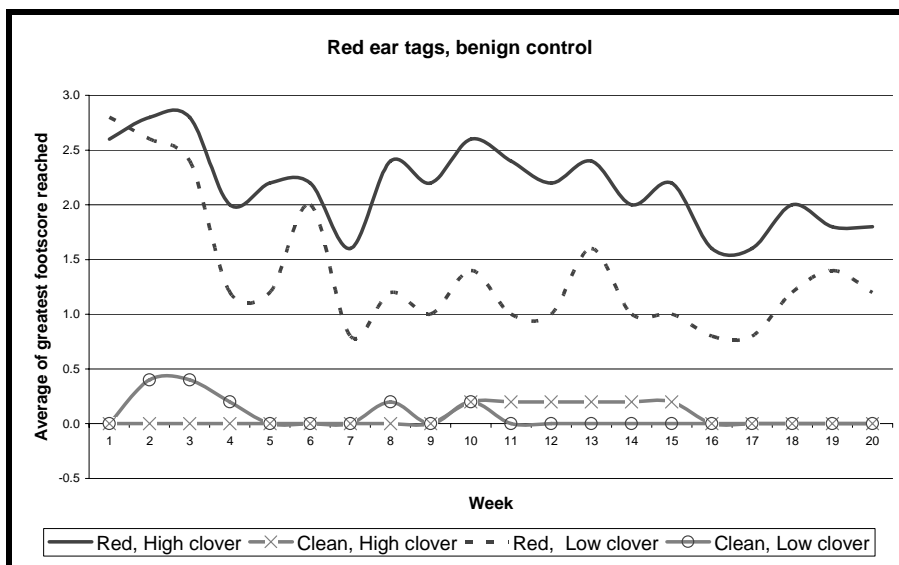
**Figure 2, Green ear tags, gel stable, field virulent, high and low clover paddocks**



**Figure 3 Purple ear tags, gel stable. This graph illustrates what also occurred in the pink and blue strains under test**



**Figure 4 Red ear tags, the benign controls**



**For all footrot strains under test, the sheep in the plots with the higher clover had worse foot scores than the equivalent sheep in the plots with lower clover.**

## **2. Field behaviour of the different strains over the 20 week period**

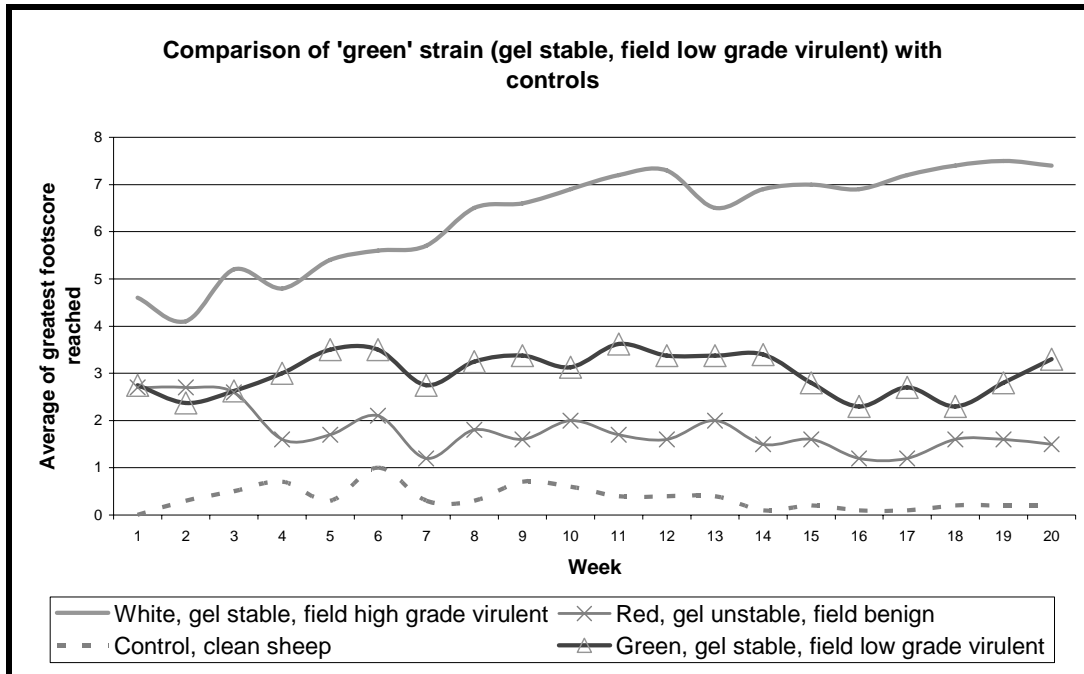
These results are shown in Figures 5 to 8. The graphs show a combined result for the coloured ear tags in both the higher and lower clover plots. An average was taken of each sheep's worst foot scores obtained each week and plotted to show the progress of the disease with all the infected strains.

The graphs show the comparison of the 4 strains under test

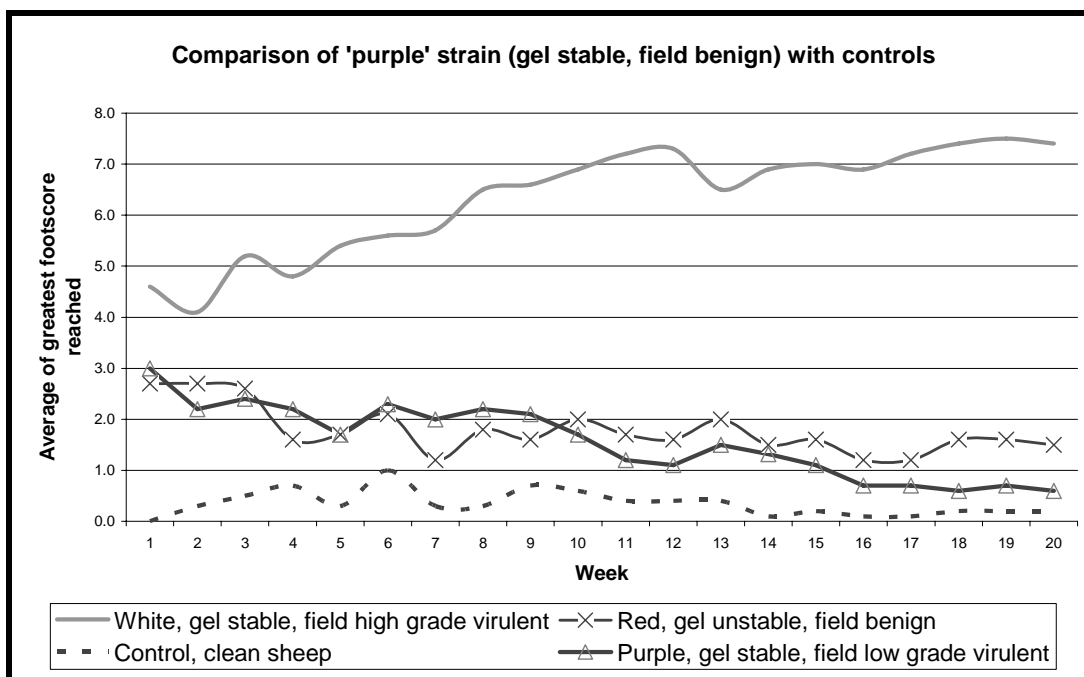
**On each graph the three controls were plotted:**

- Solid line (white ear tags) show field high grade virulent : lab stable (virulent)
- X on line (red ear tags) show field benign: lab unstable (benign)
- Dotted plain line (no ear tags) show the clean sheep

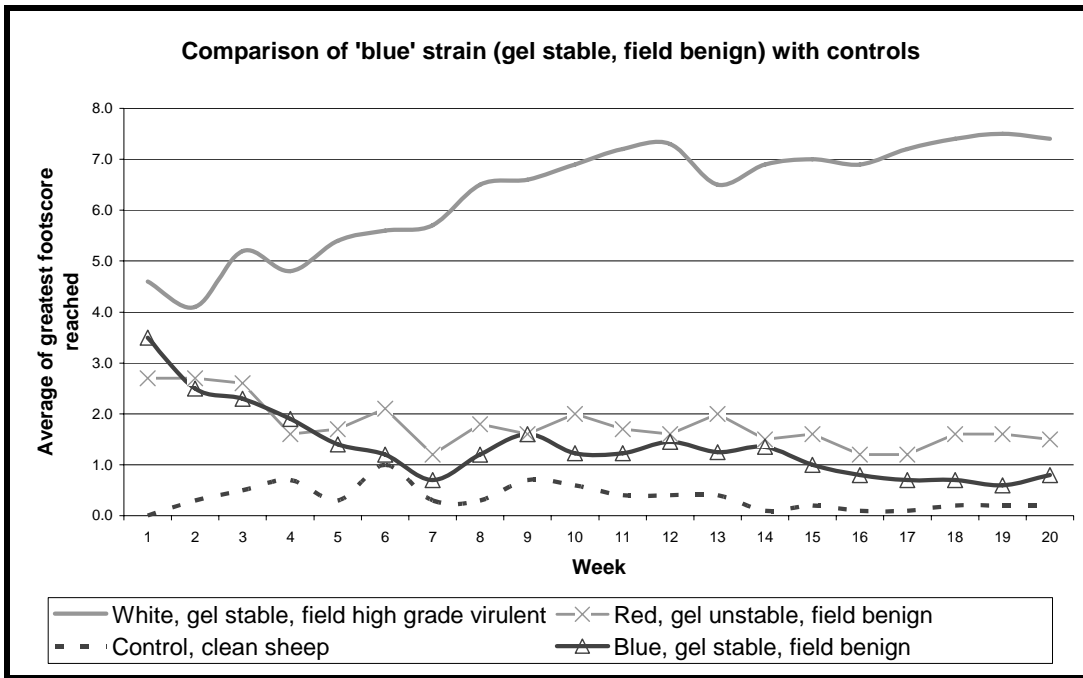
**Figure 5 The 'green' strain.** It acts in the same way as the field virulent, but to a lesser extent, showing a gradation of virulence



**Figure 6 The 'purple' strain.** It acts in the same way as the benign control despite the gel test stable lab result



**Figure 7 The 'Blue' strain. It acts in the same way as the benign control despite the gel test stable lab result**



**Figure 8 The 'Pink' strain. It acts in the same way as the benign control, this was the first time the gel results had come back as an unstable lab result, but subsequently gel stable isolates have been confirmed.**

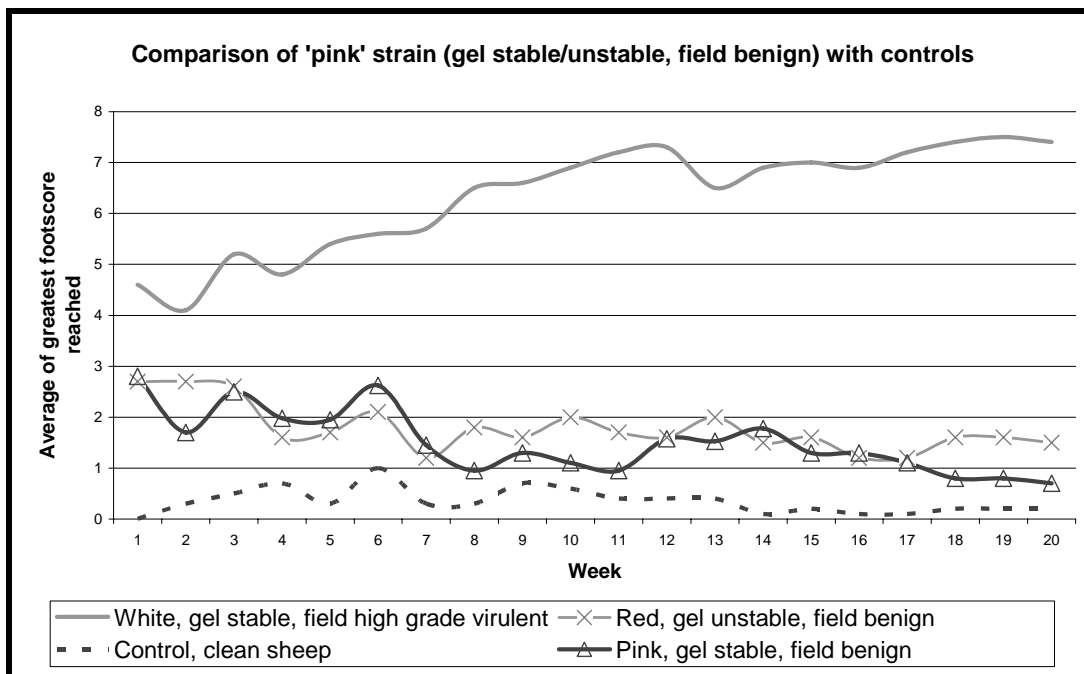
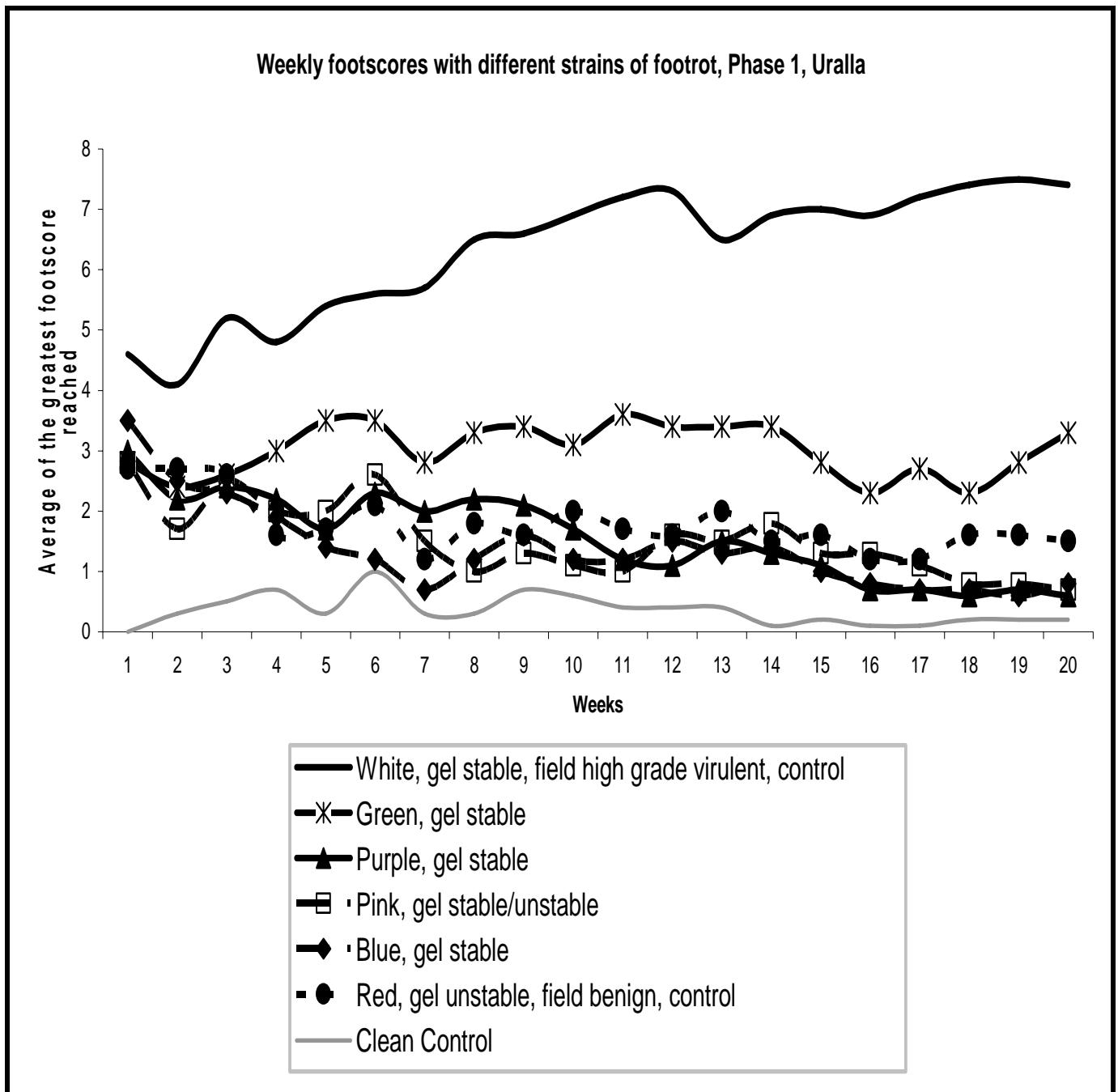


Figure 9 shows the comparison of all the strains under test at the Uralla site showing there is a gradation in virulence and some of the gel stable strains act more like the benign strain.



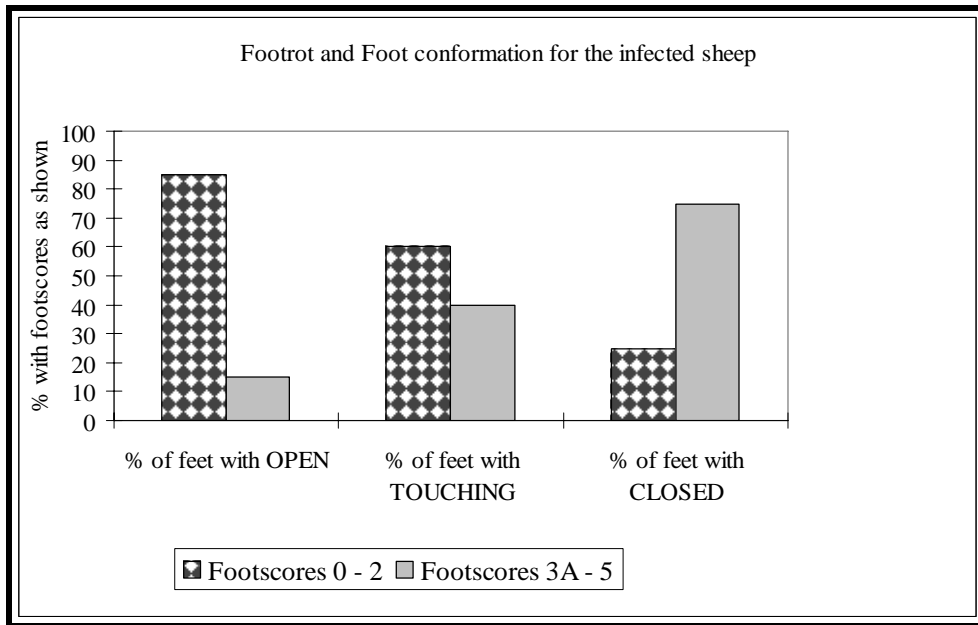
**CONFORMATION RESULTS**

We looked at the conformation of each hoof of each sheep and designated them to be

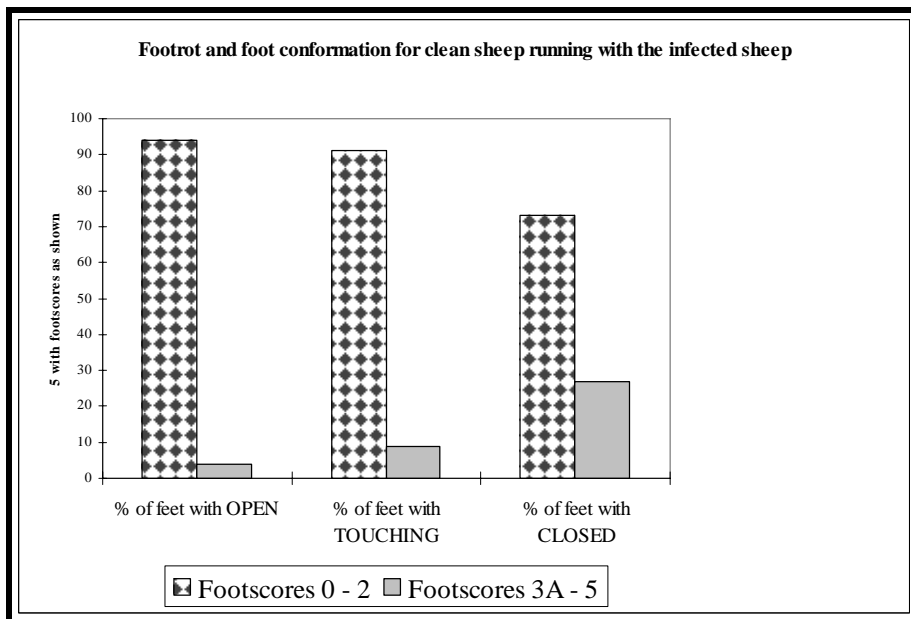
- OPEN where air could circulate as the two claws were apart when the animal walked
- TOUCHING where the two claws remained touching when the animal walked
- CLOSED where the claws were so close together they crossed over and excluded any air even when the animal put weight on its foot.

The results show the highest footrot score reached for each hoof during the course of the trial

**Figure 10** Highest footscore reached for the infected sheep which came from outside properties



**Figure 11** Highest footscore reached for the clean sheep (most had good conformation to start with) which were run with the infected sheep



**These results indicate producers should pay particular attention to the conformation of the feet of any sheep they may purchase and it should also be considered when culling is taking place.**

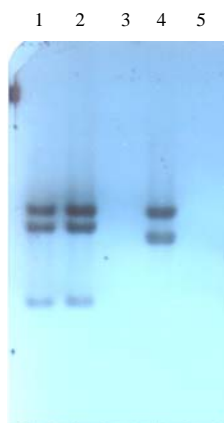
## DNA FINGERPRINTING

## Background

At the University of New England, Dr Brian Cheetham together with Dr Margaret Katz has been studying genes associated with virulence of the footrot pathogen, *Dichelobacter nodosus*. They have identified a number of genes found in virulent strains but absent from most benign strains. The existence in the New England area of strains which are gel stable but benign in the field led them to analyse the distribution of these genes in the atypical strains. They found that one gene, which is found in all strains classified as virulent from field results, was absent from the gel stable, field benign strains. This result suggests that these strains are genetically different, and not capable of causing virulent footrot.

## Results

The results of their analysis of *D. nodosus* isolates from the groups of sheep in the CICERONE footrot trial are shown below:



Lane 1 contains DNA from the virulent reference strain A198. Lane 2- gel stable, field virulent. Lane 3 – gel stable, field benign. Lane 4 – gel stable, field low grade virulent. Lane 5 – gel stable, field benign.

It is clear from the result above that strains which are benign in the field do not show bands in this DNA fingerprint, while strains which are virulent show bands.

## Summary of DNA work

DNA fingerprinting was carried out on isolates from the groups of sheep in the CICERONE footrot trial, together with a standard virulent isolate, A198. The results confirmed the previous observation that strains which are gel stable but field benign do not show bands in the DNA fingerprint test, while strains which are field virulent always show bands. Thus, this DNA test has the potential to distinguish isolates which are benign in the field, even when they give a stable result from the gel test.

## Future work

These results must be confirmed by analysis of a much larger number of strains, both virulent and benign. In addition, it is possible that the gel stable, field benign strain may cause virulent footrot under other conditions. Although they believe that this is unlikely, it is necessary to move the sheep with the gel

stable, field benign strains to another location, away from the Tablelands, to see whether virulent footrot develops. It will be necessary to carry out DNA fingerprint analysis on isolates from these sheep to ensure that the sheep do not become infected with other strains during the experiment.

## **FIELD DAYS**

Every Friday various producers come to learn about footrot, its effects and treatment. In addition specific field days were organised in December 1999 for local producers (25 attended), District Veterinary Officers and Footrot Advisory Officers (25 attended); in March 2000 Cicerone hosted Year 11 and 12 Agriculture students and staff (100 attended) and also a second Field day for local producers when 80 attended. In April a field day was organised on site by the producers of Radicate and 10 producers attended.

## **VIDEO**

A video of the field trial was taken. Laboratory work and graphs may be included when the voice over is added and the video edited in the future.

**These results were published in Newsletter 8, June 2000**

### **Summary of the Field results for the Footrot Trial Phase II**

During the spring and summer of 1999 the first phase of the Producer Initiated Research and Development (PIRD) and WoolMark funded Footrot trial took place. Our results were published in the April 2000 issue of the Cicerone Newsletter (number 6).

The sheep from this phase of the trial were over-wintered in their plots in Armidale. The two virulent strains, white and green ear tags, were removed from the trial on welfare grounds. We kept the red ear-tags (field benign, lab benign, benign control), the orange ear-tags (clean control), the pink, blue and purple 'test' strains. These sheep were drenched and shorn in their plots and foot baths of disinfectant were used by dogs and people whenever they entered and left a plot.

During this time it was finally agreed that the sheep should go to a different climatic area and see what happened with these 'odd' strains of footrot. We have had great support from John Seaman, Program Leader, Flock Health in NSW Agriculture and he was instrumental in finding a suitable site at Toogong in the Molong Rural Lands Protection Board (RLPB).

On 1<sup>st</sup> September the sheep were transported to Toogong for Phase II of the work. To ensure no cross-contamination occurred, each animal was physically carried onto the truck and they were transported in separate straw covered pens according to ear-tag colour. At Toogong they were individually carried off the truck to keep the footrot strains apart and then placed in separate plots.

A new local set of virulents were included in Phase II of the trial and new clean monitor sheep were added.

The plots in Toogong were very lush with plenty of white clover, sub clover and lucerne. A wet spring continued to ensure ideal conditions for the development and transmission of footrot.

The sheep were footscored each week by Footrot Advisory officers from the local RLPB and other nearby Boards who were interested in the results. This took place from their arrival in September to the middle of December.

The results are shown in the following graph. Footscores 3A, 3B and 3C do not translate well onto graphs, so the following conversion was used (as in the presentation of the Phase I results)

Footscore 1	= Graph score 1
Footscore 2	= Graph score 2
Footscore 3A	= Graph score 3
Footscore 3B	= Graph score 4
Footscore 3C	= Graph score 5
Footscore 4	= Graph score 6
Footscore 5	= Graph score 7

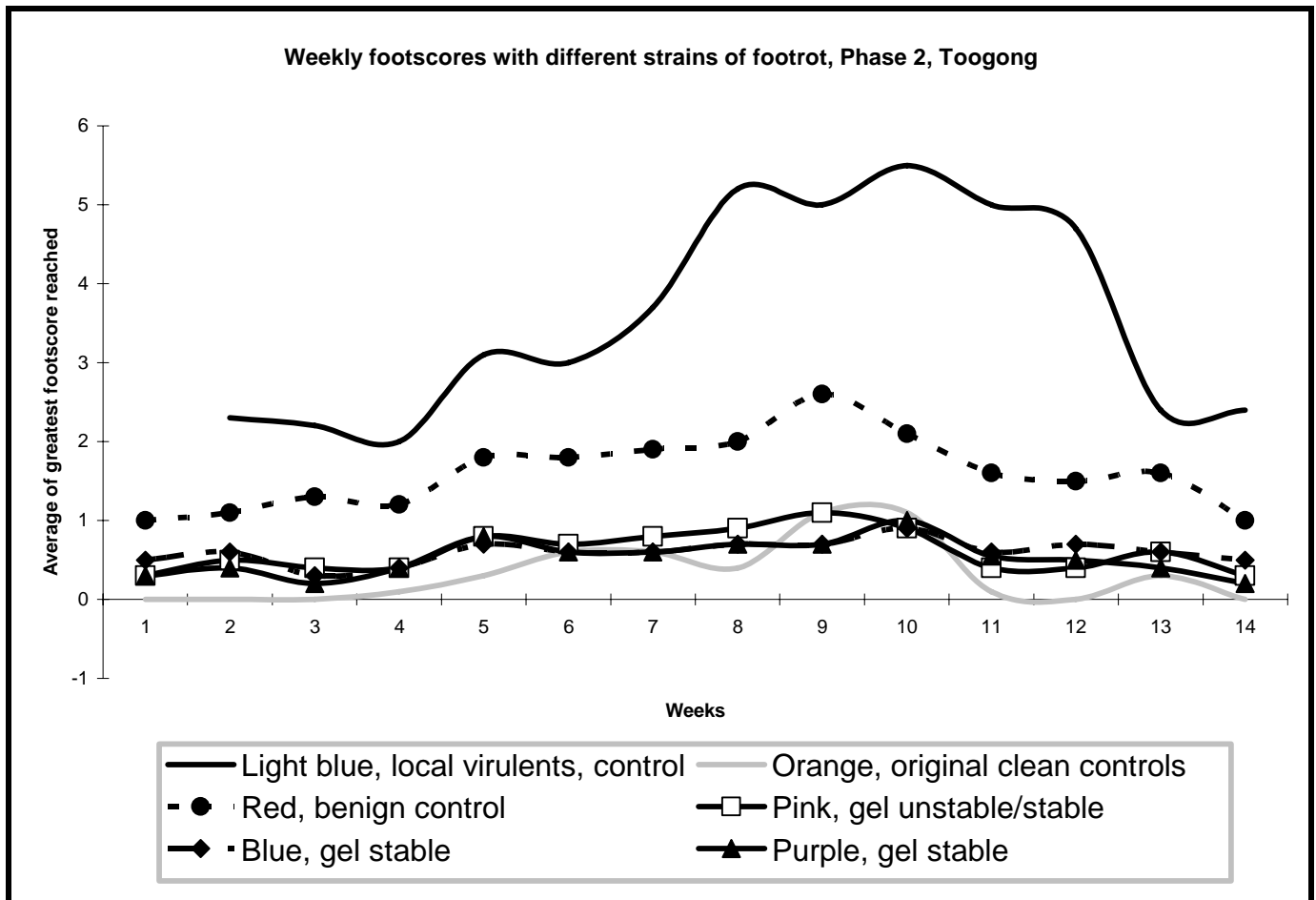
A Field Day was held for RLPB advisory officers, local producers and Cicerone members on Friday 10<sup>th</sup> November. On this day the animals with the virulent strain were footpared and treated to keep flies out of their feet. On the graph the marked drop in the virulent footscores indicates this time. The general trend downwards indicates the start of the drying off period in the pasture.

However the results still show that the three 'test' strains of pink, blue and purple ear-tags remained behaving in a benign fashion, despite the lush conditions they did not 'break out' into full blown virulent footrot. This backs up the findings from Phase I of the trial.

Dr. Brian Cheetham of the Department of Molecular and Cellular Biology at the University of New England (UNE) will be doing his DNA analysis on swabs taken from these sheep and we keenly await his results.

The sheep have now all been given injections of antibiotics and will be foot-bathed and then transported to the Elizabeth McArthur Agricultural Institute where Dr Richard Whittington of NSW Agriculture will be doing blood tests and antibody work on them.

We are most grateful to the funding from Producer Initiated Research and Development (PIRD) and WoolMark which allowed us to do this work.



These results were published in Newsletter 11, page 1

**ACKNOWLEDGEMENTS**

**PIRDs and WoolMark** for the essential funding  
**NSW Agriculture** for financial help with the Laboratory tests  
**Les Gallagher**, Farm manager at the time of the trial, for care of the sheep  
**Paul Berdar and Bruce Floyd**, rangers with Armidale RLPB, for their time scoring sheep  
**John Macfarlane**, DVO with Armidale RLPB, for his support and advice  
**Brian Cheetham**, UNE, for his advice and supporting laboratory work.  
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**Steve Atkinson**, Animal Welfare Officer with the ACEC, for his advice.  
**District producers** for the many discussions we have all held  
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**PIRD Project W99 / NO1**

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