Epidemiol. Infect. (1993), **111**, 1–8

SPECIAL ARTICLE

Interrelationships between strains of *Salmonella enteritidis*

INTRODUCTION

The decade from 1980 witnessed the emergence of *Salmonella enteritidis* as the most commonly isolated serotype from cases of human food poisoning in England and Wales. In 1981 the Laboratory of Enteric Pathogens (LEP) identified 1087 strains of this serotype which had been isolated from humans whereas in 1991, 17460 strains were identified [1], an increase of approximately 17-fold over the 11-year period. In the current epidemic, phage typing has demonstrated that strains of *S. enteritidis* belonging to phage type (PT) 4 are most frequently isolated and in 1991, 84% of isolates belonged to this phage type [1].

Chickens are a major reservoir for this organism and underlying the human epidemic has been a widespread dissemination of *S. enteritidis* PT 4 throughout poultry flocks. Poultry products have proved important vehicles of infection, in particular poultry meat [2] and whole shell eggs [3, 4] and the latter vehicle has provided a new dimension. Although not as common as PT 4, other *S. enteritidis* PTs have also been associated with poultry. In particular, *S. enteritidis* PT 8 is known to be poultry-associated and from 1981–90 was the second most common *S. enteritidis* PT from humans in England and Wales [4]. A second phage type of increasing importance is PT 13a and in 1991, 188 of 17460 *S. enteritidis* from humans (1%) belonged to this phage type. Both *S. enteritidis* PTs 8 and 13a have been responsible for extensive outbreaks in the USA since 1988 [5] but in contrast to the situation in the UK, *S. enteritidis* PT 4 has only rarely been isolated in the USA and when isolated, has been associated with infections contracted in Europe [5]. Other phage types which have caused infections in humans in England and Wales and which have been associated with poultry include 7, 7a, 23, 24 and 30 [6]. A further *S. enteritidis* phage type which has been associated with poultry is PT 1. In contrast to *S. enteritidis* PT 4, this phage type is uncommon in England and Wales but is common in countries in Eastern Europe and in recent years has been the most frequently isolated phage type in broilers in Denmark (D. J. Brown, personal communication).

In this article we describe the methods used for the typing and differentiation of *S. enteritidis*, and the plasmid-mediated and LPS-mediated interrelationships between several of the phage types known to be associated with poultry and poultry products.

TYPING METHODS

Phage typing

Phage typing is used to subdivide strains within a particular serotype and phage typing schemes have been developed for the most common salmonella serotypes in the UK, namely *S. enteritidis*, *S. typhimurium* and *S. virchow*, and also for other serotypes of clinical and epidemiological importance such as *S. typhi* [7]. The

development in LEP of a phage typing scheme for *S. enteritidis* [8] has provided an invaluable means of differentiating strains of this organism, and has proved particularly useful for epidemiological studies. The phage typing scheme of Ward and colleagues [8] has recently been extended by the addition of a further 4 phages and currently recognizes 44 different phages types [6]. Using this extended scheme, it has been demonstrated that in addition to PT 4, strains of *S. enteritidis* belonging to PTs 7, 7a, 8, 13, 13a, 23, 24 and 30 have also been associated with poultry and poultry products [6, 9, 10].

Plasmid typing

Plasmid typing is a method of strain discrimination based on the numbers and molecular weights (MWs) of carried plasmids and the resultant 'plasmid profile' of a strain can be used to supplement the more traditional methods of strain identification. The technique has proved useful for the discrimination of serotypes such as *S. gold-coast* [11] and *S. berta* [12] for which phage typing schemes are not available, and also for the differentiation of certain phage types of *S. typhimurium* [13]. However, when applied to *S. enteritidis* PT 4, of 247 strains examined which were isolated between 1981 and 1987, only 9 plasmid profile types were identified. Moreover, 78% of isolates of this phage type were characterized by a single plasmid of 38 MDa and belonged to one profile type designated SE38 [14].

Plasmid fingerprinting

Fingerprinting of plasmids using a variety of restriction endonucleases is a technique which may extend the results of plasmid profile typing [15]. However, fingerprinting of plasmids in the type strains of the *S. enteritidis* phage types, using the restriction endonucleases *Hind* III and *Pst* I has demonstrated a remarkable degree of homogeneity amongst the plasmids of 38 MDa, identified in 18 of the 27 phage type strains studied [16]. Restriction fragment length polymorphism was only observed in the 38 MDa plasmid in the type strain of *S. enteritidis* PT 15 and it was concluded that for *S. enteritidis*, plasmid fingerprinting was unlikely to significantly extend the degree of discrimination achieved by plasmid profile typing.

Drug resistance typing

In contrast to *S. typhimurium* and *S. virchow*, the second and third most prevalent serotypes from humans in England and Wales between 1981 and 1990 [17], antimicrobial drug resistance is uncommon in *S. enteritidis*. In 1990 only 11% of isolates of *S. enteritidis* from humans were drug-resistant and 1% multiresistant (= resistance to 4 or more antimicrobials) [18] and these figures have not changed significantly since 1981 [19]. Drug resistance is particularly uncommon in *S. enteritidis* PT 4 and differentiation of this phage type on the basis of antibiogram has provided only a limited degree of discrimination. Nevertheless, subdivision by antibiogram has proved useful in some instances, in for example, the identification of an outbreak of *S. enteritidis* PT 4 of R-type CSSuTTm (C, chloramphenicol; S, streptomycin; Su, sulphonamides; T, tetracyclines; Tm, trimethoprim) in the City of London in 1990 [18].

Since 1981 the only resistances to have increased significantly in incidence in *S. enteritidis* have been ampicillin (A), S and T [18, 19]. For the most part

these increases have been caused by the appearance and spread of a strain of *S. enteritidis* belonging to PT 24 and resistant to these three antimicrobials (= R-type AST) [20]. Genetic studies have demonstrated that *S. enteritidis* PT 24 of R-type AST could be derived from *S. enteritidis* PT 4 following the acquisition of a plasmid of approximately 34 MDa which coded for AST and for resistance to some of the *S. enteritidis* typing phages. The plasmid belonged to incompatibility group (*Inc*) N and the plasmid-mediated phage restriction property was responsible for the change in phage type from *S. enteritidis* PT 4 to *S. enteritidis* PT 24 [20].

In 1990, in the few strains of *S. enteritidis* with multiple antimicrobial resistance, the most common patterns were ASTFu (Fu, furazolidone) and CSSuTTm. Strains of R-type ASTFu belonged to the drug-resistant clone of *S. enteritidis* PT 24 described above but had acquired additional chromosomally-encoded resistance to furazolidone. In contrast the few strains of R-type CSSuTTm belonged to *S. enteritidis* PT 4 and were associated with the outbreak in the City of London mentioned above [18].

In 1991 a small number of strains of *S. enteritidis* belonging to PT 23 and of R-type AS were identified. Like the strains of *S. enteritidis* PT 24 described above, these strains of *S. enteritidis* PT 23 were also found to possess an *Inc* N plasmid of 34 MDa which coded for resistance to ampicillin and streptomycin, but not to tetracyclines [6].

LPS typing

Most strains of *S. enteritidis* express long-chain lipopolysaccharide (LPS) which gives a 'ladder' pattern in SDS-PAGE gels [9]; however, certain strains of *S. enteritidis*, such as those of PTs 7 and 23 do not express long-chain LPS [9, 21]. Strains of *S. enteritidis* belonging to PT 30 express either trace amounts of LPS or no O-antigen [10]. Strains of *S. enteritidis* belonging to PT 7a comprise a group of organisms which either express long-chain LPS, or do not express LPS [6] (Fig. 1). Strains of *S. enteritidis* belonging to PT 30 are unusual in expressing trace amounts of long-chain LPS, or no LPS [10].

Virulence properties

The 38 MDa plasmid carried by the majority of strains of *S. enteritidis* [21] has been shown to encode mechanisms which are essential for establishing experimental infections in mice [21, 22]. Because of its widespread distribution in strains of *S. enteritidis* from widely separated geographical areas, this 38 MDa plasmid has been designated the *S. enteritidis* 'serotype-specific' plasmid (SSP) [21]. Spontaneous loss of the SSP from strains of *S. enteritidis* PT 4 results in a million-fold increase in the Lethal Dose-50 (LD_{50}) [22]. Although the mechanisms involved in mouse virulence have not been determined, much speculation has focussed on the ability of SSP-carrying strains to survive intracellularly in mouse phagocytes.

The *S. enteritidis* SSP has classically been termed the 'virulence' plasmid [21]. However, recent studies have demonstrated that although this plasmid is undisputably involved in the virulence of *S. enteritidis* for mice, it does not appear to be involved in the pathogenicity of the organism for poultry [23]. Furthermore, strains of *S. enteritidis* carrying the SSP are only pathogenic for strains of inbred

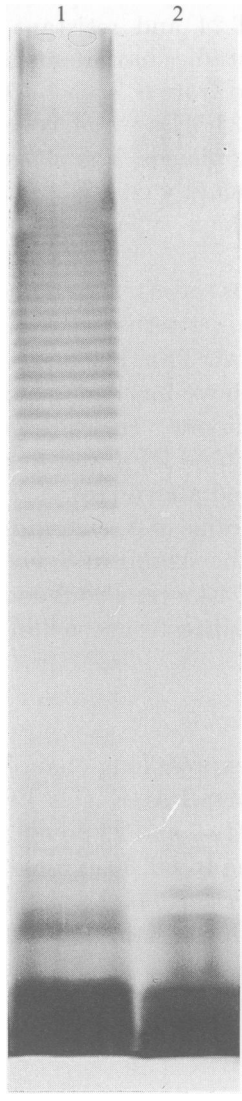

Fig. 1. SDS-PAGE of *S. enteritidis* LPS stained with silver. Certain strains of *S. enteritidis*, for example those belonging to PT 4, expressed long-chain LPS which migrated to give a typical 'ladder' pattern (lane 1). Other strains of *S. enteritidis*, for example those of PT 7, did not express LPS and only core-LPS, migrating with the dye-line, was observed (lane 2).

mouse, for example BALB/c, which do not evoke an immune response to bacterial LPS [24]. It is also noteworthy that strains of *S. enteritidis* PT 4 which do not carry the SSP have been isolated from humans with gastroenteritis, and an SSP$^-$ strain of *S. enteritidis* PT 4 has been identified as the causative agent in a hospital outbreak of food-poisoning in Wales in 1990.

DNA–DNA hybridization studies performed in several laboratories have demonstrated the existence of considerable homology in the SSPs of *S. enteritidis*, *S. typhimurium*, *S. dublin* and *S. cholerae-suis* [25, 26, 27] and the regions responsible for virulence in the *S. typhimurium*, *S. dublin* and *S. cholerae-suis* SSPs

have been sequenced [28, 29, 30]. In these plasmids the genes responsible for virulence for BALB/c mice, now designated the *S*almonella *p*lasmid *v*irulence (*Spv*) genes, are encoded on a 3·5 kb *Hin*d III fragment which has been shown to be carried on 38 MDa plasmids found in 18 of the *S. enteritidis* phage type strains, on 59 MDa plasmids identified in 6 of the phage type strains and on a 45 MDa plasmid found in the type strain of *S. enteritidis* PT 19 [16].

The ability of strains of *S. enteritidis* to express LPS has also been recognized as a virulence property. Strains of *S. enteritidis* which express long-chain LPS and carry the SSP have an LD_{50} for BALB/c mice of between 10 and 20 bacteria [22]; in contrast, strains of *S. enteritidis* which carry this plasmid but are unable to produce LPS are avirulent for BALB/c mice [22].

Strains of *S. enteritidis* have been shown to express an enterobactin-mediated iron uptake system [22, 31]. The possession of a high affinity iron sequestering system makes strains able to compete with chelators, such as egg ovotransferrin, for ferric ions [31].

IS200 typing

IS*200* is a salmonella-specific insertion element first described by Lam and Roth [33] and distributed on conserved loci on the chromosome of many salmonella serotypes [34]. Certain characteristics of IS*200* favour its use as a probe for strain discrimination and using IS*200*, three 'clonal lineages', designated SEClI, SEClII and SEClIII have been identified within the chromosomes of 27 of the *S. enteritidis* phage type strains [35]. The type strain of *S. enteritidis* PT 4 belonged to SEClI and, following chromosomal digestion with the restriction endonuclease *Pst* I, was characterized by two IS*200* loci of 4·5 and 5·2 kb [35]. However IS*200* typing was unable to differentiate 29 strains of *S. enteritidis* PT 4 isolated from humans, poultry and poultry products in five countries over a 20-year period [36, 37], although some of these strains could be subdivided on the basis of plasmid profiles [37].

INTERRELATIONSHIPS BETWEEN STRAINS OF *SALMONELLA ENTERITIDIS*

The possible existence of interrelated strains of *S. enteritidis* belonging to different phage types was indicated when smooth (LPS^+) strains of *S. enteritidis* belonging to PT 4 were shown to spontaneously mutate to rough (LPS^-) strains of *S. enteritidis* belonging to PT 7 [32]. This irreversible mutation occurred at a rate of approximately 1 in 10, and converted strains of *S. enteritidis* PT 4, virulent for BALB/c mice to avirulent strains of *S. enteritidis* belonging to PT 7 [32].

Phage type conversion was also demonstrated by experiments involving plasmid transfer. Strains of *S. enteritidis* belonging to PT 4 were shown to convert to strains of *S. enteritidis* belonging to PT 24 following the acquisition of an *Inc* N drug resistance plasmid [20]. More recent studies have shown that transferring pDEP 44, a 34 MDa *Inc* N plasmid coding for resistance to A and S, to strains of *S. enteritidis* belonging to PTs 7, 7a, 8, 13, 13a, 24 and 30 demonstrated that the phage type designation of certain other phage types of *S. enteritidis* could also be altered by the acquisition of *Inc* N plasmids (Figure 2).

The acquisition of pDEP 44 by strains of *S. enteritidis* PT 4 caused these strains to convert to smooth strains of *S. enteritidis* PT 24. Acquisition of pDEP 44 also

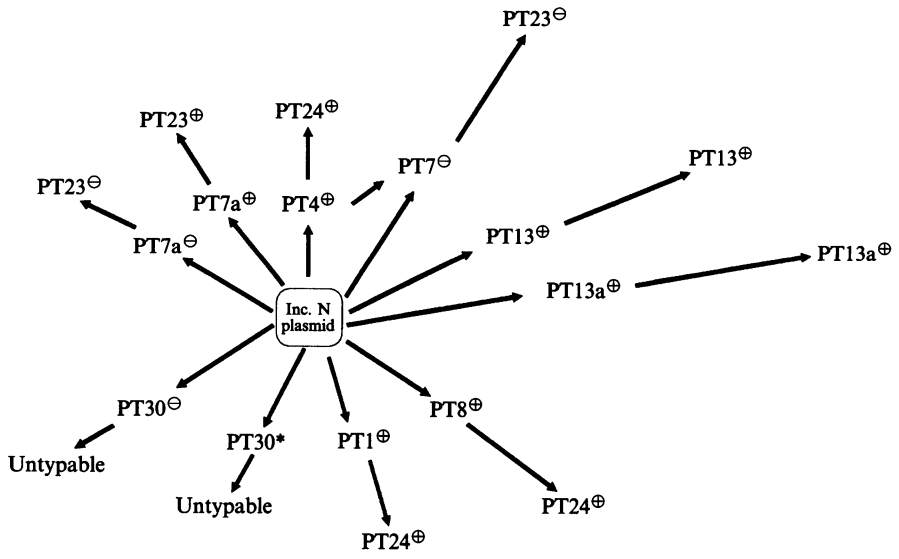

Fig. 2. Diagramatic representation showing the interactions between strains of *S. enteritidis*, and in particular the role of *Inc* N plasmids in changing phage type (PT). Strains of *S. enteritidis* belonging to PT 4 were shown to convert to strains of *S. enteritidis* belonging to PT 24 following the acquisition of pDEP 44, an *Inc* N drug resistance plasmid coding for resistance to A and S. Also, transferring pDEP 44 to strains of *S. enteritidis* belonging to PTs 1, 7, 7a, 8, 13, 13a, 24 and 30 demonstrated that the phage type designation of certain other phage types of *S. enteritidis* could also be altered by the acquisition of *Inc* N plasmids. Strains of *S. enteritidis* with long-chain LPS (⊕), with no LPS (⊖) or trace amounts of LPS (*).

resulted in the conversion of strains of *S. enteritidis* of PT 1 to PT 24 (unpublished), *S. enteritidis* of PTs 7 and 7a to *S. enteritidis* PT 23, *S. enteritidis* PT 8 to PT 24 and *S. enteritidis* PT 30 to a strain of *S. enteritidis* which did not react with the typing phages and was therefore designated untypable. The reactions of the strains of *S. enteritidis* PTs 13 and 13a with the phages which lyse strains of these phage types were lowered but the reduction in lysis was not sufficient to warrant alteration in phage type designation. Acquisition of pDEP 44 did not result in the displacement of the *S. enteritidis* SSP nor of any low MW plasmids found in some of the wild-type isolates of the respective phage types [6].

Both wild-type and exconjugant strains of *S. enteritidis* PTs 1, 4, 8, 13 and 13a into which pDEP 44 had been introduced expressed LPS. In contrast neither the wild-type strain of *S. enteritidis* PT 7, nor the exconjugant strain of *S. enteritidis* PT 23 derived from *S. enteritidis* PT 7a, expressed LPS. Strains of *S. enteritidis* PT 7a are comprised of strains which either express long-chain LPS or do not express LPS [6]. Rough and smooth strains of *S. enteritidis* PT 7a have been isolated from humans and ducks but in contrast, only rough strains of this phage type have been obtained from chickens. Transfer of pDEP 44 to rough strains of *S. enteritidis* PT 7a, result in the formation of rough strains of *S. enteritidis* which phage type as PT 23. In contrast, transfer of pDEP 44 into a smooth strain of PT 7a, results in the formation of smooth strains of *S. enteritidis* which also phage type as PT 23 and express LPS [6].

S. enteritidis PT 30 is comprised of strains which either make trace amounts of long chain LPS or do not express LPS. Regardless of whether strains express LPS

or not, acquisition of pDEP 44 results in the formation of strains of *S. enteritidis* which become untypable by phage typing. The role of pDEP 44 in changing binding sites for typing phages remains to be elucidated. The importance of long-chain LPS in determining phage type in strains of *S. enteritidis* has been reported [32]. However carriage of pDEP 44 may alter expression of LPS, although profiles so far obtained using SDS-PAGE do not suggest this.

SUMMARY

In contrast to *S. typhimurium* [19], *S. enteritidis* is a serotype which has its primary food-animal reservoir in poultry. To date, phage typing has been of paramount importance in studying the epidemiology of this serotype and in particular, has demonstrated the involvement of both poultry meat and whole shell eggs in the transmission of *S. enteritidis* PT 4 to humans. The findings discussed above describe various aspects of the serotype, particularly in relation to the involvement of both LPS and plasmids in its virulence and phage type identity (Fig. 2). These findings have led to an increased understanding of the biology of this serotype, which is of major importance in human food-poisoning in England and Wales at the present time.

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