

ABSTRACT

***Salmonella* Enteritidis in the Caribbean: An Epidemiological and Molecular Study in Trinidad and Tobago, Barbados and Jamaica**

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Background: *Salmonella* serotype Enteritidis (SE) is a foodborne pathogen of serious public health concern in the English-speaking Caribbean. SE emerged as a major cause of foodborne illness in the Caribbean in the mid to late 1990s and it is currently the leading cause of human salmonellosis and of all reported foodborne diseases (FBD) in this region. It is also a main cause of FBD outbreaks in hotels, frequently involving tourists. Since the epidemiology of SE has not yet been defined in the Caribbean, this study was undertaken to investigate and define the epidemiology and molecular characteristics of SE infections in this region, specifically in Trinidad and Tobago (T&T), Barbados and Jamaica (which together account for >90% of the reported SE infections), and to provide information for effective prevention and control measures for this pathogen.

Methods: A multidisciplinary approach of epidemiological, microbiological and environmental studies was used to define the epidemiology of SE infections in each country. Retrospective and prospective descriptive studies were conducted to determine the occurrence, demographic and clinical features, and potential risk factors for SE in the three countries. Matched case-control studies were conducted in T&T and Barbados to elucidate the sources and risk factors in sporadic SE cases. SE outbreaks were investigated by cohort studies and by environmental methods to determine the causes and vehicles of infection. Phenotypic (serotyping, phage typing, antimicrobial typing) and molecular (pulsed field gel electrophoresis) laboratory methods were used to identify, type and discriminate SE strains from humans, eggs and foods; to determine their relatedness, phenotypic and genotypic diversity; to trace the source of infection and to provide evidence to support epidemiological findings. Traceback studies of egg-associated SE infections and microbial surveys, were conducted in implicated and other farms, to detect the prevalence of SE in eggs, to identify environmental sources and to assess the role of farm sanitation and management practices in relation to the presence of *Salmonella*. Data was analyzed in Epi Info 6.04c, SAS and Molecular Analyst Fingerprinting Plus software.

Results: This study demonstrated that the epidemiology of *Salmonella* infections in the Caribbean has changed since the emergence of SE, the pathogen surpassing *S. typhimurium* to become the leading cause of salmonellosis. Shell eggs, in particular raw and undercooked eggs and their dishes, were found to be the major source of SE in all three countries. Different egg food vehicles and/or risk practices were identified, which were largely a reflection of the different cultural practices in each country. In T&T, the main SE vehicles were raw egg-containing foods, including cake batter, and raw egg-containing drinks. Undercooked eggs (mainly soft boiled) were the major SE vehicles in Barbados followed by under-cooked chickens. In Jamaica, a significant proportion of the SE cases was linked to outbreaks in hotels and traced to egg-containing foods that were prepared by pooling in bulk quantity. Having an underlying illness, handling raw eggs, and pooling of eggs, were risk factors for acquiring SE. Refrigeration of eggs was protective against SE infection. Risk practices, including pooling of eggs, non-refrigeration of eggs, undercooking, improper preparation and holding of egg-containing

dishes, poor sanitation and hygiene, poor egg-handling and cross contamination, were likely contributory factors to the occurrence of SE infections, especially outbreaks.

Different SE strains [defined by phage type (PT) and genotype (PFP)] were responsible for SE infections in each country, and one or two PTs accounted for >80% of the SE infections in a country. PT4 was predominant in T&T, PT8 was dominant in Barbados, and PTs 4bvar and 4b were most common in Jamaica. The PFPs correlated to a significant extent with the PT patterns of the SE strains. In particular, PTs 4, 8, 4b and 4var were genetically distinct, indicating that they were of different clonal lines and likely to have originated from different sources. Thus, genetically distinct PT-specific SE strains were responsible for most of the SE infections in each country. This confirmed that different epidemiological patterns of SE strains existed in each country, and suggested that the initial entry of SE in these countries may be from different international imported sources of hatching eggs, chicks, or feed.

Differences were also observed in the occurrence of SE with peak infections in the December-January festive seasons in T&T, in contrast to the May-August dominated in children <10 years in all three countries. Of the local SE cases interviewed, >70% were from the lower income/ socio-economic grouping. Some did not have refrigerators, and many did not know of food safety practices. Over 60% of the SE cases in T&T and Jamaica purchased eggs from un-refrigerated sources and did not refrigerate at home.

In all three countries, SE was detected in eggs from farms implicated as sources in egg-associated SE infections. The dominant PTs and their PFPs found in human SE strains in each country, were also found in the eggs and farm SE strains, at a similar prevalence to humans. The identical PT and PFP were also found in all epidemiologically-implicated SE strains (human, implicated food and eggs) from a particular SE infection, for all SE infections investigated by these methods. These results demonstrated that epidemiologically-related strains were also phenotypically and genetically related, hence the same strain. This also provided microbiological evidence to confirm that shell eggs and layer flocks were the major sources and reservoirs for SE in each country. It also explained in part the dominance of the specific SE strain(s) in human infections, and provided phenotypic and genetic evidence to confirm that the dominant PT causing SE infection in each country, originated from its respective local flocks.

The presence and prevalence of SE-contaminated eggs in implicated flocks in all three countries were established, showing a wide variation between country and farm-specific prevalence. Contamination of the internal contents of some eggs with SE was deduced to be likely through transovarian transmission. Several environmental sources of SE were identified in implicated farms in T&T, with manure/litter/faeces being most common. Unsanitary farm conditions (especially faecally contaminated wet litter, high manure/waste build-up, presence of rodents, dirty pens and environs) and poor practices (particularly cleaning eggs in stagnant water, molting, non-refrigeration, overcrowding, infrequent disinfection), were observed on all SE-positive farms, contributing possibly to the spread and continued existence of SE contamination on these farms. Farms with higher levels of unsanitary conditions had increased levels of SE contamination.

Finally, several findings from this study were described for the first time, including: the implication of raw cake batter and macaroni and eggs as food vehicles for SE infection; the identification of PTs 4bvar and 5var as new SE strains; the demonstration that different clonal lines existed within strains of PT8 and within strains of PT4bvar, and the separation of strains of PTs 4a, 4b, 4bvar, 5var, 12, 20a, 24 and (4b and 4bvar together), (6 and 21 together) and (8 and 2 together), into different clonal lines.

Conclusion: In conclusion, this study has elucidated the epidemiology of SE infections in the English-speaking Caribbean, as represented by T&T, Barbados and Jamaica. This was the first time such a comprehensive study on the epidemiology of SE infections was conducted in this region, providing new, invaluable and detailed knowledge on the epidemiology of SE, and establishing baseline data needed for developing effective prevention and control measures for this pathogen. It has demonstrated through a huge body of evidence that raw and undercooked eggs are the major source of SE infections in the Caribbean, that infected layer flocks are the major reservoir for SE in each country, and that certain poor food cultural, food preparation, food safety and farm practices contribute to the problem. This research has also highlighted that the epidemiology of SE, although egg-associated, differed in the countries, shown by different egg-containing food vehicles, trends and genotypically distinct SE strains. A multidisciplinary, integrated 'farm to table' approach, encompassing the entire continuum from production to consumption is thus recommended for effective prevention and control of SE in the Caribbean. These include: on-farm interventions (*Salmonella*-free chicks, feed, improved farm sanitation, rodent control, quality assurance programs); educational interventions on food safety practices for SE (emphasizing safe egg handling and storage practices, thorough cooking of all egg-containing dishes, avoiding the use of raw eggs and pooling of eggs, cooking eggs singly, refrigerated storage of shell eggs), personal hygiene, food sanitation measures, enhanced and continuous surveillance for *Salmonella*, research and legislation.