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PROGRESSION OF *SALMONELLA* ENTERITIDIS PHAGE TYPE 4 STRAINS IN SÃO PAULO STATE, BRAZIL

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SUMMARY

A total of 574 *S.* Enteritidis strains (383 from human sources and 191 from non-human sources) isolated between 1975-95, in São Paulo State, Brazil, were phagetyped. Among the strains isolated during the period of 1975-92, 80.9% of them belonged to phage type 8 (PT-8), but in 1993 strains of PT-4 accounted for 65.2% of all the *S.* Enteritidis isolates. In the following years, PT-4 strains accounted for 99.7% and 98.4% of phagetyped *S.* Enteritidis strains. The results obtained suggested that the current epidemic of *S.* Enteritidis in São Paulo State is clearly associated with the progression of PT-4 strains.

KEYWORDS: *Salmonella*; *S.* Enteritidis; Phage types.

INTRODUCTION

The increase of *Salmonella* Enteritidis as well as the factors associated with food borne outbreaks caused by this serovar is well documented worldwide[2, 4, 5, 17, 18].

Although in Europe and in the United States the increase of *S.* Enteritidis is well known since the eighties, in São Paulo State, Brazil, the changing pattern of this serovar, according to FERNANDES et al.[7], began to be noted in 1993.

Large food borne outbreaks of *S.* Enteritidis, rarely reported before 1993, has become common events in the following years both in the metropolitan area and in several inland parts of São Paulo State.

The Laboratory of Enteric Pathogens, Instituto Adolfo Lutz, São Paulo, Brazil, identified 115 *S.* Enteritidis strains among 29.369 *Salmonella* strains from human sources serotyped during the period 1950-90. Over a period of 40 years, *S.* Enteritidis accounted for only an average of 0.4% of all salmonellae[22].

The increasing frequency of isolation of this serovar in our State since 1993 from food borne gastroenteritis, ranking first of all salmonellae isolated recently, led us to undertake the phage typing to detect if the increase of *S.* Enteritidis was associated with the introduction of a new phage type.

MATERIAL AND METHODS

Bacterial strains – A total of 574 *S.* Enteritidis strains isolated in São Paulo State, Brazil, were phage typed. Among them, 383 were isolated from sporadic cases of human infections or from gastroenteritis associated with food borne outbreaks (majority linked to the consumption of homemade mayonnaise, desserts as mousse, and salad dressing, all of them containing raw eggs), and 191 were from non-human sources. These strains had been isolated as described elsewhere[3, 7, 13] or sent by regional laboratories to the Laboratory of Enteric Pathogens – Instituto Adolfo Lutz for serotyping which was done according to POPPOF & LE MINOR[16].

Phage typing – All strains, after testing for purity, were phage typed according to WARD et al.[25]. Briefly, each strain cultivated in nutrient broth for 2 hours at 37°C with agitation was poured onto a dry nutrient agar plate (double strength Bacto Nutrient Broth plus 1.3% of Bacto Agar, Difco). The excess fluid was removed, and 10 µl of each 10 phages was applied on the lawns. After overnight incubation the plates

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were examined with oblique lighting. *S.* Enteritidis type strains representative of phage types 1, 4 and 8 were included as controls.

RESULTS

Among the 574 *S.* Enteritidis strains analysed in this study, 97.6% were typable and 6 phage types were defined (Table 1). PT-8 was the predominant phage type identified among strains isolated from 1975 to 1992, accounting for 80.9%. The only one outbreak (unknown source) reported in 1975 was due to this phage type. In the same period, PT-22 strains, isolated from poultry carcass, was the second most frequent phage type. Striking change in phage type patterns was seen in 1993 when PT-4 accounted for 65.2% of all isolates of *S.* Enteritidis. The progression of this phage type is remarkable, accouting for 99.7% and 98.4%, respectively, in 1994 and 1995 (Table 1).

The frequencies of the phage types according to the sources of isolation are given in Tables 2 and 3.

DISCUSSION

Phagetyping is an useful method to differentiate *S.* Enteritidis strains, and has been largely used[1, 10, 14, 19, 24].

Although *S.* Enteritidis had been identified in São Paulo State since the nineteen fifties[3, 13, 21, 22], its isolation started to increase in 1993.

In the present study, comparing the phage types of *S.* Enteritidis strains isolated from 1975 to 1995, we could observe that while PT-8 predominated until 1992, the increase, since 1993, of *S.* Enteritidis in our State, is clearly related to the progression of PT-4 strains, one of the poultry associated phage types[9, 23].

TABLE 1

Phage types of *Salmonella* Enteritidis from human and non-human sources isolated between 1975-95, in São Paulo, Brasil.

Phage types	No (%) of strains isolated in				Total
	1975-92	1993	1994	1995	
PT-2	1 (1.2%)	–	–	–	1 (0.2%)
PT-4	2 (2.4%)	15 (65.2%)	340 (99.7%)	124 (98.4%)	481 (83.8%)
PT-6	–	–	–	1 (0.8%)	1 (0.2%)
PT-8	68 (80.9%)	8 (34.8%)	1 (0.3%)	1 (0.8%)	78 (13.6%)
PT-22	8 (9.5%)	–	–	–	8 (1.4%)
PT-23	1 (1.2%)	–	–	–	1 (0.2%)
UT[1]	4 (4.8%)	–	–	–	4 (0.6%)
Total	84	23	341	126	574

1 – Untypable (strains with no reaction with any of ten typing phages)

TABLE 2

Phage type of *Salmonella* Enteritidis strains isolated from human sources between 1975-95.

Source	Total	No. of strains belonging to phage types isolated in							
		1975-92					1993-95		
	No. of strains	PT-2	PT-4	PT-8	PT-23	UT[1]	PT-4	PT-6	PT-8
stool	334	1	1	44	–	2	283	1	2
blood	26	–	1	3	1	–	17	–	4
c.s.f[2]	7	–	–	2	–	–	5	–	–
urine	7	–	–	2	–	–	3	–	2
abscess	3	–	–	1	–	–	2	–	–
catheter	1	–	–	1	–	–	–	–	–
unknown	5	–	–	5	–	–	–	–	–
Total	383	1	2	58	1	2	310	1	8

1 Untypable (strains with no reaction with any of ten typing phages)
2 cerebrospinal fluid

TABLE 3
Phage types of *Salmonella* Enteritidis strains isolated from non-human sources between 1975-95.

Source	Total	No. of strains belonging to phage types isolated in				
		1975-92			1993-95	
	No. of strains	PT-8	PT-22	UT[1]	PT-4	PT-8
hens	48	3	–	–	44	1
environment	21	6	–	2	13	–
poultry carcass	8	–	8	–	–	–
feed	15	–	–	–	15	–
food:						
desserts	22	–	–	–	22	–
mayonnaise	21	–	–	–	21	–
meat	15	–	–	–	15	–
shell eggs	12	–	–	–	11	1
salad dressing	8	–	–	–	8	–
canapes	6	–	–	–	6	–
fish	3	–	–	–	3	–
other foods	12	1	–	–	11	–
Total	191	10	8	2	169	2

1 untypable (no reaction with any of ten typing phages)

In Western European countries, mainly in the United Kingdom, PT-4 is the most frequent phage type, whereas in the United States PT-8 and PT-13a are the major ones[1, 8, 11, 12, 15, 20]. It is likely that the introduction of *S.* Enteritidis PT-4 in São Paulo State is related to the increase of international trade with developed countries by the imported breeding and commercial chicks. Similar results were observed with the introduction of *Salmonella* Agona in many countries, including Brazil, through contaminated feeds[6, 26].

Several factors can be associated with the expansion of PT-4 strains in our State. The rapid process of urbanization, changes of the food habits, and the fact that poultry has become a popular food in our country as an important source of animal protein, among other social and environmental changes, are some examples.

Almost all strains isolated from sporadic cases as well as from outbreaks were associated with this phage type, and its predominance among both human and non-human sources showed its wide dissemination in our State.

The presence of *S.* Enteritidis PT-4 strains in the environment and in animal feed is a continuous challenge for Brazilian poultry industry, expanded substantially in the last decades and ranking second in the world.

On the other hand, the increase of extraintestinal infections (bacteremia, meningitis, etc.) by *S.* Enteritidis PT-4, particularly in children, is a major concern for public health, considering the risk of outbreaks of nosocomial salmonellosis.

RESUMO

Disseminação de cepas de *Salmonella* Enteritidis do fagotipo 4 (PT-4) em São Paulo, Brasil.

Um total de 574 cepas de *Salmonella* Enteritidis isoladas no período 1975-95, em São Paulo, Brasil, foram fagotipadas. Entre estas cepas, 383 eram de origem humana e 191 isoladas de materiais de origem não humana. Verificou-se que a grande maioria (80.9%) das cepas isoladas até 1992 pertencia ao fagotipo 8 (PT-8) e que cepas do fagotipo 4 (PT-4) começaram a predominar a partir de 1993. Cepas deste fagotipo corresponderam em 1993, 1994 e 1995, respectivamente, a 65.2%, 99.7% e 98.4% das amostras fagotipadas. O aumento da *S.* Enteritidis em São Paulo está claramente associado à disseminação de cepas PT-4.

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