**Salmonella** serovars of human origin identified in Pará State, Brazil from 1991 to 2008

Sorovares de **Salmonella** de origem humana identificados no Estado do Pará, Brasil, no período de 1991 a 2008

Serotipos de **Salmonella** de origen humano identificados en el Estado de Pará (Brasil) entre 1991 y 2008

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**ABSTRACT**

Salmonellosis presents a cosmopolitan distribution and affects all age groups, not only in developed countries, but also in developing ones. This study aimed to identify the serovars of **Salmonella** isolated from human infections occurring in 43 areas of Pará State from 1991 to 2008. Eight hundred and ninety samples of **Salmonella** isolated in coprocultures and blood cultures were analyzed, including 55 isolations of **S. Typhi** from feces and blood of symptomatic individuals, simultaneously. The cases of **Salmonella** infections were distributed into 13 serogroups. The majority of them were in group **O:9** (68.1%), and 47 serovars of **Salmonella** were identified, including **S. Typhi** (58.9%), **S. Enteritidis** (5.4%) and **S. Saintpauli** (2.5%). **S. Typhi** was the most prevalent (58.9%) among the 47 identified serovars, which demonstrates that typhoid fever is a serious public health problem in northern Brazil and requires increased attention from health agencies regarding epidemiological and environmental surveillance as effective measures for its prevention and control.

**Keywords:** **Salmonella; Serovars; Salmonella Infections.**

**INTRODUCTION**

The genus **Salmonella** belongs to the Enterobacteriaceae family and comprises Gram-negative, glucose-fermenting bacilli. Most of these organisms move by means of peritrichous flagella. The current classification is based on phenotypic and genotypic studies, which divide the genus into two species: **Salmonella enterica**, which consists of six subspecies, and **Salmonella bongori** in accordance with the following written convention: **Salmonella enterica** subspecies enterica serovar Typhimurium, or simply, **Salmonella Typhimurium**, with the genus name in italics and the serovar in Roman type. Routinely, the Kauffmann-White scheme is used for the antigenic characterization of **Salmonella** by identifying the representative polysaccharide antigens (O) and those associated with flagella (H), which are proteinaceous in nature.

Salmonellosis has a cosmopolitan distribution, affecting all age groups, both in developed and developing countries, and is an important public health problem. Clinical presentations most commonly include acute gastroenteritis and enteric fevers (typhoid and paratyphoid).

Some **Salmonella** serovars, such as **Salmonella Typhi** and **Salmonella Paratyphi A, B** and **C** are exclusively adapted to human hosts, whereas other serovars, such as **S. Pullorum**, **S. Gallinarum**, **S. Abortusovis** and **S. Choleraesuis**, are more adapted to domestic or wild animals that can transmit infection to humans. 

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Salmonellosis, therefore, is classified as a zoonosis, and is characterized by its spread by food consumption, particularly raw or undercooked20.

An estimated 1.4 million cases of salmonellosis occur annually in the United States1, with the most recent outbreaks attributed to serovar S. Enteritidis and milk, meat and chicken eggs are its main vehicles of transmission2,25.

In Brazil, food-borne disease epidemics caused by S. Enteritidis have been registered in São Paulo1,24, Brasilia4, Blumenau26 and Curitiba27. In the state of São Paulo, 3,554 cases of human salmonellosis were studied from 1996 to 2003, and 68 serovars were identified. Of these, S. Enteritidis was the most commonly found serovar9.

Studies conducted in the state of Pará from 1975 to 1986 indicate the importance of typhoid fever in northern Brazil where, overall, 59 human infectious Salmonella serovars were identified, the most common being S. Typhi (14.6%), followed by S. Typhimurium (9.6%), S. Give (9.0%), S. Agona (7.4%) and S. Newport (6.2%)17.

Typhoid fever affects about 20 to 30 million people in developing countries. The highest incidence is found in African, Asian, Caribbean and Central and South American countries. In the year 2000, more than 2.16 million cases of typhoid fever were estimated to occur worldwide and resulted in 216,000 deaths, with more than 90% of these occurring in Asia629.

In Brazil, typhoid fever is endemic, with overlapping epidemics occurring especially in the north and northeast regions17, and is associated with low socioeconomic backgrounds and poor basic sanitation.

In the Amazon region, the state of Pará has registered the greatest number of cases of typhoid fever. A total of 890 cases of typhoid fever were identified from 1987 to 2004; originating from various municipalities in the state of Pará, including epidemics in Marabá, Óbidos, Abaetetuba, Moju, Limoeiro do Ajuru and Anajás18. In 1981, during the construction of the Tucuruí hydroelectric dam, the Tucuruí municipality registered the first outbreak of paratyphoid A fever in Brazil, with 101 cases identified19.

Knowledge of the geographical distribution of Salmonella serovars of human origin is important to assess their individual incidence and prevalence, as well as to identify any risk to the health of the population. This information constitutes an important epidemiological indicator of salmonellosis in any given community9. However, very few studies are performed in the Amazon that allow the recognition of Salmonella serovars involved in human and animal infections as well as their mechanisms of transmission. To address this problem, the objectives of the present research were to identify Salmonella serovars isolated at the Bacteriology Section of the Evandro Chagas Institute (IEC) from cases of human infection that occurred between 1991 and 2008 in various municipalities of the State of Pará.

MATERIALS AND METHODS

SAMPLES

A total of 890 Salmonella isolates were obtained from 10,709 stool cultures (663 positive samples) and 6,285 blood cultures (227 positive samples) of symptomatic individuals living in different areas of the state of Pará. Identification was performed in the Bacteriology Section of the IEC from 1991 to 2008.

STOOL CULTURE

Naturally collected stool samples were stored in appropriate containers and sent to the laboratory for stool culture. No more than 2 h after the collection, the feces were plated onto MacConkey (MC) agar or Salmonella-Shigella (SS) agar and cystine selenite broth, followed by plating onto the SS agar after incubation at 37°C for 24 h. The suspected colonies (lactose negative) were then seeded onto triple-sugar-iron (TSI) agar, followed by biochemical identification according to the recommendations of Ewing1. Confirmation of the Salmonella genus was performed by seroagglutination testing, using somatic and flagellar polyvalent antisera (Difco and Bio-Rad).

BLOOD CULTURE

Immediately after collection, 10 mL of peripheral blood were used to inoculate flasks containing 50 mL of tryptose phosphate broth, which were then incubated at 37°C. Inoculated cultures were monitored daily for the presence of growth and/or turbidity for up to 15 days. Cultures from turbid flasks were used to inoculate SS agar plates, upon confirmation of Gram-negative bacilli growth by bacterioscopy (Gram staining).

SEROLOGICAL IDENTIFICATION

The characterization of Salmonella serovars was performed at the Enterobacteria Laboratory of the Instituto Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, by detection of somatic and flagellar antigens and using polyvalent and monovalent antisera with or without flagellar phase induction.

RESULTS

Firstly, it was determined that the 890 Salmonella isolates from human infections occurred in 43 municipalities of the state of Pará, with the highest prevalence rate in Belém (65.5%), followed by Abaetetuba (5.7%), Ananindeua (4.9%) and Anajás (2.9%). These samples represented 79% (704 samples) of the total analyzed (Table 1).

Overall, the identified Salmonella serovars were distributed among 13 serogroups, with group O9 comprising 68.1% of isolates, with emphasis on the serovars S. Typhi (492), S. Enteritidis (45) and S. Panama (18) (Table 2).
The importance of Salmonella serovar characterization should be noted as it allowed the recognition of 492 (58.9%) cases of typhoid fever out of the total of 835 samples analyzed within the same period.
In the analysis of the annual distribution of *Salmonella* spp species isolates from 1991 to 2008, 2004 had the greatest number of cases (98), followed by the years 2006 (64), 1994 (63) and 1998 (61) (Figure 1). Cases of typhoid fever occurred in all years, and the highest incidences were observed in 2004 (51 cases), 1998 (46), 1994 (44) and 2008 (39), comprising 36.6% of all reported cases (Figure 1).

Of the cases of typhoid fever, 53.9% were detected in stool cultures, 34.9% in blood cultures and 11.2% in both stool cultures and blood cultures (Table 3).

<table>
<thead>
<tr>
<th>Table 3 – Percent distribution of <em>Salmonella</em> Typhi isolates in stool cultures and blood cultures from 1991 to 2008</th>
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</thead>
<tbody>
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<tr>
<td>Stool culture</td>
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<td>Blood culture and stool culture</td>
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<td><strong>Total</strong></td>
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The analysis method used for these samples allowed the recognition of 47 *Salmonella* serovars. As can be seen in tables 4 and 5, *S. Typhi* (58.9%), *S. Enteritidis* (5.4%) and *S. Saintpaul* (2.5%) were the serovars most frequently detected from 1991 to 2008.

**DISCUSSION AND CONCLUSION**

In the epidemiological surveillance of human salmonellosis, it is important to know the prevalent and/or incident serovars, as well as the transmission routes, so that the health services involved in surveillance can intervene with more effective measures of disease prevention and control. At the same time, regular identification of serovars reveals the introduction of new serological types into a region.

This study analyzed 835 strains of *Salmonella* responsible for human infections over a period of 18 years, and demonstrated its occurrence in 43 municipalities in the state of Pará. Cases appeared to be clustered, especially in those municipalities with larger populations, such as Belém, with 65.5% of the isolates, followed by Abaetetuba (5.7%), Ananindeua (4.9%) and Anajás (2.9%) (Table 1). It is likely that these occurrences result from poor urban sanitation conditions. However, the observed higher incidence may result from more frequent diagnoses and laboratory testing, given the greater number of medical facilities in these areas. Overall, from the 47 *Salmonella* serovars identified (Tables 4 and 5), *S. Typhi* (65.3%), *S. Enteritidis* (6.0%) and *S. Saintpaul* (2.8%) were the most prevalent. From 1999 to 2003, *S. Enteritidis* was identified in 67.4% of cases of gastrointestinal and extraintestinal infections in the State of São Paulo, followed by *S. Typhimurium* (5.2%). In recent years, *S. Enteritidis* has been a concern to health authorities because it is the most common serovar in human infections in Africa, Asia, Europe, Latin America and the Caribbean; the *S. Typhi* serovar was the third most frequent in Africa, Latin America and the Caribbean. It is interesting to note that in the regions studied, the predominant *Salmonella* serovars found belong to serogroup O:9 (Table 2), comprising 68.1% of the cases analyzed. Within this group, it is important to highlight the occurrence of *S. Typhi* (58.9%).
Table 4 – Distribution of Salmonella serovars identified from 1991 to 1999

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Total: 17, 39, 22, 56, 35, 47, 60, 32, 322

S.I. S. enterica subsp. enterica; S.IIa S. enterica subsp. arizonae; S.IIIb S. enterica subsp. houtenae; Conventional sign used: – Numeric data equal to zero is not due to rounding.

Table 5 – Distribution of Salmonella serovars identified from 2000 to 2008

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<td>S. subsp. III*</td>
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Total: 33, 47, 49, 23, 91, 32, 56, 45, 56, 432

S.I. S. enterica subsp. enterica; S.II. S. enterica subsp. salamae; S.IIIa S. enterica subsp. arizonae; S.IIIb S. enterica subsp. arizonae; S.IV S. enterica subsp. houtenae; Conventional sign used: – Numeric data equal to zero is not due to rounding.
In the state of São Paulo, S. typhi was the fourth most frequent serovar and the second most isolated Salmonella strain from blood cultures, whereas in the state of Pará, all 227 cases of blood culture isolates were characterized as S. Typhi, and they were detected in all the years of the study (Tables 4 and 5). It is important to note the occurrence detected in 2004 (Figure 1) when, among the 98 isolates, 51 (52%) were from cases of typhoid fever, distributed among 15 municipalities in the state of Pará, with the highest incidence in Belém (52.9%), followed by Anajás (13.7%) and Ananindeua (7.8%). This highlights the importance of the problem of typhoid fever in the state of Pará, considering that of the 835 cases of salmonellosis that occurred from 1991 to 2008 in 43 municipalities, typhoid fever was diagnosed by laboratory testing in 34 (79.1%) locations. In light of this, we emphasize that typhoid fever was, and continues to be, endemic in Belém during the 18 years of this study, and is, to some extent, undoubtedly directly related to the precarious conditions of basic sanitation and low levels of education in the community.

These results are consistent with other studies previously conducted in the region, which have demonstrated the high prevalence of infection by S. typhi compared to other studied serovars. In developed countries, there are few reported cases of typhoid infection in contrast to the high frequency of typhoid fever in the developing countries of Africa and Asia.

The cases of typhoid fever originating from routine medical visits and those referred by Brazil’s Unified Health System (known as SUS) comprise the majority of patients treated at the IEC. At the time of admission, they had been ill for an average of 20 to 25 days. These data may explain the greater success in isolating S. typhi from stool samples (53.9%) as compared to blood samples (34.9%), as was found in this study (Table 3). However, although the stool samples showed a higher positive rate during the third week of illness, it should be noted that this study showed significant positive incidence rates during the first and, particularly, second weeks.

Considering that humans are the only natural host of S. typhi, prophylactic measures should be directed towards water treatment, basic sanitation, adequate personal hygiene and health education, as well as enlightening the population about the disease and vaccination. Undoubtedly, the implementation of these measures will allow minimization of the incidence of typhoid fever in the Amazon region, which will thereby help to prevent social and economic losses.

**Sorovares de Salmonella de origen humana identificados no Estado do Pará, Brasil, no período de 1991 a 2008**

**RESUMO**

A salmonelose é de distribuição cosmopolita, acometendo todas as faixas etárias, tanto nos países desenvolvidos, como naqueles em desenvolvimento. Este estudo tem como objetivo identificar os sorovares de Salmonella isolados de casos de infecção humana ocorridos em 43 municípios do Estado do Pará no período de 1991 a 2008. Foram utilizadas 890 amostras de Salmonella em coproculturas e hemoculturas, aí incluídos 55 isolamentos de S. Typhi nas fezes e sangue, simultaneamente, de indivíduos sintomáticos. Os casos de infecção por Salmonella foram distribuídos em 13 sorogrupos, com destaque para o grupo O:9 (68,1%). Foram identificados 47 sorovares de Salmonella, destacando-se S. Typhi (58,9%), S. Enteritidis (5,4%) e S. Saintpaul (2,5%). Ressalta-se a maior prevalência de S. Typhi (58,9%) dentre os 47 sorovares identificados, o que demonstra que a febre tifoide representa um sério problema de saúde pública na Região Norte do País, e que necessita de maior atenção dos serviços de saúde quanto à vigilância epidemiológica e ambiental, assim como medidas efetivas para a prevenção e controle.

**Palavras-chave:** Salmonella; Sorovares; Infeção por Salmonella.

**Serotipos de Salmonella de origen humano identificados en el Estado de Pará (Brasil) entre 1991 y 2008**

**RESUMEN**

La salmonelosis es de distribución cosmopolita y afecta a todos los grupos de edad, tanto en países desarrollados como países en desarrollo. Este estudio pretende identificar los serotipos de Salmonella aislados de casos de infección humana entre 1991 y 2008 que se produjeron en 43 municipios del Estado de Pará. Se utilizaron 890 muestras de Salmonella en cultivo de heces y en cultivos de sangre, incluyendo 55 aislamientos de S. Typhi en heces y sangre simultáneamente, de individuos sintomáticos. Los casos de infección por Salmonella fueron distribuidos en 13 serogrupos, especialmente para el grupo O:9 (68,1%). Fueron identificados 47 serotipos de Salmonella, especialmente S. Typhi (58,9%), S. Enteritidis (5,4%) y S. Saintpaul (2,5%). Destaca la mayor prevalencia de S. Typhi (58,9%) entre los 47 serotipos identificados, lo que demuestra que la fiebre tifoidea representa un grave problema de salud pública en la Región Norte de Brasil, y requiere mayor atención de los servicios de salud en lo que se refiere a la vigilancia epidemiológica y ambiental, así como medidas eficaces para la prevención y control.

**Palabras clave:** Salmonella; Serotipos; Infecciones por Salmonella.
REFERENCES


