DNA-FINGERPRINTING AND PULSED-FIELD GEL ELECTROPHORESIS OF *SALMONELLA ENTERICA* SEROTYPE *INFANTIS* STRAINS ISOLATED FROM POULTRY

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Summary

Salmonella Infantis has been the most common serovar in Hungary in the last two years, both in the fields of human and animal health. Pulsed field gel electrophoresis (PFGE) using restriction enzyme *Xba*I, and enterobacterial repetitive intergenic consensus (ERIC-PCR) were compared with respect to their ability to detect genetic differences among 31 *Salmonella Infantis* isolates from 21 poultry farms in Hungary. Results of ERIC-PCR showed that the isolates were indistinguishable. In addition PFGE analysis distinguished *Salmonella* Infantis strains into two clusters. The results of this work demonstrate the genetic diversity and expansion of *Salmonella Infantis* associated with epidemic changes.

Salmonella enteritidis and *Salmonella typhimurium* are the two most important agents causing salmonellosis worldwide. Salmonella reduction of broilers has been initiated in 2001 to be focused first on *Salmonella typhimurium* and *Salmonella enteritidis*, resulting a decrease of these serovars. In contrast, the number of infections and diseases caused by the serotype *Salmonella infantis* started to increase in the last year.

Serotyping was the standard for classification of *Salmonella* isolates in outbreak investigations prior to the development of molecular genotyping methods. However, serotyping has limited utility for epidemiologic analysis of *Salmonella* transmission, because it has poor discriminative ability for closely related isolates Olsen et al (5).

Various typing techniques have been used in epidemiological studies to differentiate isolates of *Salmonella* serovars, but only a few of them have been used to discriminate *Salmonella Infantis* strains. The applied epidemiological tools include biotyping, phage typing, antimicrobial susceptibility testing, plasmid profiling, restriction endonuclease analysis of whole chromosomal DNA by pulsed field gel electrophoresis (PFGE), repetitive extragenic palindromic (REP) sequences analysis by PCR, enterobacterial repetitive intergenic consensus (ERIC) analysis by PCR, restriction fragment length polymorphism (RFLP)

Pulsed-field gel electrophoresis (PFGE) is an established method for the analysis of large fragments generated by restriction endonuclease digestion of

genomic DNA and is currently considered to be one of the most reliable typing procedures Murase et al. (4).

In the present study, we examined strains of *Salmonella infantis* isolated in Hungary from the faeces of broiler chickens by ERIC-PCR (enterobacterial repetitive intergenic consensus PCR) and pulsed-field gel electrophoresis (PFGE).

Materials and methods

Bacterial strains: 31 isolates of Salmonella Infantis isolated from faeces of broiler chickens during 2006 in Hungary were examined in this study. Bacteria were identified to specie level by conventional methods. All isolates were maintained on nutrient agar and serologically identified as serotype *Infantis* according to the standard international scheme for serotyping *Salmonella.*

DNA extraction: Template DNA was extracted from each strain grown overnight on Colombia blood agar using the Chelex 100 method (Bio-Rad laboratories).

Enterobacterial repetitive intergenic consensus (ERIC) PCR: ERIC-PCR was carried out by the method described by BEYER *et al*.(1):1 initial cycle at 94 ºC for 1 min, 30 cycles of denaturalization at 95 ºC for 1 min, annealing at 52 ºC for 1 min, and extension at 65ºC for 8 min, with a single final extension step at 65 ºC for 16 min. We used the ERIC2 primer (5'-AAG TAA GTG ACT GGG GTG AGC G-3')

Samples of each PCR end-product were analysed on agarose 2% gels containing ethidium bromide 0.5 µg/ml.

PFGE: PFGE was performed using the method of Bo¨hm and Karch (2). Electrophoresis was carried out in 1% agarose gels made by using pulsed-field certified agarose, in SeaKem Gold agarose. The PFGE buffer was 0.53 Tris-borate-EDTA made from 53 Tris-borate-EDTA buffer concentrate. Gels were run at a temperature of 14°C and a voltage of 6 V/cm. Gels were stained in 0.5 mg of ethidium bromide per ml, and the DNA was visualized with a KODAK Gel Documentation System. All isolates tested were analyzed using *Xba*I enzyme.

Fingerprints interpretation: Analysis of the patterns was performed by visual inspection. Two isolates were said to have the same electrophoretic profile when their band patterns were identical. Minor differences in band intensity were not considered. PFGE patterns were interpreted according to the criteria suggested by TENOVER *et al*.(6)

Results

Fingerprinting with ERIC1 primer generated identical patterns between isolates that may indicate dissemination of a single clone (picture 2). Molecular typing by ERIC-PCR showed that all *Salmonella infantis* strains were genetically related.

All the 69 isolates could be typed by PFGE and XbaI digestion and two main clusters was noted (fig. 1). The main cluster consisted of twenty-four isolates, whilst the second cluster consisted of six isolates. One isolate was uniquely different from the rest.

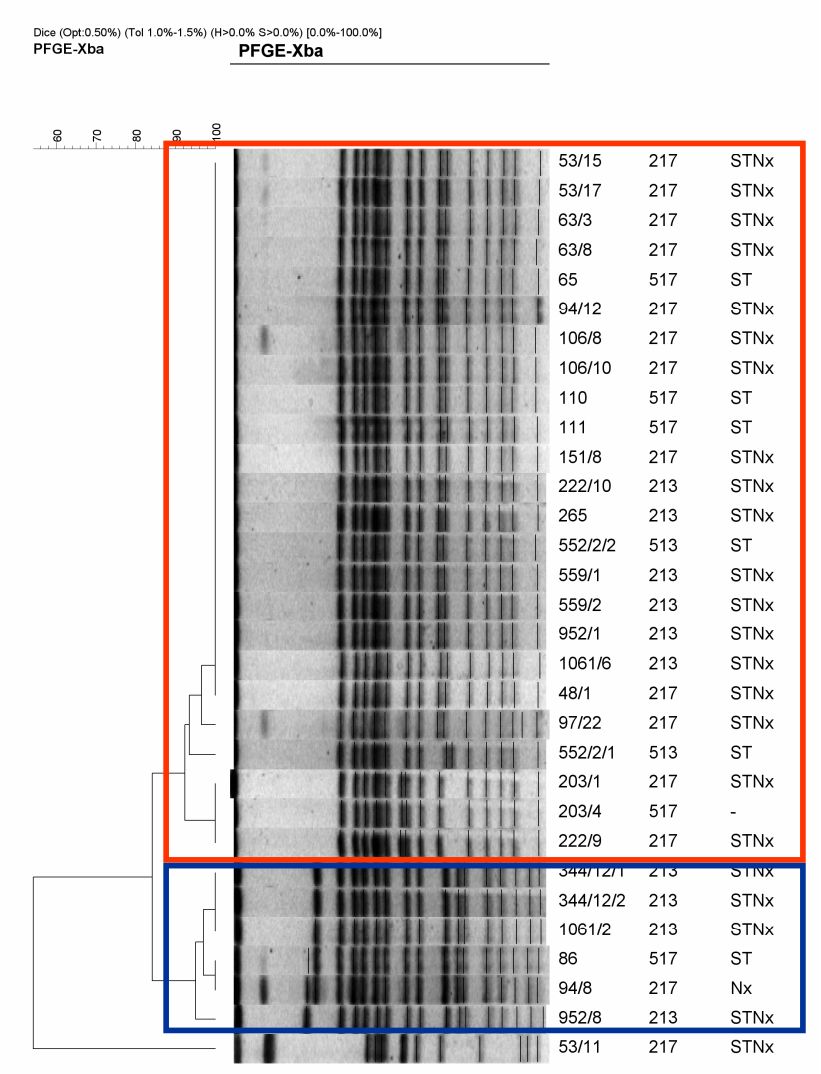

Fig. 1. Agarose gels of the PFGE profiles of *Salmonella* serovar *infantis*

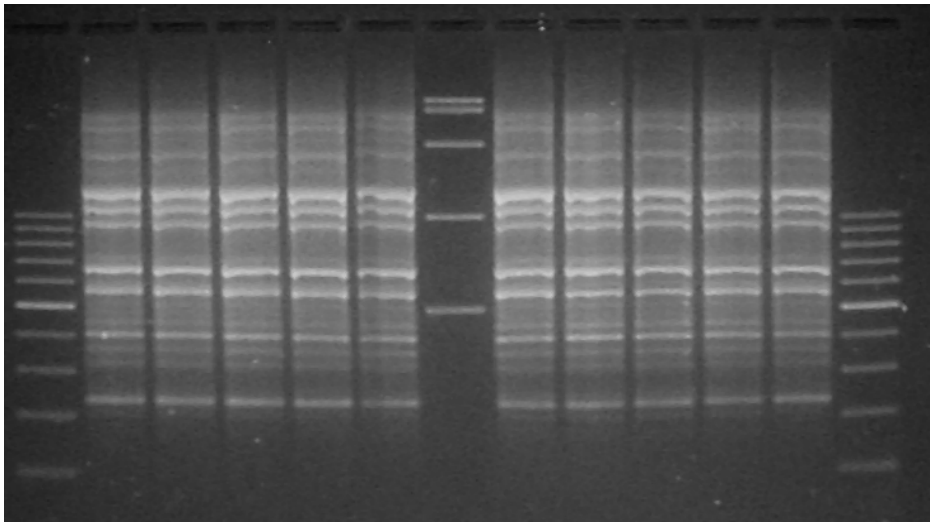

Fig.2: ERIC-PCR profile of *Salmonella infantis* isolates

Discussions

Numerous papers about the clonal relationship between endemic *Salmonella* strains can be encountered in scientific publications. The most powerful tool for discrimination of even closely related bacterial isolates has been reported to be the macrorestriction analysis of whole DNA by pulsed field gel electrophoresis (PFGE).

MURAKAMI *et al*.(3) studied the genetic diversity among human and environmental *Salmonella Infantis* strains by PFGE, obtaining 35 distinct profiles and WEGENER & BAGGESEN(7) obtained 21 different PFGE profiles among *Salmonella Infantis* strains when studying 135 isolates from various sources. These findings support the fact of the clonal variability of *Salmonella Infantis* isolates.

ERIC-PCR method was applied by several authors with good results among other serovars of *Salmonella,* but not among *Salmonella Infantis* strains. We found that this method, as it has been described previously, was not able to differentiate the isolates of the same serotype.

Electrophoresis of *Xba*I-digested genomic DNAs from the 31 isolates showed two main clusters with minor differencies between them.

Conclusions

In conclusion, the results presented suggest that ERIC-PCR is not sensitive enough to distinguish effectively among these *Salmonella Infantis* isolates.

In contrast, PFGE was able to discriminate between isolates of *Salmonella* Infantis.

The results of this work paint a picture that includes genetic diversity and expansion of specific *Salmonella Infantis* in Hungary. Two independent molecular methods were used in this work for typing *Salmonella infantis* isolates, and diversity could be observed only with macrorestriction analysis

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References

1. **Beyer, W.; Mukendi, F.M.; Kimming, P. & Böhm, R.** - Suitability of repetitive- DNA-sequence-based PCR fingerprinting for characterizing epidemic isolates of *Salmonella enterica* serovar Saintpaul. **J. clin. Microbiol., 36**: 1549-1554, 1998.
2. **Böhm, H., and H. Karch.** 1992. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. J. Clin. Microbiol. **30:** 2169–2172.
3. **Murakami, K.; Horikawa, K. & Otsuki, K**. - Genotypic characterization of human and environmental isolates of *Salmonella choleraesuis* subspecies *choleraesuis* serovar Infantis by pulsed-field gel electrophoresis. **Microbiol. Immunol., 43**: 293- 296, 1999.
4. **Murase, T., T. Okitsu, R. Suzuki, H. Morozumi, A. Matsushima, A. Nakamura, and S. Yamai.** 1995. Evaluation of DNA fingerprinting by PFGE as an epidemiologic tool for *Salmonella* infections. Microbiol. Immunol. **39:**673–676.
5. **Olsen, J.E., Brown, D.J., Skov, M.M., Christensen, J.P**., 1993. Bacterial typing methods suitable for epidemiological analysis. Applications in investigations of salmonellosis among livestock. Vet. Quart. 15, 125–135.
6. **Tenover, F.C.; Arbeit, R.D.; Goering, R.V**. - Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. **J. clin. Microbiol., 33**: 2233-2239, 1995.
7. **Wegener, H.C. & Baggesen, D.L**. - Investigation of an outbreak of human salmonellosis caused by *Salmonella enterica* ssp. *enterica* serovar Infantis by use of pulsed field gel electrophoresis. **Int. J. Food Microbiol., 32**: 125-131, 1996.