Molecular characterization of *Salmonella enterica* subsp. *enterica* serovar Typhimurium DT1 isolates

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SUMMARY

Salmonella Typhimurium DT1 is endemic to Finland and has caused human outbreaks since the 1960s. Domestic DT1 isolates ($n = 235$) from 1972 to 1999 from human cases, animals and other sources, as well as foreign DT1 isolates from human cases ($n = 20$) were analysed by molecular methods. Pulsed-field gel electrophoresis (PFGE) yielded 38 *Xba*I profiles. Of these, *Xba*I profile 10 was seen in 49% (125/255) of the isolates. Twelve IS*200* profiles were obtained; the most common IS*200* profile D was seen in 64% (33/52) of the isolates. Two clusters were formed by compilation of the *Xba*I–, *Bln*I– and *Spe*I–PFGE and IS*200* profiles and possession of the serovar-specific virulence plasmid. The major cluster contained eight IS*200* profiles, including IS*200* profile D and *Xba*I profile 10, and had no virulence plasmid, and can be regarded as typical of the endemic Typhimurium DT1 infection.

INTRODUCTION

Most cases of non-typhoidal human salmonella infections of worldwide significance are caused by only two serovars, Typhimurium and Enteritidis of *Salmonella enterica* subspecies *enterica*. Serovar Typhimurium is a globally distributed zoonotic serovar which is common in both cattle and poultry. Phage typing defining the definitive types (DTs) of Typhimurium is the internationally standardized subtyping method for epidemiological investigations of Typhimurium isolates from various sources [1]. More than 200 DTs have been identified for this serovar [2]. Further differentiation within phage types has been achieved by other phenotypic methods, such as antibiogram, and by molecular methods, including plasmid profiling and restriction fragment length polymorphisms [3, 4].

Salmonellosis in humans is relatively rare in Finland. The incidence has lately varied between 54 and 65 cases/100 000 inhabitants per year. Since generally 60–80% of the cases are of foreign origin, the number of domestic cases per year since the 1970s has varied between 500 and 1300. The most common serovars causing salmonellosis in humans in Finland are Enteritidis and Typhimurium. Where most cases of Enteritidis are of foreign origin, the cases of Typhimurium are mainly of domestic origin. Typhimurium phage-type DT1 is considered endemic in Finland. Depending on the number and size of the outbreaks it has caused, its annual share from all indigenously acquired Typhimurium infections in humans has varied between 20 and 75% since the 1970s [5, 6]. In 1983, Typhimurium DT1 spread to broiler farms in Finland, and sporadic isolations have occurred since then. DT1 has become the most common Typhimurium phage type among cattle farms,

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Table 1. *Origin, year of isolation and number of the analysed* Salmonella *serovar Typhimurium DT1 isolates*

| | Number of isolates with the following origin | | | | |
| | | | | | |
Year	Total	Human, domestic*	Human, foreign†	Cattle	Chicken, turkey	Pig, other‡
1972–1980	2	2	0	0	0	0
1981–1988	91	31	0	27	28	5
1990–1999	162	87	20	28	8	19
Total	255	120	20	55	36	24

* Human isolates from outbreaks or sporadic cases in Finland. Annual number of outbreaks 0–4.

† Human isolates with recent history of foreign travel. The isolates were from 13 different countries and four continents: Europe, 13; Asia, 4; North America, 2; Africa, 1.

‡ Other isolates: pig, 3; sheep, 1; horse, 2; dog, 4; wild bird or hedgehog, 8; slaughterhouse, 3; feed, 2; sewage water, 1.

and the proportion of DT1 among the Typhimurium-infected farms in Finland has varied between 16% (5/31) in 1981 and 82% (9/11) in 1992 [7]. Although Typhimurium DT1 is relatively common in Finland, it is not commonly involved in international outbreaks.

In this study we have analysed the molecular epidemiology of endemic Typhimurium DT1 infection in animals and humans during 1972–1999. We wished to differentiate between domestic and foreign infections, and to assess the relative importance of various animal sources for human infections. For this purpose we thoroughly characterized, by several molecular methods, Typhimurium DT1 isolates of both domestic and foreign origin.

MATERIALS AND METHODS

Serovar Typhimurium isolates

The isolates ($n=255$) were obtained from the National Public Health Institute (KTL), Helsinki, Finland ($n=140$) and from the National Veterinary and Food Research Institute, Helsinki, Finland ($n=115$). The isolates were from humans, animals, feed, or the environment (Table 1) from 1972 to 1999. The isolates from humans were classified either as domestic (patient had not been abroad during the month preceding the time when the specimen was taken) or foreign, based on the travelling history of the patient. The domestic isolates were from outbreaks during 1972–1988 and from outbreaks or sporadic cases during 1990–1999. All isolates had been serologically confirmed to be *S.* Typhimurium, phage-typed as DT1 and stored at –70 °C.

Pulsed-field gel electrophoresis (PFGE)

Chromosomal DNA was prepared in gel blocks, and the agarose plugs were treated as described previously [8]. Restriction enzyme and S1-nuclease analyses (see below) were performed using slices from the same plugs. The slices were digested at 37 °C for 16–18 h with either 20 U of *Xba*I or 10 U of *Spe*I in the reaction buffer supplied by the manufacturer (New England Biolabs, Beverly, MA, USA). Digestion with 10 U of *Bln*I was performed at 37 °C for 4 h in the reaction buffer supplied by the manufacturer (Boehringer–Mannheim GmbH, Mannheim, Germany). All 255 isolates were digested with *Xba*I, and 68 isolates representing each PFGE profile detected by *Xba*I restriction and various origin of isolation were digested also with *Spe*I and *Bln*I enzymes. PFGE was performed as described previously [8]. Briefly, for the *Xba*I-treated plugs the electrophoretic conditions were electrophoresis for 19 h at 14 °C with a pulse ramp time of 10–30 s, a voltage of 6 V/cm, and a reorientation angle of 120°. The pulse ramp time was changed to 5–15 s for the *Spe*I-digested plugs and to 10–40 s for the *Bln*I-digested plugs. The molecular weights of the linearized plasmids (see below) and the restriction fragments were determined by comparison with the size of the bacteriophage λ molecular-size ladder.

DNA profiles differing by at least one fragment larger than 20 kb were assigned a PFGE profile

number. The coefficient of similarity values (F) between the PFGE profiles were calculated as described previously [9]. After visual analysis of the PFGE profiles, a computer program for analysis of electrophoretic patterns (GelCompar, Applied Maths, Kortrijk, Belgium) [10] was used to generate dendrograms.

Ribotyping and IS200 typing

Ribotyping and IS200 typing were performed as described in ref. [11]. Bacterial DNA was isolated essentially as described previously [12] and digested with *Bam*HI, *Ban*I, *Pst*I, *Pvu*II, or *Sma*I. *Pvu*II, which lacks restriction sites within the 16S *rrn* gene, provided the optimal resolution of bands and was used for ribotyping. *Pst*I, which lacks restriction sites within IS200, provided the clearest resolution of IS200 bands and was used for the IS200 analysis. Altogether 52 isolates of Typhimurium DT1 of different origin and year of isolation were analysed by ribotyping and IS200 typing. For ribotyping, a 1·3 kb PCR product of the *Escherichia coli* 16S *rrn*B operon was amplified and purified as described previously [11]. For IS200 typing, a 557-bp PCR product of the IS200 insertion sequence was amplified from our own *Salmonella* serovar Infantis isolate K1469 with the primer pair 5′-CCTAACAGGCGCATACGATC-3′ and 5′-ACATCTTGCGGTCTGGCAAC-3′ [13]. A 30-cycle programme (1 min at 94 °C, 0·5 min at 54 °C and 2 min at 72 °C) was used. The PCR product was electrophoresed through 1% agarose gel and purified with a QIAquick-spin extraction kit (Qiagen, Chatsworth, CA, USA). The 16S rRNA and IS200 probes were labelled with DIG-11-dUTP by using a DIG-High Prime labelling kit (Boehringer–Mannheim). Then 2 μg of *Salmonella* DNA was digested with *Pst*I or *Pvu*II restriction enzyme (New England Biolabs) and electrophoresed through 0·8% agarose gel in 1× Tris–acetate–EDTA buffer [14]. Denatured DNA was transferred to a nylon membrane (Hybond-N, Amersham International plc, Amersham, UK) in 20× SSC [14] and fixed to the membrane with microwaves [15]. Hybridization and detection were performed according to the DIG Nucleic Acid Detection kit instructions (Boehringer–Mannheim). Weak bands detected by ribotyping differed between the gels, while strong bands were fully reproducible; therefore, only strong bands were scored. For IS200, the bands detected were fully reproducible. DNA profiles differing by at least one

band were assigned a profile name (capital letter). After visual analysis of the profiles, a computer program for analysis of electrophoretic patterns (GelCompar) [10] was used to generate dendrograms.

Plasmid analyses

Plasmids smaller than 20 kb were analysed by the alkaline lysis method as described by Grinsted and Bennett [16]. The preparations were analysed in 0·9% agarose gels (SeaKem LE, FMC Bioproducts, Rockland, ME, USA) at 4 V/cm for 1·5 h in 1× Tris–acetate–EDTA buffer [14] and the gels were stained with 0·5 μg ethidium bromide/ml. *Escherichia coli* strains V517 (plasmids of 35·6, 4·8, 3·7, 3·4, 1·8 and 1·4 MDa) [17] and 39R861 (plasmids of 98·0, 42·0, 23·9 and 4·6 MDa) [18] were used as plasmid reference strains in all alkaline lysis isolation procedures. Plasmids larger than 20 kb were analysed by PFGE [8] using S1 nuclease.

All 255 Typhimurium isolates were analysed by S1-nuclease treatment whereas 173 isolates were analysed by alkaline lysis. All isolates of the less common PFGE profiles detected by *Xba*I (35 profiles, 82 isolates), and of the three most common *Xba*I profiles (profiles 10, 11 and 20), 62, 17 and 12 isolates, representing different origin of isolation, were analysed by alkaline lysis.

Detection of the *spvC* virulence gene by PCR

PCR was performed with DyNAzyme DNA Polymerase kit (Finnzymes, Espoo, Finland) according to the manufacturer's guidelines. The reaction contained 20 pmol of the primers spvc-F ACT CCT TGC ACA ACC AAA TGC GGA and spvc-R TGT CTC TGC ATT TCG CCA CCA TCA [19]. Bacterial suspensions were lysed for 5 min at 95 °C and amplified 25 cycles for 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C in a UNO II thermocycler (Biometra, Göttingen, Germany). The PCR product was analysed in 1·5% agarose gels (SeaKem LE) with 0·5 μg ethidium bromide/ml at 4·7 V/cm for 50 min in 1× Tris–acetate–EDTA buffer [14].

To confirm the presence of the serovar-specific plasmid, the *spvC* gene was localized to a plasmid by hybridization. The 571-bp PCR product was amplified from a mixture of Typhimurium isolates: DT104 (IH 59841), DT12 (IH 68594, IH 69493) and DT120 (2671), electrophoresed through 1·5% agarose gel and purified with a High Pure PCR Product Purification
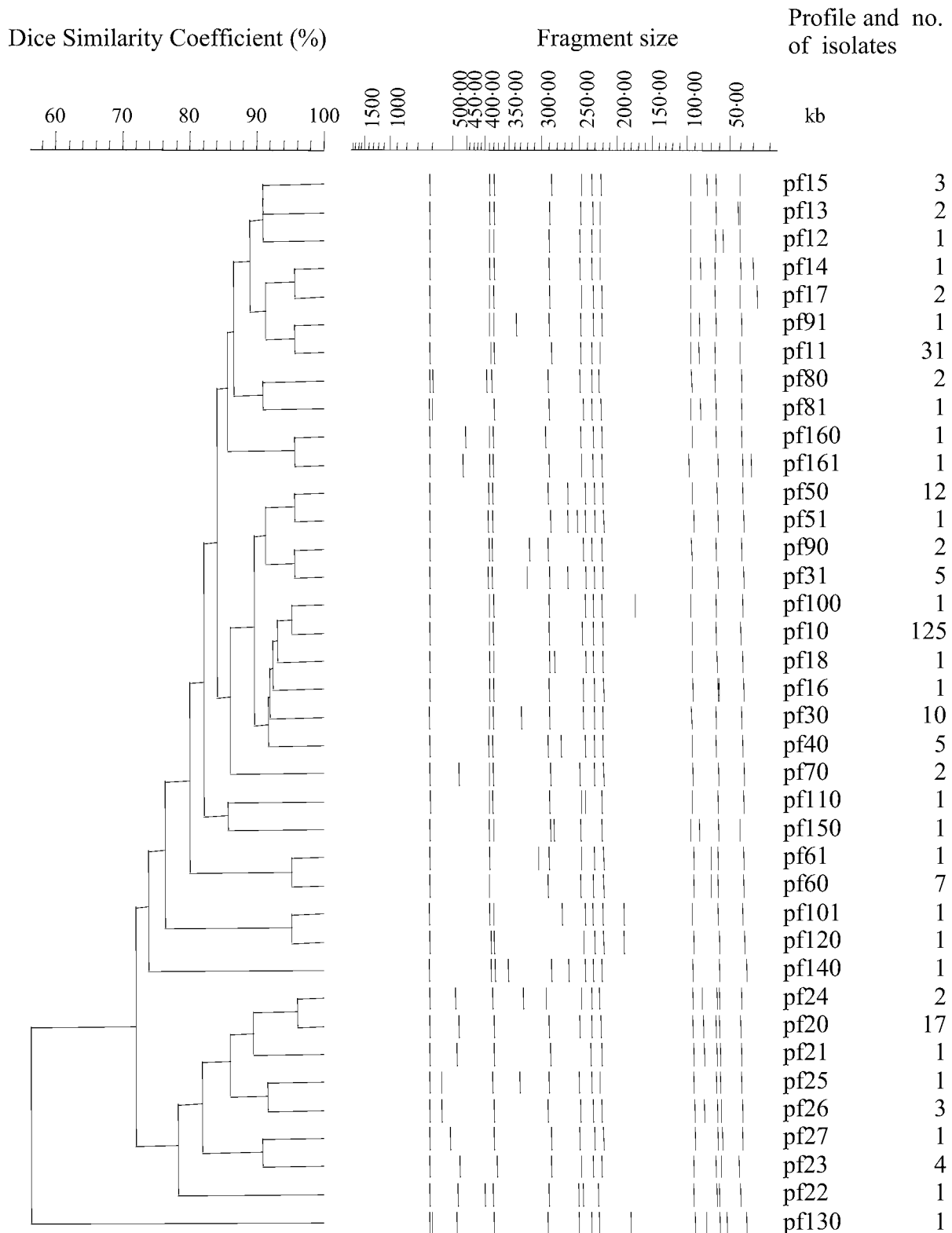
Fig. 1. Dendrogram showing 38 PFGE profiles of *Salmonella* serovar Typhimurium DT1 after *Xba*I restriction of chromosomal DNA followed by PFGE. Dice similarity coefficients are shown at the top left, the molecular sizes (in kilobases) of the fragments are shown at the top middle. PFGE profiles 10–161 indicated with letters pf, followed by numbers, and the number of isolates (1–125) with the particular PFGE profile are indicated at the top right.

Table 2. *Distribution of the 38* Xba*I–PFGE profiles (10–161) among 255* Salmonella *serovar Typhimurium DT1 isolates with respect to origin and year of isolation*

| Year | Profile for isolates with the following origins | | | | |
	Human, domestic*	Human, foreign	Cattle†	Chicken, turkey	Other‡
1972–1980	11 (2)§	—	—	—	—
1981–1988	10 (14), 11 (4), 16, 20 (6), 26 (2), 30, 101, 120, 130	—	10 (5),11 (4), 14, 15 (2), 20 (4), 25, 50, 51, 60 (2), 61, 70, 100, 110, 160, 161	10 (6), 11 (16), 50 (2), 60 (2), 81, 91	10, 11 (2), 15, 60
1990–1999	10 (55), 11, 13, 17 (2), 18, 20 (3), 23 (3), 24, 30 (4), 31, 40 (2), 50 (6), 60, 70, 80 (2), 90 (2), 140	10 (14), 20, 21, 22, 23, 26, 60	10 (17), 12, 15, 20 (4), 24, 30 (2), 31, 50	10 (2), 13, 30, 40 (2), 50 (2)	10 (11), 11 (2), 27, 30 (2), 31(2), 40
Total no. of isolates	120	20	55	36	24

* All isolates from 1972 to 1988 were from outbreaks. Profile 10 was seen in both outbreak and sporadic isolates from 1994, 1995 and 1999.
† One isolate per farm.
‡ For 1981–1988: slaughterhouse, profile 10; hedgehog, 11; horse, 11; bird, 15; pig, 60. For 1991–1999: dog, profile 10 (4 different years); hedgehog, 10 (3); pig, 10; wild bird, 10; feed, 11; horse, 27; sewage water, 30; sheep, 30; slaughterhouse, 31; pig, 40.
§ The number of isolates with a particular profile, if more than one.

kit (Boehringer–Mannheim). The PCR product was labelled with DIG-11-dUTP by using a DIG-High Prime labelling kit (Boehringer–Mannheim) and used as a probe for hybridization of S1-nuclease-digested plasmids of Typhimurium DT1. These plasmids were transferred to nylon membranes after PFGE. Hybridization and detection were performed according to the DIG Nucleic Acid Detection kit instructions (Boehringer–Mannheim). All isolates harbouring plasmids larger than 20 kb, as shown by S1 nuclease, as well as all IS200-typed and ribotyped isolates were analysed by PCR for the presence of the *spvC* gene (altogether 127 isolates). To confirm the presence of a *spv* plasmid, 20 *spvC* PCR-positive and 8 PCR-negative isolates were analysed by hybridization.

RESULTS

Profiles obtained by PFGE

Digestion of all 255 isolates with *Xba*I yielded 38 different *Xba*I–PFGE macrorestriction profiles differing by one or more bands. The number of fragments generated varied between 10 and 14 in the 20–500 kb range (Fig. 1). The most common PFGE profile (profile 10) was seen in 125 out of 255 isolates (49%) (Fig. 1). Profile 10 was seen in human isolates from

the outbreaks in the 1980s and 1990s (Table 2). The *Xba*I profiles 11, 20, 50 and 30 represented 31, 17, 12 and 10 isolates respectively (Fig. 1). A majority of the analysed isolates (76%; 195/255) had 1 of these 5 profiles. All other profiles were seen in less than 10 isolates each (Fig. 1). Of the seven *Xba*I profiles (10, 20, 21, 22, 23, 26 and 60) seen in the 20 human isolates of foreign origin, only two (21 and 22) were not seen among all domestic isolates (Table 2). Of the 38 *Xba*I–PFGE profiles, 27 (71%) were represented by only one or two isolates. The Dice coefficient of similarity (*F*) values between the PFGE profiles varied between 0·56 and 0·96 (Fig. 1). Profiles 20–27 formed a cluster and differed from profile 10 and others at a level of 0·75.

Isolates representing the 38 *Xba*I–PFGE profiles and different sources were analysed by *Bln*I and *Spe*I (Table 3). Among these 68 isolates, digestion with *Bln*I yielded 25 different PFGE profiles. The number of fragments generated varied between 7 and 10 in the 40–600 kb range. The *F* values between the *Bln*I–PFGE profiles ranged from 0·43 to 0·93 (data not shown). Profile 7 was the most common, seen in 49% (33/68) of the analysed isolates, and among 18 different *Xba*I profiles. Digestion with *Spe*I yielded 29 PFGE profiles. The number of fragments generated varied between 17 and 21 in the 20–300 kb range, and the *F* values between 0·59 and 0·97 (data not shown).

Table 3. *Grouping of* Salmonella *serovar Typhimurium DT1 according to* Xba*I,* Bln*I, and* Spe*I restriction profiles, IS*200 *types and* spv*C PCR results. The analysed 68 isolates represented all the* Xba*I–PFGE profiles and various origin and years of isolation*

No. (origin) year of isolation	*spvC* PCR	IS*200* type	*Xba*I–PFGE profile	*Bln*I–PFGE profile	*Spe*I–PFGE profile
25 (human d)* 1981–1995 2 (human f)† 1992–1994 15 (cattle) 1983–1994 6 (broiler) 1983–1994 6 (other)‡ 1986–1995	− n.d. (6)§	D, F, G, H, I, K, L, M n.d. (14)	10, 11, 12, 13, 14, 15, 16, 17, 18, 30, 31, 40, 50, 51, 60, 61, 70, 80, 81, 90, 91, 100, 101, 110, 120, 140, 150, 160, 161	1, 4, 6, 7, 9, 12, 13, 15, 17, 27, 37, 40, 41, 70	1, 3, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 22, 23, 24, 25, 26, 27, 30
3 (human d) 1987–1995 4 (human f) 1993–1995 4 (cattle) 1984–1994	+	A, C n.d. (1)	20, 21, 23, 24, 25, 26	3, 10, 11, 16, 18, 20, 28, 162, 184	2, 6, 20, 21
1 (human f) 1994	−	B	22	2	5
1 (horse) 1994	−	E	27	5	4
1 (human d) 1982	+	n.d. (1)	130	8	13

* Human domestic.
† Human foreign.
‡ One isolate each from pig, sheep, turkey, hen, wild bird, slaughter waste from broiler pig.
§ n.d., Not determined (no. of isolates).

Profile 1 was the most common, seen in 29 % (20/68) of the analysed isolates, and among seven *Xba*I profiles.

Ribotypes and IS*200* types

All the 52 isolates analysed belonged to only one ribotype using *Pvu*II restriction (data not shown). Twelve different IS*200* profiles (A–M) were detected with *Pst*I (Table 3, Fig. 2). The number of IS*200* copies varied between 8 and 14 and only one copy, in a 4·3 kb fragment, was common to all *Pst*I profiles (Fig. 2). Profile D was the most common, both in the 1980s (53 %; 8/15 isolates) and 1990s (68 %; 25/37 isolates), and appeared in isolates of various origin (Table 3). The second most common profile, A, was seen in 17 % (9/52) of the isolates. It appeared only in isolates of human or cattle origin (Table 3). Two clusters of IS*200* profiles were seen: in dendrogram profile A was closely related to C, whereas seven profiles, F, G, H, I, K, L and M, were related to profile D (data not shown).

Plasmid analyses

S1-nuclease analysis combined with PFGE showed that 104 (41 %) of the 255 isolates harboured plasmids in a size range (50–211 kb) which might contain the virulence plasmid. Of these 104 isolates, 26 % were positive for *spvC* by PCR. All 52 ribotyped and IS*200*-typed isolates were also included in the *spvC* analysis. Of these 23 did not harbour large plasmids, and were negative for *spvC* by PCR (data not shown). All isolates ($n = 10$) of the IS*200* profiles A or C and 27 isolates of the *Xba*I profiles 20, 21, 23, 24, 25, 26 or 130 had the *spvC* gene. In contrast, isolates of the IS*200* profiles D, E, F, G, H, I, K, L and M did not have the *spvC* gene (Table 3). The *spvC* probe hybridized to plasmids in a size range of 90–108 kb in all of the *spvC* PCR-positive analysed isolates (20/27). The probe did not hybridize to plasmid-free, PCR-negative isolates of IS*200* profiles D (4 isolates), B (1) or I (1) nor to two isolates of *Xba*I profiles 31 and 51 (data not shown). Based on S1-nuclease analysis and *spvC* PCR, a total of 11 % (27/255) of the DT1 isolates had the serovar-specific plasmid.

Plasmids larger than 108 kb were present in only three isolates; one each of the *Xba*I–PFGE profiles 10, 30 and 60. Only 8 % (10/125) of the isolates of the most common *Xba*I–PFGE profile 10 harboured plasmids larger than 20 kb, compared to all other isolates harbouring large plasmids in 72 % (94/130) of the isolates (data not shown).

Plasmids smaller than 20 kb were seen in 27 % (47/173) of the analysed isolates. Of the analysed isolates of the most common *Xba*I profile 10, 15 % (9/62) contained small plasmids. Small plasmids were present in 90 % (28/31) of the isolates of the *Xba*I profiles 31, 40, 50, 51, 60 and 61 (data not shown).
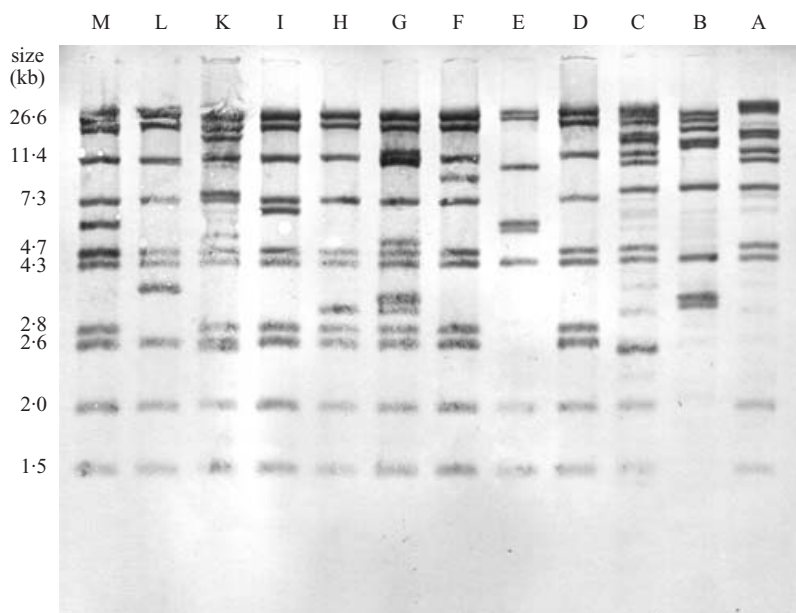
Fig. 2. IS*200* profiles of *Salmonella* serovar Typhimurium DT1 after *Pst*I restriction. Molecular weight in kilobases is shown on the left-hand side. Lanes A–M represents IS*200* profiles A–M.

Compilation results

The compilation of the *Xba*I–, *Bln*I– and *Spe*I–PFGE results gave 52 different combination profiles among the 68 analysed isolates; 48 profiles among the 61 domestic and 7 profiles among the foreign isolates respectively (Table 3). Some combination profiles, such as *Xba*I10–*Bln*I7–*Spe*I1 and *Xba*I50–*Bln*I7–*Spe*I7, were common for different infection sources. In dendrogram analysis, performed separately for each of the three enzymes used for PFGE, two major clusters were seen among the *Xba*I–, *Bln*I– and *Spe*I–PFGE profiles respectively (Fig. 1, data not shown). *Xba*I profiles 20–27 formed one cluster, and the other profiles (except profile 130) another (Fig. 1). The *Bln*I profiles 8, 10, 11, 18, 28 and 184, and *Spe*I profiles 2, 6, 13, 20 and 21, also formed one cluster respectively (data not shown).

When the PFGE results were compiled with those of IS*200* typing and *spvC* PCR, two clusters were obtained (Table 3). There was no overlapping of IS*200* types and PFGE profiles between the clusters; they contained profiles either with or without the *spvC* gene. The isolates (*n* = 54) of the larger cluster had IS*200* profiles D, F, G, H, I, K, L or M, no *spvC* gene, and 1–3 PFGE profiles in common. Isolates of the most common *Xba*I profile 10 belonged to this cluster. The isolates (*n* = 11) of the smaller cluster had IS*200* profiles A or C, the *spvC* gene, and *Xba*I profiles 20, 21, 23, 24, 25 or 26. These profiles were seen in human

foreign and domestic isolates, and in cattle. Three isolates were not placed in either of the two clusters because of their divergent IS*200* profile (B and E) or *Xba*I profile (130).

DISCUSSION

In this study we have analysed the molecular epidemiology of endemic Typhimurium DT1 infection in animals and humans during 1972–1999. The aim was to differentiate domestic infection from foreign infection, and to assess the relative importance of various animal sources for human infections. Of the molecular genetic typing methods used, PFGE was the most discriminative. Because of the high frequency of plasmid-free isolates, the presence of the serotype-specific *spvC* virulence plasmid was analysed.

*Xba*I–PFGE has been successfully applied for many serotypes of salmonellae, e.g. Agona [20, 21], Brandenburg [22], and Javiana [23], and also within phage type, e.g. Enteritidis [24], and Typhi [25]. However, in a study on Typhimurium DTs 1, 9, 126 and 135 [26] *Xba*I–PFGE had limited discriminatory power both between and within phage type. In our previous study on Infantis [8], *Xba*I–PFGE in combination with S1-nuclease analysis was discriminatory. Also in our current study, 38 different *Xba*I profiles were seen among all 255 isolates of Typhimurium DT1. Of these, 36 profiles associated with

isolates of domestic origin. However, in the majority of the analysed isolates, 1 of the 5 most common *Xba*I profiles (10, 11, 20, 30, 50) was seen. The most common *Xba*I profile 10 has been frequent among isolates of both human and animal origin since the 1980s, and can be considered typical of our endemic reservoir and indigenous infections. Profile 10 was also seen in isolates from hedgehogs and wild birds, which might act as reservoirs, keeping a certain baseline level of Typhimurium in the environment. Of the seven *Xba*I profiles seen among isolates of foreign origin, five were also found among domestic isolates.

Among our 52 isolates of Typhimurium DT1 restricted by *Pst*I for IS*200* analysis, only one band (4·3 kb) was conserved. Stanley et al. [27] observed three common *Pst*I bands of 4·8, 2·4 and 1·9 kb in their 25 isolates of Typhimurium. Baquar et al. [3] observed six conserved *Pst*I bands (24, 22, 9·4, 4·8, 2·4 and 1·9 kb) among their 15 Typhimurium DT204c strains. Millemann et al. [28] observed six common *Pst*I bands (20, 9, 4·8, 1·9 and 1 kb) among their 56 Typhimurium strains. The other bands we observed of approximately 24, 22, 19, 9·4, 4·7, 2·6 and 2 kb could correspond to the conserved *Pst*I bands reported in those previous studies [3, 27, 28]. This suggests that there are certain conserved *Pst*I bands among isolates of Typhimurium, regardless of the phage type.

In a study of 38 strains of Typhimurium DT1, which initially were assumed to be similar because they appeared to be epidemiologically related, three different IS*200* profiles by *Pvu*II digestion were detected [26]. Among the 52 isolates of Typhimurium DT1 we analysed by IS*200* typing, 12 different IS*200* profiles were detected by *Pst*I digestion. However, all except nine isolates were of profiles A or D. Profile D seemed to be typical of our endemic isolates from humans and animals since the 1980s. Profile A was fairly common among the analysed human isolates of foreign origin. It was also seen among human isolates of domestic origin and Finnish cattle isolates.

Ribotyping is generally considered a valuable tool for the typing of salmonella [29, 30]. In our study of Typhimurium DT1, it was not discriminatory: all the analysed isolates were of the same ribotype. In a study by Jeoffreys et al. [26], two different ribotypes were seen among 38 isolates of DT1. Millemann et al. [28] and Nastasi et al. [31] obtained several different ribotypes for Typhimurium, but no data were given on the phage types of their isolates.

Only 41% of the 255 analysed isolates contained plasmids in the size range of the serovar-specific virulence plasmid, and only 10% of the 255 isolates possessed the *spvC* gene on a plasmid. One would have expected a higher proportion of plasmid-carrying Typhimurium, since this serotype has been connected with a serotype-specific virulence plasmid of a distinct molecular weight. Helmuth et al. [32] showed that 88% of the 60 analysed isolates of Typhimurium carried a plasmid of approximately 60 MDa and only one of the strains did not carry a plasmid. Further analysis of the 60 MDa plasmid showed an increased virulence in plasmid-carrying strains. Woodward et al. [33] analysed the distribution of virulence plasmids within salmonellae. They tested both various serotypes and phage types, and all three isolates of Typhimurium DT1 showed homology to the 10 MDa virulence region of Dublin. In our study, the widely distributed *Xba*I profiles associated with IS*200* profile clusters D, and F–M did not carry the *spvC* plasmid.

In this study, the analysed isolates were chosen among all available isolates of Typhimurium DT1 based on details of origin (animal species, date or year of isolation, geographical location) in order to obtain as representative material as possible. The isolates from humans were either domestic or foreign, and the domestic isolates were either from outbreaks or sporadic cases. However, in judging whether the origin of an isolate is domestic or foreign, there is always the risk of misclassification. Most of the isolates of foreign origin were from countries to which tourism from Finland is common, and travellers might have already been carrying the salmonella bacteria when leaving home. The salmonella finding might have been performed after returning to Finland, and the isolate classified as foreign based on the history of recent foreign travel. Further, in many countries the rare occurrence of DT1 as an infection source may increase the rate of misclassification for this particular phage type.

Outbreaks of Typhimurium DT1 among humans in Finland involved unpasteurized milk in the 1960s and 1970s. In the 1980s, human carriers of Typhimurium DT1 appeared to spread the infection to others [6]. In 1999, an outbreak involving 120 humans was caused by homemade cheese produced from unpasteurized milk. Typhimurium DT1 was detected in both the farmer and most of his cattle [34]. The *Xba*I–PFGE profile 10 was seen in analysed isolates from this outbreak, as well as in isolates from previous DT1 outbreaks during the 1980s and 1990s.

An increase in cases of domestic human salmonellosis caused by Typhimurium DT1 has been seen in late summer and autumn since the 1960s [5, 6]. The cause of this increase is unknown, just as the source for human infections – both sporadic and endemic – is also unknown in most cases.

In conclusion, the molecular genetic types of Typhimurium DT1 were distributed among different infection sources and over a long period of time. No particular genotype can be assigned as being solely associated with a certain infection source or time. However, the strains of the major cluster, including those with the IS200 profile D and XbaI profile 10 and no virulence plasmid, can be regarded as typical of the endemic Typhimurium DT1 infection.

ACKNOWLEDGEMENTS

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