Research Note

Salmonella Serotypes Isolated from Nonhuman Sources in São Paulo, Brazil, from 1996 through 2000

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ABSTRACT

A total of 4,581 Salmonella strains isolated from nonhuman sources, including foodstuffs associated with foodborne Salmonella outbreaks, from January 1996 through December 2000 were serotyped at the Enteropathogens Laboratory, Instituto Adolfo Lutz, São Paulo, Brazil. Among the 123 different serotypes identified, Salmonella enterica subsp. enterica serotype Enteritidis (Salmonella Enteritidis) was the most prevalent (32.7%), ranking first for almost every kind of source. The next most common serotypes were Salmonella Senftenberg (10.3%), Salmonella Hadar (6.8%), Salmonella Agona (5.1%), and Salmonella Typhimurium (2.4%). Rough strains belonging to the subspecies S. enterica subsp. enterica (4.8%), S. enterica subsp. arizonae (<1%), S. enterica subsp. diarizonae (<1%), and S. enterica subsp. houtenae (<1%) were also detected. Foodstuffs (including poultry meat for consumption) contained 38.1% of the studied Salmonella strains, poultry flocks (from several farms under salmonellosis control by the owners) contained 21.7%, the environment contained 10.6%, sewage contained 9.4%, water contained 6.6%, animal feed contained 4.4%, chill water from poultry-processing operations contained 2.2%, and other sources contained 7.0%. Foodstuffs extensively contaminated with Salmonella strains were poultry meat (40%), cow meat (11%), desserts (8%), mayonnaise (6%), sausage (5%), and unpasteurized shell eggs (4%), and there were several other food sources (26%). Homemade mayonnaise was the most common vehicle for Salmonella foodborne outbreaks, and Salmonella Enteritidis was the serotype most isolated (95%) from that source. According to these data and previously published data concerning Salmonella strains isolated in São Paulo State, almost the same serotypes have predominated among nonhuman sources for the last decade.

Salmonellae are zoonotic enterobacteria that are responsible for outbreaks of both human and animal clinical diseases and have important worldwide hygienic and economic significance. There are several transmission routes for salmonellosis, but the majority of human infections are derived from the consumption of contaminated foods (especially those of animal origin) and water (19). International or multistate outbreaks caused by a range of foodstuffs contaminated with different *Salmonella* serotypes have been reported (3–5, 11, 13, 15).

Although Salmonella strains may be isolated from a wide range of sources and animal reservoirs, poultry are the most important reservoir, particularly for Salmonella enterica subsp. enterica serotype Enteritidis (Salmonella Enteritidis). This serotype and several others have often been associated with foodborne outbreaks and/or sporadic cases of human salmonellosis and have often been isolated from foodstuffs of animal origin and other sources. Raw eggs and raw-egg-containing foods and poultry and other

meats are the foods of animal origin that have most commonly been implicated in such infections (3–5, 11, 13, 19).

Salmonella Enteritidis has been reported to be the main cause of foodborne Salmonella outbreaks in several developed countries over the last 3 decades (5, 12, 13). In Brazil, this serotype started spreading in 1993, being largely isolated from poultry sources, and thereafter became the prevailing serotype for human salmonellosis, particularly in foodborne outbreaks but also in outbreaks attributable to different nonhuman sources (1, 10, 16, 18).

An important role of a public health laboratory is to contribute to salmonellosis surveillance by reporting data from *Salmonella* serotyping, which is the important initial step in the detailed characterization of *Salmonella* strains. Thus, this study was carried out to evaluate the distribution and prevalence of *Salmonella* serotypes isolated from nonhuman materials, including foodstuffs associated with foodborne outbreaks and those not associated with foodborne outbreaks, from 1996 through 2000 in São Paulo State, Brazil.

MATERIALS AND METHODS

* Author for correspondence. Fax: 55 (21 11) 3085.3505; E-mail: atavecch@hotmail.com. A total of 4,581 *Salmonella* strains isolated from nonhuman sources from January 1996 through December 2000 were serotyped at the Enteropathogens Laboratory, Instituto Adolfo Lutz, São Paulo, Brazil. These strains were sent to the Enteropathogens Laboratory by regional laboratories of Instituto Adolfo Lutz, public universities, and private laboratories of São Paulo State.

Isolation and identification of strains at the genus level were performed as described by Ewing (6). Salmonella subspecies determination and serotyping were carried out according to Popoff and Le Minor (14). Every somatic and flagellar antiserum routinely used for Salmonella serotyping has been produced and maintained by the Enteropathogens Laboratory, Instituto Adolfo Lutz.

RESULTS

Among the 4,581 Salmonella strains studied, 123 different serotypes were identified, and the 10 most common ones, accounting for 71% of all isolates, are shown in Table 1. Of these serotypes, the five most prevalent were Salmonella Enteritidis (32.7%), Salmonella Senftenberg (10.3%), Salmonella Hadar (6.8%), Salmonella Agona (5.1%), and Salmonella Typhimurium (2.4%). Each of the other 113 different serotypes was detected at <2%.

Almost 92% of all *Salmonella* Enteritidis strains were isolated from foodstuffs (including poultry meat for consumption) and poultry flocks (from several farms under salmonellosis control by the owners), which were contaminated by 88 and 51 different serotypes, respectively. *Salmonella* Hadar was the second most prevalent serotype among foodstuffs (12%) and was isolated mostly from poultry meat (including ready-to-eat dishes and carcasses). *Salmonella* Senftenberg was the prevailing serotype (65%) among the 37 serotypes detected in environmental samples, and *Salmonella* Agona was the prevailing serotype (26%) among the 35 serotypes isolated from sewage.

Among the several different foodstuffs contaminated by *Salmonella*, poultry meat accounted for 40% of all strains, cow meat accounted for 11%, desserts accounted for 8%, mayonnaise accounted for 6%, sausage accounted for 5%, unpasteurized shell eggs accounted for 4%, and other miscellaneous foods accounted for 26%. *Salmonella* Enteritidis ranked first in almost every kind of food.

For the period under study, the poultry samples (including those from the poultry flocks and the poultry meat intended for consumption) accounted for 63% of all *Salmonella* Enteritidis strains. Other foodstuffs (including unpasteurized shell eggs, homemade mayonnaise, and several other foods) accounted for 34%, whereas a wide range of other materials accounted for 3%.

DISCUSSION

Among the several serotypes identified in this study, Salmonella Enteritidis ranked first for every year except 1999, when it was surpassed by Salmonella Senftenberg because a large number of these strains were isolated from environmental samples and sent in by one private laboratory. Most of the 170 foodborne Salmonella outbreaks detected over the period under study were caused by homemade mayonnaise, and Salmonella Enteritidis accounted for 95% of the isolates from this source (unpublished data). Among the 4% of all Salmonella strains isolated from un-

Salmonella serotype	Foodstuffs ^a	Poultry flocks ^b	Environment	Sewage	Water	Animal feed	Chill water ^c	Other animals ^d	Viscera	Stool	Unknown	Total (%)
Enteritidis	854	526	30	7	5	2	29	3	17	ю	22	1,498 (32.7)
Senftenberg	12	94	317	1	ю	12		2		21	8	470 (10.3)
Hadar	204	26			18		60	2		l	б	313 (6.8)
Agona	57	18	4	111	10	16		б	1	11	2	233 (5.1)
Typhimurium	29	13	10	8		2		15	19	9	6	111 (2.4)
Infantis	53	22	S	2		33			1		9	92 (2.0)
Mbandaka	35	31		7	9	9				l	2	87 (1.9)
Albany	36	ю		20	24					-		84 (1.8)
Ohio	12	б		63		4				I	1	83 (1.8)
Rubislaw	9				72							78 (1.7)
l Rough strain ^{e}	55	85	11	51	4	33	С	2		l	S	219 (4.8)
113 other serotypes	391	172	109	159	162	154	7	25	19	52	63	1,313 (28.7)
Total	1,744	993	486	429	304	202	66	52	57	94	121	4,581 (100.0)
(%)	(38.1)	(21.7)	(10.6)	(9.4)	(9.9)	(4.4)	(2.2)	(1.1)	(1.2)	(2.0)	(2.7)	
¹ Foodstuffs include pou	ultry meat for cc	onsumption	(ready-to-eat dish	les and carcas	sses).							

 TABLE 1. Numbers of Salmonella serotypes isolated from different nonhuman sources from 1996 through 2000

⁵ Originating from several farms under salmonellosis control by the owners.

^c From poultry processing operations.

pasteurized shell eggs, around 60% were Salmonella Enteritidis strains.

Salmonella Hadar, the third most prevalent serotype in this study and the fifth most prevalent from 1991 through 1995 (18), was extensively detected in poultry meat, particularly carcasses, as well as in isolates from chill water used for processing of these carcasses. This finding is in agreement with those of previous reports describing the presence of this serotype in such sources, as well as in connection with foodborne outbreaks (2, 7).

Salmonella Agona, one of the most prevalent serotypes until the beginning of the 1990s (17), was isolated mostly from sewage samples, despite its presence in the majority of the different sources over the period studied. These data indicate the steady circulation of this important serotype through the years. The detection of the other 34 serotypes in sewage, even without periodic monitoring of this source with regard to the presence of Salmonella spp., corroborates the importance of proper sanitation of this significant contamination vehicle.

Four of the 10 serotypes most commonly found in nonhuman sources, *Salmonella* Enteritidis, *Salmonella* Agona, *Salmonella* Typhimurium, and *Salmonella* Infantis, were also among the top 10 serotypes associated with human disease over the period under study. On the other hand, almost 80 different serotypes have been found at different incidences in material of nonhuman origin but not in human infection isolates.

Apart from the 123 different serotypes identified in this study, rough (serologically untypeable) strains belonging to the subspecies *S. enterica* subsp. *enterica* (4.8%), *S. enterica* subsp. *arizonae* (<1%), *S. enterica* subsp. *diarizonae* (<1%), and *S. enterica* subsp. *houtenae* (<1%) were also identified. In addition, some changes in normal biochemical characteristics displayed by *Salmonella* spp. were detected in a few strains, such as lysine decarboxylase–negative *Salmonella* Emek and sucrose-fermenting *Salmonella* Idikan and *S. enterica* subsp. *enterica* 54:-:-. Such strains probably survived under high environmental stress, which could explain these findings.

It often happens that the list of different *Salmonella* serotypes detected in a specific region increases with the addition of some serotypes that had not been detected yet. Five serotypes, namely *Salmonella* Bracknell, *Salmonella* Uccle, sucrose-fermenting *Salmonella* Idikan, *S. enterica* subsp. *diarizonae* 60:r:e,n,x,z₁₅, and *S. enterica* subsp. *diarizonae* 65: z_{53} :k, were isolated for the first time from non-human sources in our geographic area.

Studies focusing on the determination of the prevalent serotypes in a region, particularly those associated with foodborne outbreaks, allow further detailed characterization of such serotypes. The results of such studies provide information for tracing the vehicle origin, which could distinguish local outbreaks from widespread outbreaks (11, 15). Thus, the characterization of Salmonella Enteritidis by other phenotypic and molecular methods has been applied in our laboratory (8, 9).

By comparing the results of the present study with those of previous studies (18), it can be seen that over the

last decade, almost the same *Salmonella* serotypes have predominated among nonhuman isolates in São Paulo State. These results also provide a baseline for future comparisons for *Salmonella* epidemiological surveillance.

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