Epidemiol. Infect. (1991), **106**, 11–16
Printed in Great Britain

Epidemiological study of *Salmonella enteritidis* strains of animal origin in Belgium

P. POHL[1], P. LINTERMANS[1], M. MARIN[1] AND M. COUTURIER[2]

[1] *Institut National de Recherches Vétérinaires, Groeselenberg 99 – B-1180, Bruxelles*
[2] *Université Libre de Bruxelles, rue des Chevaux 67 – B-1640, Rhode-St-Genèse*

(*Accepted 3 September 1990*)

SUMMARY

Since 1987, the number of cases of salmonellosis caused by *Salmonella enteritidis* has considerably increased in Western Europe. Comparison of endemic animal strains isolated in Belgium from 1976–84 with strains isolated from 1987 on shows that the strains which cause the current epidemic have no features distinguishing them from the previously-isolated strains and that furthermore, they do not constitute a bacterial clone. They belong to 13 different lysotypes and in most cases remain sensitive to antibiotics. Nevertheless, the lysotype 33 (which belongs to the phage type 4 [1] has increased significatively. It encompasses 37% of the animal strains isolated in Belgium from 1987–9, but only 7% of the strains isolated from 1976–84.

It is worth noting that the endemic as well as the epidemic strains contain a virulence plasmid sharing sequence similarities with the FIB and FIIA plasmid replicons and with the VirA and VirB virulence regions of the *S. typhimurium* virulent plasmid: pIP1350.

INTRODUCTION

Since 1987 the number of cases of salmonellosis caused by *Salmonella enteritidis* has considerably increased in Western Europe [2, 3]. This serotype occurs particularly in poultry and can infect man through contaminated food [4]. In Belgium in 1986 only 0·4% of all salmonella strains isolated from animals were of this serotype but in 1987 the proportion rose to 7% and in 1988 to 14% (Pohl, personal communication).

In considering the underlying causes of the epidemic one suggested hypothesis was that a new clone of *Salmonella enteritidis*, more pathogenic than the strains isolated the previous years, might have first appeared in Belgium in 1987.

To test this hypothesis, we have compared the lysotypes and plasmid contents of endemic strains isolated before 1987 with those of epidemic strains isolated since.

MATERIALS AND METHODS

Fifty-four *S. enteritidis* strains, 27 of which were isolated between 1978 and 1984, and 27 between 1987 and 1989 were studied. Forty-five of these strains were

Table 1. *Properties of 34 strains of* S. enteritidis *isolated from animals in Belgium*

Strain	Year	Animal	Resistance to antimicrobial agents*	Lysotype	Mol. weight of plasmid carried	Hybridization with probes†
53 S	1976	Guinea-fowl	—	39	None	None
169 D	1976	Colt	Sm Tc Cm Km Ap	39	±40 MDa	FIB; FIIA; H6; H8
696 S	1976	Chicken	—	39	±40 MDa	FIB; FIIA; H6; H8
355 Sa	1981	Horse	—	Atypical	±40 MDa	FIB; H6; H8
373 Sa	1981	Guinea-pig	—	29	±60 MDa	FIB; FIIA; H6; H8
374 Sa	1981	Chicken	—	77	±40 MDa	FIB; FIIA; H6; H8
425 Sa	1981	Chicken	—	77	±40 MDa	FIB; FIIA; H6; H8
437 Sa	1981	Turkey	—	77	±40 MDa	FIB; FIIA; H6; H8
438 Sa	1981	Turkey	—	77	±40 MDa	FIB; FIIA; H6; H8
439 Sa	1981	Turkey	—	77	±40 MDa	FIB; FIIA; H6; H8
468 Sa	1981	Chicken	—	77	±40 MDa	FIB; FIIA; H6; H8
3 Sa	1988	Chicken	—	77	±40 MDa	FIB; FIIA; H6; H8
58 Sa	1988	Chicken	—	39	±40 MDa	FIB; FIIA; H6; H8
59 Sa	1988	Chicken	—	39	±40 MDa	FIB; FIIA; H6; H8
62 Sa	1988	Chicken	—	41	None	None
63 Sa	1988	Chicken	—	74	±40 MDa	FIB; FIIA; H6; H8
66 Sa	1988	Chicken	—	74	±40 MDa	FIB; FIIA; H6; H8
68 Sa	1988	Chicken	—	32	±40 MDa	FIB; FIIA; H6; H8
76 Sa	1988	Turkey	—	33	±40 MDa	FIB; FIIA; H6; H8
81 Sa	1988	Chicken	—	31	None	None
83 Sa	1988	Chicken	—	32	None	None
84 Sa	1988	Chicken	—	38	None	None
85 Sa	1988	Chicken	—	33	±40 MDa	FIB; FIIA; H6; H8
107 Sa	1988	Chicken	—	72	±40 MDa	FIB; FIIA; H6; H8
108 Sa	1988	Chicken	—	74	±40 MDa	FIB; FIIA; H6; H8
109 Sa	1988	Chicken	—	33	±40 MDa	FIB; FIIA; H6; H8
110 Sa	1988	Chicken	—	33	±40 MDa	FIB; FIIA; H6; H8
111 Sa	1988	Chicken	—	33	±40 MDA	FIB; FIIA; H6; H8
112 Sa	1988	Chicken	—	33	±40 MDa	FIB; FIIA; H6; H8
113 Sa	1988	Chicken	—	33	±40 MDa	FIB; FIIA; H6; H8
118 Sa	1988	Fowl	Tc Tp	74	±40 MDa	FIB; FIIA; H6; H8
119 Sa	1988	Fowl	Tc	72	±40 MDa	FIB; FIIA; H6; H8
136 Sa	1988	Fowl	—	29	±40 MDa	FIB; FIIA; H6; H8
140 Sa	1988	Egg	—	33	±40 MDa	FIB; FIIA; H6; H8

* Antimicrobial agents used: streptomycin (Sm); tetracycline (Tc); chloramphenicol (Cm); kanamycin (Km); ampicillin (Ap); trimethoprim (Tp); gentamycin (Gm) and nalidixic acid (Nal).

† Hybridization was performed with the following probes: FIA, FIB, FIIA, rep 9, I1, HI.1, HI.2, L/M, P, O, V, W, Y, H6 and H8.

from poultry, 4 from horses, 2 from guinea-pigs, 1 from a dog, 1 from a man and 1 from meat in a slaughterhouse. All were isolated in Belgium.

The strains were identified by their biochemical and serological properties [5]. The antibiograms were performed, using disks supplied by the Institut Pasteur de Paris, according to the manufacturer's recommendations. Lysotypes were determined by the enteric lysotyping department of the Institut Pasteur de Paris.

Plasmids harboured by 34 of the strains (Table 1) were identified by the replicon typing test [6]. Replicon typing is based on the demonstration of sequence similarities between plasmid replicons by nucleic acid hybridization. The plasmid content of the strain to be tested (target nucleic acid) is immobilized on a solid matrix (filters or agarose gels) and hybridized with probes made from purified DNA fragments isolated from plasmid replicons and labelled by incorporation of radioactive nucleotides. Colony hybridization was performed with the following rep probes: FIA, FIB, FIIA, 9, 1I, HI.1, HI.2, L/M, P, Q, U, W and Y.

After separation of the plasmids by electrophoresis, hybridization on agarose gels, using the technique of Dalbadie-McFarland and colleagues [7], was performed with the rep probes. A positive response was obtained on colony hybridization (FIB and FIIA), and with the virulence probes H6 and H8. H6 and H8 are *Hin*d III restriction fragments of virulence plasmid pIP1350 of *S. typhimurium*, [8] which correspond to the virulence regions VirA and VirB [9, 10].

RESULTS

Sensitivity to antimicrobial agents

Of the 54 studied strains, 50 were sensitive to all agents tests, namely, streptomycin (Sm), tetracyline (Tc), chloramphenicol (Cm), kanamycin (Km), ampicillin (Ap), trimethoprim (Tp), gentamicin (Gm) and nalidixic acid (Nal). A strain isolated from a colt in 1976 was resistant to Su, Sm, Tc, Cm, Km, Ap. A strain isolated from man in 1984 was resistant to Su, Sm, Tc and Cm. Lastly, a strain isolated from poultry in 1988 was resistant to Tc, Tp and another also isolated from poultry in 1988 was resistant to Tc.

Thus, no progression of resistance to antibiotics in strains isolated between 1976 and 1989 was demonstrable.

Lysotypes

The lysotypes of the strains isolated from 1976–84 and of those isolated from 1987 on are presented in Table 2. The former strains were scattered among 7 lysotypes and the latter among 10.

Lysotype 33 was more frequently isolated in the 1987–9 survey than in the earlier one. The increase in incidence of this phage type is significant ($P = 0{\cdot}02$). Nevertheless, there was no indication that from 1987 a new lysotype of *S. enteritidis* had appeared in Belgium, supplanting previously observed lysotypes.

Plasmid content

The plasmid content of 11 strains isolated from 1976–81, and 23 isolated in 1988 were studied. Among the former a plasmid with an approximate molecular weight (MW) of 40 MDa was found in 9 strains, a plasmid with an approximate MW of 60 MDa in 1 strain, and in 1 strain no plasmid was present.

Table 2. *Lysotypes of* S. enteritidis. *Strains from animals, Belgium*

Lysotypes	Isolation period (number of strains) 1976–84	1987–9
29	1	1
31	—	1
32	—	2
33*	2	10
35*	7	—
38	—	1
39*	3	2
41	—	1
66	4	—
72	—	2
74	—	6
77	6	1
Atypical	4	—
Totals	27	27

* Types 33, 35 and 39 are grouped together in Ward's type PT4 (Vieu, unpublished).

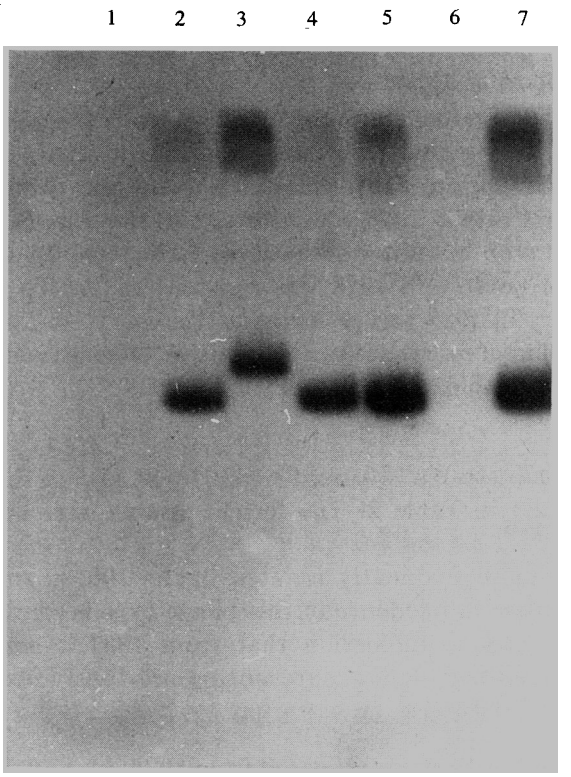

Figure 1. *Salmonella enteritidis* H8 probe. Radioautograph of agarose gel (0·6 %) after hybridization, by the technique of Dalbadie-MacFarland and colleagues 1982, with the Vir probe H8. Lines 1 and 6, strains 53S76 and 62Sa88 (no plasmid). Lines 2, 3, 4, 7, strains 169D76, 355Sa81, 373Sa81, 374Sa81, 63a88. The last two numerals of the strains code (76, 88, ...) indicate the year of isolation (see Table 1).

https://doi.org/10.1017/S0950268800056399 Published online by Cambridge University Press

Among the 1988 isolates a plasmid with an approximate MW of 40 MDa was found in 19 strains, and no plasmids in 4 strains.

Characterization of plasmids by genotyping

The five strains in which no plasmid were detected failed to hybridize with any of the test probes. The 29 others, whatever their isolation dates, all reacted weakly to the probe rep FIB and strongly to the 'vir' probes H6 and H8. Moreover, 28 of these 29, also hybridized weakly with probe rep FIIA.

Gel hybridization showed that the plasmids detected by electrophoresis were those which reacted with the various probes (Figure 1).

DISCUSSION

The frequently occurring outbreaks caused by various serotypes of salmonella in Belgium generally associated with bacterial clones which had acquired one or several plasmids which conferred increased antibiotic resistance and possibly new virulence factors. Such was the case in an epidemic caused by *S. typhi* in which strains harboured plasmid incHI.1, coding for resistance to chloramphenicol [11]. Another was due to a strain of *S. wien* harbouring plasmid inc FIme, coding for multiple resistance and for aerobactin synthesis [12, 13], and another to a strain of *S. typhimurium* belonging to phage group 49/193/204, harbouring resistance plasmids incHI.2 and incI.1, which began to spread among bovines in 1977 [14].

The situation with *S. enteritidis* is, however, different. The strains causing the current epidemic in Belgium do not all constitute a bacterial clone; they present no distinctive features when compared with previously isolated strains. The current epidemic is principally the result of the progression of the lysotype 33 (which belongs to phage type 4) which was known to be present in Belgium before 1987. The older and the more recent strains both harboured similar plasmids as far as their virulence and replication regions were concerned. The epidemic was thus not due to the appearance of a variant of *S. enteritidis* harbouring a new plasmid. Similar results had been obtained by Chart on the plasmids of nine strains of *S. enteritidis* PT4 [15]. All the lysotype 33 strains had been isolated from poultry or their products.

Finally, the relationship between the virulence plasmids of *S. enteritidis* and *S. typhimurium* is worth noting. Both contain virulence regions H6 and H8, corresponding with VirA and VirB, and they also possess replication regions with DNA sequences with similarities to replicons FIB and FIIA [16]. This virulence plasmid is likely to be the same as the most common 38 MDa plasmid found by Threlfall and colleagues [17] in the 27 *S. enteritidis* phage types.

ACKNOWLEDGEMENTS

M.C. is very grateful to Dr M. Y. Popoff for providing cloned fragments of the *S. typhimurium* virulence plasmid: pIP 1350. We thank G. Verhaegen and D. Mathy for preparing some of the DNA probes. We thank Dr J. F. Vieu for phage typing of the strains.

Work at the Université Libre de Bruxelles (M.C.) was supported by grants from Fonds de la recherche scientifique médicale and from the Belgian Government (Action de Recherche concertée).

REFERENCES

1. Ward LR, de Sa JDH, Rowe B. A phage-typing scheme for *Salmonella enteritidis*. Epidemiol Infect 1987; **99**: 291–4.
2. O'Brien JDP. *Salmonella enteritidis* infection in broilers chickens. Vet Rec 1988; **122**: 214.
3. Perales I, Audicana A. *Salmonella enteritidis* and eggs. Lancet 1988; ii: 1133.
4. Cowden JM, Chisholm D, O'Mahony M, Lynch D. Two outbreaks of *Salmonella enteritidis* phage type 4 infection associated with the consumption of fresh shell-egg products. Epidemiol Infect 1989; **103**: 47.
5. Kauffmann F. The bacteriology of *Enterobacteriaceae*. Copenhagen: Munksgaard, 1966; vol. 1: 400.
6. Couturier M, Bex F, Bergquist PL, Maas W. Identification and classification of bacterial plasmids. Microbiol Rev 1988; **52**: 375–95.
7. Dalbadie-McFarland G, Cohen LW, Riggs AD, Mloron C, Itakura K, Richards JH. Oligonucleotide – directed mutagenesis as a general and powerful method for studies of protein function. Proc Natl Acad Sci USA 1982; **79**: 6409–13.
8. Pardon P, Popoff MY, Coynault C, Marly J. Miras I. Virulence-associated plasmids of *Salmonella* serotype typhimurium in experimental murine infection. Ann Inst Pasteur Microbiol 1986; **137** B: 47.
9. Michiels T, Popoff MY, Durviaux S, Coynault C, Cornelis G. A new method for the physical and genetic mapping of large plasmids: application to the localisation of the virulence determinants on the 90 kb plasmid of *Salmonella typhimurium*. Microb Pathogenesis 1987; **3**: 109–16.
10. Norel F. Coynault C, Miras I. Hermant D. Popoff MY. Cloning and expression of plasmid DNA sequences involved in *Salmonella* serotype typhimurium virulence. Molec Microbiol 1989; **3**: 733–43.
11. Datta N. Olarte JR. R Factors in strains of *Salmonella typhi* and *Shigella dysenteriae* 1 isolated during epidemics in Mexico: classification by compatibility. Antimicrobiol Agents Chemother 1974; **5**: 310–7.
12. Colonna B, Nicoletti M, Visca P, Casalino M, Valenti P, Maimone F. Composite IS1 element encoding hydroxamate-mediated iron uptake in FIme plasmid from epidemic *Salmonella* spp. J Bact 1985; **162**: 307–16.
13. McConnell MM, Smith HR, Leonardopoulos J, Anderson ES. The value of plasmid studies in the epidemiology of infections due to drug resistant *Salmonella wien*. J Inf Dis 1979; **139**: 178.
14. Threlfall EJ, Ward LR, Rowe B. Spread of multiresistant strains of *Salmonella typhimurium* phage type 204 and 193 in Britain. Br Med J 1978; **6143**: 997.
15. Chart H, Threlfall EJ. Rowe B. Virulence of S. enteritidis phage type 4 is related to the possession of a 38 MDa plasmid. FEMS Microbiology Letters 1989; **58**: 299.
16. Pohl P, Lintermans P, Bex F. Propriétés phénotypiques et génotypiques de quatre plasmides de virulence de *Salmonella typhimurium*. Ann Inst Pasteur 1987; **138**: 523–8.
17. Threlfall EJ, Rowe, B, Ward LR. Subdivision of *Salmonella enteritidis* phage by plasmid profile typing. Epidemiol Infect 1989; **102**: 459–65.