Epidemiol. Infect. (1999), **122**, 33–39. Printed in the United Kingdom.

Molecular typing of *Salmonella* serotype Thompson strains isolated from human and animal sources

S. A. CHISHOLM, P. B. CRICHTON, H. I. KNIGHT AND D. C. OLD*

Department of Medical Microbiology, University of Dundee Medical School, Ninewells Hospital, Dundee DD1 9SY, Scotland

(*Accepted 27 August 1998*)

SUMMARY

One-hundred-and-thirteen isolates of *Salmonella* serotype Thompson from diverse sources in seven countries were characterized by *Pvu*II ribotyping and IS*200* fingerprinting. Ten *Pvu*II ribotypes were observed. The predominant *Pvu*II ribotype 1 represented a major clone of world-wide distribution but was not found in Australia; *Pvu*II ribotypes 2 and 3 represented minor clones. *Hin*cII ribotyping discriminated subtypes within *Pvu*II ribotype 1: *Hin*cII ribotype 1 was distributed widely but *Hin*cII ribotype 2 was found mainly in Scottish isolates. None of 101 isolates of *Pvu*II ribotypes 1–3 contained copies of IS*200*. All 12 isolates of *Pvu*II ribotypes 4–10 were from Australia and 7 of them contained copies of IS*200* of 5 different profiles. These results suggest the existence of at least two lineages of *Salmonella* Thompson with a different geographical distribution. The finding that most isolates from man and poultry in Scotland belonged to the same ribotype (*Pvu*II 1/*Hin*cII 2) and were IS*200*-negative suggests that poultry is an important source of human infection in Scotland.

INTRODUCTION

Salmonella enterica serotype Thompson (*S. thompson*, antigenic formula 6,7,14:k:1,5), first isolated in 1924 in Yorkshire, England [1], is often associated with human infection. In the period 1983–92, it was the sixth most frequently isolated salmonella from human sources in Canada where it accounted for 4·1% of human salmonelloses [2]. It has also featured in the top ten ranking order of salmonellae isolated from man in Scotland since 1995, but there it accounts for only 0·6% of human isolations [3, 4; Mr D. Munro, personal communication]. In contrast, *Salmonella* Thompson last appeared among the top ten salmonella serotypes isolated from man in England and Wales in 1988, when it ranked tenth with 190 isolates (Mrs L. Ward, personal communication).

In statutory food animals, incidents of *Salmonella* Thompson were uncommon in the UK before 1994 [5, 6]. However, the significant increase in poultry-associated incidents that occurred in 1995 was attributed to infections in the flocks of two poultry companies [7, 8]. Incidents of Thompson infection in other birds and spread to other statutory food animals did not occur at that time [5–8]. From 1995–7, Thompson was the predominant salmonella from poultry sources in Scotland where it accounted for 20–25% of all poultry isolations [3, 4]. Accordingly, poultry has been considered to be a likely source of human infection in Scotland [4].

Phage-typing schemes for strain discrimination have been developed for very few salmonella serotypes, generally those of clinical or epidemiological importance [9]. There are two phage-typing schemes for *Salmonella* Thompson: the one used at the Laboratory of Enteric Pathogens, Central Public Health Laboratory, Colindale, London, UK (Mrs L. Ward, personal communication); and the other developed independently at the Laboratory Center for

* Author for correspondence.

Disease Control, Ottawa, Canada [2]. These schemes have not been published, however, and are not routinely available in other countries.

Molecular typing techniques are now favoured for strain discrimination in non-phage-typable serotypes of salmonella [9–12]. For example, ribotyping and IS*200* profiling have provided information about the epidemiology and genotypic variation of *Salmonella* Eimsbuettel and Livingstone, serogroup C serotypes of particular interest in Scotland [13–15]. In this investigation, 113 isolates of *Salmonella* Thompson from human, animal and environmental sources in 7 countries, including Scotland, were analysed by ribotyping and IS*200* profiling to assess the extent of genetic variation within the serotype and to study its epidemiology in Scotland.

MATERIALS AND METHODS

Bacterial strains

All but 2 of the 113 isolates of *Salmonella* Thompson examined were obtained from the following donors: Mr D. S. Munro, Scottish Salmonella Reference Laboratory, Stobhill Hospital NHS Trust, Glasgow, Scotland; Dr C. Wray, Central Veterinary Laboratory, Weybridge, Surrey, England; Dr D. Lightfoot, Microbiological Diagnostic Unit, Melbourne University, Victoria, Australia; Mr C. J. Murray, Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia; Dr M. Y. Popoff, WHO Collaborating Center for Reference and Research on Salmonella, Unite des Entérobactéries, Institut Pasteur, Paris, France; Dr C. Poppe, Health of Animals Laboratory, O/E Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada; Dr I. Sechter, Ministry of Health, Government Central Laboratories, Jerusalem, Israel; and Dr C. E. Benson, School of Veterinary Medicine, University of Pennsylvania, PA, USA (Table 1). Two isolates from man (one each from Scotland in 1978 and England in 1997) were from the laboratory collection of one of the authors (DCO). Of the 113 isolates examined, 62 were from man and 51 were from non-human sources: poultry (24 isolates), cattle (13 isolates), other animals (6), the environment and waters (6) and one each from an unspecified food and animal feed (Table 1).

Isolates were stored on Dorset's egg slopes at room temperature (*c.* 20 °C) until subcultured for single colonies on blood agar (Oxoid Columbia blood agar base with 5% horse blood).

Table 1. *Country of origin, years of isolation and sources of 113* Salmonella *Thompson isolates examined*

Country	Years of isolation	Sources of isolates (*n*)
Scotland	1978–96	Man (33), poultry (12), environment (5), cattle (1), pig (1), sheep (1)
England	1995–7	Cattle (6), animal feed (1), dog (1), horse (1), man (1), poultry (1), sheep (1), snake (1)
Australia	1992–6	Man (12), river water (1)
Canada	1995–6	Poultry (10), cattle (5)
France	1994–6	Man (8), cattle (1), poultry (1)
Israel	1969–96	Man (6), food (1)
USA	1997	Man (2)

Extraction of DNA

DNA was extracted from salmonella isolates as described previously [16]. L broth [17] (10 ml) was inoculated with a single colony from blood agar and incubated with aeration at 37 °C for 16–18 h. After centrifugation (3000 g for 20 min), the bacterial pellet was resuspended in 650 μl of TE buffer (0·1 M Tris, 5 mM EDTA, pH 8·0) in a 2-ml screw-capped microfuge tube. SDS (10%) 40 μl and proteinase K (20 mg/ml) 4 μl were added and the suspension was mixed and incubated at 55 °C until it became clear. NaCl (5 M) 100 μl and hexadecyl trimethylammonium bromide (CTAB) solution (10% CTAB in 0·7 M NaCl) 100 μl were added and, after mixing, the solution was incubated at 65 °C for 20 min. Each of two extractions with chloroform : isoamyl alcohol (24 : 1) was followed by centrifugation (6000 g for 10 min) and the upper aqueous layer was transferred to a 1·5-ml tube. DNA, precipitated with cold (−20 °C) isopropanol (0·6 vol.) and washed by resuspension and centrifugation (12000 g for 5 min) in 70% ethanol, was dried at 37 °C, dissolved in $\leqslant$ 200 μl TE buffer and stored at −20 °C.

Ribotyping and IS*200* profile analysis

DNA (*c.* 2 μg) from each of the 113 Thompson isolates was digested for 18 h with *Pvu*II, as recommended by the manufacturer (Promega, Southampton, UK); in addition, DNA from 70 of these isolates was digested with *Hin*cII. These restriction enzymes were chosen because their target

sites are outwith the IS*200* sequence [18, 19]. DNA restriction fragments and digoxigenin (DIG)-labelled lambda DNA digested with *Eco*RI and *Hin*dIII (Boehringer–Mannheim, DIG-labelled molecular-weight marker III), were separated by electrophoresis through 0·8% agarose gels (Ultra Pure agarose from Life Technologies, Paisley, UK) with ethidium bromide 0·5 μg/ml in TBE buffer (0·089 M Tris-HCl, 0·089 M boric acid, 0·002 M EDTA) at 2 V/cm for 16–20 h. Gels were viewed on a UV transilluminator (312 nm) and photographed on Polaroid 667 film.

Before Southern blotting, gels were shaken once for 5 min in 0·25 M HCl, twice for 15 min in denaturation solution (0·5 M NaOH and 1·5 M NaCl), and twice for 15 min in neutralization solution (0·5 M Tris and 3 M NaCl, pH 7·0); gels were rinsed in distilled water after each treatment. DNA fragments were transferred to positively charged nylon membrane (Appligene-Oncor, Birtley, Chester-le-Street) by vacuum blotting (Appligene-Oncor) at 55 mbar for 75 min with 20 × SSC (1 × SSC is 0·15 M NaCl, 0·015 M sodium citrate, pH 7·0) as the transfer solution. The resultant blot was washed in 2 × SSC and the DNA fixed to the membrane by exposure to UV light for 5 min.

The 3·8-kb gene probe of *Escherichia coli* ribosomal RNA has been described elsewhere [13]; the 692-bp probe of IS*200* [18, 19] was prepared by PCR amplification as described previously [20]. Probes were labelled with DIG-11-dUTP by the High-Prime system (Boehringer–Mannheim).

Prehybridization, hybridization and detection of DIG-labelled hybrids with anti-DIG alkaline phosphatase and CSPD® were carried out as described by the manufacturer (Boehringer–Mannheim), except for the addition of boiled, sheared salmon sperm DNA 50 μg/ml to prehybridization and hybridization solutions. DIG-labelled hybrids were observed after exposure to Hyperfilm-MP (Amersham) for 1–40 min.

To remove hybridized probe, membranes were rinsed briefly in distilled water and washed twice for 15 min in preheated (37 °C) stripping solution (0·2 M NaOH and 0·1% SDS); subsequent prehybridizations and hybridizations of membranes were carried out as described above.

Numerical index of discrimination

The discrimination indices obtained after ribotyping and IS*200* fingerprinting of 113 Thompson isolates were calculated as described previously [21].

RESULTS

Ribotypes of *Salmonella* Thompson

Ten ribotypes were identified after digestion of the DNA of 113 Thompson isolates with *Pvu*II (Fig. 1, lanes 2–11). *Pvu*II ribotype 1 consisted of seven hybridized bands of 9·7, 9·0, 6·9, 6·0, 5·5, 3·5 and 2·2 kb (Fig. 1, lane 2) and was observed in 84% (95/113) of the isolates from diverse sources in six different countries (Table 2). *Pvu*II ribotype 2, closely related to *Pvu*II ribotype 1 but with an additional band of *c*. 2·0 kb (Fig. 1, lane 3), was observed in 5 (4%) of the 113 isolates; 3 of these 5 isolates were from cattle and poultry in France and Canada, 1 was from a snake in England and 1 was from an Australian patient who had travelled recently in China (Table 2). *Pvu*II ribotype 3, another variant of *Pvu*II ribotype 1 but with an additional band of 2·3 kb (Fig. 1, lane 4), was represented by a single bovine isolate from Canada (Table 2).

Another seven *Pvu*II ribotypes, 4–10, which shared bands with but clearly distinguishable from *Pvu*II ribotypes 1–3, were observed in 12 isolates from man and river-water sources in Australia, as follows: *Pvu*II ribotype 4 (1 isolate), 5 (1), 6 (1), 7 (2), 8 (2), 9 (4) and 10 (1). *Pvu*II ribotype 4 lacked the 6·9- and 5·5-kb bands present in ribotypes 1–3 but had additional bands of 12·5, 4·4, 2·3 and 2·0 kb (Fig. 1, lane 7); *Pvu*II ribotypes 5–10, with one or more bands of 12·5, 4·4 and 2·0 kb, were related to *Pvu*II ribotype 4 (Fig. 1, lanes 5, 6 and 8–11).

Seventy (62%) of the 113 Thompson isolates, selected to represent the different *Pvu*II ribotypes described above, were ribotyped after digestion of their DNA with *Hin*cII. *Hin*cII ribotypes 1 and 2 were found among 61 isolates of *Pvu*II ribotypes 1–3 from diverse sources in countries other than Australia (Table 3). *Hin*cII ribotype 1 (Fig. 2, lane 2) consisted of eight strongly hybridized bands of *c*. 4·1, 3·4, 3·3, 3·1, 2·8, 2·7 kb and a 2·65–2·60-kb doublet and 5 weakly hybridized bands in the range *c*. 2·0–2·4 kb. This ribotype accounted for 46% of the 61 non-Australian isolates (Table 3) and was recovered from man (16 isolates), cattle (6), poultry (4), sheep and snake (1 each). The other 33 (54%) of these 61 isolates were of *Hin*cII ribotype 2, which was distinguishable from *Hin*cII ribotype 1 by the absence of the 2·2-kb band and the presence of co-migrating bands of *c*. 2·15–2·3 kb (Fig. 2, lane 3). Of the 33 isolates of *Hin*cII ribotype 2, 31 were from Scotland and 2 were from England (Table 3).

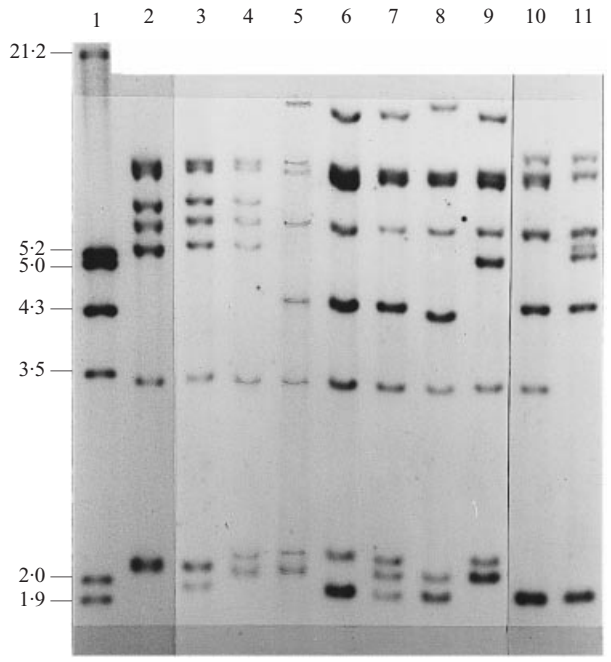

Fig. 1. *Pvu*II ribotypes of isolates of *Salmonella* Thompson. Lane 1, molecular size markers (kb); lanes 2–4, ribotypes 1–3; lanes 5 and 6, ribotypes 6 and 7; lanes 7 and 8, ribotypes 4 and 5; and lanes 9–11, ribotypes 8–10, respectively.

Table 2. *Pvu*II *ribotypes of 113 isolates of* Salmonella *Thompson*

Country	Isolates examined (*n*)	Isolates (*n*) of *Pvu*II ribotype			
		1	2	3	4–10
Scotland	53	53	0	0	0
England	13	12	1	0	0
Australia	13	0	1	0	12
Canada	15	13	1	1	0
France	10	8	2	0	0
Israel	7	7	0	0	0
USA	2	2	0	0	0

Table 3. *Hinc*II *ribotypes of 70 isolates of* Salmonella *Thompson*

Country	Isolates examined (*n*)	Isolates (*n*) of *Hinc*II ribotype		
		1	2	3–6
Scotland	35	4	31	0
England	8	6	2	0
Australia	9	0	0	9
Canada	6	6	0	0
France	5	5	0	0
Israel	5	5	0	0
USA	2	2	0	0

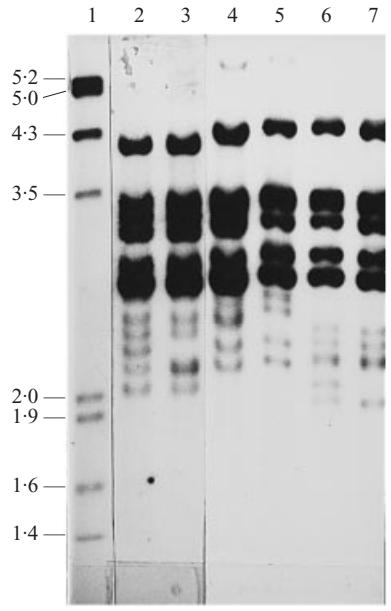

Fig. 2. *Hinc*II ribotypes of isolates of *Salmonella* Thompson. Lane 1, molecular size markers (kb); lanes 2 and 3, ribotypes 1 and 2; lanes 4 and 5, ribotypes 5 and 6; and lanes 6 and 7, ribotypes 3 and 4, respectively.

*Hinc*II ribotypes 3–6 (Fig. 2, lanes 4–7) were found in nine Thompson isolates from Australia, representative of *Pvu*II ribotypes 4–10, as follows: *Hinc*II ribotype 3 (3 isolates), 4 (1), 5 (4) and 6 (1). In addition to bands present in *Hinc*II ribotypes 1 and 2, *Hinc*II ribotype 3 contained a band of 1·9 kb and bands of 3·35 and 2·15 kb that co-migrated with those of 3·3 and 2·1 kb (Fig. 2, lane 6). *Hinc*II ribotypes 4–6, observed in six isolates from different persons in three different Australian states, were related to *Hinc*II ribotype 3: they contained the same strongly hybridized bands but showed differences in bands of low molecular size (Fig. 2, lanes 4, 5 and 7).

IS*200* profiles of *Salmonella* Thompson

Seven Thompson isolates, all from Australian sources, contained copies of IS*200*. Five distinct IS*200* profiles were observed (Fig. 3). Profile A, with one copy of IS*200* on a *Pvu*II restriction fragment of *c*. 3·7 kb, was found in two isolates from patients in different states (Fig. 3, lane 1). Profiles B, C and D contained the 3·7 kb band of profile A but differed from it in both copy number and location of other IS*200* elements (Fig. 3, lanes 2–4). Profiles B–D were each represented by single isolates that came from two patients and a river-water source in different states. In contrast, profile E, found in two isolates from human urine and

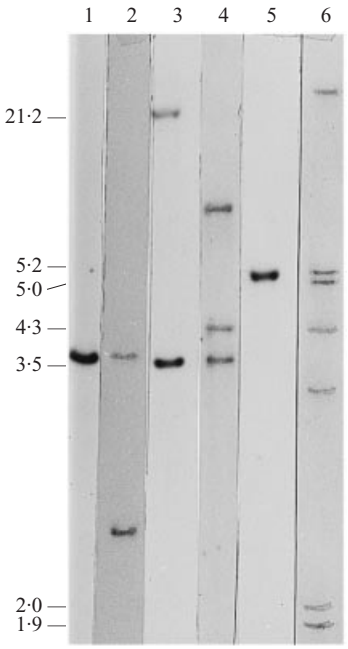

Fig. 3. *Pvu*II-generated IS*200* profiles of *Salmonella* Thompson. Lanes 1–5, profiles A–E, respectively; lane 6, molecular size markers (kb).

faeces, had a single copy of IS*200* on a restriction fragment of 5·1 kb and was clearly different from profiles A–D (Fig. 3, lane 5). The other 106 isolates were IS*200*-negative.

Discriminatory power

For 113 Thompson isolates typed by *Pvu*II ribotyping and IS*200* fingerprinting, used alone or together, discrimination indices (D) were as follows: *Pvu*II ribotyping, $D = 0{\cdot}29$; IS*200* fingerprinting, $D = 0{\cdot}12$; and *Pvu*II ribotyping/IS*200* fingerprinting, $D = 0{\cdot}29$.

DISCUSSION

The epidemiology of salmonellosis can be clarified by application of typing methods that characterize strains and allow associations to be made between human cases and food vehicles, or between animal cases and environmental sources of infection [9]. Such information is essential if appropriate measures for control and spread of salmonellosis are to be implemented.

In this study, isolates of *Salmonella* Thompson were analysed for polymorphism in two different DNA sequences, *rrn* and IS*200*. Although ribotyping of 113 Thompson isolates after digestion of their cellular DNA with *Pvu*II identified 10 different ribotypes, the discriminatory power of *Pvu*II ribotyping was low ($D = 0{\cdot}29$). The majority (84%) of isolates were of *Pvu*II ribotype 1 and had been recovered from a wide range of sources in countries other than Australia. Most had been recovered recently, in 1992–7, but 5 from Israel in 1969–72 and 1 from Scotland in 1978 also belonged to *Pvu*II ribotype 1. These findings suggest that little genetic divergence has occurred in Thompson over the last 30 years. Isolates from other countries will need to be examined to confirm that *Pvu*II ribotype 1 is the major clone.

The minor *Pvu*II ribotypes 2 and 3 accounted for only 5% of Thompson isolates. Nevertheless, isolates of *Pvu*II ribotype 2, like those of ribotype 1, were widely distributed – in England, Canada, France and Australia (Table 2). The single isolate of *Pvu*II ribotype 3 was from a bovine source in Canada. Thompson is an important cause of human infection in Canada [2]. Examination of isolates from man should establish whether their *Pvu*II ribotypes are like those from bovines and poultry.

Epidemiological information from ribotypes may be limited if DNA is digested with a single enzyme. In this study, further strain discrimination was achieved by *Hin*cII ribotyping of 70 Thompson isolates representative of the above *Pvu*II ribotypes. Interpretation of *Hin*cII ribotypes was difficult because many weakly hybridized DNA fragments of low molecular size were present (Fig. 2); nevertheless, *Hin*cII ribotyping provided subdivision within *Pvu*II ribotype 1. Isolates of the frequently observed ribotype *Pvu*II 1/*Hin*cII 1 were recovered world-wide from man and various animal sources. However, no correlation was found between *Pvu*II and *Hin*cII ribotypes and some isolates of *Hin*cII ribotype 1 belonged to *Pvu*II ribotype 2. This observation suggests that differences between these ribotypes may be due to minor variations associated with *Pvu*II recognition sequences and are not detectable after digestion with other restriction enzymes. The ribotype profile *Pvu*II 1/*Hin*cII 2 clearly distinguished Scottish isolates (Table 3). The limited geographical distribution of isolates of *Hin*cII ribotype 2 suggests that minor genetic divergence occurred recently in Scotland in isolates which have had insufficient time for wider dissemination. In recent years, Thompson has been the main serotype isolated from poultry in Scotland [3, 4]. Our data confirm that types from poultry and man are similar and suggest that poultry is a major reservoir of Thompson in Scotland, as it is in France [22, 23].

Molecular fingerprinting by IS*200* analysis has

provided effective strain discrimination in several salmonellae in serogroups B and D [12, 18, 19] and in Infantis in serogroup C [24]. It has been less helpful for other serotypes in serogroup C, such as Virchow and Bovismorbificans, because IS*200* was present either in low copy number or in minor clones only [25, 26]. In Thompson the overall discriminatory power of IS*200* fingerprinting was low ($D = 0{\cdot}12$) because the major clone of *Pvu*II ribotype 1 was IS*200*-negative. For the 12 isolates of *Pvu*II ribotypes 4–10 from Australia, however, IS*200* fingerprinting was highly discriminatory when used alone ($D = 0{\cdot}82$) or together with *Pvu*II ribotyping ($D = 0{\cdot}89$).

Despite their wide distribution, isolates of *Pvu*II ribotype 1 were not found in Australia where *Pvu*II ribotypes 4–10, not detected elsewhere, were predominant. Three national reference laboratories have confirmed independently that these Australian isolates are not serological variants of Thompson. Analysis by multilocus enzyme electrophoresis should help to establish the genetic distance of the different Australian isolates from the major Thompson clone. Results of phage typing (Mrs L. Ward, personal communication) and RAPD PCR analysis that confirm that Australian isolates are distinct and heterogeneous will be reported elsewhere.

Thompson is not a major salmonella in animals in Australia [27]; isolates of non-human origin are mainly from the environment or non-livestock-associated foods [28–31]. Because 12 of our Australian isolates were from man, examination of isolates from diverse animals might help to identify the source of human infection.

This is the first genetic study of *Salmonella* Thompson, an important cause of human and animal infection for nearly 75 years [1]. In Scotland the limited diversification of an established serotype such as Thompson contrasts with the extensive diversification of Livingstone and Eimsbuettel, serotypes of recent origin [13–15]. These findings also increase our knowledge of IS*200* distribution in salmonellae of serogroup C.

ACKNOWLEDGEMENTS

We thank the named donors who provided strains and information about them. We also thank Mr H. Mather, Scottish Salmonella Reference Laboratory, Stobhill Hospital NHS Trust, Glasgow, Scotland for assistance with serotyping.

REFERENCES

1. Scott WM. The 'Thompson' type of Salmonella. J Hyg 1926; **25**: 398–405.
2. Khakhria R, Woodward D, Johnson WM, Poppe C. Salmonella isolated from human, animal and other sources in Canada, 1983–92. Epidemiol Infect 1997; **119**: 15–23.
3. Munro DS, Girdwood RWA, Reilly WJ. Salmonellosis in Scotland 1995. SCIEH Weekly Report 1997; **31**: 146–8.
4. Surveillance report: gastro-intestinal infections. SCIEH Weekly Report 1998; **32**: 50–1.
5. Ministry of Agriculture, Fisheries and Food. Salmonella in animal and poultry production. Weybridge, Central Veterinary Laboratory, 1992: 1–27.
6. Ministry of Agriculture, Fisheries and Food. Salmonella in animal and poultry production. Weybridge, Central Veterinary Laboratory, 1994: 1–66.
7. Ministry of Agriculture, Fisheries and Food. Salmonella in livestock production. Weybridge, Veterinary Laboratories Agency, 1995: 1–55.
8. Ministry of Agriculture, Fisheries and Food. Salmonella in livestock production. Weybridge, Veterinary Laboratories Agency, 1996: 1–79.
9. Old DC, Threlfall EJ. *Salmonella*. In: Collier L, Balows A, Sussman M, eds. Topley and Wilson's microbiology and microbial infections, 9th edn, vol 2. London: Edward Arnold, 1998: 969–97.
10. Threlfall EJ, Frost JA. The identification, typing and fingerprinting of *Salmonella*: laboratory aspects and epidemiological applications. J Appl Bacteriol 1990; **68**: 5–16.
11. Threlfall EJ, Powell NG, Rowe B. Differentiation of salmonellas by molecular methods. PHLS Microbiol Digest 1994; **11**: 199–202.
12. Stanley J, Saunders N. DNA insertion sequences and the molecular epidemiology of *Salmonella* and *Mycobacterium*. J Med Microbiol 1996; **45**: 236–51.
13. Crichton PB, Old DC, Taylor A, Rankin SC. Characterisation of strains of *Salmonella* serotype Livingstone by multiple typing. J Med Microbiol 1996; **44**: 325–31.
14. Old DC, Crichton PB, Taylor A, Rankin SC. Phenotypic and genotypic discrimination of strains of *Salmonella* serotype Eimsbuettel from human and animal sources. J Med Microbiol 1997; **46**: 617–22.
15. Crichton PB, Old DC, Morphy HF. Distribution of insertion sequence IS*200* among different clonal lines of *Salmonella* of the related serotypes Livingstone and Eimsbuettel. J Med Microbiol 1998; **47**: 791–7.
16. Wilson K. Preparation of genomic DNA from bacteria. Current protocols in molecular biology. Ausubel FM, Brent R, Kingston RE, et al. eds, 2nd edn. New York: Wiley, 1987: 2.4.1–2.4.5.
17. Miller JH. Experiments in molecular genetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1972: 433.
18. Stanley J, Burnens A, Powell N, Chowdry N, Jones C. The insertion sequence IS*200* fingerprints chromosomal genotypes and epidemiological relationships in

Salmonella heidelberg. J Gen Microbiol 1992; **138**: 2329–36.
19. Stanley J, Baquar N, Burnens A. Molecular subtyping scheme for *Salmonella panama*. J Clin Microbiol 1995; **33**: 1206–11.
20. Burnens AP, Stanley J, Sechter I, Nicolet J. Evolutionary origin of a monophasic *Salmonella* serovar, 9,12:1,v:-, revealed by IS*200* profiles and restriction fragment polymorphisms of the *fli*B gene. J Clin Microbiol 1996; **34**: 1641–5.
21. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 1988; **26**: 2465–6.
22. Le Minor L, Le Minor S, Grimont PAD. Rapport quadriennal du Centre national des *Salmonella* sur l'origine et la répartition en sérotypes des souches isolées en France continentale au cours des années 1980 à 1983. Rev Epidemiol Santé Publique 1985; **33**: 13–21.
23. Carbonelle B, Becaud JP, Parvery F, Pilliot J. Recherches épidémiologiques sur les infections à *Salmonella thompson*. Rôle des volailles comme réservoir de virus. Ann Inst Pasteur 1970; **20**: 165–76.
24. Pelkonen S, Romppanen E, Siitonen A, Pelkonen J. Differentiation of *Salmonella* serovar Infantis isolates from human and animal sources by fingerprinting IS*200* and 16S *rrn* loci. J Clin Microbiol 1994; **32**: 2128–33.
25. Torre E, Threlfall EJ, Hampton MD, et al. Characterization of *Salmonella virchow* phage types by plasmid profile and IS*200* distribution. J Appl Bacteriol 1993; **75**: 435–40.
26. Ezquerra E, Burnens AP, Frith K, Costas M, Stanley J. Molecular genotype analysis of *Salmonella bovismorbificans*. Mol Cell Probes 1993; **7**: 45–54.
27. Murray CJ. *Salmonella* serovars and phage types in humans and animals in Australia 1987–1992. Aust Vet J 1994; **71**: 78–81.
28. Australian Salmonella Reference Centre. 1993 Annual Report. Adelaide, Institute of Medical and Veterinary Science: 1–126.
29. Australian Salmonella Reference Centre. 1994 Annual Report. Adelaide, Institute of Medical and Veterinary Science: 1–113.
30. Australian Salmonella Reference Centre. 1995 Annual Report. Adelaide, Institute of Medical and Veterinary Science: 1–96.
31. Australian Salmonella Reference Centre. 1996 Annual Report. Adelaide, Institute of Medical and Veterinary Science: 1–77.