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Discrimination between endemic and feedborne *Salmonella* Infantis infection in cattle by molecular typing

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SUMMARY

Salmonella enterica serovar Infantis is endemic in Finnish cattle. Feed contaminated with *S.* Infantis was distributed to cattle farms in May 1995. Following increased sampling, *S.* Infantis was detected on 242 farms in 1995. Molecular typing was used to differentiate the farms that were infected by the feed-related Infantis from those infected by other endemic strains. Twenty-three isolates from feed in 1995 and 413 from cattle (72 from 1992–4, 324 from 1995, 17 from 1996–7) were analysed. The feed-related Infantis was clonally related to the endemic infection by the ribotype, IS*200*-type and *Xba*I-profile. The feed isolates had a distinctive plasmid that appeared in pulsed-field gel electrophoresis as a 60 kb band when cleaved with *Xba*I or linearized by S1-nuclease. This plasmid appeared in cattle only since the outbreak and seemed stable on the follow-up farms. In addition to contact farms, the feedborne strain was found on 19% of the farms infected with *S.* Infantis in 1995 but not having bought suspected feedstuffs, possibly as secondary infections.

INTRODUCTION

The principal source of human salmonella infection is contaminated food, in particular food of animal origin [1]. Animal feedstuffs are a major source of salmonella for food-producing animals [2, 3], and often responsible for the flow of salmonella into the human food chain [4]. Among factors that influence the exposure of animals to salmonella are the animal management system, method of feeding, e.g. pelleting, and amount of supplement feeding [4]. Most reported feed-related outbreaks of salmonella are associated with animals other than cattle. The source of infection is difficult to trace back since the environment rapidly becomes contaminated by the organisms shed in the faeces. Furthermore, cattle herds infected by the feed but without clinical symptoms often remain undetected [5]. Certain *Salmonella* serovars (e.g. Orion, Senftenberg, Ohio, Livingstone) are more frequently recovered in feed. Such serovars might be able to survive in feed better than others [4].

A feed-borne outbreak of *Salmonella enterica* serovar Infantis occurred on cattle farms in Finland in 1995. The contaminated feed originated from a domestic feed plant. Infantis is endemic in poultry in this country since the 1970s, and it has been the most common serovar in cattle in the 1990s, also. Following a marked increase in sampling after the distribution of the contaminated feed, the prevalence of *S.* Infantis in cattle herds rose to 0·8% in 1995. In this study, we have used molecular genetic typing to identify the feed-related genotype of *S.* Infantis and the farms that might have got their *S.* Infantis infection from the contaminated feed. For this purpose we have analysed bovine *S.* Infantis isolates from 1992–5 and feed isolates from 1995. The stability of the outbreak-

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related salmonella genotype on selected farms was followed to 1997.

MATERIALS AND METHODS

Outbreak description

Contamination of cattle feedstuffs occurred in a Finnish feed factory between the 3rd and 29th May 1995. The route of contamination could not be identified in the feed factory. For preparation of compound feeds and protein concentrates, the factory uses protein rich raw materials (e.g. soya bean meal, rapeseed meal) and Finnish grain, such as barley and oat. Five different types of pelleted feed were contaminated and approximately 800 Finnish cattle farms purchased those types of cattle feed from the factory during the suspected period. All the farms were informed of the possible contamination, and the feed was withdrawn from the market. On the farms that still had feed left, samples were taken for the analysis of *S*. Infantis. Faecal samples from cattle were also analysed.

All the salmonella isolates from animals are submitted to the National Veterinary and Food Research Institute for confirmation. From the records it was possible to find out the time of the first positive salmonella diagnosis on a farm. We were informed by the feed factory which of the *S*. Infantis farms in 1995 had contacted the company regarding possible connections between contaminated feed and the salmonella infection on the farm, and which of these farms actually had purchased feed in May 1995.

Bacterial isolates

Salmonella enterica subsp. *enterica* serovar Infantis (*S*. Infantis) isolates ($n = 436$) were obtained from the National Veterinary and Food Research Institute, Helsinki, Finland, from its regional laboratories in Oulu, Seinäjoki and Kuopio. Four feed isolates were provided by the Plant Production Inspection Centre in Vantaa, Finland.

The *S*. Infantis isolates from cattle originated from farms, slaughterhouses and slaughter transportation lorries (Table 1). Apart from carcass-samples from the slaughterhouse, the isolates were mostly faecal samples. One to four isolates per farm were analysed; if available two isolates from faecal samples were taken. The isolates represented 280 cattle farms. The samples from slaughterhouses and transportation lorries were hygiene samples and therefore not to be traced to any specific farm. The feed isolates originated from the feed production plant ($n = 5$) and cattle farms ($n = 18$).

Table 1. *Origin and number of the analysed* S. *Infantis-isolates from cattle*

Origin of isolates	Year				Total no. of isolates
	1992–4	1995	1996	1997	
Farms, faecal	28	234	5	1	268
Farms, organ	22	64	11	0	97
Slaughter house	13	2	0	0	15
Transportation lorry	9	24	0	0	33
Total no. of isolates	72	324	16	1	413
Total no. of farms	40	240	14	1	295

Most of the cattle isolates (78%, 324/413) were from 1995 (Table 1). The isolates from 1992–4 ($n = 72$) were analysed to get background information on the molecular epidemiology of the *S*. Infantis infection in cattle. For the follow-up study we analysed 17 isolates from cattle farms in 1996 and 1997. All isolates had been serologically confirmed to be *S*. Infantis and stored at −70 °C. *Escherichia coli* strains V517 (35·6, 4·8, 3·7, 3·4, 1·8, 1·4 MDa) [6] and 39R861 (98·0, 42·0, 23·9, 4·6 MDa) [7] were used as plasmid reference strains.

Preparation of PFGE-samples

Chromosomal DNA was prepared in gel blocks as described previously [8], with some modifications. Bacteria were harvested from 1·1 ml of overnight culture, 1% low melting agarose (InCert Agarose, FMC BioProducts, Rockland, ME) was used for the plugs. Lysozyme treatment for 4 h at 37 °C was followed by proteinase K for 20 h at 50 °C. The plugs were stored in 0·5 M EDTA at 4 °C.

*Xba*I and S1-nuclease digestion

The agarose plugs were dialysed against 10 mM Tris, pH 7·4, 1 mM EDTA, 50 mM NaCl before treatment. Restriction enzyme and S1-nuclease analyses were performed using slices from the same plugs. The slices were digested at 37 °C for 16–18 h with 20 units of *Xba*I in the reaction buffer supplied by the manu-

facturer (New England Biolabs, Beverly, MA). The reaction was stopped with 0·5 M EDTA, pH 8·0. Other slices from dialysed plugs were treated with 2 units of S1-nuclease to linearize the plasmids [9] at 37 °C for 45 min in the reaction buffer supplied with the enzyme (Promega, Madison, WI; code M576/1, 2), after which EDTA was added.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed using clamped homogeneous electric field (CHEF) electrophoresis (CHEF-DRIII, Bio-Rad, Melville, NY). The agarose slices were loaded into 1% agarose gels (FastLane, FMC Bioproducts, Rockland, BM) and subjected to electrophoresis in 0·5 × Tris-borate-EDTA buffer [8] for 19 h at 14 °C, pulse ramp time 2–30 sec, voltage 6 V/cm, reorientation angle 120°. The gels were stained with 0·5 μg/ml ethidium bromide and photographed using a Polaroid MP-reprocamera. Bacteriophage lambda concatamers (New England Biolabs, Beverly, MA) were used as molecular weight standards. The molecular weight of the linearized plasmids and restriction fragments was determined by plotting the distance of migration against the $\log_{10}$ of the molecular size fragments [10, 11]. DNA profiles differing by one or more DNA fragments larger than 125 kb were assigned a pulsed-field (pf) type number.

Characterization of plasmids

Plasmids were isolated by alkaline lysis methods as described [12] and analysed by agarose (0·9%) gel electrophoresis (SeaKem LE, FMC Bioproducts, Rockland, ME), 4 V/cm, for 1 h in 1 × Tris-acetate-EDTA buffer [10]. The gels were stained with 0·5 μg/ml ethidium bromide. *E. coli* reference strains [6, 7] were included in all isolation procedures. For restriction fingerprinting plasmid preparations were treated with 10 units of *Hin*dIII, *Sfi*I or *Xba*I restriction enzyme (New England Biolabs, Beverly, MA) and analysed in 1·0% PFGE agarose gels, with the pulse ramp time of 0·1–2·0 s, 9 V/cm, 14 °C, 120° for 3 h in 0·5 × Tris-borate-EDTA buffer.

Ribotyping and IS*200*-typing

Ribotyping and IS*200*-typing was performed essentially as described [13] with *Ban*I-digested DNA; *Eco*RI digestion (New England Biolabs, Beverly, MA) was used to confirm some IS*200*-types [13]. The IS*200* and 16S rRNA probes were labelled with DIG-11-dUTP by using a DIG-High Prime Labelling Kit (Boehringer–Mannheim GmbH). Two μg of salmonella DNA was digested with restriction endonucleases (New England Biolabs, Beverly, MA) and electrophoresed through 0·8% agarose gel in 1 × Tris-acetate-EDTA buffer. Denatured DNA was transferred to a nylon membrane (Hybond-N, Amersham International PLC, Amersham, UK) in 20 × SSC (10) and fixed to the membrane with microwaves [14]. Hybridization and detection was performed with the DIG Nucleic Acid Detection Kit (Boehringer–Mannheim GmbH).

RESULTS

Ribotyping and IS*200*-typing

Ribotypes and IS*200*-types were determined for 26 strains. These included 7 strains isolated in 1992–4 from cattle and 19 strains isolated in 1995 from 5 official feed samples, 5 feed samples from farms, 3 cattle farms that had bought feed in May 1995 and 6 other cattle farms. All had the same ribo/IS*200*-type, 1A, which at present is the predominant combination of a ribotype and IS*200*-type among the endemic *S.* Infantis isolates [13; Lindqvist N, Pelkonen S, unpublished observations]. The feed isolates were of the endemic type, too. This suggested a domestic source of contamination. Our previous observation that the *S.* Infantis infection among Finnish cattle is very homogeneous [13] was supported by the results.

Pulsed-field gel electrophoresis

To facilitate plasmid analysis and their size determination, the PFGE gel plugs were treated with S1-nuclease which linearizes plasmids. The S1-nuclease analysis showed that 70% of the isolates harboured plasmids, 20–115 kb in size (data not shown). Thus it was likely that the plasmids would interfere with macrorestriction profiles obtained with *Xba*I digestion and PFGE analysis. Since none of the isolates contained plasmids larger than 115 kb, we decided to limit our *Xba*I-PFGE analysis in the first stage to the bands larger than 125 kb.

Among the isolates analysed from the years 1992–7, we found 35 *Xba*I-macrorestriction profiles (pulsed-

Table 2. *Pulsed-field (pf) and plasmid types of* S. *Infantis isolates from cattle and feed sources, 1992–5*

Origin of isolate	Pulsed-field and plasmid type*			Total no. of	
	39	43, 44 45, 46	Other	Isolates	Farms
Official feed sample, 1995	8	0	0	8	—
Feed sample from farm, 1995	15	0	0	15	—
Contact farm, 1995†	45‡	7‡	9	61	57
Other farms, 1995§	29	5	156	190	183
Slaughter houses, 1995	2	0	24	26	—
Farms, 1992–4	0	1‖	44	45	40
Slaughter houses, 1992–4	0	0	21	21	—
Total no. of isolates	99	13	254	366	280

* The results are based on *Xba*I-PFGE profiles. The number of isolates is shown.
† Farms that had bought feed in May 1995 (data obtained from the feed factory).
‡ Total no. of farms 50, no. of isolates 52.
§ Includes farms that had contacted the feed factory in relation to the feed-borne outbreak but had not bought feed in May 1995 (data obtained from the feed factory).
‖ Shown to be unrelated to the feed-related plasmid types by fingerprinting.

field, pf-types) that differed from the most common pf-type pf1 and from each other by one or more bands larger than 125 kb. However, only 7 out of these 35 pf-types differed from pf1 by 4 or more bands, and only 10 isolates of 286 (4%) belonged to them (data not shown). These 7 pf-types can be considered independent from pf1 [11, 15]. We observed 31 pf-types among the cattle isolates from 1995. The predominant pf-type pf1 was found in all the isolates from the feed in 1995, in 74% (179/240) of the samples from the farms in 1995 and in 61% (44/72) of the samples from the farms in 1992–4 (data not shown).

When discrimination of the *Xba*I-PFGE analysed isolates was based on all visible DNA fragments, regardless of their size, the pulsed-field (pf) types could be further divided into so called plasmid subtypes. The feed samples from 1995 ($n = 23$) were all of the same plasmid type. This subtype of the main pf-type pf1 (Table 2) was designated the number 39, and it was regarded as the feed-related pf-subtype. Its *Xba*I-macrorestriction profile had an intensive band of approximately 60 kb in size. The plasmid type 39 was found on 74 cattle farms and in 23 feed samples in 1995, but not among the older samples from 1992–4 (Table 2). Neither have we detected it among isolates from Finnish broiler chickens (300 isolates, from 1983–95) and humans (42 isolates, 1985–94) (unpublished).

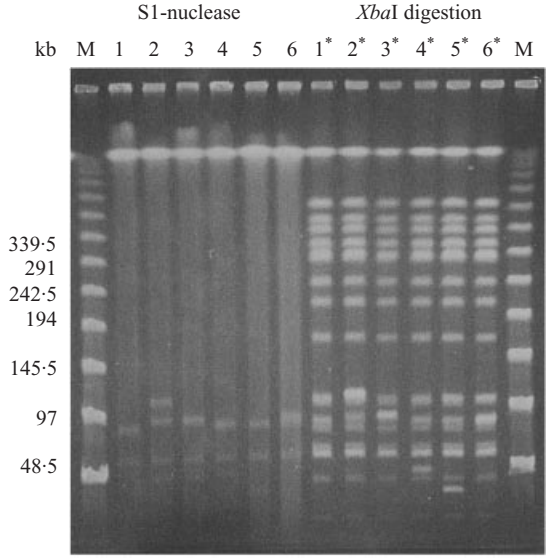

Fig. 1. Analysis of the feed-related plasmid type 39 and similar-looking plasmid types of *Salmonella* Infantis isolates in parallel by S1-PFGE and *Xba*I-PFGE. Slices of the same agarose plugs were used for both analyses. Lane M, molecular weight marker (bacteriophage lambda concatamers). Lanes 1 and 1*, plasmid type 39; lanes 2 and 2*, plasmid type 43; lanes 3 and 3*, plasmid type 44, lanes 4 and 4*, plasmid type 45, lanes 5 and 5*, plasmid type 46, lanes 6 and 6*, plasmid type 71.

Analysis with S1-nuclease showed plasmid type 39 typically to contain two plasmids, of approximately 90 and 60 kb in size (Fig. 1). According to the S1-

nuclease analysis and alkaline lysis, 69 of the 74 farm isolates of plasmid type 39 contained 2 plasmids of 90 and 60 kb. Only five of the isolates had a different plasmid profile. They all contained the 60 kb plasmid, but other plasmids of 90, 80, 70 or 40 kb as well.

Among cattle isolates from the years 1994 and 1995, we found five plasmid subtypes to the pulsed-field type pf1 that in their *Xba*I-macrorestriction profile strongly resembled plasmid type 39. No resembling plasmid subtypes were found in 1992 and 1993. The plasmid subtypes were designated numbers 43, 44, 45, 46 and 71. They all had the 60 kb band in *Xba*I-PFGE, typical of plasmid type 39, as well as other intensive bands (Fig. 1). S1-nuclease analysis showed that they shared the 60 and 90 kb plasmids with plasmid type 39, but harboured other plasmids of 105, 40 or 30 kb as well (Fig. 1).

Plasmid fingerprinting

The similarity of the plasmid types that resembled the feed-related plasmid type 39 was studied by fingerprinting of plasmid DNA. We analysed all the strains ($n = 20$; 2 from 1994, 12 from 1995 and 6 from 1996) of plasmid types 43, 44, 45, 46 and 71, and the feed isolates of plasmid type 39 ($n = 23$). The plasmids were isolated by alkaline lysis and the similarity of the plasmids was analysed with restriction fingerprinting by using *Hin*dIII, *Sfi*I or *Xba*I enzymes. The best results regarding the number of easily discriminated fragments were obtained with *Hin*dIII (Fig. 2), but the results with the other enzymes were in agreement with those of *Hin*dIII (data not shown). The plasmids of the types 43, 44 and 46 shared most of the restriction fragments with the type 39. In contrast, the isolates of the type 45 from the years 1994, 1995 and 1996 differed from each other. The isolates from 1994 and 1996 differed from the type 39, whereas the isolates of plasmid type 45 from the farms that had received the contaminated feed in 1995, shared most of their restriction fragments with the type 39. The plasmids in the type 71 were of the same size as in the type 39, but the restriction profile differed with all three enzymes from that of the type 39 (Fig. 2).

Incidence of pulsed-field types on farms

The contaminated feed had been distributed to cattle farms in May 1995. The records maintained by the Veterinary and Food Research Institute for 1995 showed the date of the first diagnosis of *S*. Infantis on the farm (Fig. 3). The feed-related plasmid type 39 was first found on one farm in April 1995. The origin of that infection remains unknown. From May onwards the number of farms with plasmid type 39 increased. A total of 74 farms with plasmid type 39 and 12 farms with the related plasmid types (43, 44, 45 or 46) were found (Table 2). Altogether 86 farms (36% of the Infantis farms) were found to be infected by the feed-related Infantis types in 1995.

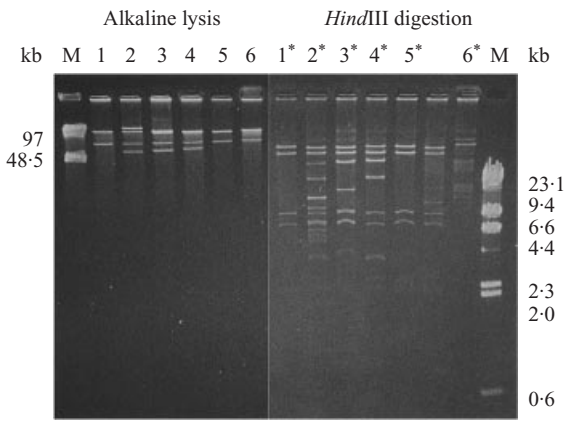

Fig. 2. Analysis of plasmids in the feed-related plasmid type 39 and similar-looking plasmid types of *Salmonella* Infantis by alkaline lysis and plasmid fingerprinting with *Hin*dIII. Lane M (alkaline lysis), molecular weight marker (bacteriophage lambda concatamers); lane M (*Hin*dIII digestion), molecular weight marker (*Hin*dIII-digested lambda DNA); lanes 1 and 1*, plasmid type 39; lanes 2 and 2*, plasmid type 43; lanes 3 and 3*, plasmid type 44; lanes 4 and 4*, plasmid type 45; lanes 5 and 5*, plasmid type 46; lanes 6 and 6*, plasmid type 71.

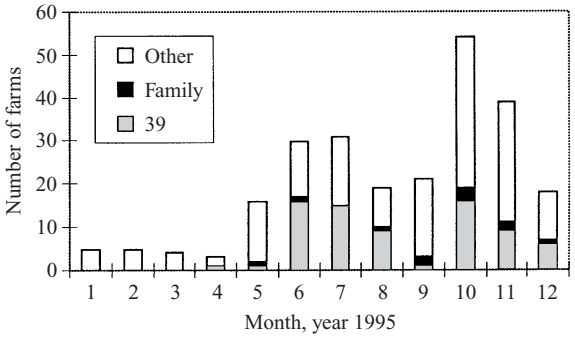

Fig. 3. Incidence of feed-related plasmid types of *Salmonella* Infantis in 1995. The bars indicate the number of *S*. Infantis-infected farms from January to December, 1995. The number 39 stands for the feed-related plasmid type 39; Family for the family of feed-related plasmid types (43, 44, 45 and 46); and Other for other pulsed-field or plasmid types.

Table 3. *Stability of the feed-related pf-type pf1, plasmid type 39 (pf 1/39) on farms*

	First isolate*		Second isolate*	
No. of farms	pf-/plasmid type	Plasmid size (kb)	pf-/plasmid type	Plasmid size (kb)
4	1/39	90, **60**	1/39	90, **60**
1	1/39	90, **60**	1/39	90, **60**
			(third) 162†	100, 90, **60**
1	1/39	90, **60**	1/43	110, 70, **60**
1	1/39	90, **60**	1/45	80, 70, **60**, 50
1	1/39	90, **60**	1/45‡	80, **60**, 50
1	1/39	90, 80, **60**, 50	143§	90, **60**, 50
1	1/44	90, **60**, 50	1/166	90, **60**, 50
1	1/46	90, **60**, 30	1/39	90, 80, **60**

* The time between the first and second isolate was 2–15 months with an average of 6 months.
† Time difference between the first isolate and † was 10 months.
‡ Time difference between the first isolate and ‡ was 15 months.
§ Time difference between the first isolate and § was 11 months.

Stability of pulsed-field types

During our study we learned that most cattle farms were cleared from the infection within 4–6 months. The stability of the 60 kb plasmid in the feed-related *S*. Infantis was followed on 11 farms. PFGE-analysis and plasmid alkaline lysis with *Hin*dIII, *Sfi*I and *Xba*I digestions was done on isolates taken at 2–15 month intervals from the farms that in 1995 were infected with plasmid type 39 or the related plasmid types 44 and 46. The analysed *S*. Infantis isolates were either from faecal samples (4 farms) or from the liver or muscle of slaughter house carcasses (8 isolates, representing 7 farms).

The 60 kb plasmid of the feed-related plasmid type 39 was stable in all the isolates ($n = 12$) and the 90 kb plasmid was stable in 75% (9/12) of them (Table 3). One farm had a pulsed-field type not seen previously (pf143). That pf-type differed from pf1 by two bands larger than 125 kb, but it still harboured the same plasmids, and an additional one, as plasmid type 39. This strain was also analysed with alkaline lysis, and it gave the same profile as plasmid type 39. One farm had a plasmid type of pf1 not previously seen (plasmid type 166). It harboured the same plasmids, and additional ones, as plasmid type 39. One farm had plasmid type 39 in one sample and a pulsed-field type not previously seen (pf162) in another. The samples were taken on different occasions. The pf-type pf162 differed from pf1 by one band larger than 125 kb and one smaller band. Plasmid analysis with S1-nuclease, alkaline lysis and *Hin*dIII, *Sfi*I and *Xba*I digestions showed that pf162 harboured the same plasmids, and additional ones as plasmid type 39.

DISCUSSION

There are only few reports on feed-borne salmonella infections in cattle. In a feed-related *Salmonella* Mbandaka-infection [5], the bacteria were recovered from the herds for less than 1 month and excreted by relatively few animals. These organisms were thought to display low virulence for cattle. In an epizootic of *Salmonella* Anatum infection in a dairy herd, the source was found to be haylage that had been contaminated by wild birds and stored improperly [16]. In a case-control study Anderson and colleagues [2] showed that the use of one particular feed mill and feeding animal fat were significant risk factors for clinical *Salmonella* Menhaden-infection. In the present study, it was known that *S*. Infantis contaminated feed had been distributed to farms. The aim was to differentiate by molecular typing of the salmonella isolates the farms that were infected by the feed-related Infantis type from those that were infected by other endemic types.

The feed-related *S*. Infantis was clonally related to the endemic infection. It resembled the endemic *S*. Infantis infection by its ribo/IS*200*-type 1A [13]. Although ribotyping and IS*200*-typing are both considered valuable tools in molecular typing of

salmonella, including *S*. Infantis [13] they do not seem to be discriminatory enough for the analysis of our endemic infection. Pulsed-field gel electrophoresis (PFGE) has often been more discriminative within salmonella serovars than IS*200*- and ribotyping [17–19; Lindqvist N, Pelkonen S, unpublished observations]. Here PFGE was not discriminatory enough, as most of the isolates from the cattle farms in 1992–5 (205 farms out of 280; 73%) and the feed isolates belonged to the predominant pulsed-field type pf1. Moreover, following the criteria of Maslow and colleagues [15] and Tenover and colleagues [11] in the interpretation of PFGE-profiles, 96% of the Infantis isolates from Finnish cattle are closely related.

Seventy percent of the Infantis isolates from cattle were found to harbour plasmids 20–115 kb in size and often visible in the *Xba*I-PFGE conditions used. Thus PFGE of *Xba*I-digested DNA proved to be very useful if combined with plasmid analysis with S1-nuclease. The feed-related isolates had in common a plasmid that appeared distinctive in *Xba*I-PFGE. Apart from one case in April 1995, this particular plasmid type 39 was not found in cattle isolates until May 1995, i.e. after the contaminated feed had already been distributed to the farms and fed to the animals. The plasmid types 43, 44, 45 and 46 from the year 1995 bore plasmids of the same size as the feed-related plasmid type 39, and also shared the plasmid restriction profile of type 39. Of the farms with these types in 1995 58% had purchased suspected feedstuffs. The switch from plasmid type 39 to 43 and 45 on the farms during the follow-up analysis (Table 3) was also observed. Based on these observations the types 43, 44, 45 and 46 were considered feed-related. The type 45 isolate from the time before the outbreak was found to be dissimilar to type 39 by fingerprinting.

Usually extrachromosomal DNA, such as plasmids, are regarded as fairly unstable genetic markers [20]. Schiaffino and colleagues [21] have shown that *Salmonella* Abortus-ovis strains with different plasmid restriction patterns had the same IS*200* hybridization pattern indicative of plasmid mobility within a genotype. Olsen and colleagues [22], on the other hand, showed that the same plasmid profile may be present in strains which are clearly different at a chromosomal level. Here the stability of the plasmids in the feed-related plasmid type was analysed on available *S*. Infantis isolates from 11 cattle farms with plasmid types 39, 44 or 46 (Table 3). The 60 kb plasmid of type 39 seems stable in this very limited material which included two farms where the pulsed-field type pf1 had changed by two bands. This change can be explained by a point mutation, and the strains can be considered closely related [11, 15]. The stability of the plasmid suggests that it may carry some useful information for the bacteria. No drug resistance was found among the feed-related isolates (data not shown), and in general, *S*. Infantis strains among Finnish cattle have been sensitive to antimicrobials [23].

Of the approximately 800 farms that had purchased feedstuffs from the factory during the suspected period, 57 farms were found to be culture positive for *S*. Infantis. The feed-related *S*. Infantis (plasmid type 39 or the related plasmid types) was present on 50 out of these farms. The seven farms with another genotype may have received the infection from another source or the feed-related salmonella strain had undergone genetic changes. Many of the culture negative farms may not have received contaminated feed at all. On the other hand, when feed samples from 26 infected farms were analysed for salmonella, only 10 of these were culture positive. Salmonella are often present in low numbers and unequally distributed in the feedstuffs, which hampers their reliable detection. In one case the animals remained salmonella negative by culture although their feed was positive.

The increase in salmonella cases (Fig. 3) seen in June and July and in October was not attributable to the feed-related infection alone. Especially subclinical cases have been detected through increased sampling. Sampling increased both after the distribution of the contaminated feed and in late autumn, when certificates of being free of salmonella were needed because of restrictions on calf distributions and the launch of an insurance programme to cover the costs of disinfection on salmonella farms. After May 1995, the number of infections caused by plasmid type 39 may have increased as a result of infections secondary to the feed-borne infection. Cattle farms that had got infected by the contaminated feed but were unaware of their infection may have spread salmonella to other farms through shared agricultural equipment, animal transport or other contacts. Among the 183 farms that had not bought the suspected feed but were found to carry *S*. Infantis during 1995, there were 34 farms (19%) that had the feed-related Infantis type. By molecular typing alone it is impossible to judge from which sources these farms had caught their infection.

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