Serotype and Phage Type Distribution of *Salmonella* Strains Isolated from Humans, Cattle, Pigs, and Chickens in The Netherlands from 1984 to 2001

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We studied serotypes and phage types of *Salmonella* strains isolated from humans and animals in The Netherlands over the period 1984 to 2001. All human strains ($n = 59,168$) were clinical isolates. The animal strains ($n = 65,567$) were from clinical and nonclinical infections. All isolates were serotyped, and *Salmonella enterica* serovar Typhimurium and serovar Enteritidis strains were further phage typed. The most prevalent serotypes were as follows: in humans, serovars Typhimurium and Enteritidis; in cattle, serovars Typhimurium and Dublin; in pigs, serovar Typhimurium; and in chickens, serovars Enteritidis, Infantis, and Typhimurium. Serovar Enteritidis phage type 4 (pt 4) was the most common phage type in humans and chickens. Serovar Typhimurium pt 510 was the most prevalent serovar Typhimurium phage type in humans and pigs, pt 200 was the most prevalent serovar Typhimurium phage type in cattle, and pt 150 was the most prevalent serovar Typhimurium phage type in chickens. Analysis of the distribution of sero- and phage types during the study period indicated that types shifted over time in humans and animals. Serovar Typhimurium DT 104 emerged in 1991 in humans, cattle, pigs, and chickens and became the most common serovar Typhimurium phage type in 2001. In general, similar sero- and phage types were found in humans and animals, although distinct types were more common in animals. Between the animal species, the sero- and phage type distributions varied considerably.

Nontyphoid salmonellosis is a major zoonotic disease in humans. In The Netherlands, which has a population of 15.8 million, 50,000 cases of salmonellosis are reported each year (incidence, 3 per 1,000 person-years) (24). Although the incidence of *Salmonella* infections in humans in The Netherlands has been decreasing during the last 2 decades, salmonellosis is still an important public health problem (22).

Salmonella infections in humans are often food borne but can also be contracted through contact with infected animals. Food containing products from farm animals, especially from poultry, pigs, and cattle, are an important source of human *Salmonella* infections. Surveillance of *Salmonella* serovars and phage types from human and animal sources is relevant for detecting outbreaks, for identifying sources of infection and for implementing prevention and control measures. Two major changes in the sero- and phage type distribution of nontyphoid salmonellosis have occurred during the last decades in many European countries and the United States of America (14). First, *Salmonella enterica* serovar Enteritidis has emerged as a major egg-associated pathogen (13). Secondly, the prevalence of multidrug-resistant *S. enterica* serovar Typhimurium phage types, such as serovar Typhimurium definite phage type 104 (DT 104) has increased. Serovar Typhimurium DT 104 initially

emerged in cattle in 1984 in England and Wales but spread to the European continent and the United States of America and has meanwhile been isolated from poultry, sheep, pigs, horses, many other animal species, and humans (3, 16, 17, 20; T. E. Besser, C. C. Gay, J. M. Gay, D. D. Hancock, D. Rice, L. C. Pritchett, and E. D. Erickson, Letter, Vet. Rec. 18:75, 1997). Its relative importance in humans and farm animals in The Netherlands is increasing (23).

The purpose of this retrospective study is to provide insight into the distribution of sero- and phage types of *Salmonella* strains isolated from humans, cattle, pigs, and chickens in The Netherlands in the period from 1984 to 2001.

MATERIALS AND METHODS

Bacterial isolates. (i) Human isolates. Since 1984 the Dutch National Institute of Public Health and the Environment (RIVM) has obtained a total of 59,168 *Salmonella* isolates from the regional Public Health Laboratories for further typing. All were the first isolates from patients suffering from salmonellosis (clinical isolates). Approximately 1.6% of these isolates originated from blood.

(ii) Animal isolates. The majority of these isolates were sent to the RIVM by the regional Dutch Animal Health Services. They were from clinical (approximately 85%) and nonclinical (approximately 15%) *Salmonella* infections from pigs ($n = 10,058$), cattle (including calves) ($n = 10,582$), and chickens ($n = 44,927$) and sampled on the farm and at the slaughterhouse.

Sero- and phage typing. Isolates were submitted to the Diagnostic Laboratory for Infectious Diseases and Perinatal Screening of the RIVM for further serotyping based on O- and H-group antigens according to the latest versions of the Kauffmann-White scheme using slide and microtiter plate agglutination. Serovar Typhimurium strains were phage typed using the Dutch phage typing system (8). For serovar Enteritidis strains the phage typing system described by Ward et al. (27) has been used since 1997.

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The Dutch phage typing system for Typhimurium was gauged in 1997 against the English system, showing that the English and Dutch types do not have one-to-one relationships. Of the phage types mentioned in this paper, the Dutch phage type 2 (pt 2) corresponds mainly with the English DT 2 and atypically reacting strains (ARS) in the English system; pt 10 corresponds mainly with DT 3 (and to a lesser extent with DT 40); pt 20 corresponds with DT 124; pt 61 corresponds with DT 12; pt 150 corresponds with ARS, DT 161, and DT 12 (and to a lesser extent DT 66, DT99, and DT 32); pt 200 corresponds with DT 208 and ARS; pt 296 corresponds with DT 12 and ARS; pt 461 corresponds with DT 1 and DT 126; pt 507 corresponds with ARS and DT 208; and pt 510 corresponds with DT 208 and ARS (and to a lesser extent DT 193 and DT195). Recently it was shown that pt 204 corresponds with DT 204 and pt 193 corresponds with DT 193. For pt 132, no comparable DT number has been encountered (W. van Pelt, personal communication).

RESULTS

Serotypes. The most prevalent serotypes during the period from 1984 to 2001 were serovars Typhimurium (44% of all *Salmonella* isolates) and Enteritidis (24%) in humans; serovars Dublin (53%) and Typhimurium (39%) in cattle; serovars Typhimurium (69%), Panama (5%), and London (4%) in pigs; and serovars Typhimurium (18%), Infantis (14%), and Enteritidis (12%) in chickens. Overall, serovar Typhimurium was the serotype most frequently isolated from humans and animals, accounting for 37% of all *Salmonella* isolates included in this study. Serotype prevalences varied considerably over time (Table 1). The relative importance of serovar Typhimurium gradually declined in humans, chickens, and cattle, but not in pigs. In humans, serovar Typhimurium was the predominant serovar until 1991. Since 1988, the number of serovar Enteritidis isolates has been increasing, and since 1993 it has been the most frequently isolated serovar from humans. In chickens, the same overall pattern was found. In pigs, serovar Typhimurium remained the predominant serovar throughout the whole period. Serovars Panama, London, Infantis, Derby, Brandenburg, and Livingstone were also found regularly. Bovine *Salmonella* infections were dominated by the serotypes Typhimurium and Dublin. Serovar Dublin has been the most-prevalent serotype since 1991. However, human infections with this serotype remained rare (224 isolates from 1984 to 2001; 0.4% of all human isolates).

Serovar Typhimurium phage types. In several periods, similar changes in serovar Typhimurium phage types were found in humans and distinct animal species. For example, the emergence and decline of serovar Typhimurium pt 150 occurred simultaneously in humans and chickens. Similarly, human infections with serovar Typhimurium pt 200 occurred in periods in which this phage type was predominantly isolated from cattle but was rarely isolated from pigs and chickens. Serovar Typhimurium pt 510 was commonly isolated from humans, pigs, and cattle (20, 20, and 9% of serovar Typhimurium isolates, respectively), but not from chickens (1% of serovar Typhimurium isolates). The multiresistant serovar Typhimurium pt 506 and pt 401 (DT 104 in the English phage typing system) was the most important emerging phage type in humans, pigs, and cattle. In 2001, this phage type was responsible for 15, 16, 10, and 3% of all *Salmonella* infections in humans, pigs, cattle, and chickens, respectively, and for 43, 25, 31, and 57% of all serovar Typhimurium infections. In humans, serovar Typhimurium pt 510, 150, 61, 20, and 296 and DT 104 prevailed (Table 2). Serovar Typhimurium pt 510 was predominant until

1995. From 1996 onwards pt 506 and 401 (DT 104) were the most prevalent phage types. In chickens, serovar Typhimurium pt 150 was the predominant phage type until 1995, but from 1996 onwards pt 506 and pt 401 (DT 104) were found most often. Other phage types were pt 461, pt 10, and pt 2 (Table 2). In pigs, pt 510, pt 506 and pt 401 (DT 104), pt 20, and pt 61 were found most often. pt 510 predominated until 1995, but in the period 1996 to 2001 DT 104 prevailed (Table 2). In cattle, pt 200 predominated until 1989, pt 204 predominated from 1990 to 1995, and DT 104 predominated from 1996 onwards. Serovar Typhimurium pt 510 was isolated frequently throughout the whole period (Table 2).

Serovar Enteritidis phage types. Serovar Enteritidis pt 4 was the major serovar Enteritidis phage type in humans as well as chickens. Other common phage types were pt 21, pt 28, pt 1, and pt 6 (Table 3). In pigs and cattle, serovar Enteritidis appeared to be of minor importance, as only 0.3 and 0.9% of all *Salmonella* isolates from pigs and cattle, respectively, carried this type.

DISCUSSION

The serotypes found in this study can be divided into host-specific, host-adapted, and non-host-specific serotypes. Serovar Typhi, which is host specific, was found only in humans. Serovar Dublin, which is host adapted, was the most-prevalent serotype in cattle but was only sporadically isolated from humans. Although infrequently found in humans, serovar Dublin appears to be a serious event in humans. Van Pelt et al. (23) found that 28% of all serovar Dublin isolates from humans in The Netherlands were cultured from blood, compared to 3% for serovar Typhimurium and 2% for serovar Enteritidis (23). This is consistent with the observation that in humans this serotype often causes severe invasive disease (9). With regard to the non-host-specific serotypes, a substantial correlation was found between serotypes from humans and animals.

A major finding from our work was that the distribution of serotypes and phage types changed considerably over the study period. This was observed for both human and animal isolates. Niches created by the decrease of one serotype or phage type often seem to be filled in by others. Often shifts in serotypes or phage types in animals and humans moved in a similar direction, although the prevalences of some of these sero- and phage types differed considerably. Possible explanations for the differences in prevalence between humans and animals range from the variable exposure of humans to the different animal sources and the different food processing procedures used for the various animal products to differences in susceptibility between hosts to certain sero- and phage types. It should also be considered that up to 10% of all *Salmonella* infections in The Netherlands have been estimated to be contracted abroad or caused by imported food products. Moreover, although most human *Salmonella* infections in The Netherlands are food borne, they can also result from direct or indirect contact with other farm animals, wildlife, or pets. Van Pelt et al. (21) noted that in The Netherlands human salmonellosis was caused in about 25% of cases by *Salmonella* types originating from pigs, was caused in about 11% of cases by *Salmonella* types originating from cattle, was caused in about 21% of cases by *Salmonella* types originating from chickens,

TABLE 1. *Salmonella* serovars isolated most frequently in The Netherlands from 1984 to 2001

Source and serotype	Data for isolates from indicated period					
	1996–2001		1990–1995		1984–1989	
	% (no.)	Rank	% (no.)	Rank	% (no.)	Rank
Human						
Enteritidis	43.6 (6,097)	1	38.8 (6,642)	1	5.4 (1,526)	2
Typhimurium	32.0 (4,470)	2	33.1 (5,666)	2	57.2 (16,049)	1
Infantis	1.8 (252)	3	1.6 (270)	6	3.3 (922)	5
Hadar	1.8 (248)	4	2.4 (403)	4	1.5 (408)	10
Brandenburg	1.7 (230)	5	1.0 (179)	9	2.5 (690)	6
Bovismorbificans	1.6 (219)	6	2.2 (380)	5	2.3 (640)	7
Virchow	1.4 (190)	7	4.7 (799)	3	4.3 (1,192)	4
Panama	0.9 (122)	8	1.0 (178)	10	4.7 (1,321)	3
Goldcoast	0.9 (120)	9	0.4 (60)		0.6 (170)	
Typhi	0.8 (108)	10	1.4 (239)	8	1.5 (409)	9
Other	13.8 (1,923)		13.5 (2,306)		16.9 (4,740)	
Total no.	13,970		17,122		28,067	
Pig						
Typhimurium	66.4 (2,384)	1	75.7 (2,378)	1	66.0 (2,197)	1
Infantis	7.8 (279)	2	1.3 (40)	7	0.8 (27)	6
London	4.6 (166)	3	1.3 (41)	6	5.4 (181)	4
Derby	4.3 (153)	4	3.3 (103)	3	3.1 (104)	5
Panama	3.9 (138)	5	3.1 (96)	4	8.5 (282)	2
Brandenburg	3.2 (114)	6	1.8 (44)	5	5.5 (184)	3
Livingstone	2.6 (93)	7	5.9 (186)	2	0.7 (23)	7
Other	7.3 (263)		8.1 (253)		9.9 (330)	
Total no.	3,590		3,141		3,328	
Chicken						
Enteritidis	20.3 (1,290)	1	15.0 (2,802)	2	5.5 (1,088)	6
Paratyphi B var. Java	14.8 (944)	2	0.1 (21)		0.2 (31)	
Infantis	12.8 (813)	3	3.5 (2,535)	4	17.8 (2,928)	2
Heidelberg	6.3 (400)	4	6.6 (1,244)	6	7.8 (1,548)	4
Mbandaka	5.6 (357)	5	2.4 (456)	7	0.8 (155)	
Typhimurium	5.3 (336)	6	14.3 (2,669)	3	26.1 (5,170)	1
Virchow	5.2 (331)	7	11.4 (2,138)	5	11.1 (2,200)	3
Indiana	4.3 (273)	8	0.3 (58)		0.6 (116)	
Agona	3.9 (245)	9	1.8 (329)	9	1.2 (234)	
Hadar	3.1 (195)	10	16.2 (3,034)	1	7.2 (1,428)	5
Other	18.6 (1,187)		18.4 (3,444)		24.9 (4,928)	
Total no.	6,371		18,730		19,826	
Cattle						
Dublin	52.8 (1,128)	1	61.7 (3,229)	1	39.8 (1,278)	2
Typhimurium	32.9 (702)	2	31.9 (1,670)	2	53.1 (1,706)	1
Other	14.3 (306)		6.4 (333)		7.2 (230)	
Total no.	2,136		5,232		3,214	

was caused in about 39% of cases by *Salmonella* types originating from eggs, and was caused in about 4% of cases by *Salmonella* types originating from other sources. These findings were based on the relative occurrence of the sero- and phage types in the animals. These figures do not correlate with the *Salmonella* contamination rate of chicken meat (21%), beef (1%), pig meat (6%), and eggs (0.03%) (percentages are from the year 2000), even when the level of contamination is taken into account (24).

A striking feature also apparent from our data is the emer-

gence of serovar Enteritidis. In the 1980s, the number of human serovar Enteritidis infections in Europe and the United States of America increased dramatically (1, 14). In The Netherlands, serovar Enteritidis emerged in 1987 and has been the predominant serovar in humans since 1993, replacing serovar Typhimurium. Chicken products, especially eggs, are considered to be the main source of the human infections, and rodents are thought to be an important reservoir (13, 14). In the present study, pt 4 was the main phage type in humans and chickens. This type has also been reported to be the most

TABLE 2. *Salmonella* serovar Typhimurium phage types isolated most frequently in The Netherlands from 1984 to 2001

Source and phage type	Data for isolates from indicated period					
	1996–2001		1990–1995		1984–1989	
	% (no.)	Rank	% (no.)	Rank	% (no.)	Rank
Human						
DT 104 (pt 506 and pt 401)	29.4 (1,315)	1	7.4 (417)	2	0.7 (115)	
pt 510	9.0 (400)	2	17.1 (971)	1	23.9 (3,839)	1
pt 296	6.2 (275)	3	1.4 (77)		0.0 (0)	
pt 20	4.0 (178)	4	6.2 (350)	3	7.3 (1,173)	4
pt 507	3.8 (169)	5	0.8 (45)		0.0 (0)	
pt 61	1.8 (80)		3.7 (207)	5	8.5 (1,359)	3
pt 150	0.7 (31)		5.6 (318)	4	11.8 (1,896)	2
Total no.	4,470		5,666		16,049	
Pig						
DT 104 (pt 506 and pt 401)	19.4 (463)	1	7.8 (185)	2	1.1 (23)	
pt 510	12.4 (295)	2	21.8 (518)	1	26.0 (572)	1
pt 507	4.5 (108)	3	1.2 (29)		0.0 (0)	
pt 80	3.6 (86)	4	4.0 (94)		2.0 (48)	
pt 351	3.5 (83)	5	3.6 (86)		1.0 (23)	
pt 296	3.3 (79)	6	0.6 (13)		0.0 (0)	
pt 61	3.3 (78)	7	2.2 (52)	8	6.7 (148)	3
Total no.	2,384		2,378		2,197	
Chicken						
DT 104 (pt 506 and pt 401)	25.9 (87)	1	2.4 (64)	3	0.04 (2)	
pt 2	8.3 (28)	2	1.0 (26)	7	1.3 (68)	4
pt 150	8.0 (27)	3	59.3 (1,582)	1	70.6 (3,648)	1
pt 10	0.3 (1)		1.5 (41)	4	2.2 (115)	2
pt 132	0.0 (0)		0.0 (0)		2.0 (104)	3
pt 461	1.2 (4)		4.5 (121)	2	0.9 (46)	6
Total no.	336		2,669		5,170	
Cattle						
DT 104 (pt 506 and pt 401)	34.8 (244)	1	9.6 (160)	2	1.5 (25)	
pt 510	8.8 (62)	2	7.7 (129)	3	10.3 (175)	3
pt 2	3.3 (23)	3	1.4 (24)		0.2 (3)	
pt 296	2.9 (20)	4	0.4 (6)		0.0 (0)	
pt 193	0.0 (0)		0.0 (0)		15.7 (267)	2
pt 204	0.0 (0)		20.1 (336)	1	0.0 (0)	
pt 200	0.3 (2)		7.5 (126)	4	29.8 (508)	1
Total no.	702		1,670		1,706	

common phage type in the United Kingdom, Germany, France, Italy, Spain, Japan, and Brazil (4, 7, 14, 15, 19, 26). In other countries, however, different phage types appear to predominate, for example, pt 6 in Denmark, pt 8 in Poland and the Slovak Republic, pt 1 in Russia and Hungary, and pt 8 and pt 13a in the United States of America (1, 6, 14). Genotyping of serovar Enteritidis isolates from the various regions revealed that there are two evolutionary lineages, one containing pt 8 and pt 13a and the other containing pt 4 and pt 1. It has been suggested that the pandemic was caused by the clonal expansion of a more virulent serovar Enteritidis strain. However, the simultaneous emergence of different phage types of serovar Enteritidis in different countries argues against this (14). Baumler et al. (2) proposed that the eradication of serovar Gallinarum in poultry resulted in loss of flock immunity against

the O9 antigen, which is shared by the serovars Gallinarum and Enteritidis, thereby enabling serovar Enteritidis to spread.

The phage type DT 104 (serovar Typhimurium pt 506 and pt 401) was isolated from humans and animals in The Netherlands for the first time in 1985. Our data indicate that from then on this phage type has emerged particularly in humans, pigs, and cattle and has become the major serovar Typhimurium phage type in these species. In Dutch chickens, serotype Typhimurium has been of minor importance during the last few years, and DT 104 is not a common phage type in this animal. In the United Kingdom in 1995, as much as 77% of *Salmonella* isolates from poultry were DT 104 (11). A major concern about the emergence of serovar Typhimurium DT 104 is the chromosomally encoded penta-drug resistance, which is a common characteristic of isolates from many different coun-

TABLE 3. *Salmonella* serovar Enteritidis phage types isolated most frequently from humans and chickens in The Netherlands from 1997 to 2001

Phage type	Data for isolates from indicated source			
	Human		Chicken	
	% (no.)	Rank	% (no.)	Rank
4	65.0 (3,137)	1	57.9 (654)	1
21	5.9 (286)	2	8.1 (91)	2
28	5.9 (283)	3	3.5 (40)	3
1	5.1 (245)	4	2.9 (33)	5
6	4.5 (218)	5	3.4 (38)	4
Total no.	4,827		1,129	

tries. In addition, infections with DT 104 seem to follow a more severe clinical course (12). In The Netherlands, 3.9% of the serovar Typhimurium DT 104 isolates were cultured from blood compared to 2.8% of other serovar Typhimurium phage types (23). However, in one similar study, Threlfall et al. (E. J. Threlfall, L. R. Ward, and B. Rowe, Letter, *Lancet* **352**:287-288, 1998) did not find this difference.

Another notable finding is the presence of serovar Paratyphi B variation Java in chickens. This serotype emerged in 1996, possibly through contaminated imported raw materials for chicken feed, and has since increased and become the predominant serotype in 2001 (30% of all *Salmonella* isolates), replacing serovar Enteritidis. Serovar Paratyphi B var. Java is almost exclusively found in broilers. Thus far, vertical transmission has not been demonstrated. Analysis of isolates from the year 2000 indicated that in The Netherlands 20% of chicken meat was contaminated with *Salmonella*, and 33% of these isolates were serovar Paratyphi B var. Java (24). The increasing frequency of isolation of serovar Paratyphi B var. Java from chickens has also been reported from Germany (5), but not from other European countries. On broiler farms this serotype has proven to be extremely difficult to eradicate. Currently, the serotype has only sporadically been found in humans (0.1% of all isolates) with gastroenteritis.

A prominent finding of our study was the considerable difference in the distribution of sero- and phage types between chickens, pigs, and cattle. The basis of these differences remains to be defined. One possible cause may be the animal feed. Raw materials for animal feed are generally thought to be an important source of salmonellae. However, although animal feed is often contaminated with *Salmonella*, the serotypes found in animal feed and its raw materials do not correspond with those found in animals (10, 18; van Pelt, personal communication). Other possible reasons for the observed different distributions of *Salmonella* types among animal species are differences in housing, husbandry, and management practices in chicken, pig, and cattle production. Established risk factors for clinical salmonellosis in cattle herds include the spreading of poultry litter on neighboring farms, the presence of rodents in cattle housing, and pasture grazing by wild geese (28). Spreading pig manure is an important risk factor favoring serovar Typhimurium DT 104 infections on Dutch cattle farms (25). In this scenario, local factors seem to play an important role in *Salmonella* epidemiology, which would be of particular

importance for studies aimed at tracing the sources and reservoirs of human *Salmonella* infections.

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