Molecular epidemiological confirmation and circumstances of occurrence of sheep (S) strains of *Mycobacterium avium* subsp. *paratuberculosis* in cases of paratuberculosis in cattle in Australia and sheep and cattle in Iceland

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**Abstract**

Distinct strains of *Mycobacterium avium* subsp. *paratuberculosis* with a tendency to segregate in either sheep, or cattle and other ruminants, have been described and are known as S and C strains, respectively. These strains can be distinguished by a polymorphism in the IS\textsuperscript{1311} element and other DNA-based methods. C strains are relatively easy to culture from tissues and faeces of animals with paratuberculosis but S strains are difficult to culture. A retrospective survey of archival formalin-fixed paraffin-embedded tissue samples from culture negative Australian paratuberculous cattle was undertaken to determine whether infection in these cases was due to S strains. Polymerase chain reaction and restriction endonuclease analysis of the amplified product was used to identify the polymorphism in IS\textsuperscript{1311}. Three cases of bovine paratuberculosis due to S strain were confirmed from three different farms. A serological survey led to the identification of a further two cases on one of these farms. S strains were also identified in archival tissues from paratuberculous sheep and cattle from Iceland, confirming epidemiological and microbiological evidence that paratuberculosis in Iceland was due to S strain following importation of infected sheep from Europe. In each bovine case in both Iceland and Australia there had been direct or indirect contact of calves with paratuberculous sheep. We were unable to determine whether S strains had established endemic infection in cattle or whether repeated infection from sheep had occurred. Limited epidemiological
evidence suggests that transmission of S strains to cattle in Australia has been uncommon under extensive grazing conditions. In Iceland, different husbandry practices appear to have favoured transmission of S strains to cattle. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Paratuberculosis or Johne’s disease is a chronic enteropathy of animals caused by Mycobacterium avium subsp. paratuberculosis. It has a global distribution and affects many species of farm livestock as well as free-living species. It was first reported in cattle in Australia in 1925 (Macindoe, 1950) and in extensively grazed sheep in Australia in 1980 (Seaman et al., 1981). It has since spread in sheep to over 400 farms in the worst affected region, New South Wales (Links et al., 1999). The origins of paratuberculosis in Australia are not possible to trace with any certainty; however, the island nation of Iceland has good records of the first introduction of the infection. This was in 1933 with a shipment of sheep from Europe, and resulted in clinical disease in local sheep from 1938, which spread to 440 farms by 1956. Cases were detected in cattle on the same farms from 1940, but these appeared to be less severe than cases in sheep (Pálsson, 1962; Fridriksdottir et al., 1999). Affected sheep flocks were slaughtered but it is thought that infected cattle acted as a reservoir of infection, resulting in the reinfection of sheep flocks (Fridriksdottir et al., 1999).

Control programmes for paratuberculosis have been developed in Australia to restrict the spread of the disease in sheep, cattle, goats and alpaca. The programme for eradication of ovine paratuberculosis requires destocking of all susceptible livestock (i.e. sheep, goats and deer), as all infected individuals cannot be detected with current tests, and then decontaminating the property over two consecutive summers. As the infection is spread mainly by the faecal–oral route and as observations suggest that ovine paratuberculosis is unlikely to transmit to cattle, a management strategy possible during the decontamination period is to graze the contaminated pasture with cattle. Calves appear to be more susceptible to infection with M. avium subsp. paratuberculosis than adult cattle (Larsen et al., 1975), so grazing with adult cattle is generally recommended. As discussed previously (Whittington et al., 2000) there is only limited evidence to support these assumptions. In contrast to the Australian situation, the control programme for paratuberculosis in Iceland is based on vaccination of lambs in endemic areas, and abattoir and serological surveillance (Fridriksdottir et al., 1999). Cattle are not vaccinated but there are movement restrictions on trade from endemic areas; a negative paratuberculosis complement fixation test or ELISA result is required prior to transport.

M. avium subsp. paratuberculosis consists of variants with limited genotypic diversity compared to other mycobacterial species. Using Southern blotting with IS900 as probe in restriction fragment length polymorphism analysis (RFLP), 28 variants have been identified using data obtained from digests with two restriction endonucleases (Pavlik
et al., 1999). However, these RFLP variants can be broadly grouped as sheep (S) and cattle (C) strains (Collins et al., 1990; Bauerfeind et al., 1996). In Australia, all isolates of *M. avium* subsp. *paratuberculosis* from sheep that have been typed by IS900 RFLP were S strains and most were strain S1 (Whittington et al., 2000). This is also the predominant strain causing ovine paratuberculosis in New Zealand (Collins et al., 1990). S strains are uncommonly associated with paratuberculosis in species other than sheep whereas C strains have been recovered from cattle and many other species with paratuberculosis (reviewed in Whittington et al., 2000). In Australia there are no published reports of paratuberculosis in cattle due to S strains and several molecular epidemiological surveys have failed to detect S strains in cattle (Cousins et al., 2000; Whittington et al., 2000). These findings are mirrored in historical and epidemiological observations of apparent lack of spread of paratuberculosis from sheep to cattle in Australia. The strains of *M. avium* subsp. *paratuberculosis* responsible for paratuberculosis in sheep and cattle in Iceland have not been determined because historical difficulty in culturing these organisms (Gunnarsson, 1979) led to culture not being undertaken for diagnosis. Paratuberculosis also occurs in goats and reindeer in Iceland and the strains of *M. avium* subsp. *paratuberculosis* involved are presumed to derive from sheep.

As control programmes for paratuberculosis in Australia assume imperfect technical knowledge the scientific basis for recommendations is openly tested wherever possible. This study was undertaken in order to test the hypothesis that S strains of *M. avium* subsp. *paratuberculosis* have not occurred in cattle in Australia. By applying molecular biological tests to archival specimens we now document the occurrence of S strains in cattle with paratuberculosis on several farms in New South Wales between 1989 and 1999. In addition, we provide the first molecular epidemiological evidence that paratuberculosis in populations of sheep and cattle in Iceland is caused by S strain. The significance of these findings for paratuberculosis control programmes in sheep and cattle is discussed.

2. Materials and methods

2.1. Study design

In Australia, retrospective evaluation of laboratory samples from cattle with paratuberculosis was undertaken. These were selected as follows. Culture media suitable for isolation of S strains of *M. avium* subsp. *paratuberculosis* have been developed only recently (Whittington et al., 1999a) but isolation of C strains from the tissues of cattle with paratuberculosis in Australia has been successful using Herrold’s egg yolk medium (HEYM) (Stephens, 1987; Whittington et al., 1999a). Thus, prior to 1998, negative culture results from cattle with histological lesions of paratuberculosis was signalment potentially consistent with infection by an S strain, although the significance of such results would not have been apparent at the time. Veterinarians were asked to recall examples of culture negative bovine paratuberculosis cases and a manual search for relevant laboratory records was undertaken to identify instances where cattle were detected with paratuberculosis by serology and histopathology but where culture of
intestinal or faecal samples for *M. avium* subsp. *paratuberculosis* had yielded negative results. In Iceland, culture of *M. avium* subsp. *paratuberculosis* from cases of paratuberculosis has been unrewarding (Gunnarsson, 1979) and is not routinely performed. Paraffin blocks containing tissues from sheep and cattle with paratuberculosis were therefore selected from laboratory archives in Iceland and referred to Elizabeth Macarthur Agricultural Institute for testing.

2.2. **Serological investigation in cattle herds with exposure to ovine paratuberculosis**

The status of adult cattle that had been exposed as calves to sheep with ovine paratuberculosis was investigated in New South Wales in 1997 in herds that satisfied the following criteria: paratuberculosis was present in sheep when cattle were <6 months old, the cattle were run as calves with sheep or on pasture contaminated by sheep, the cattle were over 2 years old at the time of sampling, sampling was undertaken to detect 2% prevalence with 95% confidence assuming sensitivity of 40% for the bovine paratuberculosis absorbed ELISA (Cannon and Roe, 1982; MacDiarmid, 1988).

2.3. **Serology**

Blood samples were collected from the tail vein, allowed to clot at room temperature and serum was tested in duplicate using a bovine paratuberculosis absorbed enzyme-linked immunosorbent assay (ELISA) for antibodies against *M. avium* subsp. *paratuberculosis* (Stephens, 1987). A ratio >2 between the optical density of the test serum and the negative control serum after absorption with *M. phlei* is regarded as positive (Yokomizo et al., 1985). A ratio >1.5 and ≤2 is regarded as inconclusive.

2.4. **Histopathology**

Intestinal tissues and associated lymph nodes were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin and by a Ziehl Neelsen method (Luna, 1968). The presence of granulomatous enteritis and/or lymphadenitis associated with intracellular acid fast bacilli was the basis of histological diagnosis.

2.5. **Culture**

Prior to 1998 tissues and faeces were cultured using HEYM (Anon., 1972). After 1998 faecal samples and samples of intestine and mesenteric lymph nodes were cultured in a radiometric system (BACTEC) and on modified 7H10 agar (Whittington et al., 1998, 1999a). Culture of samples from Iceland was not attempted.

2.6. **Identification and strain typing of *M. avium* subsp. *paratuberculosis***

DNA was extracted from thin sections cut from formalin-fixed paraffin-embedded tissues and *M. avium* subsp. *paratuberculosis* was identified using IS900 PCR–REA
(primers 150C/921) and IS\textsubscript{1311} PCR–REA (primers M56/M94) as previously described (Marsh et al., 1999; Whittington et al., 1999b). Identification and strain typing of isolates of \textit{M. avium} subsp. \textit{paratuberculosis} from BACTEC radiometric medium was achieved using the same approach but with different primer sets (P90/P91 and M56/M119). Criteria for identification of acid fast bacilli in tissue sections as \textit{M. avium} subsp. \textit{paratuberculosis} were detection of IS\textsubscript{900} PCR product of predicted size and fragment pattern after restriction analysis with \textit{Mse} I. Additional criteria for typing as \textit{S} or \textit{C} strains were detection of IS\textsubscript{1311} PCR product of predicted size and fragment pattern after restriction analysis with \textit{Hinf} I (Fig. 1).

2.7. Nucleotide sequencing

The 229 bp products from PCR reactions with primers 150C/921 were purified using 6 M guanidine thiocyanate and silica columns according to the manufacturer’s instructions (Wizard PCR Preps DNA Purification System; Promega Corporation, Madison, WI) with elution of purified DNA in 50 µl of sterile distilled water. Dye terminator sequencing reactions were undertaken with 300 ng DNA and 4.8 pmol of primer using Terminator Ready Reaction Mix on a robotic workstation (Catalyst 800 Molecular Biology Labstation, Perkin Elmer Applied Biosystems, Foster City, CA) and were analysed using a 377 DNA Sequencer (Perkin Elmer). The forward and reverse strands of the PCR products were sequenced using primers 150C and 921 and sequence was accepted for alignment only where complementary data were obtained from the two strands. Sequences were edited, aligned and compared with the known sequence for IS\textsubscript{900} (Green et al., 1989) after removing the primer regions, using the programme ESEE (Eric Cabot, University of Rochester, NY).
3. Results

3.1. Retrospective examination of animal tissues held in laboratory archives in Australia

Four cases (1–3 and 6) from the period 1989–1995 were found where cattle with confirmed paratuberculosis lesions had negative culture results from intestinal tissues and/or faeces. These cases were from farms in the Central Tablelands region of New South Wales, an area where ovine paratuberculosis is endemic and common. An additional two cases (4 and 5) were found on one of these farms in 1999 after serological investigation (see Table 1).

S strain *M. avium* subsp. *paratuberculosis* was confirmed by examining paraffin blocks from cases 1, 3 and 6 using IS900 and IS1311 PCR methods. Cases 4 and 5 were confirmed in 1999 by using these methods on cultured organisms after successful primary culture in modified BACTEC medium with subculture to modified 7H10 medium. Growth did not occur on subculture to HEYM, providing further evidence of strain identity. Case 2 predated appropriate culture methods for S strain and paraffin-embedded tissues from this animal were not retained in laboratory archives, so strain typing was not possible.

The identity of IS900 PCR product obtained from DNA extracted from paraffin-embedded tissues from Cases 1, 6 and a control bovine tissue from a previous study (Whittington et al., 1999b) was confirmed by nucleotide sequencing. Acceptable sequence was obtained over a length of at least 165 bases for each sample. Apart from a single base substitution (G for A) at the 216th base pair of the IS900 sequence in the sample from Case 6 and the control, the nucleotide sequence was identical to that for IS900.

The circumstances of infection of cattle with S strain *M. avium* subsp. *paratuberculosis* were assessed. This included revision of laboratory records for each farm. In each case there had been opportunity of contact between young cattle and paratuberculous sheep (Table 1). This was either direct contact by grazing the same pasture or indirect contact across a farm boundary fence. Farm A was destocked of cattle without further investigation after the detection of Case 1 in 1989 but it was possible to gauge the extent of paratuberculosis in cattle on other farms. Farm B1 had two positive and five inconclusive ELISA reactors among 370 cattle in 1993 and two inconclusive ELISA reactors (*n* = 168) in 1994. The nine reactors were culled from the herd but pathological examination was not undertaken. In 1993 Case 3 was thought to be bovine paratuberculosis with a false negative culture result, and at that time there were a further eight positive (ratios 7.5, 4.5, 2.2, 6.4, 11.5, 5.1, 4.4, 2.2) and 13 inconclusive ELISA reactors (*n* = 473). The positive reactors were culled but none had identifiable histological lesions of paratuberculosis and intestinal tissues were culture negative using HEYM. There were nine inconclusive ELISA reactors (*n* = 430) in 1994, no reactors (*n* = 460) in 1995, and one inconclusive reactor (*n* = 103) in 1996. All the seroreactors and their progeny were culled without laboratory examination. After retrospective identification of S strain *M. avium* subsp. *paratuberculosis* in Case 3 in 1998, serological investigation of cattle recommenced on Farm B2 in 1999 leading to the detection of seropositive Cases 4 and 5 (*n* = 422). Apart from Case 6, there were no other
Table 1

**Summary of cattle from Australia with paratuberculosis known or likely to be due to S strain *M. avium* subsp. *paratuberculosis***

<table>
<thead>
<tr>
<th>Case</th>
<th>Farm</th>
<th>Year</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical signs (reason detected)</th>
<th>Pathology</th>
<th>ELISA ratio</th>
<th>IS900 and IS1311 PCR</th>
<th>Contact of calves with paratuberculous sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>1989</td>
<td>Hereford</td>
<td>F</td>
<td>Aged</td>
<td>Chronic diarrhoea, weight loss</td>
<td>Severe granulomatous enteritis, AFB &lt;sup&gt;b&lt;/sup&gt; numerous</td>
<td>10.7</td>
<td>2/5 paraffin blocks positive</td>
<td>Yes. Cattle and 1000 crossbred ewes grazed 200 ha pasture. Paratuberculosis was detected in sheep in 1982, and caused moderate levels of mortality</td>
</tr>
<tr>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>B1</td>
<td>1993</td>
<td>Angus</td>
<td>M</td>
<td>5</td>
<td>Chronic diarrhoea, weight loss, recumbency</td>
<td>Severe granulomatous enteritis, AFB numerous</td>
<td>9.5</td>
<td>n.t.</td>
<td>Probable. Contact occurred on farm B2 between birth and 12–18 months of age when Case 2 was sold to farm B1</td>
</tr>
<tr>
<td>3</td>
<td>B2</td>
<td>1993</td>
<td>Angus</td>
<td>F</td>
<td>Mature</td>
<td>Nil (serology)</td>
<td>Mild granulomatous enteritis, AFB numerous</td>
<td>7.4</td>
<td>2/3 paraffin blocks positive</td>
<td>Probable. Although the 1100 sheep on farm B2 were not tested, sheep on two neighbouring farms had a high prevalence of paratuberculosis when tested in 1984</td>
</tr>
<tr>
<td>4 and 5</td>
<td>B2</td>
<td>1999</td>
<td>Angus</td>
<td>F</td>
<td>8 and 6</td>
<td>Nil (serology)</td>
<td>Focal granulomatous enteritis, AFB scant in 4, absent in 5</td>
<td>2.1, 2.3</td>
<td>Both positive from cultures</td>
<td>Probable. Both cases had indirect contact with neighbouring sheep flocks that had a high prevalence of paratuberculosis</td>
</tr>
<tr>
<td>6</td>
<td>C1</td>
<td>1995</td>
<td>Hereford</td>
<td>M</td>
<td>6</td>
<td>Chronic diarrhoea, weight loss</td>
<td>Mild granulomatous enteritis, AFB scant</td>
<td>3.4</td>
<td>3/3 paraffin blocks positive</td>
<td>Yes. Occurred on farm C2 between birth and 3 years of age when Case 6 was sold to farm C1. Paratuberculosis was diagnosed in sheep on farm C2 in 1996, with evidence that it had been present for many years</td>
</tr>
</tbody>
</table>

<sup>a</sup> Likely S strain case, tissue samples unavailable for confirmation.

<sup>b</sup> AFB: acid fast bacilli.

<sup>c</sup> Not tested.
Table 2

Cases of paratuberculosis in sheep and cattle from Iceland. The severity of microscopic lesions in the gut and the relative number of acid fast bacilli (AFB) in tissues are shown. The farms were located in the paratuberculosis endemic area and sheep were vaccinated

<table>
<thead>
<tr>
<th>Case</th>
<th>Year</th>
<th>Species</th>
<th>Lesion</th>
<th>AFB</th>
<th>IS900</th>
<th>IS1311</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1993</td>
<td>Sheep</td>
<td>Generalised</td>
<td>++++</td>
<td>MP^a</td>
<td>S^b</td>
<td>Two of 58 cull sheep from West Iceland confirmed with paratuberculosis based on gross examination at an abattoir and histological examination. Cattle and horses also present on farm but not assessed for paratuberculosis</td>
</tr>
<tr>
<td>2</td>
<td>1999</td>
<td>Sheep</td>
<td>Generalised</td>
<td>+++</td>
<td>MP</td>
<td>t.r.c</td>
<td>One of 15 cull sheep from North Iceland confirmed with paratuberculosis based on gross examination at an abattoir and histological examination. Paratuberculosis first diagnosed on farm in sheep in 1972. Cattle, goats and horses also present on farm but not assessed for paratuberculosis</td>
</tr>
<tr>
<td>3</td>
<td>1999</td>
<td>Sheep</td>
<td>Generalised</td>
<td>++++</td>
<td>MP</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1999</td>
<td>Sheep</td>
<td>Autolysis</td>
<td>–d</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1999</td>
<td>Sheep</td>
<td>Autolysis</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Dairy cow with clinical paratuberculosis from South Iceland. Sheep and horses also present on farm but not assessed for paratuberculosis</td>
</tr>
<tr>
<td>6</td>
<td>1992</td>
<td>Cattle</td>
<td>Multifocal</td>
<td>++++</td>
<td>MP</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1991</td>
<td>Cattle</td>
<td>Multifocal</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1992</td>
<td>Cattle</td>
<td>Autolysed</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

^a DNA consistent with *M. avium* subsp. *paratuberculosis*.

^b S strain.

^c Trace reaction, insufficient PCR product for restriction endonuclease analysis.

^d Negative result.
seroreactors on Farm C1 ($n = 160$) in 1995 and there was no serological evidence of paratuberculosis in cattle on Farm C2 in whole-herd testing in 1995 ($n = 780$), 1996 ($n = 559$) and 1997 ($n = 461$). The serological data suggest that paratuberculosis was infrequent in cattle on each farm.

3.2. Retrospective examination of animal tissues held in laboratory archives in Iceland

Eight blocks of paraffin containing tissues from five sheep and three cows with paratuberculosis were located in the archives at Department of Experimental Pathology, Reykjavik, Iceland. The sheep tissues were collected during routine abattoir surveillance for paratuberculosis while the cows were clinical cases. DNA consistent with *M. avium* subsp. *paratuberculosis* was detected in tissues from three of the sheep and one of the cows and was consistent with S strain in two sheep and the cow. In the remaining sheep strain typing could not be completed as tissue extracts gave a negative reaction in IS1311 PCR. Sheep and cattle were present on each farm (Table 2).

3.3. Serological investigation in cattle herds with exposure to ovine paratuberculosis in Australia

Cooperation was obtained from the owners of only three farms in the Central Tablelands of New South Wales where the criteria for the survey could be satisfied. Samples were tested from 426 cattle, comprising whole-herd samples from two farms ($n = 278, 97$) and a part herd sample from one other farm ($n = 51$). There was no serological evidence of paratuberculosis.

4. Discussion

The suspicion that an S strain of *M. avium* subsp. *paratuberculosis* might be the cause of several historical cases of paratuberculosis in cattle in New South Wales arose when it was realised that unsuccessful attempts to culture the organism from paratuberculous intestinal tissues of cattle were consistent with the involvement of an S strain. C strains of *M. avium* subsp. *paratuberculosis* are readily isolated on HEYM, the medium that had been used commonly in Australia, whereas isolation from sheep with paratuberculosis has not been routinely successful in Australia using this medium (Whittington et al., 1999a). Technology for strain typing from archival histological material was developed in 1998 (Marsh et al., 1999; Whittington et al., 1999b) and was applied soon afterwards to the bovine cases reported in this study.

It is difficult to be certain about the factors that led to the infection of cattle with S strain in Australia due to the time elapse since these events. However, there were two factors common to all cases: direct or indirect contact of cattle with paratuberculous sheep and affected cattle being calves when this contact first occurred. These factors are biologically plausible, particularly given the need for faecal–oral spread and the observation that calves are likely to be more susceptible to infection than older cattle (Larsen et al., 1975).
The rate of transmission of S strain from sheep to cattle on farms where it had occurred in Australia was not determined definitively; however, it did not appear to be high based on serological evidence. There was a series of negative whole-herd ELISA tests in cattle on Farm C2 between 1995 and 1997. On Farm B2 there were no clinical cases, only nine of 473 cattle reacted in the ELISA test and only one of nine reactors had histological lesions. It is not clear whether the eight histologically negative seroreactors were free of infection because the cultural method was not appropriate for infection due to S strain. Appropriate culture would be expected to be more sensitive than histopathology (Merkal, 1973; Koh et al., 1988). Furthermore, it would be unusual to obtain such a high number of false positive results in a bovine paratuberculosis absorbed ELISA, a test expected to have a specificity >99% (Collins and Sackett, 1993).

Whether initial infection of cattle with an S strain leads to endemic infection in cattle herds is also unknown. Subsequent serological tests on Farm B2 over three consecutive years after 1993 suggested absence of infection in remaining cattle but two new cases were detected by serological examination in 1999. Even though all sheep were removed from the farm in 1992, it was possible for infection of Cases 4 and 5 to have been newly acquired from a neighbour’s sheep by transmission across a fenced boundary.

There is evidence that the rate of transmission of S strain from sheep with ovine paratuberculosis to cattle was quite low in Australia, but this cannot be stated with certainty, and similarly the question of endemicity of S strain in cattle remains unanswered. There was potential for the affected cattle to spread the infection as the histological lesions in several were consistent with those seen in cattle known to be shedding \textit{M. avium} subsp. \textit{paratuberculosis} in faeces. A small serological survey did not reveal infection and broader scale surveys are indicated.

The Icelandic experience with paratuberculosis in sheep is relevant to current disease control programmes in Australia and elsewhere (Fridriksdottir et al., 1999). The predominant and traditional livestock farming method in Iceland involves mixed grazing of sheep with a small number of dairy cattle. Animals are housed for 8–9 months per year, the two species in separate facilities, but manure from both species is collected and used to fertilise the surrounding fields during the growing season. After lambing in spring, sheep graze these fields before being moved to graze common moorland and mountain pastures. Hay is then cultivated on the fields and cattle are later grazed on the stubble, to be followed by sheep when they return from mountain grazing. The use of manure as fertiliser and common grazing provides a mechanism for transmission of paratuberculosis, although in endemic areas the vaccination of sheep is thought to reduce this risk. The results of this study confirm epidemiological and bacteriological suspicions that the organism causing paratuberculosis in sheep is an S strain. This was transmitted from sheep to cattle which became a reservoir of infection and are believed to have infected healthy replacement sheep that were introduced one year after a destocking programme (Pálsson, 1962; Fridriksdottir et al., 1999). It is uncertain whether the infection is endemic in cattle or due to regular infection from sheep because cattle are commonly directly or indirectly in contact with sheep. A number of features of the epidemiology of paratuberculosis in Iceland are not applicable in Australia, for example, climate, winter housing and acid peat soils (Pálsson, 1962). However, mixed grazing of...
sheep and cattle is common to both systems and may represent a threat to disease control programmes if one species only is considered for control measures.

The results of this investigation provide reasonable evidence that strain differences in *M. avium* subsp. *paratuberculosis* do not influence the ability to diagnose the infection in cattle using conventional laboratory tests. Some cattle with S strain infection developed the usual clinical signs of paratuberculosis, all those studied reacted in the bovine paratuberculosis absorbed ELISA, had histopathological lesions typical of paratuberculosis in cattle, and after 1998 when appropriate culture methods were used, were confirmed as infected by culture of intestinal tissues.

Anecdotal evidence in Australia suggests that transmission of S strains of *M. avium* subsp. *paratuberculosis* from sheep to cattle has been uncommon but the results of this investigation confirm that such transmission has occurred several times. The frequency of transmission is uncertain, but contact of calves with infected sheep may have been required at least initially. Cattle are present on about half the farms known to have sheep with ovine paratuberculosis and further investigation will be required to disprove transmission from sheep to cattle and establishment of a reservoir of infection in cattle. The magnitude of this threat was assessed in Iceland with the benefit and wisdom of hindsight (Fridriksdottir et al., 1999). The risk is now being assessed in Australia in a larger survey.

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**References**


