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Molecular epidemiology and population genetics of *Salmonella* subspecies *diarizonae* in sheep in Norway and Sweden

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SUMMARY

Fifty-four isolates of *Salmonella enterica* subsp. *diarizonae* (IIIb) in Norway, Sweden, England, the United States, France and Australia were characterized by pulsed-field gel electrophoresis (PFGE). This study focuses on serovar 61:k:1,5,(7) [*S.* IIIb 61:k:1,5,(7)] isolated from sheep. Digestion of the bacterial DNA with restriction enzyme *Xba*I yielded 15 distinct PFGE profiles comprising 12–16 fragments in the range 48·5–630·5 kbp. Four different profiles were identified in Norwegian sheep isolates and a single profile in Swedish isolates. The spatial and temporal distribution of profiles is discussed.

INTRODUCTION

Bacteria belonging to the species *Salmonella enterica* include important human and animal pathogens that may cause severe gastroenteritis, sepsis and occasionally death. The majority of the most virulent serovars belong to subspecies *enterica* (I) [1, 2].

Subspecies II, IIIa, IIIb and IV are most commonly found in cold-blooded animals [3]. Infections in man caused by strains from subspecies *diarizonae* (IIIb) are predominantly foodborne [4], often caused by serovars found in domestic animals such as cattle [61:1, v:1,5,(7)] and sheep [61:1,v:1,5,(7), 61:k:1,5,(7)] [5, 6].

Salmonella enterica subsp. *diarizonae* serovar 61:k:1,5,(7) [*S.* IIIb 61:k:1,5,(7)] are considered as adapted to sheep [4–9]. Hall and Rowe [6] reported 2 and 44 infections in humans and sheep respectively in the United Kingdom from 1966 to 1990. Weiss et al. [5] described the serovars *S.* IIIb 61:k:1,5,(7) and *S.* IIIb 61:-:1,5,(7) separately, and 39 and 7 cases were recorded from 1967 to 1976 respectively. Twenty-seven of the 39 *S.* IIIb 61:k:1,5,(7) isolates originated from humans and the rest from sheep, food or without known origin. In Australia, the *S.* IIIb 61:k:1,5,(7) serovar has not been diagnosed in spite of the abundant numbers of sheep and reptiles [D. Davos, Institute of Medical and Veterinary Science (IMVS), personal communication], and has not been diagnosed in South Africa either (M. Henton, Onderstepoort Veterinary Institute, personal communication).

In Norway, *S.* IIIb 61:k:1,5,(7) has been isolated from a human once, from a 2-year-old boy in 1989 (National Institute of Public Health; NIPH). In Sweden, *S.* IIIb 61:k:1,5,(7) has been isolated from one person in the last 5 years (1995–2000). As that person was abroad when contracting the disease, it was not considered as a domestic case (Swedish Institute for Infectious disease Control; SMI).

The prevalence of *Salmonella* in general in domestic animals in Norway, Sweden and Finland has been very low for decades [10–15]. Norwegian and Swedish legislation has principally been based on zero

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Table. *Included* Salmonella *subspecies* diarizonae *isolates subjected to PFGE. Profile caps and isolate numbers correspond to dendrogram* (*Fig. 1*). *Serovars, sample matrices and origin of the isolates with the reference from providing laboratory are given*

Profile	No.	Serovar	Sample matrix	Origin/Lab./Country	Lab. ref.
A	1	61:k:1,5	Human, 1989	Ålesund/NIPH/western Norway	89/1514
B	2	61:k:1,5,7	Human, 1976	—/Institute Pasteur/France	76/1762
C	3	61:-:1,5,7	Equine, 1997	—/PHLS/England	S166841
D	4	61:l,v:1,(5,7)	Snake	—/CDC/USA	96-323
E	5a	61:-:1,5	Sheep, faeces	Kävlinge/SVA/Sweden	St1056/98
E	6a	61:k:1,5	Sheep, faeces	Hällefors/SVA/Sweden	St656/98
E	7a	61:-:1,5	Sheep, faeces	Falköping/SVA/Sweden	St301/99
E	7b	61:-:1,5	Sheep, faeces	Falköping/SVA/Sweden	St157/99
E	8a	61:-:1,5	Sheep, faeces	Animskog/SVA/Sweden	St697/99
E	9	61:-:1,5	Sheep, faeces	Kävlinge/SVA/Sweden	St1007/99
E	8b	61:-:1,5	Autopsy, sheep	Animskog/SVA/Sweden	St404/99
E	6b	61:-:1,5	Placenta, sheep	Hällefors/SVA/Sweden	St27/98
E	10	61:-:1,5	Sheep, faeces	Skara/SVA/Sweden	St1076/98
E	6c	61:-:1,5	Sheep, faeces	Hällefors/Swelab/Sweden	1065-4
E	11	61:-:1,5	Sheep, faeces	Uppsala/SVA/Sweden	St1058/98
E	6d	61:-:1,5	Sheep, faeces	Hällefors/Swelab/Sweden	1065-9
E	5b	61:-:1,5	Sheep, faeces	Kävlinge/SVA/ Sweden	St1055/98
F	12	48: k:1,5,7	Not known	—/Institute Pasteur/France	5243-58/180
G	13	61:-:1,5,7	Sheep	—/PHLS/England	S102256
G	14	61:k:1,5,7	Sheep	—/PHLS/England	S159348
G	15	61:-:1,5	Pony, necropsy	Kirkenær/NIPH/eastern Norway	BE98-3199
H	16	61:k:1,5,7	Human, blood	—/CDC/USA	97-150
I	17	61:-:1,5	Sheep, faeces	Tana/NIPH/northern Norway	BE98-3254
I	18	61:k:1,5	Ram, circle	Hadsel/NIPH/northern Norway	93/2748
I	19	61:-:1,5	Sheep, carcass (Bodø)	Hamarøy/NIPH/northern Norway	93/2431
I	20	61:-:1,5	Ram, circle	Hamarøy/NIPH/ northern Norway	2750/93
I	21	61:-:1,5	Sheep, carcass	Leknes/NIPH/northern Norway	93/1444
J	22	61:-:1,5	Sheep, faeces	Oppdal/NIPH/central Norway	BE98-3247
K	23	61:-:1,5	Sheep	—/WSU/USA	3047
L	24	61:k:1,5,7	Human (1997)	—/PHLS/England	S170369
M	25	61:l,v:1,5,7,z57	Human, faeces	—/IMVS/Australia	98/04467
N	26a	61:-:1,5	Sheep, faeces	Bøverbu/NIPH/eastern Norway	BE98-3244
N	26b	61:-:1,5	Sheep, faeces	Bøverbu/NIPH/eastern Norway	BE98-3233
N	27	61:-:1,5	Sheep, faeces	Koppang/NIPH/eastern Norway	BE98-3232
N	28	61:-:1,5	Sheep, faeces	Oppdal/NIPH/central Norway	BE98-3238
N	29	61:-:1,5	Sheep, faeces	Oppdal/NIPH/central Norway	BE98-3239
N	30a	61:-:1,5	Sheep, faeces	Soknedal/NIPH/central Norway	BE98-3246
N	30b	61:-:1,5	Sheep, faeces	Soknedal/NIPH/central Norway	BE98-3249
N	31	61:-:1,5	Sheep, faeces	Soknedal/NIPH/central Norway	BE98-3240
N	32a	61:-:1,5	Sheep, faeces	Hundorp/NIPH/eastern Norway	BE98-3251
N	33	61:-:1,5	Sheep, faeces	Bøverbu/NIPH/eastern Norway	BE98-3250
N	34	61:-:1,5	Sheep, faeces	Singsås/NIPH/central Norway	BE98-3236
N	35	61:k:1,5	Sheep, carcass	Mosjøen/NIPH/northern Norway	93/3380
N	36	61:-:1,5	Abattoir, bung	Mosjøen/NIPH/northern Norway	BE98-3211
N	37	61:-:1,5	Sheep, necropsy	Hønefoss/NIPH/eastern Norway	1111/92
N	38	61:-:1,5	Ram, circle	Fauske/NIPH/northern Norway	2593/93
N	39	61:-:1,5	Ram, circle	Meløy/NIPH/northern Norway	2598/93
N	40	61:-:1,5	Sheep, faeces	Neiden/NIPH/northern Norway	BE98-3253
N	41	61:-:1,5	Sheep, faeces	Oppdal/NIPH/central Norway	BE98-3237
N	42	61:-:1,5	Sheep, faeces	Berkåk/NIPH/central Norway	BE98-3248
N	43	61:-:1,5,7	Human (1990)	—/PHLS/England	S122166
N	44	61:-:1,5	Ram, circle	Mo i Rana/NIPH/northern Norway	2966/93
N	45	61:-:1,5	Sheep, faeces	Hundorp/NIPH/eastern Norway	BE98-3243
N	46	61:-:1,5	Sheep, faeces	Birtavarre/NIPH/northern Norway	10001268-1

Table (*cont.*)

Profile	No.	Serovar	Sample matrix	Origin/Lab./Country	Lab. ref.
N	47	61:k:1,5	Sheep, necropsy	Bygdøy/NIPH/eastern Norway	10000909-1
N	48	61:-:1,5	Abattoir, bung	Sandeid/NIPH/western Norway	3356/99
N	49	61:k:1,5	Abattoir, bung	Forus/NIPH/western Norway	96/2587
N	32b	61:-:1,5	Sheep, faeces	Hundorp/NIPH/eastern Norway	BE98-3252
N	50	61:-:1,5	Abattoir, bung	Kvål/NIPH/central Norway	93/3474
N	51	61:k:1,5	Sheep, faeces	Rennebu/NIPH/central Norway	BE98-3242
N	52	61:-:1,5	Sheep, carcass	Gol/NIPH/eastern Norway	2527/96
N	53	61:-:1,5	Sheep	—/WSU/USA	2756
O	54	48:i:z	Viper	Cameroun/Institute Pasteur/France	697/70

Lower-case letters (a, b, c and d) following isolate numbers indicate several isolates from the same farm.
NIPH, National Institute of Public Health, Oslo, Norway; SVA, National Veterinary Institute, Uppsala, Sweden; PHLS, Public Health Laboratory Service, London, UK; WSU, Washington State University, Pullman, USA; Institut Pasteur, WHO Collaborating Centre for Reference and Research on *Salmonella*, Paris, France; IMVS, Institute of Medical and Veterinary Science (IMVS), Adelaide, Australia; CDC, The Centre of Disease Control, Atlanta, GA, USA.

tolerance level. The legislations consider infected herds equal to those where clinical disease (salmonellosis) is diagnosed, and any finding of *Salmonella* in animals is notifiable.

The Norwegian Animal Health Authority recorded 16 incidents of salmonellosis/salmonella infection in domestic animals from 1998 to October 2000 (Anistat). However, the serovar *S.* IIIb 61:k:1,5,(7) has been sporadically isolated from sheep in Norway since 1991.

It may be difficult to demonstrate both flagellar antigens of the diphasic *diarizonae* strains, e.g. the k antigen of serovar *S.* IIIb 61:k:1,5,(7) [16], but the isolates where there is a lack of information concerning the second H antigen have been considered as *S.* IIIb 61:k:1,5,(7) due to the epidemiological picture. This serovar *S.* IIIb 61:k:1,5,(7) is one of very few endemic *Salmonella* serovars in domestic animals which have been isolated within the last decade in Norway. In regions where the serovar was found, the herd prevalence was found to range from 10 to 45%, but no positive samples were detected from southern and western Norway, where 52% of Norwegian herds are found. The infection was statistically associated with White crossbreed and large herd size in regions where the infection was prevalent. The white breeds are also abundant in the southern and western regions. Thus, it was questioned whether the infection had been imported with the Texel breed (crossed with other Norwegian white breeds) and been distributed mainly in eastern and central Norway. Large herd size is suggested to be important for persistence of the infection [17]. The sometimes high prevalence and low virulence of *S.* IIIb 61:k:1,5,(7) in sheep in Norway represent a challenge to public opinion and legislation in Norway regarding *Salmonella*.

In Sweden, *Salmonella*-infected herds are put under restrictions and actions are always taken, independent of serovar, to eliminate the infection. From 1998 to 2000 a total of 21 cases in cattle, 8 in pigs and 4 in sheep have been notified [15, 18] (Swedish Board of Agriculture; SJV).

A Swedish slaughterhouse survey of *Salmonella* in sheep was performed in 1998. Faecal samples from 604 ewes and 404 lambs were collected. *Salmonella* was isolated from three ewe samples and two lamb samples, indicating a *Salmonella* prevalence of about 0·5%. All five isolates in the study were *S.* IIIb 61:(k):1,5,(7). From 1998 to 2000 *S.* IIIb 61:(k):1,5,(7) has also been isolated in four sheep herds in Sweden [18, 19] (SJV).

The relatedness, at a DNA level, of *Salmonella* strains of different geographical and temporal origin is often investigated in order to epidemiologically distinguish strains with identical phenotypes. Pulsed-field gel electrophoresis (PFGE) is a highly reproducible and discriminatory technique that has often been used alone or in combination with other molecular techniques for this purpose [20–27].

The aim of the study was to investigate the molecular epidemiology of *S.* IIIb 61:k:1,5,(7) isolates from Norway and Sweden by PFGE to address hypotheses concerning routes of infection, and to compare the Nordic strains with related strains or serovars from the United Kingdom, United States, Australia and France.

MATERIAL AND METHODS

Bacterial isolates

Fifty-four isolates of *Salmonella enterica* subspecies *diarizonae* isolated from sheep, humans, horses and cattle in Norway ($n=34$), Sweden ($n=7$), England ($n=5$), United States ($n=4$), France ($n=3$) and Australia ($n=1$) were included. Fifty isolates were *S.* IIIb 61:(k):1,5,(7), and three additional serovars were represented (see Table). Multiple cultures from a single farm but different animals have been analysed in some cases and are indicated by a letter after the isolate number.

The Norwegian isolates of *S.* IIIb 61:k:1,5,(7) were selected from the culture library at NIPH. Five isolates originated from the first recognized outbreak with *S.* IIIb 61:k:1,5,(7) in northern Norway and from follow-up investigations in different ram circles [28] and four from a subsequent survey in Norwegian abattoirs [29].

Furthermore, isolates from 16 herds in a nationwide prevalence study in 1998 where 133 herds were randomly sampled from eleven abattoirs were included. Duplicate isolates were analysed from three of these herds, where *S.* IIIb 61:k:1,5,(7) was diagnosed in both adults and lambs [17].

The only two isolates ever recorded from western Norway originated from incidental findings in abattoir effluents. The remaining eleven Norwegian isolates originated from sporadic necropsies of sheep, necropsy of a Shetland pony (no. 15), carcass swabs and environmental samples. The isolates fairly represent the available diversity regarding geography, time and sample specimens in Norway.

The 13 Swedish cultures from seven different farms originated from a slaughterhouse survey performed in 1998 ($n=4$), from a milk-producing sheep herd situated in Hällefors in the county of Närke ($n=1$), from two different sheep herds in the county of Västergötland, Falköping ($n=1$) and Animskog ($n=1$). Multiple cultures obtained from single farms were isolated on separate sampling occasions. The isolates represented the recovered isolates from animals in Sweden during the last 5 years (1995–2000).

Details regarding the included isolates from Norway, Sweden, France, England, United States and Australia are given in the Table.

PFGE

Fresh colonies were inoculated in 2 ml Luria–Bertani (LB) broth and incubated with gentle agitation for 16 h at 37 °C. Pelleted cells of 1 ml culture were washed once in 1 ml 1× TEN buffer (1 M NaCl, 10 mM Tris–HCl (pH 8), 10 mM EDTA) and centrifuged for 6000 rpm for 6 min. Plug moulds were prepared by resuspending the cells in 250 μl of EC buffer (1 M NaCl, 10 mM Tris–HCl (pH 8), 200 mM EDTA, 0·5% sarcosyl, 0·2% sodium deoxycholate) adding 350 μl 1·5% LGT agarose (FMC Bioproducts, Rockland, ME, USA) and 30 μl lysozyme (20 mg/ml) before dispensing. The plug moulds were solidified and transferred to tubes that contained 0·8 mg/ml lysozyme in 5 ml EC buffer and incubated with gentle agitation at 37 °C. After 2·5 h incubation, the solution was replaced by a 1 mg/ml proteinase K solution in 2·5 ml EC buffer and incubated at 56 °C. After 20 h incubation, this solution was replaced by a fresh 2·5 ml EC buffer with 1 mg/ml proteinase K and further incubated at 56 °C for 20 h. The plugs were washed once in 10 ml TE buffer (10 mM Tris, 1 mM EDTA) and incubated for 2 h at 37 °C in 5 ml TE buffer containing 0·2 mM Pefablock (Boehringer–Mannheim, Mannheim, Germany). The plugs were washed twice with 7·5 and 5 ml TE buffer and finally stored at 4 °C in 1 ml TE buffer. Plugs were sliced in two and digested with 20 U *Xba*I (New England Biolabs, Beverly, MA, USA) in 100 μl NEB buffer 2 at 37 °C overnight. The DNA fragments were separated by electrophoresis (1% SeaKem GTG agarose, FMC Bioproducts) in 0·25× modified TBE buffer for 22 h at 350 V and 12 °C, with pulse times from 5 to 40 s using a Beckman GeneLine II apparatus (Beckman, Fullerton, CA, USA).

Data analysis

Scanned images of PFGE gels were stored in tagged image file format and processed with the computer software GelCompar II (Applied Maths, Kortrijk, Belgium). Similarity between fingerprints was calculated using the Dice coefficient. Band position tolerance of 1·1% was used for analysis of PFGE patterns. In data analysis, only fragments in the range 48·5–630·5 kbp were considered. Cluster analysis was performed by the unweighted-pair-group method with arithmetic averages (UPGMA). The relatedness between profiles was furthermore evaluated according to the interpretation of Tenover et al. [19] of chromosomal DNA restriction patterns produced by PFGE.

RESULTS

Digestion of DNA from the 54 isolates with the restriction enzyme *Xba*I yielded 15 distinct PFGE

profiles (A–O), comprising from 12 to 16 fragments in the range of 48·5–630·5 kbp. Two profiles (E and N) dominated, and included 47 of the isolates when each profile was defined as isolates with similarity indices (SIs) of at least 90%. The relatedness expressed as SIs are graphically shown in a dendrogram with sorted digitalized pictures of the corresponding gels (Fig. 1).

It has often proved difficult to verify the flagellar k antigen of serovar *S.* IIIb 61:k:1,5,(7). Isolates with or without the k antigen produced identical PFGE profiles in seven instances, from Norway (nos. 18 and 19; 34 and 35; 46 and 47; 48 and 49; 50 and 51), from Sweden (nos. 6a and 7a), and from the United Kingdom (nos. 13 and 14).

The spatial distribution of isolates from Norway is shown in Figure 2. The majority of Norwegian isolates clustered in two profiles; I (5 isolates) and N (31 isolates), where isolates from the eastern and central part of the country dominated the profile N cluster. However, this profile was also produced from isolates obtained from western Norway, and the far northeast close to the Russian border. This tight cluster, with a SI above 95% included 14 out of 16 isolates originating from the survey in abattoirs in 1998, four from the investigation in ram circles following the outbreak in 1993 [28] and 10 sporadically isolated strains since 1993 from abattoirs, faecal swabs and necropsies. Five isolates produced an additional 680 kbp band compared with the most common restriction pattern in cluster N, including the only two isolates from western Norway.

One isolate (no. 22) isolated from central Norway yielded a distinct PFGE profile (J), producing four fragment differences compared to isolates in cluster N from the same geographical area.

In two of the three herds from which duplicate analyses were undertaken, restriction patterns from both of the isolates were identical and part of the main group in cluster N (nos. 26a and b; 30a and b). However, the analysis of isolate no. 32a yielded an additional large band compared to isolate no. 32b.

Two foreign isolates grouped in cluster N. The PFGE profile of a British human isolate (no. 43) was identical to the pattern most common in Norwegian sheep. The ovine isolate (no. 53) from the United States was also by definition of PFGE profiles (SI > 90%) included in the N cluster, but produced a distinct restriction pattern not seen in any of the other isolates.

With one exception, the isolates belonging to cluster I originated from the 1993 outbreak in Hamarøy (northern Norway) and the subsequent follow-up investigations. The exceptional isolate (no. 17) in this cluster, was also isolated from a herd in northern Norway but from a different county. The Norwegian isolate no. 15 clustered together with a set of strains from the United Kingdom.

The Swedish isolates clustered tightly together in profile E. With one exception, they were almost identical, and the deviating isolate produced two additional fragments. Profile E was not closely related to any other clusters.

Interestingly, the strain isolated from the only human clinical case in Norway due to *S.* IIIb 61:k:1,5,(7) (no. 1) was not closely related to any of the strains isolated from sheep, and grouped together with the *S.* IIIb 61:k:1,5,(7) reference strain from the Institute Pasteur (no. 2), an equine *S.* IIIb 61:k:1,5,(7) isolate obtained from PHLS (no. 3) and reptile *S.* IIIb 61:l,v:1,5,(7) isolate obtained from CDC (no. 4). The other *S.* IIIb 61:l,v:1,5,(7) isolate from sheep in Australia (no. 25) and *S.* IIIb 48: k:1,5,7 from the Institute Pasteur (no. 12), intermingled with different profiles of *S.* IIIb 61:k:1,5,(7). Serovar *S.* IIIb 48:i:z from the Institute Pasteur (no. 54) was the strain with most deviation, with a SI of approximately 50%, while the highest SI between *S.* IIIb 61:k:1,5,(7) and the other serovars was approximately 75% (no. 25).

DISCUSSION

We are not aware of any previously published molecular epidemiological investigations of *Salmonella* subspecies *diarizonae*. Earlier publications have focused on the epidemiology of serovars. The dendrogram in Figure 1, illustrates that the genetic variation within the *S.* IIIb 61:k:1,5,(7) serovar is almost as large as between the different serovars included in the study [*S.* IIIb 61:k:1,5,(7), *S.* IIIb 61:l,v:1,5,(7), *S.* IIIb 48: k:1,5,7 and *S.* IIIb 48:i:z]. The two isolates of serovar *S.* IIIb 61:l,v:1,5,(7) were genetically distinct (SI difference = 30%). The SIs are, however, dynamic and influenced not only by restriction band pattern (number and size of fragments), but also by the number of included strains and their true relatedness. The least-related couple of strains define the range of validity. We selected serovars *S.* IIIb 61:l,v:1,5,(7), *S.* IIIb 48:k:1,5,7 and *S.* IIIb 48:i:z because their seroformulas deviated regarding both O and H antigens from the serovar of main interest.

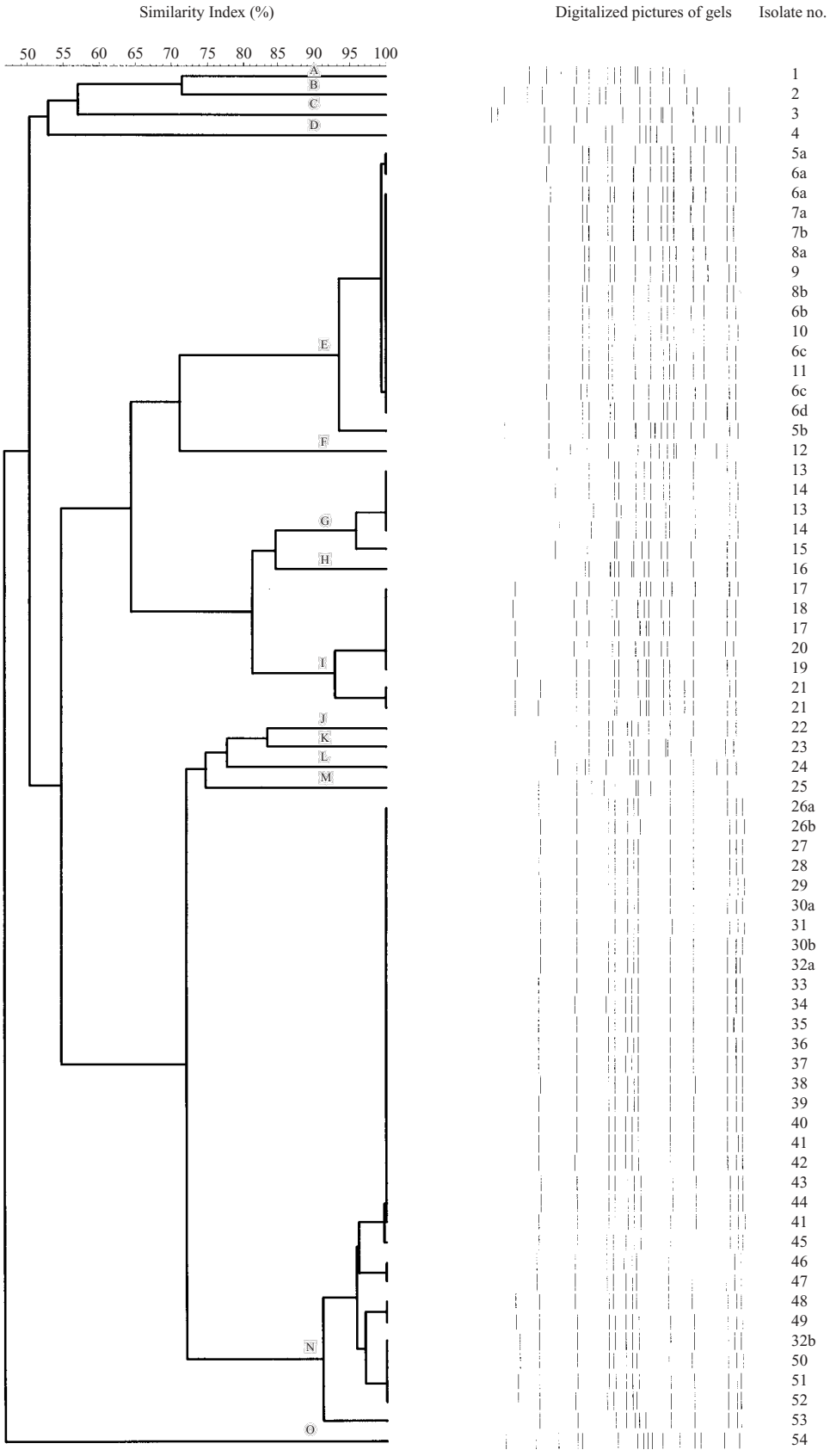

Fig. 1. Dendrogram with digitalized gels, PFGE-profiles (A–O) and isolate numbers.

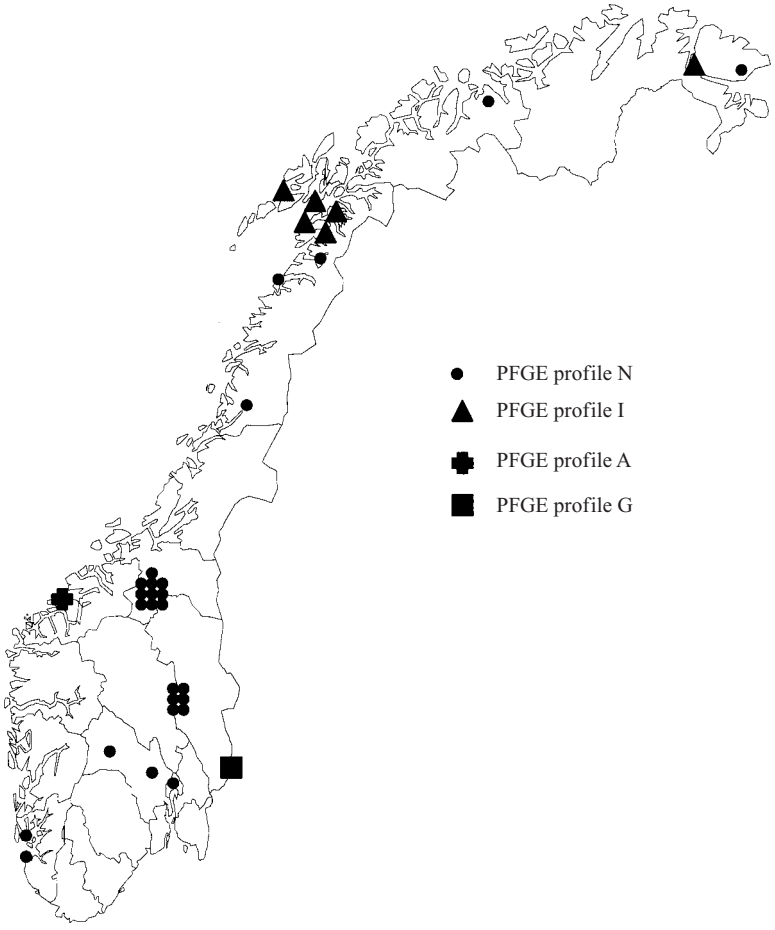

Fig. 2. Map of Norway with plots showing the spatial distribution of the isolates.

The two serovars that carried similar antigen determinants interspaced between *S.* IIIb 61:k:1,5,(7) isolates, while *S.* IIIb 48:i:z, without any common antigen determinants was the least-related strain. This inconsistent correlation between serovars and genotype is in accord with a recently published paper where 48 out of 58 serovars, representing all six subspecies of *Salmonella enterica*, were not fully differentiated, and more than one genotype was observed in seven serovars [30]. Such lack of correlation has also been described from genetic investigations of serovars in subspecies *enterica* (I) [24].

The k antigen has not been detected in many of our included isolates, and it has been questioned whether or not the two seroformulas represent distinct clones or a homogenous group. The homogenous appearances of the included strains with or without the second H antigen cannot be generalized, but demonstrate an advantage of genotypic characterization and a shortcoming of serotyping in epidemiological studies on *Salmonella*.

The Swedish isolates clustered tightly together in profile E and this profile was not closely related to any other strains in this study. The prevalence of *S.* IIIb 61(k):1,5,(7) in Swedish sheep is, according to performed studies, considerably lower than the prevalence in Norwegian sheep [15, 17]. Due to the difference in scrapie status, there has been almost no contact between the Swedish and the Norwegian sheep populations over the last decade.

Bacteria clustered in profile N may be regarded as endemic nationwide among Norwegian sheep, while the profiles G, I and J have so far appeared within distinct regions. The profile N was homogeneous and the PFGE profile has been conserved in Norwegian isolates from 1993 to 2000. Surprisingly, a British human isolate (no. 43) matched with the profile N, despite no apparent epidemiological link.

The isolate (no. 22) that produced profile J originated from central Norway. It has lost a 580-kbp and a 48-kbp band and gained a 220-kbp and a 410-kbp band compared to the isolates clustered in profile N.

Isolate no. 22 could be regarded as possibly related to strain N, but without an epidemiological link, according to the categorization of Tenover et al. [20].

Profile I was only found among isolates from northern Norway, and all isolates except one originated from the outbreak in Nordland county in 1993. The exception was a survey isolate from 1998. The stability of the profile over time indicates a stable and conserved genome of this serovar in this area during the last decade.

S. IIIb 61:k:1,5,(7) in Norwegian sheep is characterized by widespread distribution and a marked variation in prevalence between and within regions. One theory is that the serovar was introduced to Norwegian sheep with the import of the Texel breed in the early 1960s. Another hypothesis is that the infection has been endemic for a long time and that the clonal variation observed is due to genetic shift. The distribution of isolates clustered in profile I was quite consistent with the dissemination of the Texel breed, which was crossed with other Norwegian white breeds from the early 1960s. During these years, Norway imported many animals from Denmark, Sweden and perhaps directly from The Netherlands after transit through Denmark [31]. The Norwegian live animal market was not critical to the quality of the animals. Sheep of any sex, age and condition were imported. In western Norway, where *S.* IIIb 61:k:1,5,(7) has had a low prevalence the sheep-owners have been very conservative regarding breeds, and when crossing occurred it was usually strictly organized and restricted to a few rams of high quality (E. D. Eikje, personal communication).

In 1996 many herds were eradicated due to scrapie and maedi visna in western Norway. Later, new animals from eastern and central Norway replaced these animals. Isolate no. 48 was found in 1999 from effluent drains in an abattoir in western Norway, and the origin of the animals slaughtered that day could be traced back to a farm that had bought animals from a *S.* IIIb 61:k:1,5,(7)-positive herd from eastern Norway (no. 45). These isolates were closely related and both part of profile N, but no. 48 differed with an additional 680-kbp band. Such an additional band was, however, found from other closely related isolates in eastern (nos. 32b and 52) and central Norway (nos. 50 and 51). However, the only other isolate ever recovered from western Norway (no. 49) was indistinguishable in GelCompareII. This isolate was recovered in 1996, and may indicate another, and earlier, route of infection.

The F profile, a single Norwegian isolate (no. 15) clustered with two British isolates (nos. 13 and 14). Isolate no. 15 originated from necropsy of a Shetland pony that had been housed together with sheep. We assumed a route of infection from sheep to horse, but our results did not indicate a connection to any of the former isolates from sheep. The herd owner had, however, imported three ponies from Sweden. Isolate no. 15 was recovered from one of the two ponies, which had developed clinical salmonellosis. Interestingly, the Scandinavian population of Shetland ponies is often supplied with imports from the United Kingdom and The Netherlands. *S.* IIIb 61:k:1,5,(7) was subsequently recovered in samples from sheep and the environment, and our results may indicate transmission of *S.* IIIb 61:k:1,5,(7) to sheep through import of another species carrying the serovar.

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