

## Molecular Epidemiology of *Salmonella enterica* Serovar Enteritidis Strains by Pulsed-field Gel Electrophoresis Isolated in the Slovak Republic

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### Abstract

A total 44 isolates of *S. enterica* serovar Enteritidis (*S. Enteritidis*) belonged to three different phage types (PTs) 9a, 13a, 25 were analyzed by the technique of pulsed-field gel electrophoresis (PFGE). Thirty and three strains were from two outbreaks (central and southern regions of the Slovak Republic PTs 13a, 25) and 11 isolates were sporadic isolates (PT9a). These isolates showed two different patterns in PFGE with XbaI digestion. Strains of PT13a generated PFP A and isolates of PT25 showed uniform PFP B. Nine sporadic isolates of PT9a belonged to PFP A and two isolates to PFP B. The PFPs A and B were differed by only two bands. The distribution of XbaI profiles did not corresponded with PTs. We conclude that the close genetic similarity observed between epidemiologically unrelated and outbreak-related isolates of *S. Enteritidis* suggest clonal relationship of these isolates.

**Key words:** epidemiology, pulsed-field gel electrophoresis, phage types, *S. Enteritidis*

*Salmonella enterica* serovar Enteritidis is one the most common *Salmonella* serovars worldwide, particularly in developed countries. During the past 15–20 years, *S. Enteritidis* emerged as an important cause of human illness in the Slovak Republic. Nearly 90% of all sporadic cases and outbreaks are cause by this serovar. This situation reflects the importance of salmonellae as a major public health problem despite the efforts to improve food safety and to prevention measurements. Animals, particularly chicken and chicken products such as meat and eggs, are often considered as sources of *S. Enteritidis* infections (Coyle *et al.*, 1988; Humphrey *et al.*, 1988; Threlfall *et al.*, 1994; Liebana *et al.*, 2001). According to literature in Europe the predominant phage type (PT) is PT4, whereas in the United States outbreaks have been primarily linked with PT8 (Hickman-Brenner *et al.*, 1991), but in the Slovak Republic is PT8 predominant since 1995. The recent reports confirm however the presence of PT4 also in the American continent (Patrick *et al.*, 2004). The epidemiological analysis of *S. Enteritidis* strains is important to determine the source of the pathogen and improve food hygiene. Therefore, reliable and sufficiently discriminative methods of differentiating individual strains are need. Epidemiological techniques for this serovar that have been used are plasmid analysis, antibiotic resistance and phage typing (Patrick *et al.*, 2004; Gruner *et al.*, 1994; Gonzales Hevia and Mendoza, 1995; Weide-Botjes *et al.*, 1998). Phage typing represents a classic, but nevertheless highly discriminatory epidemiological typing method. A phage typing system that is used in the Slovak Republic as well as in the most countries worldwide has been developed by Ward and coworkers in the UK (Ward *et al.*, 1988). Despite the high, discriminatory power of this method, it also has some limitations: a) the availability of typing phages is limited, b) not all the patterns correspond to the limited number of recognized phage types. Pulsed-field gel electrophoresis (PFGE) has provided molecular fingerprints in epidemiological studies and has been used to define a limited diversity within several established PTs of *S. Enteritidis* (Liebana *et al.* 2001; Weide-Botjes *et al.*, 1998; Ridley *et al.*, 1998; Murakami *et al.*, 1999). In the Slovak Republic, PT8 strains are currently the predominant phage type (Majtánová, 2004). However during the past year in the Slovak Republic were recorded two outbreaks caused by strains of PT25 and PT13a, respectively, and increased number of sporadic cases caused by strains of PT9a. The strains of PT25 appeared in the Slovak Republic for the first time. For serovar Enteritidis, one problem common to molecular methods is the supposed highly clonal nature of some PTs (Helmuth and Schroeter, 1994; Stanley *et al.*, 1991) and a clonal expansion of particular phage types was also suggested (Bautsch, 1993). Therefore, in this investigation PFGE was undertaken to determine the extent of genetic diversity among these isolates.

Table I  
Genotyping of *S. Enteritidis* isolates by PFGE

No	Date of isolation	Suspected vehicle of outbreaks	Occurrence region	No of isolates	Phage type	Pulsed-field profile Xba I
1.	June/2003	Unrecorded	North western	11	9a	A, B
2.	August/2003	Cookies	Southern	11	25	B
3.	October/2003	Poppy	Central	22	13a	A

A total 44 isolates of *S. Enteritidis* were obtained from 40 patients and from four suspected vehicular foods of the two temporally different outbreaks as well as from sporadic cases in various parts of the Slovak Republic (Table I). Phage typing of the isolates was performed using standard procedures (Ward *et al.*, 1998) by the National Reference Center for Phage Typing of Salmonella in the Slovak Medical University (head Dr. Majtánová).

PFGE analysis was performed using a modification of the method described by Ridley *et al.* (1998). The agarose blocks, including DNA, were incubated overnight in 100 µl of digestion buffer containing 5–10 U of XbaI (BIO-RAD Laboratories, USA). Restricted fragments were separated on a 1.2% gel (BIO-RAD Laboratories, USA) in 0.5×TBE using a CHEF-DR III Systems (BIO-RAD Laboratories, USA). Electrophoresis was performed at 5.5 V/cm for 29 hr, with a switched pulse time of 5–50 sec at 14°C. The gels were stained with ethidium bromide and were photographed with a digital camera EPSON Photo PC 850Z. The DNA size standards used were a bacteriophage lambda ladder consisting a concatemers starting at 48.5 kB (BIO-RAD Laboratories, USA).

PEGE of XbaI-digested fragments of genomic *S. Enteritidis* DNA generated different patterns. Differences in the presence, absence or intensity of a band among strains were observed and strains were assigned to different pulsed-field profiles (PFP). Nine strains of PT9a belonged to PFP A and two strains to PFP B. The PFPs A and B were differed by only two bands (Fig. 1). These strains were isolated from sporadic cases

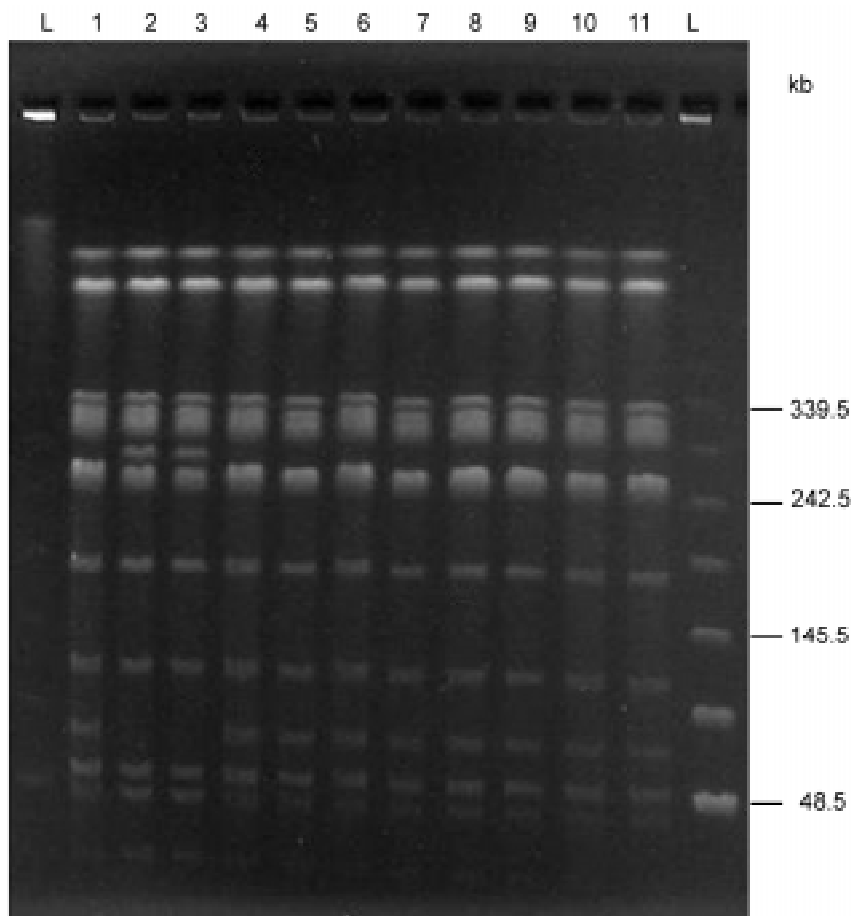


Fig. 1. PFPs of *S. Enteritidis* strains PT9a generated by use of XbaI  
Lanes L: λ (lambda) ladder (Bio-RAD Laboratories, USA). Lanes 1–11: *S. Enteritidis* strains PT9a. Lines 2, 3 – PFP B, lines 1, 4–11 – PFP A

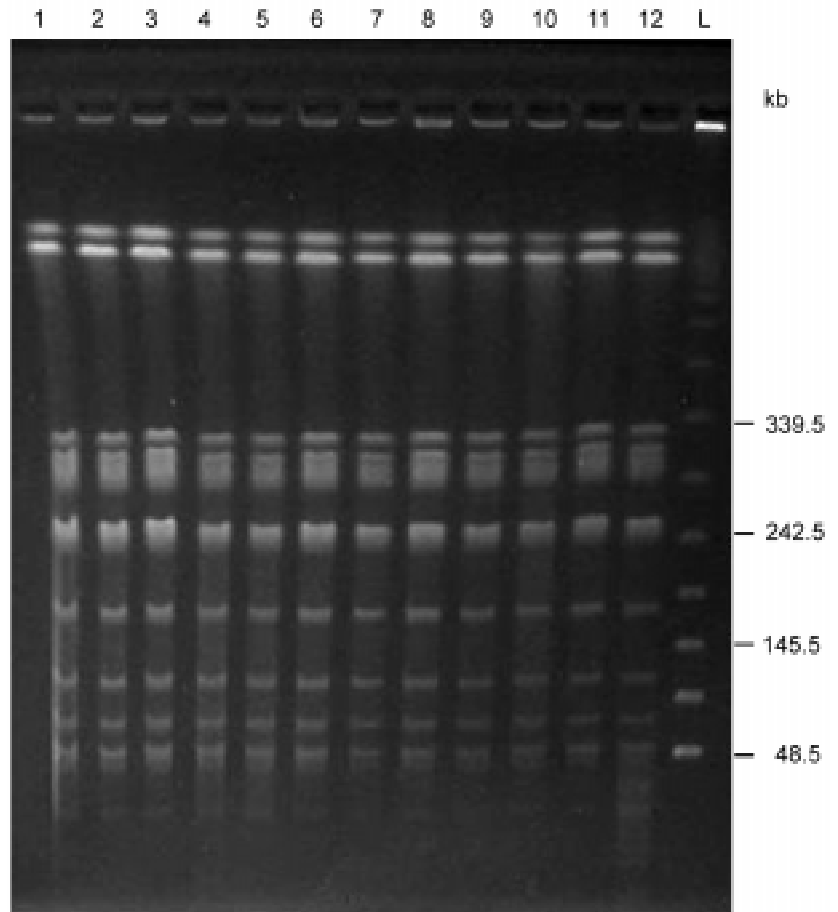


Fig. 2. PFPs of *S. Enteritidis* strains PT13a generated by use of XbaI.  
Lane L:  $\lambda$  ladder. Lanes 1–12. *S. Enteritidis* strains PT13a

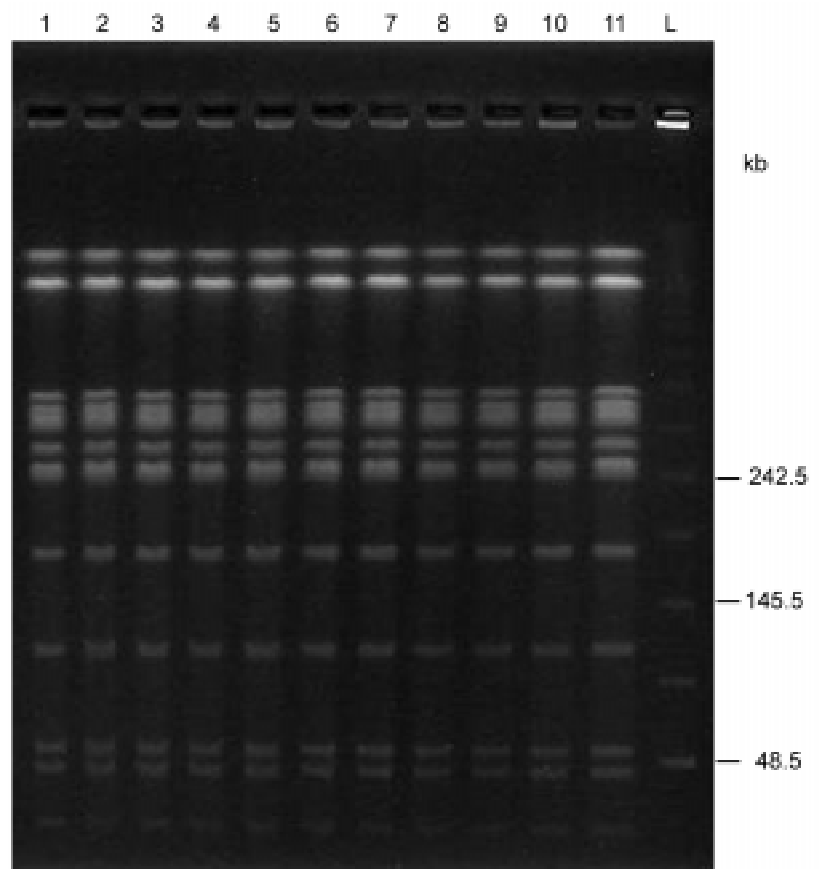


Fig. 3. PFPs of *S. Enteritidis* strains PT25 generated by use of XbaI.  
Lanes L:  $\lambda$  ladder. Lanes 1–11. *S. Enteritidis* strains PT25 (PFP B).

of salmonellosis. The strains of PT13a, which were the cause of outbreak in north of Slovak Republic generated uniform PFP A (Fig. 2). The all strains of PT25 showed the PFP B (Fig. 3). Olsen *et al.* (1994) reported that 21 of 33 phage types of *S. Enteritidis* formed one cluster by PFGE. Thong *et al.* (1995) indicated that *S. Enteritidis* strains circulating in Malaysia and Switzerland are very similar and may be clonally related. Tenover *et al.* (1995) elaborated guidelines for appropriating interpretation of the profiles obtained by PFGE analysis. According to these guidelines, various isolates are considered to be closely related with each other even when two- or three-fragment differences were shown among them, since change in the PFGE is caused by a point mutation an insertion or deletion of the chromosomal DNA of the bacteria during *in vivo* or *in vitro* growth (Coyle *et al.*, 1988). According to Tenover *et al.* (1995) and on the basis of PFGE of XbaI-digest of genome *S. Enteritidis* strains DNA our results suggest their clonal relationship, although belonging to three different PTs. This indicates that although phage typing of *S. Enteritidis* is valuable method to identify the possible source and route of infection with this organism, this typing is not sufficient but other, mainly genotyping methods also should be used. The investigation of PFGE analysis of strains *S. Enteritidis* of predominant phage types to clarification the extent of genetic diversity of *S. Enteritidis* is in progress.

### Literature

- Bautsch W. 1993. NotI macrorestriction analysis suggests a clonal relationship of *Salmonella enterica* serovar Enteritidis phage lysotype 4 strains. *Infection* **5**: 328–330.
- Coyle E.F., S.R. Palmer and C.D. Ribeiro. 1988. *Salmonella* Enteritidis phage type 4: association with hen's eggs. *Lancet* 1295–1297.
- Gonzales Hevia M.A. and M.C. Mendoza. 1995. Differentiation of strains from a food-borne outbreak of *Salmonella enterica* by phenotypic and genetic typing methods. *Eur. J. Epidemiol.* **11**: 479–482.
- Gruner E., G. Martinetti Lucchini and R.K. Hoop. 1994. Altwegg M Molecular epidemiology of *Salmonella* Enteritidis. *Eur. J. Epidemiol.* **10**: 85–89.
- Helmuth R. and A. Schroeter. 1994. Molecular typing methods for *Salmonella* Enteritidis. *Int. Food Microbiol.* **21**: 69–77.
- Hickman-Brenner F.W., A.D. Stubbs and J.J. Farmer. 1991. Phage typing of *Salmonella* Enteritidis in the United States. *J. Clin. Microbiol.* **29**: 2817–2823.
- Humphrey T.J., G.C. Mead and B. Rowe. 1988. Poultry meat as a source of human salmonellosis in England and Wales. *Epidemiol. Infect.* **100**: 175–184.
- Liebana E., L. Garcia-Migura, M.F. Breslin, R.H. Davies and M.J. Woodward. 2001. Diversity of strains of *Salmonella enterica* serotype Enteritidis from English poultry farms assessed by multiple genetic fingerprinting. *J. Clin. Microbiol.* **39**: 154–161.
- Majtánová L. 2004. Distribution of phage types of non-typhoidal *Salmonella* serovars in the Slovak Republic in 2000–2003 (in Slovakish). *Epidemiol. Mikrobiol. Imunol.* **53**: 70–73.
- Murakami K., K. Horikawa and K. Otsuki. 1999. Epidemiological analysis *Salmonella* Enteritidis from human outbreaks by pulsed-field gel electrophoresis. *J. Vet. Med. Sci.* **61**: 439–442.
- Olsen J.E., M.N. Skov, E.I. Threlfall and D.I. Brown. 1994. Clonal lines of *Salmonella enterica* serotype Enteritidis documented by IS200-, ribo-, pulsed-field gel electrophoresis and RFLP typing. *J. Med. Microbiol.* **40**: 15–22.
- Patrick M.E., P.M. Adcock, T.M. Gomez, S.F. Altekruze, B.H. Holland, R.V. Tauxe and D.L. Swerdlow. 2004. *Salmonella* Enteritidis infections, United States, 1985–1999. *Emerg. Infect. Dis.* **10**:
- Ridley A., E.J. Threlfall and B. Rowe. 1998. Genotypic characterization of *Salmonella* Enteritidis phage types by plasmid analysis, ribotyping and pulsed-field gel electrophoresis. *J. Clin. Microbiol.* **36**: 2314–2321.
- Stanley I., C.S. Jones and E.I. Threlfall. 1991. Evolutionary lines among *Salmonella* Enteritidis phage types are identified by insertion sequence IS200 distribution. *FEMS Microbiol. Lett.* **82**: 83–90.
- Tenover F.C., R.D. Arbeit, R.V. Goering, P.A. Mickelsen, B.E. Murray, D.H. Persing and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**: 2233–2239.
- Threlfall E.J., M.D. Hampton, H. Chart and B. Rowe. 1994. Use of plasmid profile typing for surveillance of *Salmonella* Enteritidis phage type 4 from humans, poultry and eggs. *Epidemiol. Infect.* **112**: 25–31.
- Thong K., Y.F. Ngeow, M. Altwega, P. Navaratnam and T. Pang. 1995. Molecular analysis of *Salmonella* Enteritidis by pulsed-field gel electrophoresis and ribotyping. *J. Clin. Microbiol.* **33**: 1070–1074.
- Ward L.R., J. de Sa and B. Rowe. 1988. A phage typing scheme for *Salmonella* Enteritidis. *Vet. Rec.* **122**: 214.
- Weide-Botjes M., B. Kobe and S. Schwarz. 1998. Inter- and intra- phage type differentiation of *Salmonella enterica* serovar Enteritidis isolates using molecular typing methods. *Zent. bl. Bacteriol.* **288**: 181–193.