Vet. Res. 38 (2007) 613–623
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DOI: 10.1051/vetres:2007013

Available online at:
www.vetres.org

Original article

Molecular genotyping of multinational ovine and caprine *Corynebacterium pseudotuberculosis* isolates using pulsed-field gel electrophoresis

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(Received 31 August 2006; accepted 21 February 2007)

Abstract – Caseous lymphadenitis (CLA) is a chronic, suppurative disease, with a worldwide distribution, caused by *Corynebacterium pseudotuberculosis*. The clinical manifestation of CLA is known to vary between different countries, and has been postulated to be due to differences in the strains present in these countries. Forty-two sheep and goat isolates of *C. pseudotuberculosis* from Australia, Canada, Eire, The Netherlands and Northern Ireland were characterized by pulsed-field gel electrophoresis (PFGE), biotyping, antimicrobial susceptibility, and production of phospholipase D. The PFGE-determined genotypes of this multicentric collection were then compared with representative ovine and caprine isolates from a previously published panel of PFGE profiles of United Kingdom isolates. Digestion with *Sfi*I generated 16–18 bands in the 48.5 and 290 kb range, and differentiated four distinct pulsotypes amongst the 36 ovine and 6 caprine strains which displayed remarkable homogeneity. Based on these results, it would appear that the genome of *C. pseudotuberculosis* is highly conserved, irrespective of the country of strain origin.

***Corynebacterium pseudotuberculosis* / antimicrobial susceptibility / PFGE pulsotypes / genome / caseous lymphadenitis**

1. INTRODUCTION

Corynebacterium pseudotuberculosis is the causative organism of a variety of chronic suppurative conditions and has been isolated from sheep, goats, cattle, horses, deer, buffaloes, camels, mules and more rarely man [1, 3, 6, 10, 26, 44]. The most significant disease syndromes are caseous lymphadenitis (CLA) in sheep and goats, ulcerative lymphangitis and contagious pustular dermatitis in horses, and ulcerative lymphangitis and mastitis in cattle and buffaloes [10]. Historically, two distinct biotypes of *C. pseudotuberculosis* have been recognized, mainly on the basis of their ability to reduce nitrate; generally, biovar ovis of sheep and goats is nitrate reductase-negative, and biovar equi of horses, cattle and buffaloes [9] is nitrate reductase-positive, although exceptions to this rule have been noted [12, 19, 44].

CLA is prevalent worldwide but its incidence is higher in areas where intensive animal husbandry is practiced [10, 17]. As European border controls have be-

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come less stringent and livestock are moved more freely between nations, countries previously free from CLA have reported outbreaks [20]. Significantly, CLA is a particular problem in Europe, the Southern hemisphere and Canada [4, 11, 20, 24, 27, 32–34, 36, 37].

Some authors have described apparent differences in the form and distribution of CLA lesions within the lymph nodes of sheep and goats in different parts of the world [7, 8, 10, 31, 43]. Such variation in the clinical manifestation of the disease has prompted speculation regarding biotype diversity, suggesting that there might be a European biotype which is distinctly different to those from other locations [38]. In Australia, CLA abscesses in sheep occur most commonly in the superficial lymph nodes of the shoulder and flank. In addition, lung abscesses are postulated to play the major role in transmission of the disease in that country, as *C. pseudotuberculosis* has been cultured from the tracheas of sheep with lung lesions and observed discharging into airways [15, 23, 37]. A similar presentation of CLA is recognized by sheep breeders in Canada, but there a visceral form of the disease, although rare in Australia, is regarded as one of the most common causes of ill thrift or "thin ewe syndrome" in adult sheep [29]. In contrast to the above, CLA in sheep in the United Kingdom most commonly manifests as lesions of the superficial lymph nodes of the head and neck [6], which is a distribution pattern more commonly associated with the disease in goats [10]. However, anecdotal reports indicate that a visceral form leading to chronic ill thrift may also be emerging. A potential difference has been previously reported in the morphological appearance of the abscess in UK sheep; a typical CLA abscess in sheep has a laminated, so-called "onion-ring" appearance [25], comprising alternating layers of fibrous tissue and caseated material, however, abscesses in UK sheep have been characterized by a thick pasty exudate with no internal structure, which is more similar to those seen in goat cases [5, 18]. However, some authors have suggested that this variation in consistency may simply be due to a maturation process [42].

In a recent review, the lack of efficient molecular tools for the genetic study of *C. pseudotuberculosis* and the requirement to develop molecular strategies to tackle this infection was highlighted [13]. Extensive variability in the cultural and biochemical characteristics of *C. pseudotuberculosis* has previously been reported [21, 35], and investigation of genotypic variation has been conducted using restriction endonuclease analysis (REA) and restriction fragment length polymorphism (RFLP) [39]. We have previously demonstrated the applicability of pulsed-field gel electrophoresis (PFGE) to the characterization of *C. pseudotuberculosis*, which demonstrated a clonal arrangement of ovine and caprine isolates in the UK and also differentiated horse isolates as being genetically distinct from the former. In order to extend our knowledge of the epidemiology of CLA, and establish the extent of genetic diversity of *C. pseudotuberculosis* at a multinational level, the present study was conducted to characterize a panel of sheep and goat isolates from Australia, Canada, Eire, The Netherlands and Northern Ireland on the basis of their biochemical, antimicrobial resistance and PFGE patterns, and compare these with representative isolates from the UK panel characterized previously.

2. MATERIALS AND METHODS

2.1. Bacterial strains, growth media and culture conditions

All bacterial strains used in this study, and the designations allocated for the purposes of our culture collection, are

Table I. Bacterial strains/isolates.

Bacterial Species	Strain designation/isolate number[a]	Description
C. pseudotuberculosis	5	Field isolate
	45 NCTC 3450	Type strain
	59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 103	Field isolates (n = 42)
C. ulcerans	NCTC 12077	
Staphylococcus aureus	NCTC 7428	
Streptococcus agalactiae	NCTC 8181	

[a] NCTC: National Collection of Type Cultures.

presented in Table I. A total of 42 *C. pseudotuberculosis* field isolates were randomly picked from different CLA outbreaks in different parts of the world; 13 ovine isolates (12 from Northern Ireland and 1 from Eire) were obtained from Frank Malone (Omagh, Northern Ireland), 4 ovine isolates were obtained from Jan Hamer (ID Lelystad, The Netherlands), 12 isolates (10 ovine and 2 caprine) were obtained from Dr John Prescott (Ontario Veterinary College, University of Guelph, Canada), and 13 isolates (9 ovine and 4 caprine) were obtained from Ms. Nicky Buller (Animal Health Laboratories, Western Australia Department of Agriculture, Perth, Australia). In addition, strains of *C. ulcerans* and *C. pseudotuberculosis* were purchased from the National Collection of Type Cultures (NCTC; PHLS, Central Public Health Laboratory, London, UK) for use as comparators throughout the biochemical and PFGE characterizations, and *Streptococcus agalactiae* and *Staphylococcus aureus* were purchased from NCTC for use in the CAMP inhibition test. All isolates were initially cultured on blood agar base (Oxoid, Basingstoke, Hampshire, England) supplemented with 5% (v/v) citrated sheep blood (blood agar; BA) and incubated at 37 °C for 48 h. Harvested cells were preserved at –70 °C in Microbank vials (Pro-lab Diagnostics, Bromborough, England), according to manufacturer's instructions. Isolates were passaged no more than six times from initial isolation to testing by PFGE.

2.2. Biotyping

All *C. pseudotuberculosis* isolates were passaged twice on BA, as described above, before biochemical characterization was conducted using the API Coryne biochemical assay (bioMérieux, Basingstoke, UK) according to manufacturer's instructions.

2.3. Antimicrobial susceptibility

Isolates were tested for antimicrobial susceptibility using the ATB-VET kit (bioMérieux). The antimicrobials used and the breakpoint concentrations were: amoxicillin (4 μg/mL), amoxicillin + clavulanic acid (4 + 2 μg/mL, respectively), apramycin (16 μg/mL), cefoperazone (4 μg/mL), cephalothin

Table II. Antimicrobial susceptibilities and PFGE typing profiles of *C. pseudotuberculosis* isolates.

MRI ref. #	Species of origin	Country of origin	Pulsotype	Antimicrobial agent[a]					
				STR	KAN	GEN	APR	SUL	PRI
5	Ovine	UK	**P3**	**R**	S	S	S	**R**	S
45	Ovine	South America	**P4**	S	S	S	S	**R**	S
59	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
60	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
61	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
62	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
63	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
64	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
65	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
66	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
67	Ovine	Eire	**P4**	**R**	**R**	**R**	**R**	S	S
68	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
69	Ovine	Northern Ireland	**P4**	**R**	**R**	**R**	**R**	S	S
70	Ovine	Northern Ireland	**P2**	**R**	**R**	**R**	S	S	S
71	Ovine	Northern Ireland	**P2**	**R**	**R**	**R**	S	S	S
72	Ovine	The Netherlands	**P2**	**R**	**R**	**R**	S	**R**	S
73	Ovine	The Netherlands	**P2**	**R**	**R**	**R**	S	**R**	S
74	Ovine	The Netherlands	**P2**	**R**	**R**	**R**	**R**	**R**	S
75	Caprine	Canada	**P2**	**R**	**R**	**R**	S	**R**	S
76	Ovine	Canada	**P4**	**R**	**R**	**R**	**R**	**R**	S
77	Ovine	Canada	**P2**	**R**	**R**	**R**	**R**	**R**	S
78	Ovine	Canada	**P4**	**R**	S	**R**	S	**R**	S
79	Ovine	Canada	**P4**	**R**	**R**	**R**	S	**R**	S
80	Ovine	Canada	**P2**	**R**	**R**	**R**	**R**	**R**	S
81	Ovine	Canada	**P2**	**R**	**R**	**R**	S	**R**	S
82	Ovine	Canada	**P2**	**R**	**R**	**R**	**R**	**R**	S
83	Caprine	Canada	**P4**	**R**	S	S	S	**R**	S
84	Ovine	Canada	**P4**	**R**	**R**	**R**	**R**	**R**	S
85	Ovine	Canada	**P4**	**R**	**R**	**R**	**R**	**R**	S
86	Ovine	Canada	**P4**	**R**	**R**	**R**	S	**R**	S
87	Ovine	Australia	**P4**	**R**	**R**	**R**	S	**R**	S
88	Ovine	Australia	**P4**	**R**	**R**	**R**	S	**R**	S
89	Ovine	Australia	**P3**	**R**	S	S	S	**R**	S
90	Ovine	Australia	**P4**	S	S	S	S	**R**	S
91	Ovine	Australia	**P4**	**R**	S	S	S	**R**	S
92	Ovine	Australia	**P5**	**R**	S	S	S	**R**	S
93	Ovine	Australia	**P4**	**R**	**R**	**R**	S	**R**	S
94	Ovine	Australia	**P4**	**R**	S	S	S	**R**	S
95	Ovine	Australia	**P4**	S	S	S	S	**R**	S
96	Caprine	Australia	**P4**	**R**	**R**	**R**	S	**R**	S
97	Caprine	Australia	**P4**	**R**	**R**	**R**	**R**	**R**	**R**
98	Caprine	Australia	**P4**	**R**	**R**	**R**	**R**	**R**	S
99	Caprine	Australia	**P2**	**R**	**R**	**R**	**R**	**R**	S
103	Ovine	The Netherlands	**P2**	**R**	**R**	**R**	S	S	S

[a] STR, streptomycin; KAN, kanamycin; GEN, gentamicin; APR, apramycin; SLF, sulfamethizole; PRI, pristinamycin.

(8 μg/mL), chloramphenicol (8 μg/mL), colistin (4 μg/mL), doxycycline (4 μg/mL), enrofloxacin (0.5 μg/mL), erythromycin (1 μg/mL), flumequine (4 μg/mL), fusidic acid (4 μg/mL), gentamicin (4 μg/mL), kanamycin (8 μg/mL), lincomycin (4 μg/mL), metronidazole (4 μg/mL), nitrofurantoin (25 μg/mL), oxacillin (2 μg/mL), oxolinic acid (2 μg/mL), penicillin (0.25 μg/mL), pristinamycin (2 μg/mL), rifampicin (4 μg/mL), spectinomycin (64 μg/mL), streptomycin (8 μg/mL), sulfamethizole (100 μg/mL), tetracycline (4 μg/mL), trimethoprim + sulphamethoxazole (2 + 38 μg/mL, respectively), and tylosin (2 μg/mL). The tests were performed according to manufacturer's instructions, with the exception of incubation time which was extended from 24 to 48 h.

2.4. Phospholipase D (PLD) production

PLD was detected by the CAMP inhibition test as described previously [12]. Isolates which were negative for PLD by this assay were further analysed by PCR amplification of the PLD-encoding gene, *pld*. For use as template in PCR, genomic DNA was extracted and purified using the NucleoSpin Tissue Kit (BD Biosciences; Franklin Lakes, NJ, USA), according to the manufacturer's instructions. The oligonucleotide primers *pld*#01 (5'-CGCGCCATATGAGGGAGAAATTTGCTTTATT-3') and *pld*#02 (5'-GCGCGGGATCCTCACCACGGGTTATCCGC-3') were designed (*Nde*I and *Bam*HI cleavage sites underlined) to amplify the entire *pld* gene, based on the publicly available sequence (accession #L16585).

2.5. Pulsed-field gel electrophoresis

PFGE was performed essentially as described previously [12] with minor modifications. Chromosomal DNA was prepared as described [16] with the exception that the bacterial concentration was increased from 1.8×10^9 cfu/mL to 9×10^9 cfu/mL, and the lysozyme concentration in the EC lysis solution was increased from 1 mg/mL to 2 mg/mL. The running time of pulsed-field gels was increased from 20 h to 24 h and gels were rinsed three times in distilled water before being stained with ethidium bromide and photographed.

The interpretation of PFGE patterns was based on the criteria proposed by Tenover et al. [40] who defined genetic events and categories of genetic and epidemiological relatedness as follows: A genetic event is caused by a point mutation or insertion/ deletion resulting in a 3- or a 2-band difference, respectively. Tenover describes four categories; indistinguishable, closely (clonally)-related, possibly-related and different. These categories are distinguishable by the presence of 0, 2–3, 4–6, and 7 or more band differences respectively. The resultant epidemiological interpretations are: isolate is related, isolate is probably related, isolate is possibly related and isolate is not related.

3. RESULTS

3.1. Biotyping

From all donated cultures, colonies were recovered with morphological characteristics consistent with that of *C. pseudotuberculosis*, i.e. white, regular, α-hemolytic, with a tendency for the entire colony to move when scraped. Approximate colony diameters after 48 h incubation were 1–2 mm. Isolates were confirmed as *C. pseudotuberculosis* using the API Coryne test, with profiles ranging from % ID = 99.9 to 90.6 (data not shown), and all isolates were unable to reduce nitrate.

3.2. Antimicrobial susceptibility

The antimicrobial susceptibilities of *C. pseudotuberculosis* isolates were determined, and only those antimicrobials for which variation in susceptibility was observed are presented in Table II. All isolates were found to be susceptible to, amoxicillin, cefoperazone, cephalothin, chloramphenicol, co-amoxyclav, cotrimoxazole, doxycycline, enrofloxacin, erythromycin, flumequine, furazolidone, fusidic acid, lincomycin, oxacillin, penicillin G, rifampicin, spectinomycin, tetracycline, and tylosin. In contrast, variation in susceptibility was observed with the antibiotics apramycin, gentamicin, kanamycin, pristinamycin, streptomycin, and sulfamethazole.

3.3. PLD production

Based on the results of the CAMP–Inhibition Test, all *C. pseudotuberculosis* isolates produced PLD (data not shown) with the apparent exception of isolates 61, 71 and 91. In order to further analyse these latter strains, a PCR test was used to confirm that the three isolates possessed the *pld* gene, encoding the PLD toxin. Cells were harvested from liquid cultures of strains 61, 71 and 91, in addition to the PLD positive strain NCTC 3450. In each case, an expected 937 bp fragment was successfully amplified, confirming the presence of the *pld* gene in the apparently PLD-deficient strains (data not shown).

3.4. PFGE

In the course of this study, the restriction endonucleases *Sfi*I and *Spe*I were investigated to determine their suitability for PFGE analysis of *C. pseudotuberculosis*. Unfortunately, *Spe*I was observed to cut frequently within the *C. pseudotuberculosis* genome; hence, the resulting DNA profiles were comprised of fragments too small to allow effective discrimination between isolates, at least under the running conditions employed in this study. In contrast, analysis of the ovine and caprine isolates following digestion of chromosomal DNA with *Sfi*I revealed, for each isolate, between 16–18 DNA bands within the 48.5–290 kb size range. Each sample was run on an average of 5 gels to test the reproducibility of the technique, and hence the accuracy of the obtained results. Differences between pulsed-field fragment patterns were first identified by eye and, subsequently, pulsotypes were confirmed using ImageMaster 1D Elite and 1D Database (Nonlinear Dynamics, Newcastle upon Tyne, England, UK). Analysis of these profiles allowed the differentiation of strains into 4 pulsotypes, which only differed from each other by between 1 and 3 bands and therefore were deemed to be closely related (Tab. II and Fig. 1). As a consequence of this study the pulsotypes of two isolates from our previous study were reassigned (see discussion) as follows: isolate 5 (previously pulsotype (P) P2) was reassigned to P3 and isolate 45 (previously P3) was reassigned to P4.

Figure 2 illustrates the computer-generated dendrogram of best-fit analysis using the Dice coefficient. Analysis of the results revealed that 28% of all *C. pseudotuberculosis* isolates clustered within the P2 pulsotype, and comprised 10 ovine and 2 caprine isolates. While P3 comprised 2 ovine isolates, P4 was determined to be the most common pulsotype (encompassing 69% of the total isolates) and comprised 25 ovine and 4 caprine isolates. Finally, P5 was represented by a single ovine isolate.

4. DISCUSSION

On the basis of the results presented here, we have shown for the first time

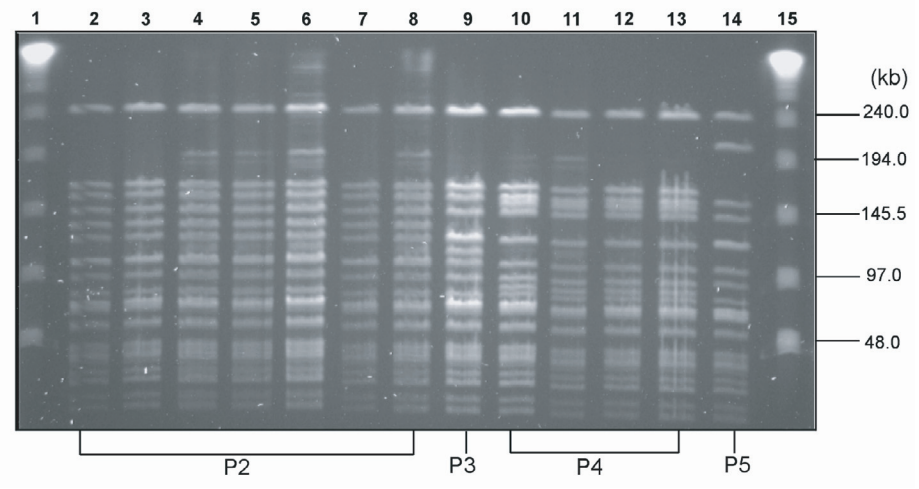

Figure 1. PFGE profiles of ovine and caprine isolates of *C. pseudotuberculosis*. Lanes 1 and 15, λ DNA ladder; lanes 2-8, pulsotype 2 (isolates 75, 70, 80, 81, 82, 99 and 103 respectively); lane 9, pulsotype 3 (isolate 5); lanes 10-13, pulsotype 4 (isolates 45, 88, 63, 66); lane 14, pulsotype 5 (isolate 92).

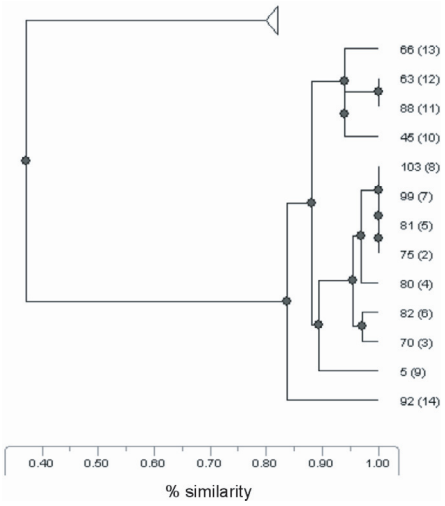

Figure 2. Dendrogram illustrating the percentage relatedness of the ovine and caprine *C. pseudotuberculosis* isolates. Isolate numbers are suffixed by the lane number of the PFGE gel (in brackets) in which they appear in Figure 1.

that, irrespective of their country of origin, *C. pseudotuberculosis* isolates are clonally related. These data support and expand on previous studies, which demonstrated a high degree of similarity amongst ovine and caprine isolates of *C. pseudotuberculosis* in several countries, including the Slovak and Czech Republics [17], France [28], Australia [38], Canada, the USA, and South Africa [35]. In this study, using PFGE, 42 non-UK isolates were grouped into four closely related (therefore clonal) pulsotypes, based on the presence or absence of 1 to 3 DNA bands at various positions; dendrogram analysis revealed that 74% (32/43 isolates) shared a 100% degree of relatedness and the minimum relatedness was still a significant 84% (data not shown). In a further analysis, the multinational pulsotypes were shown to be just as closely related to previously characterised UK pulsotypes (data not shown), further supporting the hypothesis of a clonal relationship between *C. pseudotuberculosis* isolates associated with ovine and caprine CLA. Interestingly, on the basis of our results we have found that the modal pulsotype in sheep (P4) is indistinguishable from the original caprine

outbreak strain in the UK [12] (data not shown).

We previously reported difficulties associated with the characterization of *C. pseudotuberculosis* with respect to biochemical variation, as encountered by ourselves and other groups [10, 13, 35]. Significantly, in the current study, culturing of *C. pseudotuberculosis* directly from cryogenically stored stocks was shown to result, on occasion, in an absent or weak urease reaction (unpublished observation), which in turn resulted in misdiagnosis of the organism as *C. jeikeium* (based on the API Coryne test). The urease reaction became consistently positive following subculture of the revived isolate; therefore, it is conceivable that some field isolates may be mistakenly typed in diagnostic laboratories. The absence of nitrate-reducing isolates in our test panel was consistent with previous reports of the specificity of strains belonging to biovar ovis for sheep and goats, from which only non-nitrate reducing isolates have ever been recovered [25]. In addition, the results of the antimicrobial susceptibility testing are largely in agreement with others [17, 21, 34], in that isolates were susceptible to the majority of the antimicrobial agents tested, and most were resistant to streptomycin.

It has been reported previously that all isolates of *C. pseudotuberculosis* produce PLD [28, 35]. Using the CAMP inhibition test, all except for isolates 61, 71 and 91 in the current study were found to produce an active toxin; however, PCR analysis of genomic DNA from these three apparently PLD deficient isolates resulted in the successful amplification of a product corresponding to the *pld* gene. Little is known regarding the regulation of *pld* expression, and the environmental conditions under which optimal PLD activity may be detected. While no attempt was made to quantify *pld* expression in the present study, it is possible that, under the conditions imposed by growth on artificial media, some strains fail to produce PLD, or produce it at a level lower than is detectable by the CAMP inhibition assay. Alternatively, different *C. pseudotuberculosis* isolates have been shown to produce PLD of variable activity [1, 28], and it may be that three apparently negative isolates in this study produced PLD of an insufficient activity to be detected in the standard assay.

The importance of manual analyses of PFGE data cannot be overemphasized to allow detection of incompletely-digested (ghost bands) that might otherwise be mistakenly interpreted by analytical computer software [14]. During repeat analysis of our samples, it became apparent that a variable band at 194 kb (which was also present in digest profiles of genomic DNA from isolates in the original panel) was an artefact of incomplete digestion; no pattern of gel-plug lot-to-lot variation was detected (data not shown). The reason for the inconsistency of 194 kb band remains unclear; however, its removal from subsequent analyses resulted in even greater clonality of the original panel than was first observed, and allowed the amalgamation of two pulsotypes. In this respect, we have been able to reassign what were initially thought to be distinct pulsotypes from our original panel into the four pulsotypes described here. Our panel was run on an average of eight gels in order to confirm the reassignment of pulsotypes and the lack of genomic plasticity was demonstrated in our previous study through a process of multiple passages [12].

In this study we were particularly interested to determine whether there was localised geographical strain clustering or whether *C. pseudotuberculosis* was clonally-related on a multinational level. The most common pulsotype, P4, was associated with 7/12 isolates from Canada, 10/14 from Australia, 10/12 from North Ireland, as well as the single Eire isolate (Tab. II). The second

most common pulsotype, P2, was associated with 5/12 isolates from Canada, 1/14 from Australia, 2/12 from North Ireland and 4/4 from The Netherlands. Given that there is only a single band difference between the two pulsotypes, this suggests that they are very closely clonally related, and it seems likely that *C. pseudotuberculosis* exhibits remarkable genotypic homogeneity globally. This is in direct contrast to the diversity shown by *C. jeikeium* [30], and a recent study [22] provides a possible explanation, whereby an examination was undertaken to determine the presence or absence of genes encoding recombinational repair enzymes among taxonomically-related, sequenced corynebacterial and mycobacterial species. The absence of a recombination pathway encoded by the *recBCD* genes was considered responsible for genomic stability in corynebacteria, because such genes are involved in the formation of chromosomal inversions. Significantly, it was concluded that corynebacteria have rarely undergone extensive genome arrangements and have maintained their ancestral genome structures, even after the divergence of corynebacteria and mycobacteria. While no genome sequence currently exists for *C. pseudotuberculosis*, it is intriguing to hypothesize that absence of the *recBCD* genes may contribute to its genomic stability.

Our data support the theory that the differences seen globally in the manifestation of the CLA, both in sheep and goats, are more likely to be attributed to differences in the host species, and other factors such as age or environmental pressures [2], rather than differences between the *C. pseudotuberculosis* isolates themselves. Several other studies on CLA in sheep and goats are of particular relevance to the work presented here. In Australia, Sutherland et al. compared isolates from sheep and goats with severe visceral disease, imported from North America, with isolates from indigenous merino sheep with typical superficial lesions; the *C. pseudotuberculosis* isolates were found to be identical both biochemically and genetically, which was an unexpected result as the animals were from different parts of the world and presented different clinical pictures [38]. Certainly, there appears to be no evidence of a molecular pattern that could be attributed to strains from different countries. Our findings are also in agreement with Pepin et al., who found that the occurrence and pathology of CLA can vary under different conditions [28], and Unanian et al. who reported that, in Brazil, the pattern of distribution of CLA abscesses in goats, which were found mainly in the anterior region, may be due to the type of pasture which is characterized by thorny bushes and small trees [41]. Furthermore, studies in Europe [27], and the Czech and Slovak Republics [17] have also demonstrated a high degree of similarity amongst ovine and caprine isolates of *C. pseudotuberculosis.*

In summary, we have shown for the first time that the genotype of *C. pseudotuberculosis* isolates from sheep and goats appears to be highly conserved on a multinational scale. This remarkable finding may have important implications in determining global epidemiology and ultimately future vaccine development and vaccination policies.

ACKNOWLEDGEMENTS

This work was supported by the Scottish Executive Environment and Rural Affairs Department (SEERAD). The authors wish to thank Mr. Frank Malone, Dr John Prescott, Dr Paula Menzies, Dr Daan Dercksen, and Ms. Nicky Buller for supplying the isolates of *C. pseudotuberculosis* used in this study.

REFERENCES

[1] Abou-Zaid A.A., *Corynebacterium pseudotuberculosis* in buffaloes, and es and sheep, Vet. Med. J. Giza (2001) 49:435–450.

[2] Al-Rawashdeh O.F., Al Qudah K.M., Effect of shearing on the incidence of caseous lymphadenitis in Awassi sheep in Jordan, J. Vet. Med. B Infect. Dis. Vet. Public Health (2000) 47:287–293.

[3] Aleman M., Spier S.J., Wilson W.D., Doherr M., *Corynebacterium pseudotuberculosis* infection in horses: 538 cases (1982–1993), J. Am. Vet. Med. Assoc. (1996) 209:804–809.

[4] Arsenault J., Girard C., Dubreuil P., Daignault D., Galarneau J.R., Boisclair J., Simard C., Belanger D., Prevalence of and carcass condemnation from maedi-visna, paratuberculosis and caseous lymphadenitis in culled sheep from Quebec, Canada, Prev. Vet. Med. (2003) 59:67–81.

[5] Baird G., Differential diagnosis of non-parasitic skin conditions in sheep, In Pract. (2000) 22:72–79.

[6] Baird G., Synge B., Armstrong D., Dercksen D., Caseous lymphadenitis in sheep in the United Kingdom, Vet. Rec. (2001) 149:399.

[7] Baird G., Current perspectives on caseous lymphadenitis, In Pract. (2003) 25:62–68.

[8] Batey R.G., Pathogenesis of caseous lymphadenitis in sheep and goats, Aust. Vet. J. (1986) 63:269–272.

[9] Biberstein E.L., Knight H.D., Jang S., Two biotypes of *Corynebacterium pseudotuberculosis*, Vet. Rec. (1971) 89:691–692.

[10] Brown C.C., Olander H.J., Caseous lymphadenitis of goats and sheep: a review, Vet. Bull. (1987) 57:1–12.

[11] Bruere K.N., West D.M., Caseous lymphadenitis (CLA, Cheesy Gland,Lympho), in: Bruere K.D., West D.M. (Eds.),The Sheep – Health, disease and production, Foundation for Continuing Education, Massey University, Palmerston, New Zealand, 1993, pp. 252–257.

[12] Connor K.M., Quirie M.M., Baird G., Donachie W., Characterization of United Kingdom isolates of *Corynebacterium pseudotuberculosis* using pulsed-field gel electrophoresis, J. Clin. Microbiol. (2000) 38:2633–2637.

[13] Dorella F.A., Pacheco L.G.C., Oliveira S.C., Miyoshi A., Azevedo V., *Corynebacterium pseudotuberculosis*: microbiology, biochemical properties, pathogenesis and molecular studies of virulence, Vet. Res. (2006) 37:201–218.

[14] Duck W.M., Steward C.D., Banerjee S.N., McGowan J.E. Jr., Tenover F.C., Optimization of computer software improves accuracy of pulsed-field gel electrophoresis macrorestriction fragment pattern analysis, J. Clin. Microbiol. (2003) 41:3035–3042.

[15] Ellis T.M., Sutherland S.S., Wilkinson F.C., Mercy A.R., Paton M.W., The role of *Corynebacterium pseudotuberculosis* lung lesions in the transmission of this bacterium to other sheep, Aust. Vet. J. (1987) 64:261–263.

[16] Kattak M.N., Matthews R.C., Genetic relatedness of *Bordetella* species as determined by macrorestriction digests resolved by pulsed-field gel electrophoresis, Int. J. Syst. Bacteriol. (1993) 43:659–664.

[17] Literak I., Horvathova A., Jahnova M., Rychlik I., Skalka B., Phenotype and genotype characteristics of the Slovak and Czech *Corynebacterium pseudotuberculosis* strains isolated from sheep and goats, Small Rumin. Res. (1999) 32:107–111.

[18] Lloyd S., Caseous lymphadenitis in sheep and goats, In Pract. (1994) 16:24–29.

[19] Miers K.C., Ley W.B., *Corynebacterium pseudotuberculosis* infection in the horse: study of 117 clinical cases and consideration of etiopathogenesis, J. Am. Vet. Med. Assoc. (1980) 177:250–253.

[20] Moller K., Agerholm J.S., Ahrens P., Jensen N.E., Nielsen T.K., Abscess disease, caseous lymphadenitis, and pulmonary adenomatosis in imported sheep, J. Vet. Med. B Infect. Dis. Vet. Public Health (2000) 47:55–62.

[21] Muckle C.A., Gyles C.L., Characterization of strains of *Corynebacterium pseudotuberculosis*, Can. J. Comp. Med. (1982) 46:206–208.

[22] Nakamura Y., Nishio Y., Ikeo K., Gojobori T., The genome stability in *Corynebacterium* species due to lack of the recombinational repair system, Gene (2003) 317:149–155.

[23] Paton M.W., Sutherland S.S., Rose I.R., Hart R.A., Mercy A.R., Ellis T.M., The spread of *Corynebacterium pseudotuberculosis* infection to unvaccinated and vaccinated sheep, Aust. Vet. J. (1995) 72:266–269.

[24] Paton M.W., Walker S.B., Rose I.R., Watt G.F., Prevalence of caseous lymphadenitis and usage of caseous lymphadenitis vaccines in sheep flocks, Aust. Vet. J. (2003) 81:91–95.

[25] Paton M.M., Collett G., Pepin M., Bath G.F., *Corynebacterium pseudotuberculosis* infections, in: Coetzer J.A.W., Tustin R.C. (Eds.), Infectious diseases of livestock, Oxford University press, Cape Town, South Africa, 2004, pp. 1917–1930.

[26] Peel M.M., Palmer G.G., Stacpoole A.M., Kerr T.G., Human lymphadenitis due to *Corynebacterium pseudotuberculosis*: report of ten cases from Australia and review, Clin. Infect. Dis. (1997) 24:185–191.

[27] Pepin M., Boisrame A., Marly J., *Corynebacterium pseudotuberculosis*: biochemical properties, production of toxin and virulence of ovine and caprine strains, Ann. Rech. Vet. (1989) 20:111–115.

[28] Pepin M., Paton M., Hodgson A., Pathogenesis and epidemiology of *Corynebacterium pseudotuberculosis* infection in sheep, Curr. Top. Vet. Res. (1994) 1:63–82.

[29] Renshaw H.W., Graff V.P., Gates N.L., Visceral caseous lymphadenitis in thin ewe syndrome: isolation of *Corynebacterium*, *Staphylococcus*, and *Moraxella* spp. from internal abscesses in emaciated ewes, Am. J. Vet. Res. (1979) 40:1110–1114.

[30] Riegel P., de Briel D., Prevost G., Jehl F., Monteil H., Genomic diversity among *Corynebacterium jeikeium* strains and comparison with biochemical characteristics and antimicrobial susceptibilities, J. Clin. Microbiol. (1994) 32:1860–1865.

[31] Rizvi S., Green L.E., Glover M.J., Caseous lymphadenitis: an increasing cause for concern, Vet. Rec. (1997) 140:586–587.

[32] Schreuder B.E.C., ter Laak E.A., Griesen H.W., An outbreak of caseous lymphadenitis in dairy goats: 1st report of the disease in The Netherlands, Vet. Q. (1986) 8:61–67.

[33] Schreuder B.E., ter Laak E.A., Dercksen D.P., Eradication of caseous lymphadenitis in sheep with the help of a newly developed ELISA technique, Vet. Rec. (1994) 135:174–176.

[34] Skalka B., Literak I., Michalik I., Skrivanek M., *Corynebacterium pseudotuberculosis* infection in goats in the Czech Republic, J. Vet. Med. B Infect. Dis. Vet. Public Health (1998) 45:31–35.

[35] Songer J.G., Beckenbach K., Marshall M.M., Olson G.B., Kelley L., Biochemical and genetic characterization of *Corynebacterium pseudotuberculosis*, Am. J. Vet. Res. (1988) 49:223–226.

[36] Stanford K., Brogden K.A., McClelland L.A., Kozub G.C., Audibert F., The incidence of caseous lymphadenitis in Alberta sheep and assessment of impact by vaccination with commercial and experimental vaccines, Can. J. Vet. Res. (1998) 62:38–43.

[37] Stoops S.G., Renshaw H.W., Thilsted J.P., Ovine caseous lymphadenitis: disease prevalence, lesion distribution, and thoracic manifestations in a population of mature culled sheep from western United States, Am. J. Vet. Res. (1984) 45:557–561.

[38] Sutherland S.S., Hart R.A., Buller N.B., Ribotype analysis of *Corynebacterium pseudotuberculosis* isolates from sheep and goats, Aust. Vet. J. (1993) l 70:454–456.

[39] Sutherland S.S., Hart R.A., Buller N.B., Genetic differences between nitrate-negative and nitrate-positive *C. pseudotuberculosis* strains using restriction fragment length polymorphisms, Vet. Microbiol. (1996) 49:1–9.

[40] Tenover F.C., Arbeit R.D., Goering R.V., Mickelsen B.A., Murray B.A., Persing D.H., Swaminathan B., Interpreting chromosomal DNA restriction patterns produced by pulsed-field electrophoresis: criteria for bacterial strain typing, J. Clin. Microbiol. (1995) 33:2233–2239.

[41] Unanian M.M., Feliciano Silva A.E.D., Pant K.P., Abscesses and caseous lymphadenitis in goats in tropical semi-arid north-east Brazil, Trop. Anim. Health Prod. (1985) 17:57–62.

[42] Valli V.E.O., Parry B.W., Lymphoreticular tissues, in: Jubb K.V.F., Kennedy P.C., Palmer N. (Eds.), Pathology of Domestic Animals, Academic Press, San Diego, California, 1993, pp. 238–240.

[43] Williamson L.H., Caseous lymphadenitis in small ruminants, Vet. Clin. North Am. Food Anim. Pract. (2001) 17:359–371.

[44] Yeruham I., Elad D., Van-Ham M., Shpigel N.Y., Perl S., *Corynebacterium pseudotuberculosis* infection in Israeli cattle: clinical and epidemiological studies, Vet. Rec. (1997) 140:423–427.