



## Phage types of *Salmonella enterica* serovar Enteritidis isolated in South Africa from 1991–1995

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### ABSTRACT

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A total of 615 strains of *Salmonella enterica* serovar Enteritidis (SE), received from 1991–1995 at the Onderstepoort Veterinary Institute (OVI), were phage typed. Most SE isolates (54,7%) originated from poultry followed by humans (28,5%) and poultry eggs (9,6%). Phage type 34 was the most prevalent (40,5%) of all isolates, followed by phage type 4 (33,8%). Other phage types identified were 1, 1b, 4a, 7, 7a, 9a, 14, 24, 24var and 35 (in total 2,4% of isolates). Most isolates of SE were received from the Western Cape Province (47,4%) and Gauteng (22,3%). In poultry phage type 4 was dominant, but in humans, eggs, goats, ducks, sheep, pigs and rabbits, phage type 34 was the dominant type. It appeared as if the poultry-associated epidemic of SE in South Africa that occurred from 1991–1995 originated in the Western Cape Province during 1991 amongst poultry and then spread from there to humans and eggs and then to the rest of the country, where it emerged during 1993. Results indicate that phage type 34 was the dominant phage type from 1991–1993, but during 1994–1995 its presence declined. During this latter period the presence of phage type 4 increased. This may suggest that two smaller epidemics consisting of the two different phage types might have been responsible for the epidemic that occurred from 1991–1995.

**Keywords:** Phage typing, *Salmonella enterica* serovar Enteritidis, South Africa

### INTRODUCTION

The *Salmonella* surveillance data of the World Health Organization revealed that the prevalence of *Salmonella enterica* serovar Enteritidis (SE), increased dramatically from 1980–1989 in North and South America, Europe and Africa (Anonymous 1993). During 1990 SE was isolated the most often from human samples in the UK, USA and Canada. At that stage the worldwide increase in the prevalence of SE was regarded as a pandemic. The main source of human infections was contaminated food, especially

from poultry and eggs of domestic fowls (Schroeter, Ward, Rowe, Protz, Hartung & Helmuth 1994). Given the preference of many people to eat raw, or undercooked eggs, this food was regarded as a major source of infection (Humphrey, Greenwood, Gilbert, Rowe & Chapman 1989).

The first recorded poultry-associated outbreak of SE in South Africa was in 1991 in the Western Cape Province. The Onderstepoort Veterinary Research Institute (OVI) has recorded data on the prevalence of salmonellas in South Africa since 1977, and for the period from 1977–1990 only 35 isolates of SE had been identified, mostly from cattle and pigs, but none from poultry and eggs. From 1991, a marked increase in the prevalence of SE infection in poultry and other sources was observed, and based on this, it is concluded that these outbreaks of SE infection in South Africa were linked to the pandemic in the rest of the world.

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Serotyping of *Salmonella* does not provide any information on the epidemiological relationships of strains isolated from a common outbreak. For this reason additional methods for the epidemiological differentiation of *Salmonella* strains were developed. Phage typing has been used widely to study the distribution of *Salmonella* serovars. This phage-typing scheme had been developed specifically for SE (Ward, De Sa & Rowe 1987). This paper reports on the results obtained in the phage typing of 615 South African SE isolates received at the OVI from 1991–1995.

## MATERIALS AND METHODS

### Bacterial strains and phage typing

A total of 615 isolates of SE (Table 1) isolated from various sources and which originated from eight of the nine provinces of South Africa were phage-typed at the OVI. These isolates were identified according to the biochemical identification methods described by Brenner (1984) and serotyped according to the Kauffmann-White scheme (Kauffmann 1972). Phage typing was done according to the method described by Ward *et al.* (1987). Ten typing phages were used which differentiated 53 phage types.

## RESULTS AND DISCUSSION

The prevalence of SE infections in South Africa reached a peak during 1993 and 1994, but decreased markedly during 1995 (Fig. 1). From 1996 onwards, this trend continued for the following years (Anonymous 1995). The pattern of the SE epidemic closely resembled those reported from overseas countries, i.e. a sharp increase in the number of cases from which SE was isolated, followed by a stable period of 2–3 years and then a definite decrease (Brown, Baggesen, Hansen, Hansen & Bisgaard 1994).

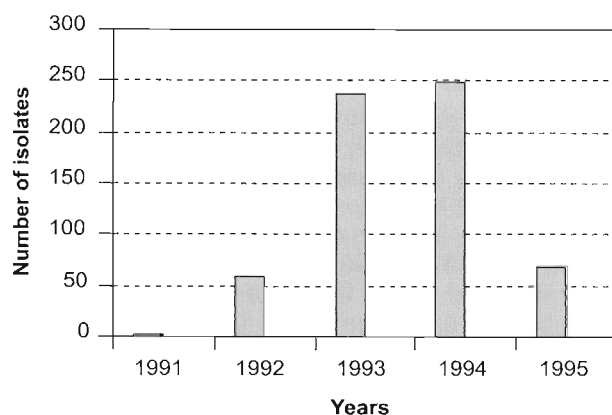


FIG. 1 The number of *Salmonella* Enteritidis isolates received at the OVI from 1991–1995

Most of the 615 samples (54,7%) received over the 5-year period were of poultry origin (Table 1). It is assumed that these samples were representative of the presence of SE for this period. During this period the awareness towards SE was very high among animal health officials, and many poultry and egg producers in the country had instituted SE screening programmes. Consequently, a large number of isolates were sent to the OVI for identification. A total of 9,6% samples were from eggs, probably due to the more intensive screening programme of many egg producers.

Samples originating from other animals were represented at very low numbers (Table 1). It is believed that these samples represented a spillover of infection from poultry to the other species. Although 28,5% of all strains isolated were from humans, it is believed that this finding is not representative from this source for the period under review. The reason is that in the majority of human diarrhoea cases in South Africa, an aetiological diagnosis of the cause is not made routinely. Hence, very few isolates of SE were made on a routine basis from humans during the study period. However, in the Western Cape Province, the Red Cross Childrens' Hospital was very active from 1991–1995 in the study of the causes of diarrhoea amongst children, and most of the human SE strains originated from that area. A small number of the human SE isolates were from cases of diarrhoea in Pretoria, Gauteng.

The prevalence of SE was the highest in the Western Cape (47,4% of all isolates) (Table 2), followed by Gauteng (22,3%), with much lower rates in the rest of the country. During 1991, only two SE isolates (phage type 34) were isolated in the Western Cape Province, and in 1992, 56 isolates were isolated from poultry and one from a human (phage type 34), in the Western Cape Province. Only from 1993 was the organism isolated from other parts of the country and from a wider source range.

In the Western Cape Province, phage type 34 was the dominant strain isolated from egg and poultry samples, but in the rest of the country, phage type 4 was the dominant one from these sources. The reason for this difference is difficult to explain. A possible explanation may be that the phage type 34 strain that was dominant in poultry in the Western Cape Province during the first part of the epidemic, was not responsible for the outbreaks in poultry during the latter part of the epidemic in Gauteng and other provinces. In total, the most dominant phage type among poultry was phage type 4, followed by phage type 34 (Table 3). For all other strains, including those isolated from eggs, phage type 34 was the most dominant. In strains isolated from humans, phage type 14 and phage type 4 (both 48/175) were present in equal numbers with, phage type 34 (59/175) being the most predominant.

TABLE 1 Source of SE isolates from 1991–1995

Source	Number of SE isolates						
	1991	1992	1993	1994	1995	Total	%
Poultry	2	56	106	131	42	337	54,7
Humans	–	1	69	79	26	175	28,5
Eggs	–	–	51	8	–	59	9,6
Feed	–	–	7	6	–	13	2,1
Goats	–	–	–	10	–	10	1,6
Environment	–	–	–	6	–	6	1,0
Cattle	–	–	–	3	1	4	0,6
Ducks	–	–	3	–	–	3	0,5
Sheep	–	–	2	–	–	2	0,3
Jackal	–	–	–	2	–	2	0,3
Parrots	–	–	–	1	–	1	0,2
Rabbit	–	1	–	–	–	1	0,2
Ostriches	–	–	–	1	–	1	0,2
Pigs	–	–	–	1	–	1	0,2
Total	2	58	238	248	69	615	100

TABLE 2 Distribution of SE isolates in the nine provinces of South Africa

Province	Total number of SE isolates received	Percentage (%) of total number of isolates	Presence of phage types 4 and 34 in poultry and eggs	
			Number of phage type 4 isolates	Number of phage type 34 isolates
Western Cape	292	47,4	6	145
Gauteng	137	22,3	47	3
Mpumalanga	50	8,1	39	0
Kwazulu-Natal	47	7,7	21	3
Free State	41	6,7	23	9
North West	34	5,5	14	3
Northern Province	10	1,6	1	5
Eastern Cape	4	0,7	4	0
Northern Cape	0	0	0	0
Total	615	100	155	168

TABLE 3 Phage types of SE occurring in the various sources

Sources	Phage types						Total
	4	34	14	7	Untypable	Other <sup>a</sup>	
Poultry	149	121	10	23	24	10	337
Humans	48	59	48	0	16	4	175
Eggs	6	47	0	1	5	0	59
Feed	1	2	6	0	4	0	13
Goats	0	10	0	0	0	0	10
Ducks	0	2	0	0	0	1	3
Ostriches	0	0	0	0	1	0	1
Environment	1	1	1	0	3	0	6
Cattle	2	2	0	0	0	0	4
Jackal	0	1	0	0	1	0	2
Sheep	0	2	0	0	0	0	2
Pigs	0	1	0	0	0	0	1
Parrots	1	0	0	0	0	0	1
Rabbit	0	1	0	0	0	0	1
Percentage	33,8	40,5	10,6	3,9	8,8	2,4	100
Totals	208	249	65	24	54	15	615

<sup>a</sup> Other phage types: 1, 1b, 4a, 7a, 9a, 24, 24var, 35

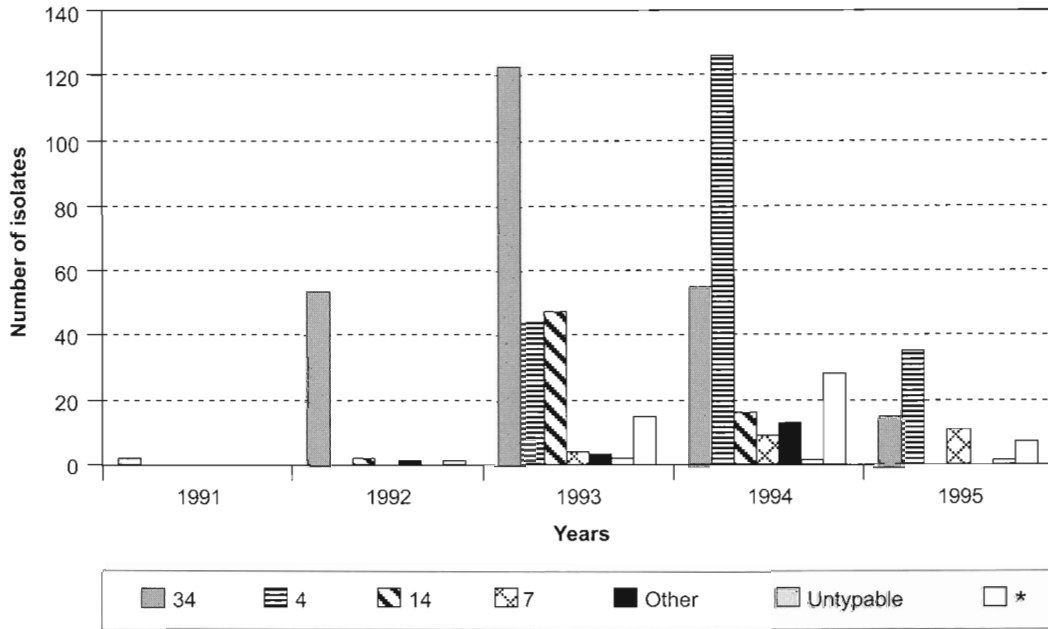


FIG. 2 The prevalence of the various phage types of *Salmonella enterica* serovar Enteritidis (SE)

\* : Isolates that could not be phage typed at the Onderstepoort Veterinary Institute  
 Other : Phage types 1, 1b, 4a, 7a, 9a, 14b, 24, 24var and 35

No consistent pattern in the distribution of the dominance of a specific phage type during the period 1993–1995 was observed (Fig. 2). From 1991–1993 phage type 34 was the most prevalent, followed by phage type 4 and 14, while from 1994–1995 its numbers decreased, and phage type 4 became the most prevalent, followed by phage type 34. A similar variation in the distribution of the dominant source of samples was not observed amongst the species, for poultry remained the most important source (Table 1), followed by humans. Thus, from 1993–1994 a change from phage type 34 to 4 as the most prevalent phage type occurred, although no change in the dominant source of the isolates was recorded. The most probable explanation for these results is that from 1991–1993 phage type 34 was the most dominant phage type in poultry and eggs, and that most of the strains originated from the Western Cape Province. The epidemic in the Western Cape Province subsided, and in the rest of the country another epidemic among poultry and eggs occurred, caused by a phage type 4 strain. If poultry and eggs are excluded, phage type 34 strains remained dominant in all parts of the country for the entire study period.

Phage type 34 is not internationally recognized as an important or very prevalent phage type, nor is it believed to be very virulent. It is present in very low numbers in other countries, and has been reported from Japan, Spain and Argentina (Caffer & Eiguer 1994; Anonymous 1993). It is not known what factors contributed to the large number of this phage

type encountered in South Africa. In addition, it is not known from where it originated, but it is related to phage type 4. A possible explanation for its high prevalence in South Africa is that initially a phage type 4 strain did enter the country, and that it mutated to phage type 34, to emerge in 1991 in poultry in the Western Cape Province. Reports from Europe, including such countries as Germany (Schroeter *et al.* 1994), Italy (Fantasia & Filetici 1994) and the United Kingdom (Khakhria, Duck & Lior 1991), indicate that phage type 4 is the most frequently encountered (Khakhria *et al.* 1991). Apart from phage type 4, none of the prominent phage types found in other countries were dominant in South Africa.

It is believed the laboratory at the OVI received during the period 1991–1995 SE isolates of poultry origin that were representative of the presence of this organism in this species. Therefore, the distribution and prevalence of the phage types in the country are believed to be representative of the situation for this period.

The data show that:

- It appears as if the epidemic of SE infections originated in poultry in the Western Cape Province in 1991, the infection probably spread to humans in the Western Cape Province in 1992, and then to eggs, other sources, and to the rest of the country, where it emerged in 1993.
- Phage type 34 was the most prevalent phage type among all strains isolated.

- Phage type 4 was more prevalent than phage type 34 in poultry.
- Phage type 34 was more prevalent during the period 1991–1993, but its numbers subsequently subsided and phage type 4 became more prevalent for the period 1994–1995.

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