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Typing of *Salmonella enterica* serovar Infantis isolates from 51 outbreaks in Germany between 1974 and 2009 by a novel phage-typing scheme

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SUMMARY

We developed a new phage-typing method and evaluated its application in combination with *Xba*I macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) as a useful tool for the long-term epidemiology of *Salmonella enterica* serovar Infantis. In this study, we investigated 1008 *S.* Infantis isolates recovered from humans, various animal species and food products from 1973 to 2009. The typing scheme is based on 17 typing phages, defining 61 different patterns within the strain collection. The experiments showed that phage typing is a reliable method for differentiation of outbreaks and sporadic clinical cases as well as for elucidation of chains of transmission. The combined analysis of phage typing and PFGE revealed the existence of epidemic clones with a high stability over time like PT29/XB27 which was identified in nosocomial salmonellosis, community outbreaks as well as in broiler chickens from 2002 to 2009.

Key words: ESBL, macrorestriction analysis, outbreak investigations, phage typing, *S.* Infantis.

INTRODUCTION

Salmonella enterica serovar Infantis (*S.* Infantis) primarily causes gastroenteritis in adults, but in newborns and small children serious infections with lethal outcome are also reported [1, 2]. Of special concern is its ability to persist in hospitals for long periods posing significant public-health problems [3–5]. Current trends in the epidemiology of *S.* Infantis show that it is increasingly involved in human infections in several countries, e.g. Hungary, Israel and Japan [6–8]. In Germany, *S.* Infantis is the third most common serovar after *S.* Enteritidis and *S.* Typhimurium in human infections in recent years [9].

The reservoirs for salmonellosis in humans are primarily seen in animals and asymptomatic human carriers associated with food preparation [10]. The European Union (EU) baseline survey on the prevalence of *Salmonella* in broiler flocks for 2005–2006 revealed that *S.* Enteritidis (37%) and *S.* Infantis (20%) were the most frequently isolated serovars [11]. Further studies of the European Food Safety Authority (EFSA) from 2008 to 2010 showed that *S.* Infantis was the most frequently reported serovar in broiler meat in the EU [12]. In Germany *S.* Infantis was mostly detected in broilers and in slaughter-age pigs [11–13]. According to regulation

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EU No. 517/2011 *S.* Infantis belongs to the zoonotic agents, which are targeted for reduction in breeding flocks of *Gallus gallus* in the European Union in the near future [14]. These facts stress the risk that *S.* Infantis of animal origin represents for human health and underlines the importance of achieving a better understanding of the epidemiology of *S.* Infantis infections.

Epidemiological typing methods are essential for identifying the source of infection and transmission pathways as well as for routine surveillance of epidemic *S.* Infantis strains. Common typing methods used include traditional serotyping and antimicrobial susceptibility testing as well as a range of molecular methods developed more recently [15]. Of these methods macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) is accepted as the 'gold standard' for *Salmonella* subtyping due to its excellent discriminatory power for surveillance and outbreak investigations. In addition, phage typing is an established method for *Salmonella* monitoring used throughout the world. The successful application of phage typing for the detection of outbreaks as well as for source attribution or transmission of epidemic strains has been demonstrated in different studies [16, 17].

The aim of our study was the development of novel phage-typing scheme for *S.* Infantis and assessment of its application in combination with PFGE analysis as a tool for routine typing.

MATERIALS AND METHODS

Bacterial strains

From 1973 to 2009 medical microbiology and veterinary laboratories throughout Germany sent 2746 *S.* Infantis strains to the National Reference Centre for *Salmonella* (NRC). Identification of *S.* Infantis was confirmed at the NRC by serotyping according to the White–Kauffmann–Le Minor scheme [18] by slide agglutination with O and H antigen-specific sera (Sifin Diagnostics, Germany) [19]. For this study we selected randomly 1008 isolates for further characterization and typing. The collection included isolates from foodborne outbreaks (187), sporadic cases (327), broiler chicken carcasses (199), pigs (71), cattle (39), layer hens (16), turkeys (2), ducks (4), geese (1), wild animals (16), dogs (6), cake (2), raw sausage (5), chicken meat (3), pork (26), beef (2), food ([16], without further information), feed (21), environmental samples (59) and autogenous vaccines for poultry (6). Isolates of German broiler chicken carcasses ($n = 169$, 85%) were sampled from slaughterhouses throughout Germany from 1996 to 2009. Isolates of Hungarian broiler chicken carcasses ($n = 30$, 15%; transported from Hungary to Germany) were originally sampled from three slaughterhouses in the German Federal States of North Rhine-Westphalia, Lower Saxony and Brandenburg during 2003–2009.

Antimicrobial susceptibility testing

All *S.* Infantis isolates were subjected to antimicrobial susceptibility testing against 14 antimicrobial substances (Sigma-Aldrich, Germany) as follows: ampicillin (AMP), mezlocillin (MEZ), cefotaxime (CTX), ceftazidime (CAZ), cefoxitin (FOX), gentamicin (GEN), amikacin (AMK), streptomycin (STR), nalidixic acid (NAL), chloramphenicol (CHL), oxytetracycline (OTE), ciprofloxacin (CIP), sulfameracin (SMZ) and sulfamethoxazole-trimethoprim (SXT) by broth microdilution according the DIN 58940 method [20]. Multidrug resistance was defined as resistance to three or more antimicrobial groups (quinolones, tetracyclines, β-lactams, aminoglycosides, sulfonamide, chloramphenicol). In strains resistant to third-generation cephalosporins (cefotaxime and/or ceftazidime) the production of extended-spectrum β-lactamases (ESBL) was confirmed by Etest® ESBL strips (bioMérieux, Germany) according to the manufacturer's instructions.

Isolation of typing phages and development of a phage-typing scheme

Fourteen typing phages were directly isolated from 14/123 lysogenic strains of *S.* Infantis by mitomycin (Sigma-Aldrich, Germany) induction [21]. Strains were cultivated at 37 °C for 5 h with agitation in 10 ml double-strength nutrient broth (Becton Dickinson GmbH, Germany) containing 2·5 μg/ml mitomycin. After centrifugation (10 000 ***g*** for 10 min) the supernatants were carefully removed and sterilized by passage through a Millipore filter (0·2 μm pore size). In order to extend the typing scheme, three additional typing phages were obtained by adaptation of three phages (nos. 12, 16, 17) on three different strains of *S.* Infantis [22, 23]. Briefly, several dilutions of the phage were spotted on different *S.* Infantis strains plated on nutrient agar (Difco, USA). From each culture a

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single plaque was removed and inoculated into 3 ml nutrient broth, followed by incubation for 5 h at 37 °C. The propagated phages, which represent new lines of phages adapted by restriction/modification or phage module exchange, were obtained from the culture supernatant and titrated on the respective sensitive strains. Routine test dilutions of each typing phage were applied to nutrient Difco agar plates with a lawn of the respective reference strains using a multipoint inoculator. They were incubated for 17 h at 37 °C until phage-induced lysis could be observed.

PFGE

Out of the 1008 selected *S.* Infantis strains 325 isolates (1999–2009) belonging to 31 frequent phage types were selected for further subtyping. These isolates from different origins [humans (206), broiler chicken carcasses (45), layer hens (10), pigs (8), cattle (5), pork (8), chicken meat (3), beef (1), raw sausage (4), cake (2), food ([11], without further information), wild animals (3), feed (6) and environmental samples (13)] were typed according to the CDC PulseNet protocol as described by Hunter *et al.* [24] using the restriction enzyme *Xba*I (Roche, Germany) and a CHEF DRIII instrument (Bio-Rad, Germany). Gel images were evaluated using BioNumerics, version 5.1 (Applied Maths, Belgium), and compared by similarity clustering using the unweighted pair-group matching algorithm (UPGMA) and the Dice correlation coefficient with a tolerance of 1·0% and an optimization of 1·5%. The PFGE patterns were designated arbitrarily by numbering according to Barrett *et al.* [25]; letters (a, b, c) in addition to the numbers were used to designate closely 'related patterns' differing only by one or two fragments from the primary pattern.

Designation of clones

Clones were designated by a number consisting of the phage type (PT01, PT02, etc.) and PFGE patterns type (XB01, XB02, etc.). For example, clone PT01/XB34 has the phage type designated PT01 and the PFGE pattern XB34.

Statistical analysis

Typability, i.e. the percentage of strains that could be assigned to a distinct phage type (e.g. PT01, PT02, etc.) was calculated as described by Struelens [26]. To evaluate the discriminatory power of phage typing and PFGE, Simpson's diversity index (DI), 95% confidence intervals (CI) and Wallace's index were calculated using Ridom EpiCompare software version 1.0 (Ridom GmbH, Germany).

RESULTS

Phage typing

The final typing scheme was established using 17 typing phages (Supplementary Table S1). In this study, we achieved a typability of 98% by the novel phage-typing scheme when typing more than 1000 isolates. Twenty-three (2%) isolates were untypable. Of the 985 isolates, 61 different phage types (DI 86·4, 95% CI 84·2–87·1) were identified. Table 1 shows the distribution of the detected phage types in various sources. The most prevalent phage types: PT29 (28%), PT01 (20%), PT11 (7%), PT09 (7%), PT24 (6%), PT04 (4%) and PT08 (3%) were found in humans and food as well as in animals. Interestingly, these frequent phage types have been detected in the majority of human sporadic cases ($n = 228$, 23%) as well as outbreaks ($n = 168$, 17%). Different phage types (e.g. PT01, PT09, PT11, PT29) have emerged in humans between 1973 and 2009, but only two phage types (PT01, PT29) account for the majority of clinical cases and most outbreaks of salmonellosis. During the 36-year study period a change in dominant phage types, e.g. PT01, PT09 and PT29 could be not observed.

Combination of PFGE and phage typing

The analysis of the selected 325 isolates revealed 58 different PFGE types (DI 95·0, 95% CI 85·2–97·5) and 31 defined phage types (DI 82·4, 95% CI 72·1–89·0). Supplementary Table S2 shows the distribution of the 325 isolates for each PFGE type and phage type. Cluster analysis of the 58 PFGE profiles revealed a similarity coefficient of 0·45. The resulting concordance showed that all outbreak isolates ($n = 89$, 27%) and the majority of sporadic isolates ($n = 150$, 46%) could be assigned to concordant phage types and PFGE types (Fig. 1). The probability of a pair of isolates with the same phage type also sharing the corresponding PFGE type was 71% (Wallace's index 0·71). Isolates of the three predominant PFGE types (XB05, XB27, XB34) showing an association with a certain phage type, e.g. isolates of XB34 ($n = 25$) were assigned to the same phage type (PT01)

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Table 1. *Distribution of phage types of* Salmonella *Infantis strains isolated from various sources*

Source	Phage type
Humans ($n=514$)	**1, 4, 8, 9, 11**, 19, **21, 24, 29, 30, 33, 38**, 51, **53**, 57, 59, 60, nt
Broilers* ($n=199$)	**1, 4**, 6, **8, 9**, 10, **11**, 14, 15, 17, **24**, 25, 26, **29**, 32, **33**, 35, **38**, 41, 46, 49, 58, 61
Chicken meat ($n=3$)	**9, 11, 24**
Layer hens ($n=16$)	**8**, 10, **29, 30**, 45, nt
Pigs ($n=71$)	**1**, 2, 3, 5, **8**, 9, **11, 21, 24**, 25, **29, 30, 33**, 39, 55, 61, nt
Pork ($n=26$)	**1**, 2, **4, 8, 11, 21, 24, 29**
Cattle ($n=39$)	**1, 9, 11**, 18, **24, 29**
Beef ($n=2$)	**29**, 39
Turkeys ($n=2$)	**8, 29**
Ducks ($n=4$)	61
Geese ($n=1$)	**29**
Wild animal ($n=16$)	**1, 4**, 5, **8, 9**, 15, 17, 40, nt
Dogs ($n=6$)	**8, 9**, 10, **11, 29**, 41
Feed ($n=21$)	**1, 9**, 10, **11**, 13, **24, 29**, 41, 42, **53**
Environment ($n=59$)	**1, 4**, 5, **8, 9**, 10, **11**, 15, 23, **24**, 26, 28, **29**, 30, **33**, 43, nt
Cake ($n=2$)	**53**
Raw sausage ($n=5$)	**1, 29, 33**
Food† ($n=16$)	**1, 8, 9, 11**, 19, 26, **29, 53**
Vaccine‡ ($n=6$)	**11**, 26, 47, 54, 56

nt, Non-typable by the present phage-typing scheme.
Bold values indicate the occurrence of some phage type in diverse sources.
* Isolates from broiler chicken carcasses.
† Isolates without further information.
‡ Isolates from autogenous vaccines for poultry.

(Supplementary Table S2). On the other hand, the results confirmed the ability of phage typing to further discriminate several frequent PFGE types, e.g. XB04, XB06 and XB16 (Supplementary Table S2). One example is isolates of PT53 ($n=11$) and PT29 ($n=8$) which were involved in two outbreaks but share a single PFGE type (XB06). Since 27 infrequent PFGE types (e.g. XB01, XB12) were represented by only single isolates the potential ability of phage typing to further discriminate within these PFGE types could not be evaluated.

Isolates belonging to clones PT29/XB27 (13%), PT29/XB05 (10%), PT01/XB34 (8%), PT53/XB06 (6%), PT24/XB02 (6%), PT29/XB27a (5%), PT04/XB04 (5%), and PT11/XB07 (5%) were epidemiologically predominant and found in humans, food, broiler chickens (Germany, Hungary) as well as in isolates from various animal species (Supplementary Table S3). Within the German broiler chicken isolates 11 clones, e.g. PT29/XB05 ($n=15$), PT29/XB27 ($n=4$), PT29/XB27a ($n=3$) and PT11/XB07 ($n=3$) were found whereas in Hungarian broiler chicken isolates only four clones, e.g. PT29/XB05 ($n=8$), PT29/XB14a ($n=1$), PT29/XB05a ($n=1$) and PT46/XB07a ($n=1$) could be detected.

Outbreak investigations

We typed 51 foodborne outbreaks with PT29/XB27 as the most prominent clone (Table 2, Supplementary Table S3). Clone PT29/XB27 was identified in hospital outbreaks, community outbreaks and in broiler chickens during 2002–2009. This clone was repeatedly detected in a rehabilitation clinic in Baden-Württemberg from 2002 to 2009 (Fig. 1). According to information from the clinic several episodes of *S.* Infantis gastroenteritis had occurred in this clinic but only in summertime, and this persisted for about 3–4 weeks every year. The sources of the latest infection in 2009 were a cross-contamination in the kitchen and carriers among the clinic personnel. These findings demonstrate the stability of the phage and PFGE types over a long period of time thereby supporting the applicability of the approach to outbreak investigations.

Two large outbreaks with 188 reported cases in two clinics (distance apart 130 km) were observed in Bavaria in 2004 and traced back to bakery products (Fig. 1). All isolates obtained from humans and food could be identified as an identical clone PT53/XB06 (Table 2, Supplementary Table S3). Two other

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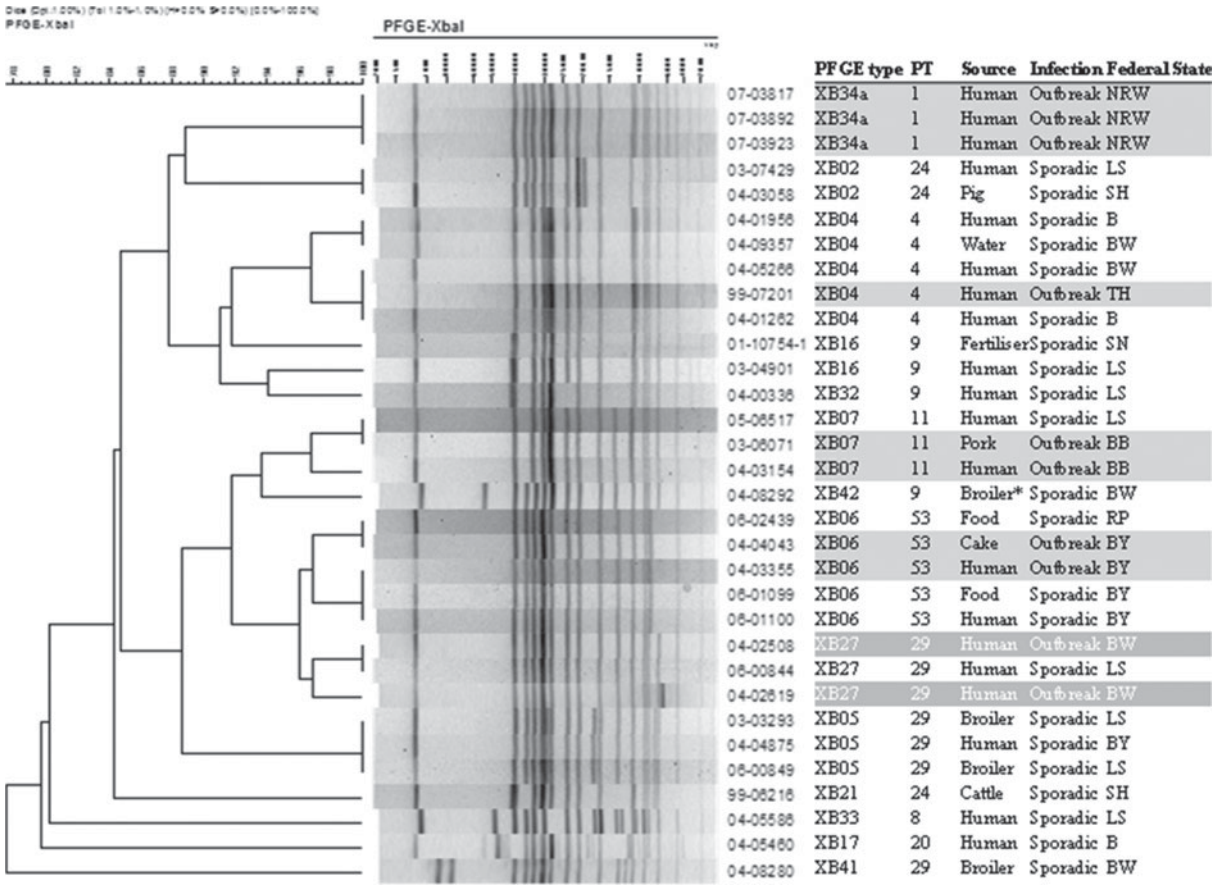

	PFGE type	PT	Source	Infection	Federal State
07-03817	XB34a	1	Human	Outbreak	NRW
07-03892	XB34a	1	Human	Outbreak	NRW
07-03923	XB34a	1	Human	Outbreak	NRW
03-07429	XB02	24	Human	Sporadic	LS
04-03058	XB02	24	Pig	Sporadic	SH
04-01956	XB04	4	Human	Sporadic	B
04-09357	XB04	4	Water	Sporadic	BW
04-05266	XB04	4	Human	Sporadic	BW
99-07201	XB04	4	Human	Outbreak	TH
04-01262	XB04	4	Human	Sporadic	B
01-10754-1	XB16	9	Fertiliser	Sporadic	SN
03-04901	XB16	9	Human	Sporadic	LS
04-00336	XB32	9	Human	Sporadic	LS
05-06517	XB07	11	Human	Sporadic	LS
03-06071	XB07	11	Pork	Outbreak	BB
04-03154	XB07	11	Human	Outbreak	BB
04-08292	XB42	9	Broiler*	Sporadic	BW
06-02439	XB06	53	Food	Sporadic	RP
04-04043	XB06	53	Cake	Outbreak	BY
04-03355	XB06	53	Human	Outbreak	BY
06-01099	XB06	53	Food	Sporadic	BY
06-01100	XB06	53	Human	Sporadic	BY
04-02508	XB27	29	Human	Outbreak	BW
06-00844	XB27	29	Human	Sporadic	LS
04-02619	XB27	29	Human	Outbreak	BW
03-03293	XB05	29	Broiler	Sporadic	LS
04-04875	XB05	29	Human	Sporadic	BY
06-00849	XB05	29	Broiler	Sporadic	LS
99-06216	XB21	24	Cattle	Sporadic	SH
04-05586	XB33	8	Human	Sporadic	LS
04-05460	XB17	20	Human	Sporadic	B
04-08280	XB41	29	Broiler	Sporadic	BW

Fig. 1. Macrorestriction patterns of 32 *Salmonella* Infantis isolates obtained with the restriction enzyme *Xba*I. The dendrogram shows the relationship of different *Xba*I patterns. Similarity analysis was performed using the Dice coefficient and the clustering was generated by UPGMA. *S.* Infantis isolates associated with outbreaks or sporadic cases could be assigned to a single combination of phage – and PFGE type (highlighted in grey). * Isolates from broiler chicken carcasses.

outbreaks in North Rhine-Westphalia in 2007 and 2008 caused by contaminated roast pork and chicken kebabs were assigned to the related clones (PT01/XB34a, PT01/XB34) (Fig. 1). Clone PT01/XB34 was also detected in isolates of broiler chickens ($n=1$), pigs ($n=2$), cattle ($n=1$), pork ($n=2$), and raw sausage ($n=1$). Moreover, 11 *S.* Infantis infections in three German Federal States: Thuringia, North Rhine-Westphalia and Lower Saxony were caused by clone PT29/XB27a; this clone was also found in three samples of broiler chicken (Supplementary Table S3). Other outbreak clones (PT11/XB07, PT21/XB01a, PT24/XB02) were also indentified in pork.

Antimicrobial susceptibility testing

The majority (809/1008, 80%) of the investigated isolates were susceptible ($n=317$), resistant to one ($n=393$) or resistant at least to two ($n=99$) of the 14 tested antimicrobial substances. The most frequent epidemic clones PT29/XB27 and PT01/XB34 were resistant only to one or two antimicrobial groups. Moreover, 199 (20%) isolates were multidrug-resistant. Notably, multidrug-resistant clones PT11/XB07 and PT29/XB27a were resistant to three antimicrobial groups (AMP-MEZ-OTE-SMZ). In this study we observed an increase of resistance to β-lactams, sulfonamides, tetracyclines, aminoglycosides and quinolones in 93 isolates of broiler chickens since 2002. The predominant multidrug-resistant clone (31 isolates; NAL-STR-SMZ-OTE) was PT29/XB05. Only four isolates of the present study (two from humans and two from broiler chickens) were resistant to cefotaxime or ceftazidime. In these isolates ESBL production was confirmed by Etest® ESBL (bioMérieux).

DISCUSSION

The present study demonstrates that the combination of a newly developed phage-typing scheme and *Xba*I

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Table 2. *Fifty-one foodborne outbreaks in Germany, 1974–2009*

Federal state	Year	Type of outbreak			Source	Phage type and/or PFGE type
		Family	Community	Nosocomial		
Baden-Württemberg	2002	x			—*	PT24
	2002			x	—	PT29/XB27
	2003			x	—	PT29/XB27
	2004			x	—	PT29/XB27
	2007			x	—	PT29/XB27
	2008			x	—	PT29/XB27
	2009			x	Food or carrier	PT29/XB27
Bavaria	1997		x		Raw sausage	PT09
	2004			x	Cake or carrier	PT53/XB06
	2004			x	Cake or carrier	PT53/XB06
Brandenburg	2004		x		Pork	PT11/XB07
Berlin	2002	x			—	PT01
Hesse	1975		x		—	PT23
	1997	x			—	PT33
Mecklenburg-West Pomerania	1998		x		—	PT29
	1999	x			—	PT01
	1999	x			—	PT01
	2002	x			—	PT01
Lower Saxony	1974		x		Rice pudding	PT01
	1998		x		Raw eggs	PT08/XB43a
	2002	x			—	PT29
	2006		x		—	PT29
	2007		x		—	PT29/XB06
	2007	x			—	PT29/XB27a
North Rhine-Westphalia	1996	x			—	PT29
	1999			x	—	PT01
	2000			x	—	PT24/XB02
	2007		x		Carrier or roast pork	PT01/XB34a
	2008		x		—	PT21/XB01a
	2008		x		Broiler chicken	PT01/XB34
Saxony-Anhalt	1996	x			Carrier	PT29
	1997	x			—	PT30
	2001	x			—	PT01
	2001	x			—	PT01
	2002	x			—	PT01
	2003	x			—	PT11
	2004	x			Carrier	PT29
	2005	x			—	PT24/XB02
	2005	x			Carrier	PT30
	2007	x			—	PT29/XB06
Schleswig-Holstein	2003		x		—	PT01
Thuringia	1999	x			—	PT04/XB04
	2000	x			—	PT19
	2000	x			—	PT09
	2002		x		—	PT11
	2003	x			—	PT24
	2006		x		—	PT29/XB27
	2007		x		—	PT29/XB27a
	2007	x			Carrier	PT29/XB27
	2007	x			—	PT29/XB27b
	2008			x	—	PT29/XB27a

* Unknown source.

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macrorestriction analysis is a reliable epidemiological tool for the routine typing of *S.* Infantis isolates at the NRC for *Salmonella.* We were able to type 985/1008 isolates with the established phage scheme, resulting in 61 different phage types. Previously only two phage-typing schemes existed for *S.* Infantis and these were developed more than 20 years ago by Laszlo *et al.* [27] and by Kasatiya *et al.* [28] in Hungary and Canada, respectively. However, in both typing systems several virulent phages or/and temperate phages were selected that cross-reacted with strains of different enterobacterial species as well as several *Salmonella* serovars (e.g. *S.* Thomson, *S.* Newport, *S.* Cerro). In contrast, phages of *S.* Infantis that we isolated for the new phage scheme are highly specific; they enable real-time monitoring of *S.* Infantis infections, and the results could be confirmed by PFGE analysis as well as by the study of Hauser *et al.* [17].

Phage typing demonstrated a high level of diversity in random isolates (61 phage types) and was also confirmed by results based on PFGE analysis with a low similarity coefficient of 0·45. In contrast, Hauser *et al.* [17] reported that the lowest observed similarity coefficient between strains was 0·72. Such heterogeneity between studies might be caused by differences in selection criteria of investigated isolates, e.g. variety of sources, number of isolates, and period of time. The combined typing showed the emergence of epidemic clones in humans (PT29/XB27, PT01/XB34) and the occurrence of the specific clone PT29/XB05 which is mainly associated with broiler chicken (Supplementary Table S3). These epidemic clones could be detected over many years in Germany indicating a highly genetic stability of these clones. Hauser *et al.* [17] reported that the *S.* Infantis clone PT29/XINF10 was predominantly found in Germany in humans, pigs, broiler chickens, and broiler meat. This clone, PT29/XINF10, is equivalent to clone PT29/XB27, according to the present study. However, at the moment there is no clear answer why this most dominant broiler chicken clone PT29/XB05 causes salmonellosis relative rarely (only four human cases). In contrast, other outbreak clones (e.g. PT01/XB34, PT29/XB27a, PT11/XB07) were also found in livestock animals or meat products (Supplementary Table S3). These findings indicate that broiler chickens or other contaminated foods are probable sources of *S.* Infantis infections in humans. A direct confirmation of the source of infection was possible for five outbreaks within the present study (Table 2).

Recent studies of the EFSA have shown that the serovar *S.* Infantis is very common in poultry flocks in the EU [11]. Furthermore, several studies suggest the occurrence of country-specific clones disseminated in humans and broilers in Hungary, Israel, Germany and Japan [6, 7, 17, 29]. Interestingly, clone PT29/XB05 was most prevalent in broiler chicken isolates from Germany and Hungary. Due to a lack of complete information on the broiler chicken strains that were sent to the NRC it is not entirely clear if this clone is in fact disseminated in German broiler flocks or if the strains transmitted in Germany are through the import of Hungarian broilers since 2003.

Two of the most prevalent clones, PT29/XB05 and PT29/XB27, are indistinguishable by phage typing but clone PT29/XB05 shows two additional bands of 70 kbp and 170 kbp in macrorestriction pattern. This is probably due to the presence of plasmids harbouring resistance genes resulting in additional resistance of clone PT29/XB05 to NAL-STR-SMZ-OTE. Corresponding to these results Nógrády *et al.* [6] reported recently that *S.* Infantis clones PT213 and PT217 were commonly found in Hungary in humans as well as in broilers. In the phage-typing scheme of Laszlo *et al.* [27], PT213 is equivalent to PT29 according to the phage-typing scheme presented in this work. Furthermore, results of other studies indicated the dissemination of an epidemic multidrug-resistant clone of *S.* Infantis from broilers in Germany, Hungary, Austria, and Poland [30]. However, it can only be hypothesized that the multidrug-resistant clone PT29/XB05 in the present study corresponds to the multidrug-resistant clone described by Nógrády *et al.* [30]. The presence of a plasmid conferring multidrug resistance could have facilitated the rapid spread of this multidrug-resistant clone in humans and livestock. Therefore, a continuous surveillance, especially of the multidrug-resistant clone PT29/XB05, is recommended.

Notably, four *S.* Infantis isolates from humans and broiler chickens belonging to epidemic clones PT01/XB34 and PT29/XB27 were found to be resistant to ceftazidime and cefotaxime due to ESBL production, although the use of cephalosporin in broilers has not been approved in Germany [31]. A possible reason for this might be the transfer of resistance plasmids from *E. coli* to *S.* Infantis since recently a high prevalence of ESBL has been described for *E. coli* from poultry in The Netherlands and Germany [32, 33]. ESBL-producing *S.* Infantis isolates have been

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increasingly reported as a source of nosocomial outbreaks posing a serious threat especially in paediatric and neonatology wards [5]. Although outbreaks with ESBL-producing *S.* Infantis are not known in Germany, continuous surveillance is required for early detection of these multidrug-resistant isolates.

The successful application of novel phage typing combined with PFGE analysis is demonstrated in the detailed analysis of outbreaks in a rehabilitation clinic in Baden-Württemberg diring 2002–2009. The cause of these outbreaks was the epidemic clone PT29/XB27, which was repeatedly identified over a period of 8 years. Retrospective investigations revealed that *S.* Infantis infections had been observed in this clinic since 1992. However, since, no patient isolates were preserved before 2002 it can only be hypothesized that clone PT29/XB27 has been endemic in the clinic for more than 8 years. Previous studies have also shown the ability of *S.* Infantis to persist in hospitals but the time spans reported were shorter than in the outbreaks described here [3, 4]. Possible explanations for the repeated isolation of clone PT29/XB27 might be readmission of carrier patients or repeated re-introduction by clinic personnel or contaminated food. A study of Murakami *et al.* [10] implied that asymptomatic carriers of *S.* Infantis associated with food preparation are a possible reservoir for infections. However, the potential duration of carriage is currently uncertain.

In conclusion, it has been shown that a novel phage-typing scheme is suitable for identification of outbreaks of *S.* Infantis as well as for long-term surveillance in Germany. The combined use of phage typing and PFGE analysis has proven to be an advantageous method for *S.* Infantis strains. The combined typing facilitates the detailed investigation of the dissemination of epidemic *S.* Infantis clones in humans, food, and livestock nationwide and internationally.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S095026881300037X.

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DECLARATION OF INTEREST

None.

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