

Original Article

Detection of *Salmonella* human carriers in Colombian outbreak areas

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Abstract

Introduction: Salmonellosis, a zoonotic and foodborne disease, is a public health problem in developing countries. With the aim of identifying human carriers of *Salmonella*, a survey was performed in five regions of Colombia with reported salmonellosis outbreaks.

Methodology: The general population and cholecystectomy surgical patients were included in this study. Stool samples from 667 volunteers and gallbladder bile samples from 199 surgical patients were examined. Detection of *Salmonella* from cultured stool and bile samples was determined by polymerase chain reaction (PCR). Multiplex PCR and biochemical and serological tests were performed to identify the serovars of the isolates.

Results: Nine (1.35%) stool samples were positive for *Salmonella*: two *S. Newport*, two *S. Anatum*, one *S. Sinfors*, and four *Salmonella* spp. A total of 11 gallbladder bile samples were positive: *S. Enteritidis* was isolated from 3 bile cultures (1.5%), and 8 samples (4%) were positive for *Salmonella* spp.

Conclusions: Our results show the presence of *Salmonella* carriers in the inhabitants of regions with reported outbreaks and suggest that these carriers are potential sources of infection in endemic and epidemic cases. Carriers also suggest *Salmonella* zoonotic transmission, since broiler and beef cattle are hosts to the *Salmonella* serotypes isolated. It is important to establish the source of infection in regions where salmonellosis is endemic in order to control transmission.

Key words: *Salmonella*; salmonellosis; carriers; zoonosis.

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Introduction

Salmonellosis, an endemic infectious disease, is considered a public health problem in developing countries. Salmonellosis is classified as a foodborne and zoonotic disease; humans acquire the infection from a variety of sources and routes of transmission [1]. However, some *Salmonella* serotypes differ based on geographical area, food sources (meat, vegetables, cheese, eggs, etc.), industry, and manipulation of foods [2,3]. Furthermore, the *Salmonella* serotype determines its ability to cause disease, as well as clinical symptoms and outbreak outcomes [4-6]. Animal and human reservoirs are responsible for the circulation of the microorganism, contributing to the occurrence of sporadic cases and outbreaks [7-10]. In addition, some food sources have higher impact on public health than do others [11-14].

Public health efforts have been made in developed countries to prevent and control foodborne and zoonotic diseases [15]. Nevertheless, the impact of these interventions is not conclusive, a fact reflected by the

variability of surveillance programs for different infective sources [7]. Several mathematic and statistical approaches, such as a Bayesian model, have been developed to quantify the contributions of each major animal-food sources to human salmonellosis [15,16]. Results have identified differing sources, based on the geographic origin of the studies, that vary among eggs, pork, and layers as the most important reservoir of human salmonellosis caused in Europe by *S. Enteritidis* [15-17]. However, in Finland and Sweden, most cases were travel related. In most other countries, the main sources were egg-laying hens or pig reservoirs, reflecting the differences in the epidemiology of *Salmonella* across the European Union [18,19]. The integrated control of farms and food processing plants have shown higher reductions in the incidence of foodborne human salmonellosis [18].

Salmonellosis in Colombia is considered an endemic infection, with sporadic cases and outbreaks [5]. The global laws of food commerce recommend implementing control measures in food chains [20].

Colombia is included in these global commerce, and several guidelines are now available for producers and processors of food, such as in the poultry industry [20]. The data of salmonellosis surveillance in Colombia shows that more developed regions of Colombia report a higher number of cases each year, a fact that is not consistent with the public health infrastructure of the regions and could be interpreted as a lack of diagnosis in rural areas where diagnostic tools are unavailable [21]. Under-reporting results in registered cases of Colombian surveillance for salmonellosis, which showed that Antioquia state and the city of Bogota (the Colombian capital) contribute 33% and 29.2% of salmonellosis cases, respectively, in a report that covered the period 2000–2013 [21]. The poorest regions of Colombia, such as Chocó state, the Amazonas region, and some states on the Atlantic coast (Sucre, Magdalena, and Guajira) reported 0.1% to 0.3% of the cases [21]. The most frequent non-typhi *Salmonella* (NTS) reported in Colombia during the period 2000–2013 among 7,219 isolates reported were *S. Typhimurium* (33.7%), *S. Enteritidis* (28.6%), *S. Dublin* (3.3%), *S. Derby* (2.1%), *S. Panamá* (2.0%), *S. Braenderup* (1.8%), *S. Saintpaul* (1.7%), *S. Uganda* (1.4%), *Salmonella* spp. (1.3%), and others (24.1%, including *S. Typhi* 9%) [21]. The current study was carried out in Colombian regions where salmonellosis outbreaks were reported during the period of 2005–2010 [20,21], with the aim detecting human carriers of *Salmonella*.

Methodology

This was a cross-sectional survey to identify *Salmonella* carriers in the general population of four towns in three states located in the west, south, and east of Colombia (Quibdó, Guachené, Caloto, and Granada). Locations were chosen based on the report of salmonellosis outbreaks from 2005–2010 [20,21]. In addition, cholecystectomy surgical patients from several cities and towns of Antioquia state were included in this study (see Figure 1).

Volunteers

The general population of the above-mentioned towns were motivated to voluntarily participate in this study after a house-to-house visit by members of the research team. Participants were given individualized instructions regarding food handling and excreta disposal in the form of a brochure designed for this purpose. Instructions and a container to collect one single stool sample per volunteer were provided.

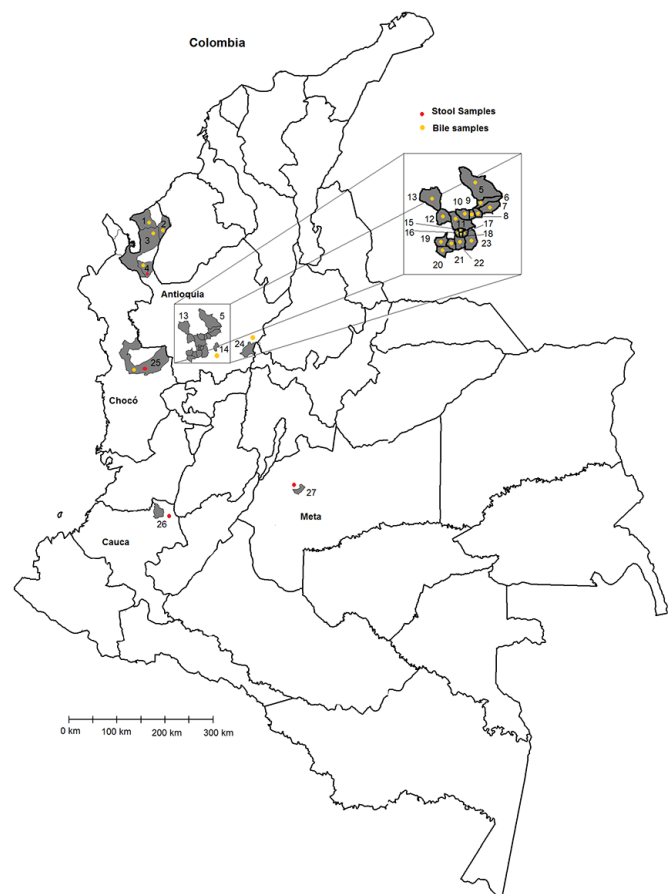
Detection of carriers in bile samples

Initially, local hospitals of the cities and towns where the stool sampling collection occurred were contacted. However, there were limitations due to the lack of surgery services at these locations. It was difficult to perform the study in rural areas. Given the difficulty of sample collection, research was conducted in two clinics in the city of Medellín that have an agreement with rural health services from Antioquia state and other Colombian regions (see Figure 1).

Sampling of bile

Patients who required a cholecystectomy because of their clinical conditions were included in the study after they signed an informed consent form. A piece of gallbladder tissue sample was stored in buffered formalin for the routine histopathology study. A separate piece of gallbladder tissue for DNA extraction was collected in 70% ethanol. The vesicular content was stored in lactose broth (Becton Dickinson, Franklin Lakes, USA).

Figure 1. Stool and bile samples studied based on the municipality of origin.



Stool and bile samples cultures

A total of 667 stool samples were collected and stored in Cary-Blair transport medium (Becton Dickinson, Franklin Lakes, USA) for culture, and in 70% ethanol for polymerase chain reaction (PCR) tests. In total, 199 bile samples were collected from patients who required a cholecystectomy.

Samples were processed at the microbiology laboratory of the Colombian Institute of Tropical Medicine (Medellin, Colombia).

Stool samples were inoculated into selenite broth (Becton Dickinson, Franklin Lakes, USA) for enrichment and incubated at 35°C for 12 hours. Then, 100 µL of the broth was plated on MacConkey and SS (*Salmonella-Shigella*) agars (Becton Dickinson, Franklin Lakes, USA) and incubated for 12 and 24 hours, respectively, at 35°C. Additionally, the colonies grown in SS agar were plated on Rambach agar (Merck, Whitehouse Station, USA) [22].

Bile samples were collected on lactose broth, incubated at 35°C for 12 hours, then plated on blood (Becton Dickinson, Franklin Lakes, USA), MacConkey, and SS agars and incubated for 12 hours at 35°C.

For both samples, biochemical tests were performed from MacConkey agar using the API 20E

commercial kit (bioMérieux, Durham, USA) on compatible colonies.

DNA extraction

DNA extraction from bacterial colonies was made using the boiling prep method [22]. Briefly, five well-isolated colonies were seeded in 200 µL of distilled water, shaken for 10 seconds, boiled for 10 minutes, and centrifuged at 11,000 rpm for 5 minutes [22]. The supernatant was stored in a fresh tube at -20°C until PCR was performed. DNA extraction from stool samples stored in ethanol (where the plate cultures were positive) underwent the process of extracting DNA directly using the UltraClean Fecal DNA Kit (MBio Laboratories, Carlsbad, USA). DNA extraction from bile samples in lactose broth was performed using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany).

PCR

All bacterial isolates, stool, and bile samples collected were tested for *hila* gene specific to *Salmonella* following the method reported [6]. PCR conditions included 40 µL of super mix (Invitrogen, Carlsbad, USA), 0.5 µL of 10 µM of forward and

Table 1. Number of bile and stool samples studied by geographical origin.

Department	City	Bile samples	Stool samples	Characteristics of the region
Antioquia	Medellín	106	-	Urban, Andean region, temperate climate, well to middle developed public health services
	Itagüi	14	-	
	Bello	12	-	
	Envigado	9	-	
	Copacabana	4	-	
	Girardota	3	-	
	Sabaneta	2	-	
	La Estrella	1	-	
	Barbosa	1	-	
	Caldas	5	-	
	Don Matías	2	-	Rural, agricultural region, temperate climate, middle to low developed public health services
	Amagá	1	-	
	Titiribí	1	-	
	Puerto Nare	1	-	
	Venecia	1	-	
	Santa Rosa de Osos	1	-	
	El Retiro	1	-	
	Ebéjico	1	-	
	Santafé de Antioquia	1	-	
Marinilla	1	-	Rural, tropical forest, low public health development	
Apartadó	2	200		
Turbo	2	-		
Chigorodó	2	-	Urban, tropical climate, poor public health development	
Chocó	Quibdó	2		144
Cauca	Guachené	-		204
Meta	Granada	-	119	
TOTAL		199	667	

reverse primers, and 9 µL of DNA to a final volume of 50 µL.

The primers used were [23]:

Forward: 5' - CGG AAG CTT ATT TGC GCC ATG CTG AGG TAG -3';

reverse: 5' - GCA TGG ATC CCC GCC GGC GAG ATT GTG -3'.

Amplification protocol included initial denaturation at 94°C for 5 minutes, 30 cycles at 94°C for 1 minute, 65°C for 1 minute and 72°C for 1 minute, and a final extension phase at 72°C for 10 minutes. Amplicon visualization was performed by electrophoresis in a 1% agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, USA) and photographed in an image analyzer EpiChemi Dark Room (UVP, Upland, USA). The presence of an 854 bp band is a positive indicator for *hliA*. DNA from *S. Enteritidis* strain ICMT10 was used as the positive control [23].

Salmonella serotyping

All the *Salmonella* isolates were serotyped using a multiplex PCR following the established protocol [24,25]. Results of this multiplex PCR were confirmed by conventional serotyping of *Salmonella* at the reference laboratory of the National Institute of Health (Bogotá, Colombia).

Ethical considerations

This study was approved by the ethical committee of the Universidad CES. Informed consent was obtained for every participant.

Results

Samples tested

A total of 667 fecal samples and 199 bile samples were tested. The geographical origins of volunteers included in this study are shown in Figure 1 and Table 1. Briefly, 200 (30%) fecal samples analyzed were from Apartadó (Antioquia state), 144 (21.6%) from Quibdó

(Chocó state), 204 (30.6%) from Guachené (Cauca state), and 119 (17.8%) from Granada (Meta state).

Stool *Salmonella* carriers

Nine (1.35%) stool samples were positive by culture and PCR. The distribution according serotype was as follows. Two samples from Quibdó were positive for *S. Newport*. From Apartadó, two samples were positive for *S. Anatum*, one sample was positive for *S. Sinstorf*, and one was only identified as *Salmonella* spp. In Granada, all the three isolates were identified as *Salmonella* spp. (see Table 2).

Bile *Salmonella* carriers

In bile samples, *S. Enteritidis* was detected by culture in three patients (1.5%) and by PCR in eight patients (4%). Table 2 shows the geographical origin of the isolates and the serotype.

By API, 34 (17.3%) bile samples were positive for bacteria: *Enterobacter cloacae* in six (17.64%), *Pseudomona* spp. in 4 (11.76%), *K. pneumoniae* in four (11.76%), and *S. Enteritidis*, *Pseudomonas fluorescens/putida*, *Raoultella terrigena* in three (8.8%) each. For *Pseudomonas aeruginosa*, *Serratia marcescens*, *Proteus mirabilis*, *Proteus penneri*, *Aeromonas* spp., *Streptococcus* group A, *Escherichia coli*, *Enterobacter sakazakii*, *Stenotrophomonas maltophilia*, and *Klebsiella oxytoca*, one isolate of each was identified.

Discussion

According to the national system of surveillance and control of Colombia (National Institute of Health), from January 2008 to August 2010, 31.7% of 102 diarrheal outbreaks were due to *Salmonella* [20]. *S. Typhimurium*, *S. Enteritidis*, *S. Branederup*, *S. Muenster*, *S. Albany*, *S. Essen*, *S. Nottingham*, *S. Give*, *S. Javiana*, and *S. Derby* were the most frequent serotypes isolated in salmonellosis outbreaks from

Table 2. *Salmonella* isolates from stool and bile culture and/or polymerase chain reaction (PCR) in patients by geographical origin.

City/department	Stool culture and PCR positive	Bile culture positive	Bile PCR
Quibdó/Chocó	<i>S. Newport</i> (2)	Ns	Ns
	<i>S. Anatum</i> (2)	Ns	Ns
Apartadó/Antioquia	<i>S. Sinstorf</i> (1)	Ns	Ns
	<i>S. enterica</i> (1)	Ns	Ns
Granada/Cauca	<i>S. enterica</i> (3)	Ns	Ns
Guachené/Meta	(0)	Ns	Ns
Medellín/Antioquia	Ns	<i>S. Enteritidis</i> (2)	<i>S. Enteritidis</i> (2)
Envigado/Antioquia	Ns	(0)	(0)
Marinilla/Antioquia	Ns	<i>S. Enteritidis</i> (1)	<i>S. Enteritidis</i> (1)
Total	9/667		11/199

NS: no sampling.

2005–2008. However, the source of the transmission was not determined [20]. The origins of the isolates from this study are rural and urban populations of the States of Nariño, Antioquia, Huila, Meta, and Cauca. Cities like Bogotá (the country's capital) and Medellín have high to moderate public health resources [21]. Some of these regions have the infrastructure to support the outbreaks (*i.e.*, laboratory resources and trained personnel). Other, poorer regions of Colombia do not report salmonellosis cases, likely due to their lack of laboratory and clinical resources to perform diagnoses.

S. Typhi outbreaks in Urabá (Antioquia) and in Quibdó (Chocó) were studied to determine the source of infection, notably by examining samples of residual waters in the neighborhoods where the outbreaks were reported. However, the source of outbreak was not determined [21]. Colombia is endemic for infections caused by *Salmonella*, with sporadic outbreaks [5,20,21]. Therefore, it is necessary to develop public health programs to detect carriers of *Salmonella* to reduce the risk of infection in communities.

In the current study, a systematic search for *Salmonella* carriers inhabiting areas where salmonellosis outbreaks occurred was performed to determine possible carriers in the affected populations. *Salmonella* was isolated from nine (1.35%) of 667 stool samples. From 199 bile samples, *S. Enteritidis* was identified by bile culture in three volunteers (1.5%). By PCR, *Salmonella* was detected in eight patients (4%). Projections made from the total population make the number of carriers important for salmonellosis transmission. A limitation of this study is that *Salmonella* serotyping was not available for five of the 8 bile/gallbladder isolates, the same limitation found in 4 of the 9 stool isolates (see Table 2).

Another limitation is the possibility that the incidence of *Salmonella* was underestimated due to the use of alternative isolation methodologies such as lactose broth used for pre-enrichment of bile samples; this has not been reported before for the culture of this kind of samples, but we used this modification to grow different types of bacteria present in bile, and this step could decrease the possibility of isolating *Salmonella*.

Public health actions, such as the active search for *Salmonella* carriers and preventive education for the control of foodborne outbreaks, are the responsibility of the governmental health institutions. However, this study shows the importance of research and academic initiatives in performing activities that influence the health and education of rural communities that might lack optimal public services and are therefore at risk of suffering foodborne outbreaks [18]. This study is

evidence of the importance of collaborative work between local government agencies and research teams to perform this type of community survey. The educational component of this work is important given that the active search for carriers is not enough to control the endemic and epidemic cases of salmonellosis in regions where the carriers were detected; an educational component may influence the behaviour of the population and therefore help to control foodborne infections. The partnership between public health agencies and research institutions identifies public health problems and develops an important educational process for the community.

Conclusions

Salmonellosis in Colombia is endemic. However, salmonellosis cases in rural regions are under-reported due to lack of laboratory infrastructures. The most frequently reported isolates in salmonellosis outbreaks that occurred between 2005 and 2008 are *S. Typhimurium*, *S. Enteritidis*, *S. Branederup*, *S. Muenster*, *S. Albany*, *S. Essen*, *S. Nottingham*, *S. Give*, *S. Javiana*, and *S. Derby*; nevertheless, the source of the transmission was not determined [20], suggesting that surveillance of salmonellosis, both as a zoonosis and as a foodborne disease, needs to be improved.

The *Salmonella* carriers identified in the same regions where the salmonellosis outbreaks were reported could be the source of transmission; however, further studies are needed in order to confirm that the isolates obtained from carriers in the present study are the same that produce the cases and outbreaks.

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