

## Distribution of *Salmonella enterica* Serovars from humans, livestock and meat in Vietnam and the Dominance of *Salmonella* Typhimurium Phage Type 90

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### Abstract

Epidemiologically unrelated non-typhoid *Salmonella* isolates from humans ( $n = 56$ ) and animal origin ( $n = 241$ , from faeces, carcasses and meat) in Vietnam were investigated. *Salmonella* Typhimurium, *S. Anatum*, *S. Weltevreden*, *S. Emek*, and *S. Rissen* were the most prevalent serovars. *S. Typhimurium* phage type 90 was predominant among *S. Typhimurium* isolates. The serotype and phage type distribution of the *Salmonella* isolates was different from that in Europe and America. Many sero- and phage types found in humans were also found in cattle, pigs, and poultry suggesting that food producing animals are an important source of human non-typhoid *Salmonella* infection in Vietnam.

### 1. Introduction

Non-typhoid *Salmonella* infections in humans continue to be a major problem, in terms of both morbidity and economic costs (Tirado and Schmidt, 2001). The majority of the 2500 *Salmonella* serovars are capable of causing infections in humans. Most human *Salmonella* outbreaks are associated with the consumption of contaminated products from animal origin (Wray and Wray, 2000) although non-foodborne *Salmonella* infection in humans may be transmitted during contact with animals, contaminated water, or the environment.

The widespread distribution of food is a global challenge in *Salmonella* control. With increasing travel and global trade, outbreaks involving widely scattered cases are occurring more frequently. Contaminated food produced in one country may cause illness in another, demonstrating the importance of national control programmes. Besides sero-typing, phage typing has played a central role in epidemiological studies in *Salmonella* Typhimurium and *Salmonella* Enteritidis (Anderson et al., 1977 and Ward et al., 1987).

The aim of this study was to examine the distribution of serovars and phage types among *Salmonella* strains isolated from humans, cattle, pigs and poultry in Vietnam in order to contribute to the understanding of the epidemiology of *Salmonella*.

## 2. Materials and methods

### 2.1. Bacterial isolates

A total of 297 epidemiologically unrelated isolates were included in this study. The isolates were from humans ( $n = 56$ ), cattle ( $n = 63$ ), pigs ( $n = 111$ ), and poultry ( $n = 67$ ). The 56 clinical human isolates of unrelated patients with diarrhoea and fever were obtained from five provincial hospitals and two Pasteur Institutes in Vietnam.

### 2.2. Sampling

The animal isolates were collected from pigs, cattle, chickens and ducks (Table 1) in 13 provinces of South Vietnam during the year 2004. Faecal samples from healthy animals were taken at the slaughterhouses (78%) and from healthy or sick animals on farms (12%). The animals sampled came from different flocks or herds. For poultry, only samples from healthy animals were collected due to the risk of avian flu infection during the study period. Rectal faecal samples were taken from pigs and cattle. Faecal samples from poultry were taken from the distal part of the intestinal tract in the slaughterhouses or from pooled samples from 5 to 10 animals on the farms. About 25 g of faeces was collected from each animal (except for poultry on the farms) and placed in a sterile sampling bag, kept in an ice-box at 4 °C and transported to the diagnostic laboratories within 24 h. For carcass and meat samples, a carcass-swabbing method using cotton surgical gauzes (10 cm × 20 cm × 5 mm) was applied to the whole carcasses of poultry, pigs or cattle in the slaughterhouses. No carcass samples were taken from animals from which rectal faecal samples were collected. Approximately 25 g of minced meat or cut meat was sampled in supermarkets, markets or restaurants and transported to the laboratory in the same conditions described for faecal samples. Five to 20 samples were taken at each animal farm, slaughterhouse, market or restaurant. Samples were analysed at Nong Lam University laboratories and two laboratories of the Provincial Departments of Animal Health of Vietnam.

Table 1.

Origins of the investigated *Salmonella* isolates

	No. samples taken	No. positive (%)	No. investigated isolates			
			From faeces	From carcasses	From meat	Total
Pigs	534	264 (49.4)	77 (14 <sup>a</sup> )	23	11	111
Cattle	390	107 (27.4)	37 (16 <sup>a</sup> )	16	10	63
Chicken	257	99 (38.5)	18	35	11	64
Ducks	34	7 (20.5)	3	0	0	3

<sup>a</sup> Isolates from animals with diarrhoea.

### 2.3. *Salmonella* isolation

The bacterial culture procedure was carried out as described in standard ISO-6579 (International Organization for Standardization, 1993). Briefly, faecal or meat samples were mixed with Buffer Peptone Water (BPW) at a 1:9 ratio, using a stomacher for 1 min and incubated at 37 °C for 18–24 h. Then, 1 and 0.1 ml of the culture was transferred to 10 ml of Müller-Kauffmann Tetrathionate Broth (MKT) and 10 ml of Rappaport Vassiliadis Soya Broth (RVS), respectively, and incubated at 42 °C for 24–48 h. The culture was then streaked onto Xylose Lysine Decarboxylase Agar (XLD) and Brilliant Green Agar (BGA). Colonies suspected of being *Salmonella* were transferred to Nutrient Agar plates and were biochemically characterized using Triple Sugar Iron Agar (TSI), Lysine Decarboxylase Broth (LDC), Urea Agar, and Tryptone Broth. All media (except Lysine Decarboxylase Broth supplied by Merck) were purchased from Oxoid (UK). If more than one sample from a slaughterhouse, farm, market or supermarket was *Salmonella* positive, only one isolate was randomly chosen and included in this study.

### 2.4. Sero typing and phage typing

*Salmonella* isolates were serotyped by slide and microtitre agglutination for O and H antigen (the antisera were purchased from Statens Serum Institut, Denmark) according to the latest version of the Kauffmann and White scheme (Popoff, 2001) at the Diagnostic Laboratory for Infectious Diseases and Perinatal Screening of the Dutch National Institute of Public Health and the Environment (RIVM).

Bacteriophage typing was used for *S. Typhimurium* and *S. Enteritidis*. The pattern of lysis produced by infection with typing phages was recorded and designated in accordance with the standard schemes. UT indicates untypeable, which means that a tested isolate was not lysed by any of the typing phages. RDNC indicates that the isolate reacted with some of the typing phages but did not conform to any recognized phage type in the typing scheme. *S. Typhimurium* phage types were determined using the method described by Anderson et al. (1977) and the interpretive guidelines supplied by the Public Health Laboratory Service (PHLS) in Colindale, United Kingdom. In addition, the Dutch phage typing system was also used (Guinee et al., 1974). *Salmonella* Enteritidis isolates were phage typed using the English phage typing system as described by Ward et al. (1987).

## 3. Results

The percentage of *Salmonella*-positive samples was 20.5, 27.4, 38.5 and 49.4% for duck, cattle, chicken and pig samples, respectively (Table 1). We found 38 serovars among the 297 *Salmonella* isolates originating from humans and animals. The 10 most common serovars represented approximately 79% of the collection (Table 2). The predominant serovars (*S. Typhimurium*, *S. Anatum*, *S. Weltevreden*, *S. Emek*, and *S. Rissen*) accounted for about 59% of the isolates. The distribution of *Salmonella* serovars among the different sources is shown in Table 2. *S. Typhimurium* (37.5%) was the most common serovar among the 56 *Salmonella* isolates from humans, followed by *S. Enteritidis* (12.5%) and *S. Weltevreden* (7.1%). In cattle, serovar *Anatum*, *Weltevreden* and *Lexington* were predominant and represented 23.8, 17.5 and 15.8%, of the 63 bovine isolates, respectively. Among the 111 porcine isolates, *S. Anatum* (26.1%) and *S. Typhimurium* (20.7%) were the most common serovars, followed by *S. Weltevreden* (15.3%), *S. Derby* (11.7%) and *S. Rissen* (11.7%). *S. Emek* (38.8%) and *S. Blockley* (20.9%) were the most prevalent serovars among 67 isolates originating from poultry. The distribution of *Salmonella* *Typhimurium*

phage types is shown in Table 3. Of the 47 *S. Typhimurium* isolates, the Dutch phage type (pt) 90 was predominant and accounted for 57.5% of all *S. Typhimurium* isolates from humans, pigs and cattle. These 27 isolates could not be typed with the English phage typing system (see Table 3). *S. Typhimurium* RDNC accounted for nearly 20% of the phage types of this serovar (by the Dutch system). The Dutch phage type 506 (corresponding with DT 104 in the English phage typing system) was found at a low rate (4.3%). Among the *S. Enteritidis* isolates, two phage types were found, pt 1 ( $n = 1$ ) and pt 7 ( $n = 2$ ). Four isolates did not react with any of the phages used.

Table 2.

Distribution of the most common *Salmonella* serovars isolated in Vietnam from humans, cattle, pigs and poultry

<b><i>Salmonella</i> serovars</b>	<b>Humans</b>	<b>Cattle (ª)</b>	<b>Pigs (ª)</b>	<b>Poultry</b>	<b>Total (%)</b>
Typhimurium	21	3	23 (3ª)		47 (15.8)
Anatum	1	15 (7ª)	29 (4ª)	1	46 (15.5)
Weltevreden	4	11 (2ª)	17 (3ª)	2	34 (11.4)
Emek	2			26	28 (9.4)
Rissen		5	13	1	19 (6.4)
Derby		3 (1ª)	13 (1ª)	1	17 (5.7)
Blockley	1			14	15 (5.1)
<i>S.</i> (I), 4, 5, 12: b, -	2	3	4 (2ª)	1	10 (3.4)
Lexington		10 (1ª)			10 (3.4)
Hadar	2			6	8 (2.7)
Newport	1	1	6	0	8 (2.7)
London		1	2	4	7 (2.4)
Enteritidis	7				7 (2.4)
Albany	1			3	4 (1.3)
Panama	1		2		3 (1.0)
Rubislaw		3 (3ª)			3 (1.0)
Kedougou	2				2 (0.7)
Schwarzengrund				2	2 (0.7)
Tallahassee				2	2 (0.7)
Others	11	8 (2ª)	2 (1ª)	4	25 (8.3)
<b>Total</b>	<b>56</b>	<b>63</b>	<b>111</b>	<b>67</b>	<b>297</b>

ª Isolates from animals with diarrhoea.

Table 3.

Distribution of *Salmonella* Typhimurium phage types

No. of isolates	Source	English phagetype	Dutch phagetype
9	Human	UT <sup>a</sup>	90
2	Cattle	UT	90
16	Pig	UT	90
1	Cattle	UT	510
1	Pig	UT	508
2	Pig	UT	507
1	Human	UT	RDNC <sup>b</sup>
1	Human	UT	UT
1	Pig	UT	UT
1	Human	104	506
1	Pig	104	506
1	Human	U302	507
2	Human	U302	RDNC
1	Pig	U302	RDNC
1	Human	195	507
1	Human	20	RDNC
2	Human	12	RDNC
2	Human	RDNC	RDNC
1	Pig	RDNC	RDNC

<sup>a</sup> UT untypeable.<sup>b</sup> RDNC reaction does not conform to any recognized phage types.

#### 4. Discussion

Surveillance of *Salmonella* serovars and phage-types from human and animal sources is relevant for detecting national and global outbreaks, for identifying the source of an infection and for implementing prevention and control measures since the distribution of *Salmonella* serovars may differ between countries. To our knowledge, this is the first study comparing the serotypes and phage types of human *Salmonella* isolates to those of farm animals in Vietnam. We observed that *S. Typhimurium*, *S. Anatum*, *S. Weltevreden*, and *S. Emek* were the most common serovars in South Vietnam. *S. Typhimurium* was the most prevalent serovar in isolates of human origin. Pigs can be considered an important reservoir of *S. Typhimurium* since this serovar was frequently isolated from pig faeces and pork. *S. Anatum* and *S. Weltevreden* were isolated from humans, cattle, pigs and chickens. A similar finding was reported from Thailand (Bangtrakulnonth et al., 2004). While *S. Typhimurium* and *S. Dublin* are known to be predominant among

isolates from cattle in Europe, Australia and America (Wray and Wray, 2000 and van Duijkeren et al., 2002) we found *S. Anatum*, *S. Weltevreden* and *S. Lexington* to be the most prevalent bovine *Salmonella* serovars in Vietnam. Among poultry isolates, *S. Emek* and *S. Blockley* predominated. *S. Enteritidis* was not cultured from poultry, although *S. Enteritidis* was often isolated from humans in Vietnam. In contrast, *S. Enteritidis* is frequently isolated from poultry in many European countries and in the USA and this animal species is an important source for human *S. Enteritidis* infections in these countries (Wray and Wray, 2000 and van Duijkeren et al., 2002). Our findings are in accordance with those of Tran et al. (2004) who conducted an investigation of *Salmonella* spp in pigs, chickens and ducks in Mekong delta provinces. They found only one *S. Enteritidis* isolate among 80 animal isolates. Thus, human *S. Enteritidis* infections in Vietnam probably originate from other sources. Another explanation for the low prevalence of *S. Enteritidis* in the present study might be that 95% of the poultry samples were taken from broilers due to the restricted access of poultry farms during the bird flu outbreak. Therefore only a small number of laying hens were sampled. The percentage of *Salmonella* positive samples in our study is much higher than the 7.1% reported by Tran et al. (2004). One possible explanation is the difference in the amount of sample used for culturing in their study, which was 1 g, whereas in our study 25 g was sampled. Overall, the data from the present study indicate that the distribution of *Salmonella* serovars in Vietnam was similar to that of other South-East Asian countries (Bangtrakulnonth et al., 2004; WHO Global Salm-Surv, 2005) but different from that of European countries and the USA (Wray and Wray, 2000, van Duijkeren et al., 2002 and Pasquali et al., 2004). One possible reason for this are the differences in animal husbandry between the continents. Determination of the distribution of *Salmonella* serovars is important in the epidemiology of salmonellosis. It provides evidence of possible sources of food borne infection in a climate of increasing international travel and trade in food products of animal origin and may lead to improved prevention and control measures.

The Dutch pt 90 was the most common phage type among the *S. Typhimurium* isolates in Vietnam and was found among bovine, porcine and human *S. Typhimurium* isolates. The dominance of this phage type is considerably different from that in European countries and America where DT 104 (corresponding to pt 506 in the Dutch phage typing system) has been common among *S. Typhimurium* isolates. The Dutch phage typing system performed better in typing our Vietnamese *S. Typhimurium* isolates than the English phage typing system (Table 3). Seventy-four percent of the *S. Typhimurium* isolates could be phage-typed with the Dutch typing system compared to 21% in the English typing system. It is important to note that although the English phage typing method was able to type most of the *S. Typhimurium* isolates from European countries and the United State of America (van Duijkeren et al., 2002, Gebreyes et al., 2004 and Pasquali et al., 2004), this system could not type 77% (10/13) of Turkish *S. Typhimurium* isolates (Ang-Kucuker et al., 2000) and 12.2% (27/221) of Japanese *S. Typhimurium* isolates (Izumiya et al., 2001).

In conclusion, the distribution of *Salmonella* serovars and phage types in Vietnam is considerably different from that in Europe and America. The same serotypes and phage types were found in humans and food animals with the exception of *S. Enteritidis*, which would suggest that farm animals are an important source of human non-typhoid *Salmonella* infection in Vietnam.

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