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INVESTIGATIONS OF THE ERADICATION OF FOOTROT

M.B. Allworth

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Animal Health
University of Sydney

August 1995
Apart from assistance stated in the acknowledgements and where reference is made in the text, this thesis represents the original work of the author.

Bruce Allworth, BVSc,MVS
4th August, 1995
SUMMARY

This study addressed some issues associated with the eradication of ovine footrot. The ability of farmers to assess the presence of footrot, and to eradicate it from affected flocks was examined. The eradicability of characterised strains of *D. nodosus* was examined in a series of experiments. The strains were further characterised by the application of molecular biology techniques. State footrot programmes were examined in relation to the findings of this study.

A survey of members of a Voluntary Footrot Group in a footrot endemic area was completed (Chapter 3). All 19 owners replied to the survey. Property visits were made to 17 of the 19 properties, to establish the footrot status of the flocks. Footrot was present in 13 of 17 sheep flocks (and both goat flocks). Infection with *D. nodosus* was detected on 16 properties, and in all sheep flocks sampled. The prevalences of virulent, intermediate and benign footrot were 23.5%, 12% and 47% respectively. There was a high correlation between the clinical diagnosis and the assessment in vitro of the virulence of *D. nodosus* isolates recovered from the flocks (0.82). Owners tended to underestimate the severity of the footrot present. Eleven owners had attempted eradication in the previous five years. Eradication was achieved in 6 flocks. Two owners who sold affected mobs eradicated footrot within one year. The nine owners who undertook eradication by inspection and culling took at least three years to eradicate footrot, and then only 4 of 9 owners were successful.

The virulence and eradicability of several strains of *Dichelobacter nodosus*, were assessed in a series of field experiments (Chapter 4). Following the successful establishment of multiple *D. nodosus* strains of different virulence within a flock, this model was used in a large scale field trial with 1450 sheep to compare the eradicability of 7 *D. nodosus* strains, using standard eradication techniques. Strains varied in their eradicability, conclusions being possible for 6 of the 7 strains. Those strains associated
with milder disease were less frequently eradicated. One virulent and three intermediate strains were eradicated from three replicated mobs, comprising 300 to 450 sheep each. A benign strain and an intermediate strain persisted following eradication, each in one of three replicates. Footrot persisted in the control flock.

Isolates collected from flocks surveyed and from the experimental flocks were further characterised by the molecular biology techniques of ribotyping and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) fingerprinting. Ribotyping following digestion with *Eco*RI produced 6 different patterns (ribotypes) from 18 isolates. PCR-RFLP fingerprints were determined for 114 *D.nodosus* isolates. *Hpa*II was the restriction enzyme used. This technique was rapid, discriminatory and reproducible. The findings from both typing methods confirmed the identity of the strains which persisted following eradication.

An examination of current State footrot programmes, in the context of the findings of this study and the principles for developing such programmes, identified a number of deficiencies. Specifically, the inclusion of mild disease in a number of programmes as a target for eradication appears to be in conflict with current knowledge.
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The work in this thesis represents work performed by myself. However, without the help of many people, this work could not have been undertaken or completed. I would therefore like to acknowledge all those who so kindly helped me during this study.

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The field work was performed in the Wantagong area near Holbrook, New South Wales. The willing co-operation of the members of the Wantagong Footrot Free Flocks group, and particularly those adjoining "Talooby", where the experimental flocks were
held, was appreciated. Both Robert Dark and Robert Shea provided assistance with inspections, and Robert Shea also assisted with sheep work and fencing.

The cultures of some of the *D. nodosus* strains used were kindly supplied by David Stewart and Jill Vaughan, CSIRO Division of Animal Health, Parkville.

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The impetus for this work came from dealing with different forms of footrot on a number of clients' properties. I would like to acknowledge the enthusiasm, dedication and commitment to better understand footrot by the many people I have dealt with. I would also like to thank my clients, who, for the past 3 years, have patiently (seemingly) allowed me to complete this study. I hope the findings from this research will be applied to the benefit of those clients dealing with footrot, and to the farming community in general.
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<tr>
<td>Bp</td>
<td>Base pair</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td><em>D. nodosus</em></td>
<td><em>Dichelobacter nodosus</em></td>
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<td>DPT</td>
<td>Degrading proteinase test</td>
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<td><em>Fusobacterium necrophorum</em></td>
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<tr>
<td>GGPTT</td>
<td>Gelatin gel protease thermostability test</td>
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<td>HA</td>
<td>Hoof agar</td>
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<td>IDS</td>
<td>Interdigital skin</td>
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<tr>
<td>OID</td>
<td>Ovine interdigital dermatitis</td>
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</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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</tr>
<tr>
<td>PE</td>
<td>Protective effectiveness</td>
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<td>PFGE</td>
<td>Pulse field gel electrophoresis</td>
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<td>PTT</td>
<td>Protease thermostability test</td>
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<tr>
<td>REA</td>
<td>Restriction endonuclease analysis</td>
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<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
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<tr>
<td>RLPB</td>
<td>Rural Lands Protection Board (NSW)</td>
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<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
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<td>SSC</td>
<td>Sodium (chloride) sodium citrate</td>
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<tr>
<td>TE</td>
<td>Tris-EDTA buffer</td>
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<td>Therapeutic effectiveness</td>
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CHAPTER 1

LITERATURE REVIEW

1.1. Introduction

Footrot is a disease of sheep's feet associated with infection by a complex mixture of bacteria. Of these, the gram negative, anaerobic rod *Dichelobacter nodosus* (formerly *Fusiformis nodosus, Bacteroides nodosus*) (Beveridge, 1941; Dewhirst et al, 1990) is the essential transmitting agent. The disease is characterised by infection of the interdigital skin (IDS), which, in susceptible sheep, may progress to separation of the soft and hard horn from the underlying hoof matrix ("underrunning").

Initially, only one form of footrot was recognised after it was distinguished from foot abscess (Gregory, 1939a). Subsequently, two forms of footrot were described (Thomas, 1962a; Egerton and Parsonson, 1969): a virulent form (virulent or progressive footrot), and a benign form (benign or non-progressive footrot). More recently, an intermediate form of footrot with clinically less severe lesions than those of virulent footrot, and more severe than benign footrot, has been recognised (Stewart et al, 1982a, 1984, 1986a, 1986d). It has been demonstrated that there is a spectrum of virulence among *D. nodosus*, and this spectrum may be associated with different forms of footrot (Stewart et al, 1982a, 1984, 1986a, 1986d).

Much of the knowledge of footrot was obtained prior to the recognition of the different forms of the disease, and therefore relates primarily to the severe and most obvious forms. Information on less severe forms is frequently extrapolated from work with virulent footrot. Whilst some aspects of the different forms of footrot have been shown to be similar, differences in their epidemiology, and in particular, their response to treatment and eradication programmes have been suggested (Alexander, 1962;
1. Literature Review

Littlejohn, 1966/67; Egerton and Parsonson, 1969; Brownrigg, 1986). Therefore, the application to less severe forms of footrot of control or eradication programmes which are effective for virulent footrot may not be appropriate, and requires further evaluation.

As the major aim of this study was to examine the eradicability of milder forms of footrot, this review will focus on those aspects of the epidemiology, pathogenesis, diagnosis, and treatment of footrot which are relevant to the eradication of the disease from flocks. Whilst much of the published information refers to virulent footrot, special emphasis will be placed on information relating to less virulent forms of the disease and those isolates of *D. nodosus* associated with those forms.

1.2. Forms of footrot - qualitative description

Recognition of different forms of footrot has led to changes in footrot terminology on a number of occasions. The term *footrot* is used as both a general term referring to any of the clinically recognisable forms of the disease, and in a narrower sense as a shortened description of severe footrot. *Virulent footrot* is used variously to mean infection with virulent or intermediate strains of *D. nodosus*, infection with protease thermostable *D. nodosus* strains, or as a clinical description. *Benign footrot* generally only refers to predominantly interdigital disease, but may be applied to diseases associated with *D. nodosus* strains which are protease thermolabile. Given the inconsistent usage of such terms as footrot, virulent, benign and intermediate, failure to define these terms adequately leads to difficulties in interpreting or applying research findings.

Throughout the literature, clinical descriptions of the disease tend to be used synonymously for infection by *D. nodosus* of different grades of virulence. For a disease where a single bacterial causal agent is implicated that may be acceptable. However, it is
not necessarily the case in footrot, where mixed populations of bacteria are invariably involved in the pathogenesis (Egerton et al., 1969).

Beveridge (1941) defined footrot as

"a contagious disease of the sheep's foot characterised by separation of a large portion of the hoof from the soft tissues due to a spreading infection immediately beneath the horn and caused primarily by *Fusiformis nodosus*." 

Alexander (1962) was the first to suggest different clinical manifestations of footrot. He proposed the use of the terms severe footrot, mild footrot and scald. Mild footrot was similar to severe footrot, but tended to self-cure, with chronic cases not being a clinical feature. Scald referred to the predominantly interdigital disease (Thomas, 1962a).

Egerton and Parsonson (1969) proposed the term benign footrot instead of scald or non-progressive footrot, and virulent footrot to describe the disease associated with extensive separation of the hoof horn.

Stewart et al. (1982a, 1984, 1986a, 1986d), following a number of challenge experiments, identified strains of *D. nodosus* of intermediate virulence which, in artificially infected sheep, resulted in a clinical expression between those of benign and virulent footrot.

At present, three clinically distinct entities of footrot are defined as follows (Stewart, 1989; Stewart and Claxton, 1993):
1. Literature Review

**Virulent footrot**
- "a persistent and chronic condition with severe extensive necrotic underrunning of the laminae of the abaxial wall in the hoof in a high percentage of sheep, causing severe production losses."
- rapid development of severe lesions occurs under favourable conditions

**Intermediate footrot**
- may be similar to virulent footrot in individual sheep
- on a flock basis it is a milder disease with only a small percentage of sheep having severe lesions extending to the abaxial edge of the sole of the hoof, and rarely underrunning the abaxial wall.
- self-cure tends to occur; however, a few severely affected sheep remain chronically affected

**Benign footrot**
- less persistent, with interdigital lesions being the predominant lesion
- underrunning to the edge of the sole is rare
- lesions heal rapidly with the onset of dry conditions.

Thus, virulent footrot is a severe, chronic disease, with the majority of affected sheep having extensive underrunning. Intermediate footrot is neither virulent nor benign, and therefore is less distinctly defined. The main clinical feature of intermediate footrot is the presence of underrunning, but with only a small proportion of sheep in the mob with extensive underrunning.

Benign footrot is a mild disease, associated with infection with less proteolytic strains of *D.nodosus* (Egerton and Parsonson, 1969). The lesions of benign footrot are predominantly interdigital (Thomas, 1962a; Egerton and Parsonson, 1969; Stewart, 1979) and characteristically there is an inability to invade epidermal tissue beyond the skin-horn
1. Literature Review

junction (Egerton and Parsonson, 1969). The IDS is inflamed and ulcerated, with a thin film of moist necrotic material (Thomas, 1962a). If separation occurs, it is generally confined to the posterior axial sole, with little necrotic material (Thomas, 1962a; Stewart, 1979). Lesions have a tendency to heal rapidly with the onset of dry conditions, and respond rapidly to topical treatment (Egerton and Parsonson, 1969).

Clinically, benign footrot closely resembles Ovine Interdigital Dermatitis (OID), a necrotising infection associated with the invasion by *F. necrophorum* (Parsonson et al, 1967). *D. nodosus* organisms are absent from smears in OID, and this appears to be the main distinguishing feature between OID and benign footrot.

Clinical descriptions of footrot have generally involved descriptions of lesions in individual feet (e.g. "separation of a large portion of the hoof from the soft tissues" (Beveridge, 1941)); or qualitative descriptions of the flock picture ("a high percentage of sheep" (Stewart, 1989), "small percentage of sheep having severe lesions", "few severely affected sheep"(Stewart and Claxton, 1993)). Categorisation of footrot may be difficult using such qualitative, imprecise definitions. The need to define the type of footrot quantitatively has been discussed (Egerton, 1989a). More quantitative definitions for diagnosis are necessary for regulatory purposes, to evaluate differences in epidemiology, and to assess responses to treatment and eradication programmes. For the evaluation of *in vitro* tests, defined descriptions of the different forms of footrot are essential.

1.3. Scoring Systems

Beveridge (1941) ranked the severity of lesions qualitatively by recording lesions as "mild", "moderate" and "severe". "Mild" lesions referred to interdigital lesions with separation of the horn of the sole of at least one digit, but with "less tissue damage than usual". "Moderate" lesions were more extensive and had "slightly" more tissue damage.
"Severe" lesions referred to severe interdigital inflammation, and separation of the horn of the entire sole, and sometimes the outer wall.

Egerton and Roberts (1971) introduced a system to score footrot lesions as follows:

- **Score 0**: normal feet
- **Score 1**: limited mild interdigital dermatitis
- **Score 2**: more extensive interdigital dermatitis
- **Score 3**: severe interdigital dermatitis and/or under-running of the horn of the heel and sole
- **Score 4**: as for 3, but with under-running extended to the wall of the hoof.

This system was based on the naturally occurring anatomical progression of the disease. Each foot was evaluated individually, with the total score of a sheep being the sum of the scores of its four feet.

This scoring system was modified by Stewart et al (1982b) as follows:

- **Score 0**: normal feet
- **Score 1**: mild interdigital dermatitis
- **Score 2**: severe interdigital dermatitis
- **Score 3a**: slight separation (0.5 cm or less) of the plantar horn of the heel
- **3b**: more advanced separation of the plantar horn of the heel
- **3c**: complete separation of the plantar horn of the heel and extension into the posterior sole region
- **Score 4**: separation of the horn of the sole and abaxial wall.
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Raadsma et al (1993) also modified the Egerton and Roberts scoring system, including a score 5, scores for healing lesions (0 to 2), and a grade (0 to 5), being a description of the overall animal grade. This more extensive scoring system was aimed at a better description of differences in the resistance of individuals.

The weighting of scores, based on the Egerton and Roberts scoring system, in favour of more severe lesions to reflect the impact of these lesions on productivity, has been proposed (Whittington and Nicholls, 1995). In this system, score 3 lesions were given a weighting of 9, and score 4 lesions a weighting of 16, with the sum of the weighted scores for the four feet giving the Total Weighted Footscore (TWFS). When compared with 15 other methods of evaluating footscores, it was considered that TWFS was the most useful method of describing footrot lesions in relation to the sheep's immunological response (Whittington and Nicholls, 1995).

Alternative scoring systems have been described by Skerman et al (1982) and Bulgin et al (1985) (0 to 4, with underrunning lesions being 2 and above), and Bagley et al (1987) (0 to 6, with underrunning lesions being 4 and above).

Despite the use of generally similar scoring systems, different criteria for defining affected and severely affected sheep have been used. In evaluating vaccines, sheep were considered affected with footrot if at least one foot had a score 3 lesion, or two or more feet had score 2 lesions (Egerton and Thorley, 1981; Thorley and Egerton, 1981; Egerton et al, 1983; Hindmarsh et al, 1989; Liardet et al, 1989; Schwartzkoff et al, 1993a). Alternatively, Stewart and workers considered sheep were footrot affected if a score 2 lesion or greater was present in at least one foot; severely affected sheep had at least a score 3c lesion in at least one foot (Stewart et al, 1983, 1984, 1985b, 1986c, 1986d; Hunt et al, 1994). The presence of underrunning (score 3) in at least one foot has also been used as a criterion for sheep being affected with footrot (Skerman et al, 1982; Mulvaney et al, 1984; Lambell, 1986b), although the presence of underrunning (score 3a or greater)
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has also been considered to be indicative of sheep being severely affected (Whittington, 1995a). Sheep with score 1 lesions have also been included as footrot affected (Casey and Martin, 1988; Marshall et al., 1991a; Glynn, 1993), although score 1 lesions may be due to other causes (Egerton et al., 1987).

The use of footscore data has been used to classify the form of footrot present. Stewart et al. (1982a, 1984, 1986a, 1986d) used both footscore data and body weight data to justify a further distinction of intermediate forms of footrot from benign and virulent footrot. Egerton (1989a) proposed assessing the percentage of severely affected sheep (at least one foot with a score 4 lesion) as one of the criteria for distinguishing the different forms of footrot: flocks with benign footrot having no more than 1% of sheep with score 4 lesions, flocks with intermediate footrot having no more than 10% of sheep with score 4 lesions, and flocks with virulent footrot having at least 10% of sheep with score 4 lesions, and usually more. Given that the main distinction between the forms of footrot is in severity of disease, distinction of the different forms of footrot on the basis of differences in prevalence and severity of lesions seems appropriate.

There is little published on the different forms of footrot and the percentage of affected sheep with score 4 lesions. Categorisation on the basis of the percentage of score 4 lesions assumes that the principal difference in severity of the different forms of footrot can be related to score 4 lesions. Whilst this may be true for distinguishing intermediate and virulent forms, it may not be appropriate when differentiating benign and intermediate footrot.

As benign footrot is principally a disease of the IDS, with little or no underrunning (Thomas, 1962a; Egerton and Parsonson, 1969; Stewart, 1979), it will be characterised by a high proportion of score 2 lesions in affected sheep, with few, if any, score 3 or score 4 lesions. Intermediate forms of footrot are also principally interdigital diseases, but unlike benign footrot, underrunning is also a feature of intermediate footrot.
in some sheep. However, the underrunning is less severe than with virulent footrot. Therefore, it is characterised by both score 2 and score 3 lesions in affected sheep, with relatively few score 4 lesions. Virulent footrot is principally a disease characterised by underrunning lesions, and these are extensive. Therefore, score 3 and score 4 lesions are predominant in affected sheep.

Given these differences, both the percentage of score 3 lesions and the percentage of score 4 lesions in affected sheep may be useful criteria in differentiating the three forms of footrot on clinical expression. The percentage of score 3 lesions in affected sheep may be a useful criterion for distinguishing benign and intermediate forms of footrot, whilst the percentage of score 4 lesions may be the most appropriate quantitative basis on which to differentiate intermediate and virulent forms of footrot.

A difficulty with the use of scoring sheep's feet to differentiate forms of footrot is the variable expression due to environmental and sheep factors (see sections 1.5.2 and 1.5.3). The use of the percentage of affected sheep (score 2 or greater) with score 4 lesions has been proposed, in recognition of the variability in expression of footrot due to non-bacterial factors (J.Egerton, pers.comm.). Use of 'affected sheep' as the denominator will in part allow for the decreased prevalence of disease likely to occur in less favourable environments. Whether a similar proportion of affected sheep become severely infected in less favourable environments remains to be established.

Definitions used in this thesis for the different forms of footrot are presented in section 2.2.2.
1.4. Pathogenesis

Footrot is a mixed infection (Beveridge, 1941; Egerton et al., 1969), with *D. nodosus* an essential but insufficient causal agent (Beveridge, 1941; Thomas, 1962b; Egerton et al., 1969). *Fusobacterium necrophorum* is also essential to the disease, and may be responsible for the majority of the tissue destruction of footrot (Egerton et al., 1969). Infection with *F. necrophorum* generally precedes the establishment of *D. nodosus* infection (Egerton et al., 1969; Roberts and Egerton, 1969). Thus, in naturally occurring cases, *D. nodosus* and *F. necrophorum* act synergistically to produce footrot. *D. nodosus* initiates invasion of the epidermal matrix (presumably due to the action of proteases), persists in lesions for long periods, and provides a heat-stable factor which increases the growth of *F. necrophorum* (Roberts and Egerton, 1969).

Other organisms associated with the footrot lesion have a secondary role, if any. The motile fusiform, frequently present in footrot lesions, is a secondary invader and unlikely to be involved in the pathogenesis of footrot (Beveridge, 1941; Thomas, 1962b; Egerton et al., 1969). Beveridge (1941) considered the spirochaete, *Spirochaeta penortha*, had a specific role as a secondary causal agent, but its role has subsequently been questioned but not examined (Thomas, 1962b; Egerton et al., 1969).

Whilst less proteolytic strains of *D. nodosus* have been shown to be associated with benign footrot, *F. necrophorum* is also present, and probably causes much of the tissue damage in benign footrot (Egerton and Parsonson, 1969).
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1.5. Epidemiology

The occurrence and clinical expression of footrot within a flock of sheep will be determined by the interaction between three main factors:

(1) microbial factors, which include:
   (i) the "net" virulence of the infecting *D.nodosus* strain(s)
   (ii) the survival of *D.nodosus* in the environment
   (iii) the survival of *D.nodosus* in the host
   (iv) possible differences in virulence of other organisms

(2) the environmental conditions

(3) host factors, which determine the susceptibility of the sheep, and include:
   (i) the integrity of the IDS
   (ii) the genetic resistance of the sheep, determined by the breed or strain, and the sheep's individual susceptibility within that strain
   (iii) acquired resistance, either naturally following exposure to footrot, or artificially following vaccination.

The clinical expressions will also be influenced by the duration of infection at the time of examination, and possibly by the influence of other components of the bacterial population.
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1.5.1. Microbial factors

1.5.1.1. Virulence of the infecting strains

The virulence of *D. nodosus* has been described as a continuum (Stewart et al, 1986d), as under similar environmental conditions, the severity of lesions varies with different infecting strains in genetically similar sheep. Virulence factors include the amount of proteolytic activity and the stability of proteases, degree of piliation, twitching motility, as well as other undefined factors (Stewart et al, 1986d; Katz et al, 1991).

The "net virulence" allows for interactions between components of multiple strain infections, and the possibility that interactions between strains may alter the clinical expression of single strains as assessed in experimental challenge systems.

1.5.1.2. Resilience of *D. nodosus* organisms in the environment

The viability of *D. nodosus* in the environment has been investigated, and the findings are summarised in Table 1.1. There appears little doubt that, under natural conditions, *D. nodosus* (associated with virulent footrot) will not remain infective away from its host for more than 7 days. In many cases, destocking of pasture ("spelling") for 1-2 days would eliminate the risk of transmission (via the pasture) to uninfected sheep. The only report of *D. nodosus* remaining infective for longer than 5 days was when lesion material was mixed with faeces or mud, and applied to scarified feet (Beveridge, 1941). Survival in this instance appeared to be favoured when *D. nodosus* organisms were mixed with faeces, and infectivity was presumably enhanced by physical disruption of the *stratum corneum* barrier.
Table 1.1. Survival of *D. nodosus* in the environment.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Challenge Method</th>
<th>0 hr</th>
<th>0-24 hr</th>
<th>24 hr</th>
<th>2-3 days</th>
<th>4-5 days</th>
<th>7 days</th>
<th>8-14 days</th>
<th>&gt;14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown, 1892</td>
<td>Pen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beveridge, 1938a</td>
<td>LM^3^</td>
<td>22/23^4^</td>
<td>24/24</td>
<td>0/4</td>
<td>0/4</td>
<td>0/1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LM+mud</td>
<td>11/12</td>
<td>5/8</td>
<td>2/16</td>
<td>1/12</td>
<td>0/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LM+faeces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gregory, 1939b</td>
<td>Plot</td>
<td>3/12^5^</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pen</td>
<td>8/10</td>
<td>0/6</td>
<td>0/32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laing &amp; Egerton, 1981</td>
<td>LM^6^</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes**
1. Number of hours / days *D. nodosus* organisms survived if infection occurs.
2. Brown, 1892, cited by Beveridge, 1941.
3. LM = Lesion material.
4. Number of feet with footrot / number of feet exposed; + = infected feet; -- = no infected feet.
5. 3/4 in winter; 0/8 in late spring.
6. Lesion material assessed by survival after culture, not challenge.
Both Beveridge (1941) and Gregory (1939b) concluded that a 7 day spelling period would be sufficient to prevent the development of footrot in sheep exposed naturally. To be conservative, a 14 day spelling period was recommended (Gregory, 1939b), although for eradication purposes Beveridge (1941) considered no spelling was necessary if pastures were dry, as sheep would not be susceptible to the disease.

Under natural conditions, spelling of paddocks for either 2 weeks or 6 weeks prevented the transfer of *D. nodosus* infection in sheep which were observed for 18-22 months subsequently (Gregory, 1939b; Beveridge, 1941). Whittington (1995a) reported the transmission of footrot via infected yards which were not spelled.

### 1.5.1.3. Survival within the host

Whilst the survival of *D. nodosus* away from the host is limited, survival of *D. nodosus* within the host can be prolonged. Virulent footrot, despite its invasive and often aggressive clinical features, is a chronic disease. Beveridge (1941) recorded a sheep which remained clinically affected with footrot for 3.5 years, and was still infective at the end of this period. He also noted lesions persisting in the IDS for up to 28 weeks, and considered infections under the horn of healed sheep a potential source of infection for other sheep.

The potential for *D. nodosus* to survive in the IDS has been discussed (Alexander, 1962; Egerton and Parsonson, 1969; Morgan et al, 1972; Glynn, 1993). *D. nodosus* organisms have been detected in smears taken from the IDS of clinically normal feet (Egerton and Parsonson, 1969; Glynn, 1993) and in the *stratum corneum* in histological sections of apparently healed feet, suggesting that the IDS is a possible site for survival of benign *D. nodosus* organisms. Topical treatments, including 5% formalin and 20% zinc sulphate / sodium lauryl sulphate, may not penetrate the IDS (Egerton and
Parsonson, 1969; Glynn, 1993), and therefore infection may persist despite footbathing. Stewart (1989) recovered benign organisms from underrun toes of sheep on three properties and suggested infection may persist under the horn of the hoof rather than the IDS.

*D.nodosus* has not been detected or isolated from lesions in ruminants other than those infections of the IDS or hoof. Experimentally, *D.nodosus* infection of the base of the horn in a sheep has been demonstrated, and when lesion material from this experimental infection of the horn was applied to a sheep's foot, typical footrot resulted (Thomas, 1962b). Beveridge (1941) failed on two occasions to infect scarified skin on the body of a sheep with *D.nodosus*. Given the role of other bacteria in footrot this is not surprising.

There is no evidence that *D.nodosus* organisms survive anywhere but in the IDS or horn matrix of feet of ruminants, except briefly on the ground. Horses have failed to become infected when challenged. The possibility of transfer of *D.nodosus* by mechanical methods (cars, boots), flies and birds is possible theoretically, but the risk is negligible (Beveridge, 1938b). There is only one recorded outbreak of virulent footrot where such transfer is suggested, with hares, ibis, flies or irrigation water being considered possible means of transfer of *D.nodosus* between groups of sheep (Stewart et al, 1984).

**1.5.1.4. Other bacteria**

Footrot is a mixed bacterial infection (Egerton et al, 1969). It might therefore be expected that microbial factors other than those associated with *D.nodosus* may influence the clinical expression of the disease, particularly as *F.necrophorum* may be responsible for the majority of the tissue destruction of footrot (Egerton et al, 1969). The pathogenicity of *F.necrophorum* varies, with biotypes A and AB being pathogenic, and
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biotype B being non-pathogenic (Clark et al, 1989). An exotoxin of *F. necrophorum* is the principal factor elaborated by pathogenic strains (Clark et al, 1989), and it is this exotoxin which is considered to be associated with the tissue destruction attributed to *F. necrophorum* in footrot (Roberts and Egerton, 1969). The type(s) of *F. necrophorum* present in footrot lesions have not been reported, nor have their role in the clinical expression of footrot been considered.

The contribution of *F. necrophorum* to the mixed infection of footrot may be influenced by a heat-stable factor elaborated by *D. nodosus* which increases the growth of *F. necrophorum* (Roberts and Egerton, 1969). Changes in the ability of *D. nodosus* to produce this factor, or to *F. necrophorum* to utilise this factor, could influence the *F. necrophorum* population, and thereby influence the clinical expression.

Microbial factors other than those directly associated with the pathogenic and transmissible capabilities of *D. nodosus* may influence the clinical outcome of footrot, and should be considered and further investigated if variations in clinical expression of footrot are to be fully understood.

1.5.2. Environmental conditions

Environmental conditions have an impact on both the transmission of the disease, and its clinical expression. Moisture (Murnane, 1933; Beveridge, 1941; Littlejohn, 1966/67; Graham and Egerton, 1968; Locke and Coombes, 1994) and temperature (Beveridge, 1941; Graham and Egerton, 1968) are important factors.

Moisture is a key factor in rendering sheep's feet susceptible to infection. Sheep with healthy normal IDS are not susceptible to infection (Beveridge, 1941; Thomas, 1962b; Egerton et al, 1969), and water maceration alone does not facilitate *D. nodosus* infection (Roberts and Egerton, 1969). However, water maceration with
faecal contamination renders sheep's feet susceptible (Roberts and Egerton, 1969). The damage to the IDS associated with prolonged moisture allows infection with *F. necrophorum*, which invariably precedes or coincides with *D. nodosus* infection (Egerton et al., 1969; Roberts and Egerton, 1969).

Moisture appears necessary for the transmission of footrot (Graham and Egerton, 1968). Whether this moisture is necessary for the maintenance of the interdigital *F. necrophorum* infection, or for the adequate survival of *D. nodosus* on pasture, or both, has not been investigated.

Graham and Egerton (1968) assessed the rainfall requirements for outbreaks of footrot. For outbreaks in spring, at least 50 mm/month during winter was necessary. For summer outbreaks, at least 125 mm/month was required, and for autumn outbreaks, an average in excess of 60 mm/month for summer and autumn was considered necessary. The lag phase between rainfall and onset of transmission could be due to the need for adequate moisture to stimulate sufficient pasture growth to provide sufficiently moist conditions to cause water maceration and render the sheep's feet susceptible to infection.

The effect of temperature, believed to be a factor by Beveridge (1941), was investigated in nine footrot outbreaks (Graham and Egerton, 1968). Outbreaks of footrot were associated with mean daily temperatures above 10°C. At lower temperatures, footrot outbreaks did not occur, even when moisture was not limiting. It was felt that the associated lowering of IDS temperatures would inhibit the multiplication of *D. nodosus*, and possibly prevent colonisation with *D. nodosus*.

Variation in pasture length and / or density has also been thought to influence the prevalence of footrot, although few quantitative data are available. Beveridge (1941) considered lush improved pastures the most favourable for footrot outbreaks. Graham and Egerton (1968) noted outbreaks of footrot on dense pastures, which appeared ideal
for ensuring maceration of sheep's feet; they also noted outbreaks on short green pastures provided there was sufficient moisture. Footrot prevalences were higher in two rotationally grazed mobs when compared to a set stocked mob (Baxter and Smyth, 1956). The pasture was shorter (and drier) in the set stocked paddock. Whittington (1995a) reported transmission on long mature pasture under moist conditions, with 96% of animals being affected. He attributed interdigital abrasion from the mature grasses as a factor in the outbreak, although moisture was also likely to be important, with a lower prevalence of footrot being recorded in sheep in a neighbouring, less dense, pasture. Paddock and sheep differences could not be separated in these observations. Paddock differences in relation to footrot prevalence have also been reported by Beveridge (1941), and Graham and Egerton (1968).

Injury to sheep's feet has been recognised as important in predisposing sheep to \(D.\text{nodosus}\) infection. Grass seeds (Beveridge, 1941; Glynn, 1993), stones (Beveridge, 1941), strongyloidosis (Beveridge, 1941; Cross, 1978a), frosts (Graham and Egerton, 1968) and paddock abrasion (Whittington, 1995a) have all been implicated, but their importance has not been verified. The reproduction of footrot with water maceration and faecal contamination (Egerton et al., 1969), and the strong association between periods of prolonged wetness, pasture suitability for maintaining wetness and the occurrence of footrot, suggest that water maceration is of primary importance in rendering sheep susceptible to \(D.\text{nodosus}\) infection.

Experimentally, sheep's feet have been predisposed to infection by both scarification of the IDS (Beveridge, 1941; Thomas, 1962b; Egerton et al., 1969) and water maceration with faecal contamination (Egerton et al., 1969). The effectiveness of the scarification method experimentally adds weight to arguments that any sufficiently severe damage to the IDS will render sheep's feet susceptible to infection. It also suggests that the \textit{stratum corneum} is an important factor in resistance.
The prevalence of footrot is thus influenced by seasonal conditions, presumably as a result of moisture and temperature changes, and resulting changes to pastures. Egerton et al (1983) found the highest incidence of footrot occurred in mid spring in outbreaks investigated in southern NSW, and the highest prevalence in late October.

The clinical expression of footrot will be determined both by the prevalence of affected sheep and the severity of infection. Whilst there is ample evidence indicating the prevalence of footrot is determined by environmental influences, there is little information specifically on the effect of the environment on the severity of lesions, as most studies only report prevalences. Where footscore data is also reported, it is difficult to interpret in terms of the effect of the environment on the severity of lesions (e.g. Glynn, 1993; Marshall et al., 1991a). Woolaston (1993) reported variation in the severity of lesions due to paddock differences. Recently, a trial in Western Australia was conducted to assess the effect of the environment on the prevalence and severity of footrot (Depiazzi et al., submitted). The strains of \textit{D. nodosus} used were poorly characterised, and the method of reporting of scores was difficult to interpret. However, the results indicated that the severity of footrot associated with a single strain infection varied between different environments. This variation was both seasonal and geographical.
1.5.3. Host factors

1.5.3.1. Variability in susceptibility to \textit{D. nodosus} infection

Variability in susceptibility to \textit{D. nodosus} infection may occur at a number of levels:

- between breeds
- between strains / bloodlines
- between sirelines
- within flocks

(Egerton and Raadsma, 1991).

Differences between breeds in susceptibility to footrot have been suggested (Beveridge, 1941; Egerton et al, 1972, Skerman et al, 1982, Emery et al, 1984, Stewart et al, 1985a; Shimshony, 1989), although the generally low numbers of sheep in the majority of these reports may mean insufficient sires have been used to generate appropriate breed samples (Egerton and Raadsma, 1991). Merinos in particular are considered more susceptible than British Breed sheep, with Merino / British Breed cross sheep showing a greater level of resistance to infection than Merinos (Egerton et al, 1972; Baker et al, 1986). Differences between strains or bloodlines of Merinos have not been adequately investigated (Egerton and Raadsma, 1991).

Within a flock, Beveridge (1941) considered rams more susceptible than ewes or wethers. All ages were susceptible, but weaners (4-12 months) appeared to be less readily and less severely infected. Littlejohn (1961) found rams were most commonly affected, and young sheep least commonly affected, although such reports can be confounded by management and / or paddock differences. Littlejohn (1966/67) reported a higher incidence in young lambs compared with ewes, but this was not the case in one study comparing lambs (mean age 139 days, 75% prevalence) with their mothers (96%
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prevalence) (Woolaston, 1993). These latter observations may have been confounded by
differences in exposure to initial infection. Woolaston (1993) observed increasing
prevalence with increasing age of ewes. Raadsma et al (1994a) found male hoggets
more likely to be infected than female hoggets in a comprehensive study of Merinos (18
months of age). Differences within flocks (mobs) of similar sheep have been reported
(Egerton et al, 1983; Raadsma et al, 1993).

Resistance to footrot is manifested by:

- Absence of clinical signs of disease following exposure
- Ability to contain infection to lesions of low severity
- Ability to heal spontaneously
- Accelerated healing after therapeutic vaccination
- Absence of clinical footrot following preventive vaccination

(Egerton et al, 1972; Egerton et al, 1983; Skerman and Moorhouse, 1987;

Thus, within a flock not all sheep will succumb to footrot when exposed
naturally, and some sheep will develop milder lesions which heal rapidly, even in more
virulent outbreaks (Egerton et al, 1983). The interaction between resistance of sheep and
virulence of infecting D. nodosus strains has not been investigated.

The integrity of the IDS appears important in the relative resistance of sheep
(Egerton and Roberts, 1969; Bulgin et al, 1988). Increasing the severity of challenge
overcame apparent differences in resistance between breeds in one study (Emery et
al, 1984).
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1.5.4. Alternative hosts

*D.nodosus* infections have been reported in cattle, goats and deer.

1.5.4.1. Cattle

Infection of cattle with *D.nodosus* has been reported (Alexander, 1962; Egerton and Parsonson, 1966; Toussaint Raven and Cornelisse, 1971; Thorley et al, 1977; Laing and Egerton, 1978; Stewart, 1979; Richards et al, 1980).

1.5.4.1.1. Clinical outcomes


The prevalence of lesions and presence of *D.nodosus* in feet of cattle reported in the reviewed literature is summarised in Table 1.2.
Table 1.2. Prevalence of lesions and presence of *D. nodosus* in cattle's feet in Australia.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of survey</th>
<th>Time of year</th>
<th>Number examined</th>
<th>Number with lesions</th>
<th>Number with <em>D. nodosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexander, 1962</td>
<td>A</td>
<td></td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Egerton &amp; Parsonson, 1966</td>
<td>A</td>
<td></td>
<td>67</td>
<td>27</td>
<td>32</td>
</tr>
<tr>
<td>Morgan, 1969</td>
<td>B</td>
<td>Sept</td>
<td>2,140</td>
<td>211</td>
<td>13</td>
</tr>
<tr>
<td>Wilkinson et al, 1970</td>
<td>A</td>
<td>Jan</td>
<td>150</td>
<td>25²</td>
<td></td>
</tr>
<tr>
<td>Laing &amp; Egerton, 1978</td>
<td>B</td>
<td>Sept</td>
<td>1314</td>
<td>356</td>
<td>72</td>
</tr>
<tr>
<td>Laing &amp; Egerton, 1978</td>
<td>C</td>
<td>Jan-Jun</td>
<td>19</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Laing &amp; Egerton, 1978⁴</td>
<td>A</td>
<td>Various</td>
<td>322</td>
<td>175</td>
<td>154</td>
</tr>
<tr>
<td>Richards et al, 1980</td>
<td>B</td>
<td>Oct-Nov</td>
<td>19,968</td>
<td>278</td>
<td>31</td>
</tr>
<tr>
<td>Mitchell et al, 1992</td>
<td>A</td>
<td>Nov-Dec</td>
<td>47</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Mitchell et al, 1992</td>
<td>B</td>
<td>Nov-Mar</td>
<td>420</td>
<td>840</td>
<td>0³</td>
</tr>
<tr>
<td>Trengove et al, 1993</td>
<td>A</td>
<td>Various</td>
<td>130</td>
<td>27</td>
<td>25</td>
</tr>
</tbody>
</table>

Notes. 1. A = Property, B = Abattoir, C = Post-Mortem specimens.
2. Average over 2 years.
3. Combined data.
4. No necrotic lesions detected; isolations of *D. nodosus* from cracks, ulcerations- numbers not reported.
Laing and Egerton (1978) found that *D.nodosus* was more prevalent in younger calves (5-12 weeks) in two herds examined. This supported a general observation by Alexander (1962) that younger animals were more likely to be infected.

Any further deductions concerning the likelihood of *D.nodosus* infections in different age groups or breeds are not possible. Only Richards et al (1980) gave any details on breeds of affected cattle, but omitted the breed composition of the sample surveyed. The distinction between beef and dairy breeds was made in one paper (Egerton and Parsonson,1966). All other reports simply referred to "cattle".

Abattoir surveys, whilst overcoming the difficulty of examining live cattle, are likely to yield less valuable information than property surveys. Problems with sample bias due to intensive sampling over a short period, and the possibility of transient infections picked up after animals have left the property limit the usefulness of the data. Nonetheless, lesion prevalence may at least give some indication of the prevalence of infection.

To date, property surveys have been confined to single properties (Egerton and Parsonson,1966; Wilkinson et al,1970; Mitchell et al,1992; Trengove et al,1993) or several properties in different regions (Laing and Egerton,1978). No property surveys to establish the prevalence of *D.nodosus* in cattle herds, or the relationship between the existence of *D.nodosus* infection in sheep and cattle on the same properties have been conducted.

1.5.4.1.2. Bacteriology

The majority of bovine isolates examined appear to be capable of causing only benign footrot in sheep. This is based on both laboratory and challenge data (Table 1.3).
Table 1.3. Analysis of virulence characteristics reported for bovine isolates.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. isolates</th>
<th>Sheep challenge data</th>
<th>In vitro characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.isolates</td>
<td>No.sheep</td>
</tr>
<tr>
<td>Egerton &amp; Parsonson, 1966</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilkinson et al, 1970</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egerton &amp; Laing, 1979</td>
<td>14</td>
<td>13</td>
<td>1-3</td>
</tr>
<tr>
<td>Stewart, 1979</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Richards et al, 1980</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stewart et al, 1982a,84,86d</td>
<td>1</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Mitchell et al, 1992</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Trengove et al, 1993</td>
<td>2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Trengove et al, 1993</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes. 1. From Stewart, 1979. +ve = clearing, with the day clearing first detected indicated in brackets; -ve = no clearing at 21 days.
2. Low PI = low proteolytic index (Egerton and Parsonson, 1969)
   Stable = stable proteases; unstable = unstable proteases in the degrading proteinase test (Depiazzi and Richards, 1979) or gelatin gel protease thermostability test (Palmer, 1993).
However, whilst the majority of isolates tested to date appear to be benign, there are suggestions that some isolates are capable of causing more severe disease in sheep. At least four isolates associated with four different outbreaks of *D. nodosus* infection in cattle have been reported as having *in vitro* characteristics consistent with more virulent isolates (thermostable proteases and/or elastase positive) (Stewart et al, 1984, 1986d; Mitchell et al, 1992; Trengove et al, 1993; R. Walker and I. Links, cited by Stewart and Claxton, 1993). Egerton and Parsonson (1966) observed lesions consistent with "typical ovine footrot" in two weaner sheep when challenged with infective bovine material. The *D. nodosus* isolate, when recovered from these sheep and used to infect a crossbred weaner sheep, caused milder lesions. The bovine isolate used in challenge experiments by Stewart et al (1984) was of intermediate virulence, based on the severity of lesions and body weight changes in sheep, when compared to apparently virulent and benign ovine isolates.

1.5.4.1.3. Transmission

Bovine isolates have been transmitted experimentally to sheep (Egerton and Parsonson, 1966; Stewart, 1979; Toussaint Raven and Cornelisse, 1971; Egerton and Laing, 1978/79; Stewart et al, 1984; Mitchell et al, 1992; Trengove et al, 1993) and to cattle (Egerton and Parsonson, 1966; Laing and Egerton, 1978). Such experiments have added weight to suggestions of transmission of *D. nodosus* organisms from cattle to sheep under field conditions (Wilkinson et al, 1970), but claims of this occurring are poorly documented (for example, Mitchell et al, 1992; Trengove et al, 1993). The transmission of virulent footrot from sheep to cattle has been unsuccessful experimentally (Laing and Egerton, 1978).
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1.5.4.2. Goats

*D.nodosus* infections associated with feet lesions have been reported in a number of breeds of goats (Claxton and O'Grady, 1986; Piriz Duran et al., 1990). Lesions were predominantly interdigital, and in some cases involved marked necrosis. Underrunning of the sole appears less frequent than in sheep (Claxton and O'Grady, 1986; Stewart et al., 1986a).

Both elastase positive and elastase negative isolates, and isolates from a range of serogroups, have been recovered from goats (Claxton and O'Grady, 1986; Ghimire et al., submitted). Isolates from one property had weak elastolytic activity (Claxton and O'Grady, 1986).

When two groups of three sheep were experimentally infected with goat isolates which were elastase positive, lesions consistent with virulent footrot developed in the sheep. Lesions in each of three goats similarly infected had predominantly interdigital lesions and were considered less severely affected than the sheep, based on the scoring system for sheep (Claxton and O'Grady, 1986). A *D.nodosus* isolate from a goat, classified as benign *in vitro*, produced mainly interdigital lesions in sheep, whilst lesions in donor goats had included more severe exudative interdigital lesions with some underrun (Claxton and O'Grady, 1986).

Similarly, when goats were challenged with ovine *D.nodosus* isolates, a benign isolate produced more severe lesions in goats than sheep, whilst a virulent isolate produced less severe lesions in goats than in sheep. In both cases, interdigital lesions in goats were generally of a severe, necrotic, exudative nature (Stewart et al., 1986a).
A proportion of goats challenged with an isolate virulent for sheep had underrun of the hard horn, whilst goats challenged with an isolate benign for sheep had no underrun of the hard horn.

The difference in clinical expression of footrot in goats and sheep has been attributed to the deeper interdigital cleft in goats, and difference in foot conformation (Claxton and O'Grady, 1986).

Goats have been implicated in transmission of *D. nodosus* infection to sheep (Egerton, 1989b), although there is only one experimental report of transmission from a goat to a sheep (Beveridge, 1941). Beveridge (1941) considered goats should be included in footrot eradication programmes, and the recovery of virulent isolates from goats (Claxton and O'Grady, 1986) supports this view. Claxton and O'Grady (1986) reported footrot in sheep in contact with footrot affected goats on three of 6 properties examined. Goat isolates from two of these properties were elastase negative (Stewart, 1979), and elastase positive from one property. No analysis of *D. nodosus* isolates from sheep on these properties was presented, nor was there any information on whether the sheep were likely to have developed footrot from the goats or *vice versa*. On the other three properties, there was no evidence of footrot in 'in-contact' sheep, despite conditions being considered favourable for footrot transmission. From each of these three properties, *D. nodosus* isolates from goats were respectively elastase positive, weakly elastase positive and elastase negative. In Nepal, isolates from sheep and goats in combined infected flocks are indistinguishable (Ghimire et al., submitted).

Therefore, whilst infection of goats with a similar range of *D. nodosus* strains to those in sheep appears likely, the clinical expression of these infections appears to differ, and the transmission of *D. nodosus* infection from goats to sheep may not always occur readily.
1.5.4.3. Deer

*D. nodosus* infection has been reported in deer, with isolation of *D. nodosus* from a hind with separation of the horn at the heel (Skerman, 1983). Isolates were elastase negative, and produced lesions consistent with benign footrot in challenged sheep (Skerman, 1983).

1.5.4.4. Other Species

Organisms morphologically similar to *D. nodosus* have been detected in smears from lesions of the IDS in pigs (Toussaint Raven and Cornelisse, 1971).

There has been no evidence of *D. nodosus* infections in horses (Toussaint Raven and Cornelisse, 1971). Experimental challenge of a hare failed to establish *D. nodosus* infection (Beveridge, 1941).

1.6. Clinical Pathology

1.6.1. Detection of *D. nodosus* infection

1.6.1.1. Examination of lesion material

Clinical diagnosis of infection with *D. nodosus* is possible because of the characteristic nature of interdigital lesions and separation of the soft and hard horn (Beveridge, 1941). The presence of *D. nodosus* organisms in these lesions can be confirmed by:
(i) microscopy

staining of smears taken from the IDS or underrun horn with the Gram stain allows visualisation of typical rod shaped Gram negative organisms (Beveridge, 1941; Stewart and Claxton, 1993). Alternatively, the fluorescent antibody technique (Roberts and Walker, 1973) may be used.

(ii) culture

characteristic spreading of *D. nodosus* colonies can be detected following anaerobic culture on 4% hoof agar (after Thomas, 1958b) at 37°C for 3-5 days. Colonies are translucent, flat, semicircular and granular, and often exhibit concentric zones with a fimbriate edge (Thorley, 1976; Stewart and Claxton, 1993).

(iii) PCR detection

recently, use of species-specific (*D. nodosus*) oligonucleotides as polymerase chain reaction (PCR) primers has provided a sensitive method of detecting *D. nodosus* organisms directly from lesion material (La Fontaine et al., 1993).

Techniques to identify the presence or absence of *D. nodosus* organisms are particularly useful to differentiate OID (Parsonson et al., 1967) from benign footrot, and in eradication programmes, where the diagnosis of a single abnormal foot may be required. The accuracy of microscopy in the diagnosis of individual cases has not been established. Studies to establish the sensitivity and specificity of the PCR-detection technique are in progress (J. Egerton, pers. comm.). Neither microscopy nor PCR-detection assist in distinguishing either the different forms of footrot or the nature of the *D. nodosus* involved, although the potential for PCR to be applied to confirmed virulence attributes of *D. nodosus* exists.
1. Literature Review

1.6.1.2. Serology

Serological tests used in footrot research have included agglutination tests for the assessment of responses to vaccines, bactericidal assays and enzyme-linked immunosorbent assays (ELISA) (Egerton and Merrit, 1970; Egerton, 1973; Stewart et al, 1982b; Fahey et al, 1983; Ferrier et al, 1988). Whittington et al (1990) found that antibody levels against D. nodosus reflected the severity of lesions, using an ELISA technique, and the test showed promise for detecting virulent footrot in young sheep (Whittington and Egerton, 1994). The use of an anamnestic response to D. nodosus protein, with the detection of specific serum antibody may allow the detection of carrier sheep (Whittington and Marshall, 1990). However, this test has not been evaluated for this purpose.

1.6.2. Assessment of Virulence of D. nodosus

The recognition of both different forms of footrot (see sections 1.1, 1.2) and a spectrum of virulence of D. nodosus (Stewart et al, 1986d) has led to in vitro characterisations of D. nodosus which may assist with the diagnosis of footrot at the laboratory level. Virulence categories adopted for D. nodosus are those of the recognised forms of footrot, namely benign, intermediate and virulent, with the implicit assumption that D. nodosus infections resulting from isolates of a determined in vitro virulence result in an outbreak of that form of footrot.

The majority of the tests have been based on differences in the proteolytic action of extracellular enzymes (proteases) (Thomas, 1962a; Egerton and Parsonson, 1969; Depiazzi and Richards, 1979; Stewart, 1979; Every, 1982; Kortt et al, 1982), although colony characteristics (Stewart, 1975; Thorley, 1976; Depiazzi and Richards, 1985), outer membrane complex proteins and the presence of fimbriae have also been analysed (Stewart et al, 1986d). More recently, the use of genetic, rather than phenotypic,
methods are being assessed with the advent of gene-probe techniques (Rood and Yong, 1989; Katz et al, 1991; Liu and Yong, 1993b; Liu, 1994).

1.6.2.1. Colony Morphology

Colony morphology has been related to the virulence of isolates (Stewart, 1975; Short et al, 1976; Skerman et al, 1981; Thorley, 1976). Colonies of virulent isolates tend to have a beaded appearance, rather than mucoid, and a fimbriate edge. The media and technique used influence colony morphology (Stewart et al, 1986d), and this may partly explain why, at times, no differences between benign and virulent isolates have been detected (Depiazzi and Richards, 1979; Stewart, 1979; Depiazzi et al, 1991). Stewart et al (1986d) considered colony morphology useful as an aid to differentiating virulent, intermediate and benign isolates.

Colony size (diameter) was considered a useful means of discriminating between benign and virulent isolates, although two isolates considered to be of intermediate virulence could not be distinguished from benign isolates on colony diameter (Depiazzi and Richards, 1985). However, Stewart et al (1986d) found considerable overlap in colony diameter when assessing virulent, intermediate and benign isolates.

Twitching motility, using a hanging drop technique, has been used to differentiate benign and virulent isolates (Depiazzi and Richards, 1985; Depiazzi et al, 1991). However, two isolates considered to be of intermediate virulence could not be differentiated from benign strains (Depiazzi and Richards, 1985). There was a high correlation between twitching motility and colony diameter. Two goat isolates, considered virulent for sheep, had a mean twitching motility similar to benign isolates (Depiazzi et al, 1991).
The combination of twitching motility and protease thermostability tests was able to differentiate two intermediate isolates, which had low twitching motility (similar to benign strains, distinct from virulent strains) and stable proteases (similar to virulent strains, distinct from benign strains) (Depiazzi and Richards, 1985).

1.6.2.2. Fimbriae (pili)

Degree of piliation was initially thought to be a useful criterion for assessing virulence (Stewart, 1975; Skerman et al, 1981). However, the degree of piliation has not enabled differentiation of virulence in a number of studies, with both benign and virulent isolates possessing fimbriae (Stewart, 1979; Depiazzi and Richards, 1985; Stewart et al, 1986d; Depiazzi et al, 1991).

1.6.2.3. Tests for Proteolysis

These tests assess, quantitatively or qualitatively, the proteolytic activity of the extracellular enzymes, their stability or their electrophoretic mobility.

i. Proteolytic Index (Egerton and Parsonson, 1969)

A casein agar plate test was evaluated in a study which involved 25 isolates (12 isolates from flocks with benign footrot, 13 isolates from flocks with virulent footrot) (Egerton and Parsonson, 1969). Isolates from benign and virulent outbreaks could be differentiated on their proteolytic activity, with benign strains having a consistently lower Proteolytic Index. These results were subsequently confirmed, although the Proteolytic Index was considered difficult to interpret, with no clear distinction between some of the benign and virulent strains (Depiazzi and Richards, 1979; Stewart, 1979).
ii. *Elastase test* (Stewart, 1979)

This test assesses the hydrolytic action of *D. nodosus* on elastin. Eighty seven isolates were initially tested, with virulent isolates producing clearing of elastin particles (positive) and benign isolates failing to cause any clearing (negative). The ability of the elastase test to differentiate virulent and benign isolates was confirmed (Skerman et al, 1981; Stewart et al, 1986d), although all intermediate strains could not be differentiated on the elastase result (Stewart et al, 1986d). Liu and Yong (1993, a, b) claimed elastase results were well correlated with virulence, and when used as a quantitative test, by recording the day clearing was first observed, differentiated benign, intermediate and virulent isolates. However, they produced no evidence about the outbreaks from which these isolates were recovered to support this latter claim.

iii. *Degrading Proteinase Test (DPT)* (Depiazzi and Richards, 1979).

This test is based on the relative stability of *D. nodosus* proteases. Initially the test was carried out at 37°C for 12 days, but was subsequently modified as a heat stability test (protease thermostability test, PTT), with samples being heated at 40°C, then 70°C for 15 minutes each (Depiazzi and Richards, 1985) or 55°C for 30 minutes (Stewart et al, 1982a; Stewart and Claxton, 1993) or at 60°C for 20 minutes (Green, 1985). Hide powder azure was used as the substrate.

Based on protease thermostability, *D. nodosus* isolates fall into one of two discrete categories - stable or unstable (Depiazzi et al, 1991).

The ability of the DPT and PTT to differentiate benign and virulent isolates has been demonstrated (Depiazzi and Richards, 1979; Stewart, 1979; Stewart et al, 1982a; Depiazzi and Rood, 1984; Depiazzi and Richards, 1985; Green, 1985; Stewart et al, 1986d; Depiazzi et al, 1991; Palmer, 1993) with virulent isolates having stable and thermostable

However, intermediate strains of *D.nodosus* have not been differentiated (Depiazzi and Richards,1985; Stewart et al,1986d). Intermediate strains generally have stable proteases (Depiazzi and Richards,1985; Gordon et al,1985), although this can be variable, with one isolate showing thermolabile proteases (Stewart et al,1986d).

iv. *Gelatin Gel Protease Thermostability Test* ("Gelatin Gel" or GGPTT) (Palmer,1993)

This test is similar to the hide powder azure thermostability test. Samples are heated at 68°C for 8 minutes, with gelatin being the test substrate. Following the testing of a large number (2965) of *D.nodosus* isolates, it was concluded that *D.nodosus* could be divided into two discrete groups - those with thermostable proteases, and those without thermostable proteases (Palmer,1993). Identical results with the PTT were recorded for 47 of these isolates (Palmer,1993). The relationship between the GGPTT result and clinical expression of disease was not given. One thermolabile isolate produced lesions consistent with benign footrot when Merino sheep were challenged.

The examination of 96 *D.nodosus* isolates indicated a strong correlation between rapid elastase clearing and protease thermostability (gelatin gel), and between failure to digest elastin and protease thermolability, with all 7 day positive isolates in the elastase test being protease thermostable, and all elastase negative isolates (at 28 days) being protease thermolabile (Liu and Yong,1993a). These authors claimed virulent isolates were thermostable, and distinguishable from benign strains which were unstable, with intermediate strains giving incomplete clearing, but the basis for these claims is not clear, due to their failure to adequately define the disease from which the isolates originated.
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Results from studies in South Australia showed a poor relationship between the occurrence of benign footrot and the presence of protease thermostable isolates, using the GGPTT. In these studies, benign footrot was defined as 1% or less of sheep having at least a score 4 lesion, and virulent footrot was defined as more than 1% of sheep having at least one score 4 lesion. In one study, 8 of the 12 isolates (from 12 different flocks) were GGPTT positive (thermostable), whilst 11 of the 12 flocks were diagnosed as having benign footrot (McFarland and Saunders, unpublished). Similarly, in a survey in 1994, 10 of 19 flocks diagnosed as having benign footrot yielded protease thermostable isolates (Cleland, unpublished). Further, in an analysis of 72 isolates recovered from 29 outbreaks of virulent footrot and 43 outbreaks of benign footrot, 28 of 29 isolates from virulent outbreaks were GGPTT positive, yet only 25 of 43 isolates from benign outbreaks were GGPTT negative (Saunders and Riley, unpublished). On this basis, the GGPTT is sensitive, but lacks specificity.

Findings were similar in Victoria, where in a survey of 37 properties, 6 of 8 outbreaks of footrot classified as virulent yielded *D. nodosus* isolates which were GGPTT positive, yet 24 of 30 GGPTT positive isolates were from clinically benign flocks (Roycroft and Harrison, unpublished). In this survey, flocks were considered to have virulent footrot if 5% or more sheep had lesions greater than score 3b lesions (underrunning halfway across heel), while benign footrot was considered present if less than 5% of sheep had lesions more severe than score 3b.


This method utilises the electrophoretic mobility of the protease isoenzymes, which give characteristic isoenzyme patterns. Zymograms have differentiated benign and virulent isolates (Every, 1982; Kortt et al, 1982; Gordon et al, 1985; Stewart et al, 1986d; Depiazzi et al, 1991; Palmer, 1993; Liu and Yong, 1993b), although intermediate isolates cannot be differentiated (Gordon et al, 1985; Stewart et al, 1986d; Liu and Yong, 1993a).
Bovine isolates have been differentiated from ovine benign and virulent isolates (Every, 1982).

Protease zymogram patterns are considered to correlate well with the virulence of *D. nodosus* isolates (Gordon et al, 1985). There is a high correlation between recognised virulent zymogram patterns and protease thermostability, and between benign zymogram patterns and protease thermolability (Stewart et al, 1986d; Depiazzi et al, 1991; Palmer, 1993), although exceptions have been reported. Stewart et al (1986d) reported an isolate of intermediate virulence as having an unstable protease yet virulent zymogram pattern. Palmer (1993) identified an isolate with a similar profile (thermolabile protease, virulent zymogram). The isolate had originated from a "benign footrot lesion", and produced benign lesions when Merino sheep were challenged. This isolate was the only anomalous result between protease thermostability and zymogram pattern in 2,965 *D. nodosus* isolates tested (Palmer, 1993).

Palmer (1993) claimed Gordon et al (1985) reported a similar anomaly, yet the isolate referred to (P343) was reported as having a single band (RF 73) which was common in both benign and virulent zymogram patterns. This protease thermolabile isolate was classified as virulent on clinical challenge data, but no records of this data are available (Liu, pers. comm.). The isolate was described as an "aberrant strain" (Gordon et al, 1985).

1.6.2.4. Gene Probes

The potential use of gene probes for differentiating *D. nodosus* strains into virulence categories has been discussed (Rood and Yong, 1989). Katz et al (1991) identified three virulence-associated gene regions which were used as three hybridisation probes to differentiate *D. nodosus* strains into different categories. The classification of
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101 *D. nodosus* isolates into three hybridisation categories was said to be correlated with the virulence of the isolates, although the basis for the original classification of these isolates was not clear. Liu and Yong (1993b) used virulent and benign specific gene probes in combination to differentiate benign and virulent isolates. Liu (1994) evaluated a further 6 virulent specific probes and one benign probe, and concluded that by using two probes, virulent, intermediate and benign strains could be differentiated. However, while the definition of the 96 isolates used in both these latter studies to evaluate the gene probes was reportedly based on a number of criteria, the presentation of results appeared to be based only on elastase results, with almost half the isolates coming from flocks with no clinical data.

Thus, the use of gene probes to differentiate the virulence of *D. nodosus* isolates appears promising, but as yet the probes have not been adequately evaluated.

1.6.2.5. Comments and Conclusions on *in vitro* Virulence Testing

From the preceding review, it is clear that protease based tests can categorise *D. nodosus* isolates. What is less clear is the relationship between the categories of *D. nodosus* isolates, and the nature of the footrot which was associated with these isolates. Failure to define adequately the footrot outbreaks from which isolates were recovered or which resulted from infection with them, the assumption that an isolate recovered from an outbreak of footrot has the same virulence as the footrot outbreak, and the inconsistent use of terminology have all resulted in difficulty in interpreting the effectiveness of *in vitro* tests for the diagnosis of benign, intermediate and virulent footrot.

In their major study of 22 isolates, Stewart et al (1986d) used pen (or field) challenge data to categorise *in vivo* 20 of the 22 isolates (two isolates were considered benign because they came from a benign flock (undefined)), with at least 7 sheep being
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challenged per isolate. On the other hand, Depiazzi et al (1991) carried out pen challenge trials on only 6 feet of Merino sheep, and used different criteria for classification of isolates from the definitions used previously by Depiazzi and Richards (1985) and Stewart et al (1986d). *D. nodosus* isolates from New Zealand were assessed by challenge data, yet neither the method of predisposition nor the number of sheep challenged were given (Skerman et al, 1981; Every, 1982; Green, 1985). Results of challenges were not presented in many cases (Skerman et al, 1981; Every, 1982; Depiazzi and Richards, 1985; Gordon et al, 1985; Green, 1985; Depiazzi et al, 1991; Liu and Yong, 1993a, b). Depiazzi and Richards (1985) used a combination of lesion severity in the field and challenge trials, and whilst sheep where the diagnosis was not apparent were assessed for 12 weeks, it is likely that sheep from different flocks were used. It is not clear whether feet were scarified prior to challenge. Similarly, Depiazzi and Richards (1979) assessed lesions on properties and used wet mats to assist with this assessment, so presumably sheep from different flocks (and different genotypes) were used. The number of sheep inspected was not given. Two sheep per isolate were used by Stewart (1979).

Liu and Yong (1993a, b) and Liu (1994), in a series of three papers, claim that clinical data and elastase results were used to assess 96 isolates, and include a table on the relationship between clinical data and elastase results. No clinical data was presented, and 45 of the 96 isolates were subsequently shown to have no clinical data. The elastase test was assessed on its relationship to the virulence of the isolates, which were reputed to have been classified on the basis of elastase results, partially, if not completely (in the absence of clinical data).

Despite these criticisms, several conclusions can be made on the use of *in vitro* tests to differentiate different forms of footrot. The *in vitro* classification of *D. nodosus* isolates into benign and virulent is related to some extent to the type of footrot outbreak which results from the associated *D. nodosus* infection. Outbreaks of virulent footrot are
generally associated with isolates classified as virulent on *in vitro* characteristics, and isolates categorised *in vitro* as benign are associated with outbreaks of benign footrot. However, isolates classified as virulent *in vitro* may be derived from benign, intermediate or virulent footrot in the field; and outbreaks of footrot considered benign may yield either benign or virulent isolates on *in vitro* classification. Intermediate footrot is generally associated with *D. nodosus* strains with virulent protease characteristics (Stewart, 1989).

At present, no single *in vitro* characteristic allows adequate virulence categorisation of *D. nodosus* isolates as intermediate. However, by using several tests in conjunction, categorisation of strains as intermediate is possible (Stewart, 1989). The claim that the use of a number of gene probes allows categorisation of virulent, intermediate and benign isolates (Liu, 1994) does not appear to be well-established and the association between *in vitro* classification and the type of footrot associated with these isolates is unclear.

Reservations over the assignment of the category of 'intermediate virulence' to isolates in some reports remain. Until the different forms of footrot are adequately defined quantitatively, and outbreaks of footrot are appropriately classified on the basis of these definitions, the ability to assess the usefulness of *in vitro* virulence characteristics of *D. nodosus* isolates will be limited. Further, until all virulence characteristics of *D. nodosus* are known, the validity of the assessment of individual isolates *in vitro* to indicate the virulence of the disease may be questioned.
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1.7. Epidemiological Markers

1.7.1. Serogrouping

Antigenic heterogeneity amongst *D. nodosus* isolates was first observed by Beveridge (1941), and has subsequently been further documented (Egerton, 1973; Thorley, 1976; Schmitz and Gradin, 1980; Claxton et al, 1983; Claxton, 1986a; Day et al, 1986; Thorley and Day, 1986; Chetwin et al, 1991; Stewart et al, 1991b). Egerton (1973) classified 33 out of 46 isolates on the basis of K (surface) antigens into types A, B, and C, using tube agglutination tests. The K antigens were subsequently shown to be the fimbriae of *D. nodosus* (Walker et al, 1973; Stewart, 1973; Stewart, 1978). English and American isolates of *D. nodosus* were differentiated into 9 and 14 (respectively) serologically distinct serotypes, using tube K-agglutination tests (Thorley, 1976; Schmitz and Gradin, 1980). Three of the 14 American isolates corresponded to Egerton's A, B and C types (Schmitz and Gradin, 1980).

The continued K-agglutination testing of *D. nodosus* isolates has led to two classification systems, with considerable overlapping between the two. The system predominantly used, and most relevant for vaccine purposes (Stewart et al, 1991b), is the serogroup classification, developed by Claxton et al (1983). Isolates are assigned to one of 8 major serogroups (A-H inclusive) on the basis of slide agglutination tests, and isolates in each group share major pilus antigens. Within serogroups, subgroups can be differentiated using cross-tube agglutination tests. Initially at least 16 subgroups were recognised (Claxton et al, 1983). Using the serogrouping system, 1260 out of 1267 Australian *D. nodosus* isolates and all of 449 British isolates were classified into one of the 8 serogroups. The 7 isolates not classified produced small non-fimbriate colonies.

This serogroup classification has been extended to include a ninth group, I (Claxton, 1986a). Further extensions to include two further serogroups, O (Stewart et al, 1991b) and M (Chetwin et al, 1991) have been advocated.
The second system, advocated by Thorley and co-workers, is based on the more specific serotypes recognised when using absorbed antisera. This grouping is based on the sharing of specific pilus antigens unique to the serotype (Stewart et al, 1991b), and 17 serotypes were recognised (A-H inclusive, J-R inclusive) (Thorley and Day, 1986; Day et al, 1986). Some of the serotypes are equivalent to subgroups identified in the serogroup system (Thorley and Day, 1986; Day et al, 1986).

In comparing the two systems, Stewart et al (1991b) considered serogroups more relevant for vaccine purposes, due to the cross-protective immunity within serogroups (Stewart, 1978; Stewart et al, 1982b; Every and Skerman, 1982; Stewart et al, 1986c; Egerton et al, 1987; Stewart and Elleman, 1987). Serotyping was considered to have more potential in epidemiological studies (Stewart et al, 1991b).

Multiple *D.nodosus* serogroups or serotypes have been demonstrated within flocks, within sheep and within sheep's feet (Schmitz and Gradin, 1980; Claxton et al, 1983; Hindmarsh and Fraser, 1985; Thorley and Day, 1986; Gradin et al, 1993). Claxton et al (1983) reported an increase in frequency and multiplicity of mixed infections as the number of colonies examined per foot increased. Serotypically distinct *D.nodosus* isolates have been detected from the same sheep at different samplings (Schmitz and Gradin, 1980; Hindmarsh and Fraser, 1985). However, no evidence for fimbrial antigenic shift *in vivo* has been detected (Moore et al, 1990). The normal multiplicity of antigenic types within flocks and unresolved sampling problems makes antigenic classification of isolates unsuitable as an epidemiological tool in naturally occurring outbreaks of footrot.
1.7.2. DNA Analysis

The use of bacterial DNA analysis as an epidemiological tool for investigating the source of outbreaks of footrot has been suggested (McGillivery et al, 1989). Using the restriction endonuclease BamHI, restriction endonuclease profiles of 29 D.nodosus isolates were examined. The authors considered this technique gave characteristic profiles for individual D.nodosus isolates, and used two case studies to demonstrate the epidemiological implications of the technique. However, few, if any, phenotypically similar but epidemiologically distinct isolates were examined in this analysis, and only one isolate per outbreak was examined in three of the four outbreaks reported.

1.8. Importance of footrot

1.8.1. Prevalence of footrot

Despite the considerable concern and interest in footrot, there have been relatively few surveys designed to determine accurately the prevalence of the various forms of the disease, nor have any definitions of these forms been generally accepted. Estimates by field officers (Anon., 1988; Walker and Plant, 1994) or numbers of properties in quarantine (Napthine, 1986a) have been used as a guide to the prevalence of footrot. Such figures have tended to underestimate the 'true' prevalence (Dobson, 1986; Locke and Coombes, 1994; Roycroft and Harrison, unpublished). Lack of definition or inconsistent definitions, and differences in methodology have also made interpretation of figures difficult.

An estimated 50 million sheep were at risk to footrot in Australia in 1986 (Donald, 1986), with 14.2 million sheep being in footrot affected flocks in New South Wales, Victoria and Tasmania (Allworth, 1988).
1. Literature Review

In New South Wales, an estimated 5.5% of the 41,000 flocks were affected with virulent footrot in 1994, compared to 7.3% in 1988 (Walker and Plant, 1994). The definition of virulent footrot was not given, but includes intermediate forms of footrot (R. Walker, pers. comm.). In an interview survey in 1989/90, the point estimate of the prevalence of virulent footrot (not defined) ranged from 4% to 49% in southern New South Wales, with 9 of the 11 districts surveyed having a higher prevalence than that estimated by field officers (Locke and Coombes, 1994). Sheep were not examined, and where the interviewee was uncertain of the footrot status, the property was excluded from analysis.

In Victoria, 46% of flocks were estimated to be affected with footrot in 1954, and in 1963, 65% of flocks in higher rainfall areas were considered affected (Tweedle, 1981). In 1967, an estimated 10% of total flocks in Western Victoria had footrot (Tweedle, 1981). In the Footrot Control Area (FCA), comprising 14 districts in western Victoria, where regulatory activities commenced in 1969/70, the prevalence was 0.5% in 1993, based on the number of properties in quarantine (Anon., 1993). Estimated flock prevalences in various regions in the rest of the state ranged from 7.3% to 15% (Anon., 1993). These figures were supported by surveys in the south-west region in 1991 and in Gippsland in 1993, indicating a prevalence for virulent footrot of 6% and 7% respectively (J. Larsen, pers. comm.). Surveys carried out in the FCA suggested that few if any infected properties were not in quarantine (Naphthine, 1986a), and the prevalence from quarantine figures was believed to be an overestimate (Naphthine, 1986b).

However, a survey in 1993 in western Victoria involving 134 properties in 7 of the original 14 shires in the FCA found the prevalence of virulent footrot to be 5.9% of flocks (Roycroft and Harrison, unpublished). In addition, 24 of 29 isolates recovered from 29 flocks with benign footrot were positive in the GGPTT.
1. Literature Review

In Tasmania, where regulatory measures had been in place from 1939 to 1975, 17.5% and 16.2% of flocks surveyed in 1982 and 1983 respectively were found to have evidence of *D. nodosus* infections (Elliott, 1986), but no distinction was made between different forms of footrot.

In South Australia, following regulatory measures introduced in 1957, the prevalence decreased from an estimated 24% of flocks in 1957 (Brownrigg, 1986) to less than 1% of flocks being quarantined in the 1980's (Dobson, 1986). A survey in 1991 on Kangaroo Island found 12 out of 63 properties with *D. nodosus* infections. *D. nodosus* isolates from 8 of these 12 flocks were positive in the GGPTT, although only one of the outbreaks was classified as virulent footrot on clinical grounds (P. Saunders, pers. comm.). A survey of 74 flocks in the South East of South Australia in 1994 found clinical signs of footrot on 39% of properties, with an estimated prevalence of 4.1% (95% confidence limit 0.8% - 11.4%) for virulent footrot (at least 1% of sheep with score 4 lesions). Isolates from 10 of the 19 flocks classified as affected with benign footrot were positive in the GGPTT.

In Western Australia, where regulation of footrot has existed since 1949, there were no known infected properties in June 1983, although outbreaks have occurred subsequently (Gwynn, 1986). At present, 209 properties (2%) are in quarantine (Anon., 1995), based on the isolation of GGPTT positive *D. nodosus* isolates on these properties.

A number of surveys have assessed the prevalence of benign footrot. In a survey of 90 flocks in western Victoria, all considered free of virulent footrot (but no definition was given), 63 of the 90 flocks had some sheep with inflammation of the IDS. Smears collected from sheep's feet on 34 of these 63 flocks had organisms consistent with *D. nodosus* (Morgan et al, 1972). This suggested 38% of all flocks without virulent footrot had benign footrot, and the remaining 29 flocks had OID (Parsonson et al, 1967).
In surveys in 1991 in the south-west region of Victoria and in 1993 in Gippsland (Victoria), 24% and 23% of surveyed flocks had footrot, respectively, with 18% and 16% of flocks considered to have benign footrot (Larsen, unpublished; Hides, unpublished). In the survey in 1993 in western Victoria involving 134 properties in 7 of the original 14 shires in the FCA, 89 properties (66%) had evidence of \textit{D. nodosus} infections, despite many farmers footbathing sheep within 48 hours prior to the property visit (Roycroft and Harrison, unpublished). Of the 89 infected properties, 81 properties were considered to have benign footrot present, although 24 of 29 isolates from these properties were positive to the GGPTT. No samples were collected from clinically normal feet in these surveys. In the 1994 survey in South Australia, 26 of 74 flocks (35%) were considered affected with benign footrot (Cleland, unpublished).

No surveys have been conducted to determine the flock prevalence of intermediate footrot, or strains of \textit{D. nodosus} considered to be of intermediate virulence. The prevalence of clinically benign, GGPTT positive isolates in western Victoria was 80% of flocks assessed, and presumably 48% of properties surveyed (Roycroft and Harrison, unpublished); on Kangaroo Island 11% of flocks clinically benign flocks yielded GGPTT positive isolates; and in South East of South Australia 53% of clinically benign flocks yielded GGPTT positive isolates. \textit{Isolates that are protease thermostable from clinically benign flocks are likely to be of intermediate virulence (D. Stewart, pers. comm.)}. Intermediate strains of \textit{D. nodosus} are believed to predominate in footrot outbreaks in South Australia (Dobson, 1986) and were considered to be mainly responsible for the footrot outbreaks in the mid 1980's in Western Australia (Gwynn, 1986). A ratio of virulent footrot to intermediate footrot of not less than 4 to 1 has been estimated in New South Wales (Egerton and Raadsma, 1991).
1.8.2. Economic Impact

Experimentally, a number of consequences of footrot on the productivity of sheep have been recorded. Loss in body weight (Stewart et al., 1984; Marshall et al., 1991a; Glynn, 1993), decreased wool production (Symons, 1978; Marshall et al., 1991a; Glynn, 1993) and myiasis (flystrike) (Marshall et al., 1991a) have been associated with footrot. In pen trials, sheep affected with virulent footrot lost 5.3% of body weight compared to unaffected sheep which gained 1.4% over an 8 week period (Symons, 1978). This would equate to a body weight difference of 3.3 kg in a 50 kg sheep. Marshall et al. (1991a) found differences of 4.1 kg, 6.4 kg and 7.3 kg after 1, 2 and 3 years, respectively, of infection with virulent footrot in Merino sheep compared to treated controls. Sheep exposed to virulent footrot (23% affected) were 6.5 kg lighter after 10-12 weeks compared to sheep exposed to benign footrot (Stewart et al., 1984). In a second experiment, sheep exposed to virulent footrot (94% affected) were 4.4 kg lighter after 13 weeks compared to sheep exposed to benign footrot (Stewart et al., 1986a). Weight loss occurred mainly during transmission periods when infections were active, with weight differences being maintained during non-transmission periods, except in treated sheep which regained the lost weight (Marshall et al., 1991a).

Lambing percentage would also be expected to decrease if body weight differences caused by footrot during a transmission period were still evident at joining. A decrease in 5 kg body weight could result in 8%-10% less lambs marked (Morley et al., 1978).

Decreases in wool production of 10% over 8 weeks in penned sheep (Symons, 1978), and 0.4 kg clean fleece weight (CFW) over 12 months in Merino sheep at pasture (Marshall et al., 1991a) have been recorded in sheep affected with virulent footrot. A decrease in fibre diameter was associated with the decrease in CFW. This
would be expected if the loss in wool production is due to decreased feed intake, as suggested by Symons (1978).

All these estimates were derived from single strain infections with isolates of high virulence. No productivity measurements have been made in flocks naturally affected with virulent footrot, although in observations from a natural outbreak of footrot in British breed sheep, footrot affected sheep were lighter than unaffected sheep (Littlejohn, 1964).

Conclusions from the limited production information available are therefore that virulent footrot may be associated with losses in the order of 5% to 10% in bodyweight, 5% to 10% in lambing percentage, and 5% to 10% in wool production (with an associated decrease in micron, and possible decrease in tensile strength), assuming results from single strain infections are representative of naturally occurring outbreaks. Based on these estimates, losses of $3 to $6 per head per year in a medium wool Merino flock would be likely in uncontrolled outbreaks of virulent footrot at current prices (1995). This represents a decrease in gross margin of 17% to 35%.

In flocks where control of footrot occurs, virulent footrot has been estimated to cost $5.00 per head per year in direct costs (Allworth, 1994). Indirect costs associated with restrictions on other management options due to footrot were estimated to be a further $4.50 per head. Higher estimates of $19.00 per affected sheep, and $14.35 per sheep on an infected property have been made (Egerton and Raadsma, 1991), but these estimates assume a high loss in sale value, which would not be realistic in most cases.

Footrot induced with intermediate strains of \textit{D. nodosus} have resulted in weight losses in affected sheep between those of virulent and benign footrot (e.g. 0, -3.0 kg, -6.5 kg, and 0, -2.3 kg, -4.4 kg for benign, intermediate and virulent strains respectively) (Stewart et al., 1984, 1986a). In a trial involving 90 sheep, 45 sheep deliberately
challenged with an intermediate strain of *D. nodosus* were 7 kg lighter than the unaffected controls at the peak of the footrot outbreak, and 5 kg lighter at the end of the autumn (Wilkinson, unpublished). Affected sheep produced 10% less wool, and the wool was finer (0.7 micron). There were no differences in tensile strength between the two groups. These results are similar to those reported above for virulent footrot. The criteria by which this strain was classified were not clear, although it was suggested that score 4 lesions did not occur. In 1986, it was estimated that this intermediate footrot cost 80c per head per year (Wilkinson, unpublished). More recently, intermediate footrot was estimated to cost between $2.40 and $3.90 per sheep if uncontrolled (Egerton and Raadsma, 1991).

Benign outbreaks of footrot have also been implicated in decreased weight gains. Sheep affected with benign footrot were 2.6 kg lighter than treated controls in November (Glynn, 1993). This difference had decreased to 0.8 kg by February. Whether this weight loss, which occurred in only one year of a three year study, was due to the effects of benign footrot or grass seed penetration could not be determined. Weight differences were greatest when grass seed penetration was most prevalent.

Sheep which had benign footrot (clinical diagnosis), but yielded 6 *D. nodosus* isolates with *in vitro* characteristics consistent with their being of intermediate virulence, produced 0.2 kg less fleece weight and produced more tender fleeces compared to treated controls (Glynn, 1993). This was estimated to cost $1.79 per sheep annually, although $0.55 per sheep would be the current (1995) market value of the loss, allowing for a decrease in fibre diameter. Benign footrot has been estimated to cost $0.05 to $0.20 if uncontrolled (Egerton and Raadsma, 1991).

It is clear from the above review that footrot can result in losses in productivity and consequently decreased farm returns. In outbreaks of virulent footrot, these losses are estimated to be substantial, although these estimates are only based on experiments.
using single strain infections. The failure to adequately define intermediate and benign footrot, the limited investigations, and the variable results (with benign footrot) mean that there is little objective information on which to assess the economic impact of the different forms of the disease.

1.10. Treatments

Considerable interest and work has been directed towards the treatment of sheep with footrot, and many preparations have been used. Interpretation of the information on treatments is limited by a number of factors:

- lack of control groups (Stewart, 1954a; Forsyth, 1957; Sinclair, 1957; Baxter and Smyth, 1956; Harris, 1968; Casey and Martin, 1988; Venning et al, 1990)
- control groups being run separately to treatment groups (Malecki and Coffey, 1987; Lambell and Chapman, unpublished; Malecki, unpublished; Shepherd and Headlam, unpublished)
- lack of replication (most trials, except Sinclair, 1957)
- variation in conditions under which trials were performed (wet versus dry, active lesions versus chronic lesions, duration of lesions, transmission versus no transmission)
- variation in breed and / or age of sheep
- new infections occurring, making assessments of therapeutic effectiveness difficult (Sinclair, 1957; Bulgin et al, 1986; Malecki and Coffey, 1987)
- variation in frequency of treatment
- variation in preparation of infected feet (paring)
- period from last treatment to assessment of therapeutic effectiveness may have been too short, especially for some topical chemicals
- variation in reporting of therapeutic effectiveness, or inaccurate reporting (Venning et al, 1990; Malecki, unpublished)
1. Literature Review

The impossibility of interpreting treatment effects without a control group is highlighted by the range in self-cure where controls have been kept (Table 1.4). Self-cures range from 0% to 82% of sheep, and 0% to 79% of feet (e.g. Stewart, 1954b). These differences may be partly due to environmental conditions, with a tendency for higher cure rates under dry conditions, or partly due to breed differences, or to paring. However, unexplained variations were apparent (for example, 0% to 35% in Merinos under similar conditions (Egerton et al, 1968)). Given the inability to assess the effectiveness of a treatment in the absence of untreated sheep, reports on the effectiveness of treatments in the absence of control sheep are excluded from this review, unless comparisons were made with other treatments.

The assessment of the therapeutic effectiveness of chemicals by examining sheep's feet 6-21 days after the last treatment is also open to question, as there is little information on the time taken for chemicals to effect an absolute cure. Beveridge (1941) recommended "at least 2 weeks" between the last treatment and detection of infected sheep, as "sheep which have recovered may carry infection on the feet for 1 or 2 weeks but not 4 weeks". Stewart (1954a), during a 4 week observation period, recorded relapses following topical treatment 3 to 21 days post treatment, although only 1 to 2 feet per treatment were assessed. The topical antibiotic treatment Chloromycetin® (10%) was the only effective topical treatment assessed where relapses did not occur. Similar findings were reported by Thomas (1958a), with relapses occurring 3-28 days post treatment. Again, no relapses were reported following Chloromycetin® application. Relapses more than 4 weeks post treatment following formalin footbathing have been suggested by Plant and Claxton (1986). Baxter and Smyth (1956) considered relapses occurred 4-6 weeks and 4-7 weeks after treatment with a 5% chloramphenicol formulation and a 10% chloramphenicol tincture respectively. Glynn (1993) detected *D. nodosus* organisms in smears from sheep with normal feet following treatment with a zinc sulphate formulation. Relapses following two one hour footbathing treatments in
1. Literature Review

this zinc sulphate formulation 5 days apart have been reported more than 40 days after the initial treatment (Atkins, 1986).

Following cessation of formalin and zinc sulphate footbathing, a higher rate of new cases developed in the footbathed groups compared with untreated controls (Skerman et al., 1983a). This may have been due to a failure of footbathing treatments to completely eliminate *D. nodosus* infection, or due to the more susceptible sheep being protected by footbathing, or a chance occurrence.

Thus, the assessment of the effectiveness of topical treatments may need to be made at least 8 weeks after treatment, to prevent including partially cured feet (or sheep) as cured.

Conversely, using parenteral antibiotics, Egerton et al. (1968) found no evidence of relapses in sheep assessed as cured 3-4 weeks post treatment when monitored for 6 to 18 months. *Similarly, no relapses were detected in sheep treated with penicillin/streptomycin or lincomycin / spectinomycin when considered cured initially at 17 days post treatment and reinspected at 50 days post treatment (Venning et al., 1990).*

Much of the work on the efficacy of treatments was undertaken prior to the full understanding of the importance of *F. necrophorum* in the pathogenesis of footrot. Treatments were deemed successful due to their efficacy on *D. nodosus*, whereas curing a footrot lesion may well be due to elimination of *F. necrophorum*, or other flora, and reducing but not eliminating *D. nodosus*.

For consistency, the term *therapeutic effectiveness (TEf)* will be used only when control groups have been kept. The TEf, expressed as a percentage, have been calculated by the formula:
1. Literature Review

Therapeutic effectiveness (TEf) (%) = \(100 \times \frac{B - A}{B}\),

A = % affected in treated groups
B = % affected in untreated groups

(Wilson and Miles, 1975).

If the TEf was calculated to be less than 0%, it is reported as 0%.

Only those preparations which are available for use at present (1995) are reviewed. Results of the effectiveness of treatments has been determined both on a sheep basis (Table 1.4a) and by reporting the number of feet cured (Table 1.4b).

1.10.1. Paring

Extensive paring of affected digits to remove all infected tissue has been widely promoted, generally in conjunction with a topical treatment (Beveridge, 1941, 1956; Pryor, 1954; Forsyth, 1957; Littlejohn, 1961; Hart et al, 1962). There have been no trials comparing severe paring alone with unpared controls. Plant and Claxton (1986) claim "to have highlighted the importance of thorough paring", but had no unpared controls.

Despite this lack of information, extensive paring is recommended as part of the treatment when using most topical treatments. Trial work with Footrite® (Hardmans Chemicals), a zinc sulphate formulation, has shown no advantage in paring under conditions conducive for the transmission of footrot (Malecki and Coffey, 1987; Casey and Martin, 1988), but paring chronically affected abnormally shaped feet is recommended (Lambell et al, 1986). Better results were achieved with paring and weekly footbathing in 10% zinc sulphate / 0.2% Teepol® (Shell Chemicals), than with no paring and twice weekly footbathing (Skerman et al, 1983a).
Table 1.4a. Summary of Trials showing the Therapeutic Effectiveness (%) for sheep for common topical and parenteral antibiotic treatments.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Breed</th>
<th>Pare²</th>
<th>Dry/Wet³</th>
<th>Freq⁴</th>
<th>Days PT⁵</th>
<th>Control cure %</th>
<th>Formalin 5%-10%</th>
<th>Zinc Sulphate 10%-20%</th>
<th>Footrite®</th>
<th>Pen/step⁶</th>
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</table>

Notes for Tables 1.4a,b:
1. Ramb = Ramboulet; Targ = Targee; Mer = Merino; BB = British breed; XB = Merino/British breed cross; Cor = Corriedale; Rom = Romney; NS = Not stated.
2. Y = pared; N = not pared; Y/N = results from both pared and unpared sheep; T/FB = trimmed, and footbathed (5% formalin).
3. Conditions when sheep treated, D = Dry; W = Wet; ? indicates not clear in text.
4. Frequency of treatment, M = multiple.
5. Days post treatment when therapeutic effectiveness assessed.
6. Penicillin / streptomycin.
7. Lincomycin / spectinomycin.
8. Erythromycin.
I. Literature Review

Trimming (removal of loose grossly underrun horn), rather than paring, was considered a beneficial adjunct to antibiotic treatment (Egerton et al, 1968), although subsequently only diagnostic paring has been suggested as necessary with antibiotic treatment (Venning et al, 1990).

1.10.2. Topical footbathing treatments

A number of formulations have been used in footbaths for mass medication of affected sheep.

1.10.2.1. Formalin

Formalin (40% formaldehyde) has been used either as a 10% solution in water, as recommended by Beveridge (1941), or as a 5% solution in water. TEfs of 0% to 73% of feet (Hart et al, 1962; Cross, 1978b; Skerman et al, 1983b) and 45% of sheep (Skerman et al, 1983a) for 10% formalin, and 0% to 60% of sheep (Egerton et al, 1968; Plant and Claxton, 1986; Lambell, unpublished; Shepherd and Headlam, unpublished) for 5% formalin have been reported.

Faecal contamination of footbaths is unlikely to affect the effectiveness of formalin solutions (Stewart, 1954b).

Regular formalin (10%) footbathing has been associated with slight hyperkeratinisation of the IDS and an increased incidence of subacute impaction of the interdigital sac (Littlejohn, 1972). Frequent treatments (alternate days for 5 weeks) or prolonged use of higher concentrations of formalin (30 minutes in 10% to 30% formalin) have led to interdigital growths, ulcers and sinuses (Pryor, 1959) and corono-pedal abscessation (Hooper and Jones, 1971).
I. Literature Review

Formaldehyde does not penetrate hoof horn, and thus probably acts as a surface antiseptic (Malecki and MacCausland, 1982). Severe paring to remove all horn overlying infected tissue has been recommended when using formalin (Beveridge, 1941; Pryor, 1954; Littlejohn, 1961). *In vitro* studies found formalin effective against *D. nodosus*, although both zinc sulphate and copper sulphate were more effective (Gradin and Schmitz, 1983). Formalin (5%) footbathing has been recommended when using antibiotics, and its effect may be due either to its evaporative or antiseptic qualities (Egerton et al, 1968). Formalin (5%) footbathing markedly increased the effectiveness of antibiotics when sheep were returned to damp pastures (Egerton et al, 1968).

In virulent footrot, *F. necrophorum* is considered responsible for the necrotic nature and severity of the lesion (Roberts and Egerton, 1969). The rapid response to formalin footbathing may be due to the elimination of *F. necrophorum* and other flora, rather than *D. nodosus* (Egerton and Parsonson, 1969), although presumably reinfection will occur readily from the environment.

1.10.2.2. Zinc sulphate formulations

Beveridge (1941) considered a 10% zinc sulphate solution had a "moderately good curative effect" but was "probably inferior to copper sulphate". TEs of 10% zinc sulphate solutions have ranged from 72% to 93% of feet (Cross, 1978b; Skerman et al, 1983b) and 0% to 80% of sheep (Skerman et al, 1983a; Skerman et al, 1984; Bulgin et al, 1986; Lambell and Chapman, unpublished). In the New Zealand trials by Skerman and others, 0.2% of a surfactant, Teepol®, was added to the zinc sulphate solution, although no advantage in adding the surfactant was demonstrated (Skerman et al, 1983b). Zinc sulphate (10%) gave comparable (Skerman et al, 1983a; Skerman et al, 1984) or slightly higher (Cross, 1978b; Skerman et al, 1983b) TEs when compared directly with 10% formalin footbathing. Formalin (5%) footbathing was superior to 10% zinc sulphate at 28 days post treatment, but there was no difference at 112 days in one trial (Plant and
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Claxton, 1986). No advantage in prolonging the 10% zinc sulphate footbath from 5 minutes to 60 minutes was detected (Plant and Claxton, 1986). Paring appeared to increase TEfs in severe footrot lesions treated with 10% zinc sulphate, and severe lesions were less likely to cure with 10% formalin than with 10% zinc sulphate (Skerman et al, 1983a).

Zinc sulphate as a 20% solution has given TEfs of 3% to 46% in sheep (Malecki and Coffey, 1987; Lambell, unpublished; Malecki, unpublished) following a one hour footbath. In comparison to formalin, a 20% zinc sulphate solution resulted in slightly higher TEfs (Lambell and Chapman, unpublished).

Footrite® (Hardmans Chemicals) is a commercially available formulation, which when diluted to "working strength", contains 20% zinc sulphate and 2% sodium lauryl sulphate (SLS). The addition of SLS was to enhance the penetration of zinc into hoof horn (Malecki and McCausland, 1982; Malecki and Coffey, 1987), thereby decreasing the need for foot paring (Malecki et al, 1983). Two, 1 hour, footbathing treatments 5 days apart is the recommended treatment regime (Footrite Grazier's Manual). TEfs of Footrite range from 0% to 100% for sheep (Malecki et al, 1983; Plant and Claxton, 1986; Malecki and Coffey, 1987; Malecki, unpublished; Lambell and Chapman, unpublished). In feet, a TEf of 32% in one trial, and 86% in another trial were reported (Bagley et al, 1987).

In comparisons of footbathing in Footrite®, 20% zinc sulphate, and 5% formalin, all as a 1 hour footbath, all performed similarly, although Footrite® appeared superior in one mob which were footpared (Lambell, unpublished). Plant and Claxton (1986) found higher TEfs with formalin at 28 days, but at 112 days there was no difference between formalin, Footrite® and 10% zinc sulphate, and all treatments were similar to pared control sheep.
Footrite® resulted in TEfs superior to 20% zinc sulphate in two trials (Malecki and Coffey, 1987; Malecki, unpublished), and to 10% zinc sulphate with 0.2% detergent in one trial (Bagley et al, 1987). Footparing did not improve the effectiveness of Footrite® treatment during wet periods conducive to footrot transmission (Malecki and Coffey, 1987; Casey and Martin, 1988). However, paring of chronically infected, misshapen feet has been recommended, as failure in Footrite® treatment was associated with chronic toe lesions, and affected feet were frequently misshapen (Lambell et al, 1986).

Recommendations for two 1 hour treatments 5 days apart appear to be based on theoretical considerations, and no field evidence comparing frequency or duration of treatment has been published. Relapses following such treatments, more than 40 days after the initial treatment, have been reported (Atkins, 1986). Apparent relapses following attempted eradication using Footrite® occurred in 3 of 38 mobs 6 months after treatment (Hinton, 1991). These mobs had been inspected 21 days after treatment and declared free of disease.

In vitro, formalin decreased the penetration of zinc, and it is recommended that formalin footbathing be discontinued at least 8 weeks prior to the use of Footrite® (Footrite Grazier's Manual). Prior treatment with formalin was associated with a generally lower response to treatments with Footrite® (Lambell et al, 1986).

Compared to formalin, less discomfort appears to be experienced by sheep footbathed in Footrite® (Lambell et al, 1986), and sheep appear to recover from lameness more rapidly following Footrite® treatment (Malecki et al, 1983).
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1.10.3. Antibiotics

Parenteral antibiotics have also been used to treat sheep with footrot. *In vitro* studies have identified a range of antibiotics to which *D. nodosus* isolates were sensitive (Stewart, 1954a; Egerton et al, 1968; Gradin and Schmitz, 1983), and a number of these have been evaluated *in vivo*.

1.10.3.1. Penicillin / streptomycin

Penicillin has been the most promising antibiotic in *in vitro* studies (Stewart, 1954a; Egerton et al, 1968; Gradin and Schmitz, 1983). Penicillin alone at low dose rates was not effective (Forsyth, 1953). However, used at higher dose rates or in combination with dihydrostreptomycin it was effective, with the combination of penicillin and streptomycin being more effective than either penicillin or streptomycin alone (Egerton et al, 1968). At a dose rate of 70,000 units / kg of penicillin, and 70 mg / kg of dihydrostreptomycin, given intramuscularly, penicillin / streptomycin was 78% to 100% curative in sheep under dry conditions and 42% to 63% curative when sheep were returned to wet pastures (Egerton et al, 1968). Antibiotic levels increase in lesions under dry conditions (Egerton et al, 1968) and this may explain the superior TEfs under dry conditions.

Concurrent supportive treatment of trimming underrun horn and 5% formalin footbathing increased the efficiency of penicillin / streptomycin treatment, and the use of 5% formalin markedly improved the result when sheep had to be returned to wet pastures (Egerton et al, 1968). Harris (1968) considered severe paring gave a better response to treatment than light paring although, recently, paring only sufficient to establish a diagnosis has been suggested (Venning et al, 1990). WebbWare et al (1994), using minimal paring and no footbathing, only achieved TEfs of 34% to 53% on three
farms, despite holding sheep on battens for 24 hours post treatment, before returning sheep to their paddocks. Footrot transmission occurred during this trial.

1.10.3.2. Lincomycin / spectinomycin

Lincomycin / spectinomycin in combination as a single intramuscular injection at 1 ml/10 kg (50 mg lincomycin and 100 mg spectinomycin per ml) gave a TEf of 91%, and gave similar cures when compared to penicillin / streptomycin (Venning et al, 1990).

1.10.3.3. Erythromycin

Erythromycin is bactericidal to *D. nodosus* organisms *in vitro*. It had similar efficacy to penicillin / streptomycin *in vivo*, achieving TEf s of 83% and 100% when used at 12 mg/kg and 18 mg/kg, respectively, in groups of 6 sheep under dry conditions in conjunction with a single formalin footbath (Egerton et al, 1968). The efficacy of erythromycin decreased when sheep were returned to wet pasture after treatment (Egerton et al, 1968). Erythromycin at approximately 7-13 mg/kg gave T Ef s of between 41% and 88%, and erythromycin performed similarly to penicillin / streptomycin (Webb Ware et al, 1994).

1.10.4. Vaccination

Vaccination, primarily a prophylactic strategy, has also been shown to be therapeutic (Egerton and Burrell, 1970; Skerman, 1971; Egerton and Morgan, 1972). Using a two strain experimental whole cell vaccine, T Ef s of 22% to 84% were achieved (Egerton and Burrell, 1970; Egerton and Morgan, 1972). Healing commenced 1-3 weeks after the second vaccine dose, and was complete 7 weeks after the second dose (Egerton and Roberts, 1971). T Ef s of 0% to 71% have been achieved with whole cell commercial vaccines (Mulvaney et al, 1984; Glenn et al, 1985; Kennedy et al, 1985; Bulgin et al, 1986;
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Plant and Claxton, 1986; Bagley et al, 1987; Shepherd and Headlam, unpublished). Higher TEfs of 46% to 100% and 81% to 100% have been reported (Reed et al, 1981; Liardet et al, 1986), but it is unclear whether these TEfs were confounded by the concurrent development of new cases in control sheep.

The above TEfs were achieved with two vaccinations. Following a single vaccination, TEfs from 29% to 60% have been reported (Kennedy et al, 1985; Plant and Claxton, 1986; Bagley et al, 1987; Hindmarsh et al, 1989; Shepherd and Headlam, unpublished). Mulvaney et al (1984) claimed to have inspected sheep four weeks after the first vaccination; and noted that "cure rates were not apparent until about four weeks after the second vaccination", so presumably healing was non-existent or very low four weeks after the first vaccination.

A single vaccination combined with footbathing in Footrite® gave higher TEfs than with single vaccination or footbathing alone (Bagley et al, 1987). However, in a comparison between combined Footrite® footbathing and a two dose vaccination treatment, and Footrite® footbathing alone, no difference in TEfs was detected (Malecki and Coffey, 1987). The combination of weekly footbathing in 5% formalin with vaccination gave higher TEfs compared to single vaccination or formalin footbathing (Shepherd and Headlam, unpublished). No differences were seen between the combined treatment and a two vaccination treatment, although only small numbers of sheep were involved (Shepherd and Headlam, unpublished).

Whilst vaccines are therapeutic, their value as a specific treatment is questioned (Egerton and Morgan, 1972; Kennedy et al, 1985; Mulvaney et al, 1984). The curative effect of commercial vaccines is therefore more likely to be useful as an adjunct to prophylaxis than as a specific treatment.
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1.10.5. Treatment of different forms of footrot

There is little or no reference to the type of footrot being treated in the majority of reports, although the severity of the lesions described are consistent with virulent footrot in most, if not all, trials. Interdigital lesions were excluded or separated from the results in several trials (Hart et al, 1962; Skerman et al, 1983a; Skerman et al, 1983b; Lambell, unpublished). Elastase results from *D. nodosus* isolates were given as positive (Malecki and Coffey, 1987) and weak positive (Lambell, unpublished) in two reports.

Where results for treatment of interdigital lesions were recorded, Footrite®, 10% formalin and 10% zinc sulphate had TEfs of 76% to 94% (Malecki and Coffey, 1987; Skerman et al, 1983a; Skerman et al, 1983b). Sheep affected with benign footrot appeared to respond less satisfactorily to penicillin / streptomycin treatment than sheep with virulent footrot, and supportive treatment (5% formalin, held on battens) did not influence the response to treatment (Egerton et al, 1968).

There appears to be no published information on the effectiveness of treatments given to sheep with intermediate footrot. Egerton and Parsonson (1969) suggest topical treatments may select for less virulent strains.

1.11. Preventive Measures

Beveridge (1941) noted that the prevalence of footrot should be below 5% in summer if eradication was to be successful, and recommended frequent footbathing in November / December to reduce the prevalence. Left uncontrolled, the prevalence of footrot is expected to reach at least 80% in suitable environments (Egerton and Morgan, 1972). Therefore, the use of prophylactic treatments to minimise the prevalence of the disease during transmission periods is highly desirable (Fitzpatrick, 1961; Egerton, 1986; Lambell, 1986; Stewart, 1989). Minimising prevalence during transmission
periods will also reduce losses in production associated with footrot (Marshall et al., 1991a). Disinfection of feet may be attempted if non-infected sheep come in contact with footrot infected sheep, or with *D. nodosus* infected material, and is recommended as a precautionary measure for purchased sheep (Walker, 1988; Anon., 1994). However, footbathing in these circumstances may disguise infections which would be better recognised without treatment. Topical agents used in a footbath, or vaccination, or a combination of both, have been used prophylactically.

In evaluating the effectiveness of preventive treatments, the protective effectiveness (PE) has been determined by the formula:

\[
\text{Protective Effectiveness (PE)} \% = 100 \times \left(\frac{B-A}{B}\right)
\]

where

- A = prevalence in treated sheep
- B = prevalence in control sheep

1.11.1. Preventive Footbathing

Beveridge (1941) recommended the use of 2% formalin or 5% to 10% copper sulphate solutions for footbathing clinically unaffected sheep during an eradication programme. Weekly footbathing in 5% formalin has given PEs of 62% to 66% (Fitzpatrick, 1961; Shepherd and Headlam, unpublished). In two trials with minimal transmission, weekly or twice weekly footbathing in 5% formalin was able to reduce the prevalence below 8%, compared to 26% to 28% in untreated or fortnightly footbathed sheep (Fitzpatrick, 1961). Increasing the duration of footbathing from a "walk through" to a 10 minute "stand-in" did not significantly alter the effectiveness of treatment (Fitzpatrick, 1961). Five minute weekly footbathing in 10% formalin gave a PE of 85% (Skerman et al., 1983b). Formalin may modify the skin horn junction, making it more resistant to *D. nodosus* infection (Malecki and MacCausland, 1982), and this may be
partly the reason for its protective effect, although it is more likely to be effective by eliminating superficial infection before underrunning occurs (J. Egerton, pers. comm.).

Zinc sulphate solutions have also been used for preventive footbathing. Weekly footbathing in 10% zinc sulphate solution with 0.2% Teepol® gave a PE of 86% to 95%, compared to 73% to 85% for 10% formalin (Skerman et al., 1983a,b). No residual protection from zinc sulphate footbathing was achieved, and footparing did not influence the protective effectiveness of the treatment (Skerman et al., 1983b). Increasing the frequency of zinc sulphate footbathing to twice weekly gave PEs of 91% to 100%, although 100% protection was achieved when treating only a small number of feet (Skerman et al., 1983a).

One hour footbathing in Footrite® every 7 days or every 2 weeks over a 12 week period was 100% and 75% protective, respectively (Marshall et al., 1991b). Footrite® had a residual effect of between 2 and 6 weeks (Marshall et al., 1991b). Two, 1 hour footbathing treatments, 5 days apart, resulted in a PE of 75% for Footrite® and 62% for 20% zinc sulphate when assessed 23-24 days after the second treatment (Malecki and Coffey, unpublished), although differences in the level of challenge between controls and treated sheep, and the method of selecting sheep may have confounded these figures.

Footbathing in Footrite® for 1 hour every 3 weeks was 70% to 75% protective with benign strains at the peak of the epidemic (Glynn, 1993). Weekly or twice weekly footbathing in 10% zinc sulphate with 0.2% Teepol® (Skerman et al., 1983a,b) or weekly footbathing in 10% formalin (Skerman et al., 1983b) was highly protective against the development of "footscald".
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1.11.2. Vaccination

The use of vaccination to prevent the development of footrot was demonstrated with the use of experimental single strain and two strain whole cell vaccines (Egerton, 1970; Egerton and Burrell, 1970; Egerton and Roberts, 1971; Egerton et al., 1972; Egerton and Morgan, 1972). Following footrot challenge, vaccinated sheep had less footrot, lesions were less severe, and the duration of infection was shorter (Egerton et al., 1972). Using experimental vaccines, PEs at the peak of the footrot epidemic of 30% to 100% were achieved when sheep were infected with strains of *D. nodosus* homologous with those in the vaccine (Egerton and Burrell, 1970; Skerman, 1971; Egerton et al., 1972; Egerton and Morgan, 1972). When assessed in summer, PEs were 55% to 90% (Egerton et al., 1972; Egerton and Morgan, 1972).

Fimbriae have been demonstrated to be the major protective antigens in vaccines (Stewart 1978; Every and Skerman, 1982; Stewart et al., 1982b), and are also the basis for serogroup classification (Walker et al., 1973; Stewart 1973, 1978). The failure of vaccines to protect against heterologous challenge was recognised (Egerton, 1974) and commercial vaccines which included strains of *D. nodosus* representative of the majority or all of the known serogroups were developed.

These commercial whole cell vaccines have given PEs of 70% to 100% (Reed et al., 1981; Glenn et al., 1985; Bulgin et al., 1986; Lambell, 1986; Hindmarsh et al., 1989; Liardet et al., 1989). Vaccination gave protection for 16-20 weeks in Romneys, but only 4-5 weeks in Merinos (Skerman et al., 1982). The protective period was at least 10 weeks in Merino / Border Leicester cross ewes (Lambell, 1986).

Recombinant *D. nodosus* pili vaccines, produced from pili subunit genes inserted and grown in *Pseudomonas aeruginosa* have recently been shown to be both prophylactic and therapeutic (Egerton et al., 1987; Stewart and Elleman, 1987; Elleman
and Stewart, 1988; Raadsma et al, 1990). No field trials with commercial recombinant vaccines have been reported.

Using recombinant pili vaccines, antigenic competition has been investigated. Increasing the number of antigens within a vaccine has decreased the effectiveness of vaccination (Schwartzkoff et al, 1993a; Hunt et al, 1994; Raadsma et al, 1994b). Agglutinating antibody levels fell when six or more specific pili were included (Hunt et al, 1994), and monovalent vaccination was more protective for longer than decavalent vaccination (Hunt et al, 1994; O'Meara et al, 1993). Increasing the number of antigens in the vaccine resulted in a linear decrease in protection and agglutinating antibody titres (Raadsma et al, 1994b).

Increasing the interval between the primary sensitising dose and second booster dose from 2 to 52 weeks resulted in increasing agglutinating antibody titres for a recombinant multivalent pilus vaccine (Schwartzkoff et al, 1993b). These authors also found higher agglutinating titres following a third dose 6 or 12 months after the initial two doses, compared with titres resulting from the two dose regime. No difference in titres was found in an earlier study which examined increasing the interval between primary and booster doses from 6 to 16 weeks with whole cell vaccines (Chetwin et al, 1986).

Local reactions to vaccination (primarily sterile abscesses) have been reported (Stewart et al, 1983; Mulvaney et al, 1984; Ross and Titterington, 1984; Bulgin et al, 1985; Lambell, 1986; Stewart et al, 1986c; Hindmarsh et al, 1989), although these reactions generally presented few problems (Bulgin et al, 1985; Lambell, 1986; Hindmarsh et al, 1989). Problems with secondary infections occurred in one trial (Bulgin et al, 1985). Body weight losses in vaccinated sheep compared with unvaccinated sheep prior to footrot challenge have been reported (Stewart et al, 1985a; Stewart et al, 1986c). Whole cell *D. nodosus* vaccines with oil adjuvants gave more local reactions than those with
alum adjuvants (Ross and Titterington, 1984). Oil adjuvant vaccines have given a higher level of protection and higher agglutinating antibodies than alum adjuvanted vaccines (Egerton and Thorley, 1981). The occurrence of a local reaction has been related to the protectiveness of vaccination, with sheep with local skin reactions having significantly less footrot than sheep without local reactions (Lambell, 1986). Stewart et al (1983, 1986c) found less local reaction to a purified pili, oil adjuvanted, vaccine than in either an alum-oil adjuvanted pili vaccine or whole cell vaccines (oil or alum-oil adjuvants), and the purified pili-in-oil vaccine was the least cross-protective.

1.12. Eradication

1.12.1. Definition

Eradication has been defined in a number of ways. Most definitions assume a specific agent is responsible for a disease, and eradication is then defined in relation to that specific agent. Whilst the general concept of eradication is well understood, differences arise over questions of 'how and when to decide an agent has been eradicated' (Schnurrenberger et al, 1987). Definitions differ in the extent of the population considered, and the method of determining that a specific agent is no longer present within the defined population. Definitions of eradication include:

- extinction of an infectious agent either from a defined area or population or in total (Cockburn, 1963, cited by Thrusfield, 1986; Anderson et al, 1978, cited by Hanson, 1983; Smith 1991)

- reduction of disease prevalence to a level at which transmission does not occur (Andrews and Langmur, 1963, cited by Thrusfield, 1986; Yekutiel, 1980;
Schnurrenberger et al, 1987). Such lack of transmission must occur in the absence of control measures (Schnurrenberger et al, 1987).

- reduction of disease prevalence to a level at which the disease ceases to be a major health problem, although some transmission may still take place (Maslakov, 1968, cited by Thrusfield, 1986). The term 'eliminated' has also been used for this description (Payne, 1963, cited by Thrusfield, 1986).

Eradication needs to be contrasted with 'control' - the reduction in prevalence, but not the elimination, of disease or the infective agent. Control is concerned with the first 99.9% of cases, whilst eradication is interested in the last 0.1% (Gelfand, 1973, cited by Schnurrenberger et al, 1987).

The definition used for eradication for a particular disease is likely to depend on the epidemiology of the disease, and techniques available for detecting the disease or causative agent. If eradication of a disease that spreads rapidly, and is easily detectable, is desirable, extinction of the infectious agent, either globally or regionally, will be the objective. On the other hand, a disease with restricted or slow transmission, and where detection of the infectious agent is difficult, may be deemed eradicated when no new cases are detected over a specific period, rather than by intensive sampling to try and demonstrate absence of the infectious agent.

Application of these general definitions of eradication to footrot is complicated by its multifactorial aetiology. Furthermore, the recognition of mild forms of footrot which are likely to result in only small losses in productivity has led to the decision to eliminate severe forms of footrot, but not the mild ones (Anon., 1988; Egerton, 1989a).

Footrot eradication programmes were originally based on the eradication of *D. nodosus* (Beveridge, 1941), because at that time no variation in types of footrot or in
D.nodosus was recognised and D.nodosus was considered the primary causal and transmitting agent. However, most claims of eradication of footrot have been based primarily on the failure to detect clinical disease, rather than testing to indicate the absence of D.nodosus (Gregory, 1939b; Littlejohn, 1961; Hayman and Triffitt, 1964; Fitzpatrick, 1986; Hinton, 1991). Because benign footrot is not a target for eradication, assessment of success requires a distinction between benign and virulent disease. The failure to establish adequate relationships between forms of footrot and in vitro characteristics of D.nodosus (section 1.6.2.5) means that, while the identification of strains of D.nodosus with certain in vitro characteristics would be a desirable criterion by which to determine the need for, and judge the success of, footrot eradication, the use of in vitro characteristics of D.nodosus for this purpose is currently limited.

For the purposes of eradication, the distinction between the different forms of footrot is generally made on clinical grounds (Anon., 1988; Egerton, 1989a; Anon., 1993). However, in vitro protease tests on D.nodosus isolates recovered from affected flocks have been used as the criterion for determining the need for, and assessing the success of, footrot eradication programmes (Anon., 1994; R. Mitchell, pers. comm.).

1.1.2.2. Footrot Eradication Programmes

Beveridge (1938b, 1941) first demonstrated that footrot could be eradicated from sheep flocks, and there have subsequently been a number of published reports, as well as many unpublished testimonials, indicating that virulent footrot can be eradicated from sheep flocks (Gregory, 1939b; Thomas, 1957; Littlejohn, 1961; Clark, 1962; Hayman and Triffitt, 1964; Fitzpatrick, 1986; Atkins, 1986; Hinton, 1991).

The eradication programme recommended by Beveridge was based on the removal of infected animals over the dry summer period. It assumed a simple
relationship between the presence of *D. nodosus* and presence of footrot, and importantly, it relied on the ability to detect all infected sheep by clinical examination.

The Beveridge Plan for winter rainfall areas consisted of the reduction in prevalence (to 5% or less) by intensive footbathing in November/December; and the inspection of all feet of all sheep in summer (at least 2 weeks after the last treatment), with the removal of affected sheep. Healthy ('clean') sheep were footbathed (5% or 10% copper sulphate or 2% formalin) and returned to the paddock. Infected and suspected infected sheep ('suspects') were sold for slaughter or treated. Treatment involved the severe paring of feet to remove all infected tissue, and footbathing or treating feet individually with 10% formalin or 30% copper sulphate. If treated, sheep had to pass two inspections at least one month apart as healthy before returning to the flock.

For irrigated pastures, or green summer conditions, it was considered necessary to return healthy sheep to a paddock that has been spelled for preferably two weeks, or at least one week, or alternatively, footbath healthy sheep every 2-3 days for 1-2 weeks.

An alternative to the inspection/segregation method was disposal of the infected flock, and restocking with healthy sheep after spelling the property for two weeks.

Beveridge (1941) stipulated that goats should be included as part of the programme. Once free of footrot, precautions to avoid re-introduction should be instigated. These precautions included examination of all feet of introduced sheep, and to maintain vigilance with travelling stock.

Whilst the recommendations for treating sheep have been modified, and other chemicals are available, the basic eradication programme outlined by Beveridge is still followed and recommended (Egerton, 1986; Stewart, 1989).
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Some modifications to the programme have been recommended:

- inspecting clean sheep on several occasions (Pryor, 1954; Hayman and Triffitt, 1964; Egerton, 1986)
- use of vaccination to reduce prevalence in the preceding spring (Egerton, 1986).

Littlejohn (1961) found eradication was achieved more quickly in Britain when attempted in the autumn which was driest, compared with spring or summer.

Another strategy for eradication involves intensive footbathing with Footrite® during the transmission period (Atkins, 1986; Hinton, 1991). One programme involved extensive footparring, with two footbathing treatments of one hour's duration 5 days apart and inspection of sheep's feet 18-21 days after the initial treatment with removal of sheep with footrot lesions at this inspection. Two further inspections at approximately 21 day intervals were carried out. Following a further inspection, presumably 3-4 months later, footrot was claimed to have been eradicated from three small flocks (Atkins, 1986).

An alternative programme using Footrite® during the transmission period involved one hour footbathing every 21 days until there is no evidence of the disease (Hinton, 1991). Initially, all sheep were footbathed for one hour, and moved to a clean paddock. Twenty one days later, sheep's feet were inspected and diagnostically pared. Sheep with underrunning lesions were segregated and sold for slaughter or treated. Clean sheep were footbathed for one hour and moved to a clean paddock. Sheep were re-inspected and footbathed every 21 days until a clean inspection was achieved (i.e. no sheep were detected with footrot). A further inspection 21 days later was recommended. Eradication in 36 of 40 mobs in the first year, and on 11 of 13 properties by the end of the following spring was achieved using this method (Hinton, 1991).
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In this latter programme, all flocks had only recently been infected with footrot. The virulence of the strains of D. nodosus involved on each property was not recorded. The only reference to virulence was to "extensive underrunning", but whether this was of soft or hard horn was not stated, nor was the percentage of sheep so affected. No chronic lesions were detected, and this was assumed to be due to the recent introduction of disease (presumably supported by property history).

Whilst the eradication of virulent footrot is well documented, there is little or no published information on the eradicability of benign or intermediate strains of D. nodosus or the milder disease associated with them. Benign strains of D. nodosus appear to be difficult to eradicate (Alexander, 1962; Littlejohn, 1966/67), although no trials have been set up to assess this. Benign footrot may be present on properties after eradication of virulent footrot (Egerton and Parsonson, 1969), but whether this was due to failure in eradication, or from reintroduction of the disease with introduced sheep or cattle was not discussed. Cattle have been implicated in acting as a reservoir of infection of benign footrot for sheep (Wilkinson et al, 1970).

Both Gwynn (1986) and Robinson (1986) claimed to have eradicated intermediate strains of D. nodosus, but no information was presented to support their claims. Less virulent strains (presumably intermediate strains) appeared more difficult to eradicate in South Australia (Brownrigg, 1986). The cost-effectiveness of eradicating less virulent strains has been questioned (Egerton, 1986; Dobson, 1986; Egerton and Raadsma, 1991).
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1.13. State Footrot Programmes

Programmes for the control and/or eradication of footrot are current (1995) in New South Wales, Victoria, South Australia and Western Australia (Walker and Plant, 1994; Mercy and Mitchell, 1995; Tolson, 1995; Turner, 1995). In Tasmania, where a programme existed from 1939 to 1975, consideration is being given to the reintroduction of a control programme (Lloyd-Webb, pers. comm.). The New South Wales, Victorian and South Australian footrot programmes aim at control of footrot (on a state basis) by eradicating footrot from individual properties and reducing the number of footrot affected flocks to a low prevalence across the state (Anon., 1988; Anon., 1993; J. Tolson, pers. comm.). In Western Australia, eradication of non-benign footrot from the State is the objective (Mercy and Mitchell, 1995).

In designated areas of the New South Wales and Victoria, and throughout South Australia and Western Australia, flocks determined to have footrot are placed in quarantine, with restrictions on the movement and sale of stock being enforced and a footrot eradication programme being mandatory. Quarantine restrictions are removed only after the flock has been inspected following the eradication programme and deemed to be free of footrot. In New South Wales and Victoria, flocks with footrot outside designated areas are not normally subject to regulatory control.

The State footrot programmes vary in the method and criteria for determining which forms of footrot are targeted for eradication from individual flocks.

New South Wales. The New South Wales Footrot Strategic Plan commenced in 1988 (Anon., 1988), and its progress has been reviewed recently (Walker and Plant, 1994). The original objective of the programme was to reduce the prevalence of footrot to less than 2% of flocks by the year 2000, although this now appears to be 1% (Walker and Plant, 1994). Based on estimates of the flock prevalence of footrot within Rural Land
Protection Boards (RLPBs), RLPBs are designated as Protected (less than 1% prevalence), Control (1%-10% prevalence) or Residual (above 10% prevalence) Areas. Flocks in Control and Protected Areas are part of the regulatory programme, with quarantine and enforcement of eradication programmes occurring for flocks considered affected with footrot. For flocks in Residual Areas, the eradication of footrot from affected flocks is encouraged through advisory programmes and farmer "self-help" groups (Voluntary Footrot Groups), but no regulatory controls are normally applied.

Both virulent and intermediate footrot are targeted for eradication from individual flocks. The diagnosis of footrot is currently based on clinical examination of the flock, and no specific criteria are given to provide cut-off points between intermediate and benign footrot (R. Walker, pers. comm.). The use of the GGPTT is recommended as an aid to diagnosis, but is not to be used as the criterion for quarantine in Control and Protected areas, except in the case of goats. Release from quarantine can occur three months after an inspection which fails to demonstrate the presence of footrot, and does not necessarily require sheep to be exposed to conditions conducive for the expression or transmission of disease.

The flock prevalence of footrot was estimated to be 7.3% in 1988, and 5.5% in 1994, with 43% of sheep flocks being in Protected Areas. These figures are likely to underestimate the actual prevalence (Locke and Coombs, 1994), and no on-farm surveys with inspection and/or sampling of feet have been conducted.

Victoria. The Footrot Control Strategy for Victoria has as its aim to reduce "the prevalence of virulent footrot to less than 1% of all sheep flocks" (Anon., 1993). This is to be achieved by the progressive expansion across the State of the existing Footrot Control Area, a region originally formed in 1970 in western Victoria, where the flock prevalence of footrot is considered less than 1% (Anon., 1993). The diagnosis of footrot
is based on clinical inspections, with flocks having 3% or more sheep in a flock with score 4 lesions being considered to have "quarantinable" footrot.

The prevalence in the existing Footrot Control Area was considered to be 0.5% (Anon., 1993), but results from a property survey in 1993 indicated a prevalence of 5.9% (Roycroft and Harrison, unpublished).

**South Australia.** In South Australia, the programme aims at controlling footrot by eradication of the disease from known infected properties. Infected properties are those where any sheep has a score 4 lesion. Release from quarantine may occur after the next likely transmission period (spring), with flocks being examined for evidence of lameness, rather than the inspection of all sheep for evidence of footrot.

A survey of 74 properties in 1994 indicated 4% of flocks in the South West region of South Australia were infected with virulent footrot (at least one sheep having a score 4 lesion), and a further 63% of flocks examined with clinically benign footrot had protease thermostable *D. nodosus* isolates (Cleland, unpublished).

**Western Australia.** In Western Australia, virulent footrot is considered present on a property if any *D. nodosus* isolates recovered are protease thermostable, irrespective of clinical signs. At 30 June, 1994, 209 properties (2%) were in quarantine due to footrot (Anon., 1995). Proposals to increase resources to achieve eradication within 10 years, and form a compensation fund are being considered (R. Mitchell, pers. comm.).

Thus, in those States where a high level of control of footrot (on a State basis) is the objective, clinical criteria are used to determine which flocks are subject to regulatory control. Where eradication (on a State basis) is the objective, the GGPTT is used to determine the footrot status of flocks, regardless of clinical signs.
Economic analyses of footrot programmes have been performed for the New England area in NSW (Carmody et al, 1984), the Footrot Control Area in Victoria (Stott, 1989), and in Western Australia (Thomson, 1993). All analyses have produced figures to support the extension or maintenance of the various programmes, based on the benefit of government inputs for community gains. However, assumptions on the impact of footrot have been based on those for fully virulent footrot, and therefore may overestimate benefits for programmes if a large percentage of flocks placed in quarantine have milder forms of disease. Such analyses do not estimate the number of producers economically penalised.

The inclusion of intermediate footrot as a target for eradication in State footrot programmes (Anon., 1988) does not appear to have been evaluated on either economic or epidemiologic criteria, the criteria used to exclude benign footrot as a target for eradication (Anon., 1988; Anon., 1994). Production losses associated with intermediate footrot have been estimated to be "nil to moderate" (Anon., 1988; Anon., 1993). Eradication of benign footrot was considered "not feasible" (Anon., 1988), presumably due to the exclusion of cattle from footrot programmes, and the potential role of cattle to act as a reservoir for benign footrot (Wilkinson et al, 1970). The eradicability of intermediate strains of *D. nodosus*, and the role of cattle in the transmission of intermediate footrot, have not been investigated.
1. Literature Review

1.14. Conclusions

The epidemiology, pathogenesis, treatment, control and eradication of virulent footrot have been extensively investigated. Much published material describes investigations which were poorly designed and there is much anecdotal material in the literature. Nevertheless, there is convincing evidence of the impact of virulent footrot and that it can be eradicated from flocks of sheep.

However, in the case of milder forms of footrot, there is a paucity of information. The economic impact of benign footrot is generally considered negligible, although no comparisons of the effect on productivity between different forms of footrot have been published. Suggestions that benign and intermediate footrot may be more difficult to eradicate have been made, but not substantiated, nor have the reasons for any such difficulty been addressed. Eradication programmes are based on experiences with virulent footrot, and their applicability to milder forms has not been evaluated.

State footrot programmes aim at reducing or eliminating both virulent and intermediate footrot, despite the lack of information on the economic impact and eradicability of less virulent strains. The inclusion of less virulent forms of footrot in such programmes appears to be on the basis that it will be shown, through experience, that they resemble virulent footrot in being able to be eradicated. This does not appear to hold for benign footrot.

As a result of regulations involving intermediate footrot, there is clearly a need to investigate its behaviour, particularly in relation to its economic impact and eradicability. More precise quantitative definitions of the different forms of footrot are necessary to facilitate the investigation of intermediate footrot, and enable the results of research to be applied with confidence. Such definitions are also essential to evaluate the role of in
vitro testing of *D. nodosus* isolates in the assessment of footrot outbreaks, particularly if such tests are to be used in State footrot programmes.

Information on the prevalence of the different forms of footrot, outcomes of control and eradication strategies for intermediate footrot, and the comparative economic impact of the various forms of footrot would allow a more rational approach to the formulation of footrot programmes, both for individual producers and at the regional level.

In recognising the current paucity of information on milder forms of footrot, this study endeavoured to investigate some aspects of the control and eradication of intermediate footrot, thereby providing part of the information necessary for rational decision-making for farm and State footrot programmes. The investigations were based principally on footrot associated with clearly characterised strains of *D. nodosus*. 
CHAPTER 2
DEFINITIONS, MATERIALS AND METHODS

2.1. Introduction

Definitions used commonly in this thesis, and materials and methods which were used frequently are presented. Materials or methodology which only relate to work described in one chapter are presented in that chapter.

2.2. Definitions

The following terminology is used throughout this thesis:

footrot
the disease resulting from a mixed infection with *D. nodosus*, *F. necrophorum* and other bacteria

*D. nodosus* infection
bacteriological evidence of the presence of *D. nodosus* with or without signs of footrot

eradication, footrot eradication
the elimination of a characterised strain of *D. nodosus* from a group of sheep

challenge
the procedure of applying cultures of *D. nodosus* to sheep's feet.
2. Definitions, Materials and Methods

**inspection**

the examination and scoring of all feet of each sheep (see section 2.4.1)

**scoring / footscoring**

the allocation of a *score* to each foot of a sheep, based on the scoring system outlined by Egerton and Roberts (1971), namely

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal feet</td>
</tr>
<tr>
<td>1</td>
<td>limited mild interdigital dermatitis</td>
</tr>
<tr>
<td>2</td>
<td>interdigital dermatitis characteristic of footrot</td>
</tr>
<tr>
<td>3</td>
<td>interdigital dermatitis and/or underrunning of the horn of the heel and sole (soft horn)</td>
</tr>
<tr>
<td>4</td>
<td>as for 3, but with underrunning extended to the wall of the hoof (hard horn)</td>
</tr>
</tbody>
</table>

**sheep footscore, sheep score**

the maximum of the scores recorded for the four feet of a sheep at an inspection

(Thus, a sheep with footscores 3,2,2,2 has a sheep footscore of 3).

**lesion**

a sheep was considered to have a *lesion* if the score of any foot was 1 or greater

**affected**

a sheep was considered to be *affected* with footrot if one or more feet had a score of 2 or greater, unless otherwise stated
infected

a sheep was considered *infected* if *D. nodosus* isolates were recovered on culture from any foot of the sheep, irrespective of the score.

The different clinical forms of footrot are arbitrarily placed into one of three categories:

**virulent footrot**

a severe form of footrot, with at least 10% of affected sheep having score 4 lesions

**benign footrot**

a predominantly interdigital disease, with no more than 5% of affected sheep having score 3 or score 4 lesions at its most severe level of expression

**intermediate footrot**

a predominantly interdigital disease, but with at least 5% of affected sheep having score 3 lesions but with no more than 10% of affected sheep having score 4 lesions at its most severe level of expression.
2. Definitions, Materials and Methods

2.3. Materials

2.3.1. Sheep

All sheep used in experiments described in Chapter 4 were purchased from the property in southern New South Wales on which the field trials were conducted (referred to as the Trial property). The majority of sheep used were Polwarths, a three quarter Merino / one quarter Lincoln stabilised breed, which had been born and reared on the Trial property. Additional sheep were Merinos, which had been introduced onto the Trial property, or had been bred from these introduced sheep (see below).

A history of the Trial property, pertinent to the footrot status of the sheep used in the field trials in this study, is given below.

The Polwarth flock was essentially closed, with replacement rams being bred on the property. Introductions of outside sheep were rare, with three rams being introduced during the five years preceding the commencement of the trial work described in Chapter 4. According to the owner, footrot had been introduced into the flock in 1978 by a neighbour's stray sheep. Virulent footrot was eradicated in 1986. One mob of sheep was diagnosed as affected with intermediate footrot in December, 1986. This mob was quarantined and a further eradication programme implemented. *D. nodosus* isolates (serogroup B) recovered from sheep in this mob had *in vitro* characteristics consistent with the isolates being of intermediate virulence. Following the culling and disposal of affected sheep from this mob in February, 1987, there has been no further evidence of virulent or intermediate footrot in any Polwarth sheep. In October, 1989, the Trial property became Accredited Footrot Free following inspection of 946 of 6,500 Polwarth sheep. Subsequent sampling of lame sheep, or sheep with interdigital lesions either yielded no *D. nodosus*, or *D. nodosus* isolates with *in vitro* characteristics consistent with their being benign. These isolates were all serogroup B prior to 1992.
Three hundred and fifty Merino sheep from 7 different properties (50 per property) were introduced onto the Trial property in December, 1989. All sheep came from flocks declared free of virulent or intermediate footrot by their owners. On introduction, all sheep's feet were inspected, and evidence of intermediate footrot, based on the prevalence of score 3 lesions, was detected in sheep from one source. A footrot treatment programme (without culling) was carried out on these sheep, which were then grazed with the remainder of the introduced Merino sheep. Ninety Polwarth sheep from the Trial property were added to the mob of Merinos to form the Merino Trial Flock. Feet from sheep in this flock were inspected on four occasions from August, 1990 to December, 1994, with no evidence of virulent or intermediate footrot being detected. Lesions of score 1 and 2 were detected in some sheep at all inspections. One score 3 lesion was detected in one sheep at one inspection. Isolates recovered from these sheep in 1992 were serogroup B. In 1994, isolates of serogroup F were recovered.

Two thousand two hundred Merino sheep were introduced onto the property from two sources in 1990, 1991 and 1993. The properties of origin of these sheep were believed by their owners to be free of footrot. These sheep are referred to as Merino Flock 1 and Merino Flock 2. Merino Flock 1 comprised 1,625 sheep introduced in 1990 (520) and 1991 (1105). The owner of the sheep of Merino Flock 1 had contributed sheep to the Merino Trial Flock, with no evidence of footrot being detected in the contributed sheep. Merino Flock 2 comprised 575 sheep introduced in 1993. Half of the sheep in Merino Flock 2 were inspected in July, 1993. There was no evidence of footrot in these. Merino sheep for trial work (Chapter 4) came from Merino Flocks 1 and 2.

As the introduced Merino sheep were considered to pose a threat to the footrot status of the Polwarth flock, the Merino Trial Flock and Merino Flocks 1 and 2 (and their progeny) were kept isolated from the Polwarth flock until December, 1994. Yards, paddocks and laneways were spelled for at least 7 days following their use by the Merino
sheep. As there was no perceived footrot risk from the Polwarth sheep, no attempt was made to prevent spread of *D. nodosus* infection from Polwarth sheep to Merino sheep.

The Accredited Footrot Free status of the Trial property was maintained until the commencement of the experiments in this study in 1992, with approximately 900-1,000 sheep being inspected annually to ensure that virulent footrot had not been introduced, or had not reappeared. Inspections in 1990 and 1991 included a sample of sheep introduced in each year. Following the commencement of the work described in this study, the Accredited Footrot Free status was maintained for the main Polwarth and Merino flocks. The experimental footrot flocks and the area leased on the Trial property (Figure 2.1) for these flocks were quarantined. No evidence of virulent or intermediate footrot was detected in annual inspections in the Polwarth and Merino flocks carried out during the period the footrot experiments were in progress on the quarantined area of the Trial property. The inspection in 1993 included a sample of sheep introduced in that year (Merino Flock 2).

Cattle on the Trial property were usually grazed separately from sheep, but cattle and Polwarth sheep frequently walked over common ground, and cattle and Merino sheep occasionally walked over common ground. Thus, no attempt was made to prevent transfer of *D. nodosus* infection from cattle to sheep, or from sheep to cattle.

2.3.2. Bacteria

Experiments described in Chapter 4 involved 7 strains of *D. nodosus* being introduced into experimental groups of sheep. These strains were further characterised in work described in Chapter 5. The strains are referred to by their serogroup classification, being designated strains A, B, C, D, E, G, and H. The characteristics of these 7 strains are tabulated in Chapter 4, Table 4.1. The origin of these strains is further described here.
Figure 2.1. Map of Trial property, showing areas grazed by different groups of animals.
2. Definitions, Materials and Methods

Additional strains / isolates evaluated in Chapter 5 are described in that chapter. However, for clarity, additional *D. nodosus* isolates originating from the Trial property are also described here. The VCS number refers to the reference number for the *D. nodosus* collection of the University of Sydney’s Department of Animal Health (formerly Veterinary Clinical Studies) at Camden, New South Wales.

2.3.2.1. Strain A, VCS 1001

This is the prototype of serogroup A. It is a virulent strain that has been used extensively for research on the pathogenesis and immunity of footrot (Egerton and Burrell, 1970; Egerton, 1974; Stewart, 1978; Egerton and Thorley, 1981; Thorley and Egerton, 1981; Skerman et al., 1982; Every and Skerman, 1982; Stewart et al., 1982a,b, 1983, 1984, 1985b, 1986b,c,d; Egerton et al., 1987; Stewart and Elleman, 1987; Elleman and Stewart, 1988; Schwartzkoff et al., 1993a; Hunt et al., 1994; Raadsma et al., 1994a,b; Whittington and Nicholls, 1995). It was originally isolated from a sheep flock in the Southern Tablelands of New South Wales, and has also been referred to in some published reports by its CSIRO collection number, 198.

2.3.2.2. Strain B, VCS 1746

This strain was isolated from the Polwarth flock on the Trial property described in section 2.3.1. Clinical findings over the period 1989-1994 were consistent with a diagnosis of benign footrot. Strain B was isolated from affected sheep in 1990. Isolates which resembled it closely were obtained from sheep in the Polwarth flock in 1989 and 1991, and from the Merino Trial Flock in 1992.
2. Definitions, Materials and Methods

2.3.2.3. Strain C, VCS 1744

This strain was isolated from a sheep flock in western Victoria in 1990. Initial inspections of sheep from this property were made in 1986, and continued until the isolation of Strain C in 1991. From 1986-1989, examination of different mobs of sheep on a number of occasions indicated that benign footrot was present. There were insufficient records to allow categorisation on the criteria given for intermediate and benign footrot above (section 2.2), but affected feet had interdigital lesions, or underrunning confined to horn of the heel (score 3a or 3b, Stewart et al, 1982b). *D. nodosus* isolates of serogroup A recovered from this flock in 1987 had *in vitro* characteristics consistent with their being of intermediate virulence (D. Stewart, pers. comm.).

In 1990, an outbreak of virulent footrot occurred in some mobs on the property. Isolates of serogroup I and D were recovered from affected sheep on two occasions, and the *in vitro* characteristics (protease thermostable, elastase positive, large fimbriate colonies) suggested both were virulent. A stringent eradication programme, based on culling for slaughter of affected animals, was carried out in summer 1990/1991. In spring 1991, no evidence of virulent footrot was found in any sheep on the property at any of three inspections, despite conditions appearing appropriate for the transmission and expression of the disease. At one of these inspections, strain C and a serogroup E isolate were recovered from affected sheep. *In vitro* characteristics of both isolates indicated both were of intermediate virulence (D. Stewart, pers. comm.). At the time of the recovery of these isolates, clinical signs of benign footrot only were observed in the mobs from which they were recovered.
2. Definitions, Materials and Methods

2.3.2.4. Strain D, VCS 1748

This strain was isolated from a sheep flock in southern New South Wales. Prior to its recovery, there had been a history of sheep in wetter paddocks on the property having benign footrot. In autumn, 1989, following early rains, above average pasture growth and continued warm weather, an outbreak of footrot appeared in several mobs, with lesions progressing to score 3 in more than 5% of affected sheep, although the majority of lesions were score 2, and no score 4 lesions were detected. Based on the history and clinical findings, intermediate footrot was diagnosed. Strain D was recovered from such affected sheep. Mobs were footbathed to reduce lameness, but no eradication programme was implemented, due to the perceived mildness of the disease. Sheep inspected in August 1990 on this property showed no clinical evidence of intermediate or virulent footrot. *D.nodosus* isolates of serogroup B isolates were recovered from sheep at this inspection. These isolates were elastase negative and protease thermolabile and thus considered benign.

2.3.2.5. Strain E, VCS 1742

This strain was isolated from a sheep flock in southern New South Wales. It was one of the flocks surveyed in this study (Chapter 3). During inspections of three mobs of sheep in the period between spring, 1991 and autumn, 1992, intermediate footrot was diagnosed. Of the 1,427 sheep inspected, 235 sheep (17%) had lesions which would have resulted in the culling of sheep in a footrot eradication programme. Of these 235 sheep, 44 (19%) had foot abnormalities other than footrot lesions, and 68 (29%) had score 1 lesions. Of the remaining 123 affected sheep, 75 (61%) sheep had score 2 lesions, 37 (30%) sheep had score 3 lesions, and 11 (9%) sheep had score 4 lesions. *D.nodosus* of 4 serogroups (A, B, C and E) were recovered from these sheep. The in vitro characteristics of the serogroup A, B, C and some of the E isolates suggested that
they were intermediate in virulence. One E isolate had benign characteristics *in vitro*. Strain E was one of the serogroup E isolates considered to be of intermediate virulence.

2.3.2.6. Strain G, VCS 1745

This strain was isolated in 1989 from a sheep flock in western Victoria which had intermediate footrot. The strain was recovered from samples collected following an outbreak of footrot in sheep introduced onto the property. Score 3 lesions were present in 33% of affected sheep, the remaining affected sheep having score 2 lesions. Only isolates of serogroup G were identified among *D. nodosus* cultured from this flock.

2.3.2.7. Strain H, VCS 1743

Strain H was cultured from affected sheep in a flock in south-eastern New South Wales in 1992. Inspections of several mobs on the property indicated that intermediate footrot was present. In the period December, 1992 to April, 1993, 875 sheep were examined from this flock. Of the 655 (75%) affected sheep, 461 (71%) sheep had score 2 lesions, 172 (26%) had score 3 lesions, and 22 (3%) had score 4 lesions. In addition to isolates of serogroup H, serogroup B was also recovered from affected sheep.

2.3.2.8. Trial property isolates

As part of a continuing surveillance of the *D. nodosus* status of the Trial property, sheep and cattle, excluding animals described in Chapter 4, were sampled on 9 occasions in the period 1989-1994. *D. nodosus* was recovered on 7 of these 9 attempts. All isolates assessed for virulence were elastase negative and/or GGPTT negative. The identity and source of the isolates is tabulated below (Table 2.1).
2. Definitions, Materials and Methods

Table 2.1 Serogroup classification of isolates recovered from animals on the Trial property.

<table>
<thead>
<tr>
<th>Source</th>
<th>Serogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polwarth Flock</td>
<td>B, C, E</td>
</tr>
<tr>
<td>Merino Trial Flock</td>
<td>B, F</td>
</tr>
<tr>
<td>Merino Flock 1</td>
<td>A, C</td>
</tr>
<tr>
<td>Merino Flock 2</td>
<td>A</td>
</tr>
<tr>
<td>Cattle</td>
<td>B, C</td>
</tr>
</tbody>
</table>

2.3.3. Hoof Agar Medium (HA)

Hoof agar medium (Thomas, 1958b) was made with agar either at a 2% concentration (2% HA) or a 4% concentration (4% HA). Other ingredients and preparation techniques were otherwise identical for both 2% HA and 4% HA. Four per cent HA was used to isolate \(D. nodosus\) from primary lesion material or lyophilised samples, and for subculturing purposes. Two per cent HA was also used for subculturing pure \(D. nodosus\) isolates and for subculturing immediately prior to lyophilising isolates. The ingredients were:

- Polypeptone Peptone (BBL) \(10.0\) g
- Sodium Chloride (NaCl) \(5.0\) g
- Lab-Lemco meat extract (Oxoid L29) \(4.0\) g
- Yeast Extract (Oxoid L 21) \(1.0\) g
- Washed, ground ovine hoof horn \(15.0\) g
- Agar (Difco) for 2% \(20.0\) g
- Agar (Difco) for 4% \(40.0\) g
- Distilled water \(1000\) ml.
The peptone, NaCl, meat extract and yeast extract were dissolved in 1000 ml distilled water, and the pH adjusted to 7.8-8.0 using 10 M sodium hydroxide (NaOH).

Agar and hoof powder were weighed into conical flasks and the dissolved ingredients added. The flasks were plugged with cotton wool and autoclaved for 20 minutes at 100 Kpa. After cooling to 50-70°C, the medium was dispensed aseptically into sterile plastic Petri dishes (25-30 ml per dish), with constant stirring to ensure hoof particles were evenly distributed. Following setting, plates were dried at 56°C for 30-60 minutes, and then stored in sealed plastic bags at 4°C until used.

2.3.4. Tris -EDTA buffer (TE buffer)

This buffer contained, as final concentration, 10 mM Tris and 1 mM EDTA (1 x TE). The pH was adjusted to 8.0 with concentrated HCl, unless otherwise stated. The buffer was made by mixing 10 ml of 1 M Tris (121.1 g Tris in 1 litre distilled water) and 2 ml of 0.5 M EDTA (186.1g EDTA in 1 litre distilled water) in 1 litre of distilled water (after adjusting pH). The buffer was autoclaved and stored at room temperature.

2.3.5. Phosphate Buffered Saline (PBS)

A stock solution of 20 x PBS was made up as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>170.0 g</td>
</tr>
<tr>
<td>Na₂HPO₄.12H₂O</td>
<td>41.0 g</td>
</tr>
<tr>
<td>NaH₂PO₄.2H₂O</td>
<td>3.12 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>to 1000 ml</td>
</tr>
</tbody>
</table>

The pH was adjusted to 7.4 using concentrated NaOH.

The stock solution was diluted 1/20 with distilled water for use.
2.4. Methods

2.4.1. Inspections

Sheep were tipped conventionally, or in sheep handling devices which presented the sheep so that all four feet were able to be examined with the operator being in a normal standing position. If claws were overgrown or abnormally shaped, or had evidence of any abnormality (footrot related or otherwise) and the status of the foot could not be readily determined, paring with secateurs or a sharp knife was carried out only to the extent that an adequate assessment of the foot could be made. In the majority of cases, inspection required no paring. At two eradication inspections, all feet of all sheep were lightly pared (Inspection 3, Chapter 4; Inspection 9, Mob 2, Chapter 4).

Each foot was carefully examined to assess the normality of the shape of the two digits, and then by parting the claws, the interdigital skin was inspected, with particular attention being paid to the skin-horn junction. If evidence of underrunning was present, attention was paid to whether the underrunning extended to beneath hard horn (score 4). Following examination, scores for each foot were recorded, together with the sheep's tag number. In addition, any other foot abnormalities, such as foot abscess, toe abscess, scabby mouth, grass seed infestation and interdigital injury were recorded. If a lesion appeared to be healing, this was recorded, although for analysis of scores, the sheep was assumed still to have the pre-healing score. Completely healed lesions were considered to be score 0 for analysis.

The inspection of the Virulence Assessment Flock (described in Chapter 4) 6 weeks after *D. nodosus* challenge was carried out by an assistant and the author jointly. All other inspections were done by the author.
2. Definitions, Materials and Methods

Sheep were inspected at the rate of approximately 60 sheep per hour, with one person assisting the author by pushing sheep up to the handler and recording the eartag number, scores and any special remarks for each sheep.

2.4.2. Collection of lesion material

Lesion material for bacteriological culture was collected from a foot of a sheep by scraping a blunt wooden swabstick across the IDS, and under the horn if underrunning was present. The swabstick was then streaked directly onto 4% HA, and incubated anaerobically at 37°C for 4 to 7 days. Anaerobic conditions were achieved using GasPak Plus envelopes (BBL Microbiological Systems).

Sampling of sheep’s feet refers to the above procedure. Where score 2 lesions were present, the inflamed or necrotic interdigital skin was always scraped. If underrunning was present, lesions were scraped under the horn, unless it was dry or affected with myiasis. In the case of toe and heel abscesses, the detected focus of infection was scraped. For feet detected with other abnormalities (grass seeds, interdigital injury, healed lesions), the abnormal tissue was scraped. For normal feet, the interdigital skin was scraped (without the addition of water).

For samples other than culture, feet were scraped as described above. For air dried smears, the wooden swabstick was rubbed onto a microscopic slide. A drop of water was added if the lesion material was not moist. For polymerase chain reaction (PCR) analysis, lesion material from the wooden swabstick was transferred into 100 - 200 μl of either Tris-EDTA (TE) or PBS in an Eppendorf tube.
2.4.3. Culture Procedures

An electric water jacketed incubator was set up in the kitchen attached to the Trial property’s shearing quarters. All preliminary isolations, subcultures and serogrouping were done in this field laboratory (Plate I).

Following anaerobic incubation at 37°C for 4 to 7 days on 4% HA, plates were examined for the characteristic spreading colonies of *D. nodosus*. *D. nodosus* colonies, or colonies suspected of being *D. nodosus*, were selected and subcultured onto 4% HA. A number of separate colonies was usually subcultured from each lesion plate, up to a maximum of 10. These subcultures were incubated anaerobically using Gas Pak Plus envelopes (BBL) at 37°C for 3 to 5 days. Depending on their purity, they were either harvested in formol PBS (1/80 formalin in stock PBS) for serogrouping by the slide agglutination method, or further subcultured until sufficiently pure for use as antigen. Immediately prior to serogrouping, *D. nodosus* isolates which were to be lyophilised were further subcultured on 2% HA. Care was taken to ensure individual colonies were subcultured.

2.4.4. Serogrouping - slide agglutination method

The slide agglutination method was used for all provisional serogrouping. The method has been described by Claxton et al (1983). Antigens for serogrouping were prepared either by:

i. harvesting *D. nodosus* cells grown for 3-4 days from at least one quarter of a 4% HA plate by the addition of 2-3 drops of formol PBS (1/80 formalin in stock PBS) to the area of the plate to be harvested, and scraping the cells with a new scalpel blade. The resulting cell suspension was collected with a Pasteur pipette;

or

ii. reconstituting lyophilised samples with 30-50 µl distilled water.
2. Definitions, Materials and Methods

Rabbit antisera for serogroups A - I were prepared by the method of Claxton et al (1983), using the following prototype *D. nodosus* strains (VCS reference no.): A - 1001; B - 1006; C - 1008; D - 1172; E - 1137; F - 1017; G - 1270; H - 1215; I - 1623.

Suspended antigen was placed in a 1.5 ml Eppendorf tube, and mixed. One drop or loopful (10μl) of suspended antigen for each serogroup tested was placed on a microscope slide. One smaller loopful (5μl) of rabbit antisera for the test serogroup was then placed on the slide adjacent to the antigen, and the slide gently rocked to facilitate mixing of antigen and antiserum. The resulting mixture was observed for characteristic flocculation appearing within 5 seconds of mixing. If flocculation occurred, a positive result for the test antigen with the antiserum was recorded.

Antigens of known serogroup were regularly tested with specific antisera to provide positive and negative control systems.
The shearers’ kitchen on the Trial property, which was used as the field laboratory.
CHAPTER 3

SURVEY OF A VOLUNTARY FOOTROT GROUP

3.1. Introduction

Footrot programmes under the supervision of Departments of Agriculture are in place in most states in Australia. Such programmes aim to eliminate or reduce the prevalence of the more virulent forms of footrot (on a property basis) by encouraging and/or enforcing the eradication of footrot from sheep and goats in designated areas. For such programmes to be successful, a sound understanding of the epidemiology of footrot, knowledge of the prevalence of the various forms of footrot, and demonstration of economic benefits to individual producers and the community as a result of the decreased prevalence are essential.

The occurrence of different forms of footrot, resulting in differences in severity of clinical signs, has both complicated the assessment of which flocks should be targeted in some programmes, and emphasised the need for property and State eradication and control programmes to be properly evaluated. The lack of information on both the prevalence and economic impact of less virulent forms of footrot is a limitation to such evaluations. A number of property surveys have been carried out recently to assess the prevalence of footrot in Victoria and South Australia (Cleland, unpublished; Roycroft and Harrison, unpublished; J. Larsen, pers. comm.; P. Saunders, pers. comm.). In New South Wales there is little information on the prevalence of the various forms of footrot in sheep flocks in areas considered favourable for the disease. Flock prevalence data have been derived from estimates by field officers (Anon., 1988; Walker and Plant, 1994), or by postal or interview surveys (Jordan et al., 1988; Locke and Coombes, 1994) which relied primarily on owner assessment.
The implementation of State footrot programmes assumes eradication can be achieved at the flock level. Further, it assumes that such eradication will generally be cost-effective. There has been little effort directed towards analysing the success of farm eradication programmes in the past 10 years, or to review techniques used by farmers in eradication programmes, both of which will influence the cost-effectiveness of eradication programmes. The risks of introducing footrot will also influence the benefits derived from eradication, and these risks have not been adequately quantified. Strategies to prevent such introduction have been recommended (Walker, 1988; Anon., 1994), but their adoption by farmers has not been assessed.

Voluntary Footrot Groups have been formed in New South Wales following the initiation of the New South Wales Strategic Plan (Anon., 1988). These groups generally comprise a group of properties within a given district. They have as their aim the eradication of footrot from properties within the group. They are of particular importance in high prevalence (Residual) areas because of the risk of re-infection from neighbouring properties. Their development has been encouraged throughout New South Wales as part of the advisory approach to footrot eradication (Walker and Plant, 1994). There are approximately 300 Voluntary Footrot Groups in New South Wales involving 8,500 producers (Scott Orr, 1995).

A survey of properties within one of these Footrot Groups was carried out in spring, 1992, and the survey is described here. The aim of the survey was to assess the footrot status of the sheep and goat flocks on the 22 contiguous properties which formed the group, to ascertain footrot eradication strategies which were used on these properties, and to determine the outcome of these.
3.2. Methods

3.2.1. Area

The survey was carried out in the Holbrook area in southern New South Wales. This is a predominantly winter rainfall area, with an annual rainfall of approximately 780 mm. Pastures are a mixture of subclover with annual grasses, or subclover with perennial pastures. Phalaris is the main perennial pasture.

3.2.2. Property selection

The properties surveyed were those in a voluntary footrot eradication group which had been formed in September, 1988. All property owners had expressed support for the group and its objective, which was to eradicate footrot from all properties within the group. Benign footrot was excluded as a target, but not explicitly defined. The group was based on the existing Bush Fire Brigade boundaries, and included all 22 properties within these boundaries. Ten meetings had been held between the formation of the group and this survey, with strategies for footrot control and eradication being discussed at these meetings. Since the formation of the group, two of the properties had been amalgamated under the one ownership, and were treated as one property in this survey. Two properties were run jointly, and were also treated as one property. One property had no sheep or goats, and was excluded from the survey, giving a total of 19 properties (flocks) surveyed.

3.2.3. Survey technique

A form (Appendix I) was sent to all owners, requesting information on flock size and composition, their current sheep (and/or goat) footrot status, footrot eradication strategies if footrot had been present over the past 5 years, and cattle numbers. Property visits were carried out between November, 1992 and January, 1993, with the exception of
two properties (Properties 5 & 13), where large numbers of sheep had been examined in
the preceding 8 months by the author, and the author was confident of the footrot status
of the sheep on these properties. Sufficient sheep were examined on each property to
enable the author and the owner to identify and agree on the most severe form of footrot
present on the property. Selection of sheep for inspection was generally biased towards
lame sheep, sheep from mobs with lame sheep, or sheep from mobs known to have, or
suspected of having, footrot.

Inspection of sheep's feet and sampling for bacterial culture were carried out by
the methods described in sections 2.4.1 and 2.4.2. Five samples were collected from
each flock, unless insufficient affected sheep were present, or mob variation justified
additional sampling.

Samples were immediately streaked onto 4% hoof agar (HA), and the plates
were incubated anaerobically at 37°C within 6 hours of the samples being collected.
Plates were examined 3 to 5 days later for growth characteristic of \textit{D. nodosus}
colonies. Colonies were progressively subcultured on 4% HA until pure growths
from individual colonies were achieved (see section 2.4.3).

Isolates were serogrouped using the slide agglutination method (Claxton et
al, 1983), as described in section 2.4.4. Isolates were further subcultured onto 2% HA
and then lyophilised for storage. Subsequently, isolates representative of all serogroups
recovered from each property were assessed for virulence, using the elastase test
(Stewart, 1979) and the gelatin gel protease thermostability test (GGPTT) (Palmer, 1993),
the latter being done at the Wagga Wagga Regional Veterinary Laboratory. Results for
the elastase test were reported as positive if there was evidence of clearing of elastin on
or before 21 days, and the day that clearing was first detected was also reported.
Negative results indicated no clearing at 21 days. Results for the GGPTT were reported
as positive (thermostable proteases) or negative (thermolabile proteases).
3.2.4. Classification of Footrot Status

The owner's assessment of his / her property footrot status was made prior to, or at the commencement of, the property visit. A clinical assessment was made after inspection of sheep and goat feet on the property. The criteria used for clinical assessment were as given in section 2.2.

The presence of footrot lesions in goats was considered indicative of the presence of footrot on the property, but a classification of the type of footrot was not made based on lesions observed in goats.

3.2.5. Analysis

Correlation coefficients were determined after ranking the data, by Spearman's Rank Correlation method.
3.3. Results

3.3.1. Survey Forms

Survey forms were completed for all properties.

3.3.1.1. Property statistics

According to the survey replies, there were 51,100 sheep on the 19 properties (average flock size of 2,700). Two properties had goats, with 380 goats in total. Fifteen of the 19 properties had cattle, with 8,050 cattle in total. The four properties without cattle had more than 1,500 sheep. The distribution of flock sizes is set out in Table 3.1.

Table 3.1. Flock sizes (sheep and goats)

<table>
<thead>
<tr>
<th>Flock size</th>
<th>&lt;50</th>
<th>50-100</th>
<th>100-1000</th>
<th>1000-2000</th>
<th>2000-5000</th>
<th>&gt;5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flocks (n=)</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

3.3.1.2. Owner assessment of footrot status

The owners' assessment of the footrot status of the sheep (and goats) present at the time of the survey is shown in Table 3.6. The footrot history over the past 5 years (excluding benign footrot) was, according to the owners, as follows:

- virulent footrot had been eradicated from 4 properties
- intermediate footrot had been eradicated from 2 properties
- virulent footrot was still present on 2 properties
- intermediate footrot was still present on 2 properties
- 8 properties had remained free of footrot
- 1 owner was unsure of the current status, but virulent footrot had been present within the past 5 years
The 8 properties considered free of footrot included two properties which had been free of footrot since purchase by current owners, but had had virulent footrot present prior to the purchase, with all sheep from the previous owners being sold prior to the property purchase. One property was Accredited footrot free.

3.3.1.3. Precautions adopted with purchased sheep

Sheep had been purchased and introduced onto 18 of the 19 properties within the past 5 years. On two properties, only rams had been purchased. On the other 16 properties, one or more mobs (more than 20 sheep) had been bought. Precautions taken to avoid introducing footrot were recorded for 17 properties, and in 13 cases were numerous and included some of the following actions by owners:

- 10 footbathed sheep off the truck
- 10 kept sheep isolated for varying periods
- 4 inspected lame sheep
- 8 attempted to purchase sheep from footrot free properties
- 2 vaccinated sheep (both these properties were infected at the time of purchasing sheep)
- 1 inspected all sheep (6 rams only).

The most common precautions were a combination of purchasing from free properties and footbathing introduced sheep immediately after they were unloaded off the truck (four producers); footbathing introduced sheep, isolation of sheep, and inspecting lame sheep (three producers); and purchasing from properties considered free of footrot and keeping sheep isolated (three producers).
3.3.1.4. Origin of footrot on infected properties

On 11 properties where footrot had occurred over the last 5 years, four owners said the footrot came from purchased sheep, two owners stated footrot had been present on the property during that period, and two owners stated that footrot had come from neighbours' sheep. Three owners were unsure where the footrot came from (Table 3.2).

Table 3.2. Owners' opinions of the source of footrot in their flocks (for flocks experiencing footrot in the previous 5 years)

<table>
<thead>
<tr>
<th>Source</th>
<th>Purchased sheep</th>
<th>Neighbour's sheep</th>
<th>Always present</th>
<th>Unsure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flocks (n=)</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

3.3.1.5. Eradication programmes

Footrot eradication programmes had been undertaken on 11 properties within the last 5-7 years. Owners were asked to complete details for their most recent eradication programme. Spring control treatments had been carried out on 9 of the 11 flocks, with all 9 owners using some form of footbathing. Formalin (5%) was used on one property, Footrite® or a 20% zinc sulphate solution was used on four properties and footbathing in 10% zinc sulphate was used on the other four properties. Sheep were foot pared during spring in addition to footbathing on four properties. Vaccination in conjunction with footbathing was used on one property.

On the two properties where spring treatments were not undertaken, the method of eradication was selling of all affected mobs.
The method of eradication during summer on the 9 properties where affected mobs were not sold involved inspection of sheep's feet. Following inspection of sheep:

- infected sheep were culled for slaughter on 7 properties
- infected sheep were treated (footbathed) on the other two properties.

Some sheep were treated with antibiotics on three of the 7 properties where inspection and culling affected sheep was nominated as the method of eradication.

Sheep were inspected 1-2 times over the summer on two properties, 2-3 inspections were carried out on four properties, 3- 4 inspections were carried out on two properties, and more than 4 inspections were made on one property (Table 3.3).

<table>
<thead>
<tr>
<th>Inspections (n=)</th>
<th>1-2</th>
<th>2-3</th>
<th>3-4</th>
<th>&gt;4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Properties (n=)</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

3.3.1.6. Outcomes of eradication programmes (owner assessment)

For the 11 properties where eradication programmes were undertaken, 7 owners claimed they were successful in eradicating either virulent (5) or intermediate (2) footrot. Footrot eradication failed on four properties. Footrot was considered eradicated from two properties in 1 year. On both these properties, the technique used was to sell all affected mobs. This group included one property with intermediate footrot. On two properties eradication took 3 years, and in both cases the final eradication programme consisted of the inspection of sheep 2-3 times over the summer, and the culling of infected sheep. Antibiotics were also used in one of these flocks, although whether
3. Survey of a Voluntary Footrot Group

treated sheep were retained or sold was not recorded.

Eradication took more than 3 years on the other three properties. Inspection and culling of infected sheep was given as the method in the most recent year on all three properties. Sheep were treated with antibiotics on two of these properties, whilst some mobs were culled on the third property.

On those four properties where footrot eradication failed, the most recent approach was given as inspection and culling of affected sheep (2), and inspection and treatment of affected sheep by paring and footbathing (2). Zinc sulphate (20%) was used to footbath sheep on one of these, and 5% formalin was used on the other. The number of inspections for these four properties was given as 1-2 times on two properties, 2-3 times on one property, and more than 4 times on one property.

One of the flocks from which intermediate footrot was claimed to have been eradicated was still considered infected at the property inspection (see section 3.3.2 below). The eradication programme for this flock involved spring footbathing in 10% zinc sulphate and footparing, 2-3 summer inspections, culling affected sheep, and the use of antibiotics. Eradication had been attempted for more than 3 successive years.

3.3.2. Property Inspections

Clinical assessments of the footrot status were made on 17 of the 19 properties (Table 3.4). One property (Property 3) had less than 10 sheep present in December, 1992, but purchased 3,000 sheep in January, 1993, and an accurate clinical assessment was not considered possible. On Property 19, all sheep were being sold at the time of the survey, and an inspection could not be arranged.

The footrot status of the flock was determined by consideration of the history of
the flock, the existing pasture and climatic conditions, the knowledge of any treatment of sheep prior to the inspection, and the inspection of sheep. Footrot lesions were present in 13 of the 17 (76%) sheep flocks, and both goat flocks, examined. On one property sheep had been grazed under conditions which were considered unsuitable for the expression of footrot (Property 12). No lesions were observed in 30 sheep examined, and the flock was considered free of footrot, although this may not have reflected the true status. On all other properties, conditions were judged to be suitable for the expression of footrot, based on the prevailing pasture conditions and temperature. No footrot was detected in either flock with less than 50 sheep (Properties 8 and 21).

Virulent footrot was present in 4/17 (23.5%) sheep flocks examined; intermediate footrot was detected in 2/17 (12%) flocks; and benign footrot was diagnosed in 8/17 (47%) flocks. Benign and virulent footrot were considered present in separate mobs on one property (Property 9). The mob with benign footrot had only been introduced recently, and had been kept separate from other sheep.

Sheep had been footbathed at least once in the 2 months preceding the property inspections on 7 properties. Based on owner assessment, two of these properties were believed to be free of footrot, and one flock had benign footrot. One owner was unsure of the footrot status, and a further two flocks were believed to have intermediate footrot. The remaining flock had virulent footrot. Sheep were footbathed in 10% zinc sulphate on 5 of the 7 properties. Formalin (5%) was the chemical used on the other two properties. Sheep's feet had also been pared by the owner on one of these two properties. Either virulent or intermediate footrot was diagnosed on four of the 7 properties which had footbathed sheep prior to the property inspections, whilst benign footrot was considered present on two of the other three properties. The seventh property (Property 19) was not inspected. The prior footbathing was not considered to have affected the flock assessment on any of these properties.
Table 3.4. Clinical assessment of property status for 17 of 19 properties surveyed.

<table>
<thead>
<tr>
<th>Property No.</th>
<th>Flock size category (^1)</th>
<th>No. sheep examined / no. in mob</th>
<th>Sheep footscore</th>
<th>Sheep with lesions (%)</th>
<th>Clinical assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50-100</td>
<td>10/50 **</td>
<td>2</td>
<td>20</td>
<td>Virulent</td>
</tr>
<tr>
<td>2</td>
<td>2000-5000</td>
<td>6/70</td>
<td>0</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>100-1000</td>
<td>35/200</td>
<td>28</td>
<td>1</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>&gt;5000</td>
<td>60/390</td>
<td>30</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>100-1000</td>
<td>78/650</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>&lt;50</td>
<td>11/11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>2000-5000</td>
<td>(a) 28/300</td>
<td>2</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>(b) 30/420</td>
<td>27</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>100-1000</td>
<td>85/675 *</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>2000-5000</td>
<td>6/6</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>1000-2000</td>
<td>30/1330 *</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>&gt;5000</td>
<td>1450/1450</td>
<td>75</td>
<td>37</td>
<td>11</td>
</tr>
<tr>
<td>14</td>
<td>100-1000</td>
<td>30/350 *</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>100-1000</td>
<td>30/300 *</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>&gt;5000</td>
<td>34/250</td>
<td>6</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>18</td>
<td>1000-2000 (^3)</td>
<td>10/55</td>
<td>0</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>100/1100</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>110/350 (^4)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1000-2000</td>
<td>30/180</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>35/100</td>
<td>7</td>
<td>7</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>40/130</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>&lt;50 (^5)</td>
<td>11/11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes:
1. Flock sizes as determined for Table 3.1.
2. Two separate flocks examined
3. Includes goats
4. Goat flocks
5. ND = not determined. Score 2 lesion indicative of the presence of footrot, but form of footrot not determined as only detected in goat.

* = all lame sheep in mob examined

** = footbathed in week before inspection
Owner and clinical assessment were in agreement on only 10 out of 17 sheep flocks. The flocks in which there was agreement included Property 6. The owner of this property believed the flock to be free of footrot, but because he owned cattle he believed he should state his assessment as benign footrot, as this was more likely to be the true status. For 6 of the 7 flocks where owner and clinical assessment did not agree, owners underestimated the severity of footrot present. Four owners were unaware of the presence of benign footrot in their flocks, a flock with virulent footrot was considered by the owners to have intermediate footrot, and one flock with intermediate footrot was considered to have benign footrot. The seventh flock had virulent footrot, with the owner being unsure of the status. Benign footrot was also diagnosed on this property.

3.3.3. Bacteriology

Samples were collected from sheep or goats with lesions if lesions were present in the animals examined. Lesions were not detected in three flocks (Properties 6, 8 and 12). For Property 6, samples were collected from the IDS of two sheep with suspicious but not inflamed IDS. For Property 8, a sample was collected from a lame cow being examined by the owner at the time of the property visit. No samples were collected from any animals on Property 12, as the sheep were being held on pastures considered unsuitable for the expression or assessment of footrot. For Property 21, the one goat present had a lesion and was sampled. No lesions were observed in any sheep, and no sheep were sampled. Properties 8 and 21 had less than 50 sheep in total.

Thus, samples for bacteriology were collected from 16 (of the 17) properties visited, 15 flocks (includes sheep and goats) and 14 sheep flocks.

*D. nodosus* was detected on all 16 properties from which samples were collected, and 94% of properties visited (16/17). *D. nodosus* was present in all sheep flocks sampled (14/14), including one flock (Property 6) where no lesions were present, and
82% (14/17) of sheep flocks inspected. *D. nodosus* was detected in both goat flocks examined.

*D. nodosus* isolates representing different serogroups were recovered from 8 of the 14 (57%) sheep flocks, and 8 of the 10 flocks where isolates from more than one sheep were serogrouped.

### 3.3.4. *In vitro* virulence testing

*In vitro* virulence testing was carried out on *D. nodosus* isolates from the 16 properties from which *D. nodosus* was isolated. Two or more isolates were tested from 6 flocks. Only one isolate was tested from each of the other 10 properties. Protease thermostable isolates were recovered from 7 of 14 sheep flocks, and 8 of 14 flocks had protease thermolabile (unstable) isolates. Protease thermostable and thermolabile isolates were recovered from sheep in two clinically different mobs on one property (Property 9). Both goat isolates tested were protease thermostable, and the single bovine isolate had unstable proteases. Elastase tests were conducted on *D. nodosus* isolates from 14 properties, these isolates being the same as those tested in the GGPTT. Elastase tests were not performed on isolates from Properties 8 (bovine thermolabile isolate) or 11 (benign footrot, thermolabile isolate). Isolates from 7 of the 14 sheep flocks were elastase positive, and 7 of 14 were elastase negative (at 21 days). All isolates from sheep flocks which were protease thermostable were elastase positive, and all isolates which were protease thermolabile were elastase negative. One goat isolate gave an anomalous result, being protease thermostable but elastase negative (at 21 days).

A comparison of clinical and laboratory findings could be made for 13 flocks (comparisons for Properties 6, 8, and 21 were not considered appropriate due to lack of clinical evidence of footrot in sheep). Clinical and laboratory assessments were in agreement for 10 flocks. This included all four flocks diagnosed with virulent footrot.
and 6 of the 7 flocks considered to have benign footrot. Isolates from the two flocks in which a clinical assessment of intermediate footrot was made could not be distinguished from virulent isolates using laboratory testing alone, although the day clearing was first detected on the elastase test tended to be later for those isolates. For one property (Property 20), the laboratory findings indicated a virulent strain of *D. nodosus* was present, whilst clinical observations suggested only benign footrot. The correlations for clinical assessment and GGPTT result, GGPTT result and elastase test result, and clinical assessment and elastase test result were 0.825, 0.86 and 0.82 respectively. If the two properties with intermediate footrot were considered to have virulent footrot, the correlations were 0.85 for clinical assessment and GGPTT result.

Owner assessment, clinical diagnosis and laboratory results were compared (Tables 3.5, 3.6). In the context of the group surveyed, where the objective was to eliminate virulent and intermediate footrot, the sensitivity and specificity of the GGPTT, using the clinical assessment as the 'gold standard', were 100% (6/6) and 89% (8/9) respectively.
Table 3.5. Owner assessment, clinical diagnosis, and laboratory results for 17 properties surveyed.

<table>
<thead>
<tr>
<th>Property No.</th>
<th>Owner Assessment</th>
<th>Clinical Diagnosis</th>
<th>Protease Thermostability result&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Elastase result&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virulent</td>
<td>Virulent</td>
<td>+</td>
<td>+(4)</td>
</tr>
<tr>
<td>2</td>
<td>Virulent</td>
<td>Virulent</td>
<td>+</td>
<td>+(4)</td>
</tr>
<tr>
<td>4</td>
<td>Nil</td>
<td>Benign</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Benign</td>
<td>Benign</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Benign</td>
<td>Nil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Nil</td>
<td>Nil</td>
<td>-&lt;sup&gt;3&lt;/sup&gt;</td>
<td>not tested</td>
</tr>
<tr>
<td>9</td>
<td>Unsure</td>
<td>(a) Virulent</td>
<td>+</td>
<td>+(4-7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Benign</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Benign</td>
<td>Benign</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Benign</td>
<td>Benign</td>
<td>-</td>
<td>not tested</td>
</tr>
<tr>
<td>12&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Nil</td>
<td>Nil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Intermediate</td>
<td>Intermediate</td>
<td>+</td>
<td>+(10-14)</td>
</tr>
<tr>
<td>14</td>
<td>Nil</td>
<td>Benign</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>Nil</td>
<td>Benign</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Intermediate</td>
<td>Virulent</td>
<td>+</td>
<td>+(11)</td>
</tr>
<tr>
<td>18</td>
<td>Benign</td>
<td>Intermediate</td>
<td>+</td>
<td>+(11)</td>
</tr>
<tr>
<td>20</td>
<td>Nil</td>
<td>Benign</td>
<td>+</td>
<td>+(7)</td>
</tr>
<tr>
<td>21&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Nil</td>
<td>Nil (sheep)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA&lt;sup&gt;6&lt;/sup&gt; (goat)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: 1. Result from GGPTT, + = thermostable, - = thermolabile (unstable)
2. + = clearing observed (days clearing first observed in brackets), - = no clearing at 21 days
3. Isolate from cow.
4. No samples collected for bacteriology.
5. Goat only animal with lesion, or evidence of* D. nodosus* infection
6. Not applicable.

Table 3.6. Summary of assessments of footrot status in 19 sheep / goat flocks

<table>
<thead>
<tr>
<th></th>
<th>No Footrot</th>
<th>Benign Footrot</th>
<th>Intermediate Footrot</th>
<th>Virulent footrot</th>
<th>Unsure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous 5 yrs</td>
<td>8&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Not asked</td>
<td>4</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Owner assessment</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Clinical Assessment&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>1&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Laboratory Assessment&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Not</td>
<td>8</td>
<td>Not</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

applicable determined

Notes: 1. Includes 2 properties where footrot had occurred in previous owners' flocks.
2. Only 17 properties visited.
3. One goat was the only affected animal in this flock.
4. Only 15 flocks assessed, one flock having both benign and virulent isolates.
3.4. Discussion

3.4.1. Prevalence

The high prevalence of both footrot lesions (76% of sheep flocks examined) and *D. nodosus* infection (82% of sheep flocks; 94% of properties) are higher than recorded in most property surveys, where 19% to 39% of flocks have had lesions indicative of footrot or *D. nodosus* organisms present (Morgan et al., 1972; Cleland, unpublished; J. Larsen, pers. comm.; P. Saunders, pers. comm.). However, 66% of flocks were considered to have footrot in a recent survey in Victoria (Roycroft and Harrison, unpublished). The high prevalence of footrot in this survey may be due to more favourable environmental conditions, bias in the sheep inspected, bias in property selection, or the time of the year the survey was conducted. It may also reflect the prevalence of footrot in districts which have conditions favourable to the disease.

The prevalence of virulent and intermediate footrot in this survey (35%) is similar to estimates for the survey region by field officers of 20% in 1988 and 40% in 1994 (Anon., 1988; Walker and Plant, 1994). It is also similar to estimates from surveys of producers (i.e. owner assessment) of 36% in 1988 (Jordan et al., 1988), and 49% in 1989 (Locke and Coombs, 1994). Based on owner assessment and the property status over the previous 5 years, the prevalence of affected flocks could have been as high as 68% in the survey group (13 properties). This is consistent with the above figures, as the area surveyed here is in a higher rainfall part of the region, and increasing rainfall has been associated with increasing number of affected flocks (Locke and Coombes, 1994).

The ratio of 2:1 for flocks affected with virulent footrot : intermediate footrot is higher than the 4:1 ratio estimated for New South Wales by Egerton and Raadsma (1991), but there are insufficient affected properties in this survey to allow any conclusions to be made. The prevalence of benign footrot (41%) was similar to the
prevalences detected in some Victorian surveys (Morgan et al., 1972; Roycroft and Harrison, unpublished) and a recent South Australian survey (P. Saunders, pers. comm.).

This survey, by its design, was not intended to define the prevalence of various forms of footrot across the region, but rather assess the footrot status of all farms within a defined group, and to compare the actual footrot property status with the owner / manager's perceptions.

The detection of *D. nodosus* in the absence of lesions (Property 6) has been reported previously (Morgan et al., 1972; Glynn, 1993; Depiazzi et al., submitted). It highlights the need to sample normal feet, as well as feet with lesions, in property surveys aimed at detecting less virulent strains of *D. nodosus*. It also indicates the need to view claims of eradication of less virulent strains of *D. nodosus*, based on clinical observations alone, with caution.

Infections with strains of *D. nodosus* from different serogroups were common in the flocks sampled. This was found despite sampling only small numbers of sheep from each flock. The detection of *D. nodosus* strains of different serogroups in a flock has been reported (see section 1.7.1).

### 3.4.2. Introduction and prevention of footrot

The owners' opinions on the sources of footrot outbreaks for properties in this survey were similar to those in a survey of properties in Victoria (Fitzpatrick, 1986). The most notable difference was the higher level of endemic disease in the Victorian survey, although this was possibly due to differences in survey technique. It was somewhat surprising that in the survey of the Holbrook group only two owners believed footrot had been introduced from stray neighbour's sheep, given that up to 13 of the 19 properties may have had footrot at some time in the 5 years preceding this survey. This figure
could have been as high as five properties, as owners on three properties were unsure of the source of footrot. Given the high property prevalence, it is understandable that this footrot group was formed, as neighbouring infected flocks are associated with an increased risk of disease for footrot free flocks (Walker, 1988; Anon., 1994).

The large proportion of owners (16 of 19) who purchased mobs of sheep highlights a further avenue for the introduction of footrot onto properties. If this is representative of properties in high rainfall areas, then the adequate identification of mobs to allow tracing of the movement of sheep will be an essential part of a State footrot programme. The need for farmers to understand the risks of introducing diseases, including footrot, with purchased sheep is also highlighted, with purchased sheep being the most frequent cause given for the introduction of footrot.

Recommendations to avoid the introduction of footrot from purchased sheep include ascertaining the status of the flock of origin, footbathing sheep on arrival, keeping sheep isolated, and inspecting any lame sheep (Walker, 1988; Anon., 1994). Whilst owners invariably adopted some of these strategies, the extent to which they were executed was not determined. Reliance on purchasing from free properties, whilst sound in theory, may be difficult in practice. Keeping sheep isolated may also not have been properly enforced. Precautions taken to minimise the introduction of footrot may not have been adequate on all properties as only four producers inspected lame sheep, and no producers purchasing mobs of sheep inspected all sheep. Footbathing of introduced sheep was commonly adopted. While this is recommended, there is no evidence that this practice prevents the introduction of footrot, and footbathing sheep may mask signs of disease, making inspection of sheep less effective. More importantly, if farmers believed footbathing minimised or prevented introducing footrot, they may have been less inclined to carry out isolation and inspection strategies properly, thereby increasing the risk of introducing footrot.
Decisions to eradicate footrot depend on costings which assume that freedom from footrot will be maintained following eradication. The risks posed by neighbour's sheep or by introduced sheep may decrease the cost-effectiveness of eradication for flocks in the group surveyed.

3.4.3. Methods of assessment of footrot status

Most owners had sought veterinary advice about lameness in sheep over the past 5 years, and had attended regular discussions on footrot in the preceding 4 years, as part of the Voluntary Footrot Group. Even so, owner assessment tended to underestimate the occurrence and severity of footrot within the flock. Owners were often unaware of the presence of benign footrot. It is likely therefore that owners with less exposure to footrot education would be even less able to assess flock footrot status, particularly if milder forms of footrot were present. These results suggest caution in the interpretation of flock prevalence data based on owner assessments.

Clinical and laboratory assessments were generally well correlated in this survey, particularly for properties with benign or virulent footrot, despite only one isolate being tested from each of 10 of the 16 properties. This correlation is in contrast to results from recent Victorian and South Australian surveys, where footrot outbreaks classified as benign frequently yielded protease thermostable isolates (Cleland, unpublished; Roycroft and Harrison, unpublished; Saunders and Riley, unpublished). The inability of protease-based tests to differentiate virulent and intermediate strains of *D. nodosus* is a limitation to the use of such tests to assess the prevalence of less virulent forms of footrot, and to assist with the diagnosis of clinical disease.

The anomalous result between the clinical and laboratory results for Property 20 could not readily be explained. Pasture quantity and quality were poorer on this property compared to pasture performance on most other properties within the Group. This may
have affected the clinical expression of disease within the flock. However, this would seem unlikely, given the prevalence of footrot lesions in at least one mob (24/40 or 60%). There was no suggestion that the disease had only been recently introduced. If a more severe form of footrot was present, the most likely source would have been from rams purchased 1 to 3 years previously from a property which was subsequently found to have intermediate footrot. The strain of Merino on the property was seen to be susceptible to footrot on other properties within the Group. No preventive measures had been practised in the mobs examined to inhibit the expression of footrot. While the possibility that the diagnosis of footrot may have been incorrect cannot be excluded, either due to insufficient sampling or other unidentified factors inhibiting the expression of footrot at the time of the flock examination, an alternative explanation would be that protease thermostable (and elastase positive) strains of *D. nodosus* may be associated with benign footrot. This has been reported previously (see section 1.6).

The correlation between clinical and laboratory assessments further suggests that in Holbrook, and presumably in areas with similar climatic conditions, the diagnosis of footrot can be confidently made on clinical criteria, in the absence of laboratory support. Alternatively, results from in vitro assessments of *D. nodosus* from affected flocks are likely to reflect the clinical severity of disease in the majority of cases.

### 3.4.4. Footrot eradication

Virulent or intermediate footrot had been eradicated from 8 properties in the preceding 5 years. This included two properties which had been sold, with the infected flocks being sold for slaughter at the time of the property sale. In both cases, the owners had failed to eradicate footrot in the year prior to selling the property. On the other 6 properties, the technique of selling footrot affected mobs was adopted on only two properties. On these two, eradication of footrot was achieved in one year. In contrast, all owners who attempted eradication of footrot by inspection and culling or inspection
and treatment of sheep took at least 3 years to eradicate footrot, and then eradication was achieved on only four of the 9 properties.

The annual success rates of footrot eradication programmes within the group were not determined. However, the success rates of between 30% and 58% appear lower than those previously reported for owner controlled summer eradication programmes (Fitzpatrick, 1986; Allworth, 1988; Mitchell, 1995). In a survey involving 105 properties where footrot eradication programmes had been undertaken, eradication of virulent footrot was apparently successful on 82 properties (80%) over a 5 year period (Egerton and Raadsma, 1993), although the method of evaluating eradication was not given, nor the methods of eradication. Eradication took more than 1 year on 53/82 (65%) properties. For farmers who fail to eradicate footrot on two attempts, the subsequent success rate of programmes was considered to be one in five (Fitzpatrick, 1986).

The reason for the low success rate for eradication in the group surveyed here was not determined. Only the most recent eradication programme was identified. The regular relapse of footrot which occurred in these failed programmes could have been due to failing to detect infected sheep at summer inspections. All owners who achieved eradication inspected sheep 2-4 times, while sheep were inspected only 1-2 times on two of the four properties which failed to eradicate footrot. Failure to detect infected sheep may also have been due to unskilled operators, or masking of infection following footbathing. Footbathing was the principal method of control during the spring, with zinc sulphate being the main chemical used recently. Given the common use of zinc sulphate footbathing and suggestions that zinc sulphate footbathing may not eliminate all D. nodosus (Atkins, 1986; Glynn, 1993), further assessment of the efficacy of this treatment and its role in eradication programmes is required.
3. Survey of a Voluntary Footrot Group

There was also a reluctance by those owners who failed to eradicate footrot to cull affected sheep. Reliance on treating sheep, rather than culling affected sheep, may have been responsible for failures on some properties. Footrot persisted in both flocks where affected sheep were treated rather than culled. In a further flock where footrot was not eradicated, inspection and culling of affected sheep was given as the method of eradication, yet some treated affected sheep were still present at the property inspection, and diagnosed as having footrot (Property 18). Insufficient culling was identified as a factor associated with low success rates in a recent survey of summer eradication programmes (Hawkins et al, 1995). Failure to effectively implement eradication programmes was also considered an important factor in this survey in Western Australia. The apparently haphazard approach to footrot on some properties in the group surveyed here, as evidenced by difficulties in completing the questions on the method of eradication, suggests that inadequacy of the programme and its application may have contributed to the failure of programmes.

The endemic nature of footrot in some flocks may have contributed to the low success rate. Success rates decreased in the first year of eradication from 58% for flocks where the disease had been recently introduced to 24% for flocks with endemic footrot (Fitzpatrick, 1986). Corresponding figures, if success rates included those properties from which footrot was eradicated within two years, were 78% for recently introduced footrot and 48% for endemic flocks (Fitzpatrick, 1986). This, combined with the lower success of programmes after two attempts, suggests that in endemic areas, eradication programmes will be less successful. This suggestion is supported by the findings in this survey. Similar conclusions were observed in the survey in Western Australia. Higher rainfall and neighbouring affected sheep, both likely to be features of endemic areas, were associated with a lower success in eradication (Hawkins et al, 1995).

The time taken to achieve eradication highlighted in this survey will influence the cost of eradication of footrot. Assuming a direct cost of $5 / head for an annual
eradication programme (Allworth, 1988), eradication programmes may have cost in excess of $15/ head. Whilst such costs may be justifiable for fully virulent footrot, they may not be appropriate for less severe forms of footrot, given the lower losses in productivity associated with such forms of footrot (Stewart et al, 1984; Anon., 1988; Egerton and Raadsma, 1991).

Further, whilst eradication of virulent or intermediate footrot was achieved on 8 properties, benign footrot was present on 6 out of 7 of these properties inspected. This supports previous observations (Egerton and Parsonson, 1969; Egerton and Raadsma, 1993). The detection of D. nodosus infection in the absence of clinical signs (Property 6) supports the hypothesis that the persistence of less virulent strains may be due to the inability to detect infected animals (Egerton and Raadsma, 1993).

3.4.5. Achievement of Group objective

The objective of the Group was to eliminate footrot (other than benign footrot) from all properties within the group. The initial number of flocks with footrot was believed to be 7 (out of 21). The results of this survey show that despite footrot being eradicated from 8 properties, the objective of the group had not been achieved, with 6 properties still having virulent or intermediate footrot. While it might be postulated that an increased awareness of footrot had been achieved, this awareness had not been translated into effective eradication or prevention programmes. The reason for the failure of the group to achieve its objective was not established, but discussions with the members suggest a number of factors might have been involved, in addition to those outlined above. Despite access to several professionals, a number of owners did not seek professional input to their footrot programme, either because they did not consider this necessary, or they did not consider footrot an important disease. Those who sought professional input did not always adopt the recommendations, mainly because they did not think that it was necessary or feasible to disrupt other farm practices to implement an
effective footrot eradication campaign. Of the four properties where eradication was achieved by an inspection and culling programme, all sought veterinary advice to assist with the formulation and completion of their footrot eradication programme in the final year. Conversely, of the five properties where eradication of footrot failed, only one owner sought individual veterinary advice to formulate an eradication strategy. This was despite access of all producers to a Rural Lands Protection Board veterinarian, who was competent to advise on footrot and whose services were available without direct fees.

Lack of motivation by owners to pursue footrot programmes was also a factor on some properties. Motivation of farmers has been considered an important aspect to the success of footrot eradication (Goodwin, 1994; Hawkins et al, 1995). The failure of the group to increase the motivation of some members may have been due to the normal outlook of those members, the perceived difficulty of dealing with footrot in endemic areas, or the lack of progress by other members within the group. Alternatively, in the case of less virulent strains, owners may not have perceived a benefit from costly and difficult eradication programmes. Some were not aware they had footrot.

3.4.6. Application of results to other groups or regions

The area surveyed was chosen because of the co-operation of the farmers, the history of footrot related activities within the group, and the suitability of the area for the expression of footrot. The difficulties experienced in this group may be associated with specific problems in the group, or alternatively, to problems associated with the particular environment. However, given the similarity in many findings to those of a survey in a different environment (Hawkins et al, 1995) and to a review of activities of other groups (Goodwin, 1994), it would seem reasonable to suggest that the findings from this survey may be applicable to much of the footrot endemic area of New South Wales and Victoria, and also to many of the 300 voluntary footrot groups in New South Wales.
3. Survey of a Voluntary Footrot Group

Losses from footrot are likely to be highest in footrot endemic areas, as not only will the disease be more likely to express itself, but more properties are likely to be affected. Surveys aimed at establishing footrot practices in such areas are likely to provide valuable information to those communities most at risk to losses from footrot, and most involved with footrot control and eradication programmes.

3.5. Conclusions

The results from this survey emphasise the difficulties faced in areas which are endemic for footrot, and in particular, the difficulty and cost of footrot eradication programmes. The presence of neighbouring infected properties, the low success rate of eradication programmes and the failure to implement effectively footrot prevention programmes resulted in the continued presence of footrot in the flocks surveyed. This was despite the existence of a group mechanism for the education and encouragement of producers.

Clearly, in such areas, there is a need to assess carefully the benefits of eradication before undertaking such time-consuming and expensive programmes. Such assessment will involve the estimation in loss of productivity from footrot, the costs of control versus eradication, and the likelihood of the success of the programme. The likelihood of success will need to take into account both the owner / property capabilities and the probability of remaining free following eradication.

The high correlation between clinical and laboratory assessments suggests that in endemic areas, the diagnosis of footrot will not be a limitation to the implementation of regional footrot programmes. However, if such programmes target milder forms of footrot, and rely on owner assessment to determine the status of properties, rather than either on-farm investigations or laboratory testing, the detection of footrot will decrease.
This further suggests that in areas where expression of footrot is limited by a less favourable environment, or on properties where the type of sheep limit the expression, owners may be unaware of more severe forms of footrot. The ability to detect footrot in this survey suggests that monitoring of sheep moved into endemic areas may be a useful technique for determining the footrot status of flocks in less favourable environments.

This survey also highlighted the ubiquitous nature of *D. nodosus* in footrot endemic areas. This was despite the application of, in some cases, successful footrot eradication programmes. These results support previous suggestions that the epidemiology of mild forms of footrot differ from that of virulent footrot, particularly in terms of eradicability. Details and results of experiments designed to evaluate the eradicability of a number of strains of *D. nodosus* are outlined and discussed in Chapter 4.
CHAPTER 4

FIELD EXPERIMENTS ON THE ERADICABILITY OF D. NODOSUS

4.1. Introduction

At the property level, decisions on the course of action following detection of any disease will depend on an assessment of the economic impact of the disease, the perception of the severity of the disease by the owner, the risk to public health, treatments and their costs, the likelihood of success of various strategies, and existing regulations. In the case of footrot, the disease may be eliminated from flocks if all cases of infection are removed by culling or effective treatment (Beveridge, 1941), as *D. nodosus* is an obligate parasite and is unable to survive for more than 7 days in the environment (Gregory, 1939b; Beveridge, 1941). Because virulent footrot can be eradicated from individual properties, and there can be economic benefits from such eradication, State footrot programmes aimed at reducing the prevalence of footrot (or eliminating it) have been implemented. By contrast, benign footrot has proved difficult to eradicate (Alexander, 1962; Littlejohn, 1966/67), and may be present on properties after eradication of virulent footrot (Egerton and Parsonson, 1969). Cattle have been implicated as a reservoir of benign footrot for sheep (Wilkinson et al, 1970), and production losses associated with the disease are less than for virulent footrot. Therefore, for practical and economic reasons benign footrot is not usually a target for eradication.

There is inadequate information on either the justification or practicability of eradicating intermediate footrot - the disease which is neither clearly benign nor obviously virulent. Anecdotal reports suggest that intermediate footrot can be eradicated (Gwynn, 1986; Robinson, 1986) but the cost-effectiveness of this has been questioned (Dobson, 1986; Egerton and Raadsma, 1991). Nevertheless, some State programmes
include all footrot other than benign as a target for eradication. The reasons for this include (i) the ability to more readily differentiate benign from "non-benign" isolates with protease-based laboratory tests, (ii) concern that the farmers may view footrot eradication a failure if intermediate forms persist, and (iii) concern that intermediate forms of footrot may become more severe if affected sheep are moved to another area (Abbott, 1994).

It is established in vitro that testing of *D. nodosus* categorises isolates into two or more groups depending on the tests used (Stewart, 1986d; Palmer, 1993; D. Stewart, pers. comm.). However, neither the relationship of those groups of isolates to the nature of disease associated with them, nor their eradicability, has been clearly established.

Accepting the premise that different strains of *D. nodosus* are responsible for different clinical manifestations of footrot, a series of experiments was designed to test the hypothesis that strains of *D. nodosus*, characterised by in vitro virulence tests and possessing a spectrum of virulence characteristics, vary in their susceptibility to eradication by conventional techniques. The main experiment was conducted in a deliberately infected flock in a footrot endemic area in southern New South Wales.

These experiments are described in this chapter.
4.2. Materials and Methods

4.2.1. Introduction

The series of experiments comprised:

(1) establishing and maintaining a multiple *D.nodosus* strain infection in a small *Donor Flock*

(2) assessment of the virulence, *in vivo*, of five of the *D.nodosus* strains included in the study

(3) the establishment and management of a large flock (referred to as the *Main Flock*) which developed footrot primarily following natural exposure to infection from sheep from the Donor Flock above (*infection phase*),

(4) the application of a programme based on conventional techniques, designed to eradicate footrot from the flock in (3) above. This programme comprised:

(a) the restriction of prevalence during the initial footrot transmission period (*the control phase*)

(b) the culling of footrot affected sheep to eliminate infection (*the eradication phase*)

(c) regular whole flock inspections over the following 18 months (*the surveillance phase*)
4. Field Experiments on the Eradicability of *D. nodosus*

A mob of affected sheep identified at the start of eradication was maintained in isolation during the eradication and surveillance phases so that the persistence of *D. nodosus* strains in sheep in the environment of the experiment could be assessed. A proportion of these sheep was specifically vaccinated in an attempt to recover *D. nodosus* strains which might be persisting at low frequency. In addition, recurrence of footrot in one mob during the surveillance phase led to further culling of clinically affected sheep (*additional eradication /surveillance phase*). Some of the sheep culled from this secondary outbreak were also vaccinated in a further attempt to recover additional *D. nodosus* strains that may have been present.

4.2.2. Bacteria

The strains of *D. nodosus* used in this study were selected to represent a spectrum of virulence principally determined by field observations of outbreaks of footrot. The strains were selected from a number of candidates so that each strain was from a different serogroup (Claxton et al, 1983), and the strains represented a spectrum of virulence determined by a number of criteria. Again the assumption was that each of the strains used was primarily responsible for the nature of the outbreak from which they came.

They will be referred to by their serogroup classification, namely as strains A, B, C, D, E, G and H. Their virulence ranking was independent of their serogroup. Clinical data from the flock of origin and *in vitro* characteristics for each strain are summarised in Table 4.1. A full description of the origin of these strains is given in section 2.3.2.

The capacity of five of these strains (A, B, C, E and H) to cause footrot in single strain infections was assessed in a field virulence assessment trial (see section 4.2.3.3).
Table 4.1. Characteristics of 7 *D. nodosus* strains.

<table>
<thead>
<tr>
<th>Strain VCS No.</th>
<th>1001</th>
<th>1746</th>
<th>1744</th>
<th>1748</th>
<th>1742</th>
<th>1745</th>
<th>1743</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Assessment&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Vir</td>
<td>Ben</td>
<td>Ben / Int</td>
<td>Ben / Int</td>
<td>Int</td>
<td>Int</td>
<td>Int</td>
</tr>
<tr>
<td>% Score 4&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&gt;50</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Serogroup</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>G</td>
<td>H</td>
</tr>
<tr>
<td>Elastase&lt;sup&gt;3&lt;/sup&gt;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin gel PT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gene Probe</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Notes: 1. Clinical assessment at time of isolation, based on number of severely affected sheep, and flock history. Vir = virulent footrot; Int = intermediate footrot; Ben = benign footrot.
2. Percentage of affected sheep with at least one score 4 lesion on property of origin; NA = not available.
3. Elastase test result; + = clearing by day 21, - = no clearing by day 21.

4.2.3. Sheep flocks, the establishment of *D. nodosus* infections within these flocks and trial design / procedures

The origin of the sheep used has been described in section 2.3.1.

4.2.3.1. Donor Flock

The Donor Flock consisted of 50 shorn five year old Polwarth ewes, considered free of footrot. In addition, one month prior to challenge, they were treated with penicillin / dihydrostreptomycin (PenStrep<sup>®</sup>, Ilium, 1 ml/3.5 kg), and held on battens for 4 days, before being placed in a paddock which had been destocked for 10 days.
were transported to the laboratory (Camden, New South Wales) for challenge with *D.nodosus*.

Donor sheep were artificially exposed to 5 strains of *D.nodosus* (strains A, B, C, D and G) by the method of Egerton et al (1969). Sheep were predisposed to *D.nodosus* infection by being placed on wet mats 5 days prior to the challenge. On the day of challenge (Day 0), all sheep's feet were inspected, and all sheep were individually tagged. Sheep were systematically allocated to one of 21 Challenge groups (2 sheep per group) (Table 4.2) or to a Control group (8 sheep). Sheep were challenged with 1-3 *D.nodosus* strains in the left front and left hind feet by application of a cotton swab containing cells scraped from half a TAS blood agar plate (J.Vaughan,pers.comm.) for each strain. Where more than one isolate was applied, the isolates were applied on the one piece of cotton wool. Front and hind feet received the same strain(s). Right feet were not challenged or bandaged. Left feet of Control sheep were bandaged but not challenged.

<table>
<thead>
<tr>
<th>Challenge group</th>
<th>Strain(s)</th>
<th>Challenge group</th>
<th>Strains</th>
<th>Challenge group</th>
<th>Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>8</td>
<td>AD</td>
<td>15</td>
<td>DG</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>9</td>
<td>AG</td>
<td>16</td>
<td>ABC</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>10</td>
<td>BC</td>
<td>17</td>
<td>ADG</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>11</td>
<td>BD</td>
<td>18</td>
<td>BCD</td>
</tr>
<tr>
<td>5</td>
<td>G</td>
<td>12</td>
<td>BG</td>
<td>19</td>
<td>BCG</td>
</tr>
<tr>
<td>6</td>
<td>AB</td>
<td>13</td>
<td>CD</td>
<td>20</td>
<td>BDG</td>
</tr>
<tr>
<td>7</td>
<td>AC</td>
<td>14</td>
<td>CG</td>
<td>21</td>
<td>CDG</td>
</tr>
</tbody>
</table>
Sheep were challenged with four strains (B, C, D and G) on Day 0, and with the fifth strain (A) on Day 2. Bandages were removed from sheep's feet 3 days after challenge. Sheep were held on wet mats until day 7, when they were put into an irrigated subclover-based pasture.

Sheep were inspected 28 days post challenge. The degree of lameness was recorded on a scale of 0 (no lameness) to 4 (severely lame):

Score 0  No lameness detectable
Score 1  Mild lameness, only apparent on careful inspection, no obvious discomfort
Score 2  Moderate lameness, some head nodding when walking
Score 3  Severely lame in one foot, obvious head nodding when walking, difficulty in moving
Score 4  Severely lame in more than one foot, walking appears difficult, appears to be "walking on hot bricks".

All feet were scored (see section 2.4.1) and sampled (see section 2.4.2). Samples were collected by scraping the IDS with a swabstick, and streaking this directly on to a 4% HA plate. Plates were incubated anaerobically at 37°C for 3-4 days. *D. nodosus* isolates were subcultured on 4% HA, and serogrouped by the slide agglutination method (see section 2.4.4). Generally 6-10 isolates per foot were serogrouped.
4. Field Experiments on the Eradicability of *D. nodosus*

### 4.2.3.2. Main Flock

Initially the Main Flock consisted of 1,450 recently shorn Polwarth wethers, aged 1-3 years.

All sheep were identified with numbered cartags, and inspected to establish freedom from clinical signs of footrot (Inspection 1). At this initial inspection, sheep were systematically allocated to one of five control phase treatment groups and one of four paddock groups (replicates). Sheep were stratified by age for both treatment and paddock groups. Mob numbers were set to provide a similar stocking density in each of the four paddocks, with equal numbers of sheep for each treatment group being allocated to each of the four paddocks. Sheep were colour branded (Siromark, IAMA) to signify treatment and paddock group. Initially they were kept as a composite flock to allow exposure to footrot during the infection phase.

This flock was grazed on the Trial Property in southern New South Wales. Paddocks consisted of predominantly annual grasses and subclover. Daily rainfall for the period January, 1992 to December, 1994 was recorded. Temperature data was available from a weather station 60 km from the Trial property. Mean daily and monthly temperatures were calculated by averaging the daily and mean monthly maximum and minimum temperatures, respectively.

The timetable for the main events for the Main Flock is summarised in Table 4.3, and a flow diagram for the Main Flock is presented in Figure 4.1.
4. Field Experiments on the Eradicability of *D. nodosus*

Table 4.3. Calendar of main events in Footrot Trial for Main Flock

<table>
<thead>
<tr>
<th>Phase</th>
<th>Date</th>
<th>Inspection Number</th>
<th>Mob(s)</th>
<th>Procedure(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept,'92</td>
<td>1</td>
<td>All</td>
<td>Initial inspection</td>
<td></td>
</tr>
<tr>
<td>Sept,'92</td>
<td>All</td>
<td></td>
<td>Introduce infected donor ewes</td>
<td></td>
</tr>
<tr>
<td>Oct/Nov,'92</td>
<td>All</td>
<td></td>
<td>Divide into 4 mobs, additional</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>challenge</td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>Nov/Dec,'92</td>
<td>All</td>
<td>1st eradication inspection, formation of Mobs 1, 2, 3 and 4</td>
<td></td>
</tr>
<tr>
<td>Jan,'93</td>
<td>2</td>
<td>All</td>
<td>Assess spring control, footbath 90% sheep</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Commenge footbathing, additional vaccination</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Assess spring control, footbath 90% sheep</td>
<td></td>
</tr>
<tr>
<td><strong>Eradication</strong></td>
<td>Feb,'93</td>
<td>3</td>
<td>All</td>
<td>Shearing</td>
</tr>
<tr>
<td>Mar,'93</td>
<td>4</td>
<td>Mobs 1,2,3</td>
<td>1st eradication inspection</td>
<td></td>
</tr>
<tr>
<td>April,'93</td>
<td>5</td>
<td>Mobs 1,2,3</td>
<td>2nd eradication inspection</td>
<td></td>
</tr>
<tr>
<td>April,'93</td>
<td></td>
<td></td>
<td>3rd eradication inspection</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1st vaccination (A,E,H) V1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>October,'94</td>
<td>PV1</td>
<td>Mob 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inspection 4 weeks post V1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>February,'94</td>
<td>8</td>
<td>Mob3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3rd surveillance inspection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>February,'94</td>
<td></td>
<td>Mob 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2nd vaccination (A,E,H) V2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>March,'94</td>
<td>PV2</td>
<td>Mob 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inspection 6 weeks post V2</td>
<td></td>
</tr>
<tr>
<td>Mar/Apr,'94</td>
<td>8</td>
<td>Mobs 1,2</td>
<td>Inspect sample at shearing,</td>
<td></td>
</tr>
<tr>
<td>Nov,'94</td>
<td>11</td>
<td>Mobs 1,3</td>
<td>Surveillance inspection</td>
<td></td>
</tr>
<tr>
<td>Additional eradication/surveillance phase (Mobs 2, 5)</td>
<td>April,'94</td>
<td>Mob 2</td>
<td>1st vaccination (C) VC1</td>
<td></td>
</tr>
<tr>
<td>May,'94</td>
<td>9</td>
<td>Mob 2</td>
<td>1st eradication inspection,'94; culls and vaccinates to Mob 5</td>
<td></td>
</tr>
<tr>
<td>May,'94</td>
<td>PVC1</td>
<td>Mob 5</td>
<td>Inspection 6 wks post VC1, 2nd vaccination (C) VC2</td>
<td></td>
</tr>
<tr>
<td>June,'94</td>
<td>10</td>
<td>Mob 2</td>
<td>2nd eradication inspection,'94</td>
<td></td>
</tr>
<tr>
<td>July,'94</td>
<td>PVC2</td>
<td>Mob 5</td>
<td>Inspection 6 weeks post VC2</td>
<td></td>
</tr>
<tr>
<td>Sept,'94</td>
<td>11</td>
<td>Mob 2</td>
<td>Spring,'94 surveillance inspection</td>
<td></td>
</tr>
<tr>
<td>Oct,'94</td>
<td>11</td>
<td>Mob 5</td>
<td>Inspection 22 weeks post VC2</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.1 Flow Chart of Procedures in Main Flock.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Event</th>
<th>Sheep on Trial Property</th>
<th>Removed from trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inspect 1</td>
<td></td>
<td>MAIN FLOCK</td>
<td></td>
</tr>
<tr>
<td>Inspect 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection phase</td>
<td>Donors (50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MAIN FLOCK</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inspect 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eradication phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inspect 3</td>
<td>Paddock 1</td>
<td>Clean</td>
<td>Donors (20)</td>
</tr>
<tr>
<td></td>
<td>Paddock 2</td>
<td>Clean</td>
<td>Culled (surplus)</td>
</tr>
<tr>
<td></td>
<td>Paddock 3</td>
<td>Clean</td>
<td>Culled (affected)</td>
</tr>
<tr>
<td></td>
<td>Paddock 4</td>
<td>Clean</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surveillance phase</td>
<td>Inspect 6</td>
<td>Mob 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inspect 7</td>
<td>Mob 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inspect 8 PV1</td>
<td>Mob 1</td>
<td>Mob 4</td>
</tr>
<tr>
<td></td>
<td>Inspect 8 PV2</td>
<td>Mob 1</td>
<td>Mob 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vaccinated</td>
</tr>
<tr>
<td>Additional eradication / surveillance</td>
<td>Inspect 9 Mob 2</td>
<td>Affected + Vaccinates</td>
<td>Mob 5</td>
</tr>
<tr>
<td></td>
<td>Inspect 10 Mob 2</td>
<td>Affected</td>
<td>Mob 5</td>
</tr>
<tr>
<td></td>
<td>PVC2 Mob 2</td>
<td>Clean</td>
<td>Mob 5</td>
</tr>
</tbody>
</table>

4. Field Experiments on the Eradicability of D. nodosus
4. Field Experiments on the Eradicability of *D. nodosus*

**Figure 4.2.** Map of Quarantine Area for Main Flock on Trial property.

Numbers 1 - 5 refer to Paddocks 1 - 5. Mobs 1, 2 and 3 were kept in Paddocks 1, 2 and 3 respectively. Mob 4, and later, Mob 5, were retained in Paddock 5.

- c = Cattle yard facility; used for sheep in Paddocks 3, 4 and 5
- S = Sheep yards, used for sheep in Paddocks 1 and 2.
- W = Woolshed
4.2.3.2.1. Infection phase

One of the objectives of this study was to establish as many of the characterised *D. nodosus* strains as possible in the Main Flock. Towards this end, *D. nodosus* strains were introduced into this flock on several occasions.

The 50 ewes in the Donor Flock were transported to the Trial property following the assessment of the mixed *D. nodosus* challenge of the donor flock. Additional bacteriological sampling of these ewes resulted in only three (strains A, C, and G) of the five strains used being recovered. At this stage, a sixth strain (strain E), suitable for inclusion, had been identified and characterised. Therefore, donor ewes were additionally challenged with strains B, D and also with strain E (grown on 2% HA) by the application of strains singly to the IDS of feet in the manner described above (section 4.2.3.1). The nature of the pastures on which these ewes were being grazed and the appearance of the interdigital skin suggested these sheep were adequately predisposed to *D. nodosus* infection, and so no artificial predisposition was considered necessary.

Seventeen days after this additional challenge, the donor ewes were inspected and added to the Main Flock of 1,450 wethers, at a time when, based on previous experience, conditions were considered suitable for the transmission of footrot (September, 1992). All wethers were maintained as one mob with the 50 donor ewes for 28 days, when the wethers were segregated into their allocated paddocks (one of four). The donor ewes were again scored, and 20 donor ewes were additionally challenged with a seventh strain (strain H, grown on 2% HA), in the manner described above, without prior predisposition, with 5 ewes being introduced into each paddock. Bandages were removed 5 days after challenge. The remaining 30 donor ewes were removed from the Main Flock and transported back to the laboratory for intensive sampling.
Despite conditions being apparently suitable for footrot transmission, there was little clinical evidence of disease in either the donor ewes or a sample of wethers in the Main Flock inspected at the time of segregation of the wethers to their allocated paddocks. Therefore, it was decided also to deliberately infect 56 wethers (14 per paddock) with strains A, B, C, D, E and G. This additional introduction of \textit{D. nodosus} strains occurred 3 weeks after wethers were segregated. Each of the 56 wethers had three strains applied (as above) to the IDS, one strain being applied to each foot. The remaining foot was not challenged. An equivalent number of feet was challenged with each strain for each of the four groups of 14 wethers. Bandages were removed 3-5 days after challenge.

\textbf{4.2.3.2.2. Control Phase}

The control phase aimed at reducing footrot prevalence in the Main Flock to between 5\% and 15\%, so that eradication by inspection and culling was a reasonable option during the summer. Due to the desire to mimic on-farm methods during the control phase so that possibly different carrier states were represented in sheep for the eradication phase, a number of control methods was compared. These methods included commonly recommended procedures.

Sheep in each of the five groups were to receive one of five treatments during the control phase:

1. nil treatment (\textit{NIL}, \textit{n}=290)

2. weekly walkthrough footbathing in 10\% zinc sulphate or Footrite\textsuperscript{\textregistered}, a 20\% zinc sulphate / sodium laural sulphate formulation (\textit{WWF}, \textit{n}=290)

3. one hour standing in a footbath every 3 weeks in Footrite\textsuperscript{\textregistered} (\textit{SIF}, \textit{n}=290)

4. commercial whole cell footrot vaccine (\textit{VWC}, \textit{n}=290)

5. experimental footrot vaccine (\textit{VEX}, \textit{n}=290).
4. Field Experiments on the Eradicability of \textit{D. nodosus}

Details for these control procedures were:

1. Nil: sheep in this group received no treatments during the control phase.

2. WWF: sheep in the weekly footbathing group received 6 - 7 treatments, which consisted of walking through an 8 m long footbath containing 10\% zinc sulphate or walking through a 4m x 4m footbath containing Footrite\textregistered every 7 days. Sheep in this group spent less than 1 minute, and generally less than 30 secs, in the footbath.

3. SIF: sheep in the 1 hour stand-in footbathing group received 3 one-hour footbathings in Footrite\textregistered at intervals of 3 weeks.

4. VWC: sheep in this group received three 1 ml doses of a commercial whole cell \textit{D. nodosus} vaccine (Vaxall NoRot\textregistered, SmithKline Beecham). The first two doses were administered 4-5 weeks apart, with the second dose being given in the week prior to the introduction of the donor ewes. A third dose was given 10 weeks after the second dose, when footbathing treatments commenced. Each dose of vaccine was administered subcutaneously.

5. VEX: sheep in this group received three 1 ml doses of an experimental recombinant \textit{D. nodosus} vaccine. The treatment regime and method of administration was as for sheep in the VWC group.

There were representatives of each of the treatment groups in each of the four replicate paddocks.
4. Field Experiments on the Eradicability of *D. nodosus*

Following these spring control measures, 25-35 days prior to the commencement of the eradication phase, all sheep were footscored (Inspection 2). Because of the high prevalence of footrot, 90% of systematically selected sheep in each paddock from each of the five treatment groups were footbathed in the zinc sulphate formulation for at least 15 minutes to reduce the prevalence to a manageable level. The remaining 10%, representing all spring treatment groups, were not treated so that adequate sampling material for bacteriology would be available at the commencement of the eradication phase.

4.2.3.2.3. Eradication Phase

The eradication phase commenced in February, 1993. The method of eradication was identification by inspection and culling of those sheep with clinical signs of footrot or with abnormalities of the IDS or hooves which would normally lead to culling in a footrot programme (Beveridge, 1941).

Three whole flock eradication inspections were carried out in this phase (Inspections 3, 4, and 5) at approximately monthly intervals. At Inspection 3, all feet of all sheep were trimmed, and inspected for evidence of footrot (Inspection 3). Sheep with apparently normal feet (referred to as 'clean' sheep) were allocated to one of three replicates (paddocks) on the following basis:
• sheep from Paddock 1 in the control phase were retained in Paddock 1;

• sheep from Paddock 2 in the control phase were retained in Paddock 2;

• sheep from Paddock 3 in the control phase were retained in Paddock 3;

• sheep from Paddock 4 in the control phase were allocated to Paddocks 1 (tag numbers ending in 1, 2 and 3), 2 (tag numbers ending in 4, 5, 6, 7) and 3 (tag numbers ending in 8, 9).

The proportion of sheep from Paddock 4 going to each of the other three paddocks was adjusted to maintain similar stocking densities in each of the three eradication replicates. Sheep in Paddocks 1, 2 and 3 are subsequently referred to as Mobs 1, 2 and 3, respectively. At Inspection 3, clean sheep were footbathed in Footrite® for 2-5 minutes, and returned to their appropriate paddock.

A Control group of 48 sheep (Mob 4) was retained from among the affected sheep culled at Inspection 3. The remaining culled sheep and those sheep with tag numbers ending in "0" from Paddock 4 during the control phase were then excluded from the trial.

At Inspections 4 and 5, sheep in Mobs 1, 2 and 3 were inspected. Little or no foot paring was carried out at these inspections, and no footbathing was done. Sheep culled for footrot or other foot abnormalities were added to Mob 4.

Sheep were shorn in April, 1993 when conditions in the yards were still dry. Mob 4 was shorn separately. Clean mobs did not come into contact with any area possibly contaminated by Mob 4.
4. Field Experiments on the Eradicability of *D. nodosus*

4.2.3.2.4. Surveillance Phase

After shearing in April, Mobs 1, 2 and 3 were maintained in their respective paddocks. They were inspected in yards adjacent to these, yards being spelled for at least 9 days prior to any subsequent inspections. Routine husbandry procedures (drenching, crutching, jetting, fly inspections) were generally carried out in conjunction with inspections.

Mobs 1, 2 and 3 were inspected and footscored twice in the spring / early summer following the eradication inspections (Inspections 6 and 7). One mob (Mob 3) was inspected a third time (Inspection 8, February, 1994).

Sheep in Mob 4 were inspected initially in October and November, 1993 (Inspections 6 and 7). In an attempt to identify additional *D. nodosus* strains present in Mob 4, 34 of the 45 sheep received 2 doses of a trivalent recombinant *D. nodosus* vaccine, containing antigens for serogroups A, E and H, 7 weeks apart (V1, V2) in December, 1993 and February, 1994. All sheep in Mob 4 were inspected in January, 1994 (Inspection PV1) and March, 1994 (Inspection PV2), 4 weeks after V1 and 6 weeks after V2, respectively.

All mobs were shorn separately in autumn, 1994, with the yards, laneways and shearing shed being spelled for at least 9 days between mobs. A sample of sheep was inspected at shearing from Mobs 1 and 2 (Inspection 8), but not Mob 3.

Surveillance of Mobs 1, 2 and 3 continued to spring, 1994. Mobs 1 and 3 were inspected in November, 1994 (Inspection 11). Surveillance and additional eradication procedures for Mob 2 are detailed below.
4. Field Experiments on the Eradicability of *D. nodosus*

4.2.3.2.4.1. Additional Eradication /Surveillance Phase (Mob 2 only)

Following the detection of clinical footrot in Mob 2 during the surveillance phase (Inspections 7 and 8), an additional eradication programme was applied to this mob. No control measures were undertaken. Eradication inspections, as described for Inspection 3, were carried out in May and June 1994 (Inspections 9 and 10, respectively). Sheep culled (as for Inspections 3-5) were removed from Mob 2 at these inspections. A group of 68 of these culled sheep (referred to as Mob 5) were retained in a separate paddock, and the remainder were consigned to slaughter. Clean sheep received no treatment after either Inspection 9 or 10, and were returned to Paddock 2 on each occasion.

In addition to this eradication programme, it was decided to vaccinate a sample of sheep in Mob 2 in an attempt to identify additional *D. nodosus* strains which might have been present. Nineteen affected sheep were given two subcutaneous 1 ml doses of an experimental monovalent recombinant *D. nodosus* vaccine, containing antigens for serogroup C, 6 weeks apart in April, 1994 (VC1) and May, 1994 (VC2). These vaccinated sheep were initially in Mob 2 at VC1, but were all transferred to Mob 5 at Inspection 9, and were subsequently retained in Mob 5. Mob 5 was inspected 6 weeks after VC1 (May, 1994, Inspection PVC1), 6 weeks after VC2 (July, 1994, Inspection PVC2), and in October, 1994, 22 weeks after VC2 (Inspection 11). All feet with lesions were sampled for bacteriology at these three inspections.

Mob 2 was inspected finally in September, 1994 (Inspection 11).

4.2.3.2.5. Sample collections

At the commencement of the eradication phase (Inspection 3), and for all surveillance inspections (Inspections 6, 7, 8, 11), material was collected for bacteriology from at least 8 sheep in each of Mobs 1, 2 and 3. All affected sheep were sampled in
4. Field Experiments on the Eradicability of *D. nodosus*

Mob 4 at Inspections 6 and 7, and at the post vaccination inspections for both Mob 4 and Mob 5. For Mobs 1 and 2, 6 and 33 sheep were sampled respectively in April 1994 after shearing. For Mob 2, all sheep culled at Inspection 10, and all sheep consigned to slaughter, were sampled.

Samples were collected from sheep with lesions (score 1 or greater) or otherwise abnormal feet where possible. Samples were usually collected by scraping a swabstick across the IDS, and streaking this material directly on to a 4% HA plate. Isolates were subcultured on 4% HA, and serogrouped by the slide agglutination method (see section 2.4.4).

4.2.3.3. Virulence Assessment Flock

The *Virulence Assessment Flock* comprised 50 Polwarth wethers selected from the Main Flock, above, and 100 Merino sheep from Merino Flocks 1 and 2 (see section 2.3.1). The Polwarth wethers had been removed from the Main Flock at Inspection 3, treated with antibiotics and inspected and sampled regularly in the 18 months between removal from the Main Flock and inclusion in the Virulence Assessment Flock. They were considered free of *D. nodosus* infection.

The Merino sheep were considered free of virulent and intermediate forms of footrot. Eight weeks prior to the commencement of the trial, they were treated with penicillin / dihydrostreptomycin (PenStrep®, Ilium, 1 ml / 3.5 kg), footbathed in 10% zinc sulphate (Hardman Chemicals) and held on battens for 24 hours, before being placed in a paddock which had been destocked for 5 days.
4. Field Experiments on the Eradicability of *D. nodosus*

At the commencement of the trial, all sheep were individually tagged, body weights were recorded, and all feet were inspected. The 150 sheep were systematically allocated to one of 15 groups, with stratification for breed and sex.

A 12 ha predominantly subclover / perennial ryegrass paddock was subdivided into 15 equal plots (0.8 ha). The paddock was spelled for 6 weeks prior to the commencement of the trial.

A randomised block design was used with three blocks comprising the first, middle and last 5 plots, respectively. Five *D. nodosus* strains of the 7 used to infect the Main Flock (A, B, C, E and H) were selected. These five were randomly allocated within each block of 5 plots. In each plot, 10 sheep were challenged with a single strain. Thus, each group of 10 sheep was infected with a single *D. nodosus* strain (of the 5 tested) and there were three replicates of 10 sheep for each strain.

All sheep had been grazed together for 2 months prior to *D. nodosus* challenge, and no additional predisposition of sheep's feet occurred other than naturally from the prevailing pasture conditions. Cultures of each strain harvested from 2% HA plates were bandaged onto the IDS of three feet of each sheep. The remaining foot was not challenged (or bandaged). Bandages were removed after 3 days.

Sheep were inspected 6 weeks and 10 weeks after challenge. They were re-weighed 5 days after the final inspection. Samples for bacteriology and PCR analysis (see section 2.4.2) were collected from three sheep per plot 6 weeks post challenge, and two sheep per plot 10 weeks post challenge.
4. Field Experiments on the Eradicability of *D. nodosus*

4.2.4. Interpretation of footscores

Generally, a sheep was considered affected with footrot if a score 2 lesion or greater was present in at least one foot. For the Main Flock, at inspections in the eradication phase (Inspections 3, 4 and 5) and Inspections 9 and 10 (Mob 2), sheep with lesions (score 1 or greater) were culled as having footrot. For the Virulence Assessment Flock, the total footscore for each sheep (TFS) was used to compare strains. TFS is the sum of the scores of all four feet of a sheep.

4.2.5. Recovery of Isolates

Samples collected from sheep from mobs with *D. nodosus* present in the Donor and Main Flocks were analysed to assess the recovery rates of *D. nodosus* from different categories of abnormal feet. Categories for sampled feet were:

- No abnormality (score 0)
- Score 1
- Score 2
- Score 3 or score 4
- Abscess - lesions considered to be due to foot abscess or toe abscess
- IDS abnormality - any abnormality of the interdigital skin other than those associated with scores 1-4
- Grass seed - lesions associated with grass seed infestation, but not concurrently with scores 1-4

Samples were considered culture positive if colonies with the typical colonial morphology of *D. nodosus* were present on lesion plates, and isolates were able to be serogrouped following subculture. Samples were considered culture negative if no colonies consistent with *D. nodosus* morphology were seen on the lesion plate. Results
4. Field Experiments on the Eradicability of *D. nodosus*

for colonies which were considered to have *D. nodosus* colonial morphology on lesion plates but were subsequently "lost" on subculture, or were not able to be serogrouped, were excluded.

4.2.6. Analysis of data

Results of proportions of sheep or feet affected and proportions of isolates were analysed using the chi-square test. For the Virulence Assessment Flock, TFS data was transformed by taking the square root of (TFS + 0.5), and an Analysis of Variance performed (Minitab, Minitab Inc.). Body weight data was analysed without transformation. Initially, a split plot analysis was performed, but as between plot variability was similar to within plot variability, data was pooled and a factorial analysis performed. Differences between means for different strains were analysed by Tukey's method.
4. Field Experiments on the Eradicability of *D. nodosus*

4.3. Results

4.3.1. Donor flock

4.3.1.1. Clinical Findings

Footrot was successfully established in the Donor flock. Twenty eight days after challenge, 34/50 (68%) sheep had footrot, with 44/84 (52%) feet challenged being affected. Clinically, the footrot induced by the multiple strain challenge resembled an intermediate form (Table 4.4). Score 2 lesions were the most prevalent, and were usually severe. Only one sheep (3% of affected sheep) had a score 4 lesion, despite the inclusion of a virulent strain of *D. nodosus* (strain A) in the group of challenge strains.

<table>
<thead>
<tr>
<th>Sheep (n=)</th>
<th>Prevalence(^1)</th>
<th>Score 2(^2)</th>
<th>Score 3</th>
<th>Score 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>68%</td>
<td>56%</td>
<td>41%</td>
<td>3%</td>
</tr>
</tbody>
</table>

Notes:  
1. Percent of sheep with at least one score 2 lesion or greater.  
2. Percent of affected feet

Lameness (scores 1-4) was recorded for sheep with score 2 lesions and greater. Lesion scores were not correlated with degree of lameness observed in affected sheep. Of the 34 sheep with footrot, 17 showed no sign of lameness. The highest lameness scores were associated with severe interdigital lesions, which by the criteria used, were score 2.

For challenged feet, hind feet were more frequently affected (*p*<0.001), with lesions being present in 14/42 front feet and 30/42 hind feet. There was evidence of spread of footrot to the feet not deliberately challenged, with 18/116 unchallenged feet
being affected. Prior bandaging of naturally exposed feet favoured the subsequent development of footrot, with 7/16 bandaged feet being affected compared to 11/100 unbandaged feet (p<0.001).

4.3.1.2. Bacteriology

Multiple strains of *D. nodosus*, as determined by multiple serogroups, were established within the Donor Flock. Of the 62 affected feet, 50 (81%) yielded *D. nodosus* isolates on culture. A total of 269 isolates was serogrouped. Multiple strain (serogroup) infections were present in 27 feet, with isolates from three serogroups being recovered from 5 feet, and four serogroups from one foot. Recovery rates, cross contamination and new infections varied between strains (Table 4.5).

Table 4.5. Results for Donor Flock 28 days after challenge.¹

<table>
<thead>
<tr>
<th>Strain</th>
<th>Feet challenged (n=)</th>
<th>Recovery rate² (proportion)</th>
<th>Feet cross-contaminated³ (n=)</th>
<th>New infections⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>28</td>
<td>8/15</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>36</td>
<td>0/9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>36</td>
<td>9/16</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>36</td>
<td>2/16</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>G</td>
<td>36</td>
<td>16/20</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

Notes: 1. Numbers in table cannot be reconciled due to some feet being challenged with multiple strains.
2. *Number of feet with isolates of indicated serogroup*  
   Number of feet positive for *D. nodosus*.
3. Number of feet challenged with other strains that were infected with the strain indicated.
4. Naturally exposed feet.
4. Field Experiments on the Eradicability of *D. nodosus*

In feet challenged with multiple isolates, the establishment of strains appeared to follow a "dominance" pattern: G > A > C > D > B. Thus if A and G were applied to a foot, G was more likely to be recovered. Whilst serogroup A, C, D and G isolates spread to naturally exposed feet, only C and G isolates became established in feet challenged with other strains. No serogroup B isolates were recovered from any feet.

4.3.2. Main Flock

There was no evidence of any lesions consistent with footrot in any wethers at the commencement of the trial (Inspection 1). Cultures from 17 sheep with otherwise abnormal feet were negative for *D. nodosus*, there being no colonies suggestive of *D. nodosus* on any primary isolation plates.

4.3.2.1. Climatic data

Above average rainfall in both 1992 and 1993 resulted in lush pasture conditions considered suitable for the expression and transmission of footrot in both spring / early summer, 1992 and spring / summer, 1993. Below average rainfall in 1994 resulted in an abnormally dry spring, with poor pasture growth and drier conditions which were considered less conducive to footrot transmission. Rainfall and temperature records suggested transmission of footrot would commence for the winter / spring periods on 22 September, 1992 and 25 August, 1993, based on at least 50 mm rainfall per month during winter and mean daily temperatures being consistently above 10°C (at least 7 consecutive days) (Graham and Egerton, 1968). For the 1994 winter / spring period, temperatures were consistently above 10°C after 25 August, but rainfall between March and September was less than 50 mm for all months except July, suggesting footrot transmission would not occur. Monthly rainfall totals, mean monthly temperatures and predicted periods of footrot transmission, based on the rainfall and temperature
requirements hypothesised by Graham and Egerton (1968) are presented in Figure 4.3 for the period January, 1992 - December, 1994.

**Figure 4.3.** Rainfall and temperature data for the Trial property, for the period January, 1992 - December, 1994, and predicted periods of footrot transmission (Graham and Egerton, 1968).
4.3.2.2. Infection phase

4.3.2.2.1. Clinical findings

The introduction of the *D. nodosus* strains into the Main Flock via the Donor Flock ewes and the artificially challenged wethers resulted in the development of an outbreak of footrot. At Inspection 2, 9 weeks after the predicted date of onset of footrot in 1992, 18/19 (95%) donor ewes and 46/52 (88.5%) artificially challenged wethers present had footrot; and 165 of 225 (73%) naturally exposed wethers in the NIL spring treatment group were affected, indicating conditions had been suitable for the transmission of footrot from the donor sheep. In the NIL spring treatment group, 104/156 (68%) artificially challenged feet developed footrot, whilst 460/952 (48%) naturally exposed feet were affected. Significantly more hind feet were affected (262/471, 55.6%) than front feet (198/481, 42%) for naturally exposed feet (*p*<0.001), but for artificially challenged feet, proportions of front and hind feet affected did not differ significantly (44/73, 60%; 60/83, 72% respectively).

Despite the deliberate introduction of strain A which is renowned for its virulence, the footrot in the Main Flock was clinically intermediate, with predominantly severe score 2 lesions, and few (7/211, 3.3%) score 4 lesions (Table 4.6). Score 3 lesions tended to be limited, with underrunning rarely extending right across the heel.
4. Field Experiments on the Eradicability of *D. nodosus*

**Table 4.6.** Distribution of footrot lesions in NIL spring treatment groups at Inspection 2.

<table>
<thead>
<tr>
<th>Mob</th>
<th>Number</th>
<th>Prevalence</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddock 1</td>
<td>88</td>
<td>82%</td>
<td>82%</td>
<td>12.5%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Paddock 2</td>
<td>79</td>
<td>87%</td>
<td>81%</td>
<td>16%</td>
<td>3%</td>
</tr>
<tr>
<td>Paddock 3</td>
<td>59</td>
<td>80%</td>
<td>89.5%</td>
<td>8.5%</td>
<td>2%</td>
</tr>
<tr>
<td>Paddock 4</td>
<td>51</td>
<td>45%</td>
<td>69.5%</td>
<td>30.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>277</td>
<td>76%</td>
<td>82%</td>
<td>15%</td>
<td>3%</td>
</tr>
</tbody>
</table>

Notes: 1. Percent of sheep with at least one score 2 lesion or greater.  2. Percent of affected feet.

4.3.2.2.2. Bacteriology

*D. nodosus* isolates of serogroups A, B, C, D, E and G were recovered from the 30 donor ewes removed from the trial in October, 1992. At Inspection 3, 104 sheep were sampled from the four mobs of the Main Flock and 274 resulting *D. nodosus* isolates from 73 infected sheep were serogrouped. Isolates of serogroups A (44 isolates), E (118 isolates), and H (78 isolates) were recovered from cases in all four paddocks, and serogroup G isolates (36) were recovered from wethers in three of the four paddocks. The proportion of isolates recovered differed significantly (*p*<0.001), serogroup E being present more frequently than other serogroups, and serogroup H was more often recovered than serogroups A and G.

Thus, the exposure of the Main Flock to donor ewes and artificially challenged wethers resulted in the establishment of a multiple strain *D. nodosus* infection.

4.3.2.3. Control phase

The control phase was the period from late November, 1992 to late January, 1993, during which *D. nodosus* infection was established in the Main Flock. It resulted in 76% of untreated sheep developing footrot.
4.3.2.3.1. Comparison of Control techniques

Preventive footbathing, either weekly (6-7 treatments) or every 3 weeks (three treatments), restricted the prevalence to 6/283 (2%) and 18/275 (6.5%) respectively. This was significantly lower than the prevalence either in untreated or vaccinated groups (p<0.001). Weekly footbathing resulted in significantly less affected sheep than footbathing for one hour every 3 weeks (p<0.05) (Table 4.7).

Vaccination with either whole cell or the experimental vaccines significantly (p<0.001) reduced the prevalence (142/280 (51%), 114/278 (41%) respectively) compared with Nil treatment. The experimental vaccine regime resulted in significantly less affected sheep than whole cell vaccination (p<0.05) (Table 4.7).

Table 4.7. Proportion of affected sheep and mean sheep footscores for the five Spring Control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>NIL 1</th>
<th>WWF</th>
<th>SIF</th>
<th>VVC</th>
<th>VEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddock 1</td>
<td>72/88 (82%)</td>
<td>0/85 (0%)</td>
<td>9/86 (10%)</td>
<td>47/85 (55%)</td>
<td>40/87 (46%)</td>
</tr>
<tr>
<td>Paddock 2</td>
<td>69/79 (87%)</td>
<td>5/83 (6%)</td>
<td>6/77 (8%)</td>
<td>50/82 (61%)</td>
<td>36/79 (46%)</td>
</tr>
<tr>
<td>Paddock 3</td>
<td>47/59 (80%)</td>
<td>0/61 (0%)</td>
<td>1/58 (2%)</td>
<td>30/62 (48%)</td>
<td>25/60 (42%)</td>
</tr>
<tr>
<td>Paddock 4</td>
<td>23/51 (45%)</td>
<td>1/54 (2%)</td>
<td>2/54 (4%)</td>
<td>15/51 (29%)</td>
<td>13/52 (25%)</td>
</tr>
<tr>
<td>Total 2</td>
<td>211/277a (76%)</td>
<td>6/283b (2%)</td>
<td>18/275c (6.5%)</td>
<td>142/280d (51%)</td>
<td>114/278e (41%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean sheep footscore</th>
<th>1.7</th>
<th>0.08</th>
<th>0.2</th>
<th>1.2</th>
<th>0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE (%) 3</td>
<td>97%</td>
<td>91%</td>
<td>33%</td>
<td>46%</td>
<td></td>
</tr>
</tbody>
</table>

Notes: 1. Includes artificially infected wethers.
2. Treatments with different superscripts differ significantly (p<0.05).
3. PE=Protective effectiveness.
4.3.2.3.2. Paddock and Age Effects

Mobs in each of paddocks 1, 2, 3 and 4 were made up of replicates of the 5 treatment groups. Even so, sheep in Paddock 4 had a significantly lower proportion of affected sheep (p<0.05) and lower mean sheep footscores than sheep in Paddocks 1, 2 and 3 (Table 4.8).

Table 4.8. Sheep affected and mean sheep footscores for paddocks during the spring control phase.

<table>
<thead>
<tr>
<th>Paddock Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep affected / sheep present¹</td>
<td>168 / 435ᵃ</td>
<td>166 / 400ᵃ</td>
<td>103 / 300ᵃ</td>
<td>54 / 262ᵇ</td>
</tr>
<tr>
<td>(39%)</td>
<td>(41.5%)</td>
<td>(34%)</td>
<td>(21%)</td>
<td></td>
</tr>
<tr>
<td>Mean sheep footscore</td>
<td>0.95</td>
<td>0.97</td>
<td>0.80</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Notes: 1. Differences are significant if superscripts differ.

One year old sheep had a significantly lower proportion of affected sheep (p<0.05) and lower mean sheep footscores than two year old or three year old sheep (Table 4.9).

Table 4.9. Sheep affected and mean sheep footscores for different age classes during the spring control phase.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep affected / sheep present¹</td>
<td>90 / 328ᵃ</td>
<td>230 / 615ᵇ</td>
<td>170 / 449ᵇ</td>
</tr>
<tr>
<td>(27%)</td>
<td>(37%)</td>
<td>(38%)</td>
<td></td>
</tr>
<tr>
<td>Mean sheep footscore</td>
<td>0.65</td>
<td>0.87</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Notes: 1. Differences are significant if superscripts differ.
4.3.2.3.3. Prevalence Reduction Treatment

The single Footrite® treatment at Inspection 2 included the treatment of 445 affected sheep. Forty seven affected sheep were not treated. The Footrite® treatment reduced the prevalence to 12% (53/445) at Inspection 3, 25-35 days following treatment, compared to a prevalence of 57% (27/47) for untreated sheep. The therapeutic effectiveness was 79%.

4.3.2.4. Eradication phase

During this phase, which included Inspections 3, 4 and 5, eradication inspections were carried out. These inspections involved the culling of any sheep with lesions, or sheep with otherwise abnormal or suspicious feet. At Inspection 3, Mobs 1, 2 and 3 were established from those sheep in the Main Flock apparently free of footrot at this time. These mobs were maintained in Paddocks 1, 2 and 3 respectively (Figure 4.1). Mob 4 comprised culled affected sheep which were thereafter maintained in isolation (Paddock 5). Sheep culled at Inspections 4 and 5 were added to Mob 4.

Conditions were hot and generally dry. Pastures had senesced, and conditions were considered unsuitable for the transmission of footrot.

4.3.2.4.1. Clinical findings

At Inspection 3, 175/1,417 wethers (12%) were culled. Of these, 150 had lesions and were therefore "culled for footrot" (Table 4.10). The other 25 had otherwise abnormal feet. Score 2 lesions were the most prevalent (84/150 or 56%).
Table 4.10. Numbers of sheep culled with lesions at Inspection 3.

<table>
<thead>
<tr>
<th>Total</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>37 (25%)</td>
<td>84 (56%)</td>
<td>19 (13%)</td>
<td>10 (6%)</td>
</tr>
</tbody>
</table>

A significantly lower proportion of sheep (135/1276 or 11%) which had received the single Footrite® treatment at Inspection 2 were culled compared with those sheep which had not been treated (40/141 or 28%) (p<0.001). Despite differences in the protective effectiveness of the various preventive measures applied during the control phase, and significantly more NIL treatment sheep being culled (p=0.001), approximately equal proportions of sheep from each of the five spring treatment groups were present in Mobs 1, 2 and 3 (Table 4.11).

Table 4.11. Numbers of sheep from each spring treatment group in eradication mobs (Mobs 1, 2 and 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>NIL</th>
<th>WWF</th>
<th>SIF</th>
<th>VWC</th>
<th>VEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mob 1</td>
<td>84</td>
<td>97</td>
<td>93</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td>Mob 2</td>
<td>68</td>
<td>83</td>
<td>91</td>
<td>83</td>
<td>85</td>
</tr>
<tr>
<td>Mob 3</td>
<td>61</td>
<td>64</td>
<td>65</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>213</td>
<td>244</td>
<td>249</td>
<td>238</td>
<td>241</td>
</tr>
</tbody>
</table>

At Inspections 4 and 5, no sheep were considered to have footrot, although 6/1,222 and 8/1,208 sheep were culled as suspicious, respectively. Details for individual mobs for the eradication inspections are given in Table 4.14.
4.3.2.4.2. Bacteriology

Cultures from sheep culled because of footrot at Inspection 3 yielded isolates of serogroups A, E, G and H (see section 4.3.2.2.2). Isolates of these four serogroups were recovered from sheep which would have been in Mobs 1 and 2 if they had not been culled. Serogroups A, E and H were recovered from sheep provisionally allocated to Mob 3. All four serogroups were recovered from sheep which were removed to Mob 4.

Cultures from three of the sheep culled for abnormalities other than footrot at Inspection 5 were negative for *D.nodosus*.

4.3.2.5. Surveillance Phase

This phase commenced in September, 1993, 4 months after the last eradication inspection. Mobs 1, 2, 3, and 4 were maintained in isolation for the duration of this phase, which continued until the sheep were consigned to slaughter in November, 1994.

4.3.2.5.1. Clinical findings

*Inspections 6 and 7, Spring 1993.* After being free of any sign of footrot at two successive eradication inspections 4 weeks apart, footrot was detected in both Mobs 1 and 2 in the spring following the eradication phase i.e. 7-9 months after removal of the last obviously affected sheep. Mob 3 remained free of footrot, although score 1 lesions were observed. In Mob 1, 4/457 and 5/457 sheep had footrot at Inspections 6 and 7 respectively. Other sheep had score 1 lesions (13 and 30 respectively). Mob 2, which had been free of footrot at Inspection 6, had 8 of its 398 sheep with footrot at Inspection 7. There were 26 other sheep with score 1 lesions. In Mob 3, score 1 lesions only were observed in a total of 26 sheep at Inspections 6 and 7 (Table 4.12) (5 sheep had score 1
lesions at both inspections). Footrot had apparently been eradicated from this mob. This was confirmed at Inspections 8 and 11.

Table 4.12. Distribution of lesions at whole mob surveillance inspections for Mobs 1, 2 and 3.

<table>
<thead>
<tr>
<th>Inspection</th>
<th>Mob 1</th>
<th></th>
<th>Mob 2</th>
<th></th>
<th>Mob 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score 1</td>
<td>Score 2</td>
<td>Score 1</td>
<td>Score 2</td>
<td>Score 1</td>
<td>Score 2</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>5</td>
<td>26</td>
<td>8</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes: 1. Includes one score 3 lesion.
2. Whole mob inspection only carried out on Mob 3.
3. Inspection carried out in September for Mob 2 and November for Mobs 1 and 3.

At Inspections 6 and 7, samples for bacteriology were collected from 62 sheep in Mob 1, 39 sheep in Mob 2 and 33 sheep in Mob 3 (Table 4.15).

Footrot persisted in Mob 4 from February, 1993 until they were consigned to slaughter in April, 1994. At Inspections 6 and 7, 87% of sheep were affected. Score 3 lesions were present in 51% of affected sheep, and 3/47 (6%) had score 4 lesions (Table 4.13).

Table 4.13. Number of sheep, and prevalence of footrot lesions for Mob 4 (untreated controls) at Inspection 6.

<table>
<thead>
<tr>
<th>Mob/Inspection</th>
<th>Number</th>
<th>Prevalence</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mob 4, Inspection 6</td>
<td>54</td>
<td>87%</td>
<td>42.5%</td>
<td>51%</td>
<td>6.5%</td>
</tr>
</tbody>
</table>

Notes: 1. Percent of sheep with at least one score 2 lesion or greater.
2. Percent of affected feet
Inspection 8, Summer / Autumn 1994. Due to the absence of footrot in Mob 3 at Inspections 6 and 7, all sheep in the mob were inspected for a third time (Inspection 8). As footrot had been detected in Mobs 1 and 2, it was decided to examine only a sample of sheep at shearing in March / April, 1994 (Inspection 8), to monitor the progress of the disease. Thirteen sheep in Mob 1 which had had lesions at Inspection 7 (and had been infected at either Inspections 6 or 7) were deliberately selected for examination at Inspection 8. Thirty two other sheep selected at random were also examined. In Mob 2, 16 sheep which had had lesions at Inspection 7 were re-examined.

Among the sheep examined from Mob 1, only score 1 lesions (3) were observed. There were no sheep with footrot. Samples were collected from the three sheep with lesions and three others which had been infected previously. In Mob 2, 9 of 16 sheep examined had footrot, and another four had score 1 lesions. In 10 sheep, more severe lesions were observed at Inspection 8 than at Inspection 7. All 16 sheep were sampled.

Inspection 11, November, 1994 (Mobs 1 and 3). Mobs 1 and 3 were not re-examined until Inspection 11. At this time, they were free of footrot. In Mob 1, 14 of 408 sheep had score 1 lesions, while 11 of 292 sheep had score 1 lesions in Mob 3 (Table 4.12). Samples for bacteriology were collected from 29 sheep in Mob 1 and 10 sheep in Mob 3 (Table 4.15). Both mobs were then consigned for slaughter.
### Table 4.14. Proportion of sheep with lesions at eradication and surveillance inspections for Mobs 1, 2, 3 and 4.

<table>
<thead>
<tr>
<th>Inspection number</th>
<th>Date</th>
<th>Mob 1</th>
<th>Mob 2</th>
<th>Mob 3</th>
<th>Total</th>
<th>Mob 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ERADICATION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Feb '93</td>
<td>45 / 521</td>
<td>76 / 521</td>
<td>29 / 375</td>
<td>150 / 1,417</td>
<td>48 / 48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(AEGH)¹</td>
<td>(AEGH)</td>
<td>(AEH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mar '93</td>
<td>0 / 460</td>
<td>0 / 430</td>
<td>0 / 332</td>
<td>0 / 1,222</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Apr '93</td>
<td>0 / 458</td>
<td>0 / 425</td>
<td>0 / 325</td>
<td>0 / 1,208</td>
<td></td>
</tr>
<tr>
<td><strong>SURVEILLANCE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sep/Oct '93</td>
<td>17 / 457</td>
<td>6* / 410</td>
<td>20* / 318</td>
<td>47 / 54²</td>
<td>(AEH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(B)</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Nov/Dec '93</td>
<td>35 / 457</td>
<td>34 / 398</td>
<td>11* / 316</td>
<td>47 / 54</td>
<td>(AEH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(B)</td>
<td>(C)</td>
<td>(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Feb-Apr '94</td>
<td>3* / 45</td>
<td>9 / 16</td>
<td>0 / 299</td>
<td></td>
<td>(AEGH)³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>(C)</td>
<td>(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ERADICATION '94</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>May '94</td>
<td>62 / 382</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>June '94</td>
<td>11 / 304</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SURVEILLANCE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Sep-Nov '94</td>
<td>14* / 408</td>
<td>0 / 287</td>
<td>11* / 292</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>(C)</td>
<td>(-)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes:
1. Serogroups of *D. nodosus* isolated are shown in brackets; (-) indicates all samples collected were negative for *D. nodosus*.
2. Includes sheep culled at Inspections 4 and 5 for abnormalities other than footrot.
3. Proportion of sheep not presented, as majority of sheep were vaccinated.

* denotes all score 1 lesions
4. Field Experiments on the Eradicability of *D. nodosus*

4.3.2.6. Additional eradication / surveillance (Mob 2)

Because of the recurrence of footrot in Mob 2 and its persistence at Inspection 8, it was decided to attempt eradication. At Inspection 9, 78 of 382 sheep were culled. Of these, 62 had lesions of score 1 or greater, 12 sheep had other feet abnormalities, and 4 sheep with normal feet were culled for other reasons. Of the 62 sheep with lesions, 58 (93.5%) had interdigital lesions (23 score 1, 35 score 2); three sheep had score 3 lesions, and one sheep had a single score 4 lesion (2.5% of affected sheep, 1.6% of sheep with any lesions). Thus, 4/39 (10%) of affected sheep had score 3 or 4 lesions. Samples were collected for bacteriology from 32 sheep.

At Inspection 10, 4 weeks later, a further 17 sheep were culled from Mob 2 (10 score 1, 1 score 2, and 6 with other feet abnormalities). Samples for culture were collected from these 17 sheep.

There were 287 sheep remaining in Mob 2 at Inspection 11 (September, 1994). No lesions were observed in any sheep at this inspection. This may have been due to the abnormally dry conditions. Samples for bacteriology were collected from 8 sheep. These sheep had either normal feet (4), damage to the IDS (3), or otherwise abnormal feet (1).

4.3.2.6.1. Bacteriology

*Mobs 1, 2 and 3.* *D. nodosus* isolates of serogroup B were recovered from 15 of 57 sheep sampled from Mob 1 at Inspections 6 and 7. No *D. nodosus* isolates were recovered from samples collected from Mob 1 sheep at Inspections 8 (post-shearing) or 11. Serogroup C isolates were recovered from 47 of 95 sheep of Mob 2 sampled at Inspections 7, 8, 9, 10 and 11 (Table 4.14). In the two mobs, the infecting isolates, B and C, were recovered from both non-specific (score 1) and specific (score 2) lesions.
4. Field Experiments on the Eradicability of \textit{D. nodosus}

The single infected sheep in Mob 2 at inspection 11 had no lesions present, but the infected foot was sampled because of an apparent injury in the IDS. Two other sheep sampled with similarly injured feet yielded no \textit{D. nodosus} at this inspection.

All 55 attempts at culturing \textit{D. nodosus} from Mob 3 sheep failed.

\textit{Mob 4.} A total of 108 \textit{D. nodosus} isolates was serogrouped from sheep in Mob 4 from samples collected at Inspections 6 and 7. These 108 isolates came from 42 of the 54 sheep sampled. Isolates of serogroups A (6), E (82) and H (20) were recovered (Table 4.15). The proportion of isolates recovered differed significantly (p<0.001), serogroup E being present more frequently than other serogroups. Of the 42 sheep, 37 were infected with isolates of serogroup E. Combinations of two serogroups were isolated from 12 sheep. Overall, it seemed that either serogroup E was most numerous or easiest to isolate.

Following treatment with a vaccine containing A, E and H antigens, \textit{D. nodosus} serogroups A, E, and H were recovered from both vaccinated and unvaccinated sheep. However, serogroup G isolates were recovered from one vaccinated sheep at both inspections following vaccination (PV1 and PV2). In a determined attempt to identify serogroups not previously recovered following their introduction, a total of 276 isolates was serogrouped from samples collected at these two inspections. Serogroup E isolates predominated (209 isolates) in vaccinated and unvaccinated sheep (Table 4.15).

\textit{Mob 5.} Serogroup C \textit{D. nodosus} isolates continued to be recovered from unvaccinated sheep in Mob 5, 6 weeks and 22 weeks after vaccination (Inspections PVC2 and II). No \textit{D. nodosus} isolates were recovered from any sheep treated with the monovalent C vaccine at any of the inspections following vaccination in this mob. This treatment had apparently eliminated what was a single strain infection.
### Table 4.15. Number of sheep sampled and isolates serogrouped during the surveillance phase.

<table>
<thead>
<tr>
<th>Mob</th>
<th>Inspection</th>
<th>Sheep sampled</th>
<th>Sheep infected</th>
<th>Isolates serogrouped</th>
<th>Serogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mob 1</td>
<td>6</td>
<td>18</td>
<td>6</td>
<td>23</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>44</td>
<td>9</td>
<td>14</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>29</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mob 2</td>
<td>6</td>
<td>11</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>28</td>
<td>17</td>
<td>31</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>16</td>
<td>9</td>
<td>41</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>32</td>
<td>20</td>
<td>65</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>17</td>
<td>9</td>
<td>22</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td>Mob 3</td>
<td>6</td>
<td>12</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>21</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>14</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mob 4</td>
<td>6 &amp; 7 (combined)</td>
<td>54</td>
<td>42</td>
<td>108</td>
<td>A(6),E(82),H(20)</td>
</tr>
<tr>
<td></td>
<td>PV1&amp;PV2, vaccinates</td>
<td>30</td>
<td>20</td>
<td>160</td>
<td>A(4),E(116), G(7),H(33)</td>
</tr>
<tr>
<td></td>
<td>PV1&amp;PV2, controls</td>
<td>9</td>
<td>9</td>
<td>116</td>
<td>A(13),E(69), H(34)</td>
</tr>
<tr>
<td>Mob 5</td>
<td>PVC1</td>
<td>22</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVC2</td>
<td>14</td>
<td>5</td>
<td>19</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>C</td>
</tr>
</tbody>
</table>
4. Field Experiments on the Eradicability of *D. nodosus*

No serogroup D isolates were recovered from any sheep for the duration of the trial.

Further investigations indicated the serogroup B and C isolates, recovered from Mobs 1 and 2 respectively, had similar protease thermostability reactions to strains B and C, respectively, which had been used to infect the sheep. Therefore, on the basis of serogroup and virulence assessment, these two strains were considered to have persisted in the flock in spite of the eradication programme. The closer identity of the isolates recovered from Mobs 1 and 2 during the surveillance phase to strains B and C was further evaluated in work described in Chapter 5.

4.3.3. Virulence Assessment Flock

Virulence assessment of strains used in this investigation was conducted in paddock conditions considered to be marginally suitable for footrot, due to below average winter and spring rainfall, and consequent poor pasture growth. The number of affected sheep (and feet) 6 weeks and 10 weeks after challenge was similar, with 40/150 (23%) sheep and 51/453 (11%) artificially challenged feet developing footrot. This was significantly less than the number of affected feet for artificially challenged wethers in the Main Flock (104/156 or 68%) or for ewes in the Donor Flock (44/84 or 52%) (p<0.001). Strain A, considered to be the most virulent of those tested, caused a higher prevalence of more severe footrot than any of the other strains. For sheep challenged with strain A, 18/30 (60%) sheep and 24/120 (20%) feet developed footrot.

Only 5/147 (3.4%) naturally exposed feet developed footrot, which was significantly less (p<0.001) than the corresponding figures for naturally exposed feet in either the NIL treatment wethers in the Main Flock (460/952 or 48%) or the ewes in the...
Field Experiments on the Eradicability of *D. nodosus*

Donor Flock (11/100 or 11%, for unbandaged feet) (p<0.001). Significantly more hind feet (50/300, 16.7%) were affected than front feet (6/300, 2%) (p<0.001).

There were discernible differences in the nature of the footrot between strains (Table 4.16). Differences in mean total footscore were statistically significant, being greater for sheep challenged with strain A than for sheep challenged with other strains (p<0.05). Interaction between *D. nodosus* strain and breed occurred, with Polwarth sheep being significantly more severely affected by strain E than Merino sheep (mean total footscore 1.5 for Polwarths compared to 0.2 for Merinos, p<0.05). In addition, whilst Polwarths tended to be more severely affected by strain H (mean total footscore 0.8 versus 0.5 respectively), Merino sheep tended to be more severely affected by strain A than were Polwarths (mean total footscore 3.0 versus 1.8 respectively). For sheep challenged with strain A, 2/5 affected Polwarths had score 4 lesions, whilst 11/13 affected Merinos had score 4 lesions. Merino sheep challenged with strain A had significantly lower weight gains than Merino sheep challenged with the other 4 strains (p<0.05).

All *D. nodosus* isolates recovered from sheep in the Virulence Assessment Flock were of the same serogroup as the strain used to infect sheep in the plot from which they were recovered. Additional tests were carried out to confirm the identity of these isolates (see Chapter 5).
Table 4.16. Results of Virulence Assessment trial with *D. nodosus* strains 6 weeks after challenge.

<table>
<thead>
<tr>
<th>Strains</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>E</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep (n=)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Sheep affected (n=)</td>
<td>18</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Score 4 lesions</td>
<td>13 (72%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Score 3 lesions</td>
<td>1 (5%)</td>
<td>0</td>
<td>0</td>
<td>1 (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Group mean total footscore/sheep</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body weight gain (kg)</td>
<td>8.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes: Differences in footscores and body weight gain are significant if superscripts differ.

1. Retransformed data.
2. Weight gain at 10 weeks post challenge for Merino sheep.

4.3.4. Additional Findings

4.3.4.1. Recovery of isolates

The recovery rates of *D. nodosus* from different categories of lesions throughout the investigation were determined from samplings from the Donor Flock, and from the Main Flock at the following inspections: Inspection 3 (all mobs), Inspection 6 (Mob 1), Inspection 7 (Mobs 1 and 2), and Inspections 8, 9, 10 and 11 for Mob 2 (Table 4.17). The proportion of culture positive feet was significantly less (*p*<0.05) for those with score 1 lesions than for those with lesions of score 2 or greater. The proportion of culture positive feet for sheep with score 2 lesions compared to those of scores 3 or 4 were not significantly different.
Table 4.17. Recovery rates of *D. nodosus*.

<table>
<thead>
<tr>
<th>Foot Category</th>
<th>Culture negative</th>
<th>Culture positive</th>
<th>Total</th>
<th>Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>159</td>
<td>1</td>
<td>160</td>
<td>0.6%</td>
</tr>
<tr>
<td>Score 1</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>50%</td>
</tr>
<tr>
<td>Score 2</td>
<td>30</td>
<td>111</td>
<td>141</td>
<td>79%</td>
</tr>
<tr>
<td>Score 3/4</td>
<td>17</td>
<td>50</td>
<td>67</td>
<td>75%</td>
</tr>
<tr>
<td>Abscess</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>12.5%</td>
</tr>
<tr>
<td>ID abnormality¹</td>
<td>18</td>
<td>3</td>
<td>21</td>
<td>14%</td>
</tr>
<tr>
<td>Grass seed²</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>87.5%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>272</strong></td>
<td><strong>213</strong></td>
<td><strong>485</strong></td>
<td></td>
</tr>
</tbody>
</table>

Notes: 1. ID abnormality = interdigital abnormality (includes injury, healed lesions).
2. Grass seed = grass seed injury, with no apparent footrot lesion.
Inspection of Mob 4 (PV1). Cattle yards adjacent to Paddock 5 were used for inspections of sheep in Paddocks 3, 4 and 5.
4. Field Experiments on the Eradicability of *D. nodosus*

Plate 3

A. Sheep yards at Paddocks 1 and 2.

A. Footbath facility used for one hour treatments of sheep in Footrite® (SIF group).

B. Sheep in Mob 1.
4. Field Experiments on the Eradicability of *D. nodosus*

Plate 4

A. Paddock immediately prior to commencement of trial.

B. Sheep in plots (10 sheep pr plot).

Virulence Assessment Trial (see section 4.2.3.3).
4. Field Experiments on the Eradicability of *D. nodosus*

Plate 5

A.

B.

Transfer of sheep to plots (Virulence Assessment Flock)
4. Field Experiments on the Eradicability of *D. nodosus*

Plate 6

A. *D. nodosus* challenge of a sheep in Virulence Assessment Flock

B. *D. nodosus* challenge of a sheep in Virulence Assessment Flock
4. Field Experiments on the Eradicability of *D. nodosus*

Plate 7

A. Virulence Assessment Trial (see section 4.2.3.3).

A. Paddock 6 weeks after the commencement of trial.

B. Paddock at completion of trial (10 weeks).
4. Field Experiments on the Eradicability of *D. nodosus*

4.4. Discussion

4.4.1. General comments

The establishment and maintenance of footrot associated with multiple strains of *D. nodosus* and its natural transmission to a large flock of sheep was achieved. The footrot established in the Donor and Main Flocks was an intermediate form, with severe interdigital lesions being the predominant clinical feature. Relatively few sheep had severe underrun, despite the introduction and recovery of strain A, a virulent strain of *D. nodosus* (see section 2.3.2.1). In the absence of treatment, footrot persisted for at least 13 months in appropriate control animals.

The prevalence of footrot associated with multiple *D. nodosus* strain infection, achieved by both artificial challenge and natural exposure, was greater than those achieved by Raadsma et al (1994a) using single virulent strains of *D. nodosus*. The higher prevalence of footrot in hind feet compared to front feet has not been reported previously, with no differences in the prevalence between feet being found in one study (Raadsma et al, 1993).

Transmission of footrot occurred during the spring / early summer period in both 1992 and 1993, and was consistent with predicted periods of footrot transmission based on rainfall and temperature data (Graham and Egerton, 1968). The transmission of footrot to 73% of untreated sheep in the Main Flock in the first transmission period was consistent with observations of uncontrolled footrot outbreaks in the same district (Egerton and Burrell, 1970; Egerton et al, 1983; Egerton and Allworth, unpublished).

In spring, 1994, the low level of transmission of footrot in the Virulence Assessment Flock, and the lack of evidence of transmission in Mobs 1 and 2, despite the presence of *D. nodosus* (Mob 2) or its likely presence (Mob 1), was probably associated
with the below average rainfall in the preceding months (Graham and Egerton, 1968). Despite this, 60% of sheep challenged with strain A, without prior artificial predisposition of feet, developed footrot, which is similar to the prevalence achieved by natural exposure using the same strain in another study (Raadsma et al, 1994a). This suggests that a higher degree of predisposition is required for natural transmission than for deliberate artificial infection.

4.4.2. Recovery of *D. nodosus*

The probability of recovery of *D. nodosus* from feet lesions of different severity (Table 4.17) has implications for eradication programmes and for the assessment of their success. Score 2, 3 and 4 lesions are generally considered indicative of *D. nodosus* infection, and recovery rates of 75%-79% support the premise that such lesions are infected with *D. nodosus*. These recovery rates are similar to those from footrot affected flocks in South Australia (Cleland, unpublished). Workers in Western Australia used *D. nodosus* culture positive feet as the standard to assess both smears and lesions, and found lesions had a specificity of 94%. This suggests that 6% of feet with lesions were not culture positive (Depiazzi et al, submitted), indicating a higher recovery rate than achieved in the experiments here, especially as score 1 lesions were included in the Western Australian data. However, the results presented in section 4.3.2.1 only include feet from which *D. nodosus* was subcultured and then serogrouped. Thus, culture positive feet did not include those from which colonies resembling *D. nodosus* were recovered but lost subsequently on subculture. Therefore, the recovery rates in this study may be lower due to the stringent criteria used. If feet with footscores 2 or greater were affected with footrot, then the sensitivity of culturing as a diagnostic method was 77% in this study. It is of interest that in this series of experiments score 2 lesions were at least as likely to yield *D. nodosus* as underrun lesions.
4. Field Experiments on the Eradicability of *D. nodosus*

Recovery of *D. nodosus* isolates from a high proportion of feet affected with grass seeds was not expected. Feet were only classed as "grass seed" affected if there was no evidence of footrot. A number of feet had grass seeds and footscores, and these were excluded from the grass seed category (Table 4.17). Thus, feet classified as "grass seed" affected had a generally discrete deep hole in the interdigital skin (centrally). The results presented suggest the majority of, if not all, "grass seed" affected feet will be infected with *D. nodosus* if *D. nodosus* is present within a flock. The association between footrot lesions and grass seeds has been commented on previously (Glynn, 1993).

The proportion of score I lesions from which *D. nodosus* was recovered emphasises the need to classify all sheep with any lesions as infected if they are in a known infected flock. Given the apparent sensitivity of culture in this study, it is possible that over 65% of score 1 lesions were infected with *D. nodosus*. The infection rate of score I lesions may have been higher in this series of experiments due to the predominance of less virulent *D. nodosus* strains, or to the breed of sheep, or both. It may be that in less severe disease score I lesions are more likely to be infected with *D. nodosus*. It could not be determined whether positive isolations from score I lesions were due to infection as such or surface contamination.

However, the presence of score 1 lesions in sheep did not necessarily mean *D. nodosus* infection was present. In Mob 3, no *D. nodosus* isolates were recovered at samplings from score 1 lesions during the surveillance phase, and it was considered that this mob was free of *D. nodosus* infection. Despite this, score 1 lesions were detected in 31 sheep. This is consistent with the occurrence of OID (Parsonson et al, 1967). In a survey of 90 flocks believed to be free of virulent footrot, Morgan et al (1972) found that, in 63 flocks, there was inflammation of the IDS in some sheep. *D. nodosus* organisms were absent from smears taken from sheep from 29 of these 63 flocks.
Feet with toe or foot abscess, or interdigital abnormalities other than grass seed infestation, were less likely to be infected with *D. nodosus* in this series of experiments, and culling of such sheep in eradication programmes will result in the removal of some uninfected sheep. However, the fact that at least 10%-15% of such feet were positive for *D. nodosus* reinforces the need to cull rigorously all sheep with feet abnormalities in footrot eradication programmes (Beveridge, 1941). Sheep culled on the presence of an abscess or other foot abnormality represented 2.5% of sheep for Inspections 3 to 5 (35 sheep from an initial 1,417), and 4.7% for Inspections 9 and 10 in Mob 2 (18 sheep from an initial 382).

Monovalent and trivalent vaccination was undertaken in an attempt to suppress those strains represented in the vaccines and facilitate isolation of other strains present at low frequency. In the case of Mob 4, despite examining 108 isolates pre-vaccination, only serogroups A, E and H were recovered, although *D. nodosus* serogroup G had been present in the sheep 8 months earlier. Following vaccination, the prevalence and severity of footrot significantly decreased (data not presented). Serogroups E and H persisted but A could not be isolated after the second vaccination. Serogroup G, which could not be demonstrated prior to vaccination, was isolated from a vaccinated sheep.

It is possible that additional *D. nodosus* strains were present in Mob 4 but were not recovered due to the presence of either serogroup E or H or both. The continued presence of E and H strains following vaccination, despite the decrease in footrot, suggests that vaccination was less effective against these strains than against A. Vaccination may have decreased the influence of strain A on the footrot lesions, thereby decreasing the prevalence and severity of footrot but without affecting the prevalence of E and H strains. It should be noted that the vaccine contained A antigens derived from Strain A, while E and H antigens were derived from strains not homologous with strains E and H. Vaccination of affected animals with a monovalent E vaccine facilitated the
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recovery and identification of additional serogroups in one study (Ghimire et al, submitted).

In Mob 5, no *D. nodosus* isolates were recovered from vaccinated sheep, but the low recovery rates from unvaccinated sheep meant that the possibility that other *D. nodosus* strain(s) were present in this mob could not be excluded. However, given the ease with which serogroups E, H and A were recovered both prior to eradication, and in Mob 4, it would appear unlikely that these serogroups were present. In the Donor Flock there was some evidence that strain C may have dominated strains B and D, and so it is possible that the latter strains were present but in very low frequency.

4.4.3. Virulence Assessment

The grading of virulence of strains of *D. nodosus* is poorly defined (Whittington, 1995b). Stewart et al (1984, 1986a,d) used bodyweight changes and footscores in challenged sheep to distinguish virulent, intermediate and benign strains of *D. nodosus*. In the present study, fewer than anticipated affected feet and sheep occurred following artificial infection in the Virulence Assessment Flock, and so the information obtained was limited. Even so, a gradation in severity of footrot associated with different strains of *D. nodosus* was evident, and the strain selected originally as most virulent resulted in reduced body weight gains in the sheep challenged with it.

Of the strain characteristics in Table 4.1, clinical assessment and gene probe category were most closely correlated with severity of footscores in the virulence trial. Elastase and protease thermostability tests were less well related to severity of footscores, and use of day of clearing for elastase did not improve the relationship (data not shown).
Valid comparisons of the virulence of *D. nodosus* strains *in vivo* are only possible in well-designed trials, where possible sheep and environmental differences are minimised, and the environmental conditions reflect those likely to be experienced in the field. Additional assessments of the virulence of the strains is possible by considering results from Mobs 1 and 2, given that infection with a single strain was the outcome of eradication in these mobs. There are, however, paddock differences, and so judgements must be made carefully. The possibility of the presence of other undetected *D. nodosus* strains, which may have influenced the clinical findings, must also be considered. If Mob 2 was only infected with strain C from Inspection 7 to Inspection 9, then the clinical findings are consistent with intermediate footrot, although the low flock prevalence at Inspection 9 qualifies this assessment. All findings in Mob 1 during the surveillance phase were consistent with the persistence of benign footrot.

Breed differences in susceptibility to footrot, as expressed by the severity of lesions, have been discussed (Egerton and Raadsma, 1991), and recently differences between bloodlines of Merino sheep in susceptibility to footrot have been demonstrated (H. Raadsma, pers.comm.). The possibility of interactions between breed (or strain) of sheep and strain of *D. nodosus* has not been discussed, although recent research suggests differences in heritability of response to vaccination to different antigens of *D. nodosus* (H. Raadsma, pers.comm.). The issue of host and *D. nodosus* strain interaction has been raised in comparisons between the severity of footrot lesions in goats and sheep (Claxton and O'Grady, 1986; Stewart et al, 1986a). Goats developed more severe lesions when infected with a *D. nodosus* strain considered benign for sheep, and less severe lesions when infected with a virulent *D. nodosus* strain, when compared to sheep. In the Virulence Assessment trial, breed differences could not be compared, due to some bias in the selection of the Polwarth sheep, and the limited number of infected feet. Even so, the finding that the Merino sheep were more severely affected by a virulent strain (strain A), whilst the Polwarth sheep were more severely affected by strain E (and possibly strain
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H), raises the possibility that breed / strain interactions may occur, and warrants further investigation. Such an interaction would have important implications on the use of artificial challenge of small groups of sheep for judging the virulence of *D. nodosus* strains. It may also decrease the usefulness of clinical assessment as a criterion for determining the presence of targeted strains of *D. nodosus* in flocks for State footrot programmes.

In the Donor Flock, bandaging of feet significantly increased the likelihood of feet developing footrot. This suggests that artificial challenge methods which rely on the application of bandages may result in more severe outbreaks of footrot than would be experienced under natural conditions. If this is true, the use of artificial pen tests to assess the virulence of strains may not be appropriate if the integrity of the IDS is disrupted by other than natural means.

4.4.4. Criteria for assessing form of footrot

In this study, three forms of footrot were arbitrarily defined using the percentage of affected sheep with score 3 and score 4 lesions (section 2.2). Despite paddock differences (Table 4.6), the criteria used classified the outbreak of footrot in the Main Flock during the Control phase as intermediate in all four replicates. This is in contrast to the classification based on the percentage of score 4 lesions only (Egerton, 1989a; Anon., 1993), where the diagnosis would have been benign for the mob in paddock 4, despite all outbreaks being associated with the same strains of *D. nodosus*. Similarly, an outbreak of footrot of the severity of the outbreaks in the mobs in paddocks 2, 3 and 4 would have been classified as benign in Victoria. The criteria used in this study gave apparently inconsistent results for footrot associated with strain C as judged by the prevalence in Mob 2 at Inspection 9 and for the Virulence Assessment Flock (see section 4.4.7). However, the application of these criteria to footrot resulting from an artificially
contrived outbreak of footrot was not appropriate, as the criteria were developed for the diagnosis of footrot under natural conditions. Further, in both cases, only 10% of sheep were affected, which is insufficient to allow an adequate quantitative assessment (J. Egerton, pers. comm.).

These results demonstrate the difficulty of field diagnoses of footrot, and highlight the need to review the current criteria used for its differentiation. More importantly, it emphasises the need to examine different mobs on a property if there is doubt about the diagnosis, and the need for a sufficient number of sheep to be affected for an accurate diagnosis to be made.

4.4.5. Concurrent Infection with multiple strains

The results from both the Donor Flock and the Main Flock support previous findings that infections of sheep's feet with *D. nodosus* isolates of different serogroups do occur (Schmitz and Gradin, 1980; Claxton et al, 1983; Hindmarsh and Fraser, 1985; Thorley and Day, 1986; Gradin et al, 1993). In this series of experiments, these multiple infections were also of strains deliberately chosen to represent different virulence characteristics.

Unexpectedly, the possibility that interactions may occur between strains which alter the clinical expression of the multiple infection when compared with single strain infections was raised in the experiments conducted. When sheep were challenged with strain A only (Virulence Assessment Flock), 72% of affected sheep developed score 4 lesions. In circumstances where strain A was a component of an infective mixture, lesions were predominantly score 2 and 3. In Mob 4, again when strain A was consistently present in lesions, only 6% of sheep had score 4 lesions, despite the high prevalence and chronicity of footrot, and bias towards susceptible sheep in the
construction of this group. This low prevalence of score 4 lesions persisted in untreated sheep for the duration of the experiment.

The differences in severity of infection observed may have been due to environmental or breed differences, or interactions between strains. Environmental differences would seem unlikely, as conditions experienced during the Virulence Assessment trial, when more severe lesions occurred in sheep challenged with strain A, were unfavourable for footrot. This was reflected by the low level of transmission within plots, and the low proportion of infection following artificial challenge, presumably due to inadequate predisposition of the IDS.

Both the Donor and Main Flocks, where multiple strain infection resulted in less severe footrot, comprised Polwarth sheep. In the Virulence Assessment Flock, where lesions in animals challenged with strain A were more severe, only one-third of the sheep were Polwarths, and two-thirds were Merinos. If Polwarths are more susceptible than Merinos to strains E and H, and less susceptible to strain A (section 4.4.3), then in concurrent infection with strains A, E and H, strains E and H may have established in the majority of sheep. The clinical expression may then reflect the higher prevalence of the less virulent strains.

Also, the apparent lack of expression of strain A may have resulted from the method and sequence of introduction of strains to the Main Flock, and possibly in the Donor Flock. In the Main Flock, strain E was added to the donors (together with strain B) just prior to the transfer of donors to the Main Flock. It may have established more effectively in the donor sheep, as lesions from the initial multiple strain infection had regressed. Wethers in the Main Flock may therefore have been challenged initially with a mixture dominated by strain E. Similarly, strain H was added to donor ewes and then these ewes were immediately put back with wethers. This may also have resulted in a
more effective challenge (compared to other strains) with strain H. Infections with strains E and H may have been sufficiently established so that, when wethers were artificially challenged with strains A, B, C, D, E and G, these further challenges did not alter the infection rates within sheep. Similarly, in the Donor Flock, strain A was introduced 2 days after the other strains. This may have resulted in an advantage for establishment by other strains. Nevertheless, there was continuing evidence of the presence of strain A in lesions throughout the investigation and judging by the high prevalence of disease, conditions in 1992 were obviously favourable for the expression of footrot.

For whichever reason, E and H predominated in cultures from samples collected at Inspection 3 and from Mob 4. If the recovery rates of different strains represented the percentage of feet affected with specific strains, it is possible that the clinical expression of mixed infections reflected the fact that the majority of infected feet contained strains E and / or H.

Alternatively, interaction between strains may have occurred which resulted in the inhibition of the expression of virulence of strain A in mobs, when it was present in mixed infections.

In the Donor Flock, differences were observed in the relative capacity of strains to establish and to infect existing lesions. The reasons for these differences were not investigated. The failure to recover any serogroup B isolates from the Donor Flock, despite challenging equal numbers of feet at the same time with strains C, D and G, suggests that strain B may have failed to become established due to low viability of the cultures used or relative lack of infectivity of this strain. Alternatively, competition or inhibition from other strains may have decreased the establishment or recovery rate for strain B isolates. However, 26 feet were challenged with strain B either singly or in
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combination and all were negative on culture. It therefore seems that competition was unlikely to be the only reason for its failure to infect, and for the consequent failure to recover it. However, it is possible that strain B was present and not recovered, although this also seems unlikely, given the intensity of sampling.

Competition and/or inhibition between strains, resulting in differences of expression of virulence (as assessed by lesion severity) compared to single strain infections, have important implications. Most current research on the virulence of *D. nodosus* is based on the assumption that results from single strain challenges will indicate the virulence potential of an isolate. If outcomes from infection with mixtures of *D. nodosus* do not reflect predictions based on outcomes with single strains, then relationships between clinical and laboratory data, or between pen challenge data and field assessments, will need to be re-assessed. If strains which are potentially more virulent as single strain infections are transmitted from these clinically milder mixed infections, and subsequently cause more severe disease, then the ability of clinical criteria to identify footrot associated with strains of *D. nodosus* which should be eradicated will also need to be re-assessed. Further work is required to investigate the possibility that mixed *D. nodosus* infections can alter the clinical expression of some *D. nodosus* strains.

In addition, a novel approach to the control of footrot may exist, if benign strains of *D. nodosus* occur which inhibit the expression of virulent and intermediate strains. Given the already likely high property prevalence of benign footrot (in the absence of virulent or intermediate footrot) (Chapter 3), the introduction of benign strains of *D. nodosus* into flocks would not be expected to be an unacceptable proposition to producers, particularly if such a strain reduced the impact of more virulent forms. This technique is known as *niche filling* (Thrusfield, 1986). Furthermore, the study of the properties of strains which inhibit or compete with other strains may provide important information relevant to the epidemiology, pathogenesis, control or eradication of footrot.
Further investigations of the possible interactions between strains of *D. nodosus* would therefore appear justified.

### 4.4.6. Control Measures

There have been few trials comparing control options for less virulent forms of footrot, and the effectiveness of control strategies may have implications on the decision to eradicate. Despite the presence of a strain with an established record for virulence in all mobs, the clinical expression of the disease in the untreated group during the control phase was consistently intermediate. Thus, comparisons made of the effectiveness of the control methods are applicable to this form of the disease.

Footbathing had a protective effectiveness of 97% for a weekly walk-through footbath in 10% zinc sulphate or Footrite®, and 91% for a one hour soak in Footrite® every 3 weeks. These are similar to those reported by Skerman et al (1983a,b). The protection given by the one hour Footrite® footbathing was higher than that reported by Marshall (1991b) for one hour footbathing every 2 weeks when dealing with virulent footrot (induced with strain A, alone). It was also greater than Glynn (1993) achieved using a similar regime in flocks with benign footrot.

Two different multistrain vaccines had a protective effectiveness of 33% and 46%. This protection was lower than that reported for commercial vaccines against virulent footrot (70% to 100%) (Reed et al, 1981; Glenn et al, 1985; Bulgin et al, 1986; Lambell, 1986; Hindmarsh et al, 1989; Liardet et al, 1989). Lambell (1986) considered sheep affected if lesions were underrunning (score 3 or greater), whilst both Hindmarsh et al (1989) and Liardet et al (1989) used the criteria of affected sheep either having at least one score 3 lesion or at least two score 2 lesions. On these criteria, whole cell and recombinant vaccination were 20%-43% and 51% protective respectively; so the lower
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protection rates found in this trial could not be explained by differences in classification of affected sheep.

These results suggest that vaccination may be less than 50% protective against intermediate footrot. However, the relatively poor performance of the vaccine may have been due to the presence of strains in this trial against which the vaccines afforded poor protection. Even though protection is said to be serogroup specific (see section 1.7.1), there is evidence that some strains within serogroups are not protected (Chetwin et al., 1991; Stewart et al., 1991b). The persistence of strains E and H in sheep in Mob 4 vaccinated with prototype A, E and H antigens supports this possibility. Further investigations of these strains to assess this are therefore warranted, to ensure that current vaccines comprise antigens protective against the majority or all of the strains likely to be encountered in the field.

The single Footrite® treatment was 76% effective, which was greater than the effectiveness achieved with a one hour Footrite® treatment (Malecki and Coffey, 1987), and similar to that achieved with two Footrite® treatments (Malecki and Coffey, 1987). The better result in this study may have been due to the predominance of interdigital lesions. A 10% zinc sulphate solution used at regular intervals cured more feet with interdigital lesions than with underrun lesions (Skerman et al., 1983a), although this was not the case when two one hour Footrite® treatments were applied (Malecki and Coffey, 1987).

There was no evidence in this experiment that zinc sulphate footbathing was likely to result in the carrier state, a conclusion which has been inferred from previous reports (Atkins, 1986; Plant and Claxton, 1986; Glynn, 1993). Such an hypothesis is difficult to evaluate, due to the practical impossibility in identifying carrier sheep. However, in Mobs 1 and 2, there was no suggestion that sheep which had been
footbathed regularly during the spring control phase (SIF, WWF groups) were more likely to develop lesions during the surveillance period. Sheep which had been in footbathing groups in the previous spring were not over-represented in sheep with lesions at Inspections 6 and 7. In any case, a higher rate of new cases developing in the footbathed groups may have been due to the more susceptible sheep being protected by footbathing in the previous spring, rather than a failure of footbathing treatments to completely eliminate *D. nodosus* infection.

Further, mobs 1, 2 and 3 comprised 93% of sheep which had been footbathed in Footrite® following Inspection 2. The failure to detect any footrot, or *D. nodosus*, in Mob 3, despite apparently suitable conditions for the expression of footrot, suggests that at least in these sheep, a carrier status was not induced by this treatment. Thus, a single Footrite® treatment, which resulted in previously affected sheep being considered healthy during the eradication phase, resulted in either the elimination of *D. nodosus* from these sheep or the reduction of *D. nodosus* organisms to a level which prevented their persistence in the mob. It is possible, of course, that a carrier or carriers undetected at the end of the experiment could have relapsed at some time in the future.

### 4.4.7. Eradicability of *D. nodosus*

Normally, eradication of footrot is assessed by absence of clinical evidence of the disease. The successful establishment and maintenance of footrot associated with multiple strains of *D. nodosus* and its natural transmission to a large flock of sheep provided an opportunity to examine the comparative eradicability of different characterised strains, this being the primary objective of these experiments.

*D. nodosus* strains A, E and H, which originated from virulent or intermediate footrot outbreaks, were apparently eliminated in each of three mobs of sheep by an
"eradication-by-culling" programme. Strain G, recovered originally from an outbreak of intermediate footrot, was eradicated from two of three mobs. Strains which persisted in 2 of 3 mobs were from flocks with either a clear history of benign footrot (strain B) or a history of either benign or intermediate footrot (strain C). The two persisting strains differed in their in vitro test characteristics. Strain B was, by all criteria, a benign strain. Strain C was, however, positive in both elastase and protease thermostability tests. In the gene probe test and virulence assessment by sheep challenge, the two persisting strains were indistinguishable (Tables 4.1 and 4.16).

Thus, the results from this study do not support the commonly held view that strains of _D.nodosus_ of different virulence and, particularly those which possess thermostable proteases, are of similar eradicability. This study suggests that the least virulent strains are less likely to be eradicated by current methods.

There must always be some uncertainty, albeit small, about the absolute eradication of disease as this requires proof that a particular organism (in this case, a strain of _D.nodosus_) does not exist in a prescribed population. No level of testing can provide that proof. However, in the trials undertaken here, a combination of factors provides evidence for the eradication of some strains from the Main Flock:

i. environmental conditions were judged to be highly conducive to the development and expression of footrot in spring 1993 i.e. conditions favoured the re-emergence of disease and associated _D.nodosus_

ii. the persistence of footrot in untreated sheep and the continued isolation of _D.nodosus_ in those sheep provided evidence that the disappearance of strains was a result of the eradication programme, and that the systems for detecting _D.nodosus_ were adequate. It also supported the conclusions drawn in (i) above
iii. intensive inspection and sampling of mobs on four occasions increased the probability that infection would be detected, and ensured sheep were examined under a number of different environmental conditions.

In particular, the ease with which serogroup E isolates were recovered both prior to eradication (data not shown) and in Mob 4 during the surveillance phase, and the failure to detect any serogroup E isolates in any of Mobs 1, 2 and 3 post eradication, suggests that strain E was eradicated from all three mobs. Similarly, although less frequently isolated than serogroup E isolates, serogroup H isolates were easily detected both pre-eradication and in untreated sheep during the initial surveillance phase, again suggesting with some certainty that strain H was eradicated from all three mobs. Whilst serogroup A isolates were less frequently isolated from Mob 4 during the surveillance phase compared to both E and H isolates, they were still isolated from a number of sheep, and had been easily detected in all mobs at Inspection 3 pre-eradication. Given its virulence, and the apparent appropriate conditions for footrot expression, it seems that the virulent strain A was eradicated from all three mobs in the trial.

Serogroup G isolates were less frequently identified in sheep pre-eradication, and were isolated from sheep originating from Mobs 1 and 2 only. Serogroup G isolates were detected in only one sheep in Mob 4 and that was following vaccination with A, E and H antigens. In the absence of the E and H strains, strain G was frequently isolated in the presence of strains A, C and D in the Donor Flock, appearing a relatively "dominant" strain in those circumstances. It therefore seems reasonable that, had strain G survived the eradication programme, it would have been detected in Mobs 1, 2 and 3 after the elimination of strains A, E and H. Whilst it was not possible to demonstrate the presence of strain G following eradication, the eradicability of strain G was not as well established as that of strains A, E and H.
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No conclusions on the eradicability of strain D can be drawn. The failure to detect any serogroup D isolates, either pre-eradication or post-eradication in any wethers, suggests that the failure to detect strain D isolates post eradication may have been due to its failure to become established in the first place. Alternatively, as with strains B and C, strain D may have been present but not detectable. In this case, it may have been eradicated in 1, 2 or 3 mobs. Given the absence of any other *D. nodosus* isolates in Mob 3 after the eradication programme, it is likely that, had strain D been present, it would have been detected, given the level of sampling, and the apparent lack of other *D. nodosus* strains to mask its presence or inhibit its expression. In Mobs 1 and 2, the possibility that strain D was present but not detected exists, but again this seems unlikely given the extent of sampling. There was no evidence, when strain D was grown in the laboratory for the initial challenge of sheep, that it was less likely to grow than other strains on the medium used. On the property of origin of strain D, in the year following its isolation, sampling of three sheep on that property revealed only serogroup B isolates with benign *in vitro* characteristics. No footrot eradication programme had been undertaken.

The presence of serogroup B isolates in Mob 1 and serogroup C isolates in Mob 2 provides evidence that strains B and C each survived the footrot eradication procedure in at least one mob, despite the successful eradication of other *D. nodosus* strains from those mobs. Neither strain was detected in wethers in the trial pre-eradication, or in Mob 4 during the surveillance phase. This suggests that in mixed infections they were suppressed or present in such low numbers that culture was unlikely.

The *in vitro* characteristics of the isolates recovered and the clinical features of the resulting footrot outbreaks were both consistent with the survival of strain B in Mob 1, and strain C in Mob 2. In Mob 1, score 1 or score 2 lesions were present in the spring (both early and late), with score 2 lesions being mild, rather than severe (data not
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shown). These lesions had apparently disappeared in the absence of any treatment or culling by the autumn; and only reached score 1 in the following spring when conditions were judged to be less favourable than normal for footrot. The response of the disease to the environment is consistent with that of benign footrot.

In Mob 2, score 2 lesions were not apparent until Inspection 7, when *D. nodosus* isolates were recovered for the first time following the original eradication procedures. The severity of footrot increased in the majority of affected sheep during the summer period, but regressed in untreated sheep (Mob 5) under less favourable conditions. Lesions were predominantly score 2, most being severe. A small number of score 3 lesions, and one score 4 lesion were also detected at the autumn inspection. Such a description is consistent with intermediate footrot (albeit a mild form).

The reason for failing to eradicate these two strains could not be determined. It is possible that one or two sheep with lesions were inadvertently not culled. However, this seems unlikely, as each mob had two complete inspections where no lesions were recorded in any sheep. An alternative explanation is that relatively avirulent strains are more likely to persist in clinically normal feet, and therefore infected animals are not detectable in the summer inspections. These carriers may occur infrequently, and so footrot may be eradicated by chance from a number of mobs, or smaller mobs, but not from all mobs on a property. In this trial, mob sizes were greatest for Mobs 1 and 2 (n>400), and least for Mob 3 (n=318).

No evidence was found to suggest that footbathing in the spring/summer period prior to eradication resulted in carriers (see section 4.4.6), although this possibility cannot be excluded.
In Mobs 1 and 2, the prevalence of interdigital lesions increased during the spring surveillance phase, whilst the prevalence decreased in Mob 3, in the apparent absence of \(D.\ nodosus\). In Mobs 1 and 2, \(D.\ nodosus\) was isolated from both mild and severe interdigital lesions. Mild lesions, in particular, are likely to heal in summer, or with topical treatment. Such healed, apparently normal, feet may still be infected with \(D.\ nodosus\).

In making assertions of the likelihood of eradication or survival of different strains in each Mob, some assumptions are made on the relative paddock differences between each Mob. Theoretically, such comparisons are invalid, as no two paddocks are likely to be identical, and therefore environmental differences could account for any of the observed differences. However, there were no paddock differences observed during the Control phase in the proportion of sheep affected for paddocks 1, 2 and 3 (where Mobs 1, 2 and 3 were kept). Paddock 2 had a number of springs and swampy areas, which may have contributed to the prevalence of footrot by maintaining conditions suitable for the survival of \(D.\ nodosus\). However there was no evidence of continued \(D.\ nodosus\) infection in Mob 2 sheep at Inspections 4 and 5, suggesting these wetter areas were not responsible for maintaining \(D.\ nodosus\) infection during the eradication phase. The wetter areas may have favoured the continued presence of lesions in Mob 2 in the second summer / autumn period.

The assessment of \(D.\ nodosus\) eradication by clinical examination relies to some extent on the presence of animals sufficiently susceptible to \(D.\ nodosus\) infection to allow clinical expression. Susceptibility of sheep to footrot within a flock varies (Egerton et al, 1983; Raadsma et al, 1990), with more resistant sheep having a decreased prevalence and a decreased severity of lesions (Raadsma et al, 1990). The removal of all susceptible animals would in theory allow eradication of footrot due to the inability for transmission to occur (Yekutiel et al, 1980). However, the objective in the Main Flock was to
eradicate footrot by an inspection and culling programme, preceded by treatment control measures to restrict the numbers of sheep culled. As a result of the trial design, it is unlikely that the culling programme resulted in removal of all sheep susceptible to footrot. The initial spring control groups were likely to have sheep with similar susceptibility. The high level of footrot control achieved in the footbathed groups in particular should have ensured the retention in the eradication mobs of a large number of susceptible sheep, given the high footrot prevalence in the NIL treatment group. The favourable response to the single Footrite® treatment following Inspection 2 would also have restricted the number of footrot susceptible sheep being culled.

Thus, for Inspections 6, 7 and 8, the failure to detect *D. nodosus* infection in Mob 3 was unlikely to be due to the prior removal of susceptible sheep. Similarly, the failure to detect some strains in Mobs 1 and 2 was unlikely to be due to the removal of sheep susceptible to those strains.

However, in the case of the subsequent attempt to eliminate *D. nodosus* infection from Mob 2, the removal of all sheep with clinically apparent lesions, without any treatment of sheep in the preceding 12 months, is likely to have resulted in the removal of the sheep most susceptible to strain C infection, if not *D. nodosus* infection. As a result, the subsequent clinical assessment of the footrot status of this mob (Inspection 11) may have been affected, particularly in view of the poorer seasonal conditions and the low virulence of the *D. nodosus* strain present. Whilst routine sampling of sheep's feet for experimental purposes was carried out in this case, and the presence of *D. nodosus* was detected in sheep with an interdigital abnormality (but no inflammation), this would not occur in normal circumstances. Therefore, incorrect conclusions about the *D. nodosus* status of a flock, as opposed to its footrot status, could easily be reached.
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The failures to detect strain D during the trial, strains B and C prior to eradication or in Mob 4, strain B in Mob 1 at Inspection 11, and clinical evidence of strain C in Mob 2 at Inspection 11 highlight the difficulties in making judgements on the presence or absence of \textit{D. nodosus} in a flock. The apparent disappearance of footrot from infected flocks in the absence of any treatment or culling, and the failure to detect \textit{D. nodosus} infection in such flocks, has been reported (Depiazzi et al, submitted). The absence of footrot in the presence of \textit{D. nodosus} infection as detected in the residual sheep in Mob 2 at Inspection 11 is consistent with other reports (Glynn, 1993; Depiazzi et al, submitted), and may explain the difficulty in eradicating less virulent strains. Unsuitable environmental conditions, or the absence of other factors essential for the development of footrot, prevented clinical expression in Mob 2, and presumably Mob 1, in the second year, emphasising that \textit{D. nodosus} alone is not sufficient for the development of footrot lesions (Egerton et al, 1969).

4.4.8. Application of results to footrot eradication programmes

Results from this study are only indicative of what may be occurring with footrot in similar flocks in similar environments. A number of factors may limit the applicability of this research. Firstly, only a limited number of strains (6) was assessed in the eradicability study. Secondly, the strains which persisted both originated from flocks which had successfully eliminated virulent footrot, whilst the strains that were eradicated came from flocks with no immediate history of footrot eradication. It could therefore be argued that such strains are not representative of the majority of \textit{D. nodosus} strains in sheep flocks. However, if the strains examined in this study represent a biased sample, it could be equally argued that strains which have survived eradication attempts will increase in prevalence with current State footrot programmes, particularly if clinical expression following infection is mild.
Thirdly, despite the eradication programmes being replicated, the results in the three replicates varied. This increases the likelihood that the findings were chance occurrences, rather than due to specific features of the *D. nodosus* strains involved, or the clinical expression resulting from infection with these strains. Further replication of the eradication programmes was not possible within the course of this study, primarily due to the size of the replicates involved. Replicate sizes were deliberately large to ensure that results were applicable to farm eradication programmes, where mob sizes are likely to be similar to those in the Main Flock.

Despite these limitations, it is relevant to consider the implications from these experiments on footrot eradication programmes, both at the *farm level*, where owners are acting independently of neighbours and in the absence of any regulation, and at the *regional level* (usually a State level) (see Chapter 6).

At the farm level, the owner (or manager) will apply certain criteria to determine whether control (in the medium to long term) or eradication is the most appropriate action to be taken when faced with an outbreak of footrot (Allworth, 1988). These criteria will depend on factors including the severity of disease, type of sheep enterprise, flock management, facilities, selling policy, attitude to footrot, likelihood of re-introduction of disease if eradication is achieved, cost of control options and their practicalities, operator ability, and available resources (both physical and financial). The results from the experiments described in this chapter suggest that the eradicability of footrot should also be considered. To determine the appropriate course of action at the farm level following an outbreak of footrot, a thorough clinical examination of sheep's feet appears to be the single most important piece of information. The inspection of at least 100-200 sheep may be necessary for such an examination. The results of the examination will facilitate the establishment of a diagnosis of the form of footrot within the flock. Importantly, it is likely to give the best information on the potential economic
4. Field Experiments on the Eradicability of *D. nodosus*

impact of the disease, as footrot characterised by severe underrunning may result in
greater losses in productivity compared to footrot characterised by predominantly
interdigital disease (Stewart et al, 1984, 1986d). It is may also give the best indication as
to the likelihood of eradication, if the hypothesis that *D. nodosus* strains associated with
less clinically severe disease are more difficult to eradicate is shown to be correct.

The cost:benefit for eradication would be expected to alter rapidly as the clinical severity
of a footrot outbreak decreases. This is due to the lower losses in productivity from less
severe infections, and the expected higher cost of eradication for less severe disease if the
probability of eradication decreases with less severe forms of footrot (Figure 4.4). In
addition, eradication not only relies on elimination of specific *D. nodosus* strains from
within a flock, but also on the prevention of introduction of footrot from outside sources,
such as neighbours' or purchased sheep. In footrot endemic regions, the likely high
property prevalence of *D. nodosus* (Chapter 3) means the probability of remaining free of
less severe disease is decreased. This is likely to decrease the cost : benefit of
eradication programmes for less severe forms of footrot.
Figure 4.4. Schematic diagram showing relationship between costs, benefit and net returns for different forms of footrot.

![Diagram showing relationship between costs, benefit and net returns for different forms of footrot.](image)

- --- Costs
- --- Benefits
- --- Net return

Virulence

Benign

Virulent
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4.5. Conclusions

The successful establishment of multiple strains of \textit{D.nodosus} within a flock represents a previously unutilised technique for assessing differences between strains of \textit{D.nodosus}, particularly in relation to their eradicability, or response to specific treatment programmes. Using this technique, evidence was obtained that strains of \textit{D.nodosus} differ in their eradicability. The strains that persisted were associated with mild footrot. One strain was protease thermostable and the other protease thermolabile. The identity of the persisting strains with those introduced to the flock was confirmed using serogroup and virulence assessments, but will be further evaluated (Chapter 5).

The possibility that clinically milder footrot is more difficult to eradicate has important implications both at the property level, and for State footrot control / eradication programmes. These latter implications are discussed in Chapter 6. If the clinical expression of the disease is a factor in the ability to eradicate infecting \textit{D.nodosus} strain(s), then the ability to eradicate a particular strain may vary in different environments and in genetically different sheep. Additionally, the use of laboratory tests designed to test for a single virulence factor of \textit{D.nodosus} may be less useful than measuring disease expression in the flock.

Further, the possibility was raised from experiments described in this chapter that mixtures of strains of \textit{D.nodosus} may result in different clinical outcomes from those suggested by virulence of single strains. Such interactions have important implications on the usefulness of research based on single strain infections, and require further investigation. They may also offer a novel approach to footrot control.
CHAPTER 5

GENETIC CHARACTERISATION OF D. NODOSUS

5.1. Introduction

Epidemiological investigations with D. nodosus, such as those assessing the eradicability of different strains of D. nodosus described in Chapter 4, require the accurate identification of the strains studied. In Chapter 4, serogrouping (Claxton et al, 1983), a phenotypic typing method, was the primary method of identification of the strains used. Virulence testing, another phenotypic typing method, was also used to identify these strains further.

Over the past decade, considerable interest has developed in the use of genotypic typing methods for epidemiological purposes. It was decided therefore, as an adjunct to the investigations described in Chapter 4, to investigate the potential of specific DNA typing methods for characterising individual isolates of D. nodosus. Two techniques, ribotyping and polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP fingerprinting), were applied to strains of D. nodosus isolated from flocks on the Trial property, and the properties surveyed in Chapter 3. These methods are reviewed, and the laboratory techniques and results from isolates analysed presented. The results are presented in relation to their implications for the findings in Chapter 4. The applicability of such techniques to other footrot investigations is discussed.
5. Genetic Characterisation of *D. nodosus*

5.2. **Molecular Typing Methods**

Subtyping by definition is the discrimination of isolates within a bacterial species (intra-species differentiation) (Swaminathan and Matar, 1993). Methods for subtyping may be broadly divided into:

(i) the identification of phenotypic markers, which includes serotyping and
(ii) molecular typing methods, which include both ribotyping and PCR-RFLP (Figure 5.1).

Serogrouping and serotyping have been used to identify different *D. nodosus* strains, principally in relation to the development of vaccines because immunity is serogroup specific. Antigenic typing has also been used for epidemiological purposes, such as identification of the source of a footrot outbreak (Stewart et al., 1991b), but its application here is limited by the fact that a multiplicity of antigenic types is normal within flocks (see section 1.7.1). Virulence testing of *D. nodosus* isolates, generally using protease-based tests such as the elastase and gelatin gel protease thermostability tests, has also been used as a guide to the success or failure of programmes designed to eradicate particular *D. nodosus* strains, or for investigations aimed at identifying strains associated with particular footrot outbreaks. Again, a range of virulence types may occur within affected flocks (Claxton, 1986b; Ghimire et al., submitted).

The limitations of both these phenotypic methods lie in their limited discriminatory ability and sampling problems associated with isolating and characterising sufficient isolates in an affected flock. Further, phenotypic methods may not enable all strains to be typed (Swaminathan and Matar, 1993).
Figure 5.1 Diagram showing the differentiation of Subtyping Methods (Swaminathan and Matar, 1992)

Species subtyping

Molecular typing methods

Nucleic-acid based
- Protein based
  - e.g. MEE
  - SDS-PAGE

Lipopolysaccharide
- Fatty-acid based
  - e.g. LPS SDS-PAGE
  - gas chromatography

DNA sequencing

Chromosomal & Extrachromosomal DNA
- Restriction Profiles
  - Hybrisation techniques
    - Probes derived from insertion sequences
    - Bacteriophage M13-based probe

PCR-based
- RAPD

Phenotypic markers
- e.g. serotyping, biotyping
- monoclonal antibody subtyping
- antimicrobial resistance pattern

Monoclonal antibody subtyping

Antimicrobial resistance pattern

Nucleic-acid based
- e.g. MEE
- SDS-PAGE

Protein based
- e.g. MEE
- SDS-PAGE

GAS Chromatography

DNA sequencing

PCR-based
- RAPD

Chromosomal DNA restriction analysis (REA)

Randomly cloned probes

Probes based on specific virulence factors

Ribosomal DNA RFLP Analysis (Ribotyping)
5. Genetic Characterisation of *D. nodosus*

The use of chromosomal DNA restriction analysis, a nucleic-acid based subtyping method, has been described for *D. nodosus*, using the restriction endonuclease *BamHI* (McGillivery et al., 1989), but this report did not adequately evaluate this method (see section 1.7.2).

Evaluation of any typing method requires the use of strains that are likely to be identical, and strains that are likely to differ, based on both epidemiological and other microbiological criteria (Swaminathan and Matar, 1993). For molecular typing methods, as with others, both the reproducibility (same result on repeat testing) and the discriminatory ability (differentiation of different strains) need to be considered. Typability (proportion of strains that can be typed) is an important trait for phenotypic tests, but is not considered important for molecular methods, as essentially all strains can be typed provided DNA is available (Swaminathan and Matar, 1993).

5.2.1. Ribotyping

Ribotyping, first described by Grimont and Grimont (1986), is based on the restriction fragment length polymorphisms (RFLPs) in the chromosomal genes that encode rRNA. It is a specific application of nucleic acid-based hybridisation, with the use of a ribosomal probe (usually *E. coli* 16S and 23S rDNA) to produce a simpler, clearer banding pattern than that achieved from chromosomal DNA restriction endonuclease analysis (REA). Interpretation of patterns is therefore easier (Nielsen et al., 1989; Snipes et al., 1989; Bingen et al., 1992f; Wolfhagen et al., 1993; Blanc et al., 1994; van Steenbergen et al., 1994). As the majority of bacteria contain multiple copies of ribosomal operons, acceptable numbers of fragments which hybridise with the probe are present (Grimont and Grimont, 1986; Grimont et al., 1989). Ribotyping involves the restriction endonuclease digestion of chromosomal DNA, a Southern transfer of the digested fragments to a membrane (nitrocellulose or nylon), probing of the membrane with a labelled rDNA probe, and the detection of the bound labelled probe.
5. Genetic Characterisation of *D. nodosus*

Ribotyping has been reported for many bacteria. Usually, its usefulness as an epidemiological tool has been examined. Organisms ribotyped at the intraspecies level include: *Actinobacillus actinomycetemcomitans* (van Steenbergen et al., 1994); *Acinetobacter* spp (Gerner-Smidt, 1992; Dijkshoorn et al., 1993); *Bacteroides ureolyticus* (Akhter and Eley, 1992); *Branhamella catarrhalis* (Denamur et al., 1991a); *Borrelia burgdorferi* (Zingg et al., 1993); *Campylobacter* spp (Hernandez et al., 1991; Kiehlbauch et al., 1991; Patton et al., 1991; Taylor et al., 1991; Russell et al., 1992; Tee et al., 1992b); *Clostridium difficile* (Wolfhagen et al., 1993; Kristjansson et al., 1994); *Enterobacter cloacae* (Garaizar et al., 1991; Lambert-ZeChovsky et al., 1992; Grattard et al., 1994); *Enterococcus* spp (Bingen et al., 1991; Hall et al., 1992; Gordillo et al., 1993; Woodford et al., 1993); *Escherichia coli* (Bingen et al., 1992d; Wasteson et al., 1992; Alos et al., 1993; Mariani-Kurkdjian et al., 1993); *Flavobacterium meningosepticum* (Colding et al., 1994); *Haemophilus* spp (Sarafian et al., 1991; Brown and Ison, 1993; Jordens et al., 1993); *Helicobacter pylori* (Linton et al., 1992; Tee et al., 1992a; Owen et al., 1994; Rautelin et al., 1994); *Klebsiella pneumoniae* (Bingen et al., 1993b); *Legionella pneumophila* (Saunders et al., 1991; Schoonmaker et al., 1992; Gomez-Lus et al., 1993; Mamolen et al., 1993; Bangsborg et al., 1995); *Leptospira interrogans* (Nielsen et al., 1989; Perolat et al., 1994); *Listeria monocytogenes* (Baloga and Harlander, 1991; Graves et al., 1991; Jacquet et al., 1992; Nocera et al., 1993; Graves et al., 1994); *Mycobacterium avium* (Arbeit et al., 1993); *Neisseria meningitidis* (Jordens and Pennington, 1991; Woods et al., 1992; Ceconi Tondella et al., 1994); *Pasteurella* spp (Snipes et al., 1989, 1990; Carpenter et al., 1991; Zhao et al., 1992; Jaworski et al., 1993; Murphy et al., 1993); *Providencia stuartii* (Rahav et al., 1994); *Pseudomonas* spp (Lipuma et al., 1988, 1990, 1991; Anderson et al., 1991; Denamur et al., 1991b; Bingen et al., 1992c, 1993c; Blanc et al., 1993; Poh et al., 1992; Gruner et al., 1993; Larsen et al., 1993; Lew and Desmarchelier, 1993; Pegues et al., 1993; Sexton et al., 1993; Smith et al., 1993; Dasen et al., 1994; Johnson et al., 1994); *Rhodococcus equi* (Lasker et al., 1992); *Salmonella* spp (Olsen et al., 1992; Pignato et al., 1992; Esteban et al., 1993; Nastasi et al., 1993b; Baquar et al., 1994; Fica et al., 1994; Milleman et al., 1995); *Serratia marcescens* (Bingen et al., 1992f; Liu et al., 1994); *Shigella*
5. Genetic Characterization of *D. nodosus*


For RFLP techniques, the use of different restriction endonucleases, either singly, or together, or using a number of restriction endonucleases singly to further classify subtypes, may alter the discriminatory power of ribotyping (for example, Jordens and Pennington, 1991; Sexton et al, 1993). Thus, there are a large number of different
combinations of restriction enzymes which can be used to optimise ribotyping within a bacterial species.

5.2.2. **PCR-RFLP fingerprinting**

PCR-RFLP fingerprinting is based on the occurrence of distinctive banding patterns produced after digestion of PCR-generated DNA fragments with restriction endonuclease(s) to characterise isolates. This is distinct from PCR fingerprinting methods, such as the use of single arbitrary primers, which rely on polymorphism of the generated PCR DNA product to specifically identify isolates.

PCR-RFLP fingerprinting requires some DNA sequence data for the targeted bacterial species, to enable the development of oligonucleotide primers (oligos) to produce appropriate DNA fragments. Ideally, the primers are selected for highly conserved genomic regions which flank highly variable regions within the bacterial species. Following PCR amplification of the designated fragment, the amplified products are digested with a restriction endonuclease (usually a 4-base cutter) and the digested products electrophoresed on an agarose gel to visualise the resulting banding patterns (fingerprints).

PCR-RFLP fingerprinting has been reported for a number of bacterial species, including *Helicobacter pylori* (Foxall et al, 1992; Clayton et al, 1993; Romero-Lopez et al, 1993; Fujimoto et al, 1994; Owen et al, 1994), *Neisseria meningitidis* (Kertesz et al, 1993; Peixuan et al, 1995) and *Leptospira interrogans* (Savio et al, 1994). The effectiveness of PCR fingerprinting techniques in the presence of only a small number of bacterial cells, and the rapidity of results, makes such a technique more applicable to epidemiological investigations than REA and ribotyping (Kostman et al, 1992; Bingen et al, 1993a; Gomez-Lus et al, 1993).
5. Genetic Characterisation of *D. nodosus*

PCR technology has been applied to the detection of the presence or absence of *D. nodosus* (La Fontaine et al., 1993) and as a means of detecting the fimbrial serogroups (John et al., 1990; Cox, 1992), but not for specific isolate genotyping (fingerprinting). During the sequencing of the region containing genes which encode for an outer membrane protein (Omp1) in the *D. nodosus* genome, a highly variable region flanked by highly conserved regions was found within the *omp1* gene (E. Moses and R. Good, pers. comm.). This gene may be present in multiple copies in *D. nodosus* (Moses, 1993). Whilst not all *D. nodosus* strains necessarily possess all four *omp1* genes, PCR amplification of a number of *D. nodosus* strains, using oligonucleotide primers specific for *omp1* gene sequences, produced variable DNA fragments (Moses, 1993). PCR amplification of a 0.5 kb region internal to *omp1* genes, followed by digestion with *Sau3AI* or *HpaII*, gave encouraging results for the application of this technique to *D. nodosus* fingerprinting. Thus, *omp1* gene fragments may, by PCR-RFLP fingerprinting, be useful genetic epidemiological strain markers (E. Moses and R. Good, pers. comm.).
5. Genetic Characterisation of \textit{D. nodosus}

5.3. Materials and Methods

5.3.1. Bacteria

During the course of the investigations described in this thesis, many different isolations of \textit{D. nodosus} were made. In this collection there were isolates derived from affected flocks which, as far as could be determined, had no contact with one another (epidemiologically unrelated). The initial characterisation of these isolates was by serogrouping using the slide agglutination test (section 2.4.4). Subsequently, proteases of some of these isolates were tested for thermostability. A number of these isolates were available for ribotyping and/or PCR-RFLP fingerprinting, in addition to the 7 experimental strains (A,B,C,D,E,G and H) described in section 4.2.1. These additional \textit{D. nodosus} isolates examined in the work described in this chapter included:

i. 54 isolates recovered from sheep in the experiments described in Chapter 4. These comprised (a) 45 isolates from the Main Flock (eradication trial) and (b) 9 isolates from the Virulence Assessment Flock

ii. 17 isolates recovered from animals which were resident on the Trial property (sheep flocks are described in section 2.31).

iii. 27 isolates recovered from the animals on properties visited for the Survey (Chapter 3)

iv. 9 isolates recovered in additional investigations (not otherwise reported) on three properties.
5. Genetic Characterisation of *D. nodosus*

5.3.2. Ribotyping

Genomic *D. nodosus* DNA was prepared from the 7 strains A, B, C, D, E, G and H, and 12 other isolates (see Table 5.1). DNA was prepared by a modification of the method of Anderson et al (1984). Cells from 2-3 day old 100 ml broth cultures (TAS (Skerman, 1975) or Eugonbroth (BBL, Becton Dickinson)) were harvested by centrifugation of the broth (10,000 rpm at 4°C for 10 minutes). Pelleted cells were resuspended in TE buffer, and centrifuged as before, with pelleted cells again being resuspended in TE buffer. The cells were incubated with lysozyme (Sigma, 4mg/ml final concentration) at 4°C for 20 minutes, and then incubated with sodium dodecyl sulphate (SDS) (1%) and Pronase (Sigma) (0.2 mg/ml) at 56°C overnight. The resulting lysate was mixed with an equal volume of phenol, and centrifuged at 10,000 rpm for 10 minutes. The upper aqueous phase was transferred to a new polypropylene tube and an equal volume of 1:1 (v/v) phenol:chloroform added, mixed and centrifuged as before. The aqueous phase was transferred to a new polypropylene tube and an equal volume of chloroform added, mixed and centrifuged as before. The upper aqueous phase was then transferred to a new tube for ethanol precipitation of the DNA.

For ethanol precipitation, sodium acetate (0.3M final concentration) was added to the DNA sample, followed by the addition of two volumes of cold (-20°C) absolute ethanol. This mixture was then centrifuged at 10,000 rpm for 30 minutes at 4°C to pellet the DNA. The supernatant was discarded; the pellet was dried, and then resuspended in TE buffer. The DNA was stored at -20°C until required.

Genomic DNA (5-10 μg) was digested with one of the following restriction enzymes: *BamHI*, *DraI*, *EcoRI*, *HindIII*, *PstI*, *PvuI*, or *XbaI*. For digestion of DNA with restriction enzymes, the appropriate buffer was added to the required volume of DNA, the mixture held at 37°C for 30 minutes before the addition of 1 μl of restriction enzyme, and the combined mixture incubated at 37°C overnight. An additional 1 μl of
5. Genetic Characterisation of *D. nodosus*

restriction enzyme was added the following morning, and the mixture incubated for a further 2 hours.

Loading buffer was added to the digested chromosomal DNA (total volume 22-35 µl) and the mixture subjected to electrophoresis in a 0.8% agarose (Progen) gel in 0.5 x TBE buffer (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) containing 0.5 µg ethidium bromide / ml at 30 volts (1 volt / cm) on a Bio-Rad DNA Sub Cell for 16 hours. Digoxigenin labelled, *HindIII* digested, lambda DNA was used as the marker. The gel was then photographed to record the RFLP patterns from the digest, and then the DNA bands transferred to a nylon membrane (Boehringer-Mannheim) via the method of Southern (1975), as follows: large fragments were depurinated in 0.25M HCl, the gel rinsed in water, then gently shaken in firstly 0.2 M NaOH / 0.6 M NaCl (45 minutes), and then twice in 10 x SSC (1.5 M NaCl, 0.15 M sodium citrate) / 0.5 M Tris-Cl (pH 7.5) (40 minutes). The gel was transferred to the Southern apparatus, and transferred overnight in a 10 x SSC solution.

Following transfer, the nylon membrane was dried and baked at 120°C for 30 minutes, and then stored at room temperature, prior to hybridisation.

The probe used for ribotyping was a 783 base pair (bp) PCR fragment, amplified using primers internal to the *D. nodosus* 16S rRNA gene (La Fontaine et al, 1993). This probe was labelled with digoxigenin by the addition of digoxigenin-11-dUTP during the course of its amplification by the addition of 2 µl of DIG DNA labelling mixture (10 x concentrated; Boehringer-Mannheim) to a standard 100µl PCR reaction mixture. The labelled PCR fragment was excised from a low gelling temperature agarose (SeaPlaque) gel and incubated at 95°C for 10 minutes prior to hybridisation.
Membranes were hybridised with the labelled probe, and bound probe detected non-radioactively using the DIG detection system (Boehringer-Mannheim) in the following manner:

the membrane was placed in a sealed plastic bag with 15 ml Hybridisation Solution (5 x SSC, 0.1% sodium-lauroylsarcosine, 0.02% SDS, 1% (w/v) Blocking Reagent), and incubated at 68°C for 1 hour. The solution was discarded, and 2.5 ml of Hybridisation Solution containing 20 ng/ml of labelled probe was added to the membrane, the bag sealed, and incubated at 68°C overnight.

Following hybridisation with the probe, the membrane was washed to remove unbound probe. This washing consisted of two 5 minute washes in 2 x SSC / 0.1% SDS, and two 15 minute washes at 68°C in 0.1 x SSC / 0.1% SDS. Detection of bound probe was performed with the DIG colorimetric detection system (Boehringer-Mannheim), used according to manufacturer's directions.

5.3.3. PCR-RFLP Fingerprinting

The 114 isolates examined by PCR-RFLP fingerprinting are listed in Table 5.3. DNA from these isolates were subjected to PCR, then digested with either HpaI or Sau3A restriction enzymes (Promega Corporation), and the resulting DNA electrophoresed on agarose gels to give a banding pattern (fingerprint).
5. Genetic Characterisation of *D. nodosus*

5.3.3.1. Preparation of DNA

Target *D. nodosus* DNA for the PCR reaction was available, or prepared, from four different sources:

i. aliquots of DNA previously prepared for ribotyping (see section 5.3.2)

ii. lyophilised preparations of cells of *D. nodosus*

iii. cells harvested from 4% HA plates which had been incubated for 3-5 days

(approximately $10^9$ cells)

iv. lesion material from feet of sheep with footrot stored in PBS or TE buffer.

DNA was prepared in the following manner. Lyophilised cells were rehydrated and suspended in 75-100 µl of sterile distilled water. Cells taken from HA plates and lesion material were suspended in PBS or TE buffer and vortexed briefly. Subsequently 50 µl of one of these three different preparations was transferred to a new Eppendorf tube containing 50 µl of lysis buffer (50mM Tris,pH 8.9; 2mM EDTA; 1% Triton X-100), mixed and heated at 95°C for 10 minutes. The mixture was then centrifuged at 13,000 rpm for 2 minutes to pellet cells. The supernatant was removed to a fresh tube, and the DNA precipitated with 0.3 M sodium acetate (final concentration) and 2.5 volumes of absolute ethanol at -20°C for at least 1 hour before centrifugation at 13,000 rpm for 30 minutes at 4°C. The supernatant was discarded, and 250 µl of 70% ethanol added to the tube to wash the DNA. The ethanol was discarded, and the tube air-dried, before the addition of 36 µl of distilled water or 0.1 x TE.

All pipetting of suspensions containing *D. nodosus* cells or DNA were performed using aerosol resistant plugged tips (ART, Molecular Bio-Products) to minimise aerosol contamination.
5.3.3.2. PCR procedure

The PCR procedures were as described by Moses (1993) as follows: the PCR reaction mixture contained 1 μM of each of two synthetic oligonucleotide primers (Oligo A: 5'--ATTCAAGGACTGAAGAA--3'; Oligo C: 5'--AATGCCGTACATTAAAGCA--3'), 200 μM of each dNTP (dATP, dCTP, dGTP, dTTP; Promega or Biotech), 2.5 - 3.0 units Taq DNA polymerase (Promega or Biotech), buffer supplied with the Taq DNA polymerase diluted to 1 x strength, 1.5mM MgCl₂ and 10 ng to 2 μg of actual target DNA, in a final volume of 100 μl. The cycle profile for amplification, using a Corbett Research Fast Thermocycler, involved 1 minute at 94°C (denaturation), 1 minute at 50°C (annealing) and 1 minute at 72°C (extension). After 31 amplification cycles, an additional extension phase of 5 minutes at 72°C was performed. A negative control (no target DNA) and a positive control (known D.nodosus DNA) were included in all groups of samples prepared for PCR.

Following amplification, 15 μl of the 100 μl reaction was subjected to electrophoresis on a 2% agarose (Progen) gel at 5 volts/cm for 1 hour to check for the presence of PCR product. The remaining 85 μl of the PCR reaction was precipitated with ammonium acetate (2.5 M) and two and a half volumes of cold (-20°C) absolute ethanol. The mixture was held at -20°C for at least 1 hour, and then centrifuged at 13,000 rpm for 30 minutes at 4°C to pellet the DNA. The supernatant was discarded; 250 μl of cold (-20°C) 70% ethanol was added to the tube to wash the pelleted DNA, the 70% alcohol was discarded and the tube air-dried. The DNA pellet was resuspended in 0.1 x TE buffer or water.

5.3.3.3. Digestion

The resuspended DNA was then digested with either HpaII or Sau3AI restriction enzymes as follows: the appropriate buffer was added to the resuspended DNA, the
5. Genetic Characterisation of *D. nodosus*

mixture held at 37°C for 30 minutes before the addition of 1 μl of restriction enzyme, and the combined mixture incubated at 37°C overnight. For *Sau3AI* digestions, and some *HpaII* digestions, an additional 1 μl of restriction enzyme was added the following morning, and the mixture incubated for a further 2 hours.

5.3.3.4. Electrophoresis and detection

Following digestion, loading buffer was added to the samples, and the mixture subjected to electrophoresis in a 2.0% agarose (Progen or Nusieve, FMC Bioproducts) gel in 0.5 x TBE buffer (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) containing 0.5 μg ethidium bromide / ml at 70 volts (5 volts/cm) on a Bio-Rad DNA Mini Sub Cell for 1.5 hours. PGEM (Promega) molecular size markers were used. The gels were photographed to record the resulting banding patterns, which were analysed visually. Each distinct banding pattern was assigned a pattern number. Comparisons of banding patterns were made both within and across agarose gels.

Nucleotide sequence data for *D. nodosus* strain A1001 held in GenBank (Dnu02462) was analysed using the restriction enzyme *HpaII* to allow the comparison of actual DNA fragment sizes detected on gel electrophoresis with those that should theoretically be present.

5.3.3.5. Terminology

The term *HpaII* fingerprint was applied to the pattern of PCR amplified *HpaII* digested DNA *omp1* gene fragments detected in the ethidium bromide stained agarose gel. The term *HpaII fingerprinting* refers to the PCR-RFLP fingerprinting technique, using *HpaII* digestion.
5. Genetic Characterisation of *D. nodosus*

5.4. Results

5.4.1. Ribotyping

5.4.1.1. Digestion with *EcoRI*

*Banding patterns:* DNA prepared from 19 isolates of *D. nodosus* were digested with *EcoRI*. For 18 of the 19 isolates, three major bands were identifiable. No result was achieved for the other isolate. The arrangement of these bands was such that 6 distinguishable patterns were observed. These patterns were designated RT1, RT2, RT3, RT4, RT5 and RT6 (Table 5.1, Figure 5.2a). There were three distinct patterns which could be identified among the 7 experimental strains A, B, C, D, E, G and H. Thus strains A and G were of RT1, strains B, C and E were of RT2, and strains D and H were of RT3.

*Patterns of epidemiologically unrelated isolates:* Sets of isolates from the same serogroup and with the same *in vitro* protease characteristics were available for ribotyping from epidemiologically unrelated isolates collected in the course of investigations described in this thesis (section 5.3.1). These were:

- 4 protease thermostable serogroup A isolates (VCS 1001, 1908, 2019, 1958)
- 2 protease thermolabile serogroup B isolates (VCS 1746, 1782)
- 2 protease thermolabile serogroup C isolates (VCS 1951, 1753)
- 2 protease thermostable serogroup E isolates (VCS 1742, 2075).

Ribotyping allowed a distinction to be made between otherwise apparently identical organisms in most cases (Table 5.2). One isolate of serogroup A (VCS 1958) which was epidemiologically unrelated to strain A could not be distinguished from it.
5. Genetic Characterisation of *D. nodosus*

Table 5.1. Results for ribotyping with *EcoRI* for 19 *D. nodosus* isolates.

<table>
<thead>
<tr>
<th>VCS No.</th>
<th>Serogroup</th>
<th>GGPTT</th>
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Notes. 1. Isolates with the same letter come from the same flock.

* indicates isolates are likely to be identical with isolates of the same letter. Thus, isolates 1746 and 1782 may be derived from the one strain. Similarly for isolates 1744 and 1775; and isolates 1742, 1883 and 1805.
Testing of isolates which persisted after eradication: Ribotypes for the serogroup B isolate recovered from Mob 1 (VCS 1782) and the serogroup C isolate (VCS 1775) recovered from Mob 2 during the surveillance phase (Chapter 4) were both RT2 i.e. the same pattern as that of strains B and C (Table 5.1). Thus, these isolates which were still present after eradication had all the characteristics of strain B and strain C respectively.

Comparison of three serogroup C isolates: During the course of work described in Chapter 4 there was always concern that there would be transfer of \textit{D.nodosus} from the experimental flocks to animals resident on the Trial property or \textit{vice versa}. During surveillance of Trial property flocks, two isolates of serogroup C were recovered: one from the Polwarth flock (VCS1753) and one from Merino Flock 1 (VCS1751). It was therefore of interest to compare these isolates with strain C which had been introduced into the Main Flock via the donor ewes. Ribotyping with \textit{EcoRI} could not distinguish strain C from these benign serogroup C isolates recovered from sheep on the Trial property (but see section 5.4.2.3).

5.4.1.2. Ribotyping using other restriction endonucleases

DNA from a number of isolates of \textit{D.nodosus} were digested for ribotyping with a number of other restriction endonucleases (Table 5.2).

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<th>Endonuclease</th>
<th>Proportion of strains with interpretable patterns</th>
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<tr>
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<td>3</td>
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<td>3</td>
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</tr>
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<td>3</td>
<td>1</td>
</tr>
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<td>\textit{PvuI}</td>
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<td>3</td>
<td>2</td>
</tr>
<tr>
<td>\textit{XbaI}</td>
<td>0 / 16</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>
5. Genetic Characterisation of *D. nodosus*

In these preliminary tests, the results following digestion with these other enzymes were not as encouraging as those achieved with *EcoRI*. Either clear bands were produced, but no distinction between different strains was possible (e.g. *BamHI*), or insufficient isolates produced clear bands to enable evaluation (e.g. *XbaI*, *HindIII*). Ribotyping following digestion of DNA with *BamHI* gave only one ribotype (with three bands) for all 16 *D. nodosus* isolates evaluated (Figure 5.2b). *DraI* digestion produced possibly two patterns from 13 isolates. The patterns were more difficult to interpret, due to a number of weaker bands. Six strains (VCS 1751, 1753, 1775, 1782, 1948, 1951), which had given three ribotypes with *EcoRI*, produced only one ribotype with *PvuI*, although differentiation of strains C and E appeared possible with *PvuI*. Strains C and E were the only strains which could be evaluated for *HindIII*, and these produced one ribotype (a similar result to *EcoRI*).

Based on these limited investigations, it was concluded that *EcoRI* showed the most promise for ribotyping to discriminate between strains derived from unrelated flocks. The ribotype patterns of *D. nodosus* isolates which persisted in flocks following the eradication programme supported the opinion that they were strains B and C, which had been introduced at the beginning of the experiment.
Ribotyping for various *D. nodosus* strains. Genomic DNA was digested with either *EcoRI* (a) or *BamHI* (b) prior to electrophoresis in a 0.8% agarose gel. The DNA fragments were transferred to a nylon membrane (Boehringer-Mannheim) via the method of Southern (1978). The membrane was dried by baking at 120°C for 30 minutes. The membrane was hybridised at 68°C overnight with a 783 bp probe (La Fontaine et al., 1993), which had been amplified in the PCR using primers internal to the *D. nodosus* 16S rRNA gene, and labelled with digoxigenin-11-dUTP. The bound probe was detected non-radioactively using the DIG colorimetric detection system (Boehringer-Mannheim). The sizes of the detected restriction fragments were determined in reference to Lamda DNA (digested with *EcoRI* and *HindIII*) markers.
(a) Ribotyping of *D. nodosus* isolates with *EcoRI* digestion. Ribotype patterns illustrated are pattern RT2 in lanes 1, 2 and 7; pattern RT1 in lanes 3, 6 and 8; pattern RT3 in lane 4; and pattern RT4 in lane 5.

(b) Ribotyping of *D. nodosus* isolates with *BamHI* digestion. Ribotype patterns were considered identical for all lanes.
5. Genetic Characterisation of *D. nodosus*

5.4.2. PCR-RFLP fingerprinting

5.4.2.1. Preliminary investigations

Initially, experiments using PCR-RFLP fingerprinting following digestion with *HpaII* were carried out on DNA prepared for ribotyping from the experimental strains A, B and C. A distinct banding pattern was observed for each strain, and these patterns were designated 1, 2 and 3 respectively. It was demonstrated with these isolates that the same pattern was produced when the test was repeated on as many as 7 different occasions from the same isolate (data not shown).

This fingerprinting technique was further applied to the other four experimental strains (D, E, G and H) and again four different patterns distinct from those from strains A, B and C were produced. The seven patterns produced from *HpaII* fingerprinting of strains A, B, C, D, E, G and H were designated 1-7 respectively (see Table 5.3).

*HpaII* fingerprints were obtained from DNA derived from genomic DNA prepared for ribotyping, lyophilised cells and cells harvested from 4% HA plates. Identical patterns were produced for the same isolate when either genomic DNA, DNA prepared from lyophilised cells or DNA prepared from fresh cells was used. It therefore appeared that the method of preparation of DNA from isolates did not affect the fingerprint pattern produced.

No *HpaII* fingerprints were produced for 58 samples collected directly from sheep (lesion material), although PCR products were detected in 2% agarose gels for two of these 58 samples. Of the 58 samples, 30 samples had been collected from sheep with clinical (a lesion of score 2 or greater), cultural or microscopic evidence of *D. nodosus* infection.
Table 5.3 Classification and *HpaI* fingerprint results for 114 *D. nodosus* isolates.

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<th>Vir² test</th>
<th>Pattern Number</th>
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Table 5.3 Classification and \textit{Hpall} fingerprint results for 114 \textit{D.nodosus} isolates (cont).

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<td>I</td>
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</tr>
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<td>1</td>
<td>B</td>
<td></td>
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</tbody>
</table>
Notes:  1. Isolate identification number
   2. Origin of isolate: Main, Mobs 1, 2, 4, 5 as described for Main Flock (Chapter 4), Inspection number isolate recovered at in brackets
   Main = Inspection 3 for Main Flock
   VAF = Virulence Assessment Flock (Chapter 4)
   1-21 indicates Property numbers as described in Chapter 3
   5 = Trial property; Polw = Polwarth flock; MerTF = Merino Trial Flock; MerF1 = Merino Flock 1, MerF2 = Merino Flock 2
   101 refers to property where strain D isolated
   * = Bovine isolate
3. Vir. test = Virulence test, + = protease thermostable or elastase positive (less than 21 days)
   - = protease thermo-unstable or elastase negative (at 21 days)
   +/- = protease thermostable and elastase negative (at 21 days)
4. Isolates from the same sheep at different samplings indicated by same S or Sheep number (S829, Sheep1230)
5. Genetic Characterisation of *D.nodosus*

### 5.4.2.2. Isolates from Experimental Flocks (Chapter 4)

The main need for a fingerprinting technique was to identify more stringently isolates recovered during the course of investigations described in Chapter 4. To this end, *HpaII* fingerprints were produced for 45 *D.nodosus* isolates from the Main Flock (Table 5.4), and 9 isolates from the Virulence Assessment Flock (Table 5.5). Among these 54 isolates there were representatives of 6 of the 7 serogroups used in the investigations. There were no serogroup D isolates. Ten *HpaII* fingerprinting patterns were produced from the 54 isolates. These patterns included patterns 1-7 and three additional patterns, which were designated patterns 3a, 5a and 8.

#### 5.4.2.2.1. Main Flock

*Isolates present post eradication:* Of most interest were the isolates which were recovered after eradication. *HpaII* fingerprints consistent with the pattern for strain B (pattern 2) were produced for all 6 serogroup B *D.nodosus* isolates tested from sheep in Mob 1 collected when footrot re-occurred (Inspection 6 and 7) (Tables 5.4,5.6; Figure 5.3a). Similarly, pattern 3 was produced by all 6 serogroup C isolates tested from sheep in Mob 2 and sheep in Mob 5 (Figure 5.3b). A serogroup C isolate recovered prior to eradication (from a donor) also had a pattern 3 fingerprint. Thus, results from fingerprinting further indicated that strains B and C persisted despite an otherwise successful eradication programme.

*Isolates from sheep not subjected to eradication:* A further 32 isolates which had been recovered from sheep either prior to eradication, or from control sheep not subjected to eradication, were tested (Tables 5.4,5.6). *HpaII* fingerprints consistent with the pattern for strain A (pattern 1) were produced for all 9 serogroup A *D.nodosus* isolates tested (Figure 5.3c).
Table 5.4. *HpaII* fingerprinting results for isolates from the Main Flock.

<table>
<thead>
<tr>
<th>Serogroup</th>
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<th>Time of Isolation</th>
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<tr>
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<td>1879</td>
<td>Control (PV2)</td>
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Notes. 1. This refers to the origin of the isolates, with inspection number given in brackets (see Chapter 4). Control = Mob 4. Vaccinate = vaccinated with A, E and H antigens; PV1 and PV2 indicate post vaccination inspections.
2. This isolate was from a Donor ewe (Inspection 3).
**Figure 5.3**

*Hpall* fingerprinting of *omp1*-related sequences of *D. nodosus* strains. Genomic DNA or DNA from cells prepared for PCR was used in PCR-based amplification reaction with the VCS1001-derived oligonucleotide primers A and C (see 5.3.3). The amplification cycle (95°C, 1 min; 50°C, 1 min; 72°C, 1 min) was repeated 31 times, prior to a final extension phase (72°C, 5 min). The amplified DNA fragments from 85% of reaction mixture were precipitated with ammonium acetate and ethanol. These DNA fragments were digested with *Hpall* prior to agarose gel electrophoresis and ethidium bromide staining. Lanes 1 and 10 contained pGEM molecular size markers (Promega), and the sizes of the *Hpall* restriction fragments were determined in reference to these markers. Pattern numbers refer to the *Hpall* fingerprint pattern assigned to each banding pattern.

A feature of the majority of patterns was the presence of a band of similar size to the undigested PCR product. This was initially thought to be a result of incomplete digestion with *Hpall*. However, adding additional *Hpall* enzyme (1 ul) did not eliminate this band. Further, all of the *Hpall* restriction fragments detected by fingerprinting for strain A (VCS1001) could be assigned to one of the four restriction maps predicted by nucleotide sequence analysis of the regions between oligo A and oligo C (data not shown), suggesting that complete digestion for strain A isolates had occurred.

A faint band (340 bp) was detected following electrophoresis in 2% agarose gels containing undigested PCR products for some isolates (Figure 5.3a). This band probably resulted from non-specific primer annealing (E. Moses, pers. comm) as a result of annealing at 50°C rather than 55°C (Moses, 1993). The band did not appear to have an internal *Hpall* site, as a faint abnormal 340 bp band appeared in *Hpall* fingerprints following digestion of products containing the band. When PCR products without these faint bands from the same isolates were digested with *Hpall*, fingerprints were similar to the first pattern, except for the absence of a 340 bp band.
(a) Serogroup B isolates. Lane 2 is derived from strain B. Lane 3 is from a serogroup B isolate from Mob 1. Lane 6 is from an isolate from the Polwarth flock (Trial property, isolated 7/2/94). Lane 7 is from an isolate from the Merino Trial flock (Trial property, isolated 7/2/92). The patterns illustrated were all designated pattern 2. An additional faint band (approx. 340 bp) is present in lanes 2 and 6.

(b) Serogroup C isolates. Lane 2 is from strain C. Lanes 3 - 7 are from serogroup C isolates from Mob 2. Lane 8 is from an isolate from the Polwarth flock (Trial property). Lane 9 is from a bovine isolate (Trial property). The patterns illustrated are pattern 3 in lanes 2-7, and pattern 2 in lanes 8 and 9.
(c) Serogroup A isolates. Lanes 2-6 are from serogroup A isolates from the Main Flock (lanes 2-4 from sheep at Inspection 3; lanes 5-6 from sheep in Mob 4). Lane 8 is from an isolate from the Merino Flock 1 (Trial property). The pattern illustrated in lanes 2-6 was designated pattern 1. Lane 8 illustrates the distinct pattern 12.
Similarly, two *D. nodosus* isolates of serogroup G (VCS 1808, 1813) collected from sheep prior to eradication (Inspection 3) produced pattern 6 fingerprints (the strain G pattern) (Figure 5.4a). Two serogroup G isolates recovered from sheep following vaccination with A, E and H antigens were also fingerprinted. One of these isolates (VCS 1763) also produced a fingerprint designated pattern 6 (Figure 5.4a). The pattern produced from the other serogroup G isolate tested (VCS 1878) was distinct from the strain G pattern and appeared similar to the second of the strain E patterns (pattern 5a, see below) (Figure 5.4a).

Three *HpaII* fingerprints were observed among serogroup E isolates recovered from sheep prior to eradication and control sheep (Table 5.4). Two of these were similar, but an additional band (460 bp) was present in one pattern (pattern 5a) (Figure 5.4b). The pattern without the 460 bp band was that of strain E (pattern 5). The three serogroup E isolates which were recovered from sheep at Inspection 3 (pre-eradication) produced pattern 5. Seven isolates were fingerprinted from the control sheep (Mob 4), and three of these were from sheep vaccinated with A, E and H antigens. Fingerprints of pattern 5 were produced for two isolates from unvaccinated sheep. Pattern 5a was observed from fingerprints of one isolate from unvaccinated sheep and from all three isolates from vaccinated sheep. The seventh isolate (VCS 1819) produced a *HpaII* fingerprint similar to some serogroup H isolates (pattern 8, see below) (Figure 5.4a).

Thus, three *HpaII* fingerprint patterns were observed from isolates which were phenotypically of serogroup E recovered from the Main Flock: patterns 5, 5a, 8.

Two distinct *HpaII* fingerprints were produced from serogroup H isolates recovered prior to eradication and from the control mob (Mob 4). These fingerprints were pattern 7 (strain H) and pattern 8 (Figures 5.4a,b). Patterns 7 and 8 were both produced from isolates collected prior to eradication (one and two isolates, respectively). 

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and from unvaccinated controls (three and one isolates respectively). Only pattern 8 was produced from fingerprinting isolates from vaccinated sheep (two isolates) (Table 5.4).

5.4.2.2.2. Virulence Assessment Flock

Nine isolates recovered from this flock were fingerprinted (Tables 5.5, 5.6). Eight of these nine isolates produced the fingerprint pattern of the strain from which, based on serogroup classification and the identity of the sheep from which they were recovered, they were derived from. The ninth isolate (VCS 1984), of serogroup C, produced a pattern similar to that of strain C, but one band (126 base pairs (bp)) was absent (Figure 5.4c). This pattern was designated pattern 3a.

Table 5.5. HpaII fingerprinting results for isolates from the Virulence Assessment Flock.

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<tr>
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<tr>
<td>C</td>
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<td></td>
<td>1894</td>
<td>3a</td>
</tr>
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<td>7</td>
</tr>
<tr>
<td></td>
<td>1892</td>
<td>7</td>
</tr>
</tbody>
</table>
Figure 5.4

HpaII fingerprinting of omp1-related sequences of *D. nodosus* strains. Genomic DNA or DNA from cells prepared for PCR was used in PCR-based amplification reaction with the VCS1001-derived oligonucleotide primers A and C (see 5.3.3). The amplification cycle (95°C, 1 min; 50°C, 1 min; 72°C, 1 min) was repeated 31 times, prior to a final extension phase (72°C, 5 min). The amplified DNA fragments from 85% of reaction mixture were precipitated with ammonium acetate and ethanol. These DNA fragments were digested with *HpaII* prior to agarose gel electrophoresis and ethidium bromide staining. Lane 1 contained pGEM molecular size markers (Promega).
(a) Lane 3 is from strain G. Lanes 4-7 are from serogroup G isolates from the Main Flock, Inspection 3 (lanes 4,5) and Mob 4 (lanes 6,7). Isolates in lanes 6 and 7 were collected from the same sheep (829) on different occasions. Lanes 8, 9 are from serogroup H isolates from the Main Flock (Inspection 3). Lane 10 is from a serogroup E isolate from Mob 4. The HpaII fingerprints illustrated are pattern 6 for lanes 3-6, pattern 5a for lane 7, pattern 8 for lanes 8 and 10, and pattern 7 for lane 9.

(b) Lanes 2, 4, 5, 6 and 8 are from serogroup E isolates. Lane 2 is from an isolate from the Main Flock (Inspection 3). Lanes 4, 5 and 6 are from isolates from Mob 4. Lane 8 is from an isolate from the Polwarth flock (Trial property). Lanes 3 and 7 are from serogroup H isolates from Mob 4. The HpaII fingerprints illustrated are pattern 5 for Lanes 2 and 5, pattern 8 for lanes 3 and 7, pattern 5a for lanes 4 and 6, and pattern 2 for lane 8.
(c) Serogroup C isolates. Lane 2 is from strain C. Lane 3 is from an isolate from the Virulence Assessment Flock challenged with strain C. Lane 4 is from an isolate from Mob 2. The HpaII fingerprints illustrated are pattern 3 for lanes 2 and 4, and pattern 3a for lane 3. Note missing band (approx. 126 bp) in lane 3 compared to lanes 2 and 4.
5. Genetic Characterisation of *D. nodosus*

Table 5.6. Summary of *HpaII* fingerprinting for isolates derived from experiments described in Chapter 4.

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Origin of isolates</th>
<th>Isolates (n=)</th>
<th>HpaII fingerprint pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Main Flock</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>VA Flock</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>Main Flock</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>VA Flock</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>Main Flock</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>VA Flock</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>3a</td>
</tr>
<tr>
<td>E</td>
<td>Main Flock</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>5a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>VA Flock</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>G</td>
<td>Main Flock</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>H</td>
<td>Main Flock</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>VA Flock</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

Notes. 1. From Chapter 4; VA Flock = Virulence Assessment Flock

5.4.2.3. Isolates from the Trial property

As the sheep in the experimental flocks (Chapter 4) came from the Trial property, and the Main and Virulence Assessment Flocks were maintained in quarantine on the Trial property, it was of interest to determine the identity of isolates collected from animals on the Trial property in addition to those in experimental flocks. *HpaII* fingerprints were produced from 17 isolates from these resident animals. In addition, PCR-RFLP banding patterns with *Sau3AI* digestion were produced from four of the 17 isolates from the Trial property. These 17 isolates were recovered from the Polwarth Flock (4), the Merino Trial flock (3), Merino Flocks 1 (7) and 2 (1) and the cattle (2).
Three *Hpa*II fingerprint patterns were observed from these isolates (Table 5.7). These were pattern 2 (the pattern for strain B, which originated in the Polwarth flock) and two additional patterns designated 11 and 12. *Hpa*II fingerprints for 10 of the 17 isolates from the Trial property were of pattern 2 (Figure 5.5a). These 10 isolates included isolates of serogroups B (5), C (3), E (1) and F (1) and were recovered from the Polwarth flock (4), cattle (2), the Merino Trial flock (3) and the other Merino sheep (1 of 8 isolates tested). Thus, isolates of different serogroup but from the same flock produced similar fingerprints. Further, the two isolates from cattle were indistinguishable from those from sheep from the Polwarth flock. Regular contact had occurred between the cattle and the Polwarth flock.

The remaining 7 *D. nodosus* isolates from the Merino flocks were from serogroups A (four isolates) and C (three isolates), and produced *Hpa*II fingerprints distinct from pattern 2. The four serogroup A isolates, and one of the serogroup C isolates appeared identical (pattern 12) and were distinct from the remaining two serogroup C isolates (pattern 11).

**Table 5.7. HpaII fingerprints of endemic isolates from the Trial property**

<table>
<thead>
<tr>
<th>Origin of isolates</th>
<th>Serogroups</th>
<th>Isolates (n=)</th>
<th>Pattern number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polwarth Flock</td>
<td>B</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Merino Trial Flock</td>
<td>B</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Merino Flock 1</td>
<td>A</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Merino Flock 2</td>
<td>A</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Cattle</td>
<td>B</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Strains A, B, C, D, E, G and H could be differentiated from all isolates from resident flocks on the Trial property (Merino and Polwarth) using HpaII fingerprinting. This suggested that the quarantine measures used for the experimental flocks had been effective at preventing the transmission of *D. nodosus* to non-experimental (residential) flocks. The results are also consistent with the quarantine practices employed on the Trial property (see section 2.3.1). Only one pattern (pattern 2) was detected from the Polwarth flock and cattle. The two additional patterns (11 and 12) detected in the Merino sheep may represent patterns from *D. nodosus* introduced with those sheep.

While the serogroup C isolates (VCS 1751, 1753) from the Polwarth flock and Merino Flock 1 could not be distinguished from strain C by ribotyping (see section 5.4.1.1), the two isolates had different HpaII fingerprints from that of strain C. Fingerprinting was therefore more discriminatory than ribotyping or serogrouping.

The finding that, in some cases, isolates of different serogroups yielded the same fingerprint was further investigated by examining banding patterns following Sau3AI digestion of PCR fragments derived from four isolates. These isolates were of serogroup B (VCS 1746, 1841) and serogroup C (VCS 1753, 1799), and had pattern 2 fingerprints following HpaII digestion. The Sau3AI patterns appeared identical for all four isolates, and were designated pattern S1.

Two of the serogroup B isolates were recovered 4 years apart, suggesting that HpaII fingerprints remained stable over time.

**5.4.2.4. Isolates from properties surveyed (Chapter 3) or investigated**

HpaII fingerprints were also produced for 37 isolates recovered from 10 surveyed flocks (Chapter 3) and three additional investigations. These isolates comprised strain E (VCS 1742) and 36 additional isolates not previously fingerprinted,
and came from 12 properties. Twenty-four different patterns were observed from these 37 isolates (Table 5.8).

Table 5.8. Results of *Hpa*II fingerprinting for 37 isolates from 12 properties.

<table>
<thead>
<tr>
<th>Property Number</th>
<th>Isolate number</th>
<th>Serogroup</th>
<th><em>Hpa</em>II pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2098</td>
<td>A</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>2099</td>
<td>A</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>2101</td>
<td>B</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>2075</td>
<td>E</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>2094</td>
<td>E</td>
<td>21</td>
</tr>
<tr>
<td>11</td>
<td>1940</td>
<td>E</td>
<td>27</td>
</tr>
<tr>
<td>12</td>
<td>1949*</td>
<td>C</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>1951*</td>
<td>C</td>
<td>18</td>
</tr>
<tr>
<td>13</td>
<td>1904</td>
<td>A</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>1908</td>
<td>A</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1916</td>
<td>A</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>1926</td>
<td>B</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>1901</td>
<td>C</td>
<td>5b</td>
</tr>
<tr>
<td></td>
<td>1909</td>
<td>C</td>
<td>5b</td>
</tr>
<tr>
<td></td>
<td>1742</td>
<td>E</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1907</td>
<td>E</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>2049</td>
<td>A</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2051</td>
<td>G</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2056</td>
<td>H</td>
<td>14</td>
</tr>
<tr>
<td>16</td>
<td>2015</td>
<td>A</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>A</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2020</td>
<td>A</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>G</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>H</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>I</td>
<td>29</td>
</tr>
<tr>
<td>18</td>
<td>2040</td>
<td>E</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>2047</td>
<td>E</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>2031</td>
<td>H</td>
<td>32</td>
</tr>
<tr>
<td>20</td>
<td>1956*</td>
<td>A</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>1958*</td>
<td>A</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>1962*</td>
<td>A</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2063</td>
<td>A</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>1959*</td>
<td>G</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>1962*</td>
<td>G</td>
<td>16</td>
</tr>
<tr>
<td>21</td>
<td>2060</td>
<td>G</td>
<td>33</td>
</tr>
<tr>
<td>101</td>
<td>1964*</td>
<td>A</td>
<td>20</td>
</tr>
<tr>
<td>101</td>
<td>1967*</td>
<td>B</td>
<td>19</td>
</tr>
</tbody>
</table>

Notes. * = denotes isolate from an additional investigation.

1. Strain D originated from this property.
From one flock (Property 13, Chapter 3), 8 isolates recovered over a 6 month period were tested. These isolates included strain E, and were of serogroups A (3), B (1), C (2) and E (2). They yielded 7 distinct banding patterns (Table 5.8). The two serogroup C isolates had \textit{HpaII} fingerprints similar to two of the patterns associated with serogroup E isolates from the footrot trials (patterns 5, 5a). This new pattern was designated 5b. It possessed a strong band slightly above 460 bp (460 bp being the additional band detected in 5a), and a weak band corresponding to the top band of pattern 5 (approximately 560 bp). The exact relationship of pattern 5b to patterns 5 and 5a was difficult to establish, as the fingerprints were not produced in the same agarose gel. The three serogroup A isolates produced three distinct patterns (patterns 14, 15 and 24) (Figure 5.5b); the serogroup B isolate yielded a pattern which was similar to pattern 24, but judged to be distinct from it (pattern 26); and the serogroup E isolate produced a pattern distinct from all other patterns (pattern 25). Thus, at least 7 distinct patterns (patterns 5b, 7, 14, 15, 24, 25, and 26) came from sheep from one property. This demonstrates the heterogeneity of \textit{D. nodosus} which may exist in flocks, and was in contrast to the findings in the Polwarth flock on the Trial property (see section 5.4.2.3). Also, on Properties 12 and 18, only one banding pattern was demonstrated in the few isolates from each property.

Heterogeneity of fingerprints of isolates from different flocks was demonstrated initially by the unique fingerprints of the experimental strains, which all originated from 7 unrelated flocks. This can be further seen from the fingerprint results from the 37 isolates described here (Table 5.8). Fingerprinting of 19 isolates from 8 properties (Properties 11, 13, 15 and 20 were excluded, as these may be related, see section 5.4.2.5) produced 13 distinct patterns, with these fingerprints being unique to each property. The same fingerprint in different flocks was detected on only one occasion (pattern 14, Properties 13 and 15). These properties adjoined, and unintentional sheep movement between these properties had occurred previously.
Figure 5.5

*Hpall* fingerprinting of *omp l*-related sequences for various *D. nodosus* strains. The methodology was as described in Figure 5.4. Lane 1 (a,b) contained pGEM molecular size markers (Promega).
(a) Lane 2 is from a serogroup C isolate from a Donor ewe. Lanes 4-6 are from serogroup C isolates from Merino Flock 1 (Trial property). Lane 7 is from strain B. Lane 8 is from a serogroup B bovine isolate (Trial property). Lane 10 is from a serogroup F isolate from the Merino Trial flock (Trial property). The HpaII fingerprints illustrated are pattern 3 for lane 2, pattern 2 for lanes 4, 7, 8 and 10, pattern 12 for lane 5, pattern 11 for lane 6.

(b) Lanes 2,3,6,7 and 9 are from isolates from one property (Property 13). Lane 2 and 7 are from serogroup C isolates. Lane 3 is from a serogroup A isolate. Lane 6 is from a serogroup E isolate. Lane 9 is from a serogroup B isolate. Lane 10 is from a serogroup E isolate from a ram purchased from Property 13. The HpaII fingerprints illustrated are pattern 5a for lanes 2 and 7, pattern 24 for lane 3, pattern 25 for lanes 6, pattern 26 for lane 9, and pattern 27 for lane 10.
(c) Serogroup A isolates. Lane 7 is from an isolate from the Merino Flock 1 (Trial property). Lane 8 is from an isolate from an introduced mob on Property 20 (isolated 1/2/94). Lane 9 is from an isolate from a resident mob on Property 20 (isolated 1/2/94). Lane 8 is from an isolate from a resident mob on Property 20 (isolated 15/11/92). The HpaII fingerprints illustrated were designated pattern 12 for lane 7, pattern 16 for lane 8, and pattern 17 for lanes 9 and 10.
Thus, fingerprinting in this study was able to distinguish isolates both between and within properties. Further, isolates indistinguishable by serogrouping but considered different on epidemiological grounds or in vitro virulence characteristics could be distinguished from other isolates within each serogroup. Thirty isolates from unrelated flocks were examined in this way (Table 5.9).

Table 5.9. Isolates from flocks believed to be unrelated, showing the ability of HpaII fingerprinting to distinguish between isolates of the same serogroup.

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Isolate number (VCS)</th>
<th>HpaII fingerprint pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1001</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1759</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>1908</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1958</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>2049</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2098</td>
<td>34</td>
</tr>
<tr>
<td>B</td>
<td>1746</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1967</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>1926</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2101</td>
<td>35</td>
</tr>
<tr>
<td>C</td>
<td>1753</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1744</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1901</td>
<td>5b</td>
</tr>
<tr>
<td></td>
<td>1951</td>
<td>18</td>
</tr>
<tr>
<td>E</td>
<td>1754</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1742</td>
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<td></td>
<td>2094</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>1907</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2075</td>
<td>28</td>
</tr>
<tr>
<td>G</td>
<td>1745</td>
<td>6</td>
</tr>
<tr>
<td></td>
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<td>14</td>
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<tr>
<td></td>
<td>1961</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2016</td>
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<tr>
<td></td>
<td>2060</td>
<td>33</td>
</tr>
<tr>
<td>H</td>
<td>1748</td>
<td>7</td>
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<tr>
<td></td>
<td>2056</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>2031</td>
<td>32</td>
</tr>
</tbody>
</table>
Isolates of different serogroups from the same flock were differentiated by \textit{HpaII} fingerprinting in 3 flocks (Properties 13, 16 and 101, Table 5.8). An isolate of serogroup A was distinguished from those of G and H by fingerprinting on a fourth property (Property 15). However, in four flocks, (Properties 1, 15, 18 and 20), isolates of different serogroups produced the same fingerprint (Table 5.8). This had also been the case for some isolates from the Trial property (see section 5.4.2.3). Thus, in five flocks, isolates of different serogroups produced the same fingerprint (Table 5.10).

\textbf{Table 5.10.} Flocks from which isolates of different serogroups were recovered which produced the same fingerprint pattern.

<table>
<thead>
<tr>
<th>Property Number</th>
<th>Serogroups</th>
<th>\textit{HpaII} pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A, B</td>
<td>35</td>
</tr>
<tr>
<td>5 (Trial Property)</td>
<td>B, C, E, F</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>G, H</td>
<td>14</td>
</tr>
<tr>
<td>18</td>
<td>E, H</td>
<td>32</td>
</tr>
<tr>
<td>20</td>
<td>A, G</td>
<td>16</td>
</tr>
</tbody>
</table>

5.4.2.5. Application of \textit{HpaII} fingerprinting to epidemiological investigations

\textit{HpaII} fingerprinting was used to examine the likely sources of footrot in two studies.

1. Flocks related to Property 13: Three flocks from which isolates were recovered for fingerprinting were epidemiologically related to that on Property 13. Rams for two flocks (11 and 20), were purchased from Property 13. The other property (Property 15) was adjacent to Property 13.
The fingerprints of isolates from those flocks which purchased rams from Property 13 were both different from those of Property 13 isolates (and from each other). An isolate recovered from recently purchased rams on Property 11 (serogroup E) produced yet another distinct HpaII fingerprint (pattern 27). Similarly, a *D. nodosus* isolate of serogroup A from sheep at the second property (Property 20) gave another distinct fingerprint (pattern 17).

Three *D. nodosus* isolates (representative of serogroups A, G and H) from Property 15 were analysed by HpaII fingerprinting. These three isolates produced two fingerprints: the serogroup G and H isolates produced fingerprints apparently identical to one of the fingerprints from Property 13 (pattern 14), and the serogroup A isolate gave a distinct pattern (pattern 22).

2. An outbreak of footrot on Property 20: Further investigations were carried out on Property 20 in the year following the survey, due to an unexpected outbreak of footrot. Five *D. nodosus* isolates obtained from this outbreak yielded two patterns:

(i) a serogroup A isolate (VCS 1956) from sheep which were resident on the property at the time of the Survey gave an identical HpaII fingerprint to the previous isolate (VCS 2063, pattern 17);

(ii) the other four isolates, from a recently introduced mob where the outbreak of footrot was observed, consisted of two serogroup A isolates (VCS 1958, 1962), and two serogroup G isolates (VCS 1959, 1961). All four isolates produced an identical fingerprint (pattern 16), which was distinct from the pattern for the *D. nodosus* isolate from the resident sheep (pattern 17) (Figure 5.5c).
Interestingly, this pattern was also distinct from that from an isolate (serogroup A, VCS 1830) recovered from Merino Flock 2 (pattern 12). Merino Flock 2 were the progeny of the mob introduced to Property 20, and were purchased at the same time from the same property. It was subsequently discovered that a ram from a completely different flock was the most likely source of the outbreak of footrot in the introduced mob on Property 20.

The identical result from two apparently similar isolates recovered from the same mob 12 months apart in (i) above represents further evidence of the stability of the HpaII fingerprint.
5.5. Discussion

Genotypic identification methods have been used either in conjunction with existing phenotypic typing methods or as an alternative typing method in bacterial epidemiological investigations (see section 5.2). The results reported in this chapter suggest genotyping methods (ribotyping and *Hpa*I fingerprinting) may be appropriate techniques for use in epidemiological investigations involving *D. nodosus*.

5.5.1. Ribotyping

Ribotyping, using a 783 bp rDNA probe and *Eco*RI digestion of total genomic DNA, allowed discrimination of isolates which were of the same serogroup and had similar virulence characteristics. It could therefore be useful in some circumstances as a means of differentiating strains. However, ribotyping was not strain specific, and could not necessarily differentiate between the *D. nodosus* strains introduced into the Main Flock (strains A, B, C, D, E, G and H), nor between at least one of these strains (C) and isolates of the same serogroup recovered from flocks on the Trial property. Thus, whilst ribotyping alone may not offer better discrimination between strains than existing phenotypic methods, it may allow additional classification when used in conjunction with these methods. These findings are consistent with the majority of studies involving ribotyping (see section 5.2.1).

Further evaluation of alternative restriction endonucleases for ribotyping of *D. nodosus*, or the use of results derived from using several restriction endonucleases, may improve the discriminatory power of ribotyping. Results from *Pvu*I digestion suggested that the use of both *Eco*RI and *Pvu*I patterns could provide additional discrimination. However, culturing of *D. nodosus* is relatively slow and sometimes unreliable, making the need to produce sufficient *D. nodosus* cells for chromosomal DNA analysis a tedious process. In addition, ribotyping itself is a time-consuming process.
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(Kostman et al, 1992; Bingen et al, 1993c; Gomez-Lus et al, 1993). The PCR (Saiki et al, 1988), which can be performed on relatively small amounts of DNA and could in theory be applied directly to lesion material, would therefore appear to be more suited to studies involving \textit{D. nodosus}.

5.5.2. PCR-RFLP Fingerprinting

In this investigation, PCR-RFLP fingerprinting, based on the digestion with \textit{HpaII} of internal \textit{omp1} gene fragments, was superior to ribotyping in the differentiation of isolates. The discriminatory ability and repeatability of the technique were considered high, with all known epidemiologically distinct strains being differentiated by PCR-RFLP patterns, and a number of strains giving identical results when re-tested.

5.5.2.1. \textit{D. nodosus} strains present 'post eradication' in the Main Flock (Chapter 4)

Results of the fingerprinting of \textit{D. nodosus} isolates collected from Mobs 1 and 2 following the footrot eradication programme were consistent with the persistence of strains B and C respectively. \textit{HpaII} fingerprints for all 6 serogroup B isolates tested from Mob 1 and all six serogroup C isolates tested from Mobs 2 or 5 during the surveillance phase had \textit{HpaII} fingerprints identical to strains B and C respectively, which had been used to infect the flock originally. Strain C could be clearly differentiated on \textit{HpaII} fingerprinting from benign serogroup C isolates recovered from resident flocks on the Trial property during the trial period. In addition, given that all 7 introduced strains (A, B, C, D, E, G and H) could be differentiated by fingerprinting, the findings support the view that the isolates collected from Mobs 1 and 2 following attempted eradication were accurately identified by serogrouping.
5.5.2.2. Stability of \textit{HpaII} fingerprinting

\textit{D. nodosus} strains appeared to have relatively stable \textit{HpaII} fingerprints, although this could not be established definitively. All isolates considered to have originated from strains A, B and C in the Main Flock had \textit{HpaII} fingerprints consistent with those for strains A, B and C, despite the exposure of infected sheep to vaccination and footbathing treatments. For strains A and C, isolates collected from sheep up to 20 months and 26 months, respectively, following introduction into the flock, were identical. For strain B, serogroup B isolates collected from the same flock 4 years apart gave identical \textit{HpaII} fingerprints. Similarly, on Property 20, isolates collected from the same flock 12 months apart gave identical fingerprints.

It would therefore appear that, under a number of different conditions, the PCR-RFLP \textit{HpaII} fingerprint of \textit{D. nodosus} isolates, which apparently originated from the same strain, remained stable. However, several anomalous results (discussed below) bring into question the stability of \textit{HpaII} fingerprints.

5.5.2.3. Anomalous results from experimental flocks

While in the main, \textit{HpaII} fingerprinting of \textit{D. nodosus} isolates in these investigations suggested that strain specific patterns existed and that these patterns were stable over time, a number of results from isolates from the experimental flocks on the Trial property were obtained which were contrary to this view. These included:
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- 2 distinct patterns (7 and 8) for serogroup H isolates which were expected to be identical
- a serogroup E isolate (VCS 1819) with a pattern 8 (serogroup H) *Hpa*II fingerprint
- a serogroup G isolate (VCS 1878) with a pattern 5a (serogroup E) *Hpa*II fingerprint
- serogroup E isolates (VCS 1792, 1845, 1848 and 1883) with an additional band
- a serogroup C isolate (VCS 1894) with a missing band

The reasons for these unexpected results were not determined. It should be noted that in some cases the fingerprints were obtained only once (e.g. VCS 1894), and therefore differences detected may represent problems in the technique, or artefacts. Other possible explanations which may explain some or all of these results include:

(i) *incorrect serogrouping.* All isolates which gave unexpected results were re-serogrouped using a separate batch of antisera (and in the majority of cases, PCR-RFLP analysis was repeated), with no change in results. Therefore, whilst incorrect serogrouping may have occurred, this would seem unlikely. Serotyping using the tube agglutination test (Claxton et al, 1983) would be necessary to remove any doubt as to the serogroup of isolates giving anomalous results.

(ii) *the presence of more than one clone in cultures used for infection, or preserved or prepared for PCR.* Contamination of isolates during subculturing, so that more than one clone was present in lyophilised samples, may have occurred, although all normal care was taken to identify and subculture individual colonies. In addition, the failure of serogrouping to identify a second isolate, and the generation of a PCR-RFLP pattern consistent with it originating from a single strain, suggest that the possibility of more than one strain being present in analysed samples is remote.
However, given the ability of the PCR to amplify small amounts of target DNA (Saiki et al, 1988), such an occurrence cannot be excluded.

(iii) interactions between strains or within strains during infection, leading to serogroup or HpaII fingerprint variation. Intra-genomic and inter-genomic interactions, such as recombination, may also be an explanation for some or all of these inconsistent results.

In considering such interactions, it is necessary to understand the role of both fimbrial and Omp1 antigens, and the effect of antigenic variation on the stability of serogrouping and HpaII fingerprinting.

It is well established that the fimbrial subunits are the antigens which determine the serogroup classification for D.nodosus strains (Walker et al, 1973; Short et al, 1976; Every, 1979), and therefore changes in genes coding for the fimbrial subunits may lead to changes in serogroup. It has also been shown that fimbrial antibodies are immunoprotective (Anderson et al, 1987; Egerton et al, 1987; Stewart and Elleman, 1987) and vaccination (either whole cell or fimbrial) is directed against fimbrial antigens (Stewart, 1978; Skerman et al, 1981; Stewart et al, 1982b). The high antibody titres elicited following vaccination, in comparison to antibody titres following natural infection (Egerton, 1973; Egerton and Merrit, 1970), suggest that vaccination could exert considerable immune pressure on genes coding for fimbrial subunits. Antigenic variation may be a consequence of such immune pressure from the host (Borst and Greaves, 1987; Seifert and So, 1988; Borst, 1991; Brunham et al, 1993). Despite this, analysis of D.nodosus strains which have been subjected to immune pressures from vaccination has revealed no evidence of antigenic variation within strains, either as a result of specific monovalent vaccination, or due to prolonged natural infection (Moore et al, 1990).
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*D. nodosus* fimbriae are classified as Type 4 (Ottow, 1975; Dalrymple and Mattick, 1987). Some bacteria with Type 4 fimbriae possess mechanisms which enable the rapid alteration of fimbrial antigenicity. *Neisseria gonorrhoeae* achieve this through recombination using minicassettes of variable DNA sequences from silent loci (Haas and Meyer, 1986), whilst DNA inversion events enable antigenic variation in *Moraxella bovis* (Marrs et al., 1988; Fulks et al., 1990). Such fimbrial intra-genomic recombination is considered unlikely in *D. nodosus*, due to the lack of evidence of the presence of silent fimbrial subunit sequences (Hobbs et al., 1991; Cox, 1992). There is evidence, however, that recombination in *D. nodosus* does occur, and at least some of the variation in serogroup classification has been attributed to a number of recombination events (Cox, 1992). The frequency of such recombination events has not been determined. Recombination to cause fimbrial antigenic variation was not detected in one 3 month study (Cox, 1992), which is consistent with other studies which have failed to detect fimbrial antigenic shift within *D. nodosus* strains (Moore et al., 1990).

Given the current knowledge of the composition of *D. nodosus* genome, it would therefore appear unlikely that intra-genomic recombination is an explanation for the presence of serogroup distinct, *omp* gene homologous strains within a flock. Rather, if genetically related strains with different fimbrial expression exist, and this is a result of changes in the fimbrial subunit locus, recombination of fimbrial subunits between strains is the most likely explanation.

*Omp1* genes, *omp1A, 1B, 1C, 1D*, code for variant outer membrane proteins (Omp1). These are antigenic (Moses et al., 1995; O'Donnell et al., 1983). The role of Omp1 has not been established, although given its antigenic properties, Omp1 could act as a decoy antigen or have a role in the survival and/or virulence of *D. nodosus* (Moses et al., 1995). It has been shown that an inversion system for the rearrangement of *omp1* genes exists in *D. nodosus* strain VCS 1001, and this intra-genomic DNA rearrangement mechanism has been suggested as a method for antigenic variation (Moses et al., 1995). If
the *omp1* gene inversion system is an effective means of achieving antigenic variation for Omp1, and the *omp1* genes themselves do not undergo changes, then the analysis of DNA fragments internal to these multiple single copy genes (Moses et al, 1995) should provide a stable and useful strain identification system.

However, if Omp1 antigenic variation is an important mechanism for the survival or proliferation of *D. nodosus*, and inversion of *omp1* genes is not the only or main mechanism for allowing antigenic variation, limitations may be found to fingerprinting. If recombinations occur which cause changes within the *omp1* gene, then *HpaII* fingerprint instability would become apparent. However, another possibility is that recombinations involving the transfer of some or all of the *omp1* genes occur, which would lead in the latter case to the same *HpaII* fingerprint in a different strain. If such a mechanism exists, and if antigenic properties of Omp1 favour the survival or proliferation of *D. nodosus*, then it is possible that a particular *omp1* genetic structure is advantageous to a number of strains under the same immune pressure. This may result in strains of *D. nodosus* evolving with similar (or identical) *omp1* genes.

Whilst further analysis of the genome, such as sequencing, restriction endonuclease and specific DNA probe analysis, of anomalous strains is necessary to substantiate or repudiate any of the above hypotheses, a more detailed discussion on the specific anomalous results is worthwhile to consider which hypotheses are the strongest.

*Two distinct patterns for serogroup H isolates.* The detection of two patterns (7 and 8) from serogroup H isolates at the first sampling of infected wethers (Inspection 3), rather than a single pattern, suggests that more than one strain may have been present in the samples used for infection of the Donor ewes i.e. strain H initially comprised phenotypically uniform cultures but which consisted of more than one fingerprint type. The lyophilised samples from which the challenge culture for the Donor ewes was grown have been subcultured subsequently. It was therefore not possible to determine if the
original cultures of strain H contained *D. nodosus* of more than one fingerprint. These subsequent subcultures were used to infect sheep in the Virulence Assessment Flock. The presence of only one fingerprint among the four isolates tested from the sheep challenged with strain H in this flock suggests homogeneity of the challenge culture for the Donor ewes and the Virulence Assessment Flock, but does not confirm it.

**Fingerprint and serogroup mismatching.** On two occasions, isolates from the Main Flock characterised by serogrouping had *Hpa*I fingerprints consistent with the patterns detected for isolates of different serogroups (see Table 5.4). If *Hpa*I fingerprints are unique and stable, and the serogroup results are accurate, then the results from isolates VCS 1819 (serogroup E, pattern 8) and VCS 1878 (serogroup G, pattern 5a) suggest that either contamination of the isolates occurred, or the strains H and E, respectively, were able to alter their fimbrial antigenicity. The sheep from which VCS 1819 was recovered had previously had a serogroup H isolate (not fingerprinted) recovered from it. Similarly, VCS 1878 was recovered from a sheep's foot which had previously had a serogroup G isolate (with a *Hpa*I fingerprint consistent with strain G) and a serogroup E isolate (not fingerprinted) recovered from it. These histories suggest that the samples collected contained more than one isolate. Alternatively, it is possible that transfer of genetic information occurred. Given that 7 epidemiologically unrelated, serogroup distinct *D. nodosus* strains were introduced into the Main Flock, recombination between serogroups (in this case E and H, and E and G) could explain a serogroup H fingerprint from a serogroup E isolate, and a serogroup E fingerprint from a serogroup G isolate. Inter-genomic transfer of the *omp1* gene locus might also explain these anomalies.

**Missing bands.** In two instances, patterns which were similar to that of the strain of the perceived origin of the isolates, but could be differentiated by the absence (or presence) of a single band, were detected. These were pattern 3a from VCS 1894, and pattern 5a from VCS 1792, 1845, 1848 and 1883. VCS 1894 was a serogroup C isolate recovered from the Virulence Assessment Flock from sheep challenged with strain C. The aberrant
pattern could be due to a problem in the repeatability of the fingerprinting technique, or to changes within the \textit{omp1} gene, there being no other strains of \textit{D.nodosus} in the single culture challenge. Similar arguments might also be made for the serogroup E isolates which yielded pattern 5a. Alternatively, for these isolates, the different banding pattern may indicate that the original strain used to infect donor sheep comprised phenotypically uniform cultures but which consisted of more than one fingerprint type, as was suggested for the two patterns detected from strain H isolates recovered from sheep in the Main Flock.

5.5.2.4. Findings from flocks other than the experimental flocks

\textit{HpaII} fingerprinting was investigated to further characterise the \textit{D.nodosus} isolates which survived eradication procedures outlined in Chapter 4. Subsequent to developing and testing \textit{HpaII} fingerprinting for this purpose, isolates which originated from 15 flocks, other than the experimental flocks, were also tested. A number of isolates tested produced interesting, and in some cases, unexpected results.

Isolates representing at least two serogroups were recovered from 9 of the 15 properties, despite the small number of isolates which were collected from some properties. Isolates representing different serogroups and which were recovered from the same flocks on 5 different properties (including the Trial Property) yielded distinct \textit{HpaII} fingerprints, and in some cases fingerprints differed even for isolates from the same flock which were of the same serogroup. For example, three serogroup A isolates from Property 13, which had similar \textit{in vitro} virulence characteristics, yielded three distinct fingerprints. From the same mob, two serogroup E isolates with different \textit{in vitro} virulence characteristics had distinct fingerprints. The evidence is then that, in flocks, strains of different genetic composition are relatively common.
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The presence of isolates representative of multiple serogroups has been reported (Schmitz and Gradin, 1980; Claxton et al., 1983; Hindmarsh and Fraser, 1985; Thorley and Day, 1986; Gradin et al., 1993). This limits the usefulness of serogrouping in epidemiological investigations, because of the intensity of sampling required to ensure identification of all serogroups present. Given the apparent absence of mechanisms which allow rapid fimbrial antigenic variation within strains (Hobbs et al., 1991; Cox, 1992), it has been assumed that the presence of multiple serogroups was indicative of multiple strain infections. The presence of distinct *HpaII* fingerprints for isolates of different serogroups from the same flock is consistent with this view. As adequate histories were not available, and the present study generally only involved one visit to a property, it could not be established whether these multiple strain infections occurred as the result of the introduction of one sheep or a mob of sheep, or whether mobs of sheep came from a single source or from multiple flocks. Clearly, the presence of apparently multiple strain infections in some flocks (and almost certainly some sheep in these flocks) could mean the possible transfer of the multiple strain infections with the unrestricted movement of mobs of sheep from these flocks.

**Serogroup distinct, *HpaII* fingerprint homologous isolates.** Distinct fingerprints were not produced for all serogroup distinct isolates from a flock. Isolates recovered from the Polwarth and Merino Trial Flocks and from cattle on the Trial property produced apparently identical fingerprints, despite the isolates representing four different serogroups. Similarly, isolates derived from four separate flocks yielded *HpaII* fingerprints apparently identical to those isolates of different serogroups from the same property (Table 5.10). Thus, on 5 occasions, epidemiologically related isolates which differed in serogroup classification appeared to possess identical fingerprints.

Further investigations of these isolates, by comparing fingerprints derived following digestion with a different restriction endonuclease, were limited to that of four isolates with *Sau3AI*. These four isolates, from the Trial property, also had
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identical banding patterns using *Sau*3AI, suggesting that the reason for identical *Hpa*II fingerprints in these isolates was due to the isolates having similar *omp*1 genes. Possible explanations for these findings are discussed here. However, given the limited nature of these investigations, and the fact that in some cases, comparisons were made between fingerprints on different agarose gels, further investigations are warranted to confirm the existence of isolates of different serogroup, but possessing identical *omp* genes, and to establish the nature of the relationship between such isolates.

The presence of serogroup distinct, *Hpa*II fingerprint homologous isolates suggests that changes in fimbrial antigenicity may have occurred, if *Hpa*II fingerprinting is generally isolate specific and stable. Alteration of fimbrial antigens in other bacteria with Type 4 fimbriae has been demonstrated (see section 5.5.2.3), and therefore the occurrence in *D. nodosus* would not be surprising. Inter-genomic transfer appears to be the most likely method of alteration in fimbrial antigenicity in *D. nodosus*. Alternatively, inter-genomic transfer of *omp*1 genes could explain these findings. In either case, the presence of multiple strains of *D. nodosus*, and hence a genetic pool, within the flock would be necessary.

Evidence of at least two strains of *D. nodosus* being present within a flock, based on serogroup and *Hpa*II fingerprint heterogeneity, was found in at least two of the 5 flocks. On Property 1, apparently identical fingerprints were observed for a serogroup A isolate and a serogroup B isolate. A second serogroup A isolate analysed from this flock had a distinct fingerprint. Similarly, for Property 15, heterology between fingerprints was also apparent for isolates, providing evidence that multiple *D. nodosus* strains were present in the flock.

No evidence was found of multiple strains on two of properties (Properties 18 and 20), but their presence could not be excluded, due to small number of isolates
examined, and the history of a footrot eradication programme (Property 18) or recently purchased sheep (Property 20).

On the Trial property, multiple strains were present, although no evidence of this was found in the Polwarth flock. Seven isolates collected from the Polwarth flock and cattle yielded only one $Hpa$II fingerprint. However, virulent footrot had been eradicated from the property 3 years prior to the isolation of strain B, and serogroup E isolates had been recovered from infected sheep 4 years prior to eradication. Further, while transfer of $D. nodosus$ was excluded from occurring directly from introduced Merino sheep to Polwarth sheep by well established quarantine procedures (Gregory, 1939b; Beveridge, 1941), indirect transfer of $D. nodosus$ via cattle was not excluded and could therefore have contributed to the genetic composition of isolates in the Polwarth flock.

Thus, whilst isolates representing at least two serogroups and having two distinct fingerprints were not detected within the Polwarth flock or cattle, their presence could not be excluded. Isolates with the pattern 2 fingerprint may have acquired fimbrial genes from strains which were present before their eradication. Alternatively, if the $ompI$ gene region associated with the B strain fingerprint provided a competitive advantage, and benign serogroup B, C and E strains had been present previously in the flock, it is possible that through recombination all surviving strains possessed this single $ompI$ gene region. In either case, it would appear that if inter-genomic recombination was responsible for the findings, it had occurred on several occasions.
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5.5.2.5. Application of *Hpa*II fingerprinting to investigating footrot outbreaks

In its restricted application in this study, *Hpa*II fingerprinting appeared to provide some useful additional information as to the possible source of footrot in several outbreaks. Thus, on Property 20, the presence of isolates with a distinct fingerprint in the outbreak investigated suggested footrot in the purchased sheep may not have resulted from infection from the resident sheep. This suggestion was subsequently supported by further investigations of the footrot status of the introduced sheep.

The availability of a collection of isolates from a group of contiguous properties (Chapter 3) provided a resource for further examining the application of *Hpa*II fingerprinting for tracing transfer of *D. nodosus* between flocks. Surprisingly, isolates with apparently homologous *Hpa*II fingerprints were demonstrated on neighbouring properties on only one occasion. This suggests that transfer of *D. nodosus* between flocks was not common in those surveyed. Alternatively, the failure to demonstrate relationships between *D. nodosus* populations on different properties may have been due to the limited number of isolates examined, and the heterogeneity of isolates which may exist on properties.

If *Hpa*II fingerprinting is to be applied to tracing footrot outbreaks, problems associated with the presence of multiple strains will need to be considered. No samples known to contain multiple strains were subjected to *Hpa*II fingerprinting. Given the likelihood of multiple *D. nodosus* strains in footrot outbreaks, the application of *Hpa*II fingerprinting directly to lesion material would not be appropriate, given the likely difficulty in evaluating the banding patterns from a mixed infection. Further, the failure to produce *Hpa*II fingerprints from samples collected directly from sheep indicates the need for the development of suitable storage and preparation techniques for such samples.
At present, the use of this fingerprinting technique would require the extensive sampling of a large number of sheep, and the testing of many isolates from those samples from each flock in an effort to ensure patterns were obtained for all isolates present. In the absence of such intensive and exhaustive sampling, errors in the conclusions drawn could be expected. For example, even if only two strains were present in an infected flock, it is possible that in the normal sampling of an outbreak, only one strain was identified, either due to different growth characteristics of the strains, or a predominance of one strain. If footrot was transmitted from this flock to, for example, a neighbouring flock, it is possible that by chance, the one or two stray sheep which transmitted the footrot only had the unidentified strain. Alternatively, both strains may have been transmitted, but in the neighbouring flock, the presence of different factors result in the unidentified strain becoming the dominant one. In both cases, investigations in the neighbouring flock may only reveal the previously unidentified strain. The incorrect conclusion would then be drawn about the source of the footrot. Even worse, the exclusion of the neighbour's flock as the source of footrot (based on what might be seen as the latest technology) may incorrectly implicate other less likely reasons for an outbreak.
5.6. Conclusions

PCR-RFLP fingerprinting, based on the digestion with HpaII of *omp1* gene fragments, appeared to be a useful epidemiological tool for *D. nodosus* investigations. The discriminatory ability and repeatability of the technique were high. HpaII fingerprinting provided additional strong evidence of the origin of the strains of *D. nodosus* which survived a conventional eradication programme. In particular, HpaII fingerprints from isolates in the Main Flock supported the findings in Chapter 4 (which were based on serogroup, *in vitro* virulence testing and clinical findings) that the *D. nodosus* isolates recovered from Mobs 1 and 2 following attempted footrot eradication were isolates from the original strains B and C respectively. In addition, HpaII fingerprints supported the findings that strains A, E, G and H persisted in untreated sheep in Mob 4.

Ribotyping did not provide additional information to serogrouping and *in vitro* virulence characteristics on the origin of the isolates persisting in Mobs 1 and 2 following the footrot eradication programme, although they were consistent with the serogroup B and C isolates in Mobs 1 and 2 originating from strains B and C respectively.

While a number of unexpected findings occurred using HpaII fingerprints as an epidemiological tool, there was no evidence that these results were due to inherent inadequacies in HpaII fingerprinting, although further investigations are necessary to confirm the usefulness of this technique as a method for tracing outbreaks of footrot. The anomalous results found during the evaluation of HpaII fingerprinting may be due to one or a number of factors. Errors associated with either serogrouping or fingerprinting are unlikely to explain these anomalies; rather, inter-genomic DNA transfer, or possibly an intra-genomic mechanism, may have occurred. As the anomalous results did not alter the findings for which the fingerprinting was performed, a more detailed investigation of the anomalous results was not carried out.
Further investigations to establish primarily whether the serogroup distinct, \textit{HpaII} fingerprint homologous strains are homologous or heterologous for the remainder of the genome not assessed by fingerprinting should provide evidence as to the likely cause of many of the unexpected results. The existence of mechanisms which enable antigenic variation within \textit{D.nodosus} strains could have important implications on possible footrot control and eradication methods, and therefore further investigations of these anomalous isolates appear warranted.

The preliminary evaluation of \textit{HpaII} fingerprinting described here suggests the technique may be a valuable tool in research where the identity of strains is required. This may be in such experiments as the effectiveness of treatments, the transmission of strains from sheep to sheep or cattle to sheep (or \textit{vice versa}), or additional eradication studies. However, at present \textit{HpaII} fingerprinting does not appear to be appropriate for investigating the source of an outbreak of footrot, given the likely multiplicity of strains in such an outbreak and the limitations of fingerprinting in the presence of multiple strains. Further evaluation of the storage and preparation of samples collected directly from sheep's feet, and the effect of the presence of multiple \textit{D.nodosus} strains, is necessary before \textit{HpaII} fingerprinting can be applied directly to lesion material.
CHAPTER 6

IMPLICATIONS OF FINDINGS FOR REGIONAL FOOTROT CONTROL AND ERADICATION PROGRAMMES

6.1. Introduction

The implications of the results from this study have been discussed in relation to farm footrot eradication programmes where owners are acting independently of neighbours and in the absence of any regulation (the farm level). At this level, the owner will apply certain criteria to determine whether control or eradication is the most appropriate action to be taken when faced with an outbreak of footrot. A clinical assessment, based on the inspection of at least 100-200 sheep, is likely to give the best information on the form of footrot present and the potential economic impact of the disease. In addition, if the hypothesis that \textit{D. nodosus} strains associated with less clinically severe disease are more difficult to eradicate is shown to be correct, this clinical assessment may also be the best indicator of the likelihood of eradication.

However, the ability or desire of farmers to operate independently from neighbours or other producers within the immediate community or State is now limited. This is because of the imposition of legislation which forces action over arbitrarily defined footrot outbreaks, or the presence of a large number of voluntary Footrot Groups, where members within a group strive for a common footrot-related objective. This objective is generally based on criteria used in legislated areas within the State where the Group exists. The recognition, by both producers and regulators, of the difficulties of working independently from neighbours in particular, and fellow producers in general, has led to both the regulatory and voluntary regional approach. Therefore the findings of this study, primarily concerned with footrot management at the farm level, need to be discussed in relation to regional programmes if they are to be relevant to the broader farming community.
The basis for a regulatory approach to disease control or eradication should be that community benefits (combined producer benefits) are more important than benefits for individual producers (Hanson, 1983). Thus, economic analysis of State footrot programmes rely on community cost : benefit ratios, not farm cost : benefit ratios (Carmody et al., 1984; Stott, 1989; Thomson, 1993). The underlying assumption with regulatory disease programmes is that individual producers may either fail to recognise the consequences of the disease, or for their particular enterprise the disease may not economically justify the measures needed to achieve regional control or eradication. As a result of this ignorance or knowledge, individuals "may act selfishly to the disadvantage of the neighbourhood or society as a whole" (Hanson, 1983). This lack of conformity, for whatever reason, which is assessed to be at the cost of the community, leads to the need for legislation, giving regulators power to force an individual producer to undertake a specific disease programme, even if it is not economically justifiable for his/her enterprise. In some cases, compensation funds are established, so that the financial losses incurred by some or all producers are offset. Compensation is a logical procedure if individual producers are forced to bear financial losses as a result of the programme, and should be available from the accrued benefits of the programme to the community.

Disease eradication programmes have been successful at the regional and national level. In the United States, 12 diseases have been eradicated since 1884 (Smith, 1991). In Australia, bovine contagious pleuropneumonia, bovine brucellosis and bovine tuberculosis have been eradicated nationally, using State eradication campaigns (Mylrea, 1990, 1991; Newton, 1992). Whilst these programmes have varied in their methodology, essential principles for disease eradication have been adhered to. These principles, together with requirements for establishing whether eradication is a rational approach to a disease, have been outlined (Yekutiel, 1980; Thrusfield, 1986).

These requirements include adequate knowledge of the epidemiology of the disease (Thrusfield, 1986). The results from work described in Chapters 3 and 4 provide
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additional information on aspects of footrot relevant to control and eradication programmes. In this chapter, the principles of, and requirements for, instigating disease control and eradication programmes, and the implications of the results obtained in this study, are discussed in relation to State footrot programmes.

6.2. Terminology

The definition of, and differences between, control and eradication have been outlined (section 1.12.1). Eradication has been considered an aspect of control (Martin et al, 1990), and many of the principles and requirements for eradication programmes are equally applicable to disease control programmes. Therefore, reference to eradication programmes in this chapter will generally be applicable to control programmes. Comparisons between control and eradication programmes will be made where this is not the case.

State footrot programmes are either aimed at eradication (Western Australia) or a high level of control (New South Wales, Victoria and South Australia) on a State basis. In New South Wales, the programme is referred to as an eradication programme (Walker and Plant, 1994). All programmes are based on the eradication of footrot from individual flocks. Therefore, these programmes should satisfy requirements for eradication programmes, at least in part.
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6.3. **Principles and Requirements of Eradication Programmes**

The phases of an eradication programme have been described as follows:

1. Preparatory
2. Attack
3. Consolidation
4. Maintenance

(Yekutiel, 1980).

The *preparatory* phase involves training personnel and setting up appropriate facilities, rather than the preliminary epidemiological and economic studies. The *attack* phase is the period when the prevalence of the disease is dramatically reduced by the interruption of transmission of disease. This phase concludes when resumption of transmission in the absence of control measures is unlikely to occur. The *consolidation* phase involves intense surveillance to identify and eliminate any remaining foci of infection. The *maintenance* phase is a less intense surveillance phase, aimed at detecting and eliminating any introduced disease.

For control programmes, the first two phases would also be undertaken, but the intensive (and expensive) surveillance of the consolidation phase would not be necessary. Rather, there would be a maintenance phase aimed at maintaining the prevalence of disease below a defined level.

The need for an intensive consolidation phase where eradication is the objective may have important implications on which type of programme is most suitable for any particular disease. Clinical and epidemiological features are the primary decisive factor in the consolidation phase (Yekutiel, 1980), due to the need to detect and eliminate disease at low prevalence levels. The difficulty and cost of this phase has frequently been
overlooked in the decision to undertake eradication programmes. As the prevalence of disease decreases, the cost of detecting and eradicating infected units increases. For example, in the United States hog cholera eradication programme, the average cost of the programme was $13,578 per case in the middle of the programme, and $217,000 per case in the final 7 years (USDA, 1981, cited by Scnurrenberger et al, 1987). The cost and technical difficulties associated with the consolidation phase must be weighed against the higher costs in the maintenance phase for control programmes, where continued inputs at a higher level are required compared to those in the maintenance phase following eradication.

The requirements and preconditions for disease programmes which need to be considered when formulating control and eradication programmes include:

1. knowledge of the epidemiology, with the need for a simple, inexpensive tool completely effective in breaking transmission in eradication programmes
2. diagnostic feasibility, with favourable epidemiological features to facilitate effective case detection and surveillance in the advanced stages of an eradication programme
3. cost:benefit analysis, and consideration of producers' and community views, with the recognition of the socio-economic importance of the disease
4. availability of resources, including trained personnel, diagnostic and research facilities, disease-free replacement stock, and funding, with adequacy of administrative, operational and financial resources essential if eradication is to be implemented
5. public health significance
6. ecological consequences, and the absence of adverse factors on the ecology
7. suitable legislation

(Yekutiel, 1980; Thrusfield, 1986).
6. Implications for Regional Footrot Programmes

A further essential precondition in eradication programmes is the existence of a specific reason for preferring eradication over control (Yekutiel, 1980).

From the above, it is clear that for a disease eradication programme to be successful and appropriate, epidemiological, economic and social factors must be favourable. The expense and magnitude of such programmes requires not only the provision of adequate funding, but also the recognition by all parties of the importance of the disease, so that the commitment to the programme to achieve success will be maintained.

6.4. State footrot programmes

When the above factors for effective control and eradication programmes are considered in relation to current State footrot programmes, a number of deficiencies are apparent. These deficiencies can be mainly, but not solely, attributed to the complex nature of the expression of footrot, and the need to discriminate different forms of footrot.

6.4.1. Knowledge of epidemiology

Footrot eradication programmes have evolved from, and are still largely based on, the original 'Beveridge plan' (1941), when a simple relationship between the presence of D. nodosus and "footrot" was the prevailing scientific understanding. The recognition of benign footrot, and the rational decision to exclude this form of footrot from State programmes on epidemiologic and economic criteria, has resulted in the implementation of programmes which require the differentiation of two categories of the same disease. The further development in knowledge of the variability in expression of footrot has not
been paralleled by the understanding of differences in epidemiology which may exist between different forms of footrot.

The epidemiological basis for eradicating virulent footrot is firstly that *D. nodosus* associated with virulent footrot does not survive for prolonged periods, except in sheep' or goats' feet, and is not harboured by cattle. As a result, the removal of infected animals, or their effective treatment, will eliminate the disease. Secondly, that an infected animal can be detected clinically, allowing the identification of all infected animals. On these bases, virulent footrot has been eradicated on many occasions, over the past 55 years (see section 1.12.2).

Destocking has also been used as a means of eradicating footrot (Fitzpatrick, 1986). This strategy only relies upon the ability to identify disease free flocks, and the limited survival of *D. nodosus*. However, State footrot programmes are based on the assumption that eradication by inspection and culling is an effective option for footrot eradication.

The inclusion of intermediate footrot as a target for eradication assumes the epidemiological factors which allow eradication of virulent footrot apply equally to these less virulent forms of disease.

The eradication of a virulent strain and three of four intermediate strains from all replicates in this study (Chapter 4) was based on knowledge of the maintenance and transmission of virulent footrot, and suggests that at least some forms of intermediate footrot (and their thermostable *D. nodosus* isolates) can be eradicated using conventional techniques. The flocks of origin of the three intermediate strains would have all been considered infected with virulent footrot in Western Australia, and two of the three flocks (those from which strains E and H were recovered) would have been placed in quarantine in Protected and Control areas in New South Wales, Victoria and South
6. Implications for Regional Footrot Programmes

Australia. The third flock (the origin of strain G) would not have been quarantined in Victoria or South Australia. Its classification within New South Wales is not clear, but it appears likely that the disease would have been classified as virulent, given the presence of underrunning.

However, the failure to eradicate strain C suggests that not all strains of *D. nodosus* associated with intermediate footrot will be eradicated based on the removal of clinically affected sheep, and that epidemiological knowledge is inadequate for such strains. State programmes which include forms of footrot associated with strains of *D. nodosus* which are similar to those of strain C may therefore experience difficulties with the programme. The diagnosis of the footrot associated with strain C, based on the expression in Mob 2 during the surveillance phase, would have been virulent in Western Australia and South Australia and benign in Victoria. In New South Wales, it is probable that it would have been classified as virulent.

Further, there is evidence to suggest that strains of footrot which result in intermediate footrot (and would be considered virulent footrot in some States) exist in cattle (Stewart et al, 1984, 1986d; Mitchell et al, 1992; Trengove et al, 1993; R.Walker and I. Links, cited by Stewart and Claxton, 1993). The role of cattle as a reservoir for intermediate footrot was not examined in this study.

The technical feasibility of a disease control programme should be established prior to its commencement (Roe, 1990). The failure to do this in relation to less virulent forms of footrot, at least in the New South Wales, South Australian and Western Australian programmes, represents a deficiency in these programmes, particularly in light of the findings described in Chapter 4.
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6.4.2. Diagnostic feasibility

Diagnosis of diseases in eradication programmes have generally relied on tests which detect the presence or absence of the causative organism, rather than on clinical grounds (Mylrea, 1990, 1991; Newton, 1992), although the use of clinical criteria proved successful in Victoria with bovine pleuropneumonia (Newton, 1992). This is because of the problem of detecting carrier (infected but clinically normal) animals. However, these eradication programmes were based on the eradication of organisms from a single species (e.g. *Brucella abortus*), and diagnostic tests were only required to distinguish between the presence or absence of that species.

The decision to eradicate only certain forms of footrot has created specific diagnostic difficulties in footrot programmes, requiring diagnostic tests to further distinguish the form of disease present, and at the bacteriological level, to differentiate within the species of *D. nodosus*.

Elimination of footrot from a flock requires the elimination of specific *D. nodosus* strains, as it is the strain (or strains) of *D. nodosus* which are transmitted, and not necessarily the clinical expression. Only in the absence of those strains can footrot be considered eradicated. Diagnostic criteria in footrot eradication programmes will therefore need to consistently identify the presence or absence of specific strains of *D. nodosus*. 
6. Implications for Regional Footrot Programmes

The main criteria for classifying outbreaks of footrot in terms of the form of footrot are:

i. clinical assessment of the footrot in that flock, either under the prevailing environmental conditions, or at subsequent inspections timed to coincide with specific environmental conditions. Such assessment generally includes the prevalence and severity of disease, by comparing actual footscores present to pre-determined "cut-off" points for different forms of footrot (Anon., 1993; J. Tolson, pers. comm.). Sheep's feet may also be re-examined within 3-4 weeks to assess the further development of the disease.

ii. assessment of the in vitro characteristics of strains of *D.nodosus* recovered from sheep in the affected flock. Currently, the gelatin gel protease thermostability test (GGPTT) is used in all States for in vitro virulence testing of *D.nodosus* isolates (Anon., 1993; Anon., 1994; Walker and Plant, 1994).

Whilst both clinical assessment and in vitro virulence testing have been shown to distinguish different forms of footrot, or strains of *D.nodosus* which differ in their virulence, the failure to establish the relationship between diagnostic criteria and the economic impact of the disease or its eradicability mean that the usefulness of these criteria to eradication programmes is unknown. Further, the failure to adequately define the different forms of footrot which have been described, either by clinical or bacteriological criteria, has limited the ability to assess their usefulness. Results from this study suggest that the use of protease-based in vitro tests may not discriminate strains of *D.nodosus* adequately in terms of eradicability. Clinically, the strains which survived eradication were less severe, and this suggests that clinical criteria may be appropriate for determining which strains of footrot can or should be eradicated.
6. Implications for Regional Footrot Programmes

There are inherent limitations to both the diagnostic methods currently being used, in addition to the lack of information on their relationship to the eradicability or economic impact of different forms of footrot. Both environmental and sheep factors will influence the clinical expression of footrot (see 1.5.2). Clinical expression of single strain *D. nodosus* infections vary in different environments (Depiazzi et al, submitted). It is therefore likely that, on the basis of clinical assessment, flocks in one district (or within a district) will be assessed as having virulent footrot, whilst other flocks within the State will be considered free of virulent footrot (and therefore not quarantinable), despite all flocks having *D. nodosus* strains of a similar virulence present. This could result in the continued existence and possible transmission of footrot if sheep are moved to areas more favourable to footrot, or local conditions change.

The results from this study also raise the possibility that interactions may occur between different breeds of sheep with different *D. nodosus* strains, and interactions may occur between *D. nodosus* strains in multiple infections, which may alter the clinical expression. This would further decrease the ability of clinical criteria to identify adequately the presence of targeted strains of *D. nodosus*.

If culling of affected sheep has been used during the eradication programme, it is possible that the susceptibility of the remaining flock to footrot has been decreased, as the culled affected sheep were likely to be the more susceptible sheep, particularly if control measures during the footrot transmission period were minimal, or based on vaccination (Raadsma et al,1990). The clinical expression of footrot, if present, may therefore differ in the flock following an eradication programme, making diagnosis of footrot, or the form of footrot, more difficult.

The use of *in vitro* virulence tests to determine the type of footrot present also presents difficulties. As the clinical expression of infection with the same *D. nodosus* strain may vary between flocks, flocks with different clinical severity are likely to be
placed in quarantine if *in vitro* characteristics of *D. nodosus* are used as the basis for quarantine. The imposition of such eradication programmes may not appear justified to some producers, where expression of the particular infection is milder. Controversy over disease programmes may occur when producers who have not been experiencing all the costs associated with a disease are forced, through legislation, to take action over the disease (Hanson, 1983). With footrot, lack of clinical disease associated with specific targeted strains in a producer's flock may cause the producer to be unwilling to take action, or resent taking action, unaware of the impact of the same disease in other flocks in the same or other regions. Thus, support for the footrot eradication may be lacking, both at the farm level and for the State programme, with producers feeling unfairly treated. This will be particularly so if no compensation is available.

Furthermore, the results in this study raise the possibility that the clinical expression of the disease may reflect the eradicability of that disease. If this is true, the eradicability of strains of *D. nodosus* may vary in different environments, so *in vitro* characteristics may not relate adequately to eradicability.

The basis for choosing appropriate *in vitro* characteristics for eradication purposes will be the relationship between the *in vitro* characteristics and clinical expression of disease, as it is the severity of clinical expression which is likely to influence the economic impact of the disease, an important criterion for justifying eradication programmes. Whilst protease thermolabile strains appear to be associated with benign footrot, the correlation with severity of disease and protease thermostability appears poor (see section 1.6.2.5). Therefore the GGPTT would appear an appropriate test for excluding flocks from the need for eradication, but not for using as a test to determine that a flock should be quarantined in a State footrot programme.

In addition, protease thermostable strains have been recovered from cattle (Stewart et al, 1984, 1986d; Mitchell et al, 1992; Trengove et al, 1993; R.Walker and I.
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Links, cited by Stewart and Claxton, 1993). One of these strains resulted in intermediate footrot in challenged sheep (Stewart et al, 1984). As cattle are excluded from quarantine in footrot programmes, it is important that criteria used to assess the strains of *D. nodosus* present within a flock do not include strains which may be harboured by cattle.

In eradication programmes, the importance of effective case detection and surveillance has been stressed (Yekutiel, 1980). The existence of strain C in the absence of clinical disease (Mob 2, Inspection 11, Chapter 4) is a further indication that the inclusion of such strains in State programmes may not be advisable. Such carrier animals have been associated with strains of *D. nodosus* or outbreaks of footrot which would have been classified as intermediate or benign in this study (Glynn, 1993; Depiazzi et al, submitted). Their existence, assuming they could subsequently be associated with the transmission of footrot, would not facilitate effective case detection or surveillance, either at the farm or regional level. Such infections would not be detected by clinical criteria, and are also unlikely to be detected using laboratory tests under the current programmes, as samples for bacteriology are generally only submitted from sheep with lesions. Large scale sampling of normal feet is not carried out.

At present, States where control is the objective generally identify infected flocks on the basis of the clinical expression within the affected flock. The limitations with clinical assessment outlined above may be less important where State control is the objective, and could be minimised by an effective traceback monitoring system, particularly based on the clinical expression of footrot in higher rainfall regions of a State (see below). This may be cheaper and more generally acceptable than eradication.
6. Implications for Regional Footrot Programmes

6.4.2.1. Method of detection of affected flocks

A further difficulty with the detection of affected flocks in current State programmes is the reliance on detection and reporting of disease by owners. At present, tests are generally applied to flocks following the report by the owner of suspicion of footrot. Thus, State programmes rely on producer recognition of disease within a flock as the initial means of disease detection. This is in contrast to some previous disease eradication programmes (Mylrea, 1990, 1991; Newton, 1992), where tests were applied to all herds, irrespective of the perceived presence of disease in the herd. Only in Western Australia is an active surveillance programme utilised to detect non-notified outbreaks of footrot (A. Mercy, pers. comm.). Results from the survey described in Chapter 3, carried out in an environment considered favourable for the expression of footrot, suggest that producers may be unaware of the presence of footrot, particularly the less severe forms. In areas less favourable for footrot, it may be expected that producers are unaware of forms of footrot that are currently targeted by State footrot programmes. Furthermore, owners may be reluctant to report disease when its presence results in quarantine of the property (Newton, 1992; More, 1993), and especially where the disease in their view is of low importance.

Mechanisms to trace the movement of affected sheep (traceback or traceforward) exist in State footrot programmes (R. Walker, pers. comm.). These mechanisms include assessing the footrot status of flocks which are suspected of being the source for footrot outbreaks (traceback) and assessing the status of flocks which have purchased sheep from properties subsequently found to be affected (traceforward). Such mechanisms may also allow additional detection of disease, particularly where movement of sheep into areas which are favourable for the expression of footrot is monitored. This monitoring will be most useful where clinical assessment is the applied test, as problems with lack of expression of footrot in some areas may be minimised. However, in New South Wales, the effectiveness of such tracing mechanisms may be limited, as
surveillance is being directed towards low prevalence districts (Control and Protected Areas), where conditions are less favourable for the expression of footrot. Conversely, higher rainfall areas associated with high prevalences of footrot do not receive the same level of surveillance, nor is the reporting of the detection of footrot mandatory in Residual areas (R. Walker, pers. comm.).

The reliance on the recognition and reporting of disease by the producer, rather than the application of tests to all flocks, in the absence of appropriate tracing and assessment of sheep associated with footrot outbreaks, limits the level of compliance with State footrot programmes. It may also lead to a perception of unfair treatment by those producers who report the presence of footrot and are quarantined, whilst flocks with similar forms of the disease go undetected.

The recognition of footrot in transferred sheep more than a few weeks after movement inevitably leads to argument about the origin of the disease. Clearly there is a need for methods which enable the accurate identification of strains involved. Based on the findings in this study, HpaII fingerprinting (Chapter 5) may provide additional information on strains in such cases, particularly as fingerprints for unrelated outbreaks were unique. The need to identify all strains present and therefore sample sheep extensively is a limitation, at present, to the use of this method for tracing the source of new outbreaks.

6.4.3. Economic and social importance

Previously major disease programmes have generally been directed at the control or eradication of zoonoses, and have probably been justifiable solely on their human health benefits (Roe, 1990). However, as footrot is not a zoonosis, the economic justification will be largely dependent on the consequences of the disease within flocks.
The increasing importance of carefully evaluating the economic outcomes of disease has been recognised (Roe, 1990; Scott Orr, 1990).

Whilst virulent forms of footrot have been shown to have adverse effects on production (Marshall et al, 1991a) and justify eradication programmes (Egerton, 1989a), there have been no measurements of the effects of benign or intermediate footrot on production to justify their inclusion or exclusion from eradication programmes. Furthermore, the adverse affects reported by Marshall et al (1991a) were derived from an outbreak of footrot in Merino sheep induced with a single virulent strain.

There is no doubt producers consider virulent footrot an important disease, and the wide support by producers for footrot programmes has been demonstrated by the formation of almost 300 Footrot Groups in New South Wales (R. Walker, pers. comm.). Support for eradicating less virulent forms of footrot is difficult to assess, as it is unlikely that, in general, producers appreciate the different impacts of the different forms of footrot, particularly those producers who have had no experience with intermediate forms of footrot. Nor is there much appreciation of the difficulties associated with eradicating footrot and maintaining freedom from it.

In north western New South Wales, economic and social factors have been identified as the main constraints in the application of the Footrot Strategic Plan (More, 1993). The lack of economic impact of footrot in drier areas, and the lack of compensation or perceived financial reward from eradicating footrot has resulted in a lack of commitment to the programme by owners in this region (More, 1993). It might therefore be assumed that such problems will occur with less severe forms of footrot, even in areas more favourable for the expression of footrot.
6. Implications for Regional Footrot Programmes

6.4.4. Ecological consequences

The benefits from footrot eradication assume freedom from all forms of footrot, with estimates of effects on productivity of virulent footrot being compared with disease free sheep. However, results from the survey in this study suggest that in higher rainfall areas at least, flocks which eradicate virulent (or intermediate) footrot will remain or become infected with benign footrot. This is not the outcome producers are likely to expect from an expensive eradication programme. The presence of benign footrot may decrease the benefit of footrot eradication, particularly in the case of less severe forms of footrot, where the difference clinically and economically between the eradicated form and benign footrot may be small.

6.4.5. Further comments

The availability of suitable tests, the payment of compensation, the use of a tail-tag identification system to allow effective abattoir monitoring and tracing of infected stock, the restriction of movement of stock, and the division of States into disease control areas have been identified as important to the success of previous eradication programmes (Mylrea, 1990, 1991; Newton, 1992). Only the last two of these strategies appears to have been utilised in current State footrot programmes.

6.4.6. The inclusion of less virulent forms of footrot in eradication programmes

From the above comments, it may be seen that both the need to differentiate between forms of footrot and the inclusion of less severe forms of footrot in State eradication programmes may have decreased the effectiveness of the programmes. There have been no published attempts to more clearly define intermediate footrot and evaluate or quantify the economic impact of this form of the disease to justify its inclusion as a target for State eradication programmes. The justification for including less severe forms
of footrot is not clear, especially as production effects have been estimated as 'nil to moderate' for intermediate forms of footrot (Anon., 1988; Anon., 1993). A possible explanation for including intermediate footrot may include the fact that the clinical expression of intermediate footrot in at least some sheep in the flock is typical of virulent footrot, and therefore the disease may have some economic impact as well as welfare implications. It has also been suggested that producers were in favour of the inclusion of intermediate footrot in the category of virulent footrot (R. Walker, pers. comm.), although this assumes that producers have a thorough knowledge of the implications and outcomes associated with intermediate footrot, an assumption which is almost certainly false, given the lack of scientific evidence or knowledge.

As intermediate strains are generally protease thermostable (Stewart and Claxton, 1993), they have been automatically included in the Western Australian footrot eradication campaign. Less virulent strains of *D. nodosus* are believed to be the predominant protease thermostable strains in Western Australia (Montgomery, 1994). However, the mild nature of some outbreaks of footrot which result in quarantine in Western Australia has been attributed to the "low expression" of potentially more virulent strains (Montgomery, 1994). The presence of protease thermostable strains has not been related adequately to the economic impact of the disease associated with them, or its eradicability.

On the criteria outlined in this chapter, there appears to be little justification for the inclusion of some forms of footrot in State footrot programmes. The lack of information on their eradicability, the possibility that their economic impact is low, the difficulty in detecting their presence, and the possible role of cattle as a reservoir all mitigate against their inclusion. The exact definition of which forms of footrot to include or exclude in State footrot programmes was not an objective of this study. Evaluation of the economic impact of different forms of footrot is critical before such decisions could be adequately made. However, on the basis of the results of this study, it would appear
that the use of the GGPTT is only appropriate for excluding those outbreaks associated with protease thermolabile strains.

A recommendation to exclude certain forms of footrot, or strains of *D. nodosus*, from State footrot programmes does not mean that such forms of footrot should not be eradicated or cannot be eradicated. Indeed, removal of regulation will allow the owner to make decisions appropriate for that property. Results from this study suggest that eradication of some strains of footrot which may be considered benign in some States may be possible in areas which favour the expression of footrot. Where owners do not consider the disease is severe, it is likely that a high level of control will be achieved by footbathing sheep for short periods of the year, the approach which is adopted to deal with benign footrot.

Whilst at the farm level the eradication of footrot may be the preferred option for intermediate footrot, it is difficult to justify the additional costs of quarantine and the difficulties associated with the identification of infected flocks associated with less virulent forms of footrot. Further investigations will be necessary to demonstrate that eradication at the regional level of less virulent forms of footrot is justifiable, and the requirements for undertaking an eradication programme outlined above can be met.

The fact that existing programmes are based on research with fully virulent footrot does not necessarily mean that the criteria for deciding which strains of *D. nodosus* are to be eradicated, and the method for detecting affected properties, are incorrect. It may be shown that protease-based tests provide the most appropriate distinction between *D. nodosus* strains which, for the community benefit, are worthwhile eradicating. It may also be shown that the single protease thermostable strain that persisted despite eradication of other *D. nodosus* strains in this study was a rare occurrence. However, the apparent lack of consideration of the eradicability of strains to date by those authorities concerned with the development of State footrot control or
6. Implications for Regional Footrot Programmes

eradication programmes suggests that variation in eradicability of *D. nodosus* strains has not been an important criterion in decision-making.

6.5. Conclusions

The need to distinguish different forms of footrot, and the inclusion of less virulent forms of footrot, in State footrot programmes, particularly in New South Wales, Victoria and South Australia, have resulted in programmes which are generally difficult to justify, particularly given the possibilities raised in this study. If the hypothesis put forward in Chapter 4, that the persistence of the disease following an eradication programme is related to the clinical expression of the disease, then the inclusion of all forms of intermediate footrot with virulent footrot for State eradication programmes is not justifiable.

The eradicability of strains of *D. nodosus* which differ in virulence appears not to have been investigated or considered as a factor in which forms of footrot, or which strains of *D. nodosus*, to target in State footrot programmes. Given the results of this study, further evaluation of the eradicability of various strains of *D. nodosus* is essential to allow a sustainable and supportable regulatory footrot programme to be implemented.

Further, the lack of information on the economic impact of the various forms of footrot or on the role of cattle as a reservoir for intermediate strains, and the reliance on owner notification are further difficulties associated with the inclusion of intermediate footrot in State footrot programmes.

The different forms of footrot have not been adequately defined, nor have the clinical or laboratory criteria used for differentiating forms of footrot been related to the economic impact or the eradicability of the disease. An adequate definition for forms of
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footrot which should be targeted for eradication will rely on the establishment of the relationship between *D. nodosus* strains and the clinical disease resulting from infection, and the ability to distinguish *D. nodosus* strains on this basis. Such definition is essential if eradication programmes in practice are to be properly formulated and evaluated.
7.1. Conclusions

Despite the existence of State footrot programmes, there are still many flocks with footrot. This is particularly so in high rainfall areas, where the impact of disease will be greatest. If progress against footrot is to continue, it will rely on the demonstration to farmers in these regions that footrot can be eradicated and that such a course is of economic benefit. It will also rely on the application of regional programmes based on adequate knowledge of the forms of footrot, and the appropriate distinction of the forms which justify inclusion in these programmes.

From a review of the literature it is apparent that the principles of current eradication programmes for all forms of footrot were derived from Beveridge's original studies with virulent footrot, when a simple relationship between the presence of a single species (D. nodosus) and the occurrence of disease was the current understanding. The development in knowledge on the aetiology and pathogenesis of footrot, and the recognition of different forms of footrot, have not been accompanied by an evaluation of the appropriateness of such eradication programmes to less severe forms of footrot. Further, the epidemiology and economic impact of these forms of disease have not been assessed, although the likelihood that these differed for benign and virulent footrot has been suggested.

The lack of adequate quantitative definitions of different forms of footrot was also identified as a major limitation to collecting information relevant to the development of footrot control and eradication programmes. The inconsistent definition has lead to
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different criteria being applied to the diagnosis of footrot in the numerous State footrot programmes. Further, the ability of the current method of characterising *D. nodosus in vitro*, the GGPTT, to define virulent footrot adequately was questioned, due to the association of protease thermostable isolates with all forms of disease.

This study was therefore undertaken to provide additional information relevant to both farm and regional footrot programmes. The objective of this study was to investigate the epidemiology of less virulent forms of *D. nodosus*, and specifically the eradicability of strains of *D. nodosus*, thus providing additional information necessary for decisions to be made on the control and eradication of these forms of footrot. The study involved (1) a survey of flocks within a Voluntary Footrot Group, to establish eradication strategies used on properties, and the flock prevalence of different forms of footrot; (2) the establishment of multiple strains of *D. nodosus* in a large flock, and the assessment of control techniques and an eradication programme to this flock; and (3) the assessment of molecular biological techniques to identify strains of *D. nodosus* in epidemiological investigations.

In Chapter 3, the low success rate of footrot eradication programmes, and the time taken to achieve eradication from flocks, were identified as problems in footrot endemic areas and highlighted the costs associated with footrot eradication. This poor success was despite virulent footrot being the initial target of eradication in most flocks. Both the high flock prevalence, and the number of flocks which introduced sheep without necessarily taking adequate precautions to prevent the introduction of footrot, appeared to be additional impediments to footrot eradication in endemic areas.

Benign footrot was present in all flocks that had eradicated virulent footrot. It was further seen that owners, whilst recognising severe disease, were often unaware of mild forms of footrot. However, careful clinical diagnosis of footrot and the assessment of *D. nodosus in vitro* were highly correlated in the flocks surveyed.
In Chapter 4, a new technique, requiring the establishment of multiple characterised strains of *D.nodosus* within a flock, was used to investigate the eradicability of these strains. It was demonstrated that the eradication of strains of *D.nodosus*, based on conventional techniques of prevalence reduction and culling of affected animals, was variable. This variability appeared to be influenced by the clinical expression of disease associated with the strain. Thus, one virulent and three intermediate strains were eradicated. A benign strain and an intermediate strain persisted. It was hypothesised that the efficiency of detecting infected sheep by visual inspection decreased with milder disease.

Unexpectedly, the possibility was raised that interactions between strains of *D.nodosus* could alter the clinical expression of disease, and this was further confounded by possible sheep and microbial interactions. It was suggested that application of results derived from experiments using artificial challenge systems and single strain infections may not be appropriate in all cases. The prospect of controlling footrot by using specific benign strains of *D.nodosus* (niche filling) was raised.

In Chapter 5, recent but established molecular biology techniques were applied to *D.nodosus* isolates to further characterise them. Both ribotyping and PCR-RFLP fingerprinting provided additional information on the strains which were present despite the application of an eradication programme. PCR-RFLP fingerprinting was the most discriminatory, and provided additional strong information that the isolates recovered post eradication were identical to strains included as part of the initial infective mixture. This technique was rapid, discriminatory and reproducible, and therefore appears to have potential as a tool for epidemiological investigations. The frequency with which multiple strains of *D.nodosus* are associated with outbreaks of naturally occurring footrot means that at present the technique is limited by the need to characterise many isolates from an affected flock.
7. Conclusions and Recommendations

In the course of this further characterisation of isolates, a number of interesting results were obtained. These preliminary findings suggested interactions between strains at the genomic level, or changes within strains, may be occurring in *D. nodosus* more frequently than previously thought. Further investigations of these identified isolates may produce a better understanding of the epidemiology of *D. nodosus* infection.

The applicability of these findings to State footrot programmes was discussed (Chapter 6). It is clear that the inclusion of less virulent forms of footrot as targets for eradication in State footrot programmes needs to be justified objectively to industry. Benign footrot is currently excluded from all such programmes. However, the criteria used to define footrot in some States will almost certainly include footrot which is not readily distinguishable from benign footrot. Thus, in Western Australia, the targeting of all outbreaks of footrot associated with strains of footrot with thermostable proteases will almost certainly include outbreaks of otherwise benign footrot. Similarly, in South Australia, the inclusion of any disease with score 4 lesions as footrot may include essentially benign outbreaks. This also applies to New South Wales, where the presence of underrunning is a principal criterion of virulent footrot.

The urgent requirement for standard acceptable quantitative definitions of the forms of footrot, the need for additional economic data, and interpretation of these in relation to additional studies on the eradicability of different forms of disease is clearly required if footrot programmes are to continue to receive widespread grower support. Such support is essential for their success. Further, the difficulties in eradicating virulent disease in endemic areas must be addressed if footrot programmes are to meet their economic and animal welfare objectives.
7. Conclusions and Recommendations

7.2. Recommendations

The continued development in the understanding of footrot is an essential requirement if progress against this disease is to continue. Advances in the following areas are likely to provide the farming community with the most useful information:

1. The agreement on an acceptable definition for the different forms of footrot based on quantitative data. This definition must initially involve at least three forms of footrot, even though the simplest objective is to identify two forms - those to be eradicated and those not.

2. The further evaluation of methods for eradication of the different forms of footrot. This work should involve both detailed study of the eradication of \textit{D. nodosus} strains as well as elimination of clinical signs of disease. The use of techniques described in this thesis can be further applied to assist in these studies. The evaluation should also involve the rigorous assessment of flocks which have successfully eradicated virulent footrot, as such investigations could identify strains which persist despite eradication.

3. The accurate determination of the prevalence of the different forms of footrot and their economic impact.

4. Further investigation of the interactions which occur between strains of \textit{D. nodosus} in the same flock. This research should be directed towards (i) the effect of such interactions on clinical expression, (ii) the identification of benign strains which may control virulent strains, and (iii) the identification of factors which may inhibit (or enhance) the expression of footrot.
7. Conclusions and Recommendations

5. The development of *in vitro* tests which will help identify those strains of *D. nodosus* which are susceptible to eradication, and for which eradication may be rationally justified.


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APPENDIX I

SURVEY QUESTIONNAIRE

AI.1 Introduction

The Questionnaire sent to owners of properties in the Voluntary Footrot Group which was surveyed (Chapter 3), together with the covering letter, is set out in this appendix.

AI.2 Letter accompanying Questionnaire

28th October, 1992.

PROPERTY STATUS SURVEY, SPRING 1992

I will be commencing the property surveys on Monday, 9th November, and hope to have completed inspections on all properties by 10th December. The excellent spring to date has meant conditions have been ideal for the spread of footrot. This means that all types of footrot should be detected if present this year.

The aim of this spring survey is to determine the type of footrot present, if any, on each property. This will be based on the history of the flock, and inspection of approximately 400 sheep per property. Samples for laboratory testing will be collected.

I have enclosed a Questionnaire which will need to be completed. It will help if this Questionnaire can be returned to me before my visit. I can then check it and go through any queries with you.
The format of the property visits will depend on sheep numbers and previous problems, but will be along the following lines:

1. Discuss recent footrot history, purchases, sheep numbers.
2. Look at property map.
3. Inspect mobs in paddock.
4. Examine 400 sheep, collect samples.

This will take 4-8 hrs per property.

The sheep to be examined will depend on any problems/concerns you have had. Lambs that have been recently weaned (since September) would be the best sheep to examine, but this will vary from property to property. A mob or mobs with lame sheep which you are concerned about would also be useful. Remember, the aim is to find out what's going on, so this is a great opportunity to sort out any problems.

I am aware how hectic November is for all of us, and this will be an extra job, but this is the month to see if any footrot is present. I will endeavour to work in with your program, so contact me as soon as possible if you are going to have sheep in the yards, or want to fix a date.

I will contact you in the next week to organise a preferred time.

Bruce Allworth.
FOOTROT SURVEY
NOVEMBER 1992

QUESTIONNAIRE

Please complete all relevant questions. Circle the appropriate response (or responses) where applicable.

1. What is your current footrot status in your sheep?
   - virulent footrot
   - an intermediate type of footrot
   - benign footrot
   - no footrot
   - dont know/unsure
   - no sheep/goats

2. What is the footrot history of the property over the past 5 years?
   - free of footrot (approx. number of years___________)
   - Accredited Free (first year Accredited___________)
   - virulent footrot eradicated (state year______)
   - an intermediate type of footrot eradicated (__________)
   - infected as stated in Question 1.

Comments:

__________________________________________________________

__________________________________________________________

__________________________________________________________

__________________________________________________________
3. If footrot has been present on the property, state source of footrot:

- purchased sheep / rams
- neighbour's sheep/goats
- travelling stock
- always present
- unknown
- other ________________________________________________

Comments:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

4. Have you purchased sheep/goats in the last 5 years? **YES**  **NO**

If YES, indicate approximate details:

<table>
<thead>
<tr>
<th>Number</th>
<th>Years</th>
<th>Source</th>
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<tr>
<td>Ewes</td>
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<td>Wethers</td>
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<td>Rams</td>
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<td>Goats</td>
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Comments:
________________________________________________________________________
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5. What precautions were taken to avoid footrot when introducing sheep?

- footbathed off the truck
- kept isolated (state for how long ________________)
- inspected all sheep's feet
- inspected lame sheep
- purchased from a known footrot free property
- vaccinated with footrot vaccine
6. If you have eradicated footrot, or attempted eradication, what methods did you use?  
(Answer for the most recent year eradication attempted)

a. SPRING

Vaccination
Footbathing - 5% formalin
Footbathing - 10% zinc sulphate
Footbathing - Footrite
Footbathing - 20% zinc sulphate "home brew"
Footparing
Nil

Other ________________________________

b. SUMMER

Inspect and cull infected sheep
Inspect and treat infected sheep
Cull whole mob/flock
Treat affected sheep with antibiotic
Pare affected sheep
Footbathing only

Other ________________________________

c. NUMBER OF SUMMER INSPECTIONS

1
1-2
2-3
3-4
more than 4

d. HOW LONG DID ERADICATION TAKE?

1 year
2 years
3 years
more than 3 years (state number of years_____)
not successful
7. Do you have cattle (own or agist)? YES NO
If YES, state approximate number _______________________

8. List sheep/goats on hand at 30/10/92
   Ewes _______________________
   Wethers _______________________
   Weaners _______________________
   Lambs _______________________
   Rams _______________________
   Others _______________________
   Goats _______________________
State breed of sheep _______________________

9. Have you undertaken any footbathing/vaccination/antibiotic treatment of sheep or pared sheep's feet this winter/spring? YES NO
If YES, complete table

<table>
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<tr>
<th>MOB</th>
<th>TREATMENT</th>
<th>REASON</th>
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Appendix 1
10. Property Visit

Please indicate if there is a specific day or week that would suit you best for the property visit, the reason, and whether you have a sheep handler for inspecting sheep's feet.

Best Time for Property Visit: ________________________________

Reason: __________________________________________________________________

Sheep handling equipment: YES NO

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE. PLEASE ADD ANY ADDITIONAL COMMENTS IN THE SPACE BELOW.

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________

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