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Distribution of Virulence Plasmids within Salmonellae

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The virulence region of the *Salmonella dublin* 50 MDa plasmid shared homology with 678 of 1021 salmonellae tested in colony hybridization experiments. The majority of *S. dublin, S. typhimurium* and *S. enteritidis* isolates tested hybridized with the region whereas, with the exception of *S. hessarek, S. pullorum* and *S. gallinarum*, other serotypes did not. Homologous virulence regions were plasmid encoded. In *S. typhimurium* a common 60 MDa plasmid was present in all phage types tested but not in DT4, DT37 and DT170. Smaller plasmids showing partial homology were found in DT12, DT18, DT193 and DT204C. In *S. enteritidis* a distinct plasmid profile for each of eight phage types was observed. Hybridizing plasmids were found in DT3, DT4, DT8, DT9 and DT11 whereas DT7, which was plasmid free, and DT10 and DT14, which harboured plasmids, did not hybridize. The extent of homology shared between *S. dublin, S. typhimurium* and *S. enteritidis* virulence plasmids was about 10 MDa and appeared conserved. Virulence plasmids from *S. typhimurium* and *S. enteritidis* did not show homology with a region of the *S. dublin* 50 MDa plasmid which was not associated with virulence functions whereas plasmids of about 24 MDa and 38 MDa in some *S. typhimurium* phage types did. The association of conserved virulence regions upon differing plasmids within salmonellae is discussed with reference to possible mechanisms of distribution and evolution of virulence genes.

INTRODUCTION

Salmonellosis is a continuing problem in man and animals, causing economic losses and public health concern (Wray, 1985). Plasmids harboured by salmonellae have been cited as encoding virulence functions. A 60 MDa plasmid harboured by several *Salmonella typhimurium* isolates (Jones *et al.*, 1982), a 50 MDa plasmid harboured by *S. dublin* (Terakado *et al.*, 1983; Baird *et al.*, 1985; Chikami *et al.*, 1985) and a 37 MDa plasmid harboured by *S. enteritidis* (Nakamura *et al.*, 1985) have been demonstrated to encode at least some virulence functions. Baird *et al.* (1985), by insertional mutagenesis with TnA, located virulence determinants encoded within a 10 MDa region of the *S. dublin* and *S. typhimurium* plasmids. These regions shared sequence homology. Furthermore, Williamson *et al.* (1988) demonstrated that sequences common to the virulence region were found in a number of other Salmonella serotypes and these sequences were functionally interchangeable after interserotype transductions.

Certain Salmonella serotypes and indeed some subgroups within specific serotypes are prevalent in animals. For example, 95% of cattle infections are attributable to *S. dublin* and *S. typhimurium* DT204C (Wray *et al.*, 1987). Whilst much importance is attached to biotyping (Duguid *et al.*, 1975), phage typing (Barker *et al.*, 1980) and plasmid profiling (Brunner *et al.*, 1983; Holmberg *et al.*, 1984; Wray *et al.*, 1987) for epidemiological studies, very little is known about the distribution and spread of factors influencing pathogenicity amongst salmonellae. In order to gain clues as to the relationship between the prevalence of certain groups of salmonellae in animals and their genetic capacity for pathogenicity, salmonellae received for serological identification at the Central Veterinary Laboratory were hybridized with a 4 kb fragment taken from within the virulence region of the *S. dublin* 50 MDa plasmid. Furthermore, to investigate

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the extent of homology shared between plasmids from different serotypes, sequences flanking the 4 kb virulence probe were used to hybridize Southern transfers of restriction digests of representative plasmids. As far as the mechanism of distribution of homologous virulence regions is concerned, an active spread by mobile vectors such as conjugative plasmids, bacteriophages and transposons, or a passive mechanism of evolution from a common ancestor, may be involved (Farrar, 1983). Assuming that presence or absence of homologous sequences on the plasmids carrying the virulence region might indicate a common evolution or an active spread respectively, the salmonellae were also hybridized with a non-virulence region derived from the *S. dublin* 50 MDa plasmid. This paper presents our initial findings.

METHODS

Bacterial strains, plasmids and plasmid vectors. These are listed in Table 1. *Escherichia coli* strains were stored in brain heart infusion broth supplemented with 15% (v/v) glycerol at −70 °C, after Silhavy *et al.* (1984). Salmonella strains were stored on Dorset egg slopes (PM5p; Oxoid) at room temperature. Bacteria were grown in complete medium (Alaeddinoglu & Charles, 1979) supplemented as described in the text.

Conjugation. The membrane filter mating described by Willetts (1984) was used.

Transposon tagging and curing of plasmids. In order to make a plasmid-free *S. dublin* derivative, the 50 MDa plasmid of *S. dublin* 1447/86 was tagged with Tn*5*.751 using the methods of Rella *et al.* (1985). Curing of the tagged plasmid was done by SDS and acridine orange treatment as described by Tomoeda *et al.* (1968) and Salisbury *et al.* (1972), respectively, and searching for kanamycin-sensitive clones by replica plating.

DNA manipulations. Crude plasmid DNA for profiling and Southern transfers was prepared by the method of Birnboim & Doly (1979). Plasmid DNA for cloning and detailed restriction mapping was further purified by centrifugation to equilibrium in a CsCl/ethidium bromide gradient (Maniatis *et al.*, 1982) for 5 h at 20 °C in a Beckman TL100 benchtop ultracentrifuge. CsCl and ethidium bromide were removed by the method of Grinsted & Bennett (1984). The method of Dazins *et al.* (1985) was used to prepare whole-cell DNA. Restriction digests,

Table 1. *Strains, plasmids and plasmid vectors*

Strain, plasmid, vector	Properties	Source or reference
Salmonella strains		
Wild-type salmonellae	Field isolates	Central Veterinary Laboratory
S. dublin 1447/86	Field isolate from bovine salmonellosis	Central Veterinary Laboratory
S. dublin 1447/86(pME9)		This work
S. dublin 1447/86 (p::Tn*5*.751)	50 MDa virulence plasmid tagged with Tn*5*.751	This work
S. dublin 1447/86 p^-	Plasmid-free derivative	This work
E. coli K12 strains		
ED8654	F^- *metB supE supF hsdM*$^+$ *hsdR*	Borck *et al.* (1976)
39R861	Strain carrying reference plasmids of known size	E. J. Threlfall, Colindale
DH5 alpha	F^- *endA1 hsdR17* (r^-m^-) *supE44 thi-1 recA1 gyrA96 relA1* ϕ80d*lacZ* M15	Gibco-BRL
Plasmids		
pME9	Cb Tc IncP1 Tra Rep(ts)::Tn*5*.751 Km Tp harboured in ED8654	Rella *et al.* (1985)
pVW1	pBR322::Tn*5*; source of *Xho*I fragment encoding kanamycin resistance	Hirsch *et al.* (1986)
pVW22	pUC8 + 4·0 kb *Hin*dIII fragment from *S. dublin* 50 MDa plasmid	This work
pVW23	pUC8 + 5·6 kb *Hin*dIII fragment from *S. dublin* 50 MDa plasmid	This work
pVW25	pUC8 + 15 kb *Xho*I fragment from *S. dublin* 50 MDa plasmid inserted at the *Sal*I site	This work
pVW26	pUC8 + 16 kb *Xho*I fragment from *S. dublin* 50 MDa plasmid inserted at the *Sal*I site	This work
pVW27	47 kb *Xho*I fragment ligated with the internal *Xho*I fragment of Tn*5* (pVW1) encoding kanamycin resistance	This work

agarose gel electrophoresis, electroelution of DNA fragments, ligations and transformations were carried out as described by Maniatis *et al.* (1982).

Hybridization. Nylon filters (Amersham) were used. Lysis of colonies onto filters was as described by Mainil *et al.* (1985). Dot blots and Southern transfers were as described by Maniatis *et al.* (1982). DNA was fixed to filters by placing air-dried filters wrapped in Saran Wrap (Dowex Corp.) inverted onto a UV transilluminator for 4 min exposure at 302 nm wavelength. Prehybridization for at least 1 h was at 68 °C in 6 × SSC (1 × SSC is 0·15 M-NaCl, 0·015 M-sodium citrate, pH 7), 5 × Denhardt's solution [1 × Denhardt's is 0·02% (w/v) bovine serum albumin, 0·02% (w/v) Ficoll, 0·02% polyvinylpyrrolidone] and 0·5% (w/v) SDS containing sonicated denatured salmon sperm heterologous DNA (20 µg ml^{-1}; Sigma). Probe DNA labelled to 1 × 10^9 c.p.m. μg^{-1} using the random sequence hexanucleotide labelling system of Feinberg & Vogelstein (1983) following the manufacturer's recommendations (Multiprime, Amersham) was heat denatured (100 °C for 2 min) and added to the filters (10 ng probe DNA per filter) in fresh hybridization solution. Hybridization was overnight at 68 °C. Filters were washed at 68 °C in 2 × SSC (2 × 15 min), 2 × SSC, 0·1% SDS (30 min), and 0·1 × SSC (30 min) sequentially. Autoradiography of filters was for 30 min to 4 h at −70 °C. Probe was removed by washing in 0·4 M-NaOH at 65 °C for at least 30 min followed by washing in 0·1 × SSC, 0·1% (w/v) SDS, 0·2 M-Tris/HCl pH 7·5 at 45 °C for 30 min.

RESULTS

Preparation of probes

Digestion of the 50 MDa plasmid of *S. dublin* 1447/87 with various restriction endonucleases gave fragments consistent with those predicted from the map of the 50 MDa plasmid of *S. dublin* 2229 (Baird *et al.*, 1985; A. Lax, personal communication). A detailed restriction map of the 50 MDa plasmid with which we agree is to be published elsewhere (A. Lax, personal communication) and, therefore, the plasmids in *S. dublin* strains 2229 and 1447/87 were considered similar, if not identical. In order to isolate DNA fragments from the 50 MDa plasmid for use as probes, *Hin*dIII fragments A–I (see Fig. 1) were cloned in pUC8 (Vieira & Messing, 1982). The choice of *Hin*dIII was made because nine fragments of suitable size (1·5–18 kb) and distribution were generated, of which one, about 4 kb in size, was located centrally within the virulence region identified by Baird *et al.* (1985). Potential probes were hybridized against subcloned fragments encompassing discrete regions of the 50 MDa *S. dublin* 1447/87 plasmid to verify site of derivation and to test for repetitive sequences. In these experiments, *Hin*dIII fragments separately were hybridized with Southern transfers of *Hin*dIII, *Eco*RI and *Sal*I digests of pVW25, pVW26 and pVW27. *Hin*dIII fragments F and G hybridized with predicted fragments only, confirming their derivation and suggesting that they lacked repeated sequences. Neither fragment F nor fragment G hybridized with whole-cell DNA of a plasmid-free derivative of *S. dublin* 1447/87 (see Methods).

Distribution of S. dublin 50 MDa plasmid sequences amongst salmonellae

Baird *et al.* (1985) demonstrated the presence of a common 10 mDa virulence region encoded by large but dissimilar plasmids in *S. dublin* 2229 and *S. typhimurium* M242. Williamson *et al.* (1988) demonstrated by colony hybridization experiments that sequences homologous to the virulence region were present in a further nine serotypes. Questions arise as to how widely distributed the virulence region is amongst salmonellae and, furthermore, what is the degree of association between the virulence region and the *S. dublin* plasmid in salmonellae other than *S.*

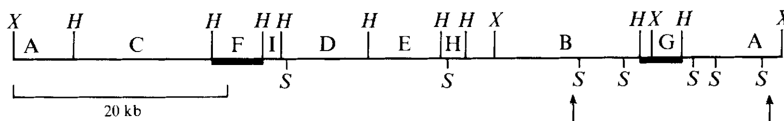

Fig. 1. Restriction map of the 50 MDa plasmid from *S. dublin* 1447/86 showing the *Hin*dIII (*H*), *Sst*I(*S*) and *Xho*I (*X*) sites. The extent of the virulence region as determined by Baird *et al.* (1985) is marked by arrows and the regions used for probe experiments are highlighted as thick lines. All *Hin*dIII fragments are designated A to I according to size, with fragment A the largest and fragment I the smallest.

Table 2. *Hybridization reaction of S. typhimurium isolates classified by phage type probed with S. dublin 50 MDa plasmid HindIII fragments F and G*

Phage type tested*	Isolates in year 1986† No.	Isolates in year 1986† %	No. tested for DNA homology by hybridization	Probe reactions F^-G^-	F^+G^+	F^-G^+	F^+G^-
1			3	0	0	3	0
2			1	0	0	1	0
6			2	0	0	2	0
8			1	0	0	1	0
9	44	1·75	8	0	0	8	0
12	121	4·82	50	3	3	44	0
13			1	0	0	1	0
18			5	0	0	5	0
41			3	1	0	2	0
44			2	0	0	2	0
49	317	12·64	55	3	0	52	0
49A			8	0	0	8	0
69			1	0	0	1	0
99			3	0	3	0	0
103			11	0	0	11	0
104	54	2·15	34	0	0	34	0
107			4	0	0	4	0
108			2	0	0	2	0
114			3	0	3	0	0
141			4	0	0	4	0
195			2	0	0	2	0
204	158	6·30	10	0	5	5	0
204A			8	0	0	8	0
204C‡	850	33·9	46	0	20	26	0
208			4	0	0	4	0
10	35	1·40	10	9	0	1	0
40			23	11	0	12	0
66			6	5	0	1	0
110	39	1·55	34	7	0	27	0
146			6	1	2	1	2
160			12	4	0	8	0
193	190	7·58	21	4	7	10	0
4			2	2	0	0	0
37			2	2	0	0	0
170	61	2·43	17	17	0	0	0

* Representative isolates of all phage types were not tested.
† Number of isolates of phage types not given if less than 1% incidence amongst *S. typhimurium*.
‡ All 204C isolates were of cattle origin.

dublin. To address these questions, *Hin*dIII fragments F and G were used as probes in colony hybridizations with 1021 Salmonella isolates taken from the 1986 Salmonella collection at the central Veterinary Laboratory.

Sequences homologous with the virulence region (*Hin*dIII fragment G) were widespread amongst those salmonellae tested (678/1021); 219 of 233 *S. dublin* isolates tested showed homology, as did 67 of 72 *S. enteritidis* isolates and 333 of 404 *S. typhimurium* isolates (Table 2). *S. hessarek* (1/1), *S. gallinarum* (2/2) and *S. pullorum* (3/3) showed homology, whereas the following did not (number of isolates tested in parentheses): *S. agama* (15), *S. agona* (17), *S. albany* (1), *S. amager* (1), *S. anatum* (18), *S. arizonae* (1), *S. bareilly* (1), *S. berta* (1), *S. binza* (1), *S. bovis-morbificans* (2), *S. brandenburg* (1), *S. bredeney* (11), *S. cerro* (1), *S. cholerae-suis* var. *kunzendorf* (1), *S. cubana* (1), *S. derby* (13), *S. durham* (1), *S. give* (11), *S. goldcoast* (1), *S. haifa* (1), *S. hardar* (10), *S. havana* (1), *S. illinois* (1), *S. indiana* (14), *S. infantis* (12), *S. kedougou* (12), *S. kentucky* (1), *S. livingstone* (11), *S. liverpool* (1), *S. london* (1), *S. manhattan* (1), *S. mbandaka* (11), *S. meleagridis* (2), *S. montevideo* (15), *S. muenchen* (1), *S. munster* (1), *S. newport* (28), *S. poona* (1),

S. ohio (2), *S. oranienberg* (3), *S. saint-paul* (5), *S. schwarzengrund* (1), *S. senftenberg* (11), *S. stanley* (17), *S. tado* (1), *S. taksony* (1), *S. tennessee* (10), *S. thielalee* (11), *S. virchow* (16), *S. worthington* (13), S4:12:D (10).

Sequences homologous with a non-virulence region (*Hin*dIII fragment F) were less widely spread amongst those salmonellae tested (265/1021); 219 *S. dublin*, 45 *S. typhimurium* and one *S. berta* showed homology. Interestingly, of the 265 isolates positive for probe F (non-virulence), all except two were also positive for probe G (virulence); a not unexpected finding if the same plasmid was involved. Of six *S. typhimurium* DT146 isolates, two hybridized with F but not G (Table 2). This finding contra-indicated, suggesting that these regions possibly resided on separate replicons.

S. dublin 50 MDa plasmid HindIII fragments F and G hybridized with dissimilar plasmids in salmonellae other than S. dublin

To determine the location of homologous sequences, probes F and G were hybridized in separate experiments with Southern transfers of plasmid profiles of 47 *S. dublin* and 71 non-*dublin* salmonellae. The results are considered below.

Significant among *S. typhimurium* infections of cattle is phage type DT204C; 52% of our isolates in 1986 were of this phagetype. Wray *et al.* (1987) have differentiated DT204C isolates on the basis of plasmid profile patterns. Southern transfers of one representative of each of 24 established plasmid profiles were hybridized in separate experiments with probes F and G. The virulence region (probe G) shared homology with a plasmid of about 60 MDa in each of 23 isolates and of about 50 MDa in one isolate. Other plasmids which were present in the profiles, including the 120 MDa *incH2* multiple resistance plasmid (Threlfall *et al.*, 1986) present in all DT204C isolates, did not hybridize with the virulence region. Probe F hybridized with 10 of the 24 profiles. The plasmids sharing homology were of approximately 38 MDa in six of the strains and 24 MDa in four.

Other prevalent *S. typhimurium* isolates in 1986 belonged to phage types DT9, 10, 12, 49, 104, 110, 170 and 193 (Table 2). The virulence region (probe G) again shared homology with a large plasmid of about 60 MDa in those *S. typhimurium* isolates positive in colony hybridization tests. Most *S. typhimurium* isolates had several other plasmids but these did not hybridize with the virulence region. One isolate of phage type DT18 harboured a hybridizing plasmid of about 90 MDa. Probe F shared homology with plasmids of about 24 MDa harboured in 3/3 *S. typhimurium* DT99 and of about 38 MDa harboured in 1/3 *S. typhimurium* DT12 and 1/3 *S. typhimurium* DT146 isolates. Hybridization of probe F with other plasmids was not observed.

S. enteritidis is of increasing concern in man and animals as a major cause of salmonellosis and, in particular, poultry are considered a common source of human infection. In our experiments, of 24 *S. enteritidis* plasmid profiles tested by Southern hybridization (Fig. 2), the virulence region hybridized with one discrete large plasmid harboured in isolates of phage types DT3, DT4, DT8, DT9 and DT11. Interestingly, phage types DT10 and DT14, each of which harboured a single large plasmid, and phage type DT7, which was plasmid-free, failed to hybridize even at lower stringencies. Within any one phage type the same size of plasmid was repeatedly observed but between phage types there was considerable size variation. Plasmids of approximately 35 MDa were found in DT4, of 55 MDa in DT3, DT9, DT11 and DT14, and of 60 MDa in DT8, whereas an untypable strain harboured two plasmids of 40 MDa and 60 MDa, both of which hybridized (Fig. 2). Probe F did not hybridize with any *S. enteritidis* plasmids.

Of 47 *S. dublin* isolates positive in colony hybridization tests taken from British outbreaks of calf salmonellosis occurring at different geographical locations during 1986, all harboured a single 50 MDa plasmid which hybridized with both probes. Furthermore, they all shared a common restriction pattern identical to *S. dublin* 1447/87 (see above). Isolates of continental origin dating back to 1972, however, showed variation in size and restriction pattern (unpublished) but, importantly, hybridized with probe G only.

Virulence plasmids in S. dublin, S. typhimurium and S. enteritidis share extensive homology

The question arose as to the extent of homology shared between plasmids hybridizing with the virulence region probe. Baird *et al.* (1985) demonstrated a tract of DNA covering the virulence

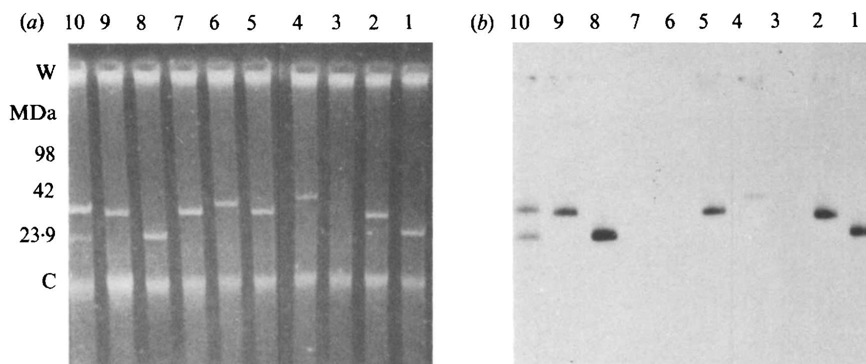

Fig. 2. (*a*) Plasmid profiles of ten *S. enteritidis* isolates of animal origin belonging to phage types DT4 (lane 1), DT3 (lane 2), DT7 (lane 3), DT8 (lane 4), DT9 (lane 5), DT10 (lane 6), DT14 (lane 7), DT4 of human origin (lane 8), DT11 (lane 9) and an untypable strain (lane 10). The distances migrated of plasmids of known molecular mass are marked. Wells are marked 'W' and chromosomal DNA is indicated by 'C'. (*b*) Autoradiograph of a Southern transfer of the plasmid profiles from (*a*) after hybridization with probe G, the virulence region of the *S. dublin* 50 MDa plasmid.

region of about 10 MDa in size common to the 50 MDa plasmid of *S. dublin* 2229 and to the 60 MDa plasmid of *S. typhimurium* 1275 as judged by restriction endonuclease digest patterns and limited hybridization experiments. To extend these findings, plasmids from 30 *S. typhimurium* isolates of phage types DT12, DT18, DT99, DT104, DT193 and DT204C, and from 7 *S. enteritidis* isolates of phage types DT3, DT4, DT8, DT9, DT10, DT11 and one untypable strain (harbouring two hybridizing plasmids), were digested with *Hin*dIII and *Sst*I together, transferred to filters and hybridized with virulence region probes. The choice of *Hin*dIII/*Sst*I cuts was made to aid comparison with the known map of the *S. dublin* 50 MDa plasmid (Fig. 1). In each test, when *Hin*dIII fragment G alone was used as probe, a single hybridization signal was located to a fragment with a mobility of about 4 kb identical to that of fragment G of *Hin*dIII-cut *S. dublin* 50 MDa plasmid. Here was evidence of conservation of this particular sequence between serotypes. After verification that vector pUC8 DNA did not hybridize with plasmids of *S. dublin*, *S. typhimurium* and *S. enteritidis*, linearized pVW25 and pVW26 together were labelled and used to probe the same filters (Fig. 3). Consistent hybrization patterns were observed for all *S. enteritidis* phage types tested, irrespective of the size of the plasmid in any one phage type. Fragments showing homology were about 1·8, 4·0, 6·5, 7·5, 8·5 and 12 kb in size. Consistent hybridization patterns were also observed for all *S. typhimurium* phage types tested. Fragments showing homology were about 1·8, 4·0, 6·0 (weak signal), 6·5, 7·5, 8·5 and 14 kb in size. In view of the high stringency of the hybridization procedure and the commonality between *S. dublin*, *S. typhimurium* and *S. enteritidis* of hybridizing fragments of about 1·8, 4·0 and 8·5 kb, a high degree of conservation of DNA associated with virulence functions is strongly supported.

DISCUSSION

Virulence regions on plasmids in salmonellae have been described previously (Jones *et al.*, 1982; Terakado *et al.*, 1983; Baird *et al.*, 1985; Chikami *et al.*, 1985; Williamson *et al.*, 1988). We have shown these regions to be widespread amongst *S. dublin*, *S. typhimurium* and *S. enteritidis*. Elimination of these plasmids does not cause absolute loss of virulence (Baird *et al.*, 1985). Also, other prevalent serotypes such as *S. anatum*, *S. indiana*, *S. montevideo* and *S. virchow* lack these virulence regions. This strongly supports the contention that virulence of salmonellae is multifactorial and different mechanisms of pathogenesis are encoded by different groups of Salmonella.

Helmuth *et al.* (1985) suggested that a given serotype would contain a serotype-specific virulence plasmid in the majority of isolates. Over 90% of *S. dublin* isolates tested hybridized with the virulence region. It is possible that the 50 MDa virulence plasmid was lost from some

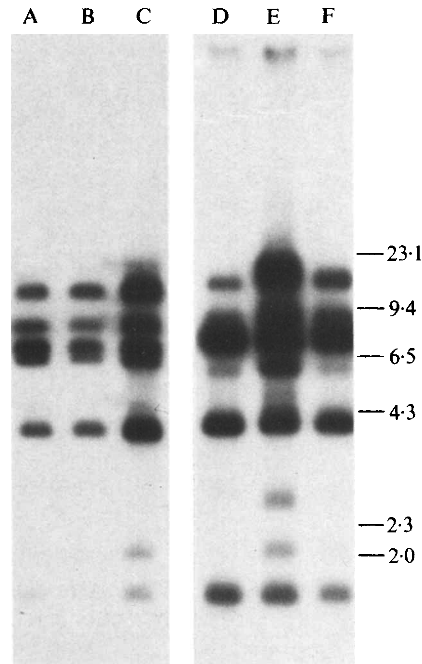

Fig. 3. Autoradiograph of ^{32}P-labelled linearized pVW25 and pVW26 DNA probed against Southern transfers of *Hin*dIII/*Sst*I digests of plasmid DNA extracted from *S. enteritidis* phage types DT3 (lane A), DT4 (lane B) and DT8 (lane C), and from *S. typhimurium* phage types DT104 (lane D), DT204C profile E (lane E) and DT99 (lane F). The sizes (kb) of λ *Hin*dIII fragments are marked.

isolates upon storage or that plasmid-free strains were relatively common. Whether those non-hybridizing *S. dublin* isolates were as virulent as those which hybridized is open to speculation. No differences between restriction pattern of the common 50 MDa plasmid in *S. dublin* of cattle origin were observed, suggesting conservation of this plasmid and possible clonal spread of *S. dublin* in Great Britain. The majority of *S. typhimurium* phage types conform to the hypothesis of Helmuth *et al.* (1985); some phage types, notably DT104, showed 100% carriage of the virulence plasmid whilst phage type DT40 showed 50% carriage and phage type DT170 showed 0% carriage. The question arises as to the fundamental differences between DT170, DT40 and other *S. typhimurium* phage types such that the virulence plasmid appears to be dispensable in one group but obligatory in another group. Each phage type of *S. enteritidis* tested, with the exception of DT7, possessed a single large plasmid the size of which correlated with phage type. However, only plasmids in phage types DT3, DT4, DT8, DT9 and DT11 hybridized with the virulence region whereas plasmids in DT10 and DT14 did not. Whether the non-hybridizing plasmids encode distinct virulence functions is open to speculation.

Similarity of plasmid size in any one serotype or phage type does not necessarily imply physical or genetic relatedness. However, probes F and G, each from physically well separated regions of the *S. dublin* plasmid and comprising some 9 kb in total, hybridized with 50 MDa plasmids in each of 47 *S. dublin* isolates, implying conservation. Interestingly, homology with probe F was shown by 38 MDa plasmids in *S. typhimurium* phage types DT12 (1/3), DT146 (1/3) and DT204C (6/24) and by 24 MDa plasmids in *S. typhimurium* DT99 (3/3). Here was evidence of disassociation between sequences contiguous on the *S. dublin* 50 MDa plasmid.

Southern blots of *Hin*dIII/*Sst*I cuts of virulence plasmids from *S. typhimurium* and *S. enteritidis* showed considerable homology with the *S. dublin* 50 MDa plasmid and conservation of restriction fragments around the virulence probe G region. Williamson *et al.* (1988) demonstrated interserotype transduction of functional virulence regions between *S. dublin* and *S. typhimurium*, thus extending the findings of Baird *et al.* (1985) that virulence regions in these

two serotypes were structurally very similar. What is surprising is the similarity of virulence regions of *S. dublin* and *S. typhimurium* with those on plasmids in *S. enteritidis*, in view of their considerable variation in size and restriction pattern (Williamson *et al.*, 1988; unpublished observation). Relatively few *S. enteritidis* plasmids have been analysed in detail and there may be a number of subsets of plasmids sharing homology; phage types DT3, DT4, DT8, DT9, DT11 and perhaps some others belong to one subset related to the *S. dublin* and *S. typhimurium* virulence plasmids. Whilst the Southern blot data indicate significant homology between virulence regions of *S. dublin*, *S. typhimurium* and *S. enteritidis* it is possible that only small regions within any hybridizing fragment shared the homology. Furthermore, considering the size of the probes pVW25 and pVW26, repeated sequences are likely to contribute to apparent homology. Whether the hybridizing sequences are contiguous is unknown.

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