

Epidemiological relationship between *Salmonella* Infantis isolates of human and broiler origin

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Introduction

Salmonella spp. are considered to be one of the most common causes of food borne bacterial infections. According to WHO estimates, *Salmonella* (*S.*) is responsible for 3 million infections of humans annually (Crump *et al.* 2004). In Germany 31.185 *Salmonella* infections were reported to the Robert Koch-Institute in 2009. Since the aetiology of enteric infections in many cases is not established and only a fraction of the cases is notified, it is assumed that actually about one million people are infected annually in Germany (Tschäpe & Bockemühl 2002).

The host-unspecific serovar *S. Infantis* ranks first among the top ten human isolates of *S. enterica* in Germany and Europe since 2001 (Galanis *et al.* 2006). Since the late 1970s this serovar has been increasingly recorded worldwide in countries like Argentina, Australia, Brazil, The Netherlands, Finland, Canada, Hungary, Japan, New Zealand and Russia. *S. Infantis* is also observed in hospitals, where primarily small children are infected, but also adults, sometimes with septicaemic symptoms and lethal outcome (Hasenson *et al.* 1995). Of special importance is that *S. Infantis* infections may persist in hospitals over a long period of time (Pessoa-Silva *et al.* 2002; Fonseca *et al.* 2006).

The reservoir for *S. Infantis* salmonellosis in humans is primarily seen in animal and especially poultry populations. A survey of the European Food Safety Authority (EFSA) indicates that *S. Enteritidis* and *S. Infantis* were mainly found in layer and broiler farms in Europe (Table 1). The highest frequency of serovar *S. Infantis* in broilers was found in Hungary (87 %), followed by Poland (19 %) and the Czech Republic (13 %) (EFSA 2007a; 2007b). In Germany the occurrence of *S. Infantis* was 3.9 % in layer flocks and 8.9 % in broiler flocks (BfR 2005, 2006; Table 1).

Table 1: Results from EFSA and BfR studies on occurrence of *Salmonella* spp. in laying and broiler farms (EFSA 2007a, 2007b; BfR 2005, 2006)

| layers | | | | broilers | | | |
|-----------------------|--------|-----------------------|--------|-----------------------|--------|-----------------------|--------|
| EU-wide 2007 | | Germany 2005 | | EU-wide 2007 | | Germany 2006 | |
| <i>S. Enteritidis</i> | 52.3 % | <i>S. Enteritidis</i> | 64.4 % | <i>S. Enteritidis</i> | 3.8 % | <i>S. 4, 12:d:-</i> | 23.0 % |
| <i>S. Infantis</i> | 8.4 % | <i>S. ssp. I R.*</i> | 18.4 % | <i>S. Infantis</i> | 22.0 % | <i>S. Paratyphi B</i> | 15.6 % |
| <i>S. Typhimurium</i> | 4.8 % | <i>S. Typhimurium</i> | 5.1 % | <i>S. Mbandaka</i> | 8.1 % | <i>S. Anatum</i> | 14.7 % |
| <i>S. Mbandaka</i> | 4.1 % | <i>S. Infantis</i> | 3.9 % | <i>S. Hadar</i> | 3.7 % | <i>S. Infantis</i> | 8.9 % |
| <i>S. Livingstone</i> | 2.7 % | <i>S. Livingstone</i> | 1.8 % | <i>S. Typhimurium</i> | 3.0 % | <i>S. Typhimurium</i> | 8.4 % |

* I R., rough form

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Another study by EFSA on the prevalence of *Salmonella* in broiler meat showed that *S. Infantis* was the most frequent serovar in the EU, averaging 55 %. In some countries as Hungary (96 %), Slovenia (55 %) and Austria (44 %) it was the most important serovar (EFSA 2007c). With an incidence of 7 % and 10 % respectively in Europe and Germany, *S. Infantis* ranked second in eggs and egg products (EFSA 2009).

The persistence of *S. Infantis* in broiler flocks is not only a problem in Hungary, but also in Japan, Iceland, France, The Netherlands, USA, Australia, Turkey, Saudi-Arabia and Algeria (Ai-Nakhli *et al.* 1999; Van Duijkeren *et al.* 2002; Sexton *et al.* 2007; Thorsteinsdottir *et al.* 2007; Cetinkaya *et al.* 2008; Elgroud *et al.* 2008; Heithoff *et al.* 2008; Rivoal *et al.* 2009).

In Germany and several other countries live vaccines against *S. Enteritidis* and *S. Typhimurium* are in use. It can be assumed that live vaccination with *S. Enteritidis* will introduce immunity against *Salmonella* of group 0:9 (D₁) and live vaccination with *S. Typhimurium* immunity against *Salmonella* of group 0:4 (B). After successful control of *S. Enteritidis* and *S. Typhimurium* apparently other serovars may enter this ecological niche and persist. It is known that *S. Enteritidis* entered poultry facilities after serovar *S. Gallinarum* had been eliminated (Rabsch *et al.* 2000).

S. Infantis of group 0:7 (C₁) is a potential candidate for a change of serovars and so far there is no licensed vaccine against *S. Infantis*. In Hungary and Japan *S. Infantis* is already the most frequent serovar in broilers, which is mirrored by an increase of human isolates in these countries (Nogrady *et al.* 2008; Shahada *et al.* 2008; Ishihara *et al.* 2009; Noda *et al.* 2010).

Suitable techniques for typing serovars are required to monitor sources and routes of infections. A method requiring relative little laboratory equipment is phage typing, which uses specific bacteriophages to differentiate strains belonging to the same serovar. Using established phage typing systems for *S. Typhimurium*, *S. Enteritidis* and *S. Agona* it has been possible to document the pathways of infections (Ward *et al.* 1987; Rabsch *et al.* 2005; Rabsch 2007). Advantages of phage typing are speed and low cost, but this method requires special expertise to interpret the phage types and strict quality control of the biological reagents (Schwarz *et al.* 2003).

Material und Methods

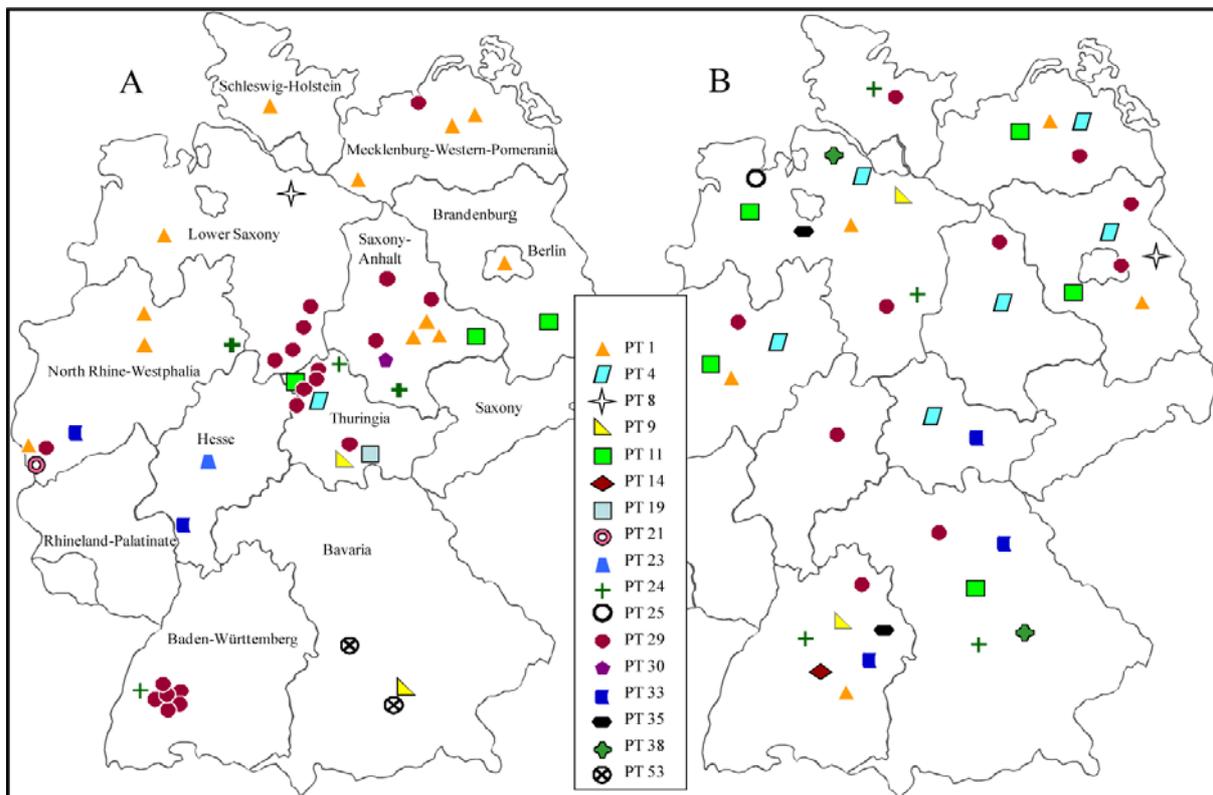
In order to monitor the serovar *S. Infantis* more effectively, a new phage typing system was established at the National Reference Center for Salmonella and other Enteric Pathogens (NRC) of the Robert-Koch-Institute. The phage typing follows the standardized NRC-protocol (Rabsch 2007). A total of 617 *S. Infantis* strains from Germany (n=542), Hungary (n=25), Iceland (n=21), and Australia (n=29) were included in this study and investigated by phage typing. The strains were isolated from outbreaks and sporadic cases of gastroenteritis in humans, from animals, food, feed and the environment during the period 2002 - 2009. For molecular subdifferentiation and examination, 255 out of the 617 *S. Infantis* isolates were analyzed by macrorestriction analysis after pulsed-field gel electrophoresis using the PulseNet CDC-protocol (Hunter *et al.* 2005).

Results and Discussion

The final typing scheme was established using 17 typing phages. Of the 617 *S. Infantis* strains analyzed, 605 could be typed with the established scheme, resulting in 61 different *S. Infantis* phage types (PT). The *S. Infantis* phage types PT 29 (30 %), PT 1 (21 %), PT 11 (7 %) and PT 9 (7 %), which are epidemiologically predominant, were found in humans and food as well as broilers.

197 strains isolated from food poisoning (40) and hospital outbreaks (11) were subtyped into 12 defined phage types (Figure 1). Remarkable was the emergence of *S. Infantis* as a nosocomial pathogen in several German hospitals. The most frequently observed clone in these outbreaks was PT 29/Xba1 27. This clone was repeatedly found in a hospital in Baden-Württemberg since 2002. In 2004 a huge outbreak with 188 cases in two clinics was investigated in Bavaria and traced back to bakery products. By phage typing and macrorestriction analysis the isolates from humans and food have been characterized to be identical (clone PT 53/Xba1 6). An outbreak with 80 cases in North Rhine-Westphalia in 2008 was caused by contaminated chicken kebab (clone PT 1/Xba1 34). A supra-

Figure 1: Distribution of *S. Infantis* phage types in Germany reported from humans (A) and broilers (B)



regional outbreak of *S. Infantis* in Thuringia, North Rhine-Westphalia and Lower Saxony was confirmed through clone PT 29/XbaI 27a which was found in isolates from humans and broilers. Additionally, some diffuse outbreaks could be detected by this complex typing system.

The EFSA studies have shown that the serovar *S. Infantis* is common in poultry. In this study broiler strains from Germany (n=143), Hungary (n=22), Australia (n=16), and Iceland (n=20) were subtyped using phage typing and macrorestriction analysis. A total of 24 phage types among these broiler strains were found.

In Germany clones PT 4/XbaI 4, PT 29/XbaI 5 and PT 29/XbaI 27 were frequently observed in isolates from broilers. The strains belonging to PT 29/XbaI 5 were associated with broilers imported from Hungary. This clone was found only in sporadic cases in Germany. Nogrady *et al.* (2007, 2008) reported recently that strains of PT 213 and PT 217 were most commonly found in Hungary, in humans as well as in broilers. The strains of PT 213 according to phage typing scheme of Laszlo correspond to PT 29 according to the phage typing scheme used at the NRC in Wernigerode. It is important to note that strains of PT 29/XbaI 27 from Germany and strains of PT 29/XbaI 5 from Hungary which are indistinguishable by phage typing differ in their macrorestriction patterns only in two bands of 70 kbp and 170 kbp, probably due to plasmids.

In contrast to the situation in Germany and Hungary, where clone PT 29 is epidemic among broilers, subtyping of *S. Infantis* strains from broilers in Iceland showed a dominance of clone PT 61, which has not yet been observed in Germany. Pelkonen *et al.* (1994) were unable to confirm a relationship between isolates from humans and from poultry in Finland. *S. Infantis* repeatedly observed in cattle could explain epidemics in Finland during the 1970s (Lindqvist *et al.* 1999; Lindqvist & Pelkonen 2007). In the EFSA study it was also stated that in 2006 *S. Infantis* was the most frequent serovar found in beef (EFSA 2007c). By phage typing and macrorestriction analysis the spreading of specific *S. Infantis* clones in broiler flocks, the circulation of defined clones between countries, and the development of country-specific epidemiologic processes were detected.

In conclusion, the data presented in this study confirmed that *S. Infantis* strains are widespread in broiler flocks worldwide. In Hungary and Japan *S. Infantis* is the most frequent serovar in broilers (Nogrady *et al.* 2008; Ishihara *et al.* 2009; Noda *et al.* 2010). After successful vaccination against *S. Enteritidis* and *S. Typhimurium* in broiler flocks apparently other serovars may enter this ecological niche to persist whereas *S. Infantis* is a potential candidate. The phage typing scheme developed can be used for laboratory-based surveillance of *S. Infantis*.

Summary

Salmonella (*S.*) *Infantis* infections in humans transmitted by food are increasingly observed around the world. *S. Infantis* strains from several origins isolated between 2002 and 2009 were subtyped by phage typing and macrorestriction analysis (MRA). It was found that the 617 *S. Infantis* isolates investigated belonged to 47 defined phage-types and the 255 isolates characterized by MRA to 45 *Xba*I-types. The combination of these typing methods revealed a great diversity of clones among the *S. Infantis* isolates as well as occurrence of identical clones (PT 1/*Xba*I 34, PT 29/*Xba*I 27) in chicken meat and in humans in Germany over several years. Several hospital infections caused by these above-mentioned clones were also identified. Circulation of clone PT 29/*Xba*I 5 in broiler flocks between Hungary and Germany has been demonstrated by complex typing. The development of country-specific epidemiologic processes was also observed as PT 61 in Iceland. The change of predominant *Salmonella* serovars in the ecologic niche "broiler", vacated by vaccination against *S. Typhimurium* and *S. Enteritidis*, may lead to an epidemic increase of *S. Infantis* in broiler flocks. Routine application of the new phage typing scheme can be used to monitor the spread of *S. Infantis*.

Zusammenfassung:

Epidemiologische Zusammenhänge zwischen *Salmonella* *Infantis*-Isolaten von Mensch und Masthähnchen

Salmonella (*S.*) *Infantis*-Infektionen des Menschen, oft durch Lebensmittel übertragen, sind weltweit von wachsender Bedeutung. *S. Infantis*-Stämme verschiedener Herkunft, die von 2002-2009 isoliert wurden, sind durch die Lysotypie und die *Xba*I- Makrorestriktionsanalyse (MRA) subtypisiert worden. 617 der mit Lysotypie untersuchten *S. Infantis*-Isolate gehörten zu 47 definierten Lysotypen, und die 255 *S. Infantis*-Isolate, die mit der MRA charakterisiert wurden, zu 45 *Xba*I-Typen. Die Kombination (komplexe Typisierung) der beiden Methoden beweist die erhebliche klonale Diversität der *S. Infantis*-Isolate, zeigt aber auch das Vorkommen von bestimmten Klonen wie z. B. PT 1/*Xba*I 34 und PT 29/*Xba*I 27 sowohl bei Isolaten aus Masthähnchenfleisch als auch bei humanen Isolaten über Jahre hin in Deutschland. Es wurden auch mehrere Krankenhausinfektionen durch die benannten Klone in Deutschland erfasst. Die komplexe Typisierung wies weiterhin die Zirkulation des *S. Infantis*-Klons PT 29/*Xba*I 5 in Broiler-Beständen in Ungarn und Deutschland nach sowie die Existenz sog. länderspezifischer epidemiologischer Prozesse wie z. B. in Island durch den Klon PT 61.

Der offenbar durch die Vakzination gegen *S. Typhimurium* und *S. Enteritidis* begünstigte Erregerwechsel in der ökologischen Nische „Masthähnchen“ könnte in der Zukunft dazu führen, dass *S. Infantis* in Masthähnchenbeständen häufiger zum Problemkeim wird. Deshalb kann der routinemäßige Einsatz des neuen Lysotypeschemas für *S. Infantis* dazu beitragen, die zeitliche und geografische Ausbreitung dieses Erregers zu überwachen.

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